North Carolina, USA). A significance level of 0.05 was applied to all two-tailed analyses.

Results

CYP2C8 variations

We reported previously the *CYP2C8* nonsynonymous variations, **5* (475delA, 159fsX18) [7], **6* (511G > A, Gly171Ser), *7 (556C > T, Arg186X), **8* (556C > G, Arg186Gly,) **9* (740A > G, Lys247Arg), **10* (1149G > T, Lys383Asn) [8], **13* (669T > G, Ile223Met), and **14* (712G > C, Ala238Pro) [9]. These variations were, however, very rare in the Japanese, and it was rather difficult to perform statistical evaluation on their in-vivo associations with altered function, because of low frequencies [9]. Therefore, we continued resequencing this gene including the promoter and intronic regions for up to 437 patients. The enhancer regions were also sequenced for 199 patients administered PTX. Table 2 summarizes the obtained data, where Genbank accession number NT_030059.12 was utilized for the reference sequence. Forty variations, including 11 novel ones, were detected in 437 patients. Because we did not find any significant differences in the genotype distributions among the three disease types ($P \geq 0.05$ by χ^2 test or Fisher's exact test), data from all patients were analyzed as one group. All detected variations were found in Hardy–Weinberg equilibrium ($P \geq 0.05$ by χ^2 test or Fisher's exact test), except for two polymorphisms IVS3−97delT and IVS3−21_−20insT. These deviations were due to the occurrence of one extra homozygote, and the existence of these homozygotes was confirmed by amplification of DNA by another set of primers and resequencing (data not shown). The overall frequencies of the previously reported nonsynonymous variations *CYP2C8*5*, **6*, *7, **8*, **9*, **10*, **12* (1382_1384del TTG, del 461Val), **13*, and **14* were 0.002, 0.002, 0.001, 0.001, 0.001, 0.001, 0.001, 0.001, and 0.001, respectively, and they were all found as heterozygotes. We also detected −271C > A (*CYP2C8*1B*) and −370T > G (**1C*) at frequencies of 0.106 and 0.330, respectively. The frequency of the **1C* allele in Japanese is approximately 5.4-fold higher than in Caucasians [6]. We did not detect any variation in the functional hepatocyte nuclear factor 4α-binding site (−155 to −137 from the translational start site on NT_030059.12) [17], and its surrounding region in 437 patients. Also no variation was found in pregnanex receptor/constitutive androstane receptor-binding site (−8807 to −8788), glucocorticoid receptor-binding site (−1930 to −1910) [17], and their surrounding regions in 199 PTX-administered patients.

Linkage disequilibrium analysis

Using the 15 detected polymorphisms greater than 0.03 in frequency, LD was analyzed for $|D'|$ and r^2 values (Fig. 1). $|D'|$ values were more than 0.9 in 89 out of 105 (85%) combinations (Fig. 1, lower left). For r^2 values (Fig. 1, upper right), strong LD ($r^2 \geq 0.80$) was observed among IVS2-64A > G, IVS2−13_−12insT, IVS3−166A > G, IVS4−150G > A, IVS4−94T > C, IVS6 + 196G > A, IVS7 + 49T > A, IVS8 + 106G > A, and 1497 (*24)C > T. These polymorphisms were also moderately linked with −411T > C and −370T > G ($r^2 \geq 0.49$). Strong LD was also observed between IVS3-21T > A and IVS4 + 151G > A ($r^2 = 0.93$), and both variations were partially linked with IVS8-204A > G ($r^2 \geq 0.57$). The r^2 values of the other combinations were below 0.33. Collectively, relatively strong LDs were observed throughout the *CYP2C8* gene, suggesting that one LD block covers the entire region analyzed (approximately 33 kb). Thus, *CYP2C8* haplotypes were analyzed as one block.

Haplotype analysis

Haplotypes determined/inferred are shown in Fig. 2. The haplotypes obtained in this study were tentatively shown as a number plus small alphabetical letter except for the haplotypes already publicized on the Human Cytochrome P450 (*CYP*) Allele Nomenclature Committee website, which are described as the number plus capital alphabetical letter (**1A*, **1B*, and **1C*). Several haplotypes were first unambiguously assigned by homozygous single nucleotide polymorphisms at all sites (**1d*-**1f*, **1j*, and **1w*) or a heterozygous single nucleotide polymorphism at only one site (**1k*, **1m*, **1t*, **1z*, **1aa*, and **8b*). Separately, diplotypes for each patient were inferred by LDSUPPORT software. The additionally inferred haplotypes were 27 **1* subtypes (**1g*, **1h*, **1l*, **1n* to **1s*, **1u*, **1v*, and other very rare 17 haplotypes), and eight haplotypes with nonsynonymous variations (**5b*, **6b*, **7b*, **9b*, **10b*, **12b*, **13b*, and **14b*). The **1* subtypes inferred in only one patient are grouped into 'others' in Fig. 2, and haplotypes with nonsynonymous variations are described with '?' except for unambiguous **8b*, since the predictability for these very rare haplotypes is known to be low in some cases. Overall, 49 haplotypes were determined and/or inferred. The most frequent haplotype was **1d* (frequency: 0.366), followed by **1e* (0.289), **1f* (0.113), and **1B* (0.085). Frequencies of the other haplotypes were less than 0.05.

Next, we performed network analysis using haplotypes found in more than two patients to clarify the relationships among the haplotypes. The results showed that the **1* subtypes could be further classified into six groups, **1A*, **1B*, **1D*, **1E*, **1G*, and **1J* groups (Fig. 3). The grouping of **1* subtypes was also shown in Fig. 2. Their frequencies were 0.435 (**1E* group), 0.381 (**1D*), 0.103 (**1B*), 0.030 (**1G*), 0.021 (**1A*), and 0.013 (**1J*). Five rare unclassified **1* subtypes were shown in '**1 others*'. Haplotypes **5b* and **6b* were shown to be derived from **1d* and **1B*, respectively.

Effects of *CYP2C8* haplotypes on PTX metabolism

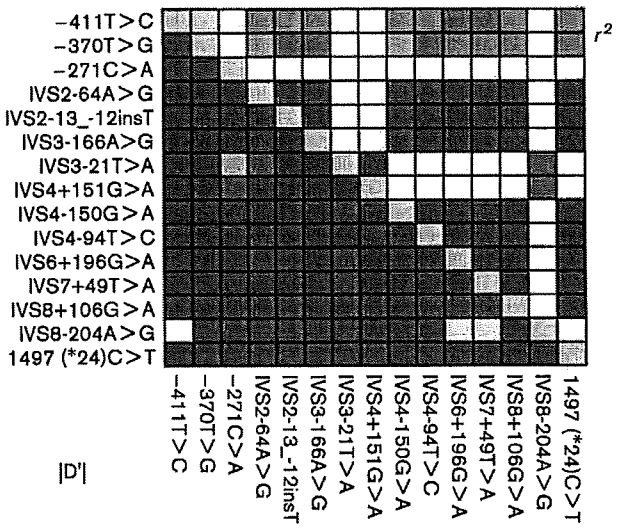
CYP2C8 catalyzes biotransformation of PTX into 6α-OH-PTX and of 3′-*p*-OH-PTX into diOH-PTX. The effects of *CYP2C8* haplotypes on PTX clearance, AUCs of PTX

Table 2 Summary of *CYP2C8* variations detected in a Japanese population

SNP ID					Position		Nucleotide change and flanking sequences (5′ to 3′)	Amino acid change	Allele name	Number of subjects			Frequency
This study	NCBI (dbSNP)	JSNP	Reference	Location	NT_030059.12	From the translational initiation site or from the nearest exon				Wild-type	Hetero-zygotes	Homo-zygotes	
MPJ6_2C8029[a]				5′-flanking	15578352_15578350	−667_−665[b]	ATAATGTAATAA**TAA/-**CACAAATATTAT			435	2	0	0.002
MPJ6_2C8030	rs7912549		[4]	5′-flanking	15578096	−411[b]	ACATTTTTTATA**T/C**ACAAAATATAGA			152	218	67	0.403
MPJ6_2C8031	rs17110453		[6]	5′-flanking	15578055	−370[b]	CAAGGTCATAAA**T/G**TCCCAACTGGTC		*1C*	201	184	52	0.330
MPJ6_2C8023	rs7909236		[6]	5′-flanking	15577956	−271[b]	AGCACATTGGAA**C/A**ACCAGGGACTT		*1B*	352	77	8	0.106
MPJ6_2C8032[a]				Intron 1	15576171	IVS1−197	CTGGGTCATTGC**G/A**TGGCACATCACA			436	1	0	0.001
MPJ6_2C8014			[7]	Intron 1	15576095	IVS1−121	ATTCAGAAATAT**C/T**GAATCTATGTGT			436	1	0	0.001
MPJ6_2C8010	rs2275622	IMS-JST071855	[4]	Intron 2	15575704	IVS2−64	TGCATGGCTGCC**A/G**AGTGTTGCAGCA			120	212	105	0.483
MPJ6_2C8001	rs11572078	IMS-JST077576	[4]	Intron 2	15575653_15575652	IVS2−13_−12	AGTTTCTGCCCC**-/T**TTTTTTTTATTAG			142	205	90	0.441
MPJ6_2C8015			[7]	Exon 3	15575497	475[b]	GAGTTGAGAAAAA**/-**CCAAGGGTGGGT	159fsX18	*5*	435	2	0	0.002
MPJ6_2C8019	rs3752988	IMS-JST105874		Intron 3	15573409	IVS3−166	AACTCATATTTA**A/G**GGTAAAAGTAAT			141	207	89	0.441
MPJ6_2C8016	rs11572091		[7]	Intron 3	15573340	IVS3−97	TTTGTAAGATATT**/-**GTTTAAAATTTC			427	9	1	0.013
MPJ6_2C8033[a]				Intron 3	15573264_15573263	IVS3−21_-20	AATAATTTTTTT**-/T**AAAAATTTTTAA			436	0	1	0.002
MPJ6_2C8004	rs7098376		[5]	Intron 3	15573264	IVS3−21	TAATAATTTTTT**T/A**AAAAATTTTTAA			409	28	0	0.032
MPJ6_2C8034			[8]	Exon 4	15573214	511[b]	ACTTTCATCCTG**G/A**GCTGTGCTCCCT	Gly171Ser	*6*	435	2	0	0.002
MPJ6_2C8035			[8]	Exon 4	15573169	556[b]	GTTTTCCAGAAA**C/T**GATTTGATTATA	Arg186X	*7*	436	1	0	0.001
MPJ6_2C8036			[8]	Exon 4	15573169	556[b]	GTTTTCCAGAAA**C/G**GATTTGATTATA	Arg186Gly	*8*	436	1	0	0.001
MPJ6_2C8037[a]				Intron 4	15573039	IVS4+44	CATTTATTCAAG**G/T**TTGTAGGGAAGA			436	1	0	0.001
MPJ6_2C8024	rs11572093		[4]	Intron 4	15572932	IVS4+151	CTTTGATTTCCT**G/A**TTCAAAATTTTC			411	26	0	0.030
MPJ6_2C8038[a]				Intron 4	15567024	IVS4−230	GACGAGTTATTG**G/A**GTGCAGTACACC			436	1	0	0.001
MPJ6_2C8039[a]				Intron 4	15567008	IVS4−214	CAGTACACCAAC**C/A**TGGCACATGTAT			436	1	0	0.001
MPJ6_2C8020	rs1926705			Intron 4	15566944	IVS4−150	AGAACTTAAAGT**G/A**TAATAAAAAATG			119	214	104	0.483
MPJ6_2C8025[a]				Intron 4	15566937	IVS4−143	AAAGTGTAATAA**A/G**AAATGTATATAT			436	1	0	0.001
MPJ6_2C8012	rs11572101			Intron 4	15566888	IVS4−94	GACATGATGTCT**T/C**ATTCATATTTAT			141	207	89	0.441
MPJ6_2C8040			[9]	Exon 5	15566768	669[b]	CCCTCTACTCAT**T/G**GATTGTTCCCA	Ile223Met	*13*	436	1	0	0.001
MPJ6_2C8041			[9]	Exon 5	15566725	712[b]	CTTAAAAATGTT**G/C**CTCTTACACGAA	Ala238Pro	*14*	436	1	0	0.001
MPJ6_2C8026			[8]	Exon 5	15566697	740[b]	ACATTAGGGAGAA**A/G**AGTAAAAGAACA	Lys247Arg	*9*	436	1	0	0.001
MPJ6_2C8027[a]				Intron 5	15566597	IVS5+21	TTAGCAACAGAT**C/T**AGTATTTTGATT			435	2	0	0.002
MPJ6_2C8042[a]				Intron 6	15553909	IVS6+184	GGAGGAGGATGA**C/G**AGAGATCAGTAG			433	4	0	0.005
MPJ6_2C8021	rs1891071	IMS-JST082397		Intron 6	15553897	IVS6+196	CAGAGATCAGTA**G/A**AAACAGTATGGC			124	215	98	0.470
MPJ6_2C8043[a]				Intron 6	15553794	IVS6+299	ATTGCCCTAGTA**T/C**TGAATGTTGGTT			436	1	0	0.001
MPJ6_2C8044			[8]	Exon 7	15551173	1149[b]	CCTCATCCCCAA**G/T**GTAAGCTTGTTT	Lys383Asn	*10*	436	1	0	0.001
MPJ6_2C8013	rs2275620	IMS-JST071852	[4]	Intron 7	15551124	IVS7+49	CTGAAATTTCCA**T/A**AGTGCTGGTTTG			124	215	98	0.470
MPJ6_2C8017			[7]	Intron 7	15551102	IVS7+71	TTGGTTCCAACC**C/T**TCTAACAACACA			430	7	0	0.008
MPJ6_2C8007			[5]	Exon 8	15547241	1230[b]	CTTTGACCCTGG**C/T**CACTTTCTAGAT	Gly410Gly		424	13	0	0.015
MPJ6_2C8008	rs1934951	IMS-JST071853	[5]	Intron 8	15547074	IVS8+106	GAATTGCTATTT**G/A**TCCATGATCAAG			143	201	93	0.443
MPJ6_2C8022	rs2275621	IMS-JST071854		Intron 8	15547050	IVS8+130	GAGCACCACTCT**T/C**AACACCCATGTG			430	7	0	0.008
MPJ6_2C8018	rs11572177		[7]	Intron 8	15545796	IVS8−204	GATAGCAAATAT**A/G**TCTCTTTTTGTA			403	33	1	0.040
MPJ6_2C8045	rs3832694	IMS-JST091412		Exon 9	15545502_15545500	1382_1384[b]	ACCTGAAATCTG**TTG/-**ATGATTTAAAGA	del 461Val	*12*	436	1	0	0.001
MPJ6_2C8009	rs1058932	IMS-JST091413	[5]	3′-UTR	15545387	1497[b](*24)[c]	CCATCTGGCTGC**C/T**GATCTGCTATCA			143	196	98	0.449
MPJ6_2C8046[a]				3′-UTR	15545208	1676[b](*203)[c]	ACTCTGTAACAC**T/-**TGTATTAATTGC			434	3	0	0.003

[a]Novel variations detected in our study.
[b]A of the translation initiation codon ATG is numbered +1.
[c]The nucleotide number from the end of translational termination codon.
SNP, single nucleotide polymorphism.

Fig. 1



Linkage disequilibrium (LD) analysis of *CYP2C8*. Pairwise LD between variations with ≥ 3% frequencies is expressed as |*D'*| (lower left) and r^2 (upper right) by 10-graded blue colors. A denser color represents a higher linkage.

and its metabolites, and metabolic ratios (ratios of metabolite AUCs to PTX AUC) were investigated in 199 PTX-administered patients.

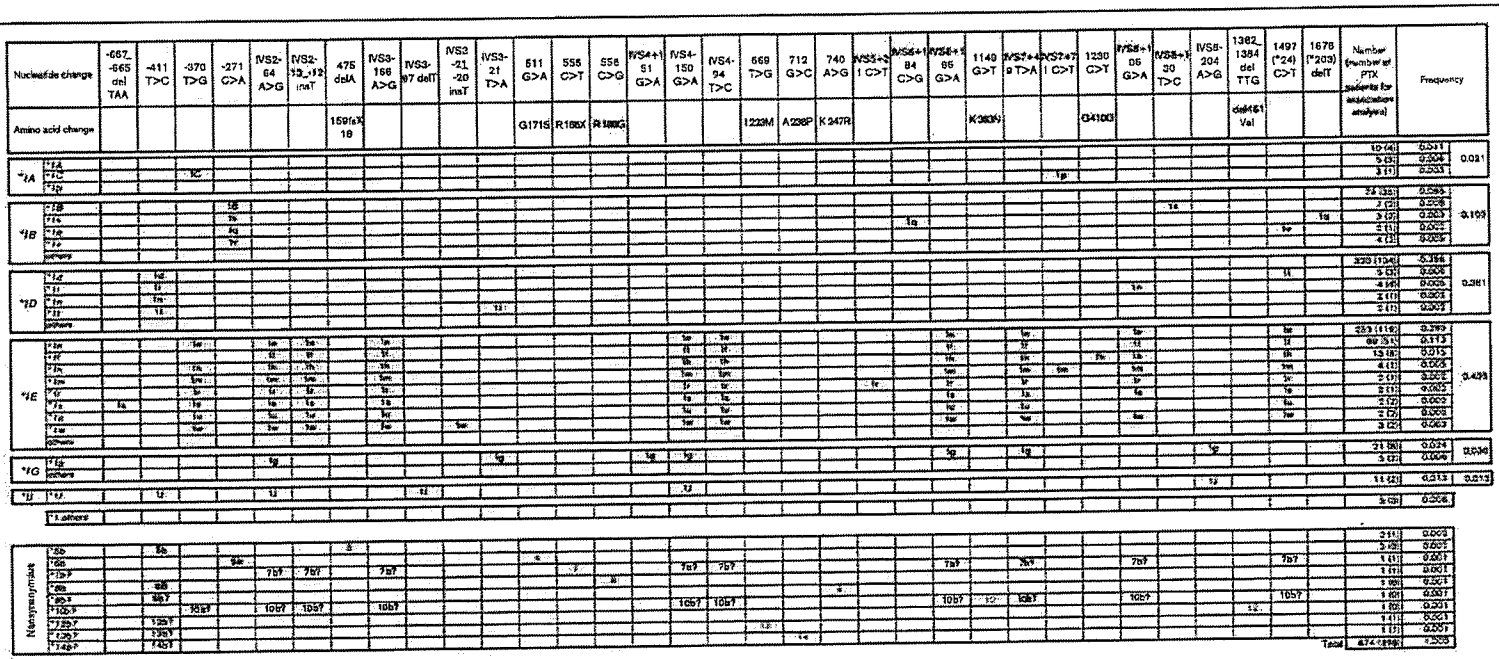
Because nonsynonymous variations were all rare, we focused on the effects of diplotypes using grouped **1* haplotypes (i.e. **1A*, **1B*, etc). No significant differences were observed in clearance of PTX, AUCs of PTX, 6α-OH-PTX and diOH-PTX, and AUC ratio of 6α-OH-PTX/PTX among the grouped **1*-diplotypes found in more than three patients (data not shown). A statistically significant deviation, however, was observed in AUC of 3′-*p*-OH-PTX among the grouped **1*-diplotypes ($n \geq 3$) ($P = 0.014$ by Kruskal–Wallis test) (Fig. 4a). Furthermore, AUC ratio of 3′-*p*-OH-PTX/PTX also showed a tendency to be different among the grouped **1*-diplotypes of $n \geq 3$ by the same test ($P = 0.071$) (Fig. 4b). Careful analysis revealed that significant differences in both parameters were observed between **1D/*1D* and **1G/*1D* patients ($P < 0.05$ for both parameters, Mann–Whitney *U*-test) and between **1E/*1E* and **1G/*1E* patients ($P < 0.001$ for AUC of 3′-*p*-OH-PTX and $P < 0.01$ for AUC ratio of 3′-*p*-OH-PTX/PTX) (Fig. 4).

Next, heterozygous **1G* diplotypes were combined into **1G/non-*1G* diplotypes ($n = 11$). Because no significant differences were observed among the other **1/*1* groups, all the other **1/*1* diplotypes were combined into one group, designated as *non-*1G/non-*1G*. As shown in Fig. 5a, the median AUC of 3′-*p*-OH-PTX was about 2.5-fold higher in the **1G/non-*1G* patients than in the *non-*1G/non-*1G* patients ($P < 0.001$ by Mann–Whitney *U*-test). The median value of 3′-*p*-OH-PTX/PTX AUC ratio was also about 64% higher in the **1G/non-*1G* patients than in the *non-*1G/non-*1G* patients ($P < 0.001$, Fig. 5b). In contrast, there were no significant differences in AUC of 6α-OH-PTX and AUC ratio of 6α-OH-PTX/PTX between the two groups (Fig. 5c and d) although the AUC ratio was about 9% lower in the **1G/non-*1G* patients than in the *non-*1G/non-*1G* patients (Fig. 5d). Considering the metabolic route of PTX, these findings suggest that CYP2C8 activity is probably reduced in the **1G*-bearing patients.

Recently, we have shown that *CYP3A4*16B* (and probably **6*, $n = 1$) decreases the AUC ratio of 3′-*p*-OH-PTX/PTX, and that no other major *CYP3A4* haplotypes significantly affect the AUC ratio and other PK parameters analyzed [9]. Therefore, we analyzed the effects of **1G* on the AUC of 3′-*p*-OH-PTX and AUC ratio of 3′-*p*-OH-PTX/PTX excluding *CYP3A4*16B*- and **6*-bearing patients and confirmed the increasing effects of **1G* ($P < 0.001$ for both by Mann–Whitney *U*-test). In addition, the significantly increasing effects of *CYP2C8*1G* were also observed within *CYP3A4*1A/*1A* patients ($P < 0.001$ for AUC of 3′-*p*-OH-PTX and $P < 0.01$ for AUC ratio of 3′-*p*-OH-PTX/PTX, Mann–Whitney *U*-test). Furthermore, distributions of *CYP3A4* diplotypes/haplotypes were not significantly different between the *CYP2C8*1G/non-*1G* patients and the *non-*1G/non-*1G* patients ($P > 0.05$ by Fisher's exact test). These results suggest that the effects of *CYP2C8*1G* are independent of the *CYP3A4* genotypes. Gender also affects the AUC ratio of 3′-*p*-OH-PTX/PTX [9]. Statistical analysis using data from men only also gave almost the same increasing effects of **1G* ($P < 0.001$ for the AUC of 3′-*p*-OH-PTX and $P = 0.001$ for the AUC ratio of 3′-*p*-OH-PTX/PTX, Mann–Whitney *U*-test).

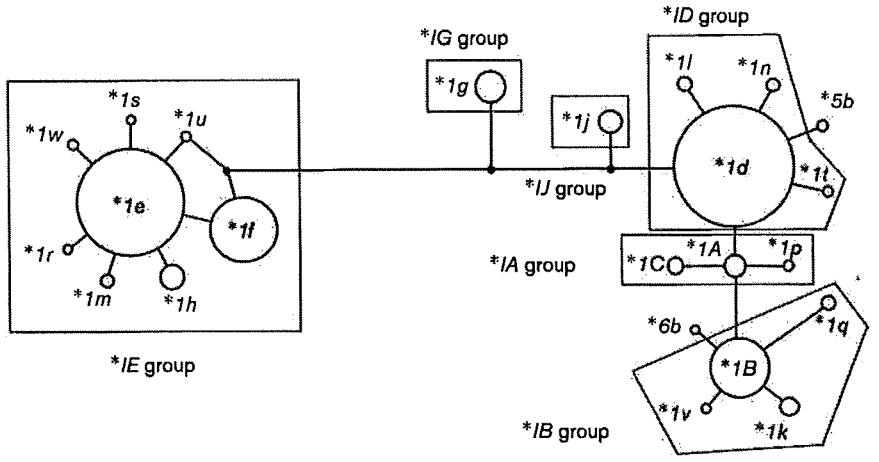
To identify further the genetic variation responsible for the increased AUC of 3′-*p*-OH-PTX and increased AUC ratio of 3′-*p*-OH-PTX/PTX, we next focused on the variations in the **1G* group. Among them, the patients bearing IVS3-21T > A showed statistically significant increases in these parameters compared with the patients without this variation ($P < 0.001$ for both parameters, Mann–Whitney *U*-test). The **1t* haplotype also harbored IVS3−21T > A, and one patient with the **1t/*1d* diplotype (grouped into **1D/*1D*) had the second highest AUC of 3′-*p*-OH-PTX (1.07 h*μg/ml) and the second highest AUC ratio of 3′-*p*-OH-PTX/PTX (0.0497) in the 24 **1D/*1D* patients (Fig. 4, grey arrowheads). These findings suggest that IVS3−21T > A might be involved in the altered CYP2C8 activity, although we cannot exclude the possibility that other identified/unidentified linked variation is causative.

Fig. 2



Haplotype structures of *CYP2C8* in Japanese population. Haplotypes are described with numbers plus alphabetical letters, and the **1* haplotypes (without amino-acid change) were grouped based on network analysis (Fig. 3). Because rare **1* haplotypes found in only one individual were grouped into 'others', the seven variations detected only in the rare haplotypes (IVS1−197G>A, IVS1 −121C>T, IVS4+44G>T, IVS4−230G>A, IVS4−214C>A, IVS4−143A>G, and IVS6+299T>C) are not shown in this figure. Numbers in parenthesis in the 'Number' section indicate patient numbers used for association analysis for pharmacokinetic parameters of PTX. White, major allele; yellow with a haplotype name, minor allele; yellow without a haplotype name in 'others,' common variations of the 'others' group. In yellow with a haplotype name, nonsynonymous variations are shown by a red number.

Fig. 3



Network analysis of *CYP2C8* haplotypes. Haplotypes found in at least two patients are shown. The areas of each circle represent the approximate frequency of each haplotype. The **1* subgroups are enclosed by red lines.

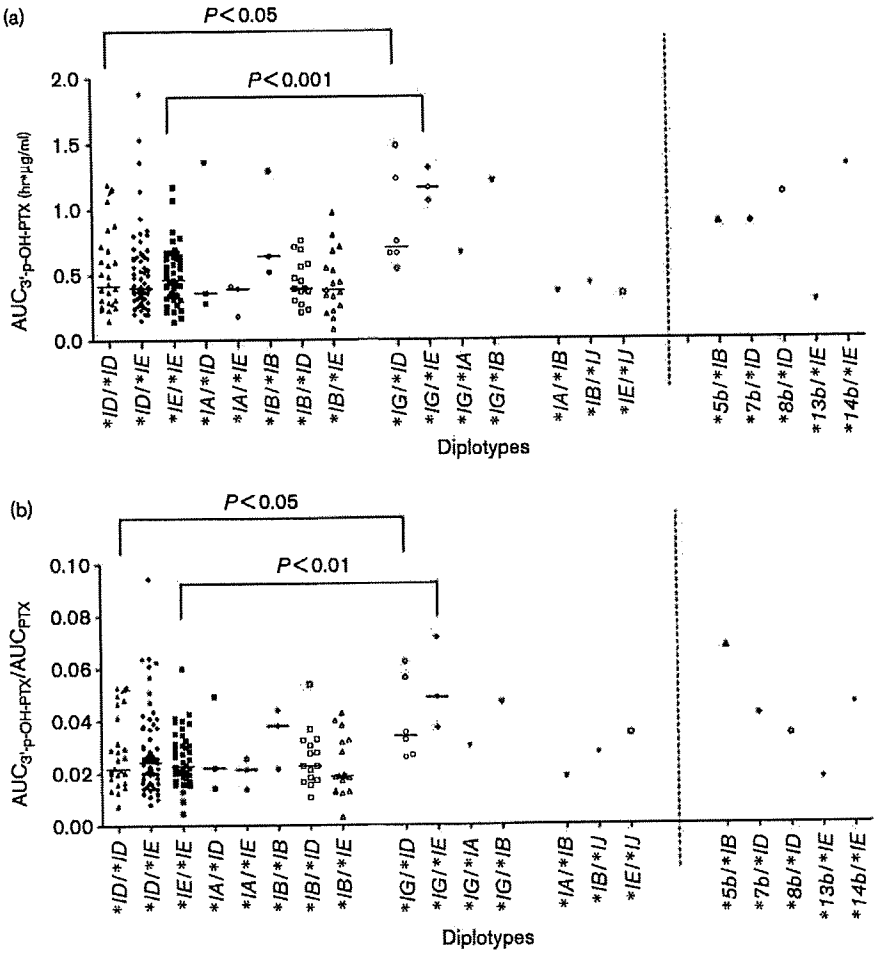
Discussion

All nonsynonymous variations of *CYP2C8* found in Japanese were rare (frequencies ≤ 0.002), and thus we could not apply statistical analysis for their associations with pharmacokinetic parameters of PTX [9]. As shown in Fig. 4b, the AUC ratio of 3′-*p*-OH-PTX/PTX of a patient with heterozygous **5b* haplotype (with 475delA, 159fsX18, no activity) was, however, the third highest (2.8-fold higher than median value) in all 199 patients analyzed. In addition, the patient with heterozygous **7b* (with 556C > T, Arg186X, no activity) had the lowest AUC ratio of 6α-OH-PTX/PTX (approximately one-fifth of the median value) (data not shown). Thus, at least some of the nonsynonymous *CYP2C8* variations described in this paper probably affect the PTX metabolism *in vivo*. These rare variations, however, cannot fully explain the interindividual differences in the CYP2C8 activity. Therefore, we focused on the **1* haplotypes without amino-acid change. The estimated *CYP2C8 *1* haplotypes could be classified into six haplotype groups (**IA*, **IB*, **ID*, **IE*, **IG*, and **IJ*) based on network analysis, and their effects on PTX metabolism were analyzed.

This study revealed that the AUC of 3′-*p*-OH-PTX and AUC ratio of 3′-*p*-OH-PTX/PTX were increased in the **IG*-bearing patients. It must be noted that AUC of 3′-*p*-OH-PTX was considerably increased (2.5-fold). The 3′-*p*-OH-PTX is generated from PTX by CYP3A4 and metabolized into diOH-PTX by CYP2C8. Thus, both CYP2C8 and CYP3A4 activities can influence the AUC of 3′-*p*-OH-PTX. In the previous study [9], we have shown that the *CYP3A4*16B* haplotype harboring 554C > G (Thr185Ser), but not the other haplotypes, increases the AUC ratio of 6α-OH-PTX/PTX and decreases the AUC ratio of 3′-*p*-OH-PTX/PTX with statistical significance. In addition, gender difference was also shown to affect both AUC ratios [9].The association of *CYP2C8*IG* group haplotypes with increased AUC of 3′-*p*-OH-PTX and AUC ratio of 3′-*p*-OH-PTX/PTX, however, could not be explained by the influence of *CYP3A4*16B* (and theoretically null haplotype **6*) or gender difference since the same conclusions were obtained even if patients with *CYP3A4*16B* and **6*, or females were excluded. Moreover, statistical analysis using data only from *CYP3A4*1A/*1A* patients also gave almost the same effects of **IG* on the AUC of 3′-*p*-OH-PTX and the AUC ratio of 3′-*p*-OH-PTX/PTX, suggesting that the effects of *CYP2C8*IG* are independent of the *CYP3A4* genotypes or gender difference. Thus, the increased AUC of 3′-*p*-OH-PTX and AUC ratio of 3′-*p*-OH-PTX/PTX can be attributed to *CYP2C8*IG*, suggesting reduced CYP2C8 activity in patients with **IG*. Moreover, transporters such as P-glycoprotein encoded by the *ABCB1* gene could contribute to the AUCs of PTX and its metabolites [20]. We reported previously that AUC of 3′-*p*-OH-PTX was slightly increased in the patients bearing **2* haplotype in block 2 of *ABCB1* (1236C > T, 2677G > T, and 3435C > T) [9]. When the frequencies of the **2* haplotype were compared between the *CYP2C8*IG/non-*IG* patients and the *non-*IG/non-*IG* patients, however, no statistically significant difference was observed ($P = 0.705$ by χ^2 test).

*CYP2C8*IG* group haplotypes harbors several variations, which are all located in introns. Thus, the mechanism for the increased AUC of 3′-*p*-OH-PTX and AUC ratio of 3′-*p*-OH-PTX/PTX is not caused by an amino-acid change. Among the variations in the **IG* group, IVS3 – 21T > A

Fig. 4



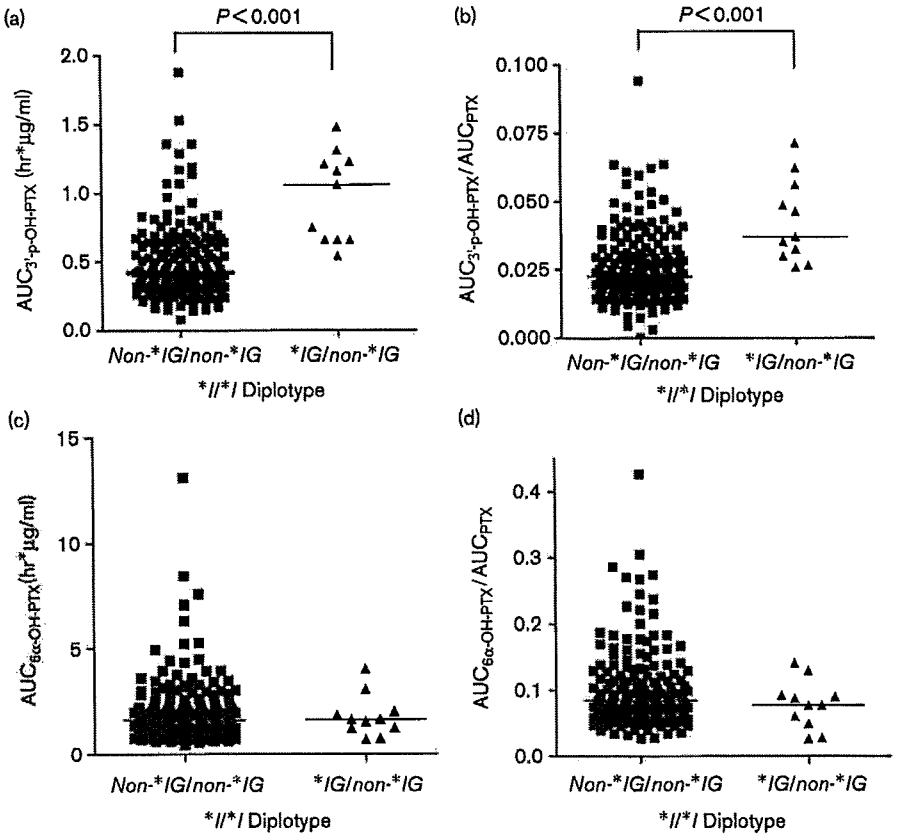
Effects of *CYP2C8* diplotypes on AUC of 3′-*p*-OH-PTX (a), and AUC ratio of 3′-*p*-OH-PTX/PTX (b). All combinations of diplotypes using grouped haplotypes for **1* are shown. Grey arrowheads indicate patients with heterozygous **1t* haplotype. Statistical significance was analyzed by the Mann–Whitney *U*-test to reveal the effects of **IG* group haplotypes. AUC, area under concentration–time curve; PTX, paclitaxel.

and IVS4 + 151G > A were relatively **IG* group specific. Because the patient with **1t* haplotype also had a high AUC of 3′-*p*-OH-PTX and a high AUC ratio of 3′-*p*-OH-PTX/PTX, it is possible that the IVS3 – 21T > A could be a functionally causing variation rather than IVS4 + 151G > A. Because IVS3-21T > A is located in the T-rich (pyrimidine-rich) region upstream of a splice acceptor site and this polypyrimidine tract is important for efficient RNA spliceosome assembly [21], this transversion could reduce the expression level of mature CYP2C8 mRNA, resulting in reduced protein expression levels. We cannot, however, exclude the possibility that other identified/unidentified linked variation could be causative.

We did not observe significant differences in the AUC of 6α-OH-PTX and AUC ratio of 6α-OH-PTX/PTX between the heterozygous **IG* patients and *non-*IG/non-*IG* patients. This is surprising because CYP2C8 is considered to be the major enzyme responsible for 6α-hydroxylation of PTX. Currently, we have no data for explaining this. It is noteworthy that the *CYP3A4*16B* haplotype more clearly affects the increase in AUC ratio of 6α-OH-PTX/PTX than the decrease in AUC ratio of 3′-*p*-OH-PTX/PTX [9]. CYP3A4- and CYP2C8-mediated disappearance processes of 6α-OH-PTX and 3′-*p*-OH-PTX, respectively, might be more influential to their AUCs than their generation from PTX. One alternative (less likely) possibility is that another unidentified enzyme also catalyzes the transformation of PTX into 6α-OH-PTX *in vivo*, and that the effect of reduced CYP2C8 activity is not clearly reflected in the parameters analyzed.

Neither the normalized clearance nor AUC of PTX was significantly influenced by *CYP2C8* diplotypes. The small effect of **IG* on PTX clearance may be partly explained

Fig. 5



Effects of *CYP2C8*IG* group haplotypes on AUC of 3′-*p*-OH-PTX (a), AUC ratio of 3′-*p*-OH-PTX/PTX (b), AUC of 6α-OH-PTX (c), and AUC ratio of 6α-OH-PTX/PTX (d). Statistical significance was analyzed by the Mann–Whitney *U*-test. AUC, area under concentration–time curve; PTX, paclitaxel.

by only small fraction of PTX to be metabolized. In fact, median AUC of 3′-*p*-OH-PTX (0.50 h/mol/l) and 6α-OH-PTX (1.85 h/mol/l) was only 2.3 and 8.5% of that of AUC of PTX (21.67 h/mol/l), respectively.

Recently, Nakajima *et al.* [13] tried to analyze the effects of *CYP2C8* polymorphisms on PTX pharmacokinetics. They genotyped 11 nonsynonymous variations including *CYP2C8*5*, but none were detected from 23 Japanese ovarian cancer patients. Also, we could not apply statistical analysis to the pharmacokinetic parameters for five nonsynonymous variations as described above since the nonsynonymous variations are all rare in Japanese. Rather, **IG* group haplotypes (and possibly **1t*) are probably important for PTX metabolism. The effect of this group haplotypes tagged by IVS3 – 21T > A on pharmacokinetics of other CYP2C8-catalyzing drugs must be clarified in the future.

In conclusion, we determined/inferred a total of 49 haplotypes using the detected variations in the *CYP2C8* gene from 437 Japanese patients. *CYP2C8*IG* group haplotypes, consisting of intronic variations, were found to be associated with significantly increased AUC of the PTX metabolite 3′-*p*-OH-PTX and the AUC ratio of 3′-*p*-OH-PTX/PTX. Thus, *CYP2C8*IG* group haplotypes may influence CYP2C8 activity, although the causative variation is not fully identified.

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References

1 Totah RA, Rettie AE. Cytochrome P450 2C8: substrates, inhibitors, pharmacogenetics, and clinical relevance. *Clin Pharmacol Ther* 2005; 77:341–352.

2 Sonnichsen DS, Liu Q, Schuetz EG, Schuetz JD, Pappo A, Relling MV. Variability in human cytochrome P450 paclitaxel metabolism. *J Pharmacol Exp Ther* 1995; **275**:566–575.
3 Baldwin SJ, Clarke SE, Chenery RJ. Characterization of the cytochrome P450 enzymes involved in the in vitro metabolism of rosiglitazone. *Br J Clin Pharmacol* 1999; **48**:424–432.
4 Dai D, Zeldin DC, Blaisdell JA, Chanas B, Coulter SJ, Ghanayem BI, *et al.* Polymorphisms in human CYP2C8 decrease metabolism of the anticancer drug paclitaxel and arachidonic acid. *Pharmacogenetics* 2001; **11**:597–607.
5 Soyama A, Saito Y, Hanioka N, Murayama N, Nakajima O, Katori N, *et al.* Non-synonymous single nucleotide alterations found in the CYP2C8 gene result in reduced in vitro paclitaxel metabolism. *Biol Pharm Bull* 2001; **24**:1427–1430.
6 Bahadur N, Leathart JB, Mutch E, Steimel-Crespi D, Dunn SA, Gilissen R, *et al.* CYP2C8 polymorphisms in Caucasians and their relationship with paclitaxel 6alpha-hydroxylase activity in human liver microsomes. *Biochem Pharmacol* 2002; **64**:1579–1589.
7 Soyama A, Saito Y, Komamura K, Ueno K, Kamakura S, Ozawa S, *et al.* Five novel single nucleotide polymorphisms in the CYP2C8 gene, one of which induces a frame-shift. *Drug Metab Pharmacokinet* 2002; **17**:374–377.
8 Hichiya H, Tanaka-Kagawa T, Soyama A, Jinno H, Koyano S, Katori N, *et al.* Functional characterization of five novel CYP2C8 variants, G171S, R186X, R186G, K247R, and K383N, found in a Japanese population. *Drug Metab Dispos* 2005; **33**:630–636.
9 Nakajima Y, Yoshitani T, Fukushima-Uesaka H, Saito Y, Kaniwa N, Kurose K, *et al.* Impact of the haplotype CYP3A4*16B harboring the Thr185Ser substitution on paclitaxel metabolism in Japanese cancer patients. *Clin Pharmacol Ther* 2006; **80**:179–191.
10 Martinez C, Garcia-Martin E, Blanco G, Gamito FJ, Ladero JM, Agundez JA. The effect of the cytochrome P450 CYP2C8 polymorphism on the disposition of (R)-ibuprofen enantiomer in healthy subjects. *Br J Clin Pharmacol* 2005; **59**:62–68.
11 Niemi M, Leathart JB, Neuvonen M, Backman JT, Daly AK, Neuvonen PJ. Polymorphism in CYP2C8 is associated with reduced plasma concentrations of repaglinide. *Clin Pharmacol Ther* 2003; **74**:380–387.
12 Henningsson A, Marsh S, Loos WJ, Karlsson MO, Garsa A, Mross K, *et al.* Association of CYP2C8, CYP3A4, CYP3A5, and ABCB1 polymorphisms with the pharmacokinetics of paclitaxel. *Clin Cancer Res* 2005; **11**: 8097–8104.
13 Nakajima M, Fujiki Y, Noda K, Ohtsuka H, Ohkuni H, Kyo S, *et al.* Pharmacokinetics of paclitaxel in ovarian cancer patients and genetic polymorphisms of CYP2C8, CYP3A4, and MDR1. *J Clin Pharmacol* 2005; **45**:674–682.
14 Judson R, Stephens JC, Windemuth A. The predictive power of haplotypes in clinical response. *Pharmacogenomics* 2000; **1**:15–26.
15 Monsarrat B, Chatelut E, Royer I, Alvinerie P, Dubois J, Dezeuse A, *et al.* Modification of paclitaxel metabolism in a cancer patient by induction of cytochrome P450 3A4. *Drug Metab Dispos* 1998; **26**:229–233.
16 Taniguchi R, Kumai T, Matsumoto N, Watanabe M, Kamio K, Suzuki S, *et al.* Utilization of human liver microsomes to explain individual differences in paclitaxel metabolism by CYP2C8 and CYP3A4. *J Pharmacol Sci* 2005; **97**:83–90.
17 Ferguson SS, Chen Y, LeCluyse EL, Negishi M, Goldstein JA. Human CYP2C8 is transcriptionally regulated by the nuclear receptors constitutive androstane receptor, pregnanex receptor, glucocorticoid receptor, and hepatic nuclear factor 4α. *Mol Pharmacol* 2005; **68**:747–757.
18 Kitamura Y, Moriguchi M, Kaneko H, Morisaki H, Morisaki T, Toyama K, *et al.* Determination of probability distribution of diplotype configuration (diplotype distribution) for each subject from genotypic data using the EM algorithm. *Ann Hum Genet* 2002; **66**:183–193.
19 Bandelt HJ, Forster P, Rohl A. Median-joining networks for inferring intraspecific phylogenies. *Mol Biol Evol* 1999; **16**:37–48.
20 Lehnert M, Emerson S, Dalton WS, de Giuli R, Salmon SE. *In vitro* evaluation of chemosensitizers for clinical reversal of P-glycoprotein-associated Taxol resistance. *J Natl Cancer Inst Monogr* 1993; **15**:63–67.
21 Roscigno RF, Weiner M, Garcia-Blanco MA. A mutational analysis of the polypyrimidine tract of introns. Effects of sequence differences in pyrimidine tracts on splicing. *J Biol Chem* 1993; **268**:11222–11229.

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Clinical Studies

Randomised phase III trial of carboplatin plus etoposide *vs* split doses of cisplatin plus etoposide in elderly or poor-risk patients with extensive disease small-cell lung cancer: JCOG 9702

H Okamoto*[,1], K Watanabe[1], H Kunikane[1], A Yokoyama[2], S Kudoh[3], T Asakawa[4], T Shibata[4], H Kunitoh[5], T Tamura[5] and N Saijo[6], on Behalf of the Japan Clinical Oncology Group (JCOG)-Lung Cancer Study Group

[1]Department of Respiratory Medicine, Yokohama Municipal Citizen's Hospital, 56 Okazawa-cho, Hodogaya-ku, Yokohama, Kanagawa 240-8555, Japan; [2]Niigata Cancer Center Hospital, Niigata-city, Japan; [3]Osaka City University Medical School, Osaka-city, Japan; [4]National Cancer Center, Tokyo, Japan; [5]National Cancer Center Hospital, Tokyo, Japan; [6]National Cancer Center East Hospital, Kashiwa, Japan

We compared the efficacy and the safety of a carboplatin plus etoposide regimen (CE) *vs* split doses of cisplatin plus etoposide (SPE) in elderly or poor-risk patients with extensive disease small-cell lung cancer (ED-SCLC). Eligibility criteria included: untreated ED-SCLC; age ⩾70 and performance status 0–2, or age <70 and PS 3. The CE arm received carboplatin area under the curve of five intravenously (IV) on day 1 and etoposide 80 mg m^{-2} IV on days 1–3. The SPE arm received cisplatin 25 mg m^{-2} IV on days 1–3 and etoposide 80 mg m^{-2} IV on days 1–3. Both regimens were given with granulocyte colony-stimulating factor support in a 21–28 day cycle for four courses. A total of 220 patients were randomised. Median age was 74 years and 74% had a PS of 0 or 1. Major grade 3–4 toxicities were (%CE/%SPE): leucopenia 54/51, neutropenia 95/90, thrombocytopenia 56/16, infection 7/6. There was no significant difference (CE/SPE) in the response rate (73/73%) and overall survival (median 10.6/9.9 mo; $P=0.54$). Palliation scores were very similar between the arms. Although the SPE regimen is still considered to be the standard treatment in elderly or poor-risk patients with ED-SCLC, the CE regimen can be an alternative for this population considering the risk–benefit balance.
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Keywords: small-cell lung cancer; carboplatin; cisplatin; etoposide; elderly; poor-risk

Approximately half of patients with small-cell lung cancer (SCLC) are older than 70 years, and the proportion of elderly SCLC patients is continuously increasing in Japan (Morita, 2002). However, since many investigators have arbitrarily excluded elderly patients from clinical trials, no standard chemotherapeutic regimen has been established for elderly patients with SCLC. The Japan Clinical Oncology Group (JCOG) has reported that carboplatin plus etoposide (CE) is an active and less toxic regimen in elderly patients with SCLC (Okamoto *et al*, 1999). However, other clinical trials have indicated that the combination chemotherapy of reduced (Souhami *et al*, 1997) or split doses of cisplatin plus etoposide (SPE) (Murray *et al*, 1998; Westeel *et al*, 1998) can be safely and effectively administered in elderly or poor-risk patients with SCLC. Therefore, we conducted a phase III trial comparing CE with SPE in elderly or poor-risk patients with SCLC. Although elderly is not the same as poor-risk, many clinical trials for the elderly have included both types of patients. Therefore, we decided to include both elderly and poor-risk patients with SCLC at the time of proposal for this phase III trial.

*Correspondence: Dr H Okamoto;
E-mail address: scyooka@alles.or.jp
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PATIENTS AND METHODS

Patient selection

Eligibility criteria included patients with histologically or cytologically confirmed SCLC who were ⩾70 years of age and had an Eastern Cooperative Oncology Group performance status (PS) of 0–2, or who were <70 years in age and had a PS of 3. Additional criteria consisted of extensive disease (ED), chemotherapy-naive, evaluable or measurable disease, expected survival ⩾2 months, adequate organ functions (leucocyte count ⩾4000 mm^{-3}, platelet count ⩾100 000 mm^{-3}, haemoglobin level ⩾9.0 g dl^{-1}, AST/ALT ⩽2 × upper limit of normal range, total bilirubin ⩽1.5 mg dl^{-1}, creatinine ⩽1.5 mg dl^{-1}, 24-h creatinine clearance (Ccr) ⩾50 ml min^{-1}, and PaO2 ⩾60 mmHg), no symptomatic pericardial or pleural effusion requiring drainage, no active concomitant malignancy, no senile dementia, and written informed consent. Exclusion criteria included brain metastases requiring radiotherapy, superior vena cava (SVC) syndrome requiring radiotherapy, serious medical or psychiatric illness, or pregnancy or lactation. Staging procedures included chest X-ray, computed tomography (CT) scan of the chest, CT scan or magnetic resonance

imaging (MRI) of the brain, CT scan or ultrasound of the abdomen, isotope bone scanning, and bone marrow aspiration or biopsy.

Treatment protocol

Patients were randomised to either the CE arm or the SPE arm. The CE regimen consisted of carboplatin area under the curve (AUC) of five intravenously (IV) on day 1 and etoposide 80 mg m^{-2} IV on days 1, 2, and 3. The SPE regimen consisted of cisplatin 25 mg m^{-2} IV on days 1, 2, and 3 and etoposide 80 mg m^{-2} IV on days 1, 2, and 3. Cycles were repeated every 3–4 weeks for up to four courses. In our previous phase II study using the CE regimen for elderly patients with SCLC, carboplatin AUC of 5 on day 1 and etoposide 100 mg m^{-2} on days 1, 2, and 3 were administered every 4 weeks (Okamoto *et al*, 1999). However, because grade 3 or 4 neutropenia occurred in 91% of the patients, in the current phase III trial we decided to reduce the etoposide dosage to 80 mg m^{-2} on days 1, 2, and 3, and repeat the cycle every 3–4 weeks instead of every 4 weeks. Twenty-four-hour Ccr was substituted for glomerular filtration rate (GFR) in Calvert's formula. Antiemetic prophylaxis with 5-HT3 antagonists plus dexamethasone was used at the treating physician's discretion. According to the Japanese approved guideline, prophylactic use of recombinant human granulocyte colony-stimulating factor (G-CSF) was recommended for daily administration after day 4 until the leucocyte (neutrophil) count exceeded 10 000 (5000) mm^{-3}. If the leucocyte (neutrophil) count decreased to less than 3000 (1500) mm^{-3}, then G-CSF was restarted. However, the actual use of G-CSF was left at the discretion of the treating physician. Subsequent courses of chemotherapy were initiated when leucocyte count $\geqslant$3000 mm^{-3}; platelet count $\geqslant$75 000 mm^{-3}; Cr$\leqslant$1.5 mg dl^{-1}; AST/ALT$\leqslant$2.5 $\times$ upper limit of normal range; and either PS$\leqslant$2 and age$\geqslant$70 years, or PS$\leqslant$3 and age$<$70 years were satisfied both after day 21 and two or more days after the discontinuation of G-CSF. If the above criteria were not satisfied by the first day of the next course, treatment was withheld until full recovery. If more than 6 weeks passed from day 1 of the last course, the patient was removed from protocol treatment. Dose modifications were made based only on grade 4 haematologic toxicities. If grade 4 leucopenia or neutropenia lasting 4 days or more was present, or grade 4 thrombocytopenia occurred, the doses for the next course were carboplatin AUC of 4 on day 1, cisplatin 20 mg m^{-2} for 3 days, and etoposide 60 mg m^{-2} for 3 days. If the same haematologic toxicity was observed after dose reduction, the patient was removed from protocol treatment. If grade 3 or 4 non-haematologic toxicities, except for nausea/vomiting and hyponatraemia, occurred, the patient was removed from protocol treatment even if the toxicities improved thereafter.

Responders after four courses were not allowed to receive further chemotherapy until progressive disease (PD) developed. Although post-protocol treatment was left at the discretion of the physician, crossover treatment was prohibited.

Evaluation

Tumour responses were evaluated according to World Health Organization criteria (World Health Organization, 1979). Toxicities were evaluated according to JCOG Toxicity Criteria (Tobinai *et al*, 1993), which are similar to the National Cancer Institute-Common Toxicity Criteria (NCI-CTC ver 1) for the grading of toxicities.

Palliation score

Study-specific eight-item palliation scores were completed by patients before treatment and 3 weeks after the third course of chemotherapy. The attending physicians were not allowed to complete the scores. The items consisted of cough, pain, anorexia, shortness of breath, well-being, nausea, diarrhoea or constipation, and sleep. The items were scored as not at all present (0), a little (1), moderate (2), and very much (3). The sum of the total score for all eight items was compared between the baseline and post-treatment assessments. If the post-treatment score was below the baseline score, the palliation score for that patient was judged as having shown improvement.

Study design and statistics

This trial was designed as a multicentre, prospective, randomised phase III trial. The study protocol was approved by the Clinical Trial Review Committee of JCOG and the institutional review board of each participating institution before the initiation of the study. The primary endpoint was overall survival (OS). In this study, the experimental arm was the CE arm and the control was the SPE arm. The MST of our previous phase II trial for elderly patients with extensive disease small-cell lung cancer (ED-SCLC) using the CE regimen was 10.1 months. The MST of the SPE regimen for a similar population was not available at the time of the study proposal. Although Westeel and co-workers in 1998 and Murray and co-workers in 1998 reported an excellent MST of SPE plus concurrent chest radiotherapy for elderly or frail patients with limited disease (LD)-SCLC, an MST of the SPE regimen for elderly or frail patients with ED-SCLC was not available at that time. The only data available on the CAV/PE regimen for elderly or poor-risk patients with SCLC using reduced cisplatin (60 mg m^{-2} IV on day 1) were reported by Souhami and co-workers in 1997 and the MST of that study was 5.9 months. Therefore, for statistical calculations in the current phase III trial, we used the MST value of the Souhami trial for the control arm instead of the MST of the SPE regimen. In addition, an individualised AUC-based dosing strategy of carboplatin was expected to have greater efficacy and less toxicity compared with the SPE regimen at that time. This trial was designed as a superiority trial and the planned sample size was 110 patients in each arm for 80% power to detect a 0.67 hazard ratio for CE to SPE in OS at an alpha of 0.025 (one sided) (Schoenfeld and Richter, 1982). Patients were randomised to receive either CE or SPE with a minimisation method for balancing centre, PS (0–1 *vs* 2–3) and age ($\geqslant$70 years *vs* $<$70 years).

Survival distributions were compared by unstratified log-rank test. Proportion of improvement in palliation score was evaluated by Fisher's exact test. The change in each symptom score by treatment arm was evaluated by the Wilcoxon rank-sum test. The relationship between the interval of each chemotherapy course and the two regimens was evaluated by the Wilcoxon rank-sum test. Multivariate analysis was performed using Cox's proportional hazards model to evaluate the importance of seven clinically selected variables (treatment arm, PS, age, sex, lactate dehydrogenase level, alkaline phosphatase level, and leucocyte count) as prognostic factors. All *P*-values in this report are two sided, excluding *P*-values for OS and progression-free survival (PFS).

The interim analysis was performed after half of the planned number of patients had been enrolled in March 2002, with adjustment for multiplicity by the alpha-spending function (DeMets and Lan, 1994) with an O'Brien-Fleming type boundary. Because the interim analysis did not meet the prespecified stopping criteria, the study was continued and the planned accrual of 220 patients was randomised in this trial.

RESULTS

Patient characteristics

Between August 1998 and February 2004, a total of 220 patients were registered from 24 institutions. Baseline characteristics were well balanced between the arms. Median age was 74 years, 92% were 70 years or older, 88% were male, and 74% had a PS of 0 or 1 (Table 1). One patient in the CE arm was found to have LD after the completion of protocol chemotherapy due to protocol violation, and this patient was considered ineligible (Figure 1).

Delivery of treatment

Reasons for termination of treatment are listed in Figure 1, and there were no major differences between the arms. Of the patients, 63% in the CE arm and 67% in the SPE arm completed four courses, and 11% in the CE arm and 8% in the SPE arm did not complete treatment because of toxicity or complications. Treatment-related death (TRD) occurred in four patients; three patients in the CE arm and one in the SPE arm. All TRDs of patients who were ⩾70 years old with a good pretreatment PS (all PS 1) were associated with neutropenic infection, which occurred after the first course of chemotherapy. Although the median interval of chemotherapy was slightly more prolonged in the CE arm than in the SPE arm, total delivered courses were similar between the arms (Table 2). One patient in the SPE arm never received chemotherapy due to the occurrence of delirium after registration. Dose reduction was more frequently observed in the CE arm than in the SPE arm: 29% *vs* 10%, $P<0.01$. Course delay, G-CSF delivery and total courses with G-CSF delivery were similar between the arms.

Toxicity and palliation score

Toxicities are listed in Table 3. Grade 3 or 4 leucopenia and neutropenia occurred in 54 and 95% of the CE arm *vs* 51 and 90% of the SPE arm, respectively. Grade 3 or 4 thrombocytopenia occurred more frequently in the CE arm than in the SPE arm: 56 *vs* 16%, $P<0.01$. Gastrointestinal toxicities including nausea or vomiting and diarrhoea were mild in both arms. There were few grade 3 or 4 toxicities and no remarkable differences between the arms. Other non-haematologic toxicities were similarly distributed between the arms. Grade 3–4 hyponatraemia, mainly caused by syndrome of inappropriate antidiuretic hormone (SIADH) secretion, occurred in 14–16% of the patients. More importantly, thrombocytopenia occurred more frequently in the CE arm, but none of the patients in either arm showed grade 3 or 4 bleeding. Only one patient in the CE arm showed grade 2 bleeding. Because no grading of febrile neutropenia was listed in JCOG toxicity criteria, the rate of the toxicity was not investigated in this study.

Baseline and post-treatment palliation scores were evaluated in 220/220 (100%) and 208/220 (95%) patients, respectively. We handled missing values by imputing the worst score. Improvement was achieved in 69 (63%) patients in the CE arm *vs* 61 (56%) patients in the SPE arm, although the difference was not statistically significant ($P=0.34$). Similarly, there were no statistical differences in the change of each symptom score between the arms (Table 4).

Objective tumour response, PFS and OS

The objective response rate of 73% was quite similar between the arms. Five CRs and 75 PRs were observed in each arm (Table 5). Progression-free survival curves and OS curves are shown in Figure 2A and B. Ninety-seven percent of the patients had progressed or died at the time of final analysis. Progression-free survival was quite similar between the arms ($P=0.20$, one sided).

Table 1 Patient characteristics

	CE ($n=110$)	SPE ($n=110$)	*P*-value
Age (years)			
Median (range)	74 (56–86)	73.5 (55–85)	0.34
⩾70 years old (%)	102 (93)	100 (91)	0.81
Sex (male/female)	95/15	98/12	0.68
ECOG PS, 0–1/2/3	81/21/8	81/19/10	0.80
⩾5% weight loss	26	38	0.18
LN metastasis			
Contralateral mediastinum	71	59	0.13
Supraclavicular	89	79	0.15
Distant metastasis			
Liver	30	30	1.0
Lung	31	30	1.0
Brain	18	18	1.0
Bone	25	17	0.23
Adrenal	13	7	0.24
Bone marrow	12	12	1.0

CE, carboplatin plus etoposide; ECOG, Eastern Cooperative Oncology Group; LN, lymph node; PS, performance status; SPE, split doses of cisplatin plus etoposide.

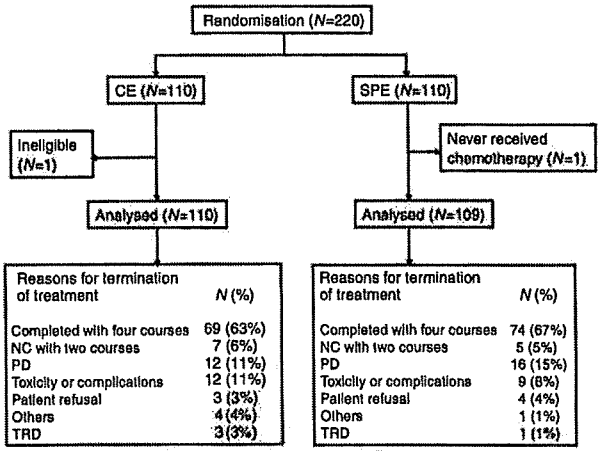

Figure 1 Flow diagram of randomised phase III trial of CE *vs* SPE in elderly or poor-risk patients with extensive disease SCLC.

Table 2 Compliance and drug delivery

	CE ($n=110$)	SPE ($n=109^a$)	*P*-value
Median interval of each chemotherapy (days) (range)			
1–2	27 (14–35)	23 (20–37)	0.02[b]
2–3	25 (21–56)	22 (20–35)	0.07[b]
3–4	27 (21–36)	24 (21–38)	0.05[b]
Total delivered courses/projected courses	353/440 (80%)	360/436 (83%)	
Dose reduction	32 (29%)	11 (10%)	<0.01[c]
Course delay	45 (41%)	40 (37%)	0.58[c]
G-CSF delivery	81 (74%)	84 (77%)	0.64[c]
No. of courses with G-CSF delivery/number of total courses	183/354 (52%)	203/362 (56%)	

CE, carboplatin plus etoposide; G-CSF, granulocyte colony-stimulating factor; SPE, split doses of cisplatin plus etoposide. [a]One patient never received chemotherapy due to delirium after registration. [b]Wilcoxon rank-sum test. [c]Fisher's exact test.

Table 3 Toxicities (JCOG Toxicity Criteria, Worst Grade of Any Course)

	CE					SPE					
	Grade					Grade					
Toxicity	**1**	**2**	**3**	**4**	**3+4 (%)**	**1**	**2**	**3**	**4**	**3+4 (%)**	**P-value**
Haematologic											
Leucopenia	5	45	46	13	(54)	8	43	49	7	(51)	0.79
Neutropenia	0	5	46	58	(95)	4	7	41	57	(90)	0.22
Anaemia	9	58	32	—	(29)	20	45	27	—	(25)	0.54
Thrombocytopenia	20	18	29	32	(56)	16	15	12	5	(16)	<.01
Non-haematologic											
Nausea/vomiting	40	24	2	—	(2)	46	28	3	—	(3)	0.68
Diarrhoea	8	9	1	0	(1)	11	3	1	0	(1)	1.0
Bilirubin	—	31	0	0	(0)	—	16	1	0	(1)	0.50
AST	47	9	3	0	(3)	30	8	6	0	(6)	0.33
ALT	40	9	2	0	(2)	38	8	4	0	(4)	0.45
Creatinine	10	2	0	0	(0)	27	3	1	0	(1)	0.50
Hyponatraemia	38	11	7	11	(16)	46	20	6	9	(14)	0.58
PaO2	39	21	7	1	(10)	44	23	2	1	(4)	0.22
Fever	15	15	0	0	(0)	21	16	0	0	(0)	—
Infection	12	15	5	3	(7)	16	7	5	1	(6)	0.78
Bleeding	8	1	0	0	(0)	4	0	0	0	(0)	—
Neurologic-sensory	2	1	0	—	(0)	3	2	0	—	(0)	—
Alopaecia	67	22	—	—		66	15	—	—		

CE, carboplatin plus etoposide; JCOG, Japan Clinical Oncology Group; PaO_2, partial pressure of oxygen; SPE, split doses of cisplatin plus etoposide.

Table 4 Palliation score

	CE		SPE		
	Change from baseline		Change from baseline		
Symptom	**Mean (s.d.)**	**Median (range)**	**Mean (s.d.)**	**Median (range)**	**P[a]**
Cough	−0.38 (1.16)	0 (−3 to 3)	−0.54 (1.06)	0 (−3 to 3)	0.51
Pain	−0.19 (1.00)	0 (−3 to 3)	−0.19 (0.96)	0 (−3 to 3)	0.96
Anorexia	−0.07 (1.16)	0 (−3 to 3)	0.08 (1.22)	0 (−3 to 3)	0.37
Shortness of breath	−0.05 (1.02)	0 (−2 to 3)	−0.31 (0.95)	0 (−3 to 3)	0.12
Well-being	−0.15 (1.13)	0 (−3 to 3)	−0.02 (1.14)	0 (−3 to 3)	0.48
Nausea	0.16 (0.84)	0 (−2 to 3)	0.26 (0.80)	0 (−1 to 3)	0.21
Diarrhoea or constipation	0.05 (1.07)	0 (−3 to 3)	0.04 (0.99)	0 (−3 to 3)	0.69
Sleep	−0.15 (1.08)	0 (−3 to 3)	−0.04 (0.89)	0 (−3 to 2)	0.10
Total	−0.80 (6.04)	−2 (−12 to 22)	−0.71 (5.35)	−1 (−15 to 21)	0.32

CE, carboplatin plus etoposide; s.d., standard deviation; SPE, split doses of cisplatin plus etoposide. [a]Wilcoxon rank-sum test.

The MST was 5.2 months in the CE arm *vs* 4.7 months in the SPE arm. OS was very similar between the arms ($P = 0.54$, one sided). The MST and 1-year survival rate was 10.6 months and 41% in the CE arm *vs* 9.9 months and 35% in the SPE arm.

Second-line chemotherapy

According to an *ad-hoc* survey (not pre-specified in the protocol), 130 (59%) patients (68 (62%) patients in the CE arm and 62 (56%) in the SPE arm) received second-line chemotherapy after relapse and the regimens were almost equally distributed between the arms. The same regimen as the initial chemotherapy, platinum-based combinations, and irinotecan regimens with or without other agents were administered in 17 (15%), 48 (44%), and 40 (36%) patients in the CE arm *vs* 10 (9%), 44 (40%), and 40 (36%) in

Table 5 Therapeutic response (WHO)

	CE	SPE	Total
CR	5	5	10
PR	75	75	150
NC	17	11	28
PD	11	16	27
NE	2	3	5
Total	110	110	220
Response rate	73%	73%	
95% CI	63–81%	63–81%	

CE, carboplatin plus etoposide; CI, confidence interval; CR, complete response; NC, no change; NE, not evaluable; PD, progressive disease; PR, partial response; SPE, split doses of cisplatin plus etoposide; WHO, World Health Organization.

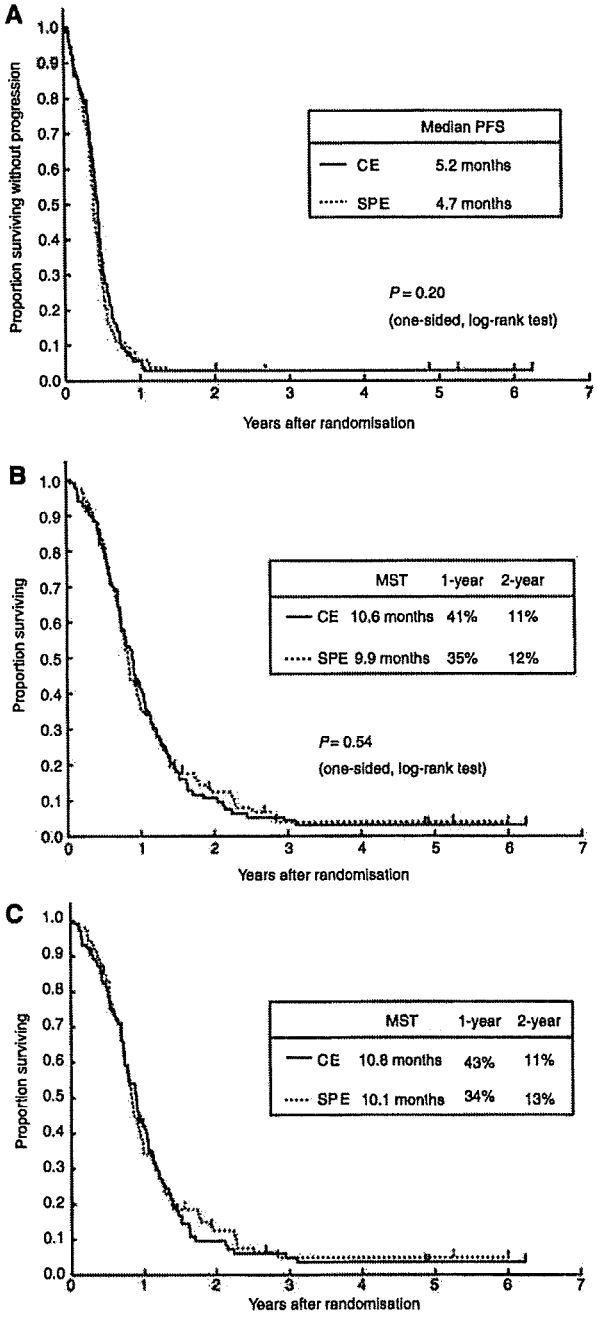

Figure 2 (**A**) PFS curves ($n=220$). (**B**) OS curves ($n=220$). (**C**) Survival curves of the patients ⩾70 years of age with a PS of 0–2 ($n=202$).

the SPE arm. Other chemotherapy regimens included topotecan monotherapy, amrubicin monotherapy, or other regimens.

Subset analysis and multivariate analysis

Subset analysis was performed according to PS and age (Table 6). There were no differences in OS between the arms in any subset; thus, an interaction between treatment and PS is unlikely. The survival curves of the patients ⩾70 years of age with a PS of 0–2 are shown in Figure 2C, and the survival curves were very similar with that of original overall populations. Even in the multivariate analysis with seven selected baseline variables, there was no difference in OS between the arms. High lactate dehydrogenase level was most strongly associated with poor prognosis (Table 7).

Table 6 Subset analysis – overall survival

		MST (months)	
Subgroup	**Number of patients (%)**	**CE**	**SPE**
PS 0–1	162 (74)	10.9	10.1
PS 2–3	58 (26)	8.3	8.1
<70 years and PS 3	18 (8)	7.1	6.9
⩾70 years and PS 0–2	202 (92)	10.8	10.0

CE, carboplatin plus etoposide; MST, median survival time; PS, performance status; SPE, split doses of cisplatin plus etoposide.

Table 7 Multivariate analysis with baseline prognostic factors

Variables	***P*-value**	**Hazard ratio**	**95% CI**
Treatment arm (CE vs. SPE)	0.99	0.99	0.75–1.33
Alkaline phosphatase level (normal vs abnormal)	0.97	0.99	0.68–1.46
Lactate dehydrogenase level (⩾ × 1.5 vs < × 1.5)	<0.001	1.69	1.23–2.26
Leucocyte count (⩾10 000/mm^3 vs <10 000/mm^3)	0.06	1.82	0.99–3.36
Age (⩾75 years vs <75 years)	0.77	1.05	0.78–1.41
PS (2–3 vs 0–1)	0.41	1.15	0.82–1.61
Sex (female vs male)	0.13	0.70	0.45–1.11

CE = carboplatin plus etoposide; SPE = split doses of cisplatin plus etoposide; PS = performance status; CI = confidence interval.

DISCUSSION

Until recently, there was no standard chemotherapeutic regimen for elderly SCLC patients. Two phase III (Medical Research Council Lung Cancer Working Party, 1996; Souhami *et al*, 1997) and two randomised phase II trials (Pfeiffer *et al*, 1997; Ardizzoni *et al*, 2005) have shown that suboptimal chemotherapies, such as oral etoposide monotherapy or attenuated doses of combination chemotherapy, may lead to reduced survival in elderly or poor-risk SCLC patients when compared with standard doses of combination chemotherapies. The CE regimen, which has acceptable toxicities and reproducible efficacy, has been used in elderly or poor-risk patients with SCLC worldwide, although there have been substantial differences in toxicities and efficacy between the reported phase II trials. Four trials demonstrated both favourable toxicities and efficacy (Carney, 1995; Evans *et al*, 1995; Matsui *et al*, 1998; Okamoto *et al*, 1999) and three showed somewhat disappointing results because of suboptimal doses of oral etoposide (Larive *et al*, 2002), greater inclusion of patients with poor prognostic factors (Samantas *et al*, 1999), and deterioration of comorbidities as a result of chemotherapy (Quoix *et al*, 2001). No phase III trial evaluating the role of the CE regimen in this population has been reported until now.

This is the first phase III trial comparing carboplatin-based CE and cisplatin-based SPE regimens in elderly or poor-risk patients with ED-SCLC. In addition, this is also the largest randomised trial specifically designed for elderly or poor-risk SCLC patients. Although there was no significant difference in the palliation scores, response rate, and OS between the arms, the efficacy of

both regimens was promising, as this study included only elderly or poor-risk patients with SCLC. Most toxicities were tolerable and the treatment compliance was also favourable in both arms. Approximately two-thirds of the patients received all four cycles of treatment. The CE arm in the current trial had more pronounced thrombocytopenia, which was considered manageable because none of the patients in the CE arm showed grade 3 or 4 bleeding, and the CE arm had a slightly prolonged course interval and a slightly greater incidence of dose reduction. However, in our opinion, these toxicities are less meaningful in clinical practice. More importantly, the CE regimen does not require hydration and can be given in an outpatient setting. Based on the results of this study, many JCOG members prefer the CE regimen to the SPE regimen and consider it to be more suitable for the control arm of future phase III trials.

The MST of each regimen (10.6 months for CE *vs* 9.9 months for SPE) was promising considering that this study included only elderly or frail patients with ED-SCLC. However, some retrospective studies have shown that fit elderly patients who have adequate organ functions, a good PS, and no comorbidity are able to tolerate intensive chemotherapy well and show a similar therapeutic response and survival rate as younger patients (Siu *et al*, 1996; Yuen *et al*, 2000). In fact, in this trial the MST of fit elderly patients $\geqslant$70 years of age with a PS of 0–1 was 10.9 months for the CE arm and 10.1 months for the SPE arm. In contrast, the MST of patients with a PS of 3 was only approximately 7 months. Furthermore, the group of fit elderly patients comprised 74% of the patients in this study. Therefore, the favourable survival rates in our trial may be attributable to patient selection. In other words, one limitation of this study is that the results of this trial cannot be extrapolated to frail elderly with a poor PS and/or comorbid illness because of the likelihood of greater inclusion of fit elderly patients in this trial.

Although the total dose in both the CE and SPE arms was slightly lower than the standard regimen, 92% of the patients showed grade 3 or 4 neutropenia, and dose reduction and course delay occurred frequently. However, the MST of both regimens was comparable with that of non-elderly or non-selected patients with ED-SCLC in historical reports (Noda *et al*, 2002; Niell *et al*, 2005). These findings suggest that both regimens are not suboptimal, but are near-full and effective doses for elderly or poor-risk patients with ED-SCLC. The CE arm in the current trial had a slightly prolonged course interval and a slightly greater incidence of dose reduction when compared to the SPE regimen. However, 95% of the patients showed grade 3 or 4 neutropenia and 56% showed grade 3 or 4 thrombocytopenia. Therefore, we believe that the dose escalation of the CE regimen may be difficult in this trial.

It remains unclear whether the elderly are able to tolerate a single modest dose of cisplatin (60–80 mg m^{-2} IV) on day 1. We feel that a fit elderly person who passes strict eligibility criteria can receive a modest dose of cisplatin IV on day 1. However, the more common situation is of elderly patients who have comorbidity and a poor PS, and cannot tolerate a standard single dose of cisplatin. Westeel *et al* (1998) and Murray *et al* (1998) reported that split doses of cisplatin were safely and effectively administered in elderly or frail patients with LD-SCLC. The SPE regimen appeared to be an appropriate treatment for elderly patients with SCLC who cannot tolerate a standard single dose of cisplatin. However, it remains unclear whether fit elderly patients in our trial can tolerate a standard single dose of cisplatin, and if so, it also remains unclear whether fit elderly patients who receive a standard single dose of cisplatin are able to achieve a more improved survival than those who receive SPE. Unfortunately, no randomised study comparing a single standard dose of cisplatin with SPE has been reported in fit elderly patients with SCLC.

There are some problems with the design in this study. The hypothesis was that carboplatin would improve survival, and the design of the trial was a superiority design with survival as the primary end point. However, this hypothesis was based on two possible misconceptions. First, carboplatin could be better dosed and might be more efficacious than cisplatin in SCLC. Unfortunately, this hypothesis could not be sustained on the basis of the available literatures. A number of clinical trials have indicated that carboplatin-based combination chemotherapy has a similar or slightly reduced efficacy compared with cisplatin-based combination chemotherapy against various tumours (Go and Adjei, 1999; Hotta *et al*, 2004). Therefore, our trial should have been designed as a non-inferiority trial. However, if this trial were planned as a non-inferiority trial, a total sample size would be about 500 to 1000 patients, with equal expected survival and a non-inferiority margin for hazard ratio ranging from 1.2 to 1.3. Second, the cisplatin dose in the control arm was an attenuated dose. Souhami *et al* (1997) used reduced dose of cisplatin (60 mg m^{-2} IV on day 1) and Murray *et al* (1998) used a single course of a split cisplatin dose in their studies. These regimens were completely different from the control arm in the present study. A standard dose of cisplatin given in 3 days is the best way of giving standard cisplatin (30 mg m^{-2} IV on days 1–3) with etoposide (130 mg m^{-2} IV on days 1–3), according the North Central Cancer Treatment Group (Maksmiuk *et al*, 1994). Had standard SPE been used for the control arm, better survival might have been achieved with increased toxicities. Another problem with the design was the inclusion of patients with a PS of 3, even if they were less than 70 years old. This made the target population heterogeneous. The number of such patients actually recruited was quite small, so emphasising the inappropriateness of their inclusion. A further limitation of this study may be a long accrual period of five-and-a-half years. Because our oncologists might have been afraid of the risk of TRD or increased toxicities in frail elderly with a poor PS and/or comorbid illness, more fit elderly patients were selectively registered and consequently the accrual rate was very slow.

In our trial, although both regimens were well-tolerated and efficacy was promising, over 90% of the patients in both arms showed grade 3 or 4 neutropenia, which may be justified and acceptable for a clinical trial involving elderly or poor risk patients with ED-SCLC, because only 6% of the patients showed grade 3 or 4 infection and TRD occurred in only four (1.8%) patients. Because all TRD occurred after the first course of chemotherapy, careful monitoring and management is necessary, particularly in the first course, if CE or SPE are administered to elderly or frail patients. Several retrospective analyses (Findlay *et al*, 1991; Radford *et al*, 1992) and a prospective study (Timmer-Bonte *et al*, 2005) have shown that standard-dose chemotherapy without G-CSF support causes more risk of early death and sepsis in the older population. Moreover, the American Society of Clinical Oncology (ASCO) guideline recommends the use of prophylactic G-CSF in patients at higher risk for chemotherapy-induced infection, such as those having a poor PS, older age, or comorbid illness (Smith *et al*, 2006). In this trial, the prophylactic use of G-CSF was recommended, but the actual use was left to the discretion of the treating physician because the use of G-CSF leads to increased drug cost. Although G-CSF was administered in only 54% of the total courses, we believe that the prophylactic use of G-CSF with CE regimen should be recommended in a new trial or clinical practice.

In conclusion, although the SPE regimen is still considered to be the standard treatment for elderly or poor-risk patients with ED-SCLC, the CE regimen can be an alternative for this population considering the risk-benefit balance. Based on the results of our trial, a phase III trial of the CE regimen *vs* amrubicin monotherapy, supported by a pharmaceutical company, is now ongoing in elderly patients with ED-SCLC in Japan, and a comparative trial of the CE regimen *vs* carboplatin plus irinotecan regimen (Okamoto *et al*, 2006) is being discussed for a future trial in our group.

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REFERENCES

Ardizzoni A, Favaretto A, Boni L, Baldini E, Castiglioni F, Antonelli P, Pari F, Tibaldi C, Altieri AM, Barbera S, Cacciani G, Raimondi M, Tixi L, Stefani M, Monfardini S, Antilli A, Rosso R, Paccagnella A (2005) Platinum-etoposide chemotherapy in elderly patients with small-cell lung cancer: results of a randomized multicenter phase II study assessing attenuated-dose or full-dose with lenograstim prophylaxis-A Forza Operativa Nazionale Italiana Carcinoma Polmonare and Gruppo Studio Tumori Polmonari Veneto (FONICAP-GSTPV) study. *J Clin Oncol* 23: 569–575

Carney DN (1995) Carboplatin/etoposide combination chemotherapy in the treatment of poor prognosis patients with small cell lung cancer. *Lung Cancer* 12(Suppl 3): S77–S83

DeMets DL, Lan KK (1994) Interim analysis: the alpha spending function approach. *Stat Med* 13: 1341–1352

Evans WK, Radwi A, Tomiak E, Logan DM, Martins H, Stewart DJ, Goss G, Maroun JA, Dahrouge S (1995) Oral etoposide and carboplatin: effective therapy for elderly patients with small cell lung cancer. *Am J Clin Oncol* 18: 149–155

Findlay MP, Griffin AM, Raghavan D, McDonald KE, Coates AS, Duval PJ, Gianoutsos P (1991) Retrospective review of chemotherapy for small cell lung cancer in the elderly: dose the end justify the means? *Eur J Cancer* 27: 1597–1601

Go RS, Adjei AA (1999) Review of the comparative pharmacology and clinical activity of cisplatin and carboplatin. *J Clin Oncol* 17: 409–422

Hotta K, Matsuo K, Ueoka H, Kiura K, Tabata M, Tanimoto M (2004) Meta-analysis of randomized clinical trials comparing cisplatin to carboplatin in patients with advanced non-small-cell lung cancer. *J Clin Oncol* 22: 3838–3852

Larive S, Bombaron P, Riou R, Fournel P, Perol M, Lena H, Dussopt C, Philip-Joet F, Touraine F, Lecaer H, Souquet PJ (2002) Carboplatin-etoposide combination in small cell lung cancer patients older than 70 years: a phase II trial. *Lung Cancer* 35: 1–7

Maksmiuk AW, Jett JR, Earle JD, Su JQ, Diegert FA, Mailliard JA, Kardinal CG, Krook JE, Veeder MH, Wiesenfeld M, Tschetter LK, Levitt R (1994) Sequencing and schedule effects of cisplatin plus etoposide in small-cell lung cancer: results of a North Central Cancer Treatment Group randomized clinical trial. *J Clin Oncol* 12: 70–76

Matsui K, Masuda N, Fukuoka M, Yana T, Hirashima T, Komiya T, Kobayashi M, Kawahara M, Atagi S, Ogawara M, Negoro S, Kudoh S, Furuse K (1998) Phase II trial of carboplatin plus oral etoposide for elderly patients with small-cell lung cancer. *Br J Cancer* 77: 1961–1965

Medical Research Council Lung Cancer Working Party (1996) Comparison of oral etoposide and standard intravenous multidrug chemotherapy for small-cell lung cancer: a stopped multicentre randomised trial. *Lancet* 348: 563–566

Morita T (2002) A statistical study of lung cancer in the annual of pathological autopsy cases in Japan, from 1958 to 1997, with reference to time trends of lung cancer in the world. *Jpn J cancer Res* 93: 15–23

Murray N, Grafton C, Shah A, Gelmon K, Kostashuk E, Brown E, Coppin C, Coldman A, Page R (1998) Abbreviated treatment for elderly, infirm, or noncompliant patients with limited-stage small-cell lung cancer. *J Clin Oncol* 16: 3323–3328

Niell HB, Herndon II JE, Miller AA, Watson DM, Sandler AB, Kelly K, Marks RS, Perry MC, Ansari RH, Otterson G, Ellerton J, Vokes EE, Green MR (2005) Randomized phase III intergroup trial of etoposide and cisplatin with or without paclitaxel and granulocyte colony-stimulating factor in patients with extensive-stage small-cell lung cancer: Cancer and Leukemia Group B Trial 9732. *J Clin Oncol* 23: 3752–3759

Noda K, Nishiwaki Y, Kawahara M, Negoro S, Sugiura T, Yokoyama A, Fukuoka M, Mori K, Watanabe K, Tamura T, Yamamoto S, Saijo N (2002) Irinotecan plus cisplatin compared with etoposide plus cisplatin for extensive small-cell lung cancer. *N Engl J Med* 346: 85–91

Okamoto H, Naoki K, Narita Y, Hida N, Kunikane H, Watanabe K (2006) A combination chemotherapy of carboplatin and irinotecan with granulocyte colony-stimulating factor (G-CSF) support in elderly patients with small cell lung cancer. *Lung Cancer* 53: 197–203

Okamoto H, Watanabe K, Nishiwaki Y, Mori K, Kurita Y, Hayashi I, Masutani M, Nakata K, Tsuchiya S, Isobe H, Saijo N (1999) Phase II study of area under the plasma-concentration-*vs*-time curve-based carboplatin plus standard-dose intravenous etoposide in elderly patients with small-cell lung cancer. *J Clin Oncol* 17: 3540–3545

Pfeiffer P, Rytter C, Madesen EL, Moeholt K, Hansen O, Bentzen S, Palshof T, Rose C (1997) Re: five-day oral etoposide treatment for advanced small-cell lung cancer: randomized comparison with intravenous chemotherapy. *J Natl Cancer Inst* 89: 1892–1893

Quoix E, Breton JL, Daniel C, Jacoulet P, Debieuvre D, Paillot N, Kessler R, Moreau L, Coetmeur D, Lemarie E, Milleron B (2001) Etoposide phosphate with carboplatin in the treatment of elderly patients with small-cell lung cancer: a phase II study. *Ann Oncol* 12: 957–962

Radford JA, Ryder WD, Dodwell D, Anderson H, Thatcher N (1992) Predicting septic complications of chemotherapy: An analysis of 382 patients treated for small cell lung cancer without dose reduction after major sepsis. *Eur J Cancer* 29A: 81–86

Samantas E, Skarlos DV, Pectasides D, Nicolaides P, Kalofonos H, Mylonakis N, Vardoulakis Th, Kosmidis P, Pavlidis N, Fountzilas G (1999) Combination chemotherapy with low doses of weekly carboplatin and oral etoposide in poor risk small cell lung cancer. *Lung Cancer* 23: 159–168

Schoenfeld DA, Richter JR (1982) Nomograms for calculating the number of patients needed for a clinical trial with survival as an endpoint. *Biometrics* 38: 163–170

Siu LL, Shepherd FA, Murray N, Feld R, Pater J, Zee B (1996) Influence of age on the treatment of limited-stage small-cell lung cancer. *J Clin Oncol* 14: 821–828

Smith TJ, Khatcheressian J, Lyman GH, Ozer H, Armitage JO, Balducci L, Bennett CL, Cantor SB, Crawford J, Cross SJ, Demetri G, Desch CE, Pizzo PA, Schiffer CA, Schwartzberg L, Somerfield MR, Somlo G, Wade JC, Wade JL, Winn RJ, Wozniak AJ, Wolff AC (2006) 2006 update of recommendations for the use of white blood cell growth factors: an evidence-based clinical practice guideline. *J Clin Oncol* 24: 3187–3205

Souhami RL, Spiro SG, Rudd RM, Ruiz de Elvira MC, James LE, Gower NH, Lamont A, Harper PG (1997) Five-day oral etoposide treatment for advanced small-cell lung cancer: randomized comparison with intravenous chemotherapy. *J Natl Cancer Inst* 89: 577–580

Timmer-Bonte JN, de Boo TM, Smit HJ, Biesma B, Wilschut FA, Cheragwandi SA, Termeer A, Hensing CA, Akkermans J, Adang EM, Bootsma GP, Tjan-Heijnen VC (2005) Prevention of chemotherapy-induced febrile neutropenia by prophylactic antibiotics plus or minus granulocyte colony-stimulating factor in small-cell lung cancer: a dutch randomized phase III study. *J Clin Oncol* 23: 7974–7984

Tobinai K, Kohno A, Shimada Y, Watanabe T, Tamura T, Takeyama K, Narabayashi M, Fukutomi T, Kondo H, Shimoyama M, Suemasu K (1993) Toxicity grading criteria of the Japan Clinical Oncology Group. *Jpn J Clin Oncol* 23: 250–257

Westeel V, Murray N, Gelmon K, Shah A, Sheehan F, McKenzie M, Wong F, Morris J, Grafton C, Tsang V, Goddard K, Murphy K, Parsons C, Amy R, Page R (1998) New combination of the old drugs for elderly patients with small-cell lung cancer: a phase II study of the PAVE regimen. *J Clin Oncol* 16: 1940–1947

World Health Organization (1979) *WHO Handbook for Reporting Results of Cancer Treatment* WHO offset publication No. 48 Geneva: World Health Organization

Yuen AR, Zou G, Turrisi AT, Sause W, Komaki R, Wagner H, Aisner SC, Livingston RB, Blum R, Johnson DH (2000) Similar outcome of elderly patients in Intergroup Trial 0096: cisplatin, etoposide, and thoracic radiotherapy administered once or twice daily in limited stage small cell lung carcinoma. *Cancer* 89: 1953–1960

Appendix

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Detection of unsuspected distant metastases and/or regional nodes by FDG-PET in LD-SCLC scan in apparent limited-disease small-cell lung cancer

Seiji Niho[a,*], Hirofumi Fujii[b], Koji Murakami[b,c], Seisuke Nagase[a], Kiyotaka Yoh[a], Koichi Goto[a], Hironobu Ohmatsu[a], Kaoru Kubota[a], Ryuzo Sekiguchi[d], Shigeru Nawano[d], Nagahiro Saijo[a], Yutaka Nishiwaki[a]

[a] *Division of Thoracic Oncology, National Cancer Center Hospital East, Kashiwanoha 6-5-1, Kashiwa, Chiba 277-8577, Japan*
[b] *Functional Imaging Division, National Cancer Center Research Center for Innovative Oncology, Chiba, Japan*
[c] *PET Center, Dokkyo Medical University, Tochigi, Japan*
[d] *Department of Radiology, National Cancer Center Hospital East, Chiba, Japan*

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Small-cell lung cancer;
Limited-disease;
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Occult distant metastasis

Summary We retrospectively investigated the clinical usefulness of fluorodeoxyglucose positron emission tomography (FDG-PET) for evaluation of patients with limited-disease small-cell lung cancer (LD-SCLC) diagnosed by conventional staging procedures. Sixty-three patients received whole body FDG-PET scans after routine initial staging procedures. The findings of FDG-PET scans suggesting extensive-stage disease were confirmed by other imaging tests or by the patient's clinical course. FDG-PET scan findings indicated distant metastases in 6 of 63 patients. Metastatic disease was confirmed in five of these six patients (8%, 95% confidence interval: 3–18%). FDG-PET scan also detected regional lymph node metastases even in nine patients (14%) in whom computed tomography images had been negative, including contralateral lymph node metastasis in three patients. FDG-PET scan detected additional lesions in patients diagnosed as having LD-SCLC by conventional staging procedures. The therapeutic strategies were changed in 8% of patients based on the results of FDG-PET. FDG-PET scan is recommended as an initial staging tool for patients with this disease.

1. Introduction

Small-cell lung cancer (SCLC) accounts for 15–20% of all lung cancers. SCLC shows more aggressive biological behaviour than non-small cell lung cancer (NSCLC). A clinical two-stage system proposed by the Veterans Administration Lung

* Corresponding author. Tel.: +81 4 7133 1111; fax: +81 4 7131 4724.
E-mail address: siniho@east.ncc.go.jp (S. Niho).

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Study Group (VALSG) distinguishes limited-disease (LD) and extensive-disease (ED) in SCLC [1]. LD is defined as limited to one hemithorax, including mediastinal, contralateral hilar and ipsilateral supraclavicular lymph nodes, while ED represents tumour spread beyond these regions. Approximately two-thirds of patients with SCLC are diagnosed as having ED at the initial staging. The current standard care for LD-SCLC is a combination of chemotherapy and chest irradiation. With current treatment, patients with LD have a median survival of 23–27 months [2,3], compared to 10–12 months for those with ED [4]. Therefore, accurate pretreatment staging is important for patients with SCLC in order to determine the appropriate therapy.

Conventional staging procedures for lung cancer consist of computed tomography (CT) of the chest and upper abdomen, bone scan, and CT scan or magnetic resonance imaging (MRI) of the brain. Recently, fluorodeoxyglucose positron emission tomography (FDG-PET) was introduced as a staging tool for NSCLC. According to the guidelines of the American Society of Clinical Oncology, PET scan is recommended for survey occult locoregional lesions and distant metastases in patients with NSCLC [5]. Two separate prospective studies demonstrated that FDG-PET detected unsuspected distant metastases in 24% of patients with apparent stage III NSCLC [6,7]. Another study showed that FDG-PET changed or influenced management decisions in 67% of patients with NSCLC. PET plays an important role in staging of NSCLC [8]. However, previous PET studies of SCLC involved only a relatively small number of patients [9–17]. In a prospective study, FDG-PET was performed for 24 patients diagnosed as having LD-SCLC by conventional staging procedures [9]. Based on FDG-PET findings, two of these 24 patients were upstaged to ED. Bone metastases were found in one patient, and contralateral supraclavicular lymph node metastasis in another. Larger studies are required to confirm the role of FDG-PET in the staging of LD-SCLC. In this study, we retrospectively investigated the usefulness of FDG-PET to detect distant metastases or unsuspected regional nodal metastases in patients with LD-SCLC diagnosed by conventional staging procedures.

2. Patients and methods

2.1. Patients

Seventy patients were newly diagnosed as having LD-SCLC by conventional staging procedures at the National Cancer Center Hospital East between July 2003 and December 2006. Conventional staging procedures included history and physical examination, chest radiography, CT scan of the chest, CT scan or ultrasound (US) of the abdomen, bone scan, and CT scan or MRI of the brain. CT scan and MR images were enhanced with contrast media. LD is defined in this study as disease limited to one hemithorax, including mediastinal, contralateral hilar and supraclavicular lymph nodes, ipsilateral pleural effusion, and pericardial effusion, while ED represents tumour spread beyond these manifestations [18]. This study included 63 patients who received whole body FDG-PET scan after the routine initial staging procedures. Fifty-seven were male and the remaining 6 were female. Median age was 64 years, range 48–80 years. Forty-two patients received FDG-PET before commencement of chemotherapy. The remaining 21 patients received FDG-PET 1 to 11 days (median: 4 days) after commencement of chemotherapy. Forty-four and 19 patients received CT scan and US of the abdomen, respectively.

2.2. FDG-PET scan

FDG-PET scans were performed before March 2005 (patients No. 1–25), and FDG-PET/CT scans were performed after April 2005 (patients No. 26–63). Three hundred MBq of F-18 FDG were intravenously injected after at least 6 h of fasting. Acquisition was initiated 60 min after the injection. FDG-PET imaging was performed using a GE Advance Scanner (General Electric Medical System, Milwaukee, WI), whose axial field of view was 15.2 cm and spatial resolution 4.9 mm of full-width-half-maximum. Scans were performed using two-dimensional acquisition mode from the thigh to the skull base with seven bed positions. Each bed position was composed of 1 min of transmission scanning and 5 min of emission scanning.

FDG-PET/CT imaging was performed using a GE Discovery LS Scanner (General Electric Medical System, Milwaukee, WI) or a GE Discovery ST Scanner (the same manufacturer). The PET component of the GE Discovery LS Scanner was the same as that of the GE Advance Scanner. For the PET component of the GE Discovery ST Scanner, the axial field of view was 15.7 cm and the spatial resolution was 6.2 mm of full-width-half-maximum. PET scans were performed with both scanners using 2-dimensional acquisition mode from the thigh to the skull base with 7 bed positions. Each bed position was composed of 4 min of emission scanning. The CT component of both PET/CT scanners was a 16-row multidetector CT scanner and CT images were acquired with a tube voltage of 140 kV, and the tube current was automatically set using the auto-exposure control function so that the number of standard deviations of noise was limited to 10. Attenuation correction of PET images was performed using the data from CT images.

Image reconstruction was performed using an ordered subsets expectation maximization (OSEM) algorithm with subset and iteration values of 14 and 2, respectively.

2.3. Image interpretation

All PET and CT images were interpreted by experienced radiologists and physicians. The 4.25 mm-thick images of axial, coronal and sagittal planes on hard copy films were reviewed. Uptake stronger than mediastinal blood pool activity was diagnosed as malignancy by the visual estimation. Symmetrical activities observed in both hilar regions were considered to be benign reactive changes. Any discrepancies between the radiologist and physician were resolved by discussion. The findings detected by FDG-PET were confirmed by other image tests or observation of the clinical course. FDG-PET was conducted after conventional staging procedures. CT, US and bone scans were interpreted without the FDG-PET findings. However, FDG-PET scan was interpreted in comparison with CT findings, while PET/CT findings were interpreted independently.

Table 1 Discrepancy between FDG-PET and conventional staging procedures (distant metastases)

Patient no.	Age (years)	Gender	CT N	PET N	PET M	Interval between conventional staging procedures and FDG-PET (days)	Comments
2	61	Male	2	2	1	20	Multiple bone metastases (PET)
6	68	Male	2	2	1	7	Lymph* node metastasis around the cardia (PET)
47	61	Male	3	3	1	28	Multiple bone metastases (PET)
55	68	Male	2	2	1	20 (CT) and 14 (bone scan)	Liver, axillary lymph node, and iliac bone metastases (PET)
59	52	Male	3	3	1	13	Adrenal, cervical and mandibular lymph node metastases (PET)
63	59	Male	3	3	1	18 (CT) and 11 (bone scan)	Multiple bone and liver metastases (PET)

FDG, fluorodeoxyglucose; PET, positron emission tomography; CT, computed tomography; N, node; M, metastasis.
* Diagnosis of lymph node metastasis was not confirmed by other imaging modalities or observation of the clinical course.

3. Results

3.1. Detection of distant metastasis

FDG-PET showed results different from those of conventional staging procedures in 17 of 63 patients. PET scan demonstrated findings suggesting distant metastases in 6 of 63 patients (Table 1). The median interval between conventional staging procedures and FDG-PET was 16 days (range: 7–28). Abnormal uptake was observed around the cardia in one of these six patients (No.6). A repeat FDG-PET study demonstrated a longer uptake stripe indicating radiation-induced oesophagitis and the diagnosis could not be established, as there was a remaining possibility of physiological uptake in the oesophagus. The diagnosis of metastatic disease was confirmed in the remaining five patients (8%, 95% confidence interval (CI): 3–18%). Among these five patients, four had bone metastases, two had liver metastases, one had adrenal metastasis, and two had lymph node metastases in the cervical or axillary region. The therapeutic strategy for these five patients was changed and they received only chemotherapy without thoracic radiotherapy. One patient (No. 47) had shown negative findings on bone scintigraphy four weeks before the FDG-PET study, but PET scan demonstrated increased FDG uptake in bones throughout the body. MRI of the spine confirmed the diagnosis of multiple bone metastases (Fig. 1). A repeat bone scan after three months detected obvious multiple bone metastases in No. 2 patient. Two hepatic lesions, as well as the primary tumour, mediastinal and hilar lymph nodes, had all increased in size after two cycles of chemotherapy in patient No. 55. A hepatic lesion, as well as the primary tumour, had decreased in size after two cycles of chemotherapy in patient No. 63. These hepatic lesions were compatible with liver metastases. Abnormal uptake by the right adrenal gland disappeared on repeat PET/CT after four cycles of chemotherapy in patient No. 59. Abnormal uptake in primary and mediastinal lesions was extremely decreased in this patient. The right adrenal gland lesion was compatible with metastasis.

FDG-PET detected liver metastasis in one of 44 patients staged by CT scan of the abdomen (No. 55), and liver or adrenal metastasis in two of 19 patients staged by US (Nos. 59 and 63). Liver and adrenal metastases not detected by US were small, such that the CT part of PET/CT could not detect them as metastases. Ratios of upstaging by FDG-PET between initial CT scan and US of the abdomen were not statistically significant (1/44 versus 2/19, $P=0.214$).

3.2. Detection of regional lymph node metastases

FDG-PET scans detected regional lymph node metastases that had been negative on CT scans in nine patients (14%) (Table 2). The median interval between CT of the chest and FDG-PET was 19 days (range: 7–34). FDG-PET scans newly detected ipsilateral supraclavicular lymph node metastasis in four patients, contralateral lymph node metastasis in three, and mediastinal lymph node metastasis in two. These nine patients all underwent curative chemoradiotherapy, and abnormal FDG uptake in mediastinal and/or supraclavicular lymph nodes disappeared or decreased on repeat PET scans after chemoradiotherapy. These lymph nodes were considered positive for metastasis.

CT scan detected swollen mediastinal lymph nodes without abnormal FDG uptake in two patients. One patient had a past history of pulmonary tuberculosis complicated by pulmonary fibrosis. The swollen pretracheal lymph node was considered negative for metastasis because the node size remained unchanged after four cycles of chemotherapy although the primary tumour shrank. This case showed false positive findings on CT whereas FDG-PET correctly diagnosed the extent of disease (No. 43). The other patient had atelectasis of the right middle lobe due to the primary tumour. Superior mediastinal and subcarinal lymph nodes were considered to be metastatic on CT, but abnormal FDG uptake was absent. After three cycles of chemotherapy the

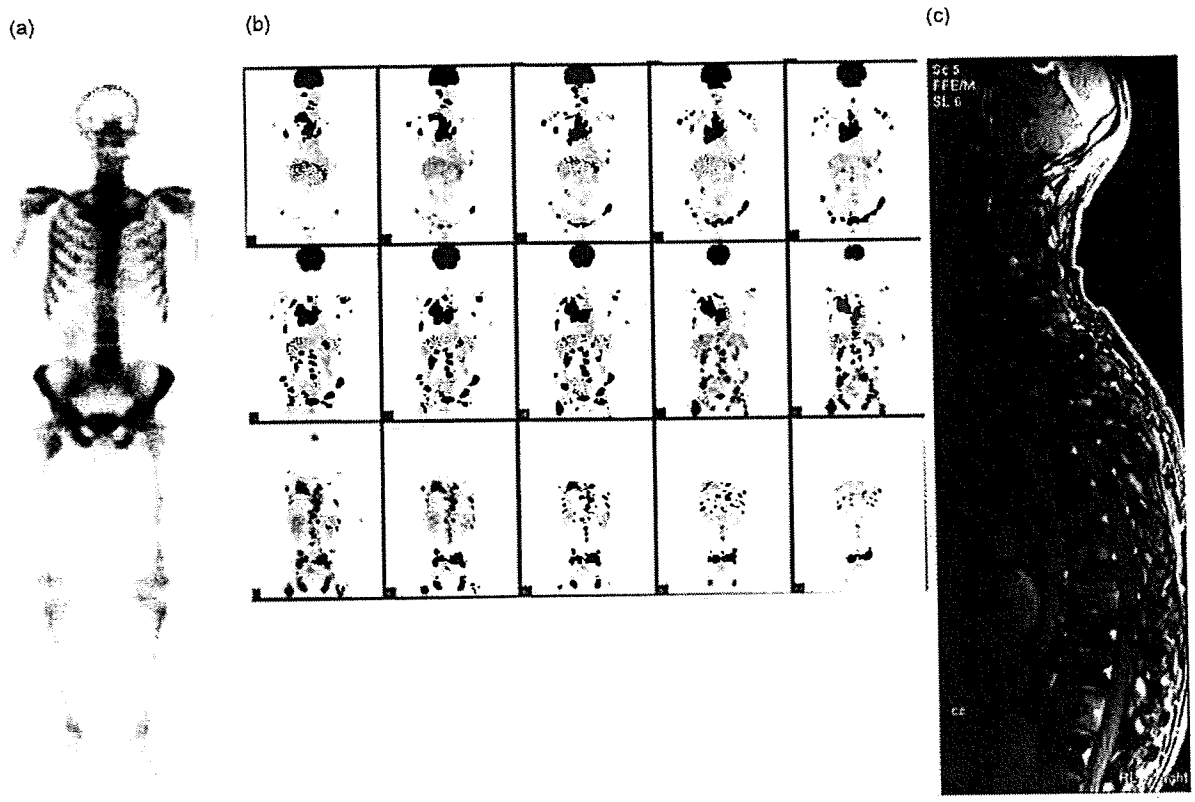

Fig. 1 A 61-year-old man with small-cell lung cancer. Bone scintigraphy was negative for osseous metastasis (a). However, PET scan demonstrated increased FDG uptake in bones throughout the body (b). MRI of the spine confirmed multiple bone metastases (c).

mediastinal lesion showed no change although the primary tumour had decreased in size and atelectasis of the right middle lobe was improved. The mediastinal lymph nodes were considered negative for metastasis (No. 61).

4. Discussion

SCLC tends to disseminate early in the disease course and displays a more aggressive clinical behaviour than NSCLC. Local treatment modalities alone such as radiotherapy or surgery are not effective in prolonging survival beyond a few weeks. Systemic chemotherapy is the mainstay of treatment for patients in all stages of SCLC. A combination of chemotherapy and thoracic irradiation can promote long-term survival for patients diagnosed as having limited disease and recent clinical trials of chemoradiotherapy for LD-SCLC obtained 5-year survival rates of 24–26% [2,3]. However, thoracic irradiation might cause severe radiation pneumonitis, resulting in respiratory failure and/or treatment-related death. Furthermore, thoracic irradiation might also cause oesophagitis which worsens patient quality of life. Accurate clinical staging is important to determine the indications for chemoradiotherapy in SCLC. Our study demonstrated that FDG-PET scan detected unsuspected distant metastases in 8% of patients with LD-SCLC based on conventional staging procedures and that the detection of these new lesions changed their therapeutic strategies. Furthermore, FDG-PET scan detected regional lymph node metastases which had not been visualized on CT scan in 14% of patients. The radiation field could be appropriately set to cover the positive nodes based on the PET study results. Our results reconfirmed those of a previous preliminary study with a smaller number of patients [9].

Is the rate of the detection of unsuspected distant metastases (8%) clinically significant? Previous studies demonstrated that FDG-PET scan detected unsuspected distant metastases in 24% of patients with stage III NSCLC [6,7]. Compared to this result, the impact of FDG-PET on the staging of SCLC seems to be weaker. SCLC tends to have more obvious distant metastases than NSCLC, because of the aggressive biological behaviour of SCLC. Therefore, FDG-PET might detect unsuspected distant metastases at a relatively low rate. The most common region for unsuspected PET-detected metastasis in NSCLC was the abdomen, with 53% of pateints having adrenal, liver, and other lesions [6]. In our study, FDG-PET detected bone metastases in four of five patients who were upstaged from LD to ED. These lesions might reflect metastasis to the bone marrow, although no pathological evidence was obtained, because neither bone marrow biopsy nor aspiration cytology was routinely conducted for the initial clinical staging.

Our retrospective analyses have several limitations. We did not confirm histologically regional lymph node or distant metastases detected by FDG-PET or CT. These lesions were not routinely biopsied and most metastatic lesions were chemosensitive and radiosensitive. Our confirmation was inevitably based on observation of the clinical course.