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.

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

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 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/Ggggctcccaaa		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/Ggagtagctttc			0.044
MPJ6_GTM1002	rs3815029	ssj0002147	5′-flanking	6137641	−540C>G	gggagtagctttC/Ggatcagaggaa			0.026
MPJ6_GTM1003	rs412302	ssj0002148	5′-flanking	6137701	−480A>G	tcccaggttgggA/Gccaccactttt			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/Gggggaaggtgg			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.)

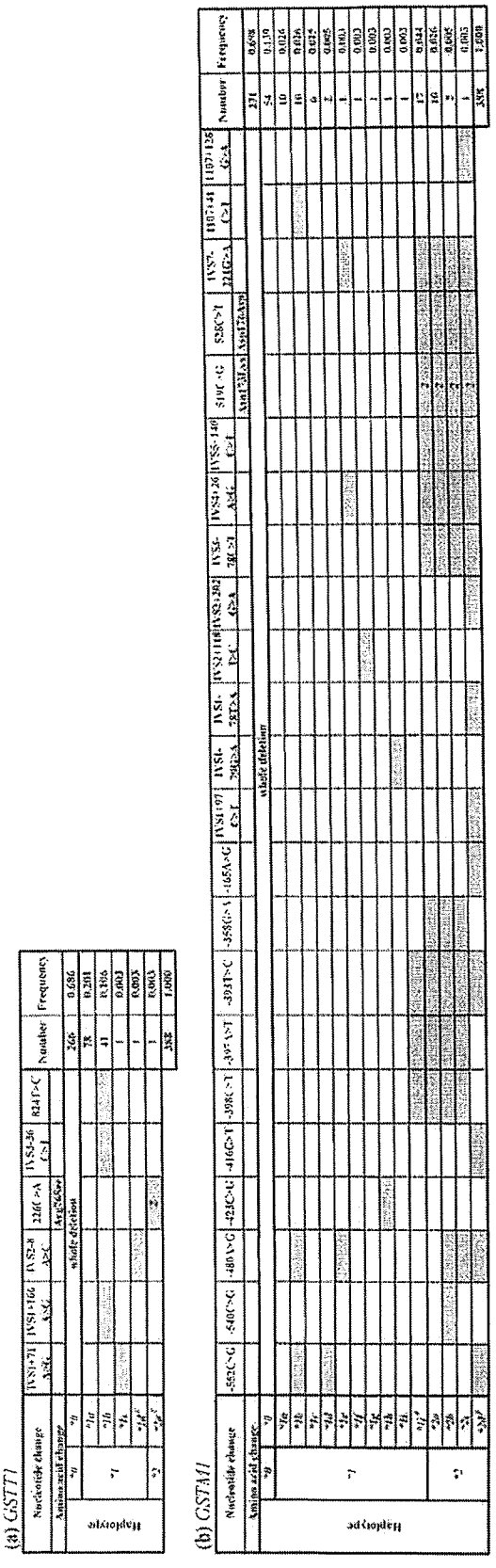

Fig. 1. *GSTT1* (a) and *GSTM1* (b) haplotypes in a Japanese population
Each haplotype is shown in the row, and the alleles are in the columns with the white cell being the major allele and gray cell the minor (nucleotide alteration). [5]Haplotypes were inferred in only one patient and were ambiguous except for the marker SNPs.

−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

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 upregulated 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 ≥ 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 $\geq$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 $\leq$ hemoglobin level $\leq$ 11.0 g/dl), an Eastern Cooperative Oncology Group performance status (PS) $\leq$2, life expectancy $\geq$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 $\geq$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 $\leq$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 ≥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

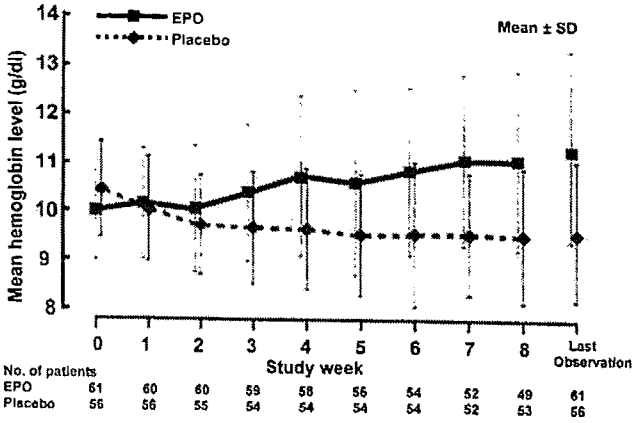

Figure 1. Hemoglobin level during the treatment period. A colour version of this figure is available as supplementary data at http://www.jjco.oxfordjournals.org. SD, standard deviation; EPO, epoetin beta.

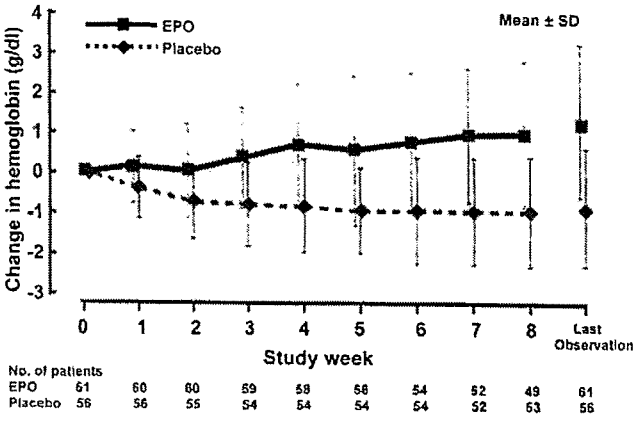

Figure 2. Change in hemoglobin level during the treatment period. A colour version of this figure is available as supplementary data at http://www.jjco.oxfordjournals.org.

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

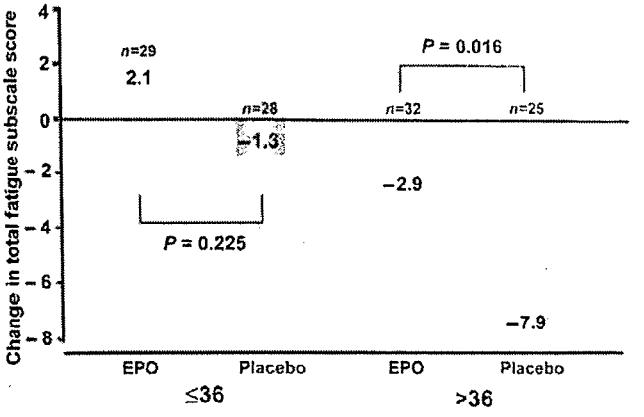

Figure 3. Mean change in FACT-An total fatigue subscale score stratified by baseline total fatigue subscale score (≤ 36, > 36). A colour version of this figure is available as supplementary data at http://www.jjco.oxfordjournals.org. FACT-An, Functional Assessment of Cancer Therapy-Anemia.

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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Authors' disclosures of potential conflicts of interest and author contributions are found at the end of this article

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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 AND METHODS

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.

RESULTS

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

Characteristic	Trial: FACS (n = 145) No	%	LC00-03 (n = 197) No	%	S0003 (n = 184) 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	86	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

Table 2. Toxicity Comparisons

Toxicity	Trial						
	FACS (n = 148)		LC00-03 (n = 197)		S0003 (n = 184)		
	No	%	No	%	No	%	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	Trial			
	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

[redacted]

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	Trial						
	FACS (n = 145)		LC00-03 (n = 197)		S0003 (n = 184)		
	No	%	No	%	No	%	P
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]

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*C*				
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

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 6. Cox Model to Compare Outcomes by Polymorphism

Outcome by Polymorphism	Analyses			
	Comparison	HR	95% CI	P
ABCB1 3425				
Overall survival	Com *v* Het/Var (CC *v* CT/TT)	1 09	0 71 to 1.67	69
PFS		1 04	0 70 to 1 56	82
Response		0 97	0 39 to 2 38	1.00
Neutropenia		0.54	0 22 to 1 30	19
CYP2C8 R139K				
Overall survival	Com *v* Het/Var (GG *v* GA/AA)	1 09	0 61 to 1 96	76
PFS		1 12	0 63 to 2 00	69
Response		1.92	0 46 to 11.11	51
Neutropenia		1 30	0 35 to 5 00	87
*CYP3A4**1B				
Overall survival	Com *v* Het/Var (AA *v* AG/GG)	0 74	0 32 to 1 72	.48
PFS		0.36	0 14 to 0.94	.04
Response		0 63	0.10 to 4.76	.84
Neutropenia		0 44	0 04 to 2 94	58
*CYP3A5**3C				
Overall survival	Com/Het *v* Var (AA/AG *v* GG)	1 64	0 95 to 2 86	07
PFS		1 56	0 93 to 2 63	09
Response		1 61	0 53 to 4 76	47
Neutropenia		1 30	0 44 to 3 85	78
ERCC1 (118)				
Overall survival	TT *v* TC/CC	1 20	0 74 to 1 96	45
PFS		1 11	0.69 to 1 82	65
Response		1 45	0 48 to 4.17	61
Neutropenia		0 57	0 20 to 1.61	35
ERCC2 K751Q				
Overall survival	Com *v* Het/Var (AA *v* AC/CC)	0 97	0 63 to 1 49	89
PFS		0 85	0 55 to 1 30	45
Response		0 33	0 13 to 0 83	02
Neutropenia		0 75	0 30 to 1 85	63
nr1I2-206 del				
Overall survival	Com *v* Het/Var 206 deletion	0.82	0 53 to 1 25	.35
PFS		0.93	0.63 to 1.39	.75
Response		0 82	0.34 to 2 00	.77
Neutropenia		0 88	0 37 to 2 08	.90

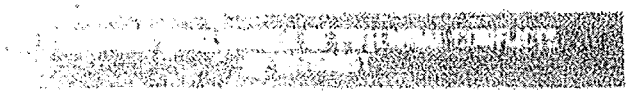
Abbreviations: HR, hazard ratio; PFS, progression-free survival; Com, common allele; Het, heterozygous allele; Var, variant allele

neutropenia ($P = .19$) for patients who harbored the ABCB1 3435 common allele.

The *ERCC2* gene, also known as xeroderma pigmentosum complementation group D, encodes a DNA helicase which complexes with TFIIH, a transcription factor essential for replication and nucleotide excision repair.[31] Several nonsynonymous SNPs have been described in this gene, including an Asp→Asn (G→A) at codon 312 in exon 10 and a Lys→Gln (A→C) at codon 751 in exon 23 and are likely in linkage disequilibrium with each other.[32,33] The functional consequences of these SNPs are still in contention, and the majority of studies indicate that variants in these alleles result in reduced DNA repair capacity.[34-41] Additionally, most studies indicate that *ERCC2* variants confer an increased risk of lung cancer.[32,34,35,42-48] In this study, 51% of patients (ie, 37 of 72 patients) from the US were homozygous wild type for the common (A) allele. These patients were significantly less likely to respond to treatment compared with US patients who had one or more variant alleles (A/C or C/C). However, no differences in overall survival were observed on the basis of *ERCC2* K751Q allele frequencies. In addition, this allele cannot account for the improved survival experienced by Japanese patients, as they uniformly harbored the common A/A genotype (and only one patient harbored A/C). The *ERCC1* 118 C→T SNP does not result in an amino acid substitution, although studies have nevertheless identified associations with patient outcome in various tumor types.[49] It has been suggested that this variant may modulate *ERCC1* mRNA and protein expression and/or may be in linkage disequilibrium with other functional SNPs.[14,50,51] However, three reports in NSCLC found no associations between the *ERCC1* 118 and patient outcome.[52-54] Here, we found a highly significant divergence in allele frequency between Japanese and US patients ($P < .0001$); however, no impact on patient outcome was observed.

In summary, the results of cancer clinical trials to test the same regimen may differ for a variety of reasons, including differences related to ethnicity. FACS, LC00-03, and S0003 were prospectively designed to facilitate a comparison of patient outcomes and pharmacogenomic results, in a setting where joint clinical trials sponsored by the US National Cancer Institute were not possible. Our

results suggest that global clinical trials (ie, those conducted internationally) should be carefully designed and conducted to account for potential genetic differences in the patient populations studied. This common-arm approach provides a model for the prospective study of population-related pharmacogenomics in which ethnic differences in antineoplastic drug disposition are anticipated.



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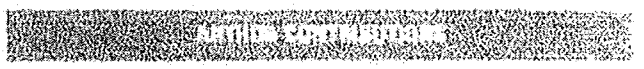

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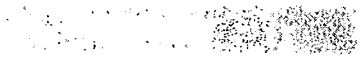

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Outcome of 93 patients with relapse or progression following allogeneic hematopoietic cell transplantation

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Relapse/progression after allogeneic hematopoietic cell transplantation (allo-HCT) remains the major cause of treatment failure. In this study, the subsequent clinical outcome was overviewed in 292 patients with leukemia/myelodysplastic syndrome who received allo-HCT. Among them, 93 (32%) showed relapse/progression. Cohort 1 was chosen to receive no interventions with curative intent (n = 25). Cohort 2 received reinduction chemotherapy and/or donor lymphocyte infusion (n = 48), and Cohort 3 underwent a second allo-HCT (n = 20). Sixty-three patients received reinduction chemotherapy, and 27 (43%) achieved subsequent complete remission (CR). The incidence of nonrelapse mortality (NRM) was similar among the three cohorts (4, 15, and 5%). The 1-year overall survival (OS) after relapse was significantly better in patients with a second HCT (58%) than in others (14%, Cohorts 1 and 2; P < .001). However, the 2-year OS did not differ between the two groups, which suggests that it is difficult to maintain CR after the second HCT. Multivariate analysis showed that reinduction chemotherapy, CR after intervention, second HCT, and longer time to post-transplant relapse were associated with improved survival. In conclusion, for patients with relapse after allo-HCT, successful reinduction chemotherapy and a second HCT may be effective for prolonging survival without excessive NRM. However, effective measures to prevent disease progression after a second HCT clearly need to be developed. Am. J. Hematol. 84:815–820, 2009. © 2009 Wiley-Liss, Inc.

Introduction

Relapse or progression of leukemia occurring after allogeneic hematopoietic cell transplantation (allo-HCT) remains the major cause of post-transplantation mortality, with a median postrelapse survival of 1.6–6 months when aggressive intervention is suspended [1–6]. The optimal treatment strategy for these patients has not yet been established. Although some patients can be reinduced into complete remission (CR) with conventional chemotherapy, only a few become long-term survivors while maintaining conventional chemotherapy [4–6], and the benefit of donor lymphocyte infusion (DLI) for acute leukemia is limited [1,3,7].

Several studies have shown that a second allo-HCT improved survival after relapse and represents a potential therapeutic option, which may increase the duration of leukemia-free survival (6–25 months) [1,6,8–14]. However, this is associated with a high rate of nonrelapse mortality (NRM) (24–75%) [8–13,15]. In many studies, the results regarding a second HCT are generally represented by heterogeneous cohorts of patients or series with relatively few patients carrying variable backgrounds. Furthermore, most studies have not compared the outcome of a second HCT with that of other interventions in the modern treatment era.

To identify the factors that influence the outcome of patients with relapse after various salvage therapies, including second HCT, we performed a retrospective single-center analysis of consecutive 292 patients.

Patients and Methods

Patients. Between January 2000 and December 2006, a total of 292 patients with leukemia or myelodysplastic syndrome (MDS) underwent allo-HCT at the National Cancer Center Hospital. Recipients of haploidentical transplants from related donors and patients aged 15 or under were not included in this study. The characteristics of the patients and transplantations are summarized in Table I. The underlying diseases were AML (n = 142), MDS (n = 73), CML (n = 34), and ALL (n = 43). The median age at the initial HCT was 50 years (range: 16–68). Of the 292 patients, 148 received an initial HCT with myeloablative conditioning (cyclophosphamide plus fractionated TBI or busulfan), and the remaining 144 received reduced-intensity conditioning (RIC; fludarabine- or cladribine-based).

Definitions. Relapse/progression after transplantation was defined as the presence of or increase in leukemic blasts as detected by morphology either in bone marrow or peripheral blood. Detection of minimal residual disease by flow cytometry, PCR, or decreasing donor chimerism did not constitute evidence of recurrence in the absence of morphological abnormalities. CR was defined as normocellular bone marrow with less than 5% blasts along with the absence of blasts in the peripheral blood [16]. Postrelapse overall survival (OS) was measured from the date of relapse or progression to the time of death or censored date of last contact. Withdrawal of immunosuppression (WIS) was defined as the cessation of immunosuppression at the diagnosis of relapse or progression. Chemotherapy was categorized into two groups: reinduction chemotherapy and less-intensive chemotherapy intended for palliative treatment. Disease-specific reinduction chemotherapy included high-dose cytarabine, idarubicin + cytarabine, aclarubicin + low-dose cytarabine [17,18], and other remission-induction therapies for myeloid and lymphoid leukemia. Imatinib mesylate for CML, all-trans retinoic acid or arsenic trioxide for acute promyelocytic leukemia (APL), gemtuzumab ozogamicin for CD33-positive AML, and intrathecal chemotherapy alone for isolated central nervous system (CNS) relapse were also included in the reinduction chemotherapy group. Less-intensive chemotherapy included oral hydroxyurea, cytarabine or 6-mercaptopurine, and the sole intravenous administration of aclarubicin or vincristine, which are not thought to be intensive enough to achieve remission, but are aimed at palliation. NRM was defined as death from toxicities related to therapy without disease recurrence.

Interventions were categorized into three cohorts: Cohort 1, WIS or less-aggressive chemotherapy; Cohort 2, reinduction chemotherapy and/or DLI; Cohort 3, second allo-HCT.

Statistical analysis. Data were retrospectively reviewed and analyzed as of August 2007. The primary endpoint of the study was OS following relapse/progression. OS was estimated by the Kaplan-Meier method.

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Conflict of Interest: Nothing to report.

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The log-rank test and generalized Wilcoxon test were used to compare the probabilities of survival over time across patient subgroups. Multiple cox regression models were used for multivariate risk-factor analysis for OS following relapse/progression. The clinical factors evaluated were diagnosis, patient age at the initial HCT, gender, conditioning in the initial HCT (myeloablative or RIC), donor in the initial HCT (HLA-matched related or others), disease status at the initial HCT, interval from the initial HCT to relapse/progression, interventions that were chosen after relapse (Cohorts 1–3), and the response to the initial intervention. We considered two-sided *P*-values of <0.05 to be statistically significant. Statistical analyses were performed with the SPSS statistics and SAS version 8.2 (SAS, Cary, NC).

TABLE I. Patient and Transplanation Characteristics

Characteristics	All patients	Relapsed patients %[a]
No. of patients	292	93 (32)
Age, year, median (range)	50 (16–68)	47 (16–68)
Diagnosis[b]		
AML	142	57 (40)
MDS	73	13 (9)
CML	34	5 (4)
ALL	43	18 (13)
Gender		
Male	173	49 (35)
Female	119	44 (31)
Matched related donor		
Yes	125	44 (31)
No	167	49 (35)
Conditioning regimen		
Myeloablative		
TBI-based	90	38 (27)
BU/CY-based	58	21 (16)
RIC	144	34 (24)
Stem cell source		
BM	125	37 (26)
PBSC	149	49 (35)
CB	18	7 (5)
Disease status at first HCT		
CR	150	42 (30)
non-CR	142	51 (36)
GVHD prophylaxis		
CSP-based	243	77 (54)
TAC-based	49	16 (11)

AML, acute myeloid leukemia; MDS, myelodysplastic syndrome; CML, chronic myeloid leukemia; ALL, acute lymphoid leukemia; TBI, total body irradiation; BU/CY, busulfan/cyclophosphamide; RIC, reduced-intensity conditioning; BM, bone marrow; PBSC, peripheral blood stem cell; CB, cord blood; CR, complete remission; GVHD, graft-versus-host disease; CSP, cyclosporin; TAC, tacrolimus.

[a] The percentage shown here indicates the proportion to relapsed patients among each category.

[b] MDS overt leukemia was categorized into AML.

Results

Relapse or progression

The characteristics of all patients and relapsed patients are shown in Table I. Overall, 93 of the 292 patients (32%) relapsed or progressed at a median of 154 days (range; 15–1,211) after the initial HCT (AML, $n = 57$; MDS, $n = 13$; CML, $n = 5$; ALL, $n = 18$). The interval from the initial HCT to relapse/progression was less than 100 days in 34 patients, 100 days to 1 year in 39 patients, and more than 1 year in 20 patients.

TABLE II. Outcomes of Interventions after Relapse

Therapy	*n*	CR (%)	NRM (%)	OS after relapse, day, median, (range)
Total	93	34 (37)	9 (10)	184 (5–1456)
No aggressive Tx	25	1 (4)	1 (4)	61 (5–245)
No therapy	7	0	0	56 (22–166)
WIS alone	10	1	1	60 (5–245)
Less- Int. CTx	8	0	0	74 (12–203)
Chemotherapy/DLI	48	18 (38)	7 (15)	194 (19–1,456)
Reinduction CTx	31	9 (29)	2 (6)	167 (19–1,456)
CTx + DLI	14	7 (50)	4 (29)	194 (52–1,254)
DLI alone	3	2 (67)	1 (33)	240 (32–243)
second HCT	20	15 (75)	1 (5)	502 (66–997)

CR, complete remission; NRM, nonrelapse mortality; OS, overall survival; Tx, therapy; WIS, withdrawal of immunosuppression; Less-Int. CTx, less-intensive chemotherapy; DLI, donor lymphocyte infusion; HCT, hematopoietic cell transplantation.

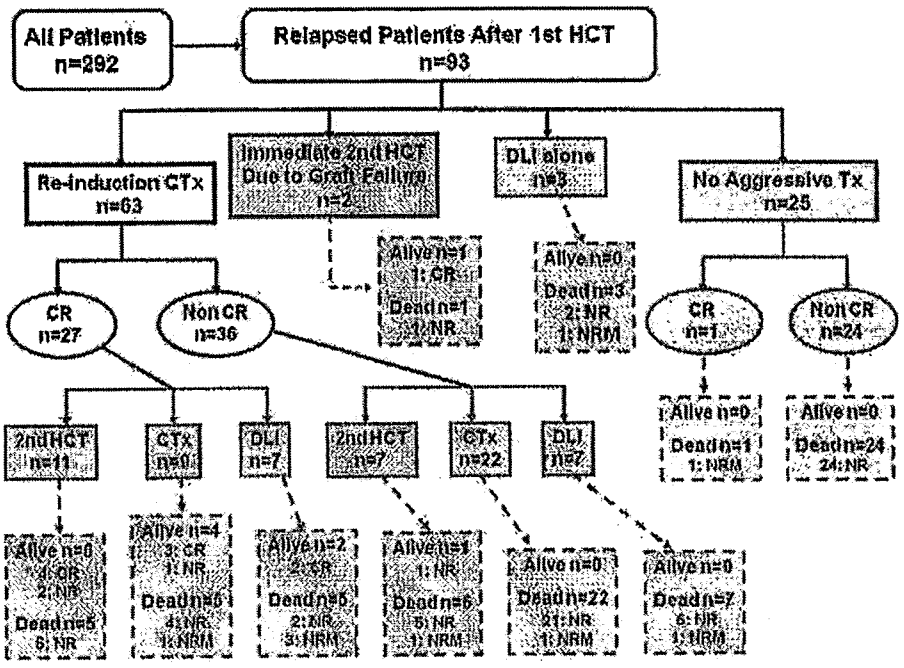

Figure 1. Summary of interventions after relapse. Abbreviations: HCT, hematopoietic cell transplantation; CTx, chemotherapy; Tx, therapy; CR, complete remission; DLI, donor lymphocyte infusion; NR, nonremission; NRM, nonrelapse mortality. [Color figure can be viewed in the online issue, which is available at www.interscience.wiley.com.]