Pennington, M. W., Owens, I. S. and Popescu, N. C.: Thirteen UDPglucuronosyltransferase genes are encoded at the human *UGT1* gene complex locus. *Pharmacogenetics*, **11**: 357–368 (2001).

4) Radominska-Pandya, A., Czernik, P. J., Little, J. M., Battaglia, E. and Mackenzie, P. I.: Structural and functional studies of UDP-glucuronosyltransferases. *Drug Metab. Rev.*, **31**: 817–899 (1999).
5) Ouzzine, M., Pillot, T., Fournel-Gigleux, S., Magdalou, J., Burchell, B. and Siest, G.: Expression and role of the human liver UDP-glucuronosyltransferase UGT1*6 analyzed by specific antibodies raised against a hybrid protein produced in *Escherichia coli*. *Arch. Biochem. Biophys.*, **310**: 196–204 (1994).
6) King, C. D., Rios, G. R., Assouline, J. A. and Tephly, T. R.: Expression of UDP-glucuronosyltransferases (UGTs) 2B7 and 1A6 in the human brain and identification of 5-hydroxytryptamine as a substrate. *Arch. Biochem. Biophys.*, **365**: 156–162 (1999).
7) Harding, D., Fournel-Gigleux, S., Jackson, M. R. and Burchell, B.: Cloning and substrate specificity of a human phenol UDP-glucuronosyltransferase expressed in COS-7 cells. *Proc. Natl. Acad. Sci. USA.*, **85**: 8381–8385 (1988).
8) Hanioka, N., Ozawa, S., Jinno, H., Ando, M., Saito, Y. and Sawada, J.: Human liver UDP-glucuronosyltransferase isoforms involved in the glucuronidation of 7-ethyl-10-hydroxycamptothecin. *Xenobiotica*, **31**: 687–699 (2001).
9) Court, M. H., Duan, S. X., von Moltke, L. L., Greenblatt, D. J., Patten, C. J., Miners, J. O. and Mackenzie P. I.: Interindividual variability in acetaminophen glucuronidation by human liver microsomes: identification of relevant acetaminophen UDP-glucuronosyltransferase isoforms. *J. Pharmacol. Exp. Ther.*, **299**: 998–1006 (2001).
10) Ciotti, M., Marrone, A., Potter, C. and Owens, I. S.: Genetic polymorphism in the human UGT1A6 (planar phenol) UDP-glucuronosyltransferase: pharmacological implications. *Pharmacogenetics*, **7**: 485–495 (1997).
11) Ouzzine, M., Magdalou, J., Burchell, B. and Fournel-Gigleux, S.: An internal signal sequence mediates the targeting and retention of the human UDP-glucuronosyltransferase 1A6 to the endoplasmic reticulum. *J. Biol. Chem.*, **274**: 31401–31409 (1999).
12) Nagar, S., Zalatoris, J. J. and Blanchard, R. L.: Human UGT1A6 pharmacogenetics: identification of a novel SNP, characterization of allele frequencies and functional analysis of recombinant allozymes in human liver tissue and in cultured cells. *Pharmacogenetics*, **14**: 487–499 (2004).
13) Kitamura, Y., Moriguchi, M., Kaneko, H., Morisaki, H., Morisaki, T., Toyama, K. and Kamatani, N.: Determination of probability distribution of diplotype configuration (diplotype distribution) for each subject from genotypic data using the EM algorithm. *Ann. Hum. Genet.*, **66**: 183–193 (2002).
14) Lampe, J. W., Bigler, J., Horner, N. K. and Potter, J. D.: UDP-glucuronosyltransferase (*UGT1A1*28* and *UGT1A6*2*) polymorphisms in Caucasians and Asians: relationships to serum bilirubin concentrations. *Pharmacogenetics*, **9**: 341–349 (1999).

Drug Metab. Pharmacokinet. 20 (2): *SNP13* (144)–*SNP20* (151) (2005).

SNP Communication

Genetic Variations and Haplotypes of UGT1A4 in a Japanese Population

Mayumi SAEKI[1], Yoshiro SAITO[1,2,*], Hideto JINNO[1,3], Kimie SAI[1,4], Akiko HACHISUKA[2], Nahoko KANIWA[1,5], Shogo OZAWA[1,6], Manabu KAWAMOTO[7], Naoyuki KAMATANI[7], Kuniaki SHIRAO[8], Hironobu MINAMI[9], Atsushi OHTSU[10], Teruhiko YOSHIDA[11], Nagahiro SAIJO[12], Kazuo KOMAMURA[13,14], Takeshi KOTAKE[15], Hideki MORISHITA[15], Shiro KAMAKURA[13], Masafumi KITAKAZE[13], Hitonobu TOMOIKE[13] and Jun-ichi SAWADA[1,2]

[1]*Project Team for Pharmacogenetics,* [2]*Division of Biochemistry and Immunochemistry,* [3]*Division of Environmental Chemistry,* [4]*Division of Xenobiotic Metabolism and Disposition,* [5]*Division of Medicinal Safety Science,* [6]*Division of Pharmacology, National Institute of Health Sciences, Tokyo, Japan* [7]*Division of Genomic Medicine, Department of Advanced Biomedical Engineering and Science, Tokyo Women's Medical University, Tokyo, Japan* [8]*Division of Internal Medicine, National Cancer Center Hospital,* [11]*Genetics Division, National Cancer Center Research Institute, National Cancer Center, Tokyo, Japan* [9]*Division of Oncology/Hematology,* [10]*Division of GI Oncology/Digestive Endoscopy,* [12]*Deputy Director, National Cancer Center Hospital East, Chiba, Japan* [13]*Division of Cardiology,* [14]*Department of Cardiovascular Dynamics Research Institute,* [15]*Department of Pharmacy, National Cardiovascular Center, Osaka, Japan*

Full text of this paper is available at http://www.jssx.org

Summary: Nineteen genetic variations, including 11 novel ones, were found in exon 1 and its flanking region of the UDP-glucuronosyltransferase (UGT) 1A4 gene from 256 Japanese subjects, consisting of 60 healthy volunteers, 88 cancer patients and 108 arrhythmic patients. These variations include −217T>G and −36G>A in the 5′-flanking region, 30G>A (P10P), 127delA (43fsX22; frame-shift from codon 43 resulting in the termination at the 22nd codon, codon 65), 175delG (59fsX6), 271C>T (R91C), 325A>G (R109G), and 357T>C (N119N) in exon 1, and IVS1+1G>T, IVS1+98A>G and IVS1+101G>T in the following intron. Among them, 127delA and 175delG can confer early termination of translation, resulting in an immature protein that probably lacks enzymatic activity. Variation IVS1+1G>T is located at a splice donor site and thus may lead to aberrant splicing. Since we did not find any significant differences in the frequencies of all the variations among the three subject groups, the data were analyzed as one group. The allele frequencies of the novel variations were 0.006 for IVS1+101G>T, 0.004 for 30G>A (P10P) and 357T>C (N119N), and 0.002 for the 8 other variations. In addition, the two known nonsynonymous single nucleotide polymorphisms (SNPs), 31C>T (R11W) and 142T>G (L48V), were found at 0.012 and 0.129 frequencies, respectively. The SNP 70C>A (P24T), mostly linked with 142T>G (L48V) in German Caucasians, was not detected in this study. Sixteen haplotypes were identified or inferred, and some haplotypes were confirmed by cloning and sequencing. It was shown that most of 142T>G (L48V) was linked with −219C>T, −163G>A, 448T>C (L150L), 804G>A (P268P), and IVS1+43C>T, comprising haplotype **3a*; haplotype **4a* harbors 31C>T (R11W); 127delA (43fsX22) and 142T>G (L48V) were linked (haplotype **5a*); 175delG (59fsX6) was linked with 325A>G (R109G) (**6a* haplotype); and −219C>T, −163G>A, 142T>G (L48V), 271C>T (R91C), 448T>C (L150L), 804G>A (P268P), and IVS1+43C>T comprised haplotype **7a*. Our results provide fundamental and useful information for genotyping *UGT1A4* in the Japanese and probably Asian populations.

Key words: UGT1A4; amino acid alteration; frameshift; splice donor site; drug metabolism

Received; December 6, 2004, Accepted; February 17, 2005

To whom correspondence should be addressed: Yoshiro SAITO, Ph.D., *Project Team for Pharmacogenetics, National Institute of Health Sciences, 1-18-1 Kamiyoga, Setagaya-ku, Tokyo 158-8501, Japan. Tel. +81-3-3700-9453, Fax. +81-3-3707-6950, E-mail: yoshiro@nihs.go.jp*

Table 1. Primers utilized for *UGT1A4* amplification and sequencing

	Direction	Primer Name	Sequences	Location[a]
Amplification	forward	UGT1A4-1stF	TTAACAAAGTAGAAGGCAGTG	135092
	reverse	UGT1A4-1stR	TGAAAACTTGAAATACACTAGGC	136460
Sequencing	forward	UGT1A4-1stF	TTAACAAAGTAGAAGGCAGTG	135092
	forward	UGT1A4seqF2	GGGCTGAGAGTGGAAAGGT	135502
	forward	UGT1A4seqF3	TCCTTCCTCCTATATTCCTAAGTT	135995
	reverse	UGT1A4seqR1-2	ATCAAATTCCTTCTGGGTCC	135698
	reverse	UGT1A4seqR2	AAGGGGCAGAAAAAGTATGG	136119
	reverse	UGT1A4-1stR	TGAAAACTTGAAATACACTAGGC	136460

[a]The 5′-end of each primer on AF297093.1.

On December 2, 2004, these variations were not found on the UDP Glucuronosyltransferase home page (http://som.flinders.edu.au/FUSA/ClinPharm/UGT/), the Japanese Single Nucleotide Polymorphisms (JSNP) (http://snp.ims.u-tokyo.ac.jp/), dbSNP in the National Center for Biotechnology Information (http://www.ncbi.nlm.nih.gov/SNP/), or PharmGKB (http://www.pharmgkb.org/do/) databases.

This study was supported by the Program for the Promotion of Fundamental Studies in Health Sciences (MPJ-1, -3, and -6) of the Pharmaceuticals and Medical Devices Agency (PMDA) of Japan, and the program for the Promotion of Studies in Health Scrences of the Ministry of Health, Labor and Welfare of Japan.

Introduction

As phase II enzymes, the UDP-glucuronosyltransferase enzymes (UGTs) play crucial roles in the detoxification and elimination of a large number of endogenous and exogenous compounds.[1] Of the UGT1 and UGT2 subfamilies expressed in humans, the genes encoding UGT1As have a unique genetic structure consisting of at least 13 different exon 1′s, including four inactive ones, and the common exons 2 to 5 clustered on chromosome 2q37.[2] One of the exon 1′s can be spliced on to the common exons. The *N*-terminal domains (encoded by the exon 1′s) of the UGT1A proteins determine their substrate-binding specificity, and the common *C*-terminal domain (encoded by exons 2 to 5) is important for UDP-glucuronic acid binding.[3]

UGT1A4 is expressed in the liver, bile ducts, colon, small intestine, and pancreas.[1,4,5] UGT1A4 catalyzes the conjugation of exogenous amines and alcohols, including nicotine, sapogenins, imipramine, trifluoperazine, and tamoxifen.[1,6-9] In addition, many androgens and progestins are reported as endogenous substrates of UGT1A4.[6] Several genetic polymorphisms of *UGT1A4* were reported in the public databases. Among them, two nonsynonymous single nucleotide polymorphisms (SNPs), 70C>A (P24T) and 142T>G (L48V), were found in German Caucasians, and they were shown to be closely associated.[10] The variant enzymes (24T and 48V) had reduced *in vitro* activities for β-naphtylamine, benzidine, *trans*-androsterone, and dihydrotestosterone in a substrate-specific manner.[10]

In spite of the clinical importance of UGT1A4, there is no report on the comprehensive sequencing analysis for the genetic polymorphisms of *UGT1A4* in Asian populations, including the Japanese. In the present study, *UGT1A4* exon 1 was sequenced from 256 Japanese subjects. Eleven novel genetic variations were identified, including 4 nonsynonymous ones.

Materials and Methods

Human genomic DNA samples: DNA was obtained from the blood leukocytes of 88 Japanese cancer patients and 108 Japanese arrhythmic patients. Written informed consent was obtained from all participating patients. DNA was also extracted from Epstein-Barr virus-transformed lymphoblastoid cells, for which blood samples were collected from 60 healthy Japanese volunteers at the Tokyo Women's Medical University under the auspices of the Pharma SNP Consortium (Tokyo, Japan). Informed consent was also obtained from all healthy subjects. The ethical review boards of all the participating organizations approved this study.

PCR conditions for DNA sequencing: First, exon 1 of *UGT1A4* was amplified from genomic DNA (100 ng) using 0.625 units of *Ex*-Taq (Takara Bio. Inc., Shiga, Japan) with 0.2 μM of amplification primers designed in the introns (**Table 1**). The PCR conditions were 94°C for 5 min, followed by 30 cycles of 94°C for 30 sec, 55°C for 1 min, and 72°C for 2 min, and then a final extension at 72°C for 7 min. These PCR products were then treated with a PCR Product Pre-Sequencing Kit (USB Co., Cleveland, OH, USA) and were directly sequenced on both strands using an ABI Big Dye Terminator Cycle Sequencing Kit (Applied Biosystems, Foster City, CA, USA) (see **Table 1** for sequencing primers). The excess dye was removed by a DyeEx96 kit (Qiagen, Hilden, Germany). The eluates were analyzed on an ABI Prism 3700 DNA Analyzer (Applied Biosystems). All variations were confirmed by repeating

Table 2. Summary of *UGT1A4* polymorphisms detected in a Japanese population

SNP ID					Position		Nucleotide change and flanking sequences (5′ to 3′)	Amino acid change	Number of subjects			Frequency			
This Study	dbSNP-NCBI database	JSNP database	Pharm GKB atabase[b]	Location	AF297093.1	From the translational initiation site or from the end of exon 1 (IVS1+)			Wild-type	Hetero-zygote	Homo-zygote	Total (n=256)	Healthy volunteers (n=60)	Cancer patients (n=88)	Arrhythmic patients (n=108)
MPJ6_U1A081	rs3732219	IMS-JST085729	O	5′-flanking	135210	−219	GGGTCAGATGAG**C/T**TTTTCAAGATAG		195	54	7	0.133	0.133	0.142	0.125
MPJ6_U1A082[a]				5′-flanking	135212	−217	GTCAGATGAGC**T/G**TTCAAGATAGGC		255	1	0	0.002	0.000	0.000	0.005
MPJ6_U1A083	rs3732218	IMS-JST085728	O	5′-flanking	135266	−163	TAACGAAAGGCA**G/A**TTATAGATTAAT		195	54	7	0.133	0.133	0.142	0.125
MPJ6_U1A084[a]				5′-flanking	135393	−36	CAGGCACAGCGT**G/A**GGGTGGACAGTC		255	1	0	0.002	0.000	0.006	0.000
MPJ6_U1A085[a]				Exon 1	135458	30	GGTTCCCCTGCC**G/A**CGGCTGGCCACA	P10P	254	2	0	0.004	0.000	0.000	0.009
MPJ6_U1A086	rs3892221		O	Exon 1	135459	31	GTTCCCCTGCCG**C/T**GGCTGGCCACAG	R11W	250	6	0	0.012	0.025	0.011	0.005
MPJ6_U1A087[a]				Exon 1	135555	127	AGCCCCTGGCTC**A/-**GCATGCGGGAGG	43fsX22	255	1	0	0.002	0.000	0.000	0.005
MPJ6_U1A088	rs2011425		O	Exon 1	135570	142	ATGCGGGAGGCC**T/G**TGCGGGAGCTCC	L48V	197	52	7	0.129	0.133	0.148	0.111
MPJ6_U1A089[a]				Exon 1	135603	175	GGCCACCAGGCG**G/-**TGGTCCTCACCC	59fsX6	255	1	0	0.002	0.000	0.000	0.005
MPJ6_U1A090[a]				Exon 1	135699	271	AAGGAATTTGAT**C/T**GCGTTACGCTGG	R91C	255	1	0	0.002	0.000	0.000	0.005
MPJ6_U1A091[a]				Exon 1	135753	325	CATCTTCTGAAG**A/G**GATATTCTAGAA	R109G	255	1	0	0.002	0.000	0.000	0.005
MPJ6_U1A092[a]				Exon 1	135785	357	AATTATGAACAA**T/C**GTATCTTTGGCC	N119N	254	2	0	0.004	0.008	0.006	0.000
MPJ6_U1A093	rs12468274		O	Exon 1	135876	448	TTTGATGTGGTT**T/C**TAACAGACCCCG	L150L	195	54	7	0.133	0.133	0.142	0.125
MPJ6_U1A094	rs2011404		O	Exon 1	135899	471	CGTTAACCTCTGC**/T**[c]GGGGCGGTGCTG	C157C	251	5	0	0.010	0.008	0.011	0.009
MPJ6_U1A095	rs3732217	IMS-JST085727	O	Exon 1	136232	804	CTACCCCAGGCC**G/A**ATCATGCCCAAC	P268P	195	54	7	0.133	0.133	0.142	0.125
MPJ6_U1A096[a]				Intron 1	136296	IVS1+1	CCACTATCTCAG**G/T**TCTGTATTGGTG		255	1	0	0.002	0.000	0.000	0.005
MPJ6_U1A097	rs2011219	IMS-JST085726	O	Intron 1	136338	IVS1+43	TTCCAGGCAAAA**C/T**ACTTTTTAAAAA		195	54	7	0.133	0.133	0.142	0.125
MPJ6_U1A098[a]				Intron 1	136393	IVS1+98	ACTTATCTTTCC**A/G**AAGATTTTATTT		255	1	0	0.002	0.000	0.006	0.000
MPJ6_U1A099[a]				Intron 1	136396	IVS1+101	TATCTTTCCAAA**G/T**ATTTTATTTTGG		253	3	0	0.006	0.008	0.006	0.005

[a]Novel variations detected in this study.
[b]The SNPs included in the PharmGKB database was shown as "O".
[c]T in the reference sequence.

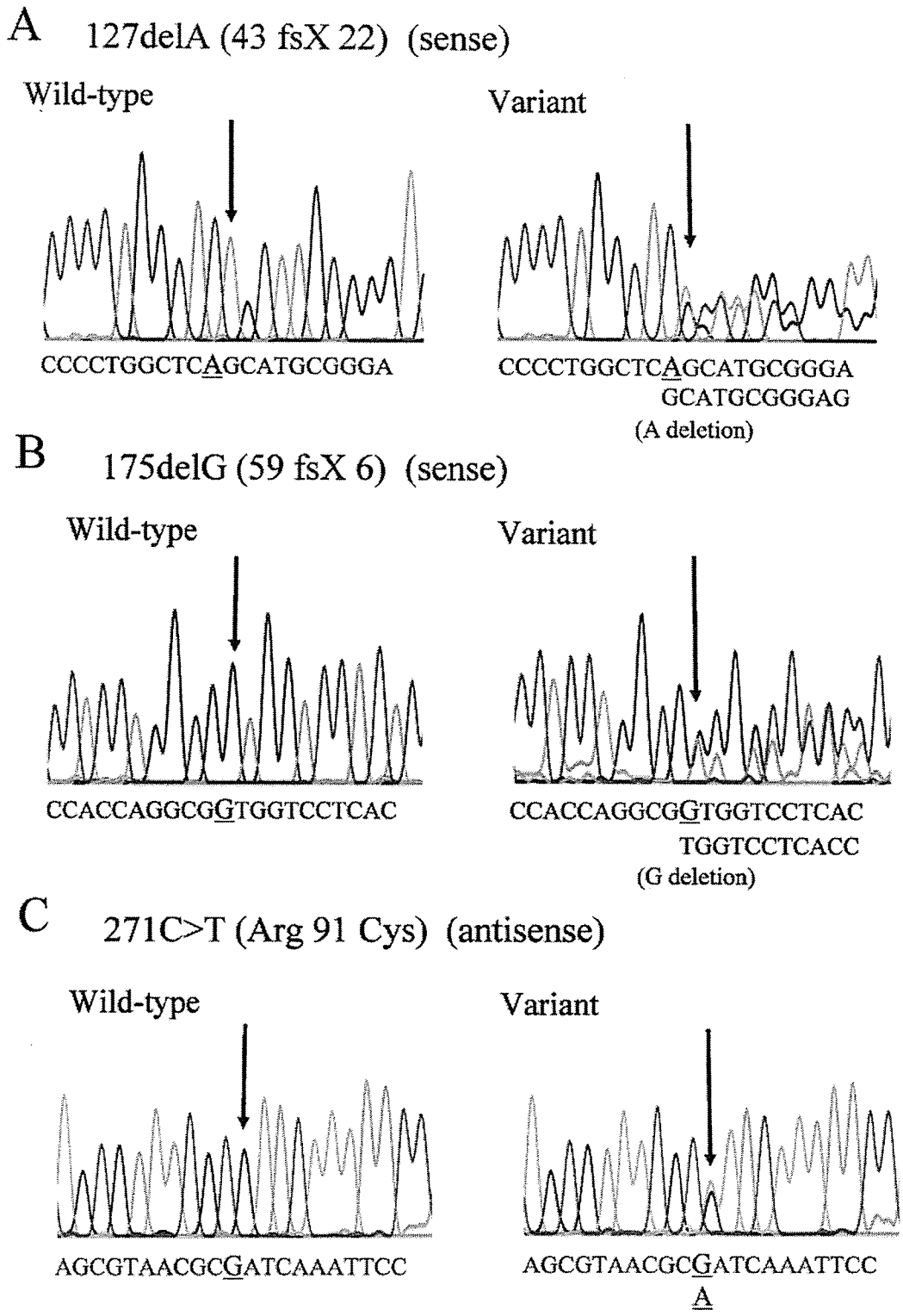
A
127delA (43 fsX 22) (sense)
Wild-type
Variant
CCCCTGGCTCAGCATGCGGGA
CCCCTGGCTCAGCATGCGGGA
GCATGCGGGAG
(A deletion)
B
175delG (59 fsX 6) (sense)
Wild-type
Variant
CCACCAGGCGGTGGTCCTCAC
CCACCAGGCGGTGGTCCTCAC
TGGTCCTCACC
(G deletion)
C
271C>T (Arg 91 Cys) (antisense)
Wild-type
Variant
AGCGTAACGCGATCAAATTCC
AGCGTAACGCGATCAAATTCC
A

Fig. 1

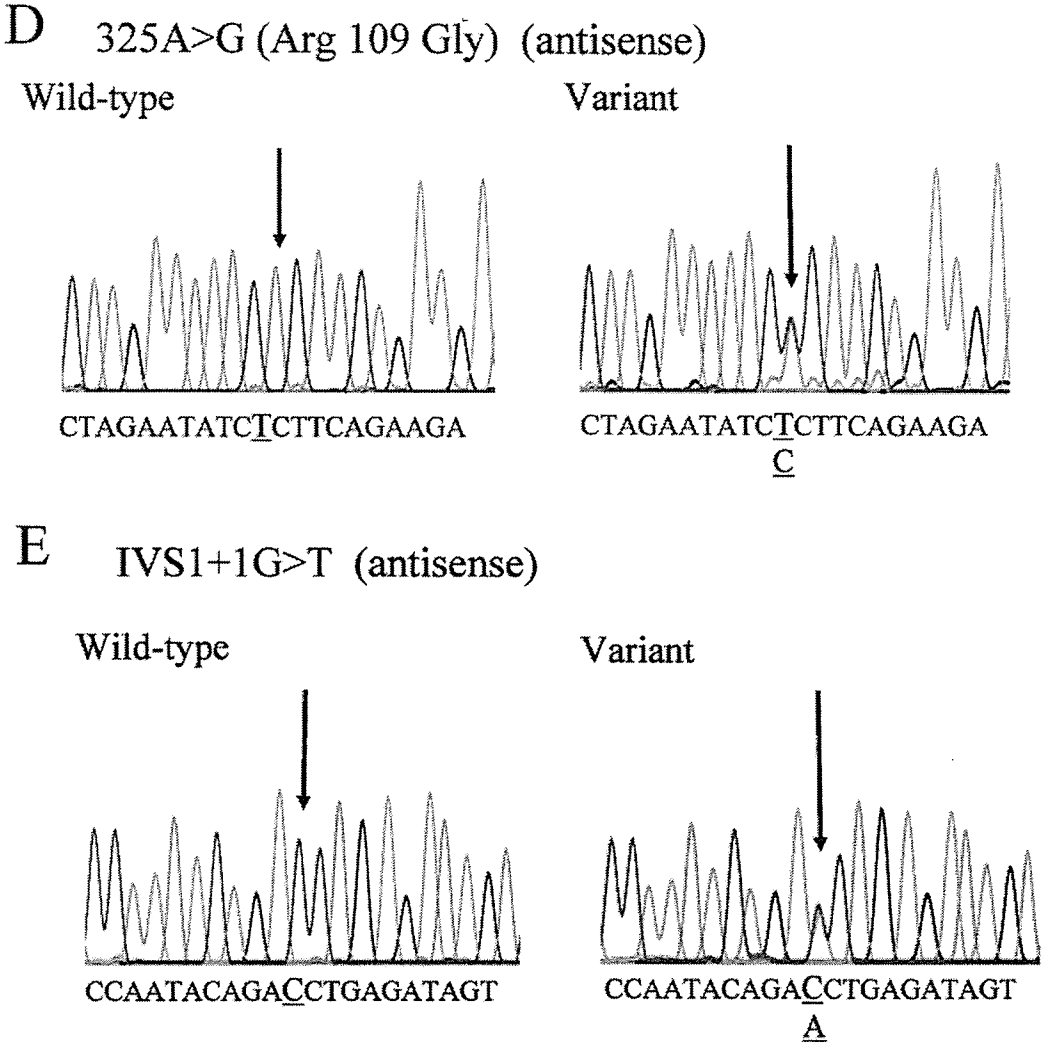

Fig. 1. The 4 novel genetic variations with amino acid substitutions and 1 splice donor site variation of human *UGT1A4*. (A) MPJ6_U1A087 (wild-type, 127A/A; variant, 127A/-). (B) MPJ6_U1A089 (wild-type, 175G/G; variant, 175G/-). (C) MPJ6_U1A090 (wild-type, 271C/C; variant, 271C/T). (D) MPJ6_U1A091 (wild-type, 325A/A; variant, 325A/G). (E) MPJ6_U1A096 (wild-type, IVS1+1G/G; variant, IVS1+1G/T). Arrows indicate the positions of the nucleotide changes.

the PCR on genomic DNA and sequencing the newly generated PCR products. Furthermore, the rare variations found in only one subject were confirmed by sequencing the PCR fragments produced by amplification with a high fidelity DNA polymerase KOD-Plus- (TOYOBO, Tokyo, Japan).

Linkage disequilibrium (LD) and haplotype analysis: Hardy-Weinberg equilibrium analysis and LD analysis were performed by SNPAlyze software (Dynacom Co., Yokohama, Japan). Pairwise LDs were shown in rho square (r^2) values. Some of the haplotypes were unambiguous from the subjects with homozygous SNPs at all sites or a heterozygous SNP at only one site. Separately, the diplotype configurations (a combination of haplotypes) were inferred by LDSUPPORT software, which determines the posterior probability distribution of the diplotype configuration for each subject based on the estimated haplotype frequencies.[11] The haplotypes were described as a number plus a small alphabetical letter.

Results and Discussion

UGT1A4 exon 1 and its flanking regions (from −286 bases upstream of the translational start site to 112 bases downstream of the end of exon 1) were sequenced from 256 Japanese subjects. Genbank accession number AF297093.1 was utilized for the reference sequence. Nineteen polymorphisms were detected, including 11 novel ones (2 were in the 5′-flanking region, 6 in exon 1, and 3 in the following intron) (**Table 2**). All of the allelic frequencies were in Hardy-Weinberg equilibrium ($p=$

0.13 or over). Since we did not find any significant differences in the frequencies of all the variations among three subject groups ($p>0.25$ by χ^2 test) and between two of the three groups ($p>0.13$ by χ^2 test or Fisher's exact test), the data for all subjects were analyzed as one group.

We found two novel nonsynonymous variations, 271C>T (R91C) and 325A>G (R109G), and two novel deletions, 127delA (43fsX22) and 175delG (59fsX6), as individual heterozygotes at a 0.002 frequency. Among them, 127delA (43fsX22) and 175delG (59fsX6) are the frameshift variations starting from codon 43 and 59, respectively, resulting in early stop codons at the 22nd (*i.e.* codon 65) and the 6th (*i.e.* codon 65) codons, respectively. It is most likely that these variations generate an immature protein that probably has null activity. The functional significance of 271C>T (R91C) and 325A>G (R109G) is currently unknown. Additionally, IVS1+1G>T, which was found at a frequency of 0.002, was located at a splice donor site and thus may lead to aberrant splicing (**Fig. 1**).

We also detected two known nonsynonymous SNPs, 31C>T (R11W) and 142T>G (L48V), at 0.012 and 0.129 frequencies, respectively. The frequency of 142T>G (L48V) was almost comparable to that of German Caucasians (0.09).[10] L48V was reported to lead to a partial decrease in glucuronidation of β-naphthylamine and benzidine, a marked decrease in the activity to *trans*-androsterone, and no activity toward dihydrotestosterone *in vitro*.[10] The functional significance of SNP 31C>T (R11W) has not been reported yet.

High linkage disequilibrium ($r^2 \geqq 0.89$) was observed among −219C>T, −163G>A, 142T>G (L48V), 448T>C (L150L), 804G>A (P268P), and IVS1+43C>T. A perfect linkage ($r^2=1$) was found between 175delG and 325A>G (R109G), but found in only one subject. The r^2 values were below 0.014 between the other pairs of polymorphisms. The SNP 70C>A (P24T), mostly linked with 142T>G (L48V) in German Caucasians,[10] was not detected in this study. Thus, it must be clarified whether the differences in the linkage of those SNPs may lead to the ethnic differences in the enzymatic activities of UGT1A4. A similar kind of ethnic difference has been found in the **IB* haplotype, which harbors the three linked SNPs in the 3′-untranslated region of UGT1A common exon 5 found in a Japanese population.[12] In Caucasian and African-American populations, this linkage of the 3 SNPs was not complete, especially in African-Americans.[13]

Using the detected SNPs, haplotype analysis was then performed (**Table 3**). Since *UGT1A4*2* [70C>A (P24T)] and **3* [142T>G (L48V)] were defined in AF465196 and AF465197 (Genbank accession numbers), respectively, the novel haplotypes with amino acid changes, frameshift variations, or splice donor site

Table 3. *UGT1A4* haplotypes in a Japanese population

Haplotypes[a]	Nucleotide change	−219 C>T	−217 T>G	−163 G>A	−36 G>A	30 G>A	31 C>T	127 delA	142 T>G	175 delG	271 C>T	325 A>G	357 T>C	448 T>C	471 C>T	804 G>A	IVS1 +1 G>T	IVS1 +43 C>T	IVS1 +98 A>G	IVS1 +101 G>T	Frequency
	Amino acid change					P10P	R11W	43fsX22	L48V	59fsX6	R91C	R109G	N119N	L150L	C157C	P268P					
*1	*1a																				0.856
	*1b																				0.010
	*1c																				0.008
	*1d																				0.006
	*1e																				0.004
	*1f																				0.004
	*1g																				0.002
	*1h																				0.002
	*1i																				0.002
*3	*3a																				0.125
	*3b																				0.002
*4	*4a																				0.012
*5	*5a																				0.002
*6	*6a																				0.002
*7	*7a																				0.002
*8	*8a																				0.002

[a] A of the translational start codon of *UGT1A4* is numbered 1. AF297093.1 was used as the reference sequence.

[b] The haplotypes were described as a number plus a small alphabetical letter.

variation, were assigned as haplotypes **4* to **8*. Several haplotypes were first unambiguously assigned by homozygous SNPs at all sites (**1a* and **3a*) or a heterozygous SNP at only one site (**1b*, **1d*-**1i*, **3b*, **4a*, and **8a*). Separately, we estimated the diplotype configuration (a combination of haplotypes) for each subject by LDSUPPORT software. The diplotype configurations of 256 subjects were inferred with probabilities (certainty) of 0.9998 or over, except for one subject. The additionally inferred haplotypes were **1c*, **5a*, **6a*, and **7a*. As for one subject with a low probability (who had heterozygous SNPs of −219C>T, −163 G>A, 31C>T, 142T>G, 448T>C, 804G>A, and IVS1+43C>T), the diplotype was determined by the cloning and sequencing of DNA fragments. One chromosome had haplotype **3a* (consisting of −219C>T, −163 G>A, 142T>G, 448T>C, 804G>A, and IVS1+43C>T) and the other had haplotype **4a* (31C>T). Moreover, the data obtained by cloning and sequencing analysis confirmed the presence of haplotypes **5a* [127delA (43fsX22) and 142T>G (L48V)], **6a* [175delG (59fsX6) and 325A>G (R109G)], and **7a* [−219C>T, −163G>A, 142T>G (L48V), 271C>T (R91C), 448T>C (L150L), 804G>A (P268P), and IVS1+43C>T] (**Table 3**). The most frequent haplotype was **1a* (frequency: 0.818), followed by **3a* (0.123), **4a* (0.012) and **1b* (0.010). The frequencies of the other haplotypes were less than 0.01. Since 325A>G (R109G) was linked with 175delG (59fsX6), the enzymatic activity of this haplotype (**6a*) is probably null. The other SNP, 271C>T confers the R91C substitution. In human UGT1A4, eight cysteine residues were located in the lumenal domain.[3,14] Though the disulfide-bond formation and its significance are not clear in the UGT1A4, it has been reported that the reduction of disulfide-bonds of rat UGT1A6 with dithiothreitol increases its enzymatic activity in the liver microsomes.[15] On the other hand, the alterations of several lumenal cysteines into serine residues seem to reduce the UGT1A6 activity when the mutant enzymes were expressed in COS cells.[15] The effect of additional cysteine residue at codon 91 in the UGT1A4 should be determined in the future.

In conclusion, we detected 19 polymorphisms, including 11 novel ones, in *UGT1A4* from a Japanese population. Using the detected polymorphisms, 16 haplotypes were identified. Our results provide fundamental and useful information for genotyping *UGT1A4* in the Japanese, and probably Asian populations.

Acknowledgments: We thank Ms. Chie Knudsen for her secretarial assistance.

References

1) Tukey, R. H. and Strassburg, C. P.: Human UDP-glucuronosyltransferase: Metabolism, Expression, and Disease. *Annu. Rev. Pharmacol. Toxicol.*, **40**: 581–616 (2000).
2) Mackenzie, P. I., Owens, I. S., Burchell, B., Bock, K. W., Bairoch, A., Belanger, A., Fournel-Gigleux, S., Green, M., Hum, D. W., Iyanagi, T., Lancet, D., Louisot, P., Magdalou, J., Chowdhury, J. R., Ritter, J. K., Schachter, H., Tephly, T. R., Tipton, K. F. and Nebert, D. W.: The UDP glycosyltransferase gene superfamily: recommended nomenclature update based on evolutionary divergence. *Pharmacogenetics*, **7**: 255–269 (1997).
3) Radominska-Pandya, A., Czernik, P. J. and Little, J. M.: Structural and functional studies of UDP-glucuronosyltransferases. *Drug Metab. Rev.*, **31**: 817–899 (1999).
4) Strassburg, C. P., Kneip, S., Topp, J., Obermayer-Straub, P., Barut, A., Tukey, R. H. and Manns, M. P.: Polymorphic gene regulation and interindividual variation of UDP-glucuronosyltransferase activity in human small intestine. *J. Biol. Chem.*, **275**: 36164–36171 (2000).
5) Ockenga, J., Vogel, A., Teich, N., Keim, V., Manns, M. P. and Strassburg, C. P.: UDP glucuronosyltransferase (UGT1A7) gene polymorphisms increase the risk of chronic pancreatitis and pancreatic cancer. *Gastroenterology*, **124**: 1802–1808 (2003).
6) Green, M. D. and Tephly, T. R.: Glucuronidation of amines and hydroxylated xenobiotics and endobiotics catalyzed by expressed human UGT1.4 protein. *Drug Metab. Dispos.*, **24**: 356–363 (1996).
7) Nakajima, M., Tanaka, E., Kobayashi, T., Ohashi, N., Kume, T. and Yokoi, T.: Imipramine *N*-glucuronidation in human liver microsomes: biphasic kinetics and characterization of UDP-glucuronosyltransferase isoforms. *Drug Metab. Dispos.*, **30**: 636–642 (2002).
8) Nakajima, M., Tanaka, E., Kwon, J. T. and Yokoi, T.: Characterization of nicotine and cotinine *N*-glucuronidations in human liver microsomes. *Drug Metab. Dispos.*, **30**: 1484–1490 (2002).
9) Kaku, T., Ogura, K., Nishiyama, T., Ohnuma, T., Muro, K. and Hiratsuka, A.: Quaternary ammonium-linked glucuronidation of tamoxifen by human liver microsomes and UDP-glucuronosyltransferase 1A4. *Biochem. Pharmacol.*, **67**: 2093–2102 (2004).
10) Ehmer, U., Vogel, A., Schutte, J. K., Krone, B., Manns, M. P. and Strassburg, C. P.: Variation of hepatic glucuronidation: Novel functional polymorphisms of the UDP-glucuronosyltransferase UGT1A4. *Hepatology*, **39**: 970–977 (2004).
11) Kitamura, Y., Moriguchi, M., Kaneko, H., Morisaki, H., Morisaki, T., Toyama, K. and Kamatani, N.: Determination of probability distribution of diplotype configuration (diplotype distribution) for each subject from genotypic data using the EM algorithm. *Ann. Hum. Genet.*, **66**: 183–193 (2002).
12) Sai, K., Saeki, M., Saito, Y., Ozawa, S., Katori, N.,

Jinno, H., Hasegawa, R., Kaniwa, N., Sawada, J., Komamura, K., Ueno, K., Kamakura, S., Kitakaze, M., Kitamura, Y., Kamatani, N., Minami, H., Ohtsu, A., Shirao, K., Yoshida, T. and Saijo, N.: UGT1A1 haplotypes associated with reduced glucuronidation and increased serum bilirubin in irinotecan-administered Japanese patients with cancer. *Clin. Pharmacol. Ther.*, **75**: 501–515 (2004).

13) Kaniwa, N., Kurose, K., Jinno, H., Tanaka-Kagawa, T., Saito, Y., Saeki, M., Sawada, J., Tohkin, M. and Hasegawa, R.: Racial variability in haplotype frequencies of UGT1A1 and glucuronidation activity of a novel SNP 686C>T (P229L) found in an African-American. *Drug Metab. Dispos.*, in press.

14) Ritter, J. K., Crawford, J. M. and Owens, I. S.: Cloning of two human liver bilirubin UDP-glucuronosyltransferase cDNAs with expression in COS-1 cells. *J. Biol. Chem.*, **266**: 1043–1047 (1991).

15) Ikushiro, S., Emi, Y. and Iyanagi, T.: Activation of glucuronidation through reduction of a disulfide bond in rat UDP-glucuronosyltransferase 1A6. *Biochemistry*, **41**: 12813–12820 (2002).

www.nature.com/clinicalpractice/onc

What phase III trials are needed to improve the treatment of advanced non-small-cell lung cancer?

Nagahiro Saijo

Platinum-based doublets are standard treatments for stage IV non-small-cell lung cancer (NSCLC). Several doublets that include new drugs improve survival, but no one regimen is clearly superior to the others, as previously discussed by Scagliotti[1] and Govindan[2] in *Nature Clinical Practice Oncology*.

Numerous molecular-target-based drugs have been introduced for the treatment of NSCLC, but can they replace or be used as an adjuvant to current therapy, and can they be combined with other chemotherapeutic agents, radiotherapy and/or surgery? We hypothesize that incorporation of novel molecular-target-based therapies into current treatment paradigms will improve outcomes. However, carefully designed clinical trials and translational science will be required to identify the subsets of patients likely to benefit. If these treatment strategies are to be used, we must first answer the following critical questions. First, will patients lacking the target still respond? It is still unclear why responses occur in those lacking the correct molecular target. Second, what expression levels of the target are sufficient for a response, and can we measure the target in a biologically relevant and/or technologically valid way? Third, does the agent inhibit the proposed target at the dose and schedule utilized? Fourth, is the target a critical driving force for cell growth in the tumor type in question?

Various molecular-target-based drugs for advanced NSCLC have been evaluated in randomized controlled trials, but the majority, including a matrix metalloproteinase inhibitor, a protein kinase C inhibitor, and trastuzumab, have yielded negative results.[3,4] Gefitinib (Iressa®) and erlotinib (Tarceva™) are orally available selective epidermal growth factor receptor (EGFR) tyrosine kinase inhibitors (TKI) that exhibit antitumor activity in patients with previously treated advanced NSCLC. However, both drugs failed to show additive or synergistic effects when combined with platinum-based chemotherapy as a first-line treatment for NSCLC. On 17 December 2004, AstraZeneca announced the preliminary results of their ISEL (Iressa® Survival Evaluation in Lung Cancer) study of 1,692 patients with advanced recurrent or refractory NSCLC. Unfortunately, gefitinib failed to prolong survival significantly compared with placebo (hazard ratio 0.89, $P=0.11$) in the overall patient population or among patients with adenocarcinoma (hazard ratio 0.83, $P=0.07$). A retrospective analysis of patients treated with gefitinib showed that tumor response was associated with distinct subgroups: women, patients with no history of smoking, patients with adenocarcinoma, and Japanese patients. Survival in the gefitinib group in the ISEL study was significantly higher for non-smokers ($P<0.01$) and Asians ($P<0.01$) than in the placebo group. The survival curves of the two treatment groups were the same for non-Asians. The results of similar randomized trials of erlotinib (the BR21 study) were presented at the American Society of Clinical Oncology meeting in 2004. Erlotinib significantly prolonged survival in patients with advanced, previously treated, refractory or recurrent NSCLC. The survival of non-smokers in the erlotinib group in the BR21 study was extremely good and contributed to the improvement in overall survival. The presence of an *EGFR* mutation has been demonstrated to be a strong predictor of a favorable response to EGFR-TKI. Mutations have recently been reported to be significantly more frequent in women, in patients with adenocarcinoma, and in those who had never smoked, and these findings are consistent with the clinical predictors of tumor response in patients treated with EGFR-TKI. Mitsudomi *et al.* reported that patients with *EGFR* mutations survived longer after the initiation of gefitinib treatment than those without mutations.[5] It can be concluded that translational studies are extremely important for the development of molecular-target-based drugs.

Numerous molecular-target-based drugs have been introduced for the treatment of NSCLC, but what is their place in current therapy?

N Saijo is an Advisory Board member of Nature Clinical Practice Oncology.

Competing interests
The author declared he has no competing interests.

www.nature.com/clinicalpractice
doi:10.1038/ncponc0199

Supplementary information, in the form of a reference list, is available on the *Nature Clinical Practice Oncology* website.

www.nature.com/clinicalpractice/onc

Is radiotherapy optimally combined with chemotherapy in elderly patients with limited-stage small-cell lung cancer?

GLOSSARY

ECOG PERFORMANCE STATUS (ECOG PS)
A scoring system to assess the wellbeing of cancer patients and their ability to perform ordinary tasks (0 = fully active to 5 = dead)

Original article Schild SE *et al.* (2005) Results of combined-modality therapy for limited-stage small cell lung carcinoma in the elderly. *Cancer* **103:** 2349–2354

SYNOPSIS

KEYWORDS cisplatin, combined-modality therapy, etoposide, radiotherapy, small-cell lung cancer

BACKGROUND

It is important to understand the effects of modern combined-modality therapy in elderly patients with lung carcinoma. Half of the patients who are diagnosed with lung carcinoma are ≥70 years of age.

OBJECTIVES

To determine the relationship between age and outcome in patients with limited-stage small-cell lung cancer (SCLC) treated with etoposide and cisplatin in addition to once-daily or twice-daily radiotherapy (QDRT or BIDRT respectively).

DESIGN AND INTERVENTION

From September 1990 to November 1996, this North Central Cancer Treatment Group phase III trial enrolled patients with limited-stage disease confirmed by pathology as SCLC, with ECOG PERFORMANCE STATUS (ECOG PS) ≤2 and sufficient organ function. Six 3-day cycles of etoposide and cisplatin were given, with a 28-day interval between cycles. Cisplatin (30 mg/m^2 given intravenously over 30–60 minutes), and etoposide (130 mg/m^2 given intravenously over 45 minutes) were administered on each chemotherapy day. After the first three cycles, the dose of etoposide was reduced to 100 mg/m^2 per cycle. Patients were randomized to receive thoracic radiotherapy (in parallel to chemotherapy cycles 4–5), either QDRT (50.4 Gy in 28 fractions) or BIDRT (48 Gy in 32 fractions).

OUTCOME MEASURES

Toxicity, disease control and survival.

RESULTS

Of 263 evaluable patients (median age 63 years, range 37–81 years), followed for a median of 8.1 years (range 4.6–11.9 years), 209 were younger than 70 years old and 54 were 70 years old or older. Baseline ECOG PS and weight loss were worse in the older group. Tumor progression rates, survival, local control, and overall, hematologic and nonhematologic toxicities did not differ according to patient age. The 2-year and 5-year survival rates were 48% and 22% respectively, in patients aged <70 years, versus 33% and 17% in older patients (P=0.14). Hematologic toxicities ≥grade 3 or ≥grade 4 did not occur more frequently in elderly patients. Grade 3 toxicity or worse occurred in 91% of patients aged <70 years compared with 94% of elderly patients (P=0.58). Toxicities of grade 4 or more occurred in 46% of patients aged <70 years compared with 50% of older patients (P=0.65). Grade ≥3 nonhematologic toxicity occurred in 46% of those aged <70 years compared with 52% of older patients (P=0.45). Grade ≥4 nonhematologic toxicity occurred in 12% of patients aged <70 years compared with 11% of elderly patients (P=1.0). Of the nonhematologic toxicities, only grade ≥4 pneumonitis occurred more frequently in elderly patients. Grade ≥3 esophagitis occurred in similar numbers of patients in the two age groups. Treatment-related toxicity caused death in 4 of 263 patients (2%)—3 in the elderly group (pneumonitis) and 1 in the younger group (infection).

CONCLUSION

Elderly patients should be encouraged to receive combined-modality therapy, especially within clinical trials.

www.nature.com/clinicalpractice/onc

COMMENTARY

Nagahiro Saijo

Cisplatin plus etoposide with concurrent thoracic radiotherapy is the standard treatment for limited-disease small-cell lung carcinoma (LD-SCLC) in the elderly.[1,2] In Intergroup study 0096, Turrisi *et al.* found that, when combined with etoposide plus cisplatin chemotherapy, a total radiation dose of 45 Gy administered as a twice-daily therapy (1.5 Gy twice daily) produced superior survival to the same total dose administered as a once-daily therapy (1.8 Gy once daily).[1] The Japan Clinical Oncology Group also obtained excellent survival data (median survival time 27 months) using concurrent chemotherapy and twice-daily irradiation (Japan Clinical Oncology Group 9104).[2] In 2004, Schild *et al.* reported that equivalent survival benefit was achieved with twice-daily and once-daily irradiation with etoposide plus cisplatin chemotherapy.[3] Once-daily radiotherapy was administered continuously, and twice-daily radiotherapy was administered with a 2.5-week intermission after 24 Gy. The treatment schedule of the Intergroup study differed from that of the present study in that concurrent radiotherapy was given from the start of chemotherapy, and radiotherapy was given without a break. The dose intensity of the combination of chemotherapy and radiotherapy in the Intergroup study was higher in the twice-daily group. Efficacy improved with increased intensity of combined-modality therapy, as did adverse events. Elderly patients usually experience more toxicity than younger patients, and cannot tolerate intensive treatment. Few studies have specifically targeted elderly populations.

The elderly patients in the present analysis (aged ≥70 years) experienced significantly greater weight loss and poorer performance status than the younger patients (aged <70 years). The 2-year and 5-year survival rates were 48% and 22% for younger patients, compared with 33% and 17% for elderly patients. The incidence of grade 4 pneumonitis was higher in the elderly patients. Grade 5 toxicity occurred in 1 of 209 younger patients versus 3 of 54 older patients. Schild *et al.* concluded that LD-SCLC patients over 70 years of age are candidates for clinical trials of aggressive treatment if they do not have severe comorbidity. Yuen *et al.* reviewed the elderly subset results from the Intergroup 0096 study.[4] Quon *et al.* also studied the influence of age on the delivery, tolerance, and efficacy of thoracic irradiation in the combined-modality treatment of limited stage small-cell lung cancer.[5] In both analyses it was suggested that an elderly subset seems to be at risk of toxicity, but that those patients completing therapy do as well as their younger counterparts. It is extremely difficult, however, to distinguish those patients who are at risk of toxicity before toxicity occurs.

LD-SCLC is curable by chemotherapy and radiotherapy without surgery. Since the average age of LD-SCLC patients will increase year by year, fit elderly patients with LD-SCLC should be encouraged to undergo combined-modality therapy. An initial cycle of chemotherapy before concurrent treatment might unveil the vulnerable subset. The role of sequential chemotherapy should be evaluated in elderly patients considered marginal, to help us to distinguish those patients that are able to tolerate aggressive therapy from those that are too easily tipped over into a less-fit category. In conclusion, it is extremely important to establish a safe and effective standard treatment for the elderly patient population.

References

1 Turrisi AT *et al.* (1999) Twice-daily compared with once-daily thoracic radiotherapy in limited small-cell lung cancer treated concurrently with cisplatin and etoposide. *N Engl J Med* **340:** 265–271

2 Takada M *et al.* (2002) Phase III study of concurrent versus sequential thoracic radiotherapy in combination with cisplatin and etoposide for limited-stage small-cell lung cancer: results of the Japan Clinical Oncology Group study 9104. *J Clin Oncol* **20:** 3054–3060

3 Schild SE *et al.* (2004) Long-term results of a phase III trial comparing once-daily radiotherapy with twice-daily radiotherapy in limited-stage small-cell lung cancer. *Int J Radiat Oncol Biol Phys* **59:** 943–951

4 Yuen AR *et al.* (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

5 Quon H *et al.* (1999) The influence of age on the delivery, tolerance, and efficacy of thoracic irradiation in the combined modality treatment of limited stage small cell lung cancer. *Int J Radiat Oncol Biol Phys* **43:** 39–45

N Saijo is Deputy Director of the National Cancer Center Hospital East, Tokyo, Japan.

Acknowledgments
The synopsis was written by Petra Roberts, Associate Editor, Nature Clinical Practice.

Competing interests
The author declared he has no competing interests.

Correspondence
National Cancer Center Hospital
Thoracic Oncology Division
Tsukiji 5-1-1
Chuo-ku
Tokyo 104-0045
Japan
nsaijo@east.ncc.go.jp

Received 26 July 2005
Accepted 16 September 2005
www.nature.com/clinicalpractice
doi:10.1038/ncponc0328

PRACTICE POINT
Further study of combined-modality therapy within clinical trials is needed to establish a safe and effective standard treatment for elderly patients with lung carcinoma

NAGAHIRO SAIJO, MD, PhD
ATSUSHI HORIIKE, MD
Division of Internal Medicine
National Cancer Center Hospital
Tokyo, Japan

Topoisomerase I Inhibitors in Small-Cell Lung Cancer

The Japanese Experience

ABSTRACT

Among patients with lung cancer, approximately 15% have small-cell lung cancer (SCLC). The clinical characteristics of SCLC tend to be more aggressive, but also more sensitive to chemotherapy and radiation therapy than those of non-SCLC. Irinotecan (Camptosar) is a derivative of camptothecin, an inhibitor of the nuclear enzyme topoisomerase I. Irinotecan has been shown to exhibit excellent antitumor activity against SCLC in monotherapy regimens and in combination with cisplatin. A phase III trial comparing irinotecan and cisplatin (IP) with etoposide and cisplatin (EP) in patients with previously untreated extensive-stage SCLC (ED-SCLC) was conducted. Patients in the IP arm responded significantly better than patients in the EP arm. In the IP arm, the response rate was 84%, and median overall survival was 12.8 months. A phase II trial of irinotecan, cisplatin, and etoposide (IPE) administered weekly (arm A) or every 4 weeks (arm B) for ED-SCLC (JCOG 9902-DI) was also performed. In arm B, the response rate was 77% and the median overall survival was 12.9 months. A randomized trial comparing IP with IPE administered every 3 weeks in patients with previously untreated ED-SCLC is presently being performed in Japan.

An estimated 75,000 new cases of lung cancer were diagnosed in Japan in 2002. Approximately 15% of these cases were diagnosed as small-cell lung cancer (SCLC), which is strongly associated with tobacco use, as is non–small-cell lung cancer (NSCLC). The clinical characteristics of SCLC tend to be more aggressive, but also more sensitive to chemotherapy and radiation therapy than those of NSCLC. Small-cell lung cancer is usually staged as either limited disease (LD) or extensive disease (ED).[1]

Platinum-based chemotherapy remains the mainstay of treatment regimens for ED-SCLC. In a meta-analysis of 19 randomized trials comparing a cisplatin-based regimen with a non–cisplatin-based regimen, patients randomized to a regimen containing cisplatin had a significantly higher probability of response and survival, with no significant increase in toxicity.[2] Berghmans et al presented a detailed analysis of the roles of etoposide and cisplatin in the treatment of SCLC.[3] Between 1980 and 1998, 36 eligible trials were performed. These trials concluded that the use of cisplatin and/or etoposide offered a significant survival advantage to patients with SCLC.

Irinotecan (Camptosar) has been semisynthesized as a water-soluble derivative of camptothecin, an inhibitor of nuclear enzyme topoisomerase I, in an attempt to reduce its toxicity and to improve its therapeutic efficacy.[4-8] In a phase II trial of irinotecan for SCLC, the response rate was 47%.[9,10] In preclinical studies, irinotecan and cisplatin exhibited synergistic activities. Their toxicity pro-

Financial Disclosure: The authors have no significant financial interest or other relationship with the manufacturers of any products or providers of any service mentioned in this article.

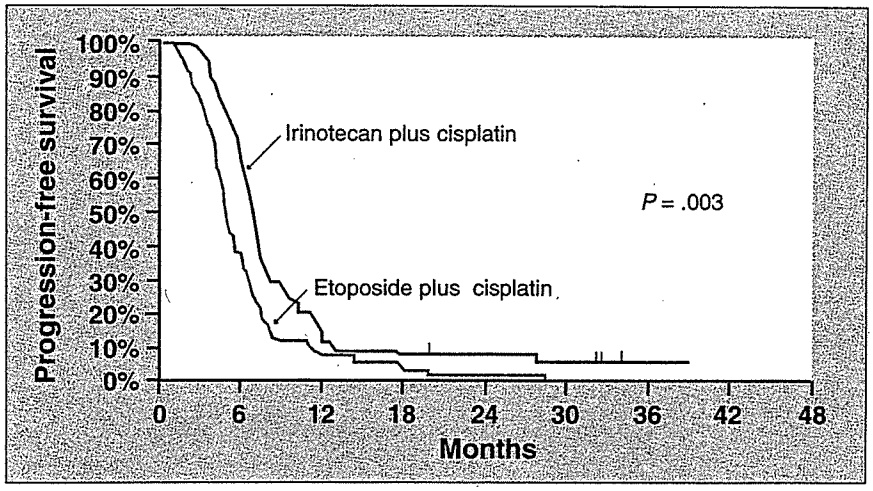

Figure 1: Progression-Free Survival—Progression-free survival of patients with extensive small-cell lung cancer who were assigned to treatment with irinotecan plus cisplatin or etoposide plus cisplatin (JCOG 9511).

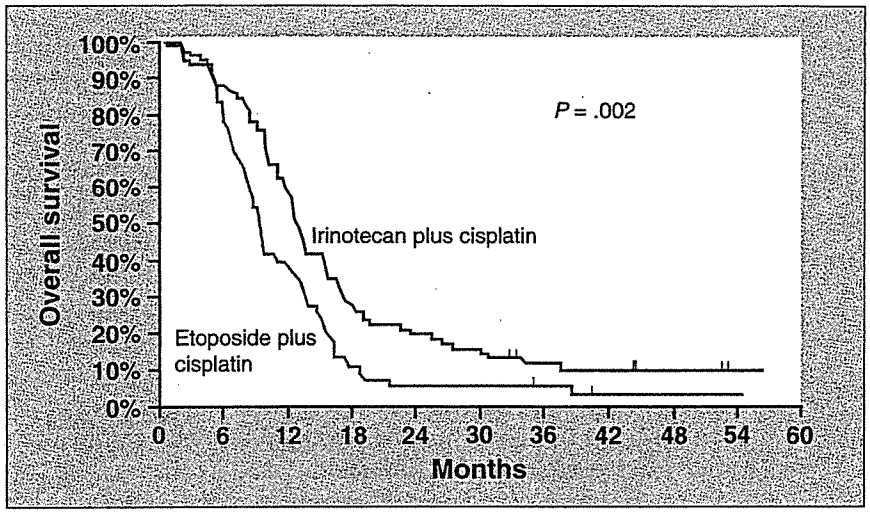

Figure 2: Overall Survival—Overall survival of patients with extensive small-cell lung cancer who were assigned to treatment with irinotecan plus cisplatin or etoposide plus cisplatin (JCOG 9511).

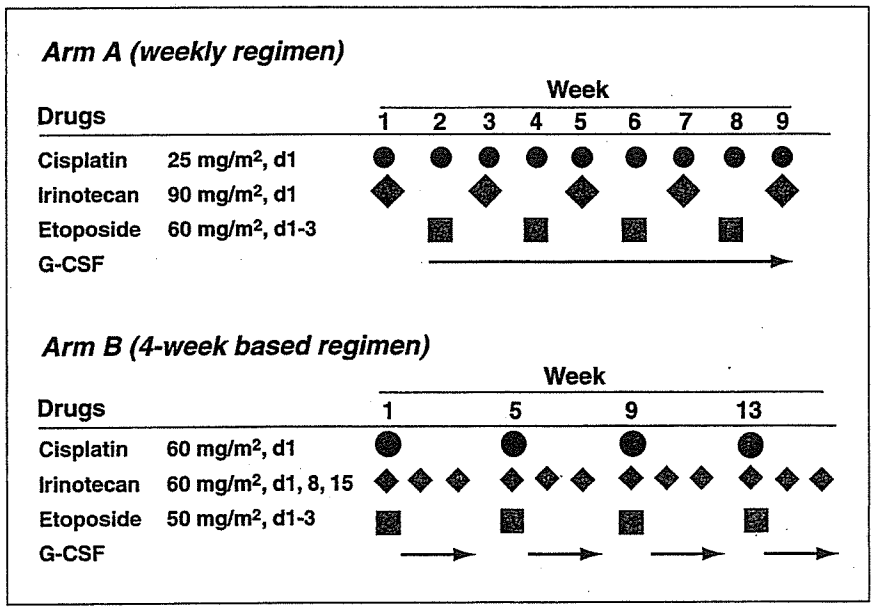

Figure 3: Study JCOG 9902-DI—Treatment schema of arm A (weekly regimen) and arm B (4-week regimen).

files also showed a minimal overlap.[11-16] In a phase II trial of irinotecan and cisplatin, the response rate was 86%.[17] In these trials, the principal toxicities were neutropenia and diarrhea.

Phase III Trial Comparing Irinotecan and Cisplatin With Cisplatin and Etoposide

Based on the results of the phase II trial, the Japan Clinical Oncology Group (JCOG) conducted a multiinstitutional randomized phase III trial (JCOG-9511) comparing irinotecan and cisplatin (IP) with cisplatin and etoposide (EP) in patients with previously untreated ED-SCLC.[18] The patient characteristics in this trial included an Eastern Cooperative Oncology Group (ECOG) performance status of 0–2 and age ≤ 70 years. Patients with symptomatic central nervous system metastases requiring radiation or corticosteroid treatment were excluded from the trial. The experimental arm consisted of irinotecan at 60 mg/m^2 administered on days 1, 8, and 15 of each 4-week cycle, along with cisplatin at 60 mg/m^2 administered on day 1 for a total of four 4-week cycles (IP). This treatment regimen was compared with a regimen of etoposide at 100 mg/m^2 administered on the first 3 days of each 3-week cycle along with cisplatin at 80 mg/m^2 administered on day 1 for a total of four 3-week cycles (EP).

The principal end point was overall survival. The projected accrual for this trial was 230 patients (115 patients per arm). An interim analysis conducted after 77 patients had been accrued in each arm showed a significant survival advantage for the IP arm. Therefore, further enrollment in the trial was discontinued.

The response rate was significantly higher in the IP arm than in the EP arm (84% vs 68%; P = .02). Additionally, the IP arm showed a statistically significant improvement in both progression-free survival (6.9 vs 4.8 months; P = .003) (Figure 1) and median overall survival (12.8 vs 9.4 months; P = .002) (Figure 2).

The results of this trial were the most exciting to be seen in patients with previously untreated SCLC. The IP regimen is thus another platinum-based combination that should be considered for the treatment of ED-SCLC. Appropriately, the combination of cisplatin and irinotecan has become the new standard treatment for patients with ED-SCLC in Japan. However, several points must be examined before the IP regimen can be fully established as the new standard treatment for ED-SCLC. Three randomized controlled trials comparing the EP regimen with the IP regimen are presently under way in Europe and the United States.

Table 1

Patient Characteristics in JCOG 9902-DI

	Arm A (n = 30)		Arm B (n = 30)	
	Number of Patients	Percentage	Number of Patients	Percentage
Sex				
Female	3	10%	3	10%
Male	27	90%	27	90%
Median age (range)	64 yr	(47–70 yr)	63 yr	(46–68 yr)
Performance status				
0	2	7%	3	10%
1	25	83%	25	83%
2	3	10%	2	7%
Body weight loss				
< 5%	23	77%	21	70%
5%–10%	6	20%	8	27%
> 10%	1	3%	1	3%

Table 2

Number of Chemotherapy Cycles Delivered in JCOG 9902-DI

Arm A			Arm B		
Number of Cycles	Number of Patients	Percentage	Number of Cycles	Number of Patients	Percentage
9	22	73%	4	21	70%
8	4	13%	3	5	17%
5	1	3%	2	2	7%
4	1	3%	1	2	7%
2	1	3%			
1	1	3%			

Table 3

Total Administered Dosage and Dose Intensity Delivered in JCOG 9902-DI

	Arm A	Arm B
	Median (Range) Total Dosage	
Cisplatin	225 mg/m² (25–225 mg/m²)	240 mg/m² (60–240 mg/m²)
Irinotecan	450 mg/m² (90–450 mg/m²)	563 mg/m² (60–720 mg/m²)
Etoposide	720 mg/m² (0–720 mg/m²)	600 mg/m² (150–600 mg/m²)
	Median (Range) Dose Intensity	
Cisplatin	21 mg/m²/wk (13–25 mg/m² wk)	15 mg/m²/wk (12–15 mg/m²/wk)
Irinotecan	40 mg/m²/wk (21–90 mg/m²/wk)	35 mg/m²/wk (15–45 mg/m²/wk)
Etoposide	68 mg/m²/wk (0–80 mg/m²/wk)	37 mg/m²/wk (28–38 mg/m²/wk)

Table 4

Toxicity in JCOG 9902-DI

	Arm A (n = 30)		Arm B (n = 30)	
Toxicity (Grade 3/4)	**Number of Patients**	**Percentage**	**Number of Patients**	**Percentage**
Leukocytopenia	15	50%	16	53%
Neutropenia	17	57%	26	87%
Anemia	13	43%	14	47%
Thrombocytopenia	8	27%	3	10%
Infection	2	7%	4	13%
Diarrhea	2	7%	3	10%
Hyponatremia	4	13%	6	20%
CRN elevation	1	3%	1	3%
Treatment-related death	1	3%	0	0%

CRN = creatinine.

Phase II Trial of Cisplatin, Irinotecan, and Etoposide Administered Weekly or Every 4 Weeks

JCOG 9511 showed that the IP regimen was significantly better than the EP regimen. However, because etoposide was still considered to be a key drug in the treatment of SCLC, a combination of these three drugs—irinotecan, cisplatin, and etoposide (IPE)—seemed to be a promising strategy for the treatment of ED-SCLC. The recommended weekly doses (JCOG 9507) and the dosages for each 4-week cycle (JCOG 9512) for IPE were decided using dose-escalation trials. For these reasons, a phase II trial of irinotecan, cisplatin, and etoposide administered weekly or every 4 weeks for ED-SCLC (JCOG 9902-DI) was performed.[19]

The purpose of this trial was to evaluate the toxicity and antitumor effect of the combination of irinotecan, cisplatin, and etoposide administered according to two schedules, weekly (arm A) and every 4 weeks (arm B), for the treatment of previously untreated ED-SCLC, and to select the appropriate arm for use in phase III trials. Patients were enrolled in this trial if they met the following criteria: (1) a histologic or cytological diagnosis of SCLC; (2) no prior treatment; (3) measurable disease; (4) extensive disease, defined as distant metastasis or contralateral hilar lymph node metastasis; (5) performance status of 0 to 2 on the ECOG scale; (6) a life expectancy of 3 months or longer; (7) age between 20 and 70 years; (8) adequate organ function; and (9) written informed consent.

The treatment schedule is shown in Figure 3. In arm A, cisplatin at 25 mg/m² was administered intravenously (IV) over 60 minutes on day 1 and at 1-week intervals for 9 weeks; irinotecan at 90 mg/m² was administered IV over 90 minutes on day 1 on weeks 1, 3, 5, 7, and 9; and etoposide at 60 mg/m² was administered by IV over 60 minutes on days 1 to 3 of weeks 2, 4, 6, and 8. Granulocyte colony-stimulating factor (G-CSF) was administered prophylactically on the days when a cytotoxic drug was not given, unless the white blood cell (WBC) count exceeded 10.0×10^9/L.

In arm B, cisplatin at 60 mg/m² was administered by IV over 60 minutes on day 1; irinotecan at 60 mg/m² was administered by IV over 90 minutes on days 1, 8, and 15; and etoposide at 50 mg/m² was administered by IV over 60 minutes on days 1 to 3. G-CSF was injected subcutaneously from day 5 until the day when the WBC count exceeded 10.0×10^9/L. This treatment was repeated every 4 weeks for a total of four cycles.

Patient characteristics are listed in Table 1. Between August 1999 and October 2000, 30 patients were entered in each arm. The last follow-up

examination was performed in February 2002. All enrolled patients were included in the toxicity, tumor response, and patient survival analyses. No differences in any of the listed characteristics were observed between the two arms.

Treatment delivery is listed in Table 2. Of the 30 patients in each arm, 22 (73%) and 21 (70%) patients in arms A and B, respectively, received full cycles of chemotherapy (nine cycles in arm A and four cycles in arm B). Therapy was stopped because of toxicity in four (13%) patients in arm A and in six (20%) patients in arm B. Therapy was stopped because of tumor progression in three (10%) patients in each arm. The need for treatment delay in arm A and treatment skipping in arm B, however, was significant. Only eight (27%) patients in arm A completed the treatment without delay, and only seven (23%) patients in arm B received all the planned doses. A total of 105 chemotherapy cycles were administered to 30 patients in arm B, but eight (8%) doses of irinotecan on day 8, and 33 (31%) doses of irinotecan on day 15 were omitted because of toxicity, according to criteria in the protocol.

The median total dosages of cisplatin and etoposide administered per patient were maintained at the planned dosage levels in both arms (Table 3). The median total dosage of irinotecan as a percentage of the scheduled dosage (the relative total dosage) was 100% in arm A, but only 78% in arm B, reflecting the doses of irinotecan that were skipped on days 8 and 15.

Dose intensity was evaluated in 29 patients in arm A and 28 patients in arm B (Table 3). The median relative dosage intensity was well maintained at a level of 80% or higher, except that of irinotecan in arm B (77%). The median actual dosage intensity of etoposide was 70 mg/m^2/wk in arm A and 37 mg/m^2/wk in arm B.

Address all correspondence to:
Nagahiro Saijo, MD, PhD
Division of Internal Medicine
National Cancer Center Hospital
Tsukiji 5-1-1
Chuo-ku, Tokyo 104-0045
Japan

Table 5

Antitumor Responses in JCOG 9902-DI

	Arm A (n = 30)		Arm B (n = 30)	
Responses	Number of Patients	Percentage	Number of Patients	Percentage
Complete	2	7%	5	17%
Partial	23	77%	18	60%
No change	1	3%	0	0%
Progressive disease	3	10%	4	13%
No effect	1	3%	3	10%
Response rate	83% (95% CI = 65%–94%)		77% (95% CI = 58%–90%)	

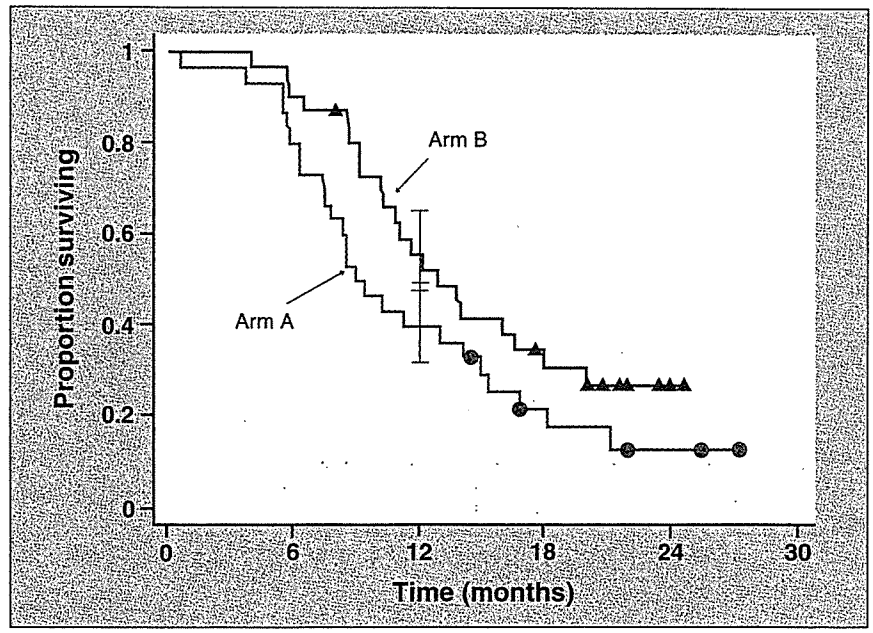

Figure 4: Study JCOG 9902-D1—Survival in treatment arms.

Toxicity was evaluated in all patients. The incidences of grade 3/4 neutropenia, anemia, thrombocytopenia, infection, and diarrhea in arm A were 57%, 43%, 27%, 7%, and 7%, respectively, and 87%, 47%, 10%, 13%, and 10%, respectively, in arm B. A treatment-related death occurred in one patient in arm A (Table 4).

Two complete responses (CRs) and 23 partial responses (PRs) were obtained in arm A, resulting in an overall clinical response rate of 83%, whereas five CRs and 18 PRs were obtained in arm B, resulting in an overall response rate of 77% (Table 5). The median time to survival and 1-year survival rate in arm A were 8.9

months and 40%, respectively, and 12.9 months and 57%, respectively, in arm B (Figure 4).

In this trial, the two IPE schedules were both effective against ED-SCLC and had an acceptable toxicity level. Arm B was adopted as the investigational arm in phase III trials.

Conclusion

The combination of cisplatin and irinotecan has become the new standard treatment for patients with ED-SCLC in Japan. However, SCLC is rarely cured, although the response rate has been improved and the survival time extended through the use of chemotherapy. Based on the results of JCOG 9511 and JCOG 9902-DI, a randomized trial comparing IP with IPE administered every 3 weeks in patients with previously untreated ED-SCLC is now being performed in Japan.

References

1. Kristjansen PE, Hansen HH: Management of small cell lung cancer: A Summary of the Third International Association for the Study of Lung Cancer Workshop on Small Cell Lung Cancer. *J Natl Cancer Inst* 82:263-266, 1990.

2. Pujol JL, Carestia L, Daures JP: Is there a case for cisplatin in the treatment of small-cell lung cancer?: A meta-analysis of randomized trials of a cisplatin-containing regimen vs a regimen without this alkylating agent. *Br J Cancer* 83:8-15, 2000.

3. Berghmans T, Paesmans M, Mascaux C, et al: A meta-analysis of the role of etoposide (VP-16) and cisplatin (CDDP) in small cell lung cancer (SCLC) with a methodology assessment (abstract). *Eur J Cancer* 35(suppl):S248, 1999.

4. Kunimoto T, Nitta K, Tanaka T, et al: Antitumor activity of 7-ethyl-10-[4-(1-piperidino)-1-piperidino] carbonyloxy-camptothecin, a novel water-soluble derivative of camptothecin, against murine tumors. Cancer Res 47:5944-5947, 1987.

5. Matsuzaki T, Yokokura T, Mutai M, et al: Inhibition of spontaneous and experimental metastasis by a new derivative of camptothecin, CPT-11, in mice. *Cancer Chemother Pharmacol* 21:308-312, 1988.

6. Tsuruo T, Matsuzaki T, Matsushita M, et al: Antitumor effect of CPT-11, a new derivative of camptothecin, against pleiotropic drug-resistant tumors in vitro and in vivo. *Cancer Chemother Pharmacol* 21:71-74, 1988.

7. Taguchi T, Wakui A, Hasegawa K, et al: Phase I clinical study of CPT-11. *Gan To Kagaku Ryoho* 17:115-120, 1990. (in Japanese)

8. Ohno R, Okada K, Masaoka T, et al: An early phase II study of CPT-11: A new derivative of camptothecin, for the treatment of leukemia and lymphoma. *J Clin Oncol* 8:1907-1912, 1990.

9. Negoro S, Fukuoka M, Niitani H, et al: Phase II study of CPT-11, new camptothecin derivative, in small cell lung cancer (SCLC) (abstract). *Proc Am Soc Clin Oncol* 10:241, 1991.

10. Masuda N, Fukuoka M, Kusunoki Y, et al: CPT-11: A new derivative of camptothecin for the treatment of refractory or relapsed small-cell lung cancer. *J Clin Oncol* 10:1225-1229, 1992.

11. Kudoh S, Takada M, Masuda N, et al: Enhanced anti-tumor efficacy of a combinatio of CPT-11, a new derivative of camptothecin and cisplatin against human lung tumor xe nografts. *Jpn J Cancer Res* 84:203-207, 1993

12. Masumoto N, Nakano S, Esaki T, et al Inhibition of cis-diamminedichloroplatinun (II)-induced DNA interstrand cross-link re moval by 7-ethyl-10-hydroxy-camptothecin i HST-1 human squamous-carcinoma cells. *In J Cancer* 62:70-75, 1995.

13. Pei XH, Nakanishi Y, Takayama K, e al: Effect of CPT-11 in combination with othe anticancer agents in lung cancer cells. *Anticancer Drugs* 8:231-237, 1997.

14. Masuda N, Fukuoka M, Kudoh S, et al Phase I and pharmacologic study of irinotecar in combination with cisplatin for advanced lung cancer. *Br J Cancer* 68:777-782, 1993.

15. Masuda N, Fukuoka M, Kudoh S, et al Phase I study of irinotecan and cisplatin with granulocyte colony-stimulating factor suppor for advanced non-small-cell lung cancer. *J Clin Oncol* 12:90-96, 1994.

16. Nakagawa K, Fukuoka M, Niitani H, e al: Phase II study of irinotecan (CPT-11) and cisplatin in patients with advanced non-small-cell lung cancer (NSCLC) (abstract). *Proc Am Soc Clin Oncol* 12:332, 1993.

17. Kudoh S, Fujiwara Y, Takada Y, et al: Phase II study of irinotecan combined with cisplatin in patients with previously untreated small-cell lung cancer. West Japan Lung Cancer Group. *J Clin Oncol* 16:1068-1074, 1998.

18. Noda K, Nishiwaki Y, Kawahara M, e al: Irinotecan plus cisplatin compared with etoposide plus cisplatin for extensive small-cell lung cancer. *N Engl J Med* 346:85-91, 2002.

19. Sekine I, Nishiwaki Y, Noda K, et al: Randomized phase II study of cisplatin, irinotecan and etoposide combinations administered weekly or every 4 weeks for extensive small-cell lung cancer (JCOG9902-DI). *Ann Oncol* 14:709-714, 2003.

Original article

Annals of Oncology 15: 45–50, 2004
DOI: 10.1093/annonc/mdh015

A phase II study of cisplatin and docetaxel administered as three consecutive weekly infusions for advanced non-small-cell lung cancer in elderly patients

Y. Ohe[1]*, S. Niho[2], R. Kakinuma[2], K. Kubota[2], H. Ohmatsu[2], K. Goto[2], H. Nokihara[1], H. Kunitoh[1], N. Saijo[1], H. Aono[3], K. Watanabe[3], M. Tango[4], A. Yokoyama[4] & Y. Nishiwaki[2]

[1]*Department of Internal Medicine, National Cancer Center Hospital, Tokyo;* [2]*Division of Thoracic Oncology, National Cancer Center Hospital, Kashiwa City;* [3]*Division of Respiratory Medicine, Yokohama Municipal Citizen's Hospital, Yokohama;* [4]*Department of Internal Medicine, Niigata Cancer Center Hospital, Niigata, Japan*

Received 20 April 2003; revised 17 August 2003; accepted 28 August 2003

Background: To evaluate the efficacy and safety of treatments for advanced non-small-cell lung cancer in elderly patients aged 75 years or older, we conducted a phase II study of cisplatin and docetaxel administered in three consecutive weekly infusions.

Patients and methods: The eligibility criteria for the study included the presence of chemotherapy-naive advanced non-small-cell lung cancer, age ≥75 years, Eastern Cooperative Oncology Group performance status of 0 or 1, a measurable lesion, adequate organ functions and signed informed consent. The chemotherapy regimen consisted of cisplatin (25 mg/m^2) and docetaxel (20 mg/m^2) on days 1, 8 and 15 every 4 weeks.

Results: Between February 2000 and March 2002, 34 elderly patients with non-small-cell lung cancer were enrolled in the study and 33 patients were treated. Two complete responses and 15 partial responses were obtained for an objective response rate of 52% in 33 treated patients. The median survival period was 15.8 months, and the 1-year survival rate was 64%. Toxicities were mild with no grade 4 toxicities. Only grade 3 leukopenia (6%), neutropenia (12%), anemia (3%), hyponatremia (3%) and nausea/vomiting (3%) were observed.

Conclusion: Cisplatin and docetaxel administered in three consecutive weekly infusions was safe and effective for the treatment of elderly patients with chemotherapy-naive non-small-cell lung cancer.

Key words: cisplatin, docetaxel, elderly patients, non-small-cell lung cancer, weekly administration

Introduction

Lung cancer is one of the most common carcinomas not only in Japan, but also in the United States and Europe. More than 55 000 patients die from lung cancer each year, and the mortality rate is still increasing in Japan [1, 2]. In particular, the number of elderly lung cancer patients is increasing in Japan [1, 2]. Surgery is the most effective curative treatment for early stage non-small-cell lung cancer (NSCLC); however, only 30% of patients with NSCLC receive a curative resection [3]. Cisplatin-based chemotherapy offers a survival benefit and symptom relief for patients with inoperable NSCLC [4]. However, we have demonstrated that classic standard cisplatin-based chemotherapy regimens such as cisplatin (80 mg/m^2) on day 1 with etoposide (100 mg/m^2) on days 1–3 or cisplatin (80 mg/m^2) on day 1 with vindesine (3 mg/m^2) on days 1 and 8 cause severe myelotoxicity in elderly NSCLC patients aged ≥75 years [5]. We used a very restricted eligibility criteria to select patients who could tolerate the cisplatin-based standard chemotherapy. Among 34 elderly patients, only 10 fitted the eligibility criteria. In spite of granulocyte colony-stimulating factor (G-CSF) support, nine of the 10 eligible patients experienced grade 4 neutropenia and six had infectious episodes [5]. Thus, we hypothesized that the recommended dose for elderly patients aged ≥75 years should be determined in a specific phase I study only for elderly patients.

Docetaxel has demonstrated antitumor activity in NSCLC patients with chemotherapy-naive lesions and tumor progression after receiving cisplatin-based regimens [6–10]. Docetaxel with cisplatin is one of the most promising chemotherapy regimens for NSCLC [11]. The commonly used dose and schedule of docetaxel is 60–100 mg/m^2 every 3 weeks; however, moderate to severe neutropenia is frequently observed [6–11]. Recent studies have shown that weekly administration of docetaxel produces a higher dose intensity and less myelotoxicity [12–14]. Thus, we conducted two independent phase I studies for elderly and non-elderly patients with NSCLC to determine the recommended dose for phase II studies and to evaluate the safety and efficacy of cisplatin and docetaxel administered as three consecutive weekly infusions in both non-elderly (≤74 years) and elderly (≥75 years) patients

**Correspondence to:* Dr Y. Ohe, Department of Internal Medicine, National Cancer Center Hospital, 5-1-1 Tsukiji, Chuo-ku, Tokyo 104-0045, Japan. Tel: +81-3-3542-2511; Fax: +81-3-3542-7006; E-mail: yohe@ncc.go.jp

[15]. Different recommended doses of docetaxel were obtained for non-elderly and elderly patients [15]. The recommended doses were 25 mg/m^2 cisplatin and 35 mg/m^2 docetaxel on days 1, 8 and 15 for non-elderly patients, and 25 mg/m^2 cisplatin and 20 mg/m^2 docetaxel on days 1, 8 and 15 for elderly patients.

Two phase II studies of cisplatin and docetaxel administered as three consecutive weekly infusions for non-elderly and elderly patients were conducted. The results of the phase II study for non-elderly patients with NSCLC have been reported elsewhere; the objective tumor response was 30% [95% confidence interval (CI) 15% to 46%] and the median survival time was 12.8 months [16]. Here, we report the promising results of a phase II study for elderly patients with NSCLC.

Patients and methods

Patient selection

Patients with histologically and/or cytologically documented NSCLC were eligible for the study. Each patient was required to meet the following criteria: clinical stage IV or IIIB (including only patients with no indications for curative radiotherapy), an Eastern Cooperative Oncology Group performance status (PS) of 0 or 1, age ≥75 years, no prior chemotherapy, measurable lesions, adequate hematological function [white blood cell count (WBC) 4000–12 000/mm^3; neutrophils ≥2000/mm^3; platelets ≥100 000/mm^3; hemaglobin ≥9.0 g/dl], adequate hepatic function (total bilirubin <1.1 mg/dl, aspartate aminotransferase and alanine aminotransferase <60 IU/l), and adequate renal function (creatinine ≤1.2 mg/dl, creatinine clearance ≥60 ml/min). Patients with active infection, severe heart disease, uncontrollable hypertension or diabetes mellitus, active concomitant malignancy and pleural and/or pericardial effusion requiring drainage were excluded. The study was approved by the Institutional Review Board at the National Cancer Center, Yokohama Municipal Citizen's Hospital and Niigata Cancer Center. Written informed consent was obtained from each patient.

Patient evaluation

The pretreatment evaluation consisted of complete blood cell count, differential count, routine chemistry measurements, a chest radiograph, a chest computed tomography (CT) scan, abdominal ultrasound or CT scan, whole-brain magnetic resonance imaging or CT scan, and an isotope bone scan. Complete blood cell count, differential, count and routine chemistry measurements were carried out at least twice a week during the first course of chemotherapy.

Treatment schedule

All patients were admitted to hospital during the first course of chemotherapy. Chemotherapy consisted of cisplatin (25 mg/m^2) on days 1, 8 and 15 and docetaxel (20 mg/m^2) on days 1, 8 and 15 every 4 weeks. Docetaxel was infused over 30 min with 16 mg dexamethasone and 3 mg granisetron administered just before the docetaxel infusion. Ninety minutes after the completion of the docetaxel infusion, 25 mg/m^2 cisplatin were administered over 15 min with 1500 ml normal saline over 3.5 h. The prophylactic administration of G-CSF was not permitted. Administration of G-CSF was permitted in patients with grade 4 neutropenia and/or leukopenia or grade 3 febrile neutropenia. The administration of both cisplatin and docetaxel were skipped on day 8 and/or day 15 if the patients met the following criteria: WBC <2000/mm^3 and/or platelets <50 000/mm^3. No dose modifications were carried out on days 8 and/or day 15 of the cisplatin and docetaxel administrations. Treatment was carried out for at least two courses, unless unacceptable toxicity or disease progression occurred.

Response and toxicity evaluation

The patients' responses were evaluated according to the World Health Organization criteria [17]. A complete response (CR) was defined as the complete disappearance of all clinically detectable tumors for at least 4 weeks. A partial response (PR) was defined as a reduction of ≥50% in the product of the largest perpendicular diameters of one or more clearly measurable lesions or as a >50% reduction in evaluable malignant disease lasting for >4 weeks with no new areas of malignant disease. No change included: the regression of indicator lesions that were insufficient to meet the criteria for PR, <25% increase in any measurable lesion and no new lesions of malignant disease. Progressive disease was defined as an increase in any measurable lesion by >25% or a new lesion of malignant disease. Survival times from the start of treatment were calculated using the Kaplan–Meier method. The toxicity grading criteria of the Japan Clinical Oncology Group (JCOG) were used to evaluate toxicity [18]. Most detailed gradings for individual organ toxicity in the JCOG Toxicity Criteria are identical to those of the National Cancer Institute Common Toxicity Criteria proposed in 1988. The only differences in the definitions used in the present study were that neutrophils were used instead of granulocytes and the definitions for nausea and vomiting were combined.

Statistical analysis

According to the minimax two-stage phase II study design by Simon [19], the treatment program was designed to refuse response rates of 20% and to provide a significance level of 0.05 with a statistical power of 80% in assessing the activity of the regimen as a 40% response rate. The upper limit for first-stage drug rejection was four responses among 18 evaluable patients; the upper limit of second-stage rejection was 10 responses among 33 evaluable patients. Overall survival was defined as the interval between enrolment in this study and death or the last follow-up visit. Median overall survival was estimated using the Kaplan–Meier analysis method [20].

Results

Patient characteristics

Between February 2000 and March 2002, 34 elderly patients with NSCLC were enrolled and 33 were treated in this study (Table 1). One patient did not receive the protocol treatment because the PS of the patient decreased before the start of the treatment and the patient no longer met the eligibility criteria. All treated patients were assessed for response, survival and toxicity. The median age of the patients was 77 years (range 75–86). The gender, PS and histology of the patients were as follows: 26 males, seven females; seven patients with PS 0, 26 patients with PS 1; 20 patients with adenocarcinoma, nine patients with squamous cell carcinoma, three patients with large cell carcinoma and one patient with NSCLC. Twenty-four patients had no prior treatment, five patients had undergone surgery, three patients had received radiotherapy for brain and/or bone metastases, and one patient had undergone both surgery and radiotherapy as prior treatments.

Treatment received and dose intensity

The total number of treatment cycles was 101 and the median was 3 (range 1–15). Two patients received only one course because of a decrease in their PS. Of the 33 treated patients, 12 patients received two courses, 13 received three and six received four or more. One patient received 15 courses; however, he received