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Appendix 1

STUDY PARTICIPANTS

The following institutions and investigators participated in the trial:

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Appendix 2

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SNP Communication

Genetic Variations and Haplotype Structures of the Glutathione *S*-transferase Genes, *GSTT1* and *GSTM1*, in a Japanese Patient Population

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Full text of this paper is available at http://www.jstage.jst.go.jp/browse/dmpk

Summary: Glutathione *S*-transferases (GSTs) play a vital role in phase II biotransformation of many synthetic chemicals including anticancer drugs. Deletion polymorphisms in *GSTT1* and *GSTM1* are reportedly associated, albeit controversial, with an increased risk in cancer as well as with altered responses to chemotherapeutic drugs. In this study, to elucidate the haplotype structures of *GSTT1* and *GSTM1*, genetic variations were identified in 194 Japanese cancer patients who received platinum-based chemotherapy. Homozygotes for deletion of *GSTT1* (*GSTT1*0/*0* or null) and *GSTM1* (*GSTM1*0/*0* or null) were found in 47.4% and 47.9% of the patients, respectively, while 23.2% of the patients had both *GSTT1* null and *GSTM1* null genotypes. From homozygous (+/+) and heterozygous (*0/+) patients bearing *GSTT1* and *GSTM1* genes, six single nucleotide polymorphisms (SNPs) for *GSTT1* and 23 SNPs for *GSTM1* were identified. A novel SNP in *GSTT1*, 226C>A (Arg76Ser), and the known SNP in *GSTM1*, 519C>G (Asn173Lys, **B*), were found at frequencies of 0.003 and 0.077, respectively. Using the detected variations, *GSTT1* and *GSTM1* haplotypes were identified/inferred. Three and six common haplotypes ($N \geq 10$) in *GSTT1* and *GSTM1*, respectively, accounted for most (>95%) inferred haplotypes. This information would be useful in pharmacogenomic studies of xenobiotics including anticancer drugs.

Keywords: *GSTT1*; *GSTM1*; nonsynonymous SNP; haplotype; haplotype-tagging SNP

Introduction

Glutathione *S*-transferases (GSTs) (EC 2.5.1.18) are dimeric phase II metabolic enzymes that mainly catalyze conjugation of reduced glutathione (GSH) with a variety of electrophilic compounds including carcinogens, therapeutic drugs and environmental toxins as well as endogenous substances.[1] In addition, GSTs possess selenium-independent GSH peroxidase activity to reduce organic hydroperoxides, and therefore, play significant roles in detoxification, occasionally toxification, and cellular protection against oxidative stress.[2] Noncatalytical-

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On April 28th, 2008, the novel variations described in this paper were not found in the Japanese Single Nucleotide Polymorphisms (JSNP) (http://snp.ims.u-tokyo.ac.jp/), dbSNP in the National Center for Biotechnology Information (http://www.ncbi.nlm.nih.gov/SNP/) or SNP500Cancer Database (http://snp500cancer.nci.nih.gov/).
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ly, GSTs modulate signaling pathways by interacting with protein kinases[3] and by binding numerous ligands for nuclear hormone receptors.[4]

Human GSTs are composed of three main families: cytosolic, mitochondrial and microsomal (or membrane-bound). The cytosolic family, which is principally involved in biotransformation of toxic xenobiotics, contains at least 17 genes subdivided into seven separate classes designated alpha, mu, pi, sigma, theta, zeta, and omega.[5,6] Increasing numbers of GST genes are identified as polymorphic.

The θ-class enzyme GSTT1 and the μ-class enzyme GSTM1 exhibit gene deletion polymorphisms (*GSTT1*0* and *GSTM1*0*, respectively).[7] The null genotype of *GSTT1* (*GSTT1*0/*0*) is found in 15–40% of Caucasians and 50–60% of Asians.[7] On the other hand, about half of both Japanese and Caucasians and 30% of Africans are homozygous for the *GSTM1* deletion (*GSTM1*0/*0*).[7] In intact *GSTM1*, alleles **A* and **B* are used to discriminate the single nucleotide polymorphism (SNP) with amino acid substitution (thereafter, nonsynonymous SNP), 519C>G (Asn173Lys) in exon 7, in which both alleles encode proteins that are catalytically identical for the substrates, 1-chloro-2,4-dinitrobenzene (CDNB), *trans*-4-phenyl-3-buten-2-one (tPBO) and 1,2-epoxy-3-(*p*-nitrophenoxy)propane (EPNP).[8] In addition, a tandem duplication in *GSTM1* associated with ultrarapid enzyme activity was observed in Saudi Arabians.[9] A gene-dose effect has been clearly established: that is, homozygously deleted (**0/*0*), heterozygously (**0/+*) and homozygously intact (+/+) *GST* genotypes correspond to non-, intermediate, and high conjugators, respectively.[10,11]

A large number of association studies on *GSTM1* and *GSTT1* null genotypes have been performed with inter-individual differences in susceptibility to environmental toxins, cancer and other diseases, and in the outcomes of anticancer treatments. Increased risk of lung, bladder, breast and colon cancers were observed in carriers of *GSTM1* or *GSTT1* null genotypes, while other studies have reported controversial findings.[5-7] As for response to anti-cancer drugs, pharmacodynamic correlations have been investigated, but the obtained results are inconsistent.[6] It should be pointed out that despite the possible gene-dose effect, most association studies were only focused on null genotypes of *GSTM1* and/or *GSTT1*. Therefore, in addition to nonconjugators, discrimination between high and intermediate conjugators would be valuable to evaluate the clinical relevance of these GST loci. Also, certain SNPs in the intact genes might affect either the expression of the gene or the activity of the encoded enzyme.

In this study, we first determined the deletion genotypes (**0/0*, **0/+*, and +/+) of *GSTM1* and *GSTT1* by conventional PCR and TaqMan real-time quantitative PCR for 194 Japanese cancer patients treated by platinum-based chemotherapy. Then, we resequenced the homozygous and heterozygous intact *GSTM1* and *GSTT1* genes. Lastly, linkage disequilibrium (LD) and haplotype analyses were performed using the detected SNPs.

Materials and Methods

Human genomic DNA samples: All 194 patients participating in this study were administered carboplatin or nedaplatin in combination with paclitaxel for treatment of various cancers (mainly non-small cell lung cancers) at the National Cancer Center. Genomic DNA was extracted from blood leukocytes from all subjects prior to the chemotherapy. The ethical review boards of the National Cancer Center and National Institute of Health Sciences approved this study. Written informed consent was obtained from all subjects.

Conventional PCR amplification of the *GSTT1* deletion junction: We used the genotyping assay described by Sprenger *et al.*,[10] in which 1460 (for **0* allele) and 466 bp (for exon 5 of the wild-type) PCR fragments were coamplified by multiplex PCR. PCR reactions were performed according to their method with minor modification.[10] Briefly, PCR mixtures contained 100 ng of genomic DNA, 0.2 μM each of the 4 primers reported previously, 0.2 mM each of four deoxynucleotide triphospates (dNTPs), and 0.75 units of HotStarTaq polymerase (Qiagen, Tokyo, Japan) in a 50 μl volume. The PCR conditions were 95°C for 15 min, followed by 30 cycles of 94°C for 30 sec, and 65°C for 1.5 min. PCR fragments were analyzed on 1% agarose gels with ethidium bromide in TAE buffer.

Conventional PCR amplification of *GSTM1*: We used the method of McLellan *et al.* (1997),[9] in which exons 3 to 5 of *GSTM1* were coamplified with β-globin as an internal standard by multiplex PCR. The PCR reactions were carried out according to their method[9] except that 100 ng of genomic DNA and 0.75 units of HotStar-Taq polymerase (Qiagen) were used in a 50 μl total volume. The PCR conditions were 94°C for 15 min, followed by 30 cycles of 94°C for 48 sec, 62°C for 48 sec, and 72°C for 1.5 min, and then a final extension for 5 min at 72°C.

Quantitative real-time PCR for *GSTM1* and *GSTT1*: Quantitative real-time PCR using the TaqMan (5′-nuclease) assay system was carried out according to the method of Covault *et al.*,[12] in which the amounts of target *GSTM1* or *GSTT1* were quantified relative to those of the reference β-2-microglobulin (*B2M*) or cannabinoid receptor 1 (*CNR1*), respectively. Briefly, triplicate reactions were performed for 5 ng of genomic DNA used as a template in 1x TaqMan Universal PCR Master Mix with Amp Erase (50 μl) (Applied Biosystems, Foster City, CA, USA). The thermal cycling conditions were 50°C for 2 min and then 95°C for 10 min, followed by 40 cycles of

95°C for 20 sec and 60°C for 1 min with the 7500 Real-Time PCR System (Applied Biosystems).

***GSTT1* DNA sequencing:** The heterozygous and homozygous samples for *GSTT1* (*0/+ and +/+), the 5′-flanking region (up to 801 bp upstream from the translation start site), all 5 exons with their surrounding introns and the 3′-flanking region were amplified by PCR and directly sequenced. For the 1st round PCR, the reaction mixtures contained 25 ng of genomic DNA, 1.25 units of Ex-Taq (Takara Bio. Inc. Shiga, Japan), 0.2 mM dNTPs, and 0.2 μM primers listed in **Table 1**. The PCR conditions were 94°C for 5 min, followed by 30 cycles of 94°C for 30 sec, 60°C for 1 min, and 72°C for 2 min; and then a final extension for 7 min at 72°C. The regions from 5′-flanking to exon 1 and from exon 4 to 3′-flanking were amplified separately by the nested PCR with Ex-Taq (1.25 units) and the primer sets (0.2 μM) listed in "2nd round PCR" of **Table 1**. The 2nd round PCR conditions were the same as described in the 1st round PCR. The 2nd round PCR products and the 1st round PCR products for exons 2 and 3 were then treated with a PCR Product Pre-Sequencing Kit (USB Co., Cleveland, OH, USA) and were directly sequenced on both strands using an ABI BigDye Terminator Cycle Sequencing Kit (Applied Biosystems) with the sequencing primers listed in **Table 1** (Sequencing column). Excess dye was removed by a DyeEx96 kit (Qiagen, Hilden, Germany). Eluates were analyzed on an ABI Prism 3730 DNA Analyzer (Applied Biosystems). All novel SNPs were confirmed by repeated sequencing of the PCR products generated by new genomic DNA amplifications. The genomic and cDNA sequences of *GSTT1* obtained from GenBank (NT_011520.11 and NM_000853.1, respectively) were used as reference sequences.

***GSTM1* DNA sequencing:** For samples with *0/+ and +/+, genetic variations were identified by resequencing. Particular attention was paid to avoid amplification of sequences of other homologous *GSTMs* because exon 8 of *GSTM1* is 99% identical to that of *GSTM2*.[13] We confirmed that PCR fragments were not amplified from samples with *GSTM1**0/*0 genotypes to evaluate primer specificities. The entire *GSTM1* gene except for the region through exon 8 to the 3′-flanking region was amplified in the 1st round of PCR from 25 ng of genomic DNA utilizing 1.25 units of Ex-Taq with 0.2 μM of primers listed in **Table 2**. Next, three regions (from 5′-flanking to exon 3, from exon 4 to 5, and from exon 6 to 7), were separately amplified in the 2nd round PCR from the 1st round PCR product by Ex-Taq (0.625 units) with 0.2 μM primers listed in **Table 2**. The region from exon 8 to the 3′-flanking was separately amplified from 25 ng of genomic DNA using 0.625 units of Ex-Taq with 0.2 μM primers (listed in **Table 2**). All PCR conditions were the same as those described for *GSTT1*. PCR products were then directly sequenced with the primers listed in

Table 1. *GSTT1* primer sequences

		Amplified and sequenced region	Forward primer		Reverse primer		PCR product (bp)
			Sequence (5′ to 3′)	Position[a]	Sequences (5′ to 3′)	Position[a]	
1st round PCR	multiplex	5′-flanking (up to −1366) to exon 1	CACTCCCGCCCCAAATTAGGTT	3776166	ATGATCCCCACCCCTTTATTCG	3774444	1723
		Exon 4 to 3′-flanking region	ATCACAAGGTCAGGAGATTG	3767902	ACTCTTGGCAAACATCAGGG	3766589	1314
		Exon 2	ACATAATCTCTTCTGCAAACTG	3773267	TGTCTCAAGGATACTCTCACCA	3772011	1257
		Exon 3	GCAAATTGTCAGAAAGGTTAAAGA	3770734	CCCACCTCCTGATTAGCTTAGAAG	3768725	2010
2nd round PCR		5′-flanking (up to −801) to exon 1	TTTCAGTGGGATTCGTTTTAGA	3775601	CCCCGTGGTCTATTCCGTGA	3774478	1124
		Exon 4 to 3′-flanking region	CATCACTAATCATTAGGGAA	3767648	CTGGGAAGGGGGTTGTCTTT	3766628	1021
Sequencing		5′-flanking (up to −801)	TTTCAGTGGGATTCGTTTTAGA	3775601	GGCTCGCTCATTTCACTTAG	3775090	
		Exon 1	GGTGGGAAATTCTGACACAC	3775162	CCCCGTGGTCTATTCCGTGA	3774478	
		Exon 2[b]	AAGGGACAAGGTAGTCAGTC	3772758	AACTGGAATAGCAGGAAGGC	3772099	
		Exon 3[b]	AAAAAAAGCGACTATGTATGAAAT	3770153	AGATAAAATGGATGAACAGATGGT	3769662	
		Exon 4	CATCACTAATCATTAGGGAA	3767648	CAGACTGGGGATGGATGGTTGT	3767204	
		Exon 5 to 3′-flanking region	CATCCCCAGTCTGTACCCTTTTCC	3767216	CTGGGAAGGGGGTTGTCTTT	3766628	

[a]The nucleotide position of the 5′ end of each primer on NT_011520.11.
[b]For exons 2 and 3, the 1st round PCR product was directly sequenced.

Table 2. *GSTM1* primer sequences

	Amplified and sequenced region	Forward primer		Reverse primer		PCR product (bp)
		Sequence (5′ to 3′)	Position[a]	Sequences (5′ to 3′)	Position[a]	
1st round PCR	5′-flanking (up to −1309) to exon 7	CCACAAACAAGTTTATTGGGCG	6136872	GTACTAGACATCAATGTCACCGTT	6141347	4476
	Exon 8 to 3′-flanking region	ACAGTGAGATTTTGCTCAGGTATT	6142766	CTCAATTCTAGAAAAGAGCGAG	6145058	2293
2nd round PCR	5′-flanking (up to −650) to exon 3	GACCACATTTCCTTTACTCTGG	6137531	TAAGAATACTGTCACATGAACG	6139231	1701
	Exon 4 to 5	TCTGTGTCCACCTGCATTCGTTCA	6139192	CTGAACACAAACTTTACCATAC	6139883	692
	Exon 6 to 7	CTAATAAATGCTGATGTATCCAAT	6140410	CCTACTATTGCCAGCTCCATCTAT	6141315	906
Sequencing	5′-flanking (up to −650)	GTCCTTCCTATACCACTGACAC	6137567	AACCGAGCAGGGCTCAGAGTAT	6138145	
	Exon 1 to 2	CCCTGACTTCGCTCCCGGAAC	6137956	GGACACCCGTCCCAATTAGACA	6138764	
	Exon 3	TCTGCCCACTCACGCTAAGTTG	6138577	TAAGAATACTGTCACATGAACG	6139231	
	Exon 4 to 5	TCTGTGTCCACCTGCATTCGTTCA	6139192	CTGAACACAAACTTTACCATAC	6139883	
	Exon 6 to 7	CTAATAAATGCTGATGTATCCAAT	6140410	CCTACTATTGCCAGCTCCATCTAT	6141315	
	Exon 8[b]	GAACTTCTGTTTCCCACATGAG	6143164	GAGTAAAGATGGGAATAAACAG	6143735	
	3′-untranslated and flanking region[b]	TCGTTCCTTTCTCCTGTTTATT	6143701	CCTTGGGGTCCTATTCAATGAG	6144362	

[a]The nucleotide position of the 5′ end of each primer on NT_019273.18.
[b]For the region from exon 8 to 3′-flanking, the 1st round PCR product was directly sequenced.

"sequencing" of **Table 2** as described above for *GSTT1*. All novel SNPs were confirmed by repeated sequencing of PCR products that were newly generated by amplification of genomic DNA. The genomic and cDNA sequences of *GSTM1* obtained from GenBank (NT_019273.18 and NM_000561.2, respectively) were used as reference sequences.

Linkage Disequilibrium (LD) and haplotype analyses: Hardy-Weinberg equilibrium and LD analyses were performed by SNPAlyze ver 7.0 (Dynacom Co., Yokohama, Japan). Pairwise LD ($|D'|$ and r^2 values) between two variations was calculated using 102 subjects bearing one or two *GSTT1* genes and 101 subjects bearing one or two *GSTM1* genes. Some haplotypes were unambiguous from subjects with heterozygous **0* alleles. Diplotype configurations were inferred based on estimated haplotype frequencies using Expectation-Maximization algorithms by SNPAlyze software, which can handle multiallelic variations. Haplotypes containing SNPs without any amino acid change were designated as **1*, and nonsynonymous SNP-bearing haplotypes were numerically numbered. Subtypes were named in their frequency order by use of alphabetical small letters.

Results

Determination of deletion polymorphisms in *GSTM1* and *GSTT1*: Both conventional PCR[10] and TaqMan real-time PCR[12] were used to identify deletion of *GSTT1*. By conventional PCR, 92 out of 194 subjects (frequency = 0.474) were assigned as *GSTT1*0/*0*. For all 92 samples with *GSTT1*0/*0*, no significant fluorescence derived from *GSTT1* amplification was detected by TaqMan real-time PCR (mean cycle threshold, Ct, 37.6). Eighty-two (frequency = 0.423) and 20 (frequency = 0.103) subjects were identified as heterozygous (**0/+*) and homozygous (*+/+*) for intact *GSTT1* by conventional PCR, respectively. In the TaqMan real-time PCR, the mean ± SD of relative amounts of *GSTT1* was 1.0 ± 0.111, and 0.448 ± 0.058 for homozygous and heterozygous *GSTT1* carriers, respectively (the mean value for the 20 homozygotes was set as 1). Since the maximum relative amount of *GSTT1* was 1.214, no gene duplication could be inferred for *GSTT1*. The assigned genotypes were consistent between both methods, and their frequencies (**Table 3a**) were in Hardy-Weinberg equilibrium ($p = 0.785$ by Pearson's chi-square test).

As for *GSTM1*, conventional PCR[9] indicated that 93 out of 194 subjects had a homozygous deletion of *GSTM1* (**0/*0*), and that the remaining 101 subjects were either heterozygotes (**0/+*) or homozygotes (*+/+*) for intact *GSTM1*. By real-time PCR, Ct values of 93 samples with the null genotypes were greater than 36.5, which exceeded the sensitivity limits (Ct = 35) of the real-time PCR detection system, indicating that both methods gave consistent results for *GSTM1*0/*0*. As for the 101 subjects with intact *GSTM1* genes (either **0/+* or *+/+*), the distribution of relative amounts of *GSTM1* was clustered into two groups with 1.0 ± 0.083 (16 homozygotes), and 0.51 ± 0.048 (85 heterozygotes) when the mean value of the 16 homozygotes was set as 1. No individuals showed relative amounts more than 1.216, suggesting that the duplication in *GSTM1*[9] was not present in our population. Thus, the frequencies of *GSTM1*0/*0*, **0/+*, and *+/+*, were 0.479, 0.438, and 0.082, respectively (**Table 3a**), and in Hardy-Weinberg equilibrium ($p = 0.576$ by the Pearson's chi-square test).

Table 3b summarizes the results of the distribution of *GSTM1* and *GSTT1* deletions in our Japanese population.

About one-fourth (45 of 194 subjects) were null for both *GSTM1* and *GSTT1* genes.

Variations found in the intact *GSTT1* gene and their LD profiles: Six variations including three novel ones were found by sequencing the 5′-flanking regions, all 5 exons and their flanking regions in the 102 Japanese subjects with *0/+ and +/+ genotypes (**Table 4**). All detected variations were in Hardy-Weinberg equilibrium ($p \geq 0.44$ by the χ^2 test or Fisher's exact test) when assuming the presence of three alleles (wild, variant and *0 alleles) at each site. One novel nonsynonymous variation, 226C>A (Arg76Ser), was heterozygous in one subject with two intact *GSTT1* genes, and its allele frequency was 0.003 (1/388). The remaining two novel variations in the intronic regions (IVS1+71A>G and IVS2−8A>C) were also rare (allele frequency = 0.003 for both).

Three known variations (IVS1+166A>G, IVS3−36C>T and 824T>C) were found at a relatively high frequency (0.106) and were perfectly linked ($r^2 = 1.0$) with each other.

Variations found in the intact *GSTM1* gene and their LD profile: We found 23 variations, including seven novel ones, in 194 Japanese cancer patients (**Table 5**). Ten variations were located in the 5′-flanking region, 2 in the coding exons, 9 in the introns, and 2 in the 3′-flanking region. All detected variations were in Hardy-Weinberg equilibrium ($p > 0.37$ by the χ^2 test or Fisher's exact test) except for 1107+41C>T in the 3′-flanking region ($p = 0.003$ by the Fisher's exact test). Deviation from Hardy-Weinberg equilibrium for this variation was due to 2 more homozygotes than expected among 16 *GSTM1* +/+ subjects.

Seven novel variations, −416G>T and −165A>G in the 5′-flanking region, IVS1+97C>T, IVS1−79G>A, IVS1−78T>A, and IVS2+202G>A in the introns and 1107+128G>A in the 3′-flanking region, were found in single subjects (allele frequencies = 0.003). No novel nonsynonymous SNPs were detected.

Sixteen other variations were already reported or publicized in the dbSNP and/or JSNP databases. They were detected in more than 10 chromosomes (allele frequencies ≥0.026) in our population except for −423C>G and IVS2+118T>C (allele frequency = 0.003).

The pairwise |D′| values between 14 common variations (N≥10) in *GSTM1* were higher than 0.95 except for the combinations between −480A>G and other variations, which showed lower |D′| values ($0.27 < |D'| < 1.0$). As for the r^2 values, strong LDs ($r^2 > 0.87$) were observed among 10 variations,

Table 3. Frequencies of *GSTT1* and *GSTM1* deletions

(a)

	Genotype	N	Frequency (%)	Allele	N	Frequency (%)
GSTT1	*0/*0	92	0.474	*0	266	0.686
	*0/+	82	0.423			
	+/+	20	0.103	+	122	0.314
GSTM1	*0/*0	93	0.479	*0	271	0.698
	*0/+	85	0.438			
	+/+	16	0.082	+	117	0.302

(b)

Genotype combination		N	Frequency (%)
GSTT1	*GSTM1*		
*0/*0	*0/*0	45	0.232
	*0/+	42	0.216
	+/+	5	0.026
*0/+	*0/*0	39	0.201
	*0/+	34	0.175
	+/+	9	0.046
+/+	*0/*0	9	0.046
	*0/+	9	0.046
	+/+	2	0.010

*0, deletion; +, intact gene

Table 4. Summary of *GSTT1* SNPs detected in a Japanese population

SNP ID			Location	Position		Nucleotide change and flanking sequences (5′ to 3′)	Amino acid change	Allele frequency (N=388)
This study	dbSNP (NCBI)	JSNP		NT_011520.11	From the translational initiation site or from the end of nearest exon			
MPJ6_GTT1001[a]			intron1	3774618	IVS1+71A>G	catagcttagggA/Gacttctcccagc		0.003
MPJ6_GTT1002	rs140313	ssj0002194	intron1	3774523	IVS1+166A>G	gatccaagagtcA/Ggggctccccaaa		0.106
MPJ6_GTT1003[a]			intron2	3770088	IVS2-8A>C	catgacccccacA/Ccccacagtgtgg		0.003
MPJ6_GTT1004[a]			Exon3	3770055	226C>A	ctctacctgacgC/Agcaaatataagg	Arg76Ser	0.003
MPJ6_GTT1005	rs140308		intron3	3767603	IVS3-36C>T	ctaactccctacC/Tccagtaactccc		0.106
MPJ6_GTT1006	rs4630	ssj0002197	3′-UTR	3766891	824(*101[b])T>C	ggaatggcttgcT/Ctaagacttgccc		0.106

[a]Novel variations detected in this study.
[b]The nucleotide that follows the translation termination codon TGA is numbered and starts as *1.

Table 5. Summary of *GSTM1* SNPs detected in a Japanese population

SNP ID			Location	Position		Nucleotide change and flanking sequences (5′ to 3′)	Amino acid change	Reported alleles	Allele frequency (N=388)
This study	dbSNP (NCBI)	JSNP		NT_019273.18	From the translational initiation site or from the end of nearest exon				
MPJ6_GTM1001	rs412543	ssj0002146	5′-flanking	6137629	−552C>G	agactaagccctC/Gggagtagctttc			0.044
MPJ6_GTM1002	rs3815029	ssj0002147	5′-flanking	6137641	−540C>G	gggagtagctttC/Gggatcagaggaa			0.026
MPJ6_GTM1003	rs412302	ssj0002148	5′-flanking	6137701	−480A>G	tcccaggttgggA/Gccaccacttttt			0.064
MPJ6_GTM1004	rs3815026		5′-flanking	6137758	−423C>G	cccttgggaactC/Gggcagcggagag			0.003
MPJ6_GTM1005[a]			5′-flanking	6137765	−416G>T	gaactcggcagcG/Tgagagaaggctg			0.003
MPJ6_GTM1006	rs4147561	ssj0002149	5′-flanking	6137783	−398C>T	aaggctgagggaC/Taccgcgggcagg			0.077
MPJ6_GTM1007	rs4147562	ssj0002150	5′-flanking	6137784	−397A>T	aggctgagggacA/Tccgcgggcaggg			0.077
MPJ6_GTM1008	rs4147563	ssj0002151	5′-flanking	6137788	−393T>C	tgagggacaccgT/Cgggcagggagga			0.080
MPJ6_GTM1009	rs28549287	ssj0002152	5′-flanking	6137823	−358G>A	gagctttgctccG/Attaggatctggc			0.075
MPJ6_GTM1010[a]			5′-flanking	6138016	−165A>G	cttactgagtgcA/Ggccccaggcgcc			0.003
MPJ6_GTM1011[a]			intron1	6138313	IVS1+97C>T	tcctcttcagggC/Ttgcccgcctcag			0.003
MPJ6_GTM1012[a]			intron1	6138398	IVS1−79G>A	ggtacgtgcagtG/Ataaactgggggc			0.003
MPJ6_GTM1013[a]			intron1	6138399	IVS1−78T>A	gtacgtgcagtgT/Aaaactgggggct			0.003
MPJ6_GTM1014	rs4147564	ssj0002153	intron2	6138670	IVS2+118T>C	ctgcaggctgtcT/Ccttccctgagcc			0.003
MPJ6_GTM1015[a]			intron2	6138754	IVS2+202G>A	ctgtctaattggG/Aacgggtgtccct			0.003
MPJ6_GTM1016	rs737497	ssj0002154	intron3	6139277	IVS3−78C>T	cccggtctcctcC/Tctgctcttgctt			0.077
MPJ6_GTM1017	rs4147565	ssj0002155	intron4	6139462	IVS4+26A>G	gctgcaatgtgtA/Gggggggaaggtgg			0.080
MPJ6_GTM1018	rs4147566	ssj0002156	intron5	6139772	IVS5+140C>T	cagttattctcaC/Tgactccaatgtc			0.077
MPJ6_GTM1019	rs1065411	ssj0002159	Exon7	6140823	519C>G	atttgagcccaaC/Gtgcttggacgcc	Asn173Lys	*B	0.077
MPJ6_GTM1020	rs1056806	ssj0002160	Exon7	6140832	528C>T	caagtgcttggaC/Tgccttcccaaat	Asp176Asp		0.077
MPJ6_GTM1021	rs4147569	ssj0002161	intron7	6143292	IVS7−221G>A	tgtagaatcttcG/Ataagtgttagct			0.080
MPJ6_GTM1022	rs4147570	ssj0002162	3′-flanking	6144093	1107(*450)+41C>T[b]	ctggccatctacC/Tcagactgtctgt			0.026
MPJ6_GTM1023[a]			3′-flanking	6144180	1107(*450)+128G>A[b]	ggattctgctggG/Acatagtaaggcg			0.003

[a]Novel variations detected in this study.
[b]The position of the 3′ end of exon 8 (1107 or *450)+the position in the 3′-flanking region. (*450 indicates the position from the termination codon TAG.)

−398C>T, −397A>T, −393T>C, −358G>A, IVS3−78C>T, IVS4+26A>G, IVS5+140C>T, 519C>G (Asn173Lys), 528C>T (Asp176Asp), and IVS7−221G>A. Of these variations, two (−398C>T and −397A>T) and four (IVS3−78C>T, IVS5+140C>T, 519C>G, and 528C>T) pairs of SNPs were in perfect LD ($r^2 = 1.0$).

Haplotype estimation and selection of haplotype-tagging SNPs (htSNPs): Based on results of the LD profiles, haplotypes of *GSTT1* and *GSTM1* were analyzed as one LD block that spans at least 7.7 kb and 6.5 kb, respectively. Using the six variations and null alleles in *GSTT1*, three common haplotypes (*GSTT1*0*, **1a* and **1b*) and three rare haplotypes (**1c*, **1d* and **2a*) were identified or inferred (**Fig. 1a**). Frequencies of the common haplotypes, **0*, **1a*, and **1b*, were 0.686, 0.201, and 0.106, respectively. Thus, the htSNPs are either one of IVS1+166A>G, IVS3−36C>T, and 824T>C for **1b* and 226C>A for **2*.

For the *GSTM1* gene, three groups of haplotypes (*GSTM1*0*, **1* and **2*), each containing 1, 10 and 4 subtypes, were identified or inferred using the 23 variations and the null allele (**Fig. 1b**). The **2* group (**2a* to **2d*) was defined as the haplotypes harboring the known nonsynonymous SNP, 519C>G (Asn173Lys), which was previously assigned **B*.[8] The most dominant haplotype was **0* (0.698 frequency), followed by **1a* (0.139), **2a* (0.044), **1b* (0.026), **1c* (0.026), and **2b* (0.026). These six haplotypes accounted for 95% of all haplotypes. The htSNPs that were able to resolve the 5 common haplotypes of the intact genes were −552C>G (**1b* and **1d*), −540C>G (**2b*), −480A>G (**1b* and **2b*), 519C>G (Asn173Lys) (**2*), and 1107+41C>T (**1c*).

Discussion

The present study provides the first comprehensive data on genetic variations of *GSTT1* and *GSTM1* in Japanese, the genes encoding the phase II metabolic enzymes important for cellular defense systems. Moreover, SNPs in intact genes were identified by resequencing, and haplotype structures and tagging SNPs were shown.

It is well recognized that **0* alleles in *GSTT1* and *GSTM1* distribute with different frequencies in several ethnicities. We have shown that 47.4% and 47.9% of our Japanese population homozygously lack *GSTT1* (*GSTT1*0/*0*) and *GSTM1* (*GSTM1*0/*0*), respectively. The *GSTT1*0/*0* frequency is comparable to that reported previously in Japanese (54.0%)[14] and east Asians such as Koreans (46–62%)[7,15] and Chinese (49–58%),[16,17] but was higher than Malay (38%),[17] Indians (16%),[17] Caucasians (15–24%),[7,18] African Americans (22–24%),[7,18] Mexican Americans (10%),[7] and Scandinavians (15%).[7] On the other hand, no marked differences are found in the frequencies of *GSTM1*0/*0* between Caucasians (42–60%)[7,18] and East Asians including Japanese, Koreans

(a) *GSTT1*

Nucleotide change		IVS1+71 A>G	IVS1+166 A>G	IVS2-8 A>C	226C>A	IVS3-36 C>T	824T>C	Number	Frequency
Amino acid change					Arg76Ser				
*0	*0	whole deletion						266	0.686
*1	*1a							78	0.201
	*1b							41	0.106
	*1c							1	0.003
	*1d†							1	0.003
*2	*2a†				2			1	0.003
								388	1.000

(b) *GSTM1*

Nucleotide change		-552C>G	-540C>G	-480A>G	-423C>G	-416G>T	-398C>T	-397A>T	-393T>C	-358G>A	-165A>G	IVS1+197 C>T	IVS1+390G>A	IVS1+787G>A	IVS2+118 T>C	IVS2+202 G>A	IVS3-78C>T	IVS4+26 A>G	IVS5+140 C>T	519C>G	528C>T	IVS7-221G>A	1107+41 C>T	1107+128 G>A	Number	Frequency
Amino acid change																				Asn173Lys	Asp176Asp					
*0	*0	whole deletion																							271	0.698
*1	*1a																								54	0.139
	*1b																								10	0.026
	*1c																								10	0.026
	*1d																								6	0.015
	*1e																								2	0.005
	*1f																								1	0.003
	*1g																								1	0.003
	*1h																								1	0.003
	*1i																								1	0.003
	*1j†																								1	0.003
*2	*2a																			2					17	0.044
	*2b																			2					10	0.026
	*2c																			2					2	0.005
	*2d†																			2					1	0.003
																									388	1.000

Fig. 1. *GSTT1* (a) and *GSTM1* (b) haplotypes in a Japanese population
Each haplotype is shown in the row, and the alleles are in the columns with the white cell being the major allele and gray cell the minor (nucleotide alteration). †Haplotypes were inferred in only one patient and were ambiguous except for the marker SNPs.

and Chinese (44–63%),[7,14–16] although these frequencies were higher than that of Africans (16–36%).[7,18] The subjects bearing neither *GSTT1* nor *GSTM1* were observed at 23.2%, the frequency of which is similar to Koreans (29.1%)[15] and Shanghai Chinese (24%),[16] but higher than Caucasians (7.5–10.4%)[7,18] and Africans (3.9%).[18]

A number of association studies of the *GSTM1* and *GSTT1* genotypes with cancer susceptibility and cancer therapy outcome have been reported; however, the results are sometimes conflicting.[5–7] In our 194 patients with mainly non-small cell lung cancers, the frequency of *GSTT1**0/*0 and *GSTM1**0/*0 was similar to those in healthy Japanese. This result is in good agreement with a body of literature where the effects of *GSTT1* and *GSTM1* null genotypes on lung cancer development were not clear unless other genetic traits affecting carcinogen metabolism such as *CYP1A1*2A* and *GSTP1*B* (Ile105Val) were combined.[7]

One novel *GSTT1* nonsynonymous variation (226C>A, Arg76Ser) was found in one subject. Arg76 is located in the α3 helix of N-terminal domain I, which forms glutathione binding sites.[19,20] In the crystal structure of human GSTT1-1, this residue closely (2.7 Å) contacts Tyr85 of another subunit (Protein Data Bank, 2C3T).[21] Arg76 is conserved among human, bovine and chicken, whereas this residue is a histidine in mouse and rat. Interestingly, rat and mouse GSTT2 have Ser at position 76.

Of the six SNPs detected in *GSTT1*, three were perfectly linked, resulting in a simple haplotype structure. One of the linked SNPs, 824T>C, was analyzed for various ethnicities in the SNP500Cancer Database (http://snp500cancer.nci.nih.gov/). Its frequency in Japanese (0.106) was comparable to that in Caucasians (0.121), but lower than that in Africans and African-Americans (0.70).

In the *GSTM1* 5′-flanking region (up to −650), eight known SNPs in the NCBI dbSNP database were also detected in this study. This was in contrast to *GSTT1*, in which no SNPs were detected in the 5′-flanking region (up to −801 bp). Murine GSTM1 is transcriptionally up-regulated by the Myb proto-oncogene protein through the Myb-binding site (−58 to −63) in the *GSTM1* promoter,[22] whereas no studies on the mechanisms of transcriptional regulation have been performed with human GSTM1. The four common SNPs, −398C>T, −397A>T, −393T>C, and −358G>A (0.075–0.080 in frequencies), were almost perfectly linked with the known SNP, 519C>G (Asn173Lys, *GSTM1*B*) in Japanese. The GSTM1a-1a isozyme (Asn173) and GSTM1b-1b isozyme (Lys173) were reported to have similar catalytic activities *in vitro*.[8] Nevertheless the association of the *GSTM1*A* alleles has been shown with a reduced risk for bladder cancer.[23] Therefore, the functional significance of promoter SNPs on *GSTM1* expression should be further elucidated.

In conclusion, deletions of *GSTT1* and *GSTM1* in Japanese were analyzed by conventional PCR and TaqMan real-time PCR. About one-fourth (0.232 in frequency) of subjects had double *GSTM1* and *GSTT1* null genotypes. In the intact *GSTT1* and *GSTM1* genes, six and 23 SNPs were identified, respectively, and three (*GSTT1*0*, **1a*, **1b*) and six (*GSTM1*0*, **1a*, **2a*, **1b*, **1c* and **2b*) common haplotypes were inferred. Only one rare nonsynonymous SNP (226C>A, Arg76Ser) was found in *GSTT1*, suggesting that this gene is highly conserved. These findings would be useful for pharmacogenetic studies that investigate the relationship between the efficacy of anticancer drugs and *GST* haplotypes.

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Weekly Administration of Epoetin Beta for Chemotherapy-induced Anemia in Cancer Patients: Results of a Multicenter, Phase III, Randomized, Double-blind, Placebo-controlled Study

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Objective: The efficacy and safety of weekly administration of epoetin beta (EPO) for chemotherapy-induced anemia (CIA) patients was evaluated.
Methods: One hundred and twenty-two patients with lung cancer or malignant lymphoma undergoing chemotherapy were randomized to the EPO 36 000 IU group or the placebo group. Hematological response and red blood cell (RBC) transfusion requirement were assessed. Quality of life (QOL) was assessed using the Functional Assessment of Cancer Therapy-Anemia (FACT-An) questionnaire.
Results: Mean change in hemoglobin level with EPO increased significantly over placebo (1.4 ± 1.9 g/dl versus -0.8 ± 1.5 g/dl; $P < 0.001$). The proportion of patients with change in hemoglobin level $\geq$2.0 g/dl was higher for EPO than those for placebo ($P < 0.001$). After 4 weeks of administration, the proportion of RBC transfusion or hemoglobin level <8.0 g/dl was significantly lower for EPO than those for placebo ($P = 0.046$). The changes in the FACT-An total Fatigue Subscale Score (FSS) were less deteriorated with EPO than those with placebo. Progressive disease (PD) did not influence the change in hemoglobin level but there was less decrease in FSS in non-PD patients. No significant differences in adverse events were observed. Thrombovascular events and pure red cell aplasia related to EPO were not observed. Retrospective analysis of survival showing the hazard ratio of EPO to placebo was 0.94.
Conclusion: Weekly administration of EPO 36 000 IU significantly increased hemoglobin level and ameliorated the decline of QOL in CIA patients over the 8-week administration period.

Key words: anemia – erythropoietin – cancer – chemotherapy-induced anemia – quality of life – survival

INTRODUCTION

One of the causes of anemia in cancer patients is myelosuppression due to chemotherapy or radiation therapy (1). Anemia occurs at a high frequency when using platinum agents, taxanes or anthracyclines often used in cancer patients, especially in patients with lung cancer and malignant lymphomas. Clinical symptoms associated with anemia such as tachycardia, palpitations, fatigue, vertigo and dyspnea are observed in patients with hemoglobin level <10.0 g/dl, and quality of life (QOL) patients is markedly reduced.

In Japan, only red blood cell (RBC) transfusions have been approved for the treatment of chemotherapy-induced anemia (CIA). However, although the safety of RBC transfusions has improved, there are still concerns about viral infections and graft-versus-host disease, as well as adverse effects on survival prognosis. Erythropoiesis-stimulating agents (ESAs) were approved for the treatment of CIA in the 1990s in the United States and in Europe, but they have still not

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been approved for this indication in Japan. It has been reported that the requirement for RBC transfusion can be reduced and QOL improved by increasing the hemoglobin level by ESA administration (2–7). In the United States, 'Use of epoetin in patients with cancer: evidence-based clinical practice guidelines of the American Society of Clinical Oncology and the American Society of Hematology' (8) (the ASH/ASCO guidelines) was published in 2002. The present placebo-controlled, double-blind, comparative study was planned in 2003 based on the ESAs guidelines and applications for ESAs in the United States and Europe for reference. Since 2003, however, several clinical studies have reported that ESAs worsened prognosis in cancer patients (9–16), and the risks of ESAs were investigated by three meetings of the Oncologic Drugs Advisory Committee (ODAC) (May 2004, May 2007 and March 2008). Since 2007, a safety alert (17) including a change in the upper hemoglobin limit has been issued, and the package inserts have been revised. The ASH/ASCO guidelines were also revised in 2007 (18). The effects of ESAs on cancer patient prognosis are not clear at present, and the US Food and Drug Administration (FDA) revised the labeling for ESAs following the 13 March 2008 ODAC's recommendations to impose additional restrictions.

As a result of a previous dose-finding study, once a week epoetin beta (EPO) 36 000 IU was recommended for Japanese cancer patients (19). In this prospective, placebo-controlled, double-blind comparative study, the efficacy and safety of weekly administration of EPO 36 000 IU was evaluated. Efficacy was assessed based on the hematological response and QOL. In addition, considering the recent regulatory conditions in the United States and in Europe, a survival survey was retrospectively performed, and survival in the EPO group and in the placebo group was compared.

PATIENTS AND METHODS

Patient population

The study protocol was approved by the institutional review board at each study site, and written informed consent was obtained before study-related procedures were begun. Patients eligible for this study were required to be patients of age ≥20 to <80 years, who had lung cancer or malignant lymphoma, were receiving a platinum-, taxane- or anthracycline-containing chemotherapy regimen with at least two cycles of chemotherapy scheduled after the first study drug administration and had CIA (8.0 g/dl ≤ hemoglobin level ≤ 11.0 g/dl), an Eastern Cooperative Oncology Group performance status (PS) ≤2, life expectancy ≥3 months as well as adequate renal and liver function. Exclusion criteria included iron-deficiency anemia (serum iron saturation <15% or mean corpuscular volume (MCV) <80 μm^3), history of myocardial, pulmonary or cerebral infarction, severe hypertension beyond control by drug therapy, pregnancy, obvious hemorrhagic lesions or other severe complications, myeloid malignancy or ESA/RBC transfusion within 4 weeks before the first study drug administration.

Study design

Patients were randomized 1:1 to receive EPO 36 000 IU or placebo subcutaneously once a week for 8 weeks. The planned number of patients was 120 (60 in each group). Randomization was conducted by central registration system and a dynamic balancing method using tumor type, PS, age and institution as the adjusting factors. Administration was terminated if the hemoglobin level reached 14 g/dl or more. Oral iron-supplementing drugs were administered if serum iron saturation fell below 15% or MCV fell <80 μm^3. Hemoglobin level and clinical laboratory tests were monitored weekly until 1 week after last study drug administration. RBC transfusion was allowed at the discretion of the investigator during the study.

Study endpoints

The primary endpoint was change in hemoglobin level from baseline, and the last evaluation was performed 8 weeks after the first study drug administration or at study discontinuation. The last observation carried forward method was used for evaluation of the change in hemoglobin level. The secondary endpoints were change in the Functional Assessment of Cancer Therapy Anemia total Fatigue Subscale Score (FSS) (0–52, where a higher score means less fatigue) from baseline to last evaluation, RBC transfusion requirement, nadir hemoglobin level, proportion of patients who achieved a hemoglobin level increase ≥2.0 g/dl from baseline, proportion of the patients with hemoglobin level <8.0 g/dl during the study and incidence of either RBC transfusion or hemoglobin level <8.0 g/dl. Safety was assessed by National Cancer Institute – Common Toxicity Criteria, ver. 2, translated by the Japan Clinical Oncology Group. Anti-erythropoietin antibodies were measured by enzyme-linked immunosorbent assay and radioimmunoprecipitation assay, and compared with the data of the first study drug administration with the data of the last observation. Detection by either method was judged as positive. A retrospective analysis of survival was performed.

Statistics

Efficacy analyses were performed using the full-analysis-set (FAS) population, comprising all eligible patients who received a study drug. In both EPO and placebo groups, changes in hemoglobin level and changes in FSS at the last evaluation were compared using Student's *t*-test. Stratified analyses in the groups with baseline FSS >36 and ≤36, respectively, were also performed.

RESULTS

Patient disposition

One hundred and twenty-two patients were recruited from February 2004 to March 2005 at 11 sites in Japan. Sixty-five patients had lung cancer and 57 had malignant lymphoma. The patients were randomly assigned to the EPO group ($n = 63$) or the placebo group ($n = 59$). One patient in each group never received a study drug, one patient in each group never received chemotherapy and one patient in the placebo group did not have laboratory data after administration. Thus, the FAS population was 117 patients (61 patients in the EPO group, 56 patients in the placebo group).

Demographics, clinical and baseline characteristics

Patient demographics were well balanced between the two groups, except for baseline hemoglobin levels and serum erythropoietin concentrations (Table 1). The mean hemoglobin level in the EPO group was slightly lower than in the placebo group (10.0 versus 10.4 g/dl). The baseline hemoglobin level did not influence the evaluation of the primary endpoint by analysis of covariance.

Hematological evaluations

Mean change in hemoglobin level at the last evaluation significantly increased in the EPO group (1.4 ± 1.9 g/dl) than in the placebo group (-0.8 ± 1.5 g/dl) ($P < 0.001$). The hemoglobin level started to elevate in the EPO group at 3 weeks after the first administration (Figs 1 and 2). After 4–8 weeks of administration, the proportion of patients who achieved changes in hemoglobin level $\geq$2.0 g/dl from baseline was 42.6% (26/61) for the EPO group and 1.8% (1/56) for the placebo group ($P < 0.001$).

During the study, the proportion of patients with the hemoglobin level increased 12.0 g/dl or more was evaluated in the patients with hemoglobin level below 12.0 g/dl at baseline, the proportion was higher in the EPO group than in the placebo group [49.2% (29/59) versus 9.6% (5/52), $P < 0.001$]. The nadir hemoglobin level was 9.4 ± 1.5 g/dl in the EPO group and 8.6 ± 1.3 g/dl in the placebo group ($P = 0.002$). The proportion of patients with hemoglobin level decreased <8.0 g/dl was evaluated in the patients with hemoglobin level >8.0 g/dl at baseline, the proportion was 18.6% (11/59) in the EPO group and 32.1% (18/56) in the placebo group ($P = 0.096$).

RBC transfusion

The incidence of RBC transfusion was not different between the EPO group and the placebo group throughout the study [11.5% (7/61) versus 12.5% (7/56), $P = 0.865$] or from Week 5 to Week 8 [8.2% (5/61) versus 12.5% (7/56), $P = 0.443$]. However, the incidence of RBC transfusion or hemoglobin level <8.0 g/dL from Week 5 to Week 8 was significantly lower in the EPO group than those in the placebo group [16.4% (10/61) vs. 32.1% (18/56), $P = 0.046$], and fewer RBC transfusion units were required in the EPO group (10 units, $n = 5$) than in the placebo group (26 units, $n = 7$).

Quality of life

At the last observation, the FSS data for two patients were missing because of progressive disease (PD). The missing scores were substituted by the maximum decrease in score

Table 1. Patient demographics of full-analysis-set population

	Placebo group ($n = 56$)	EPO group ($n = 61$)
Sex		
Male	33	34
Female	23	27
Age (years), mean ± SD	62.1 ± 9.6	61.8 ± 11.9
Tumor		
Lung cancer	30	32
Small cell lung cancer	7	8
Non-small cell lung cancer	23	24
Malignant lymphoma	26	29
Hodgkin lymphoma	0	3
Non-Hodgkin lymphoma	26	26
Chemotherapy		
1st line	38	41
2nd line	6	8
3rd line	1	1
Relapse/recurrence	11	11
ECOG performance status		
0	38	33
1	17	26
2	1	2
Weight (kg), mean ± SD	54.5 ± 8.8	55.2 ± 10.0
Hemoglobin (g/dl), mean ± SD	10.4 ± 1.0	10.0 ± 1.0
Serum endogenous erythropoetin (mU/ml), mean ± SD	49.1 ± 33.4	67.3 ± 72.0
MCV (fl), mean ± SD	93.5 ± 6.0	91.9 ± 5.5
Transferrin saturation (%), mean ± SD	29.4 ± 19.8	32.4 ± 22.0
Baseline QOL: FACT-An		
Fatigue subscale (0–52), mean ± SD	33.9 ± 10.0	35.5 ± 9.7
≤36	29	29
>36	26	32
Data missing	1	0

SD, standard deviation; ECOG, Eastern Cooperative Oncology Group; QOL, quality of life; FACT-An, Functional Assessment of Cancer Therapy-Anemia; MCV, mean corpuscular volume; EPO, epoetin beta.

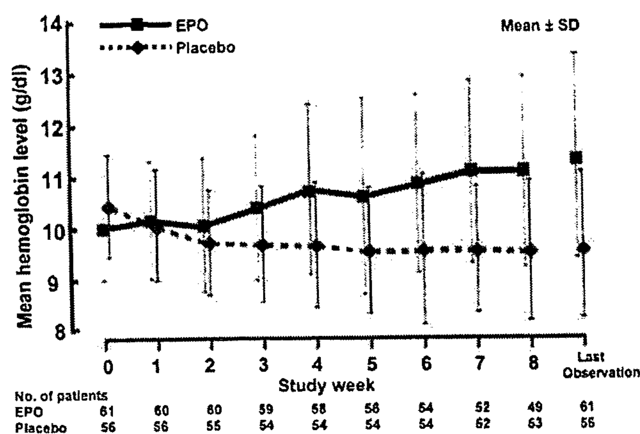

Figure 1. Hemoglobin level during the treatment period. A colour version of this figure is available as supplementary data at http://www.jjco.oxfordjournals.org. SD, standard deviation; EPO, epoetin beta.

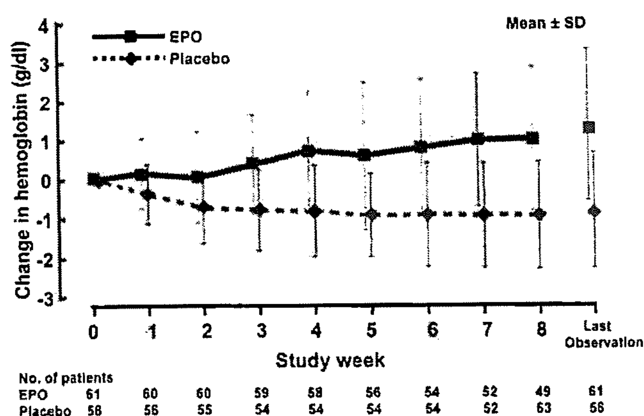

Figure 2. Change in hemoglobin level during the treatment period. A colour version of this figure is available as supplementary data at http://www.jjco.oxfordjournals.org.

for all patients. This substitution was decided before blinded data review. The changes in FSS from baseline were less in the EPO group than those in the placebo group (Mean ± SD: -0.5 ± 9.4 versus -4.5 ± 10.0, $P = 0.031$). But excluding these two patients with missing data at the last observation, the change in FSS from baseline was not significant in the EPO group and in the placebo group (-0.5 ± 9.4 versus -3.6 ± 9.0, $P = 0.082$). The factors that influenced the change in FSS were baseline FSS, change in hemoglobin level, treatment group and PS at the last observation (analysis of variance). It has been suggested that if the baseline FSS is higher than 36, the change in FSS will decrease after administration of ESA because of the high baseline and the lack of symptoms (ceiling effect and regression to the mean) (20,21). Thus, we also analyzed patients whose baseline FSS was ≤ 36. In the baseline FSS ≤ 36 patients, change in FSS was 2.1 ± 11.7 in the EPO group and -1.3 ± 9.6 in the placebo group, so the EPO group showed improvement in FSS ($P = 0.225$). However, in the baseline FSS > 36 patients, the change in FSS was -2.9 ± 5.9 in the EPO group and -7.9 ± 9.4 in the placebo group ($P = 0.016$), so the EPO group showed suppression of the decline in FSS (Fig. 3). In subset analysis of the EPO group, the mean change in hemoglobin level did not differ in PD and non-PD patients (1.3 ± 1.8 versus 1.4 ± 2.0 g/dl), but PD patients showed a more marked decrease in FSS than non-PD patients (-6.8 ± 9.4 versus 0.2 ± 9.2).

SAFETY

The incidence of adverse events was evaluated for the 120 patients who receive a study drug. Adverse events were observed in 62 patients (100%) in the EPO group and 57 patients (98.3%) in the placebo group, and no significant differences were found between the two groups ($P = 0.299$). The adverse events related to the study drug were 24 events in the EPO group (17 of 62 patients, 27.4%) and 19 events in the placebo group (11 of 58 patients, 19.0%) ($P = 0.274$). Adverse drug reactions observed in at least 3% of the patients in the EPO group were increased blood pressure (6.5%), increased lactate dehydrogenase (3.2%) and increased urinary glucose (3.2%). In the placebo group, rash (3.4%), increased blood pressure (3.4%) and decreased activated partial thromboplastin time (3.4%) were reported. Grade 3 abdominal pain and Grade 3 liver dysfunction were both observed in the same patients in the EPO group. Five patients (5 events) in the EPO group and five patients (12 events) in the placebo group experienced serious adverse events. Of these, only Grade 3 liver dysfunction was considered related to EPO treatment (Table 2). One thrombovascular event (TVE), a lacunar infarction, was reported in the EPO group. No other TVEs were reported in either group. No anti-erythropoietin antibodies were reported.

SURVIVAL

A retrospective analysis of survival was performed. The median follow-up duration was 670 days for the EPO group

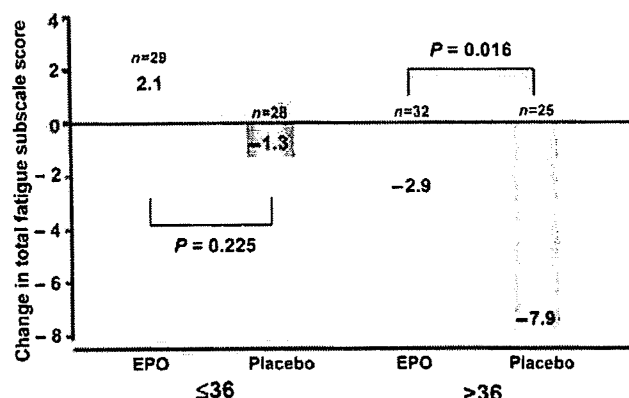

Figure 3. Mean change in FACT-An total fatigue subscale score stratified by baseline total fatigue subscale score (≤36, >36). A colour version of this figure is available as supplementary data at http://www.jjco.oxfordjournals.org. FACT-An, Functional Assessment of Cancer: Therapy-Anemia.

Table 2. Incidence of the most common adverse events

	Placebo group ($n = 58$)		EPO group ($n = 62$)	
	No. of patients	%	No. of patients	%
Adverse events	57	98.3	62	100
Adverse events with incidence ≥20% in the EPO group				
Neutropenia	47	81.0	47	75.8
Leukopenia	46	79.3	47	75.8
Thrombocytopenia	28	48.3	31	50.0
Nausea	28	48.3	27	43.5
Fatigue	26	44.8	28	45.2
Anorexia	24	41.4	27	43.5
Lymphopenia	24	41.4	32	51.6
Alopecia	17	29.3	22	35.5
Increased LDH	15	25.9	16	25.8
Constipation	10	17.2	14	22.6
Adverse drug reactions	11	19.0	17	27.4
Adverse drug reactions with incidence ≥3% in either group				
Increased blood pressure	2	3.4	4	6.5
Increased LDH	1	1.7	2	3.2
increased urinary glucose	0	0.0	2	3.2
Rash	2	3.4	0	0.0
Decreased APTT	2	3.4	0	0.0
Adverse drug reactions with severity ≥Grade 3				
Abdominal pain	0	0.0	1	1.6
Liver dysfunction	0	0.0	1	1.6

LDH, lactate dehydrogenase; APTT, activated partial thromboplastin time.

and 641 days for the placebo group. The 1-year survival population based on Kaplan–Meier estimates was 64.9% in the EPO group and 65.9% in the placebo group. The hazard ratio was 0.94 for the EPO group relative to the placebo group (95% CI: 0.57–1.53).

DISCUSSION

Improvements in hemoglobin level were observed in Japanese patients with CIA on administration of EPO 36 000 IU once a week for 8 weeks. In the evaluation of QOL, it is necessary to consider the effects of scores at baseline, such as the ceiling effect and regression to the mean (20). It has been reported that in patients with less symptoms as baseline FSS is more than 36, the change in FSS became negative (21). The results of a stratified analysis of groups with baseline FSS ≤36 and >36 (performed for reference) showed that in patients with baseline FSS ≤36 (severe anemia symptoms), the symptoms of anemia improved in the EPO group, but worsened in the placebo group. In patients with baseline FSS >36 (mild anemia symptoms), worsening occurred in both groups, but the worsening was significantly inhibited in the EPO group compared with the placebo group. In the United States, at present, the FDA has not approved the use of ESAs to improve QOL, but the results of this study suggest that EPO may be useful in the prevention of worsening of symptoms of anemia.

In the United States, it has been stressed that the purpose of using ESAs is to treat CIA in order to avoid RBC transfusions. In the present study, the incidence of RBC transfusion during administration was low and the hemoglobin level when RBC was transfused was 5.5–8.8 g/dl. In Japan, most physicians and patients are reluctant to use RBC transfusions, but in the United States and in Europe, RBC transfusions are often started when the hemoglobin level is 8.0–10.0 g/dl (22). In this study, the proportion of patients with either severe anemia requiring a RBC transfusion or hemoglobin level of <8.0 g/dl (NCI-CTC Grades 3 and 4) was examined. Evaluation of this proportion from 4 weeks after the start of administration, when ESAs exhibited hematopoietic effects (23–25), indicated that this proportion was significantly lower in the EPO group (16.4%, 10 of 61 patients) than in the placebo group (32.1%, 18 of 56 patients) ($P = 0.046$).

One TVE was observed in this study, a lacunar infarction (Grade 1) in one patient (69-year-old male with lung cancer) in the EPO group. The investigator judged without causal relationship to the study drug but by aging, because the event was observed 1 day after the first study drug administration. No other TVEs were reported. Increased blood pressure and hypertension occurred in 10 patients (six in the EPO group, four in the placebo group). Marked differences from the placebo group were not observed for other adverse events.

The FDA has issued several safety alerts regarding data that demonstrated adverse survival outcomes in ESA-treated cancer patients. In this study, however, based on the results of a survey of overall survival, the 1-year survival proportion showed no significant difference between the groups. The effects of ESAs on survival of cancer patients have been examined by the ODAC and other groups since 2007, based on new clinical trial reports. So far, the reported safety data have been insufficient to rule out the risk of mortality in chemotherapy-treated patients, but ESAs are considered a therapeutic option for the management of CIA. Clinical studies based on the doses and hemoglobin levels recommended on the labels will continue to accumulate evidence on the effects of ESAs on survival.

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Conflict of interest statement

The author, Yasuo Ohashi, receives consultation fee from Chugai Pharmaceutical Co., Ltd.: the author advises on design and data analysis of clinical trials.

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ORIGINAL REPORT

Japanese-US Common-Arm Analysis of Paclitaxel Plus Carboplatin in Advanced Non–Small-Cell Lung Cancer: A Model for Assessing Population-Related Pharmacogenomics

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Clinical Trials repository link available on JCO.org.

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A B S T R A C T

Purpose
To explore whether population-related pharmacogenomics contribute to differences in patient outcomes between clinical trials performed in Japan and the United States, given similar study designs, eligibility criteria, staging, and treatment regimens.

Methods
We prospectively designed and conducted three phase III trials (Four-Arm Cooperative Study, LC00-03, and S0003) in advanced-stage, non–small-cell lung cancer, each with a common arm of paclitaxel plus carboplatin. Genomic DNA was collected from patients in LC00-03 and S0003 who received paclitaxel (225 mg/m^2) and carboplatin (area under the concentration-time curve, 6). Genotypic variants of CYP3A4, CYP3A5, CYP2C8, NR1I2-206, ABCB1, ERCC1, and ERCC2 were analyzed by pyrosequencing or by PCR restriction fragment length polymorphism. Results were assessed by Cox model for survival and by logistic regression for response and toxicity.

Results
Clinical results were similar in the two Japanese trials, and were significantly different from the US trial, for survival, neutropenia, febrile neutropenia, and anemia. There was a significant difference between Japanese and US patients in genotypic distribution for *CYP3A4*1B* ($P = .01$), *CYP3A5*3C* ($P = .03$), *ERCC1* 118 ($P < .0001$), *ERCC2* K751Q ($P < .001$), and *CYP2C8* R139K ($P = .01$). Genotypic associations were observed between *CYP3A4*1B* for progression-free survival (hazard ratio [HR], 0.36; 95% CI, 0.14 to 0.94; $P = .04$) and ERCC2 K751Q for response (HR, 0.33; 95% CI, 0.13 to 0.83; $P = .02$). For grade 4 neutropenia, the HR for ABCB1 3425C→T was 1.84 (95% CI, 0.77 to 4.48; $P = .19$).

Conclusion
Differences in allelic distribution for genes involved in paclitaxel disposition or DNA repair were observed between Japanese and US patients. In an exploratory analysis, genotype-related associations with patient outcomes were observed for *CYP3A4*1B* and *ERCC2* K751Q. This common-arm approach facilitates the prospective study of population-related pharmacogenomics in which ethnic differences in antineoplastic drug disposition are anticipated.

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INTRODUCTION

Results may vary between different clinical trials that evaluate the same treatment regimen for many reasons, including trial design, eligibility criteria, patient characteristics, and subtle alterations in the treatment regimens themselves. An additional explanation for divergence of outcomes is host-related genetic differences associated with ethnicity, which is particularly pertinent when trials that are performed in different parts of the world are compared.

More than 10 years ago, the Southwest Oncology Group (SWOG) established a collaboration with Japanese investigators of lung cancer to provide a forum for exchange of research data, to facilitate standardization of clinical trial design and conduct, and to establish areas for joint collaboration.[1] We hypothesized that outcome differences between trials performed in Japan and the United States that evaluated similar treatment regimens in advanced-stage, non–small-cell lung cancer (NSCLC) could be explained by population-related

pharmacogenomics. To evaluate this possibility, we prospectively designed three phase III trials, (Four-Arm Cooperative Study [FACS], LC00-03, and S0003), each with similar patient eligibility criteria, staging, and treatment with a common arm of paclitaxel plus carboplatin. We have reported previously that, despite this effort at trial standardization, differences in clinical outcomes were observed in Japanese versus US patients treated on these studies.[2,3] Herein, we report the results of a clinical and pharmacogenomic analysis that involved patients from two of the three clinical trials (LC00-03 and S0003), and we report implications for additional studies by using this clinical research approach in which population-related differences in drug disposition are anticipated.

Patients

The clinical trial methodology employed was prospective design of three separate-but-equal, randomized, phase III trials in advanced-stage NSCLC, each with its own comparator regimens but linked by a common treatment arm of paclitaxel plus carboplatin. In FACS, patients were randomly assigned to a standard treatment in Japan (irinotecan plus cisplatin) versus experimental arms of paclitaxel plus carboplatin, gemcitabine plus cisplatin, and vinorelbine plus cisplatin. LC00-03 compared paclitaxel plus carboplatin to the nonplatinum regimen of sequential vinorelbine plus gemcitabine followed by docetaxel, whereas patients on S0003 were randomly assigned to paclitaxel plus carboplatin with or without the hypoxic cytotoxin tirapazamine.

Clinical results for the three trials have been previously presented and published separately.[4-6] Common elements of eligibility criteria are summarized here. All patients had histologically or cytologically confirmed chemotherapy-naïve NSCLC with stage IV (ie, no brain metastases) or selected stage IIIB disease (ie, positive pleural or pericardial effusion or multiple ipsilateral lung nodules); measurable or assessable disease, performance status (PS) of 0 or 1; and adequate hematologic, hepatic, and renal function. All patients gave written informed consent in accordance with institutional regulations, and each protocol was approved by the respective institutional review boards; trials were conducted with adherence to the Helsinki Declaration.

Treatment Schedule, Dose Modifications, and Toxicity Assessment

Study elements of S0003, FACS and LC00-03 were designed to be as similar as possible: each study contained a common arm of paclitaxel plus carboplatin, which was repeated on a 21-day schedule. In all three studies, carboplatin was dosed at an area under the concentration-time curve (AUC) of 6.0 mg/mL/min on day 1. Paclitaxel was dosed at 225 mg/m^2 in S0003 and LC00-03 and at 200 mg/m^2 in FACS because of regulatory requirements for this study; in each study, paclitaxel was delivered as a 3-hour infusion on day 1. Premedication to prevent paclitaxel-related allergic reactions were similar. Prophylactic granulocyte colony-stimulating factor was not utilized. A complete blood count and chemistries were performed on day 1 of each cycle. Dose modifications occurred as previously described.[4] Patients were evaluated every two cycles for objective response by using RECIST (Response Evaluation Criteria in Solid Tumors) criteria[7] Toxicity grading was performed in accordance with the National Cancer Institute Common Toxicity Criteria, version 2.0, in each study.[8]

DNA Extraction and Genotyping

Specimens were not available from FACS; therefore, this analysis compares pharmacogenomic results from LC00-03 with S0003. Whole-blood specimens were collected from consenting patients at the time of enrollment on to LC00-03 and S0003. For S0003, DNA was extracted from patient plasma by using the Gentra PureGene Blood Kit (Gentra, Minneapolis, MN) and the QIAamp DNA Blood midi kit (Qiagen, Valencia, CA), and DNA was reconstituted in a buffer that contained 10 mmol/L Tris (pH 7.6) and 1 mmol/L EDTA, as previously described.[9] For LC00-03, DNA was extracted from buffy coats by using the GenElute Blood Genomic DNA Kit (Sigma-Aldrich, St Louis, MO). Selected genotypic variants related to paclitaxel disposition (ie, the ABC transporter superfamily [multidrug resistance {MDR} transporter 1 P-glycoprotein, *ABCB1* 3435C→T], the pregnane X receptor (PXR, NR1I2-206 deletion), *CYP3A4* (*CYP3A4*1B* 392A→G, 5' untranslated region), CYP3A5 (*CYP3A5*3C* 6986A→G, splice variant), *CYP2C8* (*CYP2C8*3* 416G→A, R139K) or to platinum-related DNA repair enzymes *ERCC1* (118C→T, silent) and ERCC2 (XPD, K751Q) previously reported to be of functional consequence were analyzed by polymerase chain reaction (PCR) or pyrosequencing, as previously described.[9-13] Briefly, PCR was conducted by using Amplitaq Gold PCR master mix (ABI, Foster City, CA), 5 pmol of each primer, and 5 to 10 ng of DNA. Pharmacogenetic analysis was conducted by using the Pyrosequencing hsAPSQ96 instrument and software (Biotage, Uppsala, Sweden). The genotype was considered variant if it differed from the Reference Sequence consensus sequence for the single-nucleotide polymorphism (SNP) position (http://www.ncbi.nlm.nih.gov/RefSeq/). The *ERCC1* polymorphism was analyzed by PCR restriction fragment length polymorphism, as previously described.[14]

Statistical Methods

Comparison of clinical results among the three trials was prospectively planned and was coordinated through the SWOG statistical center. Pharmacogenomic results were assessed by Cox model for progression-free survival (PFS) and overall survival and by logistic regression for response and toxicity, adjusted for sex and histology.[15] Comparisons of patient demographics, toxicity, and efficacy parameters were made, when applicable, from the available data sets, by two-sample *t* tests, log-rank tests, and Wilcoxon rank sum tests.

Clinical Results Summary

Clinical results are presented for all three trials to document similarities between the two Japanese trials compared with the US S003 trial, whereas pharmacogenomic information was derived only from LC00-03 and S0003. Table 1 summarizes characteristics of patients on the paclitaxel-plus-carboplatin arms of each of the three trials. The median ages and age ranges were similar, and there were no significant differences in sex, stage, or histology. In S0003, 3% of patients self-reported Asian heritage, not additionally specified. Toxicity, efficacy, and dose delivery comparisons are listed in Table 2, which compares S0003 versus FACS/LC00-03 when applicable. Grades 3 to 4 neutropenia and febrile neutropenia were comparable

Table 1. Patient Demographic and Clinical Characteristics

	Trial						
	FACS (n = 145)		LC00-03 (n = 197)		S0003 (n = 184)		
Characteristic	No	%	No	%	No	%	*P*
Age, years							.03*
Median	63		65		63		
Range	33-74		33-81		28-80		
Female sex	46	32	61	31	68	37	.42
Disease stage IV	117	81	162	82	161	87	.20
Nonsquamous tumor type	114	79	167	85	152	83	.17

Abbreviation: FACS, four-arm cooperative study
*Two-sample *t* test to compare LC00-03 and S0003 data. Patient-level data not available for FACS

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Table 2. Toxicity Comparisons

Toxicity	FACS (n = 148) No	FACS %	LC00-03 (n = 197) No	LC00-03 %	S0003 (n = 184) No	S0003 %	P
Neutropenia grades 3-4	130	88	137	70	70	38	< 0001
Febrile neutropenia grades 3-4	27	18	24	12	4	2	< 0001
Thrombocytopenia grades 3-4	16	11	14	7	12	6 5	31
Anemia grades 3-4	22	15	16	8	12	7	03
Neuropathy grades 2-4	25	17	32	16	30	16	99

Abbreviation FACS, four-arm cooperative study

in FACS and LC00-03 and were significantly greater than in S0003. Anemia was more frequent in FACS compared with the two other trials (Table 2). Efficacy comparisons are summarized in Table 3. Response rates were similar between the three trials and ranged from 32% to 36%. Median PFS rates were 4.5, 6, and 4 months in FACS, LC00-03, and S0003, respectively. Median survival rates were higher in the Japanese studies at 12 and 14 months, versus 9 months in S0003, and 1-year survival was significantly higher in FACS and LC00-03 than in S0003 ($P = .0004$). Dose delivery, summarized in Table 4, was lower in FACS than in S0003 and LC00-03. Dose reductions were similar between LC00-03 and S0003. Dose reduction data were not available from FACS.

Pharmacogenomic Results

Table 5 lists allelic distributions of patients with common, heterozygous, and variant alleles in the Japanese (LC00-03) and US (S0003) trials. Fisher's exact test was used to determine whether allele distributions were different between the populations. There were significant differences between patients from Japan (LC00-03) and the United States (S0003) in genotype distribution for *CYP3A4*1B* ($P = .01$), *CYP3A5*3C* ($P = .03$), *ERCC1* 118 ($P < .0001$), *ERCC2* K751Q ($P < .001$), and *CYP2C8*3* ($P = .01$).

Across populations, genotypic correlations were observed between *CYP3A4*1B* for PFS (hazard ratio [HR], 0.36; 95% CI, 0.14 to 0.94; $P = .04$) and *ERCC2* K751Q for response (HR, 0.33; 95% CI, 0.13 to 0.83; $P = .02$). There were no other significant associations noted (Table 6). For grade 4 neutropenia, the HR for ABCB1 3425C→T was 1.84 (95% CI, 0.77 to 4.48; $P = .19$). The relationship between the ERCC2 polymorphism and patient response stems principally from US patients. All but one Japanese patient was homozygous for the common allele (A/A). Those who harbored one or more variant alleles were significantly more likely to respond to treatment compared with those who had the common genotype. The response rate for patients with variant alleles was 51% versus 19% for patients homozygous for the common allele $P = .004$). However, no differences were observed in overall survival when stratified by this locus.

In S0003 (ie, the US trial), there were seven African American patients who had specimens available for genotyping. African American patients accounted for all seven patients who were heterozygous or homozygous for the *CYP3A4*1B* allele (Table 5). Additionally, the three patients with the common allele for *CYP3A5*C* were African American.

Table 3. Efficacy Comparisons

Parameter	FACS (n = 145)	LC00-03 (n = 197)	S0003 (n = 184)	P
Response				55
No	47	73	61	
%	32	37	33	
PFS, months	4 5	6	4	04*
MST, months	12	14	9	0006*
1-year survival	51%	57%	37%	0004

Abbreviations FACS, four-arm cooperative study; PFS, progression-free survival; MST, median survival time
*Log-rank test to compare LC00-03 and S0003 Patient-level data not available for FACS

DISCUSSION

This report describes the culmination of a unique multinational and multistudy collaboration that explores the hypothesis that clinical differences in treatment outcomes between Japanese and US patients with NSCLC may be explained, in part, by pharmacogenomic factors. Potential differences in drug disposition related to ethnic variability in distribution of relevant single nucleotide polymorphisms are well recognized. To our knowledge, however, the current project represents the first attempt to prospectively incorporate study of this topic into a joint clinical trial design. To preplan such a multinational endeavor required a high level of collaboration and compromise among all participants, including, in the case of FACS, Japanese regulatory authorities. Nevertheless, this report demonstrates the overall feasibility of using a common-arm methodology to investigate this research topic, in which a single, prospectively planned, joint study cannot be conducted. Considering the limitations of the clinical and pharmacogenomic data sets generated in this effort, and considering the multiple comparisons generated, the results reported here should be viewed as exploratory only and as primarily useful for refining this common-arm model of multinational collaboration. Even so, the clinical results are remarkably consistent with those anticipated, in which expectations were for both improved efficacy and higher levels of toxicity in Japanese patients who received a similar treatment regimen. Observation of clinical differences despite reduced paclitaxel

Table 4. Treatment Delivered

Treatment Data	FACS (n = 145)		LC00-03 (n = 197)		S0003 (n = 184)		P
	No	%	No	%	No	%	
Median cycles delivered	3 5		4		4		07
Received > three cycles	35	24	118	60	100	54	< 0001
Received six cycles	16	11	58	29	68	36 5	< 0001
Dose was reduced	No data	No data	100	51	98	26	63*

Abbreviation: FACS, four-arm cooperative study
*Wilcoxon rank sum test to compare LC00-03 and S0003 Patient-level data not available for FACS

dosing and drug delivery of paclitaxel plus carboplatin in the FACS Japanese study highlights the contrast.

The rationale for conducting this common-arm project specifically in collaboration with Japanese investigators was based on several factors, including the established SWOG interaction described earlier, the high quality of lung cancer investigation by Japanese cooperative groups, and prior literature that suggested that overall, Japanese patients achieve better results than their US counterparts. However, the most compelling rationale was prior pharmacogenomic literature, which suggested that relevant drug disposition differences might exist between US and Japanese populations treated with cancer chemotherapeutic agents. Well recognized here are alterations in irinotecan metabolism as a result of variability in the allelic distribution of UDP-glucuronosyltransferases, particularly *UGT1A1*28* in different ethnic groups, as Asians have a much lower frequency of variant alleles. Recently, a comparative analysis of patient-level data from phase III trials in small-cell lung cancer in Japan (J9511) and the United States (S0124) demonstrated significant differences in toxicity profiles between the two groups. In addition, a pharmacogenomic analysis of S0124 showed significant associations between genotypic variants and toxicity levels.[16,17]

The genes evaluated in this study were selected on the basis of their potential to influence paclitaxel disposition or DNA damage repair. Paclitaxel is principally eliminated through multiple hydroxylation reactions mediated by cytochrome isoforms *CYP2C8*, *CYP3A4*, and *CYP3A5*.[18,19] The *CYP2C8*3* variant (R139K), which is associated with decreased metabolism of paclitaxel, occurs at a frequency of 9% to 15% in white patients but is rare in African and Asian populations.[20-23] In this study, the allele frequency in the US population was 12%, which was significantly different from the less-than-1% frequency in the Japanese cohort (P = .01). *CYP2C8* genotypic variability at R139K was not significantly associated with patient outcome. CYP3A isozymes account for 45% to 60% of paclitaxel metabolism.[24] In white patients, the *CYP3A5* allele is commonly nonfunctional as a result of a transition in intron 3 that produces a truncated splice variant.[25] Our findings are consistent with that of Hustert et al,[25] who reported frequencies of functional *CYP3A5* as 5% in white patients, 29% in Japanese patients, and 73% in African American patients. Of patients enrolled onto the S0003 trial conducted in the US, three of three with the functional allele (indicated as common in Table 5) were African Americans, as were three of the seven heterozygous patients. Although trends were observed, *CYP3A5*3C* genotypic variability was not significantly associated with patient outcome (overall survival P = .07; PFS P = .09), perhaps related to the small sample size. Similarly, the *CYP3A4*1B* allele was observed in seven of seven African American patients but was absent in white and Japanese patients. In vitro studies suggest that the *CYP3A4*1B* variant has enhanced activity over common allele.[26] An association was observed between occurrence of the *CYP3A4*1B* and PFS (P = .04); however, this association should be interpreted in the context that only African American patients harbored this allele. Thus, it remains unclear whether this potential relationship with outcome is associative or causative. The PXR (*NR1I2*-206 deletion) is a master regulator of genes involved in xenobiotic detoxification and influences transcription of *CYP3A4*, *CYP3A5*, *CYP2C8*, and *MDR-1 (ABCB1)*.[27-29] Paclitaxel can activate PXR, which enhances drug clearance through increased activity of MDR1.[30] No significant differences by genotype were observed for PXR or ABCB1, although there was a trend toward

Table 5. Genotype Profiles in Japanese and US Patients on LC00-03 and S0003

Polymorphism by Trial Location	No of Patients			P
	Com	Het	Var	
*CYP3A4*1B*				
Japan	73	0	0	.01
United States	64	4	3	
*CYP3A5*3C*				
Japan	7	16	50	.03
United States	3	7	66	
CYP2C8 (R139K)				
Japan	69	2	0	.01
United States	57	7	5	
ABCB1 (3435C→T)				
Japan	33	21	17	.11
United States	24	23	29	
NR1I2 (206 deletion)				
Japan	51	19	5	.25
United States	40	25	8	
ERCC1 (118)				
Japan	8	27	43	< 0001
United States	23	33	19	
ERCC2 (K751Q)				
Japan	73	1	0	< .001
United States	37	27	8	

NOTE LC00-03 is the trial in Japan; S0003 is the trial in the United States Fisher's exact test was used to determine whether allele distributions were different between the populations

Abbreviations: Com, common allele; Het, heterozygous allele; Var, variant allele

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