VOLUME 25 · NUMBER 1 · JANUARY 1 2007

JOURNAL OF CLINICAL ONCOLOGY

ORIGINAL REPORT

Pharmacokinetics of Gemcitabine in Japanese Cancer Patients: The Impact of a Cytidine Deaminase Polymorphism

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Submitted March 23, 2006; accepted August 25, 2006.

Supported by the Program for the Promotion of Fundamental Studies in Health Sciences (Grant No. MPJ6 and 05-25), and the Health and Labour Sciences Research Grant on Human Genome and Tissue Engineering (Grant No. H16-Genome-008) from the Ministry of Health, Labour, and Welfare of Japan.

Presented in part at the 41st Annual Meeting of the American Society of Clinical Oncology, May 13-17, 2005, Orlando, FL, and at the 13th Annual Meeting of the North American Society for the Study of Xenobiotics, October 22-27, 2005, Maui, HI.

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

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0732-183X/07/2501-32/$20.00

DOI: 10.1200/JCO.2006.06.7405

ABSTRACT

Purpose
Gemcitabine is rapidly metabolized to its inactive metabolite, 2′,2′-difluorodeoxyuridine (dFdU), by cytidine deaminase (CDA). We previously reported that a patient with homozygous 208A alleles of *CDA* showed severe adverse reactions with an increase in gemcitabine plasma level. This study extended the investigation of the effects of *CDA* genetic polymorphisms on gemcitabine pharmacokinetics and toxicities.

Patients and Methods
Genotyping of *CDA* was performed by a direct sequencing of DNA obtained from the peripheral blood of Japanese gemcitabine-naïve cancer patients (n = 256). The patients recruited to the association study received a 30-minute intravenous infusion of gemcitabine at a dose of either 800 or 1,000 mg/m^2, and eight blood samples were periodically collected (n = 250). Plasma levels of gemcitabine and dFdU were measured by high-performance liquid chromatography. Plasma CDA activities toward cytidine and gemcitabine were also measured (n = 121).

Results
Twenty-six genetic variations, including 14 novel ones and two known nonsynonymous single nucleotide polymorphisms (SNPs), were detected. Haplotypes harboring the nonsynonymous SNPs 79A>C (Lys27Gln) and 208G>A (Ala70Thr) were designated **2* and **3*, respectively. The allelic frequencies of the two SNPs were 0.207 and 0.037, respectively. Pharmacokinetic parameters of gemcitabine and plasma CDA activities significantly depended on the number of haplotype **3*. Haplotype **3* was also associated with increased incidences of grade 3 or higher neutropenia in the patients who were coadministered fluorouracil, cisplatin, or carboplatin. Haplotype **2* showed no significant effect on gemcitabine pharmacokinetics.

Conclusion
Haplotype **3* harboring a nonsynonymous SNP, 208G>A (Ala70Thr), decreased clearance of gemcitabine, and increased incidences of neutropenia when patients were coadministered platinum-containing drugs or fluorouracil.

J Clin Oncol 25:32-42. © 2007 by American Society of Clinical Oncology

INTRODUCTION

Gemcitabine (2′,2′-difluorodeoxycytidine) is a nucleoside anticancer drug that has a broad spectrum of antitumor activity against various solid tumors, such as non–small-cell lung cancer and pancreatic cancer.[1] In a randomized clinical trial, gemcitabine was confirmed to provide a survival advantage over fluorouracil in addition to symptom-relieving benefits in patients with advanced pancreatic cancer.[2] On the basis of these results, gemcitabine has generally been accepted as a standard chemotherapeutic agent for advanced pancreatic cancer.

Gemcitabine is transported into cells by concentrative and equilibrative nucleoside transporters,[3-8] where it is phosphorylated to its monophosphate form by deoxycytidine kinase. Gemcitabine triphosphate, an active form of gemcitabine, is incorporated into an elongating DNA strand, and is followed by the addition of another deoxynucleotide that leads to the halt of DNA synthesis.[9,10] Another mode of action in solid tumors, associated with the inhibition of ribonucleotide reductase, has also been suggested.[11]

Gemcitabine is rapidly metabolized to an inactive metabolite, 2′,2′-difluorodeoxyuridine (dFdU)

Table 1. *CDA* Haplotypes Estimated in This Study

Region		5'-Flanking			Exon 1 (5'-UTR)			Exon 1	Intron 1	Exon 2		Intron 2		
SNP ID		CDA001	CDA002	CDA003	CDA004	CDA005	CDA007	CDA009	CDA010	CDA011	CDA012	CDA014	CDA016	CDA017
Nucleotide change		−451C>T	−205C>G	−182G>A	−116G>A	−92A>G	−33_−31 delC	79A>C	IVS1+37 G>A	208G>A	210T>C	IVS2 +87_+88 insTCAT	IVS2+242 A>G	IVS2+296 T>A
Amino acid change								Lys27Gln		Ala70Thr	Ala70Ala			
Haplotypes														
*1	*1a													
	*1b													
	*1c													
	*1d													
	*1e													
	*1f													
	*1g													
	*1h													
	*1i													
	*1j													
	*1k													
	*1l													
	*1m													
	*1n													
	Other *1													
*2	*2a													
	*2b													
	*2c													
	*2d													
	Other *2													
*3	*3a													
	*3b													

(continued on next page)

NOTE. The haplotypes were described as a number plus a small alphabetical letter. Four single nucleotide polymorphisms (SNPs) (CDA006, 008, 013, 015) were found only in the very rare ambiguous *1 haplotypes. Since these ambiguous haplotypes were grouped and described as "Other *1" in this table, the four SNPs are not shown in the row of nuclotide change. White, major allele; gray, minor allele.

by cytidine deaminase (CDA),[9] and most of an administered dose is recovered as dFdU in the urine.[12] CDA is expressed at varying levels in the human tissues,[13] and the rapid clearance of gemcitabine can be attributed to its plentiful occurrence in the liver.[14] Two single nucleotide polymorphisms (SNPs), 79A>C (Lys27Gln) and 435T>C (Thr145Thr), have been discovered in *CDA*, the CDA-encoding gene in humans.[15,16] The 79A>C SNP reportedly reduces the deamination activity (maximum velocity/Km) toward 1-beta-D-arabinofuranosyl cytosine (cytarabine),[15] and increases Km toward gemcitabine,[17] in vitro. A recently discovered third SNP, 208G>A (Ala70Thr) displayed a decrease in deamination activity of 60% for cytidine and 68% for cytarabine when introduced into a *CDA*-null yeast strain.[18]

Toxicity of gemcitabine is generally mild,[19,20] but unpredictable severe toxicities such as myelosuppression are occasionally experienced.[21,22] Our previous case report described a patient with homozygous 208A alleles of the *CDA* gene who showed severe adverse reactions with increased plasma gemcitabine levels.[23] In addition, there has been controversy over the relationship between cellular CDA activity and the clinical effects of cytarabine.[24-27] This study examined the relationship between *CDA* polymorphisms, and the pharmacokinetics of gemcitabine, plasma CDA activity, or adverse reactions in Japanese cancer patients.

Patients and Methods

Gemcitabine and dFdU for analytic standards were supplied by Eli Lilly Japan K.K. (Kobe, Japan). Tetrahydrouridine, 3'-deoxy-3'-fluoro-thymidine (3'-dFT), cytidine and uridine (Sigma-Aldrich Chemical Co, St Louis, MO) were purchased. All other chemicals were of highest grade available.

Patients

The participants in this study consisted of 256 Japanese patients with carcinoma, including six patients described in a previous report,[23] at the National Cancer Center Hospital (Tokyo, Japan) and National Cancer Center Hospital East (Kashiwa, Japan). Two hundred fifty-one patients received a 30-minute intravenous infusion of gemcitabine at a dose of either 800 or 1,000 mg/m^2, and five patients received a fixed dose-rate (10 mg/m^2/min) infusion at a dose between 1,000 and 1,500 mg/m^2. The eligibility criteria for the study were as previously reported.[23] The ethics committees of the National Cancer Center and the National Institutes of Health Sciences approved this study. Written informed consent was obtained from each participant.

Table 1. *CDA* Haplotypes Estimated in This Study (continued)

Intron 3					Exon 4	Exon 4 (3'-UTR)					
CDA018	CDA019	CDA020	CDA021	CDA022	CDA023	CDA024	CDA025	CDA026			
IVS3+71 T>C	IVS3 −194_−193 insAlu	IVS3-56 G>A	IVS3-36 G>A	IVS3-23 C>T	435C>T	510 (*69) G>T	637_638 (*196_*197) insC	676 (*235) A>G			
					Thr145Thr						
									No.	Frequency	
									175	0.342	
									63	0.123	
									52	0.102	
									17	0.033	
									13	0.025	
									12	0.023	
									12	0.023	
									11	0.021	0.756
									8	0.016	
									5	0.010	
									4	0.008	
									4	0.008	
									2	0.004	
									1	0.002	
									8	0.016	
									84	0.164	
									11	0.021	
									5	0.010	0.207
									3	0.006	
									3	0.006	
									18	0.035	0.037
									1	0.002	
									512	1.000	1.000

Monitoring and Toxicities

A complete medical history and data on physical examinations were recorded before the gemcitabine therapy. CBC and platelet counts, as well as blood chemistry, were measured once a week during the first 2 months of gemcitabine treatment. Toxicities were graded according to the National Cancer Institute Common Toxicity Criteria, version 2.

DNA Sequencing

All four exons and the 5'-upstream region (approximately 800 base pairs [bp] from the translation initiation codon) of *CDA* were amplified from 100 ng of DNA extracted from peripheral blood, and sequenced along both strands. Polymerase chain reaction (PCR) primers[23] and sequencing and PCR conditions[28] were described previously. For detection of an approximately 300-bp Alu insertion (IVS3-194_-193insAlu), PCR was performed using a specific primer set (5'- TTGTCATAGCAGAAGGAGGTT-3' and 5'- TCAG CTCTCCACACCATAAGG-3') and 100 ng of DNA as a template. Then, sizes of the amplified fragments were determined by 1% agarose gel electrophoresis. NT_004610.17 (GenBank, National Center for Biotechnology Information, Bethesda, MD) was used as the reference sequence.

Linkage Disequilibrium and Haplotype Analyses

Hardy-Weinberg equilibrium and linkage disequilibrium (LD) analyses were performed by SNPAlyze software (Dynacom Co, Yokohama, Japan). All of the detected variations were found to be in Hardy-Weinberg equilibrium ($P \geq .05$), except for the SNP IVS1+37G>A ($P = .002$). Some of the haplotypes were unambiguously assigned from subjects with homozygous variations at all sites or a heterozygous variation at only one site. The diplotype configurations (a combination of haplotypes) were separately inferred by LDSUPPORT software,[29] which determines the posterior probability distribution of the diplotype configuration for each subject based on the estimated haplotype frequencies. The diplotype configurations of all but 11 subjects were inferred with probability of more than 0.93. All haplotypes inferred in single subjects were gathered as the groups "Other *1" and "Other *2" in Table 1.

Pharmacokinetic Study

Five patients with fixed dose-rate infusion and one patient with interruption of infusion for more than 15 minutes were excluded from the pharmacokinetic analysis described herein. Heparinized blood was collected before administration of gemcitabine and used to measure plasma CDA activity. Five milliliters of heparinized blood was also sampled for pharmacokinetic analysis before the first gemcitabine administration, and at 0, 15, 30, 60, 90, 120, and 240 minutes after the termination of the infusion. Fifty microliters of 1% tetrahydrouridine was immediately added to these samples to prevent ex vivo deamination. Plasma levels of gemcitabine and dFdU were determined using the high-performance liquid chromatography method previously reported.[23] The area under the curve (AUC) and mean residence time from 0 to infinity, peak concentration (C_{max}), clearance (CL/m^2) and distribution volume based on the terminal phase (Vz/m^2) were calculated using WINNonlin (Scientific Consultant, Apex, NC) version 4.01 (Pharsight Corporation, Mountain View,

CA). AUC and C_{max} were corrected for dose, assuming that all patients received 1,000 mg/m^2 of gemcitabine.

CDA Activities in Plasma

Determination of CDA activities was performed using the method by Richards et al[30] with slight modifications (modifications are as follows: gemcitabine was used as a substrate as well as cytidine, internal standards for analysis [3'-dFT for gemcitabine or dFdU for cytidine] were added to the mixtures at the beginning of the reaction, and high-performance liquid chromatography was used for detection of reaction products). CDA activity was expressed by unit, and one unit of enzyme activity was defined as the concentration that produces 0.1 nmol of dFdU or uridine per minute per milliliter of plasma.[30]

Statistical Analysis

Kruskal-Wallis, Mann-Whitney, and Pearson's correlation tests were performed using the JMP software (SAS Institute Inc, Cary, NC). Two ordinally scaled categoric data were subjected to χ^2 analysis for a correlation test. A significance level of .05 was applied to all two-tailed and correlation tests. Multiplicity was adjusted by the false-discovery rate,[31] if necessary.

RESULTS

Genetic Variations and Haplotype Structures of CDA

Twenty-six (14 novel) genetic variations were detected in the 256 Japanese cancer patients enrolled onto this study (Table 2). Three of the novel variations were found in the 5'-untranslated region, one in exon 2, three in the 3'-untranslated region and seven in the introns. Three known SNPs in the coding region of *CDA* were also detected. Among these, the nonsynonymous SNPs, 79A>C (Lys27Gln) and 208G>A (Ala70Thr), exhibited allelic frequencies of 0.207 and 0.037 (Table 2), respectively, and they were comparable to those reported previously.[18] One patient was found to be homozygous for the 208A polymorphism. A novel insertion of an approximately 320-bp Alu element (IVS3-194_-193insAlu) was newly found in intron 3.

The detected variations were used to analyze LD (Fig 1). Four novel variations (IVS3-56G>A, IVS3-36G>A, IVS3-23C>T and

Table 2. Variations of the *CDA* Gene Found

SNP ID				Position				
This Study	NCBI (dbSNP)	JSNP	Location	NT_004610.17	From the Translational Initiation Site or From the Nearest Exon	Nucleotide Change and Flanking Sequences (5' to 3')	Amino Acid Change	Allele Frequency
MPJ6_CDA001	rs532545	IMS-JST008767	5'-Flanking	3739514	−451‡	TGCCTCCTGCCT**C/T**GGGATGCCGCAG		0.199
MPJ6_CDA002	rs603412	IMS-JST008768	5'-Flanking	3739760	−205‡	CACACGTAGGCA**C/G**TGTCTTACACCA		0.266
MPJ6_CDA003	rs12726436		5'-Flanking	3739783	−182‡	CACACCTGCTGA**G/A**TCCAAACCATGG		0.061
MPJ6_CDA004*			Exon 1 (5'-UTR)	3739849	−116‡	CTGAGAGCCTGC**G/A**GTCTGGCTGCAG		0.059
MPJ6_CDA005	rs602950		Exon 1 (5'-UTR)	3739873	−92‡	GGGACACACCCA**A/G**GGGGAGGAGCTG		0.205
MPJ6_CDA006*			Exon 1 (5'-UTR)	3739884	−81‡	AAGGGGAGGAGC**T/C**GCAATCGTGTCT		0.002
MPJ6_CDA007	rs3215400	IMS-JST076939	Exon 1 (5'-UTR)	3739934	−33_−31‡	GCTCCTGTTTCC**C/-**GCTGCTCTGCTG		0.451
MPJ6_CDA008*			Exon 1 (5'-UTR)	3739957	−8‡	TGCCTGCCCGGG**G/A**TACCAACATGGC		0.002
MPJ6_CDA009†	rs2072671	IMS-JST008769	Exon 1	3740043	79‡	CAGGAGGCCAAG**A/C**AGTCAGCCTACT	Lys27Gln	0.207
MPJ6_CDA010	rs12059454		Intron 1	3740155	IVS1+37	CCCAGCCCAGCAG**G/A**CCTGGGTGGTGG		0.184
MPJ6_CDA011†			Exon 2	3755816	208‡	GCTGAACGGACC**G/A**CTATCCAGAAGG	Ala70Thr	0.037
MPJ6_CDA012*			Exon 2	3755818	210‡	TGAACGGACCGC**T/C**ATCCAGAAGGCC	Ala70Ala	0.004
MPJ6_CDA013*			Intron 2	3755932	IVS2+58	GCCAACATCTTCC**C/T**TTACACATATTA		0.002
MPJ6_CDA014*			Intron 2	3755961_3755962	IVS2+87_+88	TCATTCATTCAT**-/TCAT**CTGACATATGTT		0.135
MPJ6_CDA015*			Intron 2	3756043	IVS2+169	ATAAGGAGATAA**A/G**TAAGAAATGGAG		0.002
MPJ6_CDA016	rs10916825		Intron 2	3756116	IVS2+242	CATACAAGGGCC**A/G**GTATGCCCCTGT		0.289
MPJ6_CDA017	rs818194		Intron 2	3756170	IVS2+296	GTCCTACAAGATT**/A**TAACAGAAAGGC		0.217
MPJ6_CDA018	rs3738130	IMS-JST083844	Intron 3	3764805	IVS3+71	AGCCACGCCAAG**T/C**TGCAGGCATGGC		0.053
MPJ6_CDA019*			Intron 3	3769093_3769094	IVS3-194_−193	CTGTTCAGTTTC**-/(Alu)**§ACAGCATTCTTT		0.293
MPJ6_CDA020*			Intron 3	3769231	IVS3-56	CAGACCCAGTCC**G/A**TCTCAGCCCCCT		0.293
MPJ6_CDA021*			Intron 3	3769251	IVS3-36	CCCCTCAGCCAC**G/A**CTGTGTCTCTCA		0.293
MPJ6_CDA022*			Intron 3	3769264	IVS3-23	CTGTGTCTCTCA**C/T**GCCAGCTTTGCC		0.293
MPJ6_CDA023†	rs17846527		Exon 4	3769397	435‡	CCTGCAGAAGAC**C/T**CAGTGACAGCCA	Thr145Thr	0.293
MPJ6_CDA024*			Exon 4 (3'-UTR)	3769472	510 (*69)‡	CTCACAGCCCTG**G/T**GGACACCTGCCC		0.002
MPJ6_CDA025*			Exon 4 (3'-UTR)	3769599_3769600	637_638 (*196_197)‡	ACCGCCGCCCCC**-/C**TGCCCCACCTTT		0.293
MPJ6_CDA026*			Exon 4 (3'-UTR)	3769638	676 (*235)‡	GGGCCCTCTTTC**A/G**AAGTCCAGCCTA		0.010

*Novel variations detected in this study.
†Yue et al.[18]
‡A of the translation initiation codon ATG is numbered 1, and the number with * in parentheses indicates the position from the termination codon TGA.
§The sequence of the Alu insertion was as follows: 5' - (T)nGAGACGGAGTCTCGCTGTCGCCCAGGCTGGAGTGCAGTGGCGCAATCTCGGCTCACTGCAGGCTCCG CCCCCTGGGGTTCACGCCATTCTCCTGCCTCAGCCTCCCGAGTAGCTGGGACTACAGGCGCCCGCCACCTCGCCCGGCTAATTTTTTGTATTTTTAGTAGAGACGGGGTT TCACCGTGTTAGCCAGGATGGTCTCGATCTCCTGACCTCGTGATCCGCCCGCCTCGGCCTCCCAAAGTGCTGGGATTACAGGCGTGAGCCACCGCGCCCGGCCCACTG TTCAGTTTC-3' (n = approximately 25).

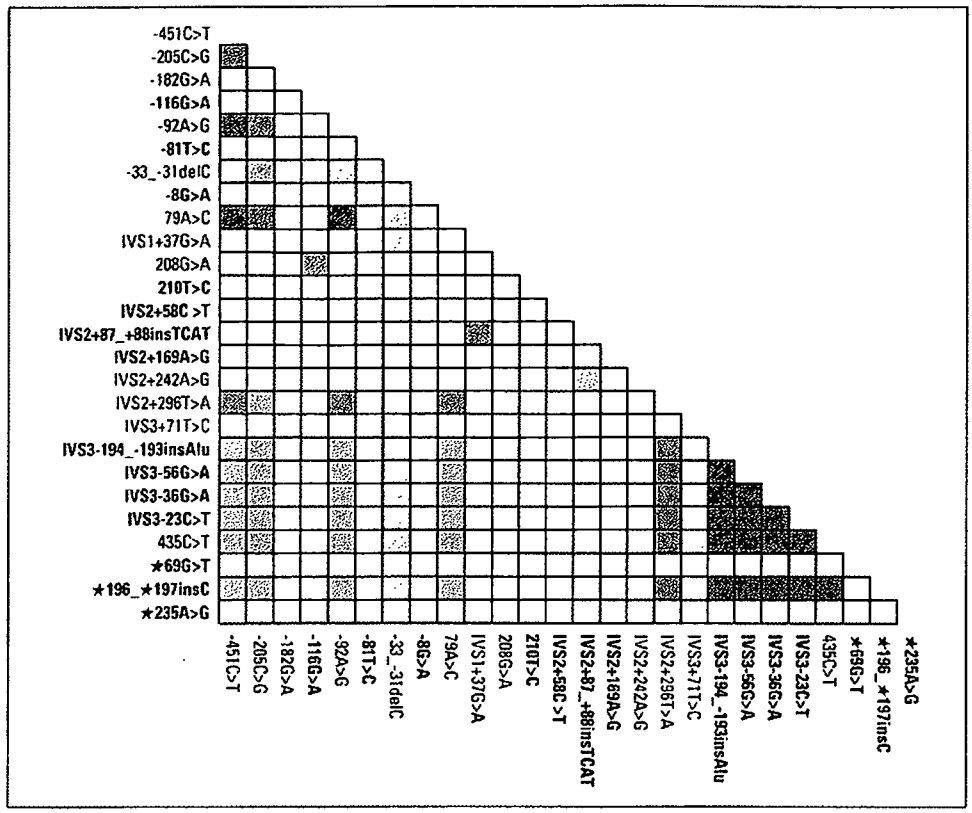

Fig 1. Linkage disequilibrium (LD) among 26 *CDA* variations. Pairwise LD as r^2 (from 0 to 1) is expressed as 10-graded blue color. The density of the blue color increases with higher linkage rates.

*196_*197insC), the Alu element insertion and a known SNP 435C>T (Thr145Thr) showed complete linkage (Fig 1) with a frequency of 0.293. Strong LD ($r^2 \geq 0.93$) was also observed among SNPs −451C>T, −92A>G, and 79A>C. Note that moderate linkages ($r^2 \geq 0.42$) were observed between the two completely and strongly linked groups (Fig 1). Because relatively close linkages were observed throughout the entire *CDA* gene spanning approximately 30 kb, the *CDA* haplotypes were analyzed as one LD block.

The haplotypes determined/inferred in this study are summarized in Table 1. Haplotypes without amino acid changes were defined as the **1* group. These harboring the nonsynonymous SNPs 79A>C and 208G>A were designated **2* and **3*, respectively. The most frequent haplotype was **1a* (frequency, 0.342), followed by **2a* (0.164), **1b* (0.123), and **1c* (0.102).

Effects of Patient Background Factors on Gemcitabine Pharmacokinetics

Characteristics of the 250 patients recruited for the pharmacokinetic study are shown in Table 3. As previously reported, the patient who was homozygous for 208A showed extraordinarily high gemcitabine and low dFdU plasma concentrations.[23] Therefore, this patient was excluded when effects of patient background factors on the pharmacokinetic parameters of gemcitabine were analyzed.

The effects of age and sex on pharmacokinetic parameters are summarized in Table 4. Vz/m^2 was significantly higher in males than in females, even after adjustments for their body surface areas (Mann-Whitney $P = .0031$). The C_{max}, AUC, CL/m^2, and Vz/m^2 of gemcitabine showed significant correlations with age ($P < .0001$ for all parameters). Values of any clinical tests, including creatinine concentration, did not correlate with pharmacokinetic parameters of gemcitabine. Although approximately 30% of patients in this study underwent combined chemotherapy, no clinically significant effects of coadministered drugs on pharmacokinetic parameter values of gemcitabine were detected.

Effects of CDA Genetic Polymorphisms on Gemcitabine Pharmacokinetics

Because age and sex were unbiasedly distributed among the patients, with the various genotypes compared in the following analysis (data not shown), the 250 patients were not further stratified.

After careful examination, the data did not identify any **1*, **2*, or **3* subtypes that showed statistically significant differences from each major subtype within the three groups (Table 5; unpublished data). Therefore, each subtype was combined into one group (the **1*, **2*, or **3* group) to investigate the association between pharmacokinetic parameters and genetic groups.

The relationships between the diplotype groups and the pharmacokinetic parameters of gemcitabine are shown in Figure 2 and summarized in Table 6. The data clearly showed a haplotype **3*–dependent decrease in clearance and increases in C_{max} and AUC values (χ^2 trend $P < .0001$ for all parameters). The values of C_{max}, AUC, and CL/m^2 observed in the patient bearing a homozygous 208G>A (**3/*3*) were two-fold, five-fold, and one-fifth of the means of the **1/*1* group, respectively (Table 6). In contrast, the pharmacokinetic parameters of gemcitabine except for mean residence time (data not shown) were not significantly influenced by the haplotype **2*.

Table 3. Characteristics of Patients Recruited to Pharmacokinetic Studies (N = 250)

Characteristic	
Sex	
Male	165
Female	85
Age, years	
Mean	62.6
Range	32-80
SD	9.2
Body surface area, m^2	
Mean	1.57
Range	1.18-1.99
SD	0.17
Weight, kg	
Mean	54.8
Range	34.4-80.3
SD	9.7
Performance status	
0	122
1	118
2	10
Primary tumor	
Pancreas	205
Lung	38
Mesothelium	7
Dose, mg/m^2	
1,000	246
800	4
Regimen	
Gemcitabine alone	180
Gemcitabine-based combination	70
Cisplatin	30
Carboplatin	16
Fluorouracil	14
Vinorelbine ditartrate	10
Previous treatment	
None	134
Surgery	66
Radiation	74
Chemotherapy	65

*Effect of Haplotypes *2 and *3 on Plasma CDA Activity*

Plasma CDA activities were measured in 121 patients of the 250 patients in this study. One patient in the **1/*2* group who showed extremely high plasma CDA activities to both gemcitabine and cytidine (43.04 and 29.04 units, respectively; far higher than the 99% upper confidence limits of plasma CDA activities for the **1/*2* group) was excluded as an outlier from the following statistical analysis, although his pharmacokinetic parameters were quite normal.

Haplotype **2* failed to show any significant effects on the plasma CDA activities toward both gemcitabine and cytidine. On the other hand, activity decreased depending on the number of haplotype **3* (Table 6; Fig 3). The plasma CDA activities in the homozygous **3* (208A) patient were 12% (gemcitabine) and 25% (cytidine) of the median activities for the **1/*1* patients. As shown in Figure 4, a statistically significant correlation between the plasma CDA activity toward gemcitabine and the AUC values of gemcitabine was observed ($r = -0.30$; $P = .0009$). However, the correlations were not remarkable.

*Effect of Haplotype *3 on Toxicities*

Then, associations of haplotype **3* with toxicities were analyzed. Nadir grades of neutrophil counts were compared between the patient groups with and without haplotype **3* under the individual therapeutic regimens. As shown in Table 7, there were no significant differences in incidences of grade 3 or higher neutropenia between the two groups under the gemcitabine monotherapy. However, when gemcitabine was administered with carboplatin, cisplatin, or fluorouracil, grade 3 or higher neutropenia was more frequently observed in the haplotype **3*–bearing group than in the group without haplotype **3*. The increases in incidences were statistically significant. AUC values were also increased in the group with haplotype **3* under concomitant therapeutic regimen as under the monotherapy.

DISCUSSION

The pharmacokinetic parameters summarized in Table 4 showed great similarity to those obtained with adult American patients.[32] The age-dependent decrease in gemcitabine clearance in Japanese patients in this study is in agreement with the description for Gemzar injections (Eli Lilly Japan K.K.), which is based on a population pharmacokinetic study performed outside Japan. The main route of gemcitabine elimination is its metabolism into dFdU, and there was no correlation between plasma creatinine level and gemcitabine clearance. Therefore, the aging effect on gemcitabine clearance is likely to result from a decrease in distribution volume or liver function. It is

Table 4. Effects of Patient Background Factors on Pharmacokinetic Parameters of Gemcitabine

Factor	C_{max} (μg/mL) Median	C_{max} (μg/mL) 1/4-3/4 Quantiles	AUC (hr · μg/mL) Median	AUC (hr · μg/mL) 1/4-3/4 Quantiles	CL/m^2 (L/hr/m^2) Median	CL/m^2 (L/hr/m^2) 1/4-3/4 Quantiles	Vz/m^2 (L/m^2) Median	Vz/m^2 (L/m^2) 1/4-3/4 Quantiles
Sex								
Male	23.1	18.4-26.1	9.9	8.6-11.8	100.3	83.7-115.9	42.4*	35.13-52.0
Female	24.0	19.8-28.8	10.2	9.0-11.5	97.6	86.1-111.2	38.7	32.7-43.5
Mann-Whitney *U* test	NS		NS		NS		$P < .005$	
Age								
Spearman r	0.32		0.39		−0.39		−0.39	
P value	< .0001		< .0001		< .0001		< .0001	

Abbreviations: C_{max}, peak concentration; AUC, area under the curve; CL/m^2, clearance; Vz/m^2, distribution volume based on the terminal phase.
*Significantly different from the value for female (Mann-Whitney *U* test $P = .0031$).

Table 5. Pharmacokinetic Parameters of Gemcitabine in Patients With Various *CDA* Diplotypes

Diplotype	No. of Patients	Median Gemcitabine PK Parameters				
		C_{max} (μg/mL)	AUC (hr · μg/mL)	CL/m^2 (L/hr/m^2)	MRT (hours)	AUC Ratio (dFdU/gemcitabine)
*1a/*1a	30	22.40	10.54	94.24	0.37	8.86
*1a/*1b	17	22.75	10.08	97.91	0.35	9.08
*1b/*1b	6	20.81	9.19	108.60	0.36	9.19
P value*		0.82	0.40	0.59	0.97	0.83
*1a/*1c	23	23.23	10.87	94.31	0.35	8.73
*1c/*1c	1	25.84	16.62	60.16	0.55	8.40
P value*		0.77	0.57	0.94	0.97	0.83
*1a/*1d	7	22.05	9.07	108.30	0.36	9.04
*1d/*1d	1	26.43	9.99	100.10	0.31	7.70
P value*		0.82	0.45	0.90	0.86	0.57
*2a/*2a	8	23.94	9.34	107.20	0.33	9.70
*2a/*2b	4	23.02	9.78	100.13	0.38	8.59
*2a/*2c	2	21.50	9.22	111.63	0.36	10.99
P value†		0.66	0.98	0.76	0.077	0.46

Abbreviations: PK, pharmacokinetics; C_{max}, peak concentration; AUC, area under the curve; CL/m^2, clearance; MRT, mean residence time; dFdU, 2',2'-difluorodeoxyuridine.

*P value of a correlation test among *1a/*1a, *1a/(*1b, *1c, or *1d), and (*1b, *1c, or *1d)/(*1b, *1c, or *1d). Multiplicity is adjusted by false-discovery rate.

†P value of a Kruskal-Wallis test among *2a/*2a, *2a/*2b, and *2a/*2c.

also indicated on the label that the elimination half-life of gemcitabine was longer in females than in males in a population pharmacokinetic study using 45 Japanese non–small-cell lung cancer patients. The present study did not reveal any significant sex-based difference in clearance. However, the distribution volume was significantly smaller in females than in males.

Human CDA is involved in the salvaging of pyrimidines,[33,34] and plays a key role in detoxifying gemcitabine. Although the activities of 27Gln or 70Thr variant (the products of 79A>C or 208G>A) toward cytidine and cytarabine were reported to be lower than those of the "prototype" in a yeast expression system,[18] the decreased CDA activity in patients bearing these SNPs has not been reported. Kreis et al[35] reported that the response of leukemic patients to cytarabine correlated with the phenotype of CDA deamination determined based on the ratio of plasma concentrations of a cytarabine metabolite and cytarabine.[35] They reported that 70% of subjects were slow metabolizers. However, the relationship between genetic polymorphisms and phenotypes remained to be clarified.

In our study, the haplotype *2 harboring 79C (27Gln) did not show clear effects on the AUC and CL/m^2 values. In contrast, the 208A (Thr70, *3) -dependent decreases in gemcitabine clearance and plasma CDA activities were clearly demonstrated in this study. These results suggest that the *CDA* variant loses its in vivo deamination activities toward gemcitabine considerably. Moreover, the decreased plasma CDA activities toward gemcitabine and cytidine ex vivo also strongly suggest that the reduced enzymatic activity was caused by the genetic variation.

In the monotherapy group, the increased AUC in the patient with haplotype *3 did not clearly augment the incidence of toxicities including neutropenia. However, the incidences of grade 3 or higher neutropenia were higher in patients heterozygous for haplotype *3 compared with in the patients without haplotype *3 when they received concomitant chemotherapy with fluorouracil or platinum compounds. As we reported recently, one patient homozygous for haplotype *3 who received both gemcitabine and cisplatin suffered from extremely severe adverse effects including grade 3 anathema.[23] However, he experienced neither of the specific toxicities associated with cisplatin, nephrotoxicity, and neurotoxicity. Abbruzzese et al[36] reported the gemcitabine dose-dependent increase in incidence of thrombocytopenia (one of seven at 525 mg/m^2/wk, three of nine at 790 mg/m^2/wk, and three of six at 1,000 mg/m^2/wk).[36] Therefore, we concluded that extremely high exposure to gemcitabine (AUC five times higher than the average) due to the decreased deamination activity caused the life-threatening severe toxicities in this patient. In contrast, the gemcitabine AUC of the patients with heterozygous haplotype *3 was only slightly (23% to 48%) increased from that of the patients having no haplotype *3 (Table 6). This finding coincides with the lack of life-threatening severe toxicities in the heterozygotes for *3, although the incidences of grade 3 or higher neutropenia in the heterozygotes in combined chemotherapy groups were higher in the group without haplotype *3.

CDA is also involved in the activation of capecitabine to its active form fluorouracil.[37] Therefore, capecitabine activation would be inefficient in patients who are homozygous for 208A. The allele frequency of the 208G>A SNP, a tagging SNP of haplotype *3, was reported to be 0.125 in Africans, while it was not detected in Europeans.[38] The frequency of homozygous carriers of the variant could be higher in Africans than in the Japanese population. However, the frequency of 208G>A in Africans is still controversial, because it was not detected in 60 African Americans in a recent report.[17] Extra attention may be necessary for patients with the allele before treatments with gemcitabine or cytarabine are initiated, especially to *3/*3 patients, although more studies are necessary to confirm the clinical importance of this allele in the treatments using gemcitabine or cytarabine.

A number of studies have investigated the associations between cellular CDA activity and drug responses to cytarabine.[24-27,39] However, correlation between plasma CDA activity and the

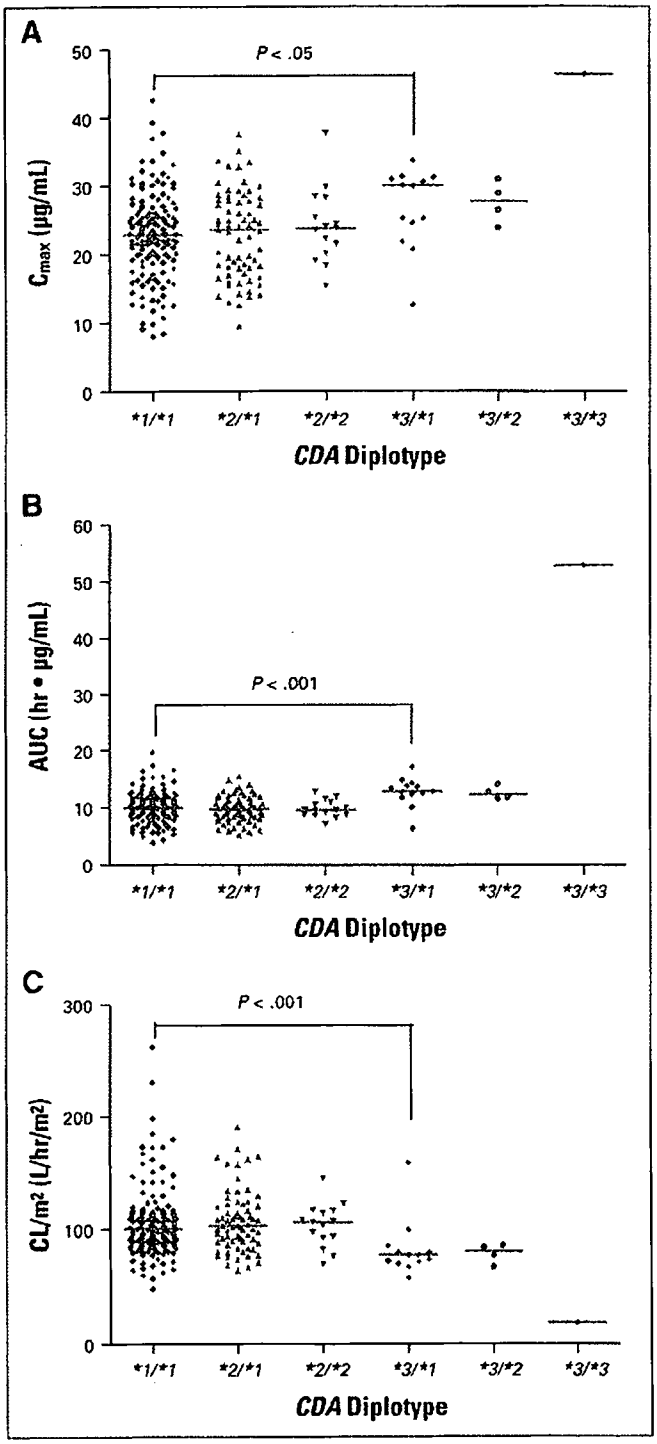

Fig 2. Effects of haplotypes *2 and *3 on the pharmacokinetic parameters of gemcitabine. (A) Peak concentration (C_{max}) and (B) area under the curve (AUC) were corrected assuming that all patients received 1,000 mg/m^2 of gemcitabine. (C) Clearance (CL/m^2). Each point corresponds to an individual patient. The bars denote the median values. *P* values are from Dunn's multiple comparison test.

pharmacokinetics of gemcitabine has not been reported. Plasma CDA activity may be a useful biomarker to screen patients with a markedly decreased metabolic CDA activity such as the patient homozygous for the *3 allele found in our study, who showed extremely low plasma CDA activity. However, a very low contribution of plasma CDA to the total clearance of gemcitabine was reported,[36] and the plasma CDA levels are increased in the inflammatory diseases.[30,40] These may account for the failure in obtaining good correlations between plasma CDA activity and the pharmacokinetic parameters of gemcitabine, as shown in Figure 4.

In conclusion, we analyzed the *CDA* genetic variations and haplotypes in Japanese cancer patients who received gemcitabine. We then investigated the associations between genetic polymorphisms and the pharmacokinetics of gemcitabine or toxicities. Depending on the haplotype *3 harboring 208A, the metabolic clearance of gemcitabine decreased, and AUC and C_{max} values were increased. Moreover, plasma CDA activities correlated well with the *CDA* genotypes. The clinical importance of the SNP 208G>A, especially of homozygotes, should be confirmed by prospective clinical studies because only one homozygous *3 patient was found in this study.

Author disclosures of potential conflicts of interest

Although all authors completed the disclosure declaration, the following authors or their immediate family members indicated a financial interest. No conflict exists for drugs or devices used in a study if they are not being evaluated as part of the investigation. For a detailed description of the disclosure categories, or for more information about ASCO's conflict of interest policy, please refer to the Author Disclosure Declaration and the Disclosures of Potential Conflicts of Interest section in Information for Contributors.

Employment: N/A **Leadership:** N/A **Consultant:** N/A **Stock:** N/A **Honoraria:** Nagahiro Saijo, Chugai, AstraZeneca, Bristol-Myers Squibb Co **Research Funds:** Nagahiro Saijo, Bristol-Myers Squibb Co **Testimony:** N/A **Other:** N/A

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Final approval of manuscript: Nahoko Kaniwa, Jun-ichi Sawada, Hideki Ueno, Nagahiro Saijo

Table 6. Pharmacokinetic Parameters of Gemcitabine and Plasma CDA Activities in the Patient Groups Categorized According to Diplotypes

Diplotype	Median Gemcitabine PK Parameters: No. of Patients	C_{max} (μg/mL)	AUC (hr·μg/mL)	CL/m² (L/hr/m²)	Median CDA Activity (units): No. of Patients	Gemcitabine	Cytidine
*1/*1	148	22.81	9.96	100.30	63	6.26	5.54
*2/*1	69	23.57	9.71	103.00	25	6.81	5.71
*2/*2	15	23.75	9.57	106.10	14	6.53	6.24
P value*		0.52	0.46	0.99		0.47	0.19
*3/*1	13	30.02	12.83	77.93	13	2.99	3.07
*3/*3	1	46.42	52.86	18.92	1	0.74	1.40
P value†		5.94E-04	6.66E-13	7.77E-04		9.35E-05	2.45E-04

Abbreviations: CDA, cytidine deaminase; C_{max}, peak concentration; AUC, area under the curve; CL/m², clearance.
*P value of a correlation test among *1/*1, *1/*2, and *2/*2. Multiplicity is adjusted by false-discovery rate.
†P value of a correlation test among *1/*1, *1/*3, and *3/*3. Multiplicity is adjusted by false-discovery rate.

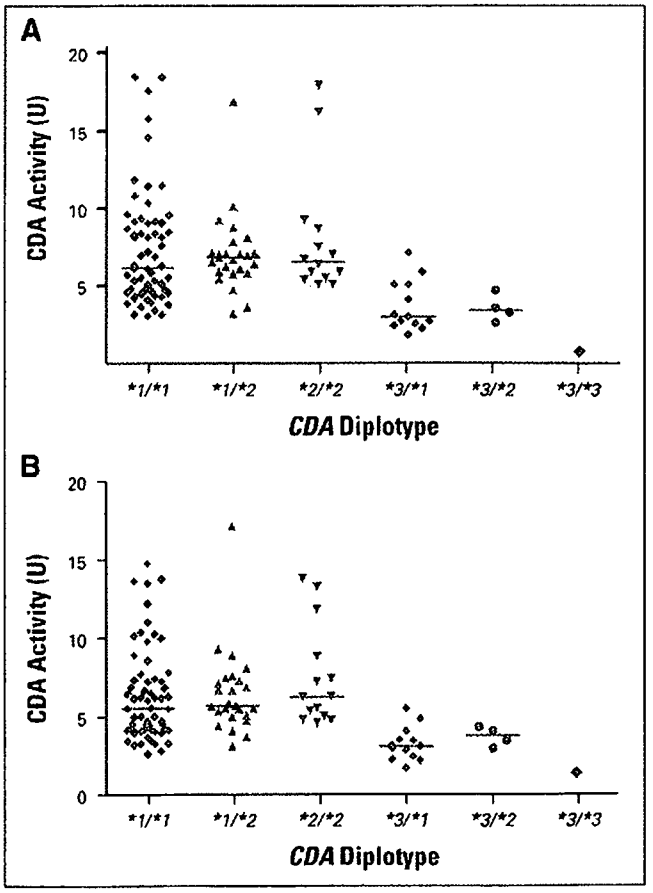

Fig 3. Effects of haplotypes *2 and *3 on plasma cytidine deaminase (CDA) activity toward gemcitabine and cytidine substrates. (A) Gemcitabine was used as a substrate, and (B) cytidine was used as a substrate. Each point corresponds to an individual patient. The bars denote the median values.

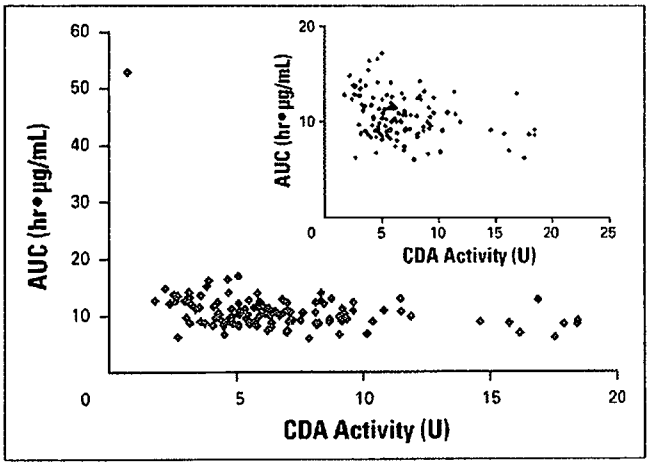

Fig 4. Correlation between plasma area under the curve (AUC) and cytidine deaminase (CDA) activity toward gemcitabine. AUC was corrected assuming that all patients received 1,000 mg/m² of gemcitabine. The inset excludes the data obtained from a homozygous *3 carrier. The correlation coefficient is −0.31 when the homozygous *3 carrier is included and −0.28 when the carrier is excluded.

Table 7. Comparison of Adverse Reaction Incidence and Pharmacokinetic Parameters of Gemcitabine Between Two Patient Groups With and Without Haplotype *3

		Incidence of Neturopenia (nadir)*						
		≥ Grade 3			≥ Grade 4			
Chemotherapy	Genotype	No. of Cases	Total No. of Patients	Probability	No. of Cases	Total No. of Patients	Probability	AUC† (hr·μg/mL)
Monotherapy	*non*3/non*3*	66	167	0.40	8	67	0.05	9.91
	*non*3/*3*	6	10	0.60	1	10	0.10	13.13
	P			0.205			0.514	**0.0017**
With fluorouracil	*non*3/non*3*	3	12	0.25	2	12	0.17	8.11
	*non*3/*3*	2	2	1.00	1	2	0.50	11.98
	P			**0.029**			0.327	0.055
With carboplatin	*non*3/non*3*	9	13	0.69	1	13	0.08	9.87
	*non*3/*3*	3	3	1.00	2	3	0.67	12.48
	P			0.163			**0.033**	**0.031**
With cisplatin	*non*3/non*3*	8	28	0.29	2	28	0.07	9.53
	*non*3/*3*	1	1	1.00	0	1	0.00	11.71
	*3/*3*	1	1	1.00	1	1	1.00	52.86
	P‡			**0.030**			0.128	0.061

Note. No analyses were performed in patients who received gemcitabine with vinorelbine, because only one patient bore the haplotype *3. Boldfacing indicates a statistically significant difference ($P < .05$).
*χ^2-test.
†Kruskal-Wallis test.
‡A *P* value for comparison between *non*3/non*3* and (*non*3/*3* + *3/*3*).

References

1. Noble S, Goa KL: Gemcitabine: A review of its pharmacology and clinical potential in non-small cell lung cancer and pancreatic cancer. Drugs 54:447-472, 1997
2. Burris HA III, Moore MJ, Andersen J, et al: Improvements in survival and clinical benefit with gemcitabine as first-line therapy for patients with advanced pancreas cancer: A randomized trial. J Clin Oncol 15:2403-2413, 1997
3. Rauchwerger DR, Firby PS, Hedley DW, et al: Equilibrative-sensitive nucleoside transporter and its role in gemcitabine sensitivity. Cancer Res 60:6075-6079, 2000
4. Mackey JR, Mani RS, Selner M, et al: Functional nucleoside transporters are required for gemcitabine influx and manifestation of toxicity in cancer cell lines. Cancer Res 58:4349-4357, 1998
5. Mackey JR, Yao SY, Smith KM, et al: Gemcitabine transport in xenopus oocytes expressing recombinant plasma membrane mammalian nucleoside transporters. J Natl Cancer Inst 91:1876-1881, 1999
6. Baldwin SA, Yao SY, Hyde RJ, et al: Functional characterization of novel human and mouse equilibrative nucleoside transporters (hENT3 and mENT3) located in intracellular membranes. J Biol Chem 280:15880-15887, 2005
7. Mangravite LM, Badagnani I, Giacomini KM: Nucleoside transporters in the disposition and targeting of nucleoside analogs in the kidney. Eur J Pharmacol 479:269-281, 2003
8. Ritzel MW, Ng AM, Yao SY, et al: Molecular identification and characterization of novel human and mouse concentrative Na+-nucleoside cotransporter proteins (hCNT3 and rCNT3) broadly selective for purine and pyrimidine nucleosides (system cib). J Biol Chem 276:2914-2927, 2001
9. Plunkett W, Huang P, Gandhi V: Preclinical characteristics of gemcitabine. Anticancer Drugs 6:S7-S13, 1995 (suppl 6)
10. Plunkett W, Huang P, Searcy CE, et al: Gemcitabine: Preclinical pharmacology and mechanisms of action. Semin Oncol 23:S3-S15, 1996 (suppl 10)
11. Heinemann V, Xu YZ, Chubb S, et al: Inhibition of ribonucleotide reduction in CCRF-CEM cells by 2',2'-difluorodeoxycytidine. Mol Pharmacol 38:567-572, 1990
12. Kiani A, Kohne CH, Franz T, et al: Pharmacokinetics of gemcitabine in a patient with end-stage renal disease: Effective clearance of its main metabolite by standard hemodialysis treatment. Cancer Chemother Pharmacol 51:266-270, 2003
13. Watanabe S, Uchida T: Expression of cytidine deaminase in human solid tumors and its regulation by 1 alpha, 2, 5-dihydroxyvitamin D3. Biochim Biophys Acta 1312:99-104, 1996
14. Ho DH: Distribution of kinase and deaminase of 1-beta-D-arabinofuranosylcytosine in tissues of man and mouse. Cancer Res 33:2816-2820, 1973
15. Kirch HC, Schroder J, Hoppe H, et al: Recombinant gene products of two natural variants of the human cytidine deaminase gene confer different deamination rates of cytarabine in vitro. Exp Hematol 26:421-425, 1998
16. Schroder JK, Kirch C, Seeber S, et al: Structural and functional analysis of the cytidine deaminase gene in patients with acute myeloid leukaemia. Br J Haematol 103:1096-1103, 1998
17. Gilbert JA, Salavaggione OE, Ji Y, et al: Gemcitabine pharmacogenomics: Cytidine deaminase and deoxycytidylate deaminase gene resequencing and functional genomics. Clin Cancer Res 12:1794-1803, 2006
18. Yue L, Saikawa Y, Ota K, et al: A functional single-nucleotide polymorphism in the human cytidine deaminase gene contributing to ara-C sensitivity. Pharmacogenetics 13:29-38, 2003
19. Aapro MS, Martin C, Hatty S: Gemcitabine: A safety review. Anticancer Drugs 9:191-201, 1998
20. Gallelli L, Nardi M, Prantera T, et al: Retrospective analysis of adverse drug reactions induced by gemcitabine treatment in patients with non-small cell lung cancer. Pharmacol Res 49:259-263, 2004
21. Bokemeyer C, Gerl A, Schoffski P, et al: Gemcitabine in patients with relapsed or cisplatin-refractory testicular cancer. J Clin Oncol 17:512-516, 1999
22. Locker GJ, Wenzel C, Schmidinger M, et al: Unexpected severe myelotoxicity of gemcitabine in pretreated breast cancer patients. Anticancer Drugs 12:209-212, 2001
23. Yonemori K, Ueno H, Okusaka T, et al: Severe drug toxicity associated with a single-nucleotide polymorphism of the cytidine deaminase gene in a Japanese cancer patient treated with gemcitabine plus cisplatin. Clin Cancer Res 11:2620-2624, 2005
24. Colly LP, Peters WG, Richel D, et al: Deoxycytidine kinase and deoxycytidine deaminase values correspond closely to clinical response to cytosine arabinoside remission induction therapy in patients with acute myelogenous leukemia. Semin Oncol 14:S257-S261, 1987 (suppl 1)
25. Steuart CD, Burke PJ: Cytidine deaminase and the development of resistance to arabinosyl cytosine. Nat New Biol 233:109-110, 1971
26. Tattersall MH, Ganeshaguru K, Hoffbrand AV: Mechanisms of resistance of human acute leukaemia cells to cytosine arabinoside. Br J Haematol 27:39-46, 1974
27. Chiba P, Tihan T, Szekeres T, et al: Concordant changes of pyrimidine metabolism in blasts of two cases of acute myeloid leukemia after repeated treatment with ara-C in vivo. Leukemia 4:761-765, 1990
28. Nakamura T, Saito Y, Murayama N, et al: Apparent low frequency of sequence variability within the proximal promoter region of the cytochrome P450(CYP)3A5 gene in established cell lines from Japanese individuals. Biol Pharm Bull 24:954-957, 2001
29. Kitamura Y, Moriguchi M, Kaneko H, et al: 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

30. Richards DA, Sherwood RA, Ndebele D, et al: Determination of plasma cytidine deaminase activity by HPLC. Biomed Chromatogr 2:148-151, 1987

31. Benjamini Y, Hochberg Y: Controlling the false discovery rate: A practical and powerful approach to multiple testing. J R Statist Soc Ser B 57:289-300, 1995

32. Bhargava P, Marshall JL, Fried K, et al: Phase I and pharmacokinetic study of two sequences of gemcitabine and docetaxel administered weekly to patients with advanced cancer. Cancer Chemother Pharmacol 48:95-103, 2001

33. Johansson E, Mejlhede N, Neuhard J, et al: Crystal structure of the tetrameric cytidine deaminase from Bacillus subtilis at 2.0: A resolution. Biochemistry 41:2563-2570, 2002

34. Costanzi S, Vincenzetti S, Vita A, et al: Human cytidine deaminase: Understanding the catalytic mechanism. Nucleosides Nucleotides Nucleic Acids 22:1539-1543, 2003

35. Kreis W, Lesser M, Budman DR, et al: Phenotypic analysis of 1-B-D-arabinofuranosylcytosine deamination in patients treated with high doses and correlation with response. Cancer Chemother Pharmacol 30:126-130, 1992

36. Abbruzzese JL, Grunewald R, Weeks EA, et al: A phase I clinical, plasma, and cellular pharmacology study of gemcitabine. J Clin Oncol 9:491-498, 1991

37. Nishida M: Pharmacological and clinical properties of Xeloda (capecitabine), a new oral active derivative of fluoropyrimidine [Japanese]. Nippon Yakurigaku Zasshi 122:549-553, 2003

38. Fukunaga AK, Marsh S, Murry DJ, et al: Identification and analysis of single nucleotide polymorphisms in the gemcitabine pharmacologic pathway. Pharmacogenomics J 4:307-314, 2004

39. Jahns-Streubel G, Reuter C, Auf der Landwehr U, et al: Activity of thymidine kinase and of polymerase alpha as well as activity and gene expression of deoxycytidine deaminase in leukemic blasts are correlated with clinical response in the setting of granulocyte-macrophage colony-stimulating factor-based priming before and during TAD-9 induction therapy in acute myeloid leukemia. Blood 90: 1968-1976, 1997

40. Sherwood RA: The measurement of nucleoside deaminases by high performance liquid chromatography and their use in clinical chemistry. Biomed Chromatogr 5:235-239, 1991

Acknowledgment

We thank Emiko Jimbo, Miho Akimoto, Atsuko Watanabe, Tomoko Chujo, Makiyo Iwamoto, and Mamiko Shimada for assistance in sample collection and management, and Chie Sudo for secretarial assistance.

Jpn J Clin Oncol 2007;37(3)175–180
doi:10.1093/jjco/hym008

Phase I Study of Cisplatin Analogue Nedaplatin, Paclitaxel, and Thoracic Radiotherapy for Unresectable Stage III Non-Small Cell Lung Cancer

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Received September 6, 2006; accepted November 1, 2006; published online April 23, 2007

Background: The standard treatment of unresectable stage III non-small cell lung cancer is concurrent chemoradiotherapy in patients in good general condition, but where the optimal chemotherapeutic regimen has not been determined.
Methods: Patients with unresectable stage III non-small cell lung cancer received nedaplatin (80 mg/m^2) and paclitaxel on day 1 every 4 weeks for 3–4 cycles and concurrent thoracic radiotherapy (60 Gy/30 fractions for 6 weeks) starting on day 1. The dose of paclitaxel was escalated from 120 mg/m^2 in level 1, 135 mg/m^2 in level 2 to 150 mg/m^2 in level 3.
Results: A total of 18 patients (14 males and 4 females, with a median age of 62.5 years) were evaluated in this study. Full cycles of chemotherapy were administered in 83% of patients in level 1, and in 50% of patients in levels 2 and 3. No more than 50% of patients developed grade 4 neutropenia. Transient grade 3 esophagitis and infection were noted in one patient, and unacceptable pneumonitis was noted in three (17%) patients, two of whom died of the toxicity. Dose-limiting toxicity (DLT), evaluated in 15 patients, noted in one of the six patients in level 1, three of the six patients in level 2 and one of the three patients in level 3. One DLT at level 2 developed later as radiation pneumonitis. Thus, the maximum tolerated dose was determined to be level 1. The overall response rate (95% confidence interval) was 67% (41–87%) with 12 partial responses.
Conclusion: The doses of paclitaxel and nedaplatin could not be escalated as a result of severe pulmonary toxicity.

Key words: non-small cell lung cancer – chemoradiotherapy – paclitaxel – nedaplatin – pneumonitis

INTRODUCTION

Locally advanced unresectable non-small cell lung cancer (NSCLC), stage IIIA disease with bulky N2 and stage IIIB disease without pleural effusion, is characterized by large primary lesions, and/or involvement of the mediastinal or supraclavicular lymph nodes, and occult systemic micrometastases (1). Concurrent chemoradiotherapy, recently shown to be superior to the sequential approach in phase III trials, is the standard medical care for this disease (2–4). Chemotherapy regimens used concurrently with thoracic radiotherapy in these randomized trials were second-generation platinum-based chemotherapy, such as combinations of cisplatin, vindesine and mitomycin, cisplatin and vinblastine, and cisplatin and etoposide. The third-generation cytotoxic agents including vinorelbine and paclitaxel, which provided a better survival rate in patients with disseminated disease than second-generation agents, must be reduced when administered concurrently with thoracic radiotherapy (5–7). Thus, the optimal chemotherapy for concurrent chemoradiotherapy has not been established.

Nedaplatin (*cis*-diammine-glycolate-O,O′-platinum II, 254-S) is a second-generation platinum derivative that has an

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antitumor activity comparable to that of cisplatin but is less toxic to the kidney as shown in preclinical experiments (8). Nedaplatin produced a promising response rate for NSCLC, especially for squamous cell lung cancer (9,10). In addition, this drug can be safely administered with full-dose thoracic radiation, as shown in patients with esophageal cancer (11). Paclitaxel is another promising drug for the treatment of stage III NSCLC, as shown by the favorable response rate and survival in phase II trials in combination with platinum and thoracic radiation (6,7).

Our previous study of the nedaplatin and paclitaxel combination in patients with systemic disease showed that the recommended dose of these drugs was 80 mg/m^2 and 180 mg/m^2, respectively, repeated every 3–4 weeks. A promising response rate of 55% was achieved in patients with squamous cell lung cancer (12). The objectives of the present study were primarily to evaluate the toxicity of nedaplatin, paclitaxel and concurrent thoracic radiotherapy and determine the recommended dose of these two drugs for a phase II trial, and secondarily to observe the antitumor effect of this regimen in patients with stage III NSCLC.

PATIENTS AND METHODS

Patient Selection

The eligibility criteria were: histologically or cytologically proven NSCLC; unresectable stage IIIA or IIIB disease indicated for curative radiotherapy; no previous treatment; measurable disease; the percentage of the normal lung volume receiving 20 Gy or more (V_{20}) (13) expected to be 30% or less; age between 20 years and 74 years; Eastern Cooperative Oncology Group (ECOG) performance status (14) 0 or 1; adequate bone marrow function (12.0 × 10^9/L ≥ white blood cell (WBC) count ≥ 4.0 × 10^9/L, neutrophil count ≥ 2.0 × 10^9/L, hemoglobin ≥ 10.0 g/dL and platelet count ≥ 100 × 10^9/L), liver function (total bilirubin ≤ 1.5 mg/dL and transaminase ≤ twice the upper limit of the normal value), and renal function (serum creatinine ≤ 1.5 mg/dL and creatinine clearance ≥ 60 mL/min); and a PaO_2 of 70 torr or more. Patients were excluded if they had malignant pleural or pericardial effusion, active double cancer, a concomitant serious illness, such as uncontrolled angina pectoris, myocardial infarction in the previous 3 months, heart failure, uncontrolled diabetes mellitus, uncontrolled hypertension, interstitial pneumonitis or lung fibrosis identified by a chest X-ray, chronic obstructive lung disease, infection or other diseases contraindicating chemotherapy or radiotherapy, pregnancy, or breast-feeding. All patients gave their written informed consent.

Pretreatment Evaluation

The pretreatment assessment included a complete blood cell count and differential count, routine chemistry determinations, creatinine clearance, blood gas analysis, electrocardiogram, lung function testing, chest X-rays, chest computed tomographic (CT) scan, brain CT scan or magnetic resonance imaging, abdominal CT scan, and radionuclide bone scan.

Treatment Schedule

Paclitaxel and nedaplatin were administered as previously described (12). Briefly, paclitaxel diluted in 500 ml of 5% glucose was administered as a 3-h intravenous infusion with premedication consisting of dexamethasone, ranitidine and diphenhydramine. Nedaplatin diluted in 250 ml of normal saline was administered in a 1-h intravenous infusion. This treatment was repeated every 4 weeks for 3–4 cycles. The dose of paclitaxel was escalated as follows: 120 mg/m^2 (level 1), 135 mg/m^2 (level 2), and 150 mg/m^2 (level 2). The dose of nedaplatin was 80 mg/m^2 through the levels 1–3.

Thoracic radiation therapy was given with photon beams from a liniac or microtron accelerator with energy between 6 and 10 MV. The total dose of 60 Gy was delivered at a single dose of 2 Gy once daily Monday through Friday for 6 weeks without interruption beginning on day 1 of the chemotherapy. Three-dimensional conformal radiotherapy technique was used in all patients. The gross target volume (GTV) included the primary lesion (GTV1) and involved lymph nodes whose short diameter was 1 cm or larger (GTV2) based on conventional chest X-ray and CT scans. The clinical target volume (CTV) consisted of CTV1 and CTV2, identical to GTV1 and GTV2, respectively, and CTV3, the ipsilateral hilum and bilateral mediastinum area. The contralateral hilum was excluded from the CTV. The supraclavicular fossa was also excluded unless it was involved. The planning target volume (PTV) for the initial dose up to 40 Gy consisted of CTV1-3 with the superior and inferior field margins extended to 1–2 cm and the lateral field margins extended to 0.5 cm for respiratory variation and fixation error. The PTV for the boost 20 Gy included only CTV1-2 based on the second CT scans with the same margins. The spinal cord dose was limited to 44 Gy by using oblique parallel opposed fields.

Toxicity Assessment and Treatment Modification

Complete blood cell counts and differential counts, routine chemistry determinations and a chest X-ray were performed once a week during the course of treatment. Toxicity was graded according to the NCI Common Toxicity Criteria version 2.0. Subsequent cycles of chemotherapy were delayed if any of the following toxicities was noted on day 1: WBC count <3.0 × 10^9/L, neutrophil count <1.5 × 10^9/L, platelet count <100 × 10^9/L, serum creatinine level ≥1.6 mg/dL, infection ≥ grade 2, elevated hepatic transaminase level or total serum bilirubin ≥ grade 2, pneumonitis ≥ grade 2, peripheral neuropathy, musculoskeletal pain ≥ grade 3, fever ≥ 38°C, or performance status ≥ 2. Chemotherapy was terminated if the toxicities did not

recover within 2 weeks. The doses of nedaplatin and paclitaxel were reduced by 25% in all subsequent cycles if any of the dose-limiting toxicities (DLTs) defined below were noted. The dose of nedaplatin was reduced by 25% in all subsequent cycles if the serum creatinine level was elevated to 2.0 mg/dl or higher. Thoracic radiotherapy was suspended if any of the following toxicities was noted: fever $\geq 38^{\circ}C$, infection $\geq$ grade 2, esophagitis of grade 3, performance status ≥ 3, or radiation pneumonitis was suspected. Thoracic radiotherapy was terminated if radiation pneumonitis that required corticosteroid administration was noted, or radiotherapy was not completed within 60 days. Both chemotherapy and thoracic radiotherapy were terminated if any of the following was noted: disease progression, any of the grade 4 non-hematological toxicities except abnormal electrolytes, performance status of 4, patient refusal to receive subsequent treatment, protocol violation, or patient death of any cause. Granulocyte colony-stimulating factor and antibiotics were administered if febrile neutropenia was noted.

DLT, Maximum Tolerated Dose (MTD), and Recommended Dose For Phase II Trials

The DLT was defined as a grade 4 leukopenia, grade 4 neutropenia lasting 7 days or longer, febrile neutropenia, platelet count $<20 \times 10^9$/L, grade 3 or a more severe non-hematological toxicity other than nausea, vomiting and transient electrolyte abnormality, and treatment termination before two cycles of chemotherapy and thoracic radiotherapy were completed. Dose levels were escalated according to the frequency of DLT evaluated during the first and second cycles of chemotherapy and thoracic radiation. Six patients were initially enrolled at each dose level. If none to two of the six patients experienced DLT, the next cohort of patients was treated at the next higher dose level. If three or more of the six patients experienced DLT, that level was considered to be the MTD. The recommended dose for phase II trials was defined as the dose preceding the MTD.

Response Evaluation

Objective tumor response was evaluated according to the Response Evaluation Criteria in Solid Tumors (RECIST) (15).

Study Design, Data Management and Statistical Analyses

This study was designed as a phase I study at the National Cancer Center Hospital. The protocol and consent form were approved by the Institutional Review Board of the National Cancer Center. Registration was conducted at the Registration Center. Data management, periodic monitoring, and the final analysis were performed by the Study Coordinator. A patient accrual period of 2 years and a follow-up period of 3 years were planned. Overall survival time and progression-free survival time were estimated by the Kaplan–Meier method (16). Overall survival time was measured from the date of registration to the date of death from any cause or last follow-up. Progression-free survival time was measured from the date of registration to the date of disease progression or death from any cause or last follow-up. Patients who were lost to follow-up without event were censored at the date of their most known follow-up. A confidence interval for the response rate was calculated using methods for exact binomial confidence intervals. Response rates among patients with squamous cell carcinoma and those with non-squamous carcinoma were assessed with the χ^2 test. The Dr. SPSS II 11.0 for Windows software package (SPSS Japan Inc., Tokyo, Japan) was used for statistical analyses.

RESULTS

Registration and Characteristics of The Patients

From October 2003 to July 2004, six patients were registered at dose level 1, eight patients at dose level 2 and five patients at dose level 3. Two patients at dose level 2 were excluded from the DLT evaluation, because they discontinued receiving the treatment early because of disease progression and anaphylactic shock, respectively. Initially, DLT was noted in only two of the six patients at dose level 2, and therefore, patient registration at dose level 3 was started. However, severe radiation pneumonitis developed 5 weeks after the end of radiotherapy in another patient at dose level 2 and this pneumonitis was counted as DLT. Thus, because DLT was finally noted in three of the six patients at dose level 2, patient registration at dose level 3 was stopped. One patient at dose level 3 was found to be ineligible because the radiation treatment planning showed that the V_{20} exceeded 30%. The patient did not receive the current treatment and was excluded from the analysis. Thus, a total of 18 patients were subjects of this study and their detailed demographic characteristics are listed in Table 1.

Treatment Delivery

The planned three to four cycles of chemotherapy were administered in 83% of patients in level 1 and in 50% of patients in levels 2 and 3. Radiation delivery was generally well maintained and it did not differ among the three dose levels (Table 2).

Toxicity, DLT and MTD

Hematological toxicity was generally mild. No more than 50% of patients developed grade 4 neutropenia, and no one developed grade 2 or higher thrombocytopenia (Table 3). Non-hematological toxicity other than lung toxicity was also well tolerated. One patient developed transient grade 3 esophagitis and grade 3 infection not associated with neutropenia, which were considered DLTs. Another patient developed grade 4 anaphylactic shock 1 min after the second cycle infusion of paclitaxel, but soon recovered with fluid

Table 1. Patient characteristics

	n	(%)
Number of patients	18	
Gender		
male	14	(78)
female	4	(22)
Age		
median (range), years	62.5	(46–69)
PS		
0	11	(61)
1	7	(39)
Body weight loss		
< 5%	15	(83)
5–9%	2	(11)
≥ 10%	1	(6)
Clinical stage		
IIIA	10	(56)
IIIB	8	(44)
Histology		
adenocarcinoma	8	(44)
squamous cell carcinoma	6	(33)
non-small cell, not specified	4	(22)

PS, performance status.

replacement and oxygen therapy. This patient was excluded from DLT evaluation. One patient in level 1 and another patient in level 2 developed grade 4 pneumonitis after completion of two cycles of chemotherapy and thoracic radiotherapy and they died of the pneumonitis. The V_{20} and mean lung dose (MLD) of these patients were 23% and 30%, and 1341 cGy and 1675 cGy, respectively.

Table 2. Treatment delivery

Dose level	Level 1	Level 2	Level 3
	(*n* = 6)	(*n* = 8)	(*n* = 4)
Number of chemotherapy cycles			
3–4	5	4	2
2	1	3	1
1	0	1	1
Total radiation dose (Gy)			
60	6	7	3
50–59	0	1	0
NE	0	0	1
Radiotherapy delay (days)			
0–4	5	7	2
5–9	1	0	1
NE	0	1	1

NE, not evaluable.

Table 3. Toxicity in all patients

Dose level	Level 1 (*n* = 6)			Level 2 (*n* = 8)			Level 3 (*n* = 4)		
Toxicity grade	2	3	4	2	3	4	2	3	4
Leukopenia	2	3	0	3	3	0	1	2	1
Neutropenia	0	4	1	2	3	1	0	2	2
Anemia	0	0	0	2	0	0	2	0	0
GPT elevation	1	0	0	2	0	0	0	0	0
Total bilirubin elevation	1	0	0	1	0	0	1	0	0
Infection	0	0	0	1	1	0	0	0	0
Allergic reaction	1	0	0	2	0	1	0	0	0
Anorexia	1	0	0	2	0	0	0	0	0
Nausea	0	0	0	1	0	0	0	0	0
Constipation	0	0	0	2	0	0	0	0	0
Esophagitis	1	0	0	2	1	0	0	0	0
Pneumonitis	0	0	1*	1	0	1*	0	0	0
Musculoskeletal pain	1	0	0	1	0	0	1	0	0
Alopecia	4	0	0	4	0	0	0	0	0

GPT, glutamic pyruvic transaminase.
*Pneumonitis was fatal in these patients.

Both patients were former heavy smokers with a smoking index of 520 and 1680, respectively. The chest CT scan of the former patient disclosed mild emphysematous, but no interstitial changes. A spirometry analysis showed a vital capacity (VC) of 3480 ml (104% of predicted), and a forced expiratory volume one second percent (FEV1.0%) of 82%. The lung diffusing capacity measurement using carbon monoxide (DL_{CO}) was not done in this patient. The PaO_2 was 93.3 torr. The serum LDH level before treatment was 241 IU/l (the upper limit of the normal value was 229 IU/l). The chest CT scan of the latter patient disclosed slight changes in the dorsal portion of the both lungs, which were considered the gravitation effect, or fibrotic changes. The VC was 3810 ml (107% of predicted), % DL_{CO} was 111%, and PaO_2 was 99.7 torr. The serum LDH level before treatment was 147 IU/l. Another patient in level 2, whose V_{20} and MLD were 15% and 822 cGy, respectively, developed grade 2 pneumonitis when he received 52 Gy of radiotherapy and the subsequent protocol treatment was stopped. The chest CT scan of this patient before treatment showed no abnormal findings except for lung cancer. Pulmonary function test values were all within normal limits. The serum LDH level before treatment was 178 IU/l. Thus, in total three (17%) of 18 patients developed unacceptable severe pneumonitis induced by the current treatment, which was counted as DLT.

To sum up, DLT was noted in one of six patients in level 1, three of six patients in level 2, and one of three patients in level 3. The DLTs were pneumonitis in three patients, grade 4 leukopenia in one patient, and grade 3 esophagitis and grade 3 infection in one patient. Thus, the MTD was determined to be level 1.

Objective Response and Survival

All patients were included in the analyses of tumor response and survival. No CR, 12 PRs, and 3 SD were noted among the 18 patients and the overall response rate (95% confidence interval) was 67% (41–87%). The response rate in patients having squamous cell carcinoma was 100%, while that for non-squamous histology was 58%. The median progression-free survival time was 9.7 months. The median overall survival time has not yet been reached and the 1-year survival rate was 78%.

DISCUSSION

The feasible doses of anticancer agents in this study were paclitaxel 120 mg/m^2 and nedaplatin 80 mg/m^2 every 4 weeks. These figures are lower than those in a randomized phase II trial for stage III NSCLC conducted in the USA, where paclitaxel 135 mg/m^2 and cisplatin 80 mg/m^2 were administered every 3 weeks concurrently with thoracic radiotherapy (6). The occurrence of severe pneumonitis hampered the dose escalation of the anticancer agents in this study. A Japanese phase I/II study of weekly paclitaxel, nedaplatin and concurrent thoracic radiotherapy for stage III NSCLC showed that the DLT was also pneumonitis and that the response rate was 75% and progression-free survival was 5.6 months, similar to the outcome of this study (17). The reasons for the frequent pneumonitis in this study remain unknown. Paclitaxel was the most frequently used anticancer agent together with thoracic radiotherapy in patients with NSCLC outside Japan. A randomized phase II study of induction chemotherapy followed by concurrent chemoradiation therapy in patients with stage III NSCLC (CALGB study 9431) showed that grade 3–4 pneumonitis during chemoradiation was noted in 14% of patients treated with gemcitabine and cisplatin, 20% of patients treated with paclitaxel and cisplatin, and 20% of patients treated with vinorelbine and cisplatin. One patient died of pneumonitis in the vinorelbine and cisplatin arm (6). Thus, incidence of pneumonitis in patients receiving paclitaxel was reported to be the same as that for other agents in this setting. Nedaplatin was a new agent but one of the platinum that has been repeatedly shown to be safely administered with radiation (1). A case series of 24 esophageal cancer patients treated with radiation therapy (60–70 Gy) combined with Nedaplatin (80–120 mg) and 5-fluorouracil (500–1000 mg for 5 days) showed that toxicity was mainly hematological and no grade 3 or higher non-hematological toxicity was observed (18). Treatment-related pneumonitis may be more readily developed among Japanese patients, because gefitinib-induced pneumonitis is more common in Japan than in other countries (19–21). Similarly, a relatively high incidence of drug-induced pneumonitis was noted among Japanese patients in association with the use of weekly docetaxel (20) and leflunomide, a newly developed disease-modifying antirheumatic drug that exhibits anti-inflammatory, antiproliferative and immunosuppressive effects (22). Further studies are needed to define ethnic or geographic variation of treatment-related pneumonitis.

Recent dose–volume histogram studies showed that the volume–dose parameters such as the V20 and MLD were significantly associated with development of severe radiation pneumonitis (23). The V_{20} and MLD in the three patients who developed unacceptable pneumonitis in this study (15–30% and 822–1675 cGy, respectively) were not so large, and therefore, the severe pneumonitis in these patients could not be fully explained by their irradiation volume alone. Patient characteristics such as age, sex, smoking habit, location of the primary tumor and pre-existing lung diseases may be associated with the development of radiation pneumonitis, but their contribution was inconclusive (24).

Radiation pneumonitis is the most common dose-limiting complication of thoracic radiation. Its incidence varies greatly from one report to another: the incidence of grade 2 radiation pneumonitis was between 2% and 33% and that of grade 3 was between 0% and 20% (25) This inconsistency among reports can be explained by the different radiation pneumonitis scoring system and follow-up duration in each study. No scoring system for radiation pneumonitis is perfect. The distinction between grade 2 and grade 3 toxicity is highly subjective. In addition, these scoring systems do not account for intercurrent symptoms from tumor, infection and chronic lung illnesses such as chronic obstructive pulmonary diseases (25).

For future trials, it is an important strategy to reduce the lung volume receiving radiation without an increase in the local recurrence rate. Elective nodal regions with potential subclinical micrometastases (CTV3 in this study) have been included in the standard irradiation volume. The advent of three-dimensional conformal treatment techniques, however, has allowed for a more precise definition of target volume and may allow the possibility of reduced toxicity and increased radiation dose delivery by the omission of elective nodal irradiation (26). We are conducting a phase I study of high-dose thoracic three-dimensional conformal radiotherapy without elective nodal irradiation concurrently combined with cisplatin and vinorelbine in patients with inoperable stage III non-small cell lung cancer.

In conclusion, the doses of paclitaxel and nedaplatin combined with thoracic radiotherapy could not be escalated owing to severe pulmonary toxicity. We do not recommend a phase II study of this chemoradiotherapy regimen.

Acknowledgements

We thank Yuko Yabe and Mika Nagai for preparation of the manuscript. This study was supported in part by Grants-in-Aid for Cancer Research from the Ministry of Health, Labour and Welfare of Japan.

Conflict of interest statement

None declared.

References

1. Vokes EE, Crawford J, Bogart J, Socinski MA, Clamon G, Green MR. Concurrent chemoradiotherapy for unresectable stage III non-small cell lung cancer. *Clin Cancer Res* 2005;11:5045s–50s.
2. Fournel P, Robinet G, Thomas P, Souquet PJ, Lena H, Vergnenegre A, et al. Randomized phase III trial of sequential chemoradiotherapy compared with concurrent chemoradiotherapy in locally advanced non-small-cell lung cancer: Groupe Lyon-Saint-Etienne d'Oncologie Thoracique-Groupe Francais de Pneumo-Cancerologie NPC 95-01 Study. *J Clin Oncol* 2005;23:5910–7.
3. Furuse K, Fukuoka M, Kawahara M, Nishikawa H, Takada Y, Kudoh S, et al. Phase III study of concurrent versus sequential thoracic radiotherapy in combination with mitomycin, vindesine, and cisplatin in unresectable stage III non-small-cell lung cancer. *J Clin Oncol* 1999;17:2692–9.
4. Curran W, Scott CJ, Langer C, Komaki R, Lee J, Hauser S, et al. Long-term benefit is observed in a phase III comparison of sequential vs concurrent chemo-radiation for patients with unresected stage III NSCLC: RTOG 9410. *Proc Am Soc Clin Oncol* 2003;22:p621 (abstr 2499).
5. Sekine I, Noda K, Oshita F, Yamada K, Tanaka M, Yamashita K, et al. Phase I study of cisplatin, vinorelbine, and concurrent thoracic radiotherapy for unresectable stage III non-small cell lung cancer. *Cancer Sci* 2004;95:691–5.
6. Vokes EE, Herndon JE, 2nd, Crawford J, Leopold KA, Perry MC, Miller AA, et al. Randomized phase II study of cisplatin with gemcitabine or paclitaxel or vinorelbine as induction chemotherapy followed by concomitant chemoradiotherapy for stage IIIB non-small-cell lung cancer: cancer and leukemia group B study 9431. *J Clin Oncol* 2002;20:4191–8.
7. Choy H, Akerley W, Safran H, Graziano S, Chung C, Williams T, et al. Multiinstitutional phase II trial of paclitaxel, carboplatin, and concurrent radiation therapy for locally advanced non-small-cell lung cancer. *J Clin Oncol* 1998;16:3316–22.
8. Kameyama Y, Okazaki N, Nakagawa M, Koshida H, Nakamura M, Gemba M. Nephrotoxicity of a new platinum compound, 254-S, evaluated with rat kidney cortical slices. *Toxicol Lett* 1990;52:15–24.
9. Furuse K, Fukuoka M, Kurita Y, Ariyoshi Y, Niitani H, Yoneda S, et al. A phase II clinical study of cis-diammine glycolato platinum, 254-S, for primary lung cancer. *Gan To Kagaku Ryoho* 1992;19:879–84.
10. Yamamoto N, Tamura T, Kurata T, Yamamoto N, Sekine I, Kunitoh H, et al. Phase I and pharmacokinetic (PK) study of (Glycolate-O, O')-diammine platinum (II) (Nedaplatin: 254-S) in elderly patients with non-small cell lung cancer (NSCLC). *Proc Am Soc Clin Oncol* 2000;19:203a (abstr 792).
11. Nemoto K, Matsushita H, Ogawa Y, Takeda K, Takahashi C, Britton KR, et al. Radiation therapy combined with *cis*-diammine-glycolatoplatinum (Nedaplatin) and 5-fluorouracil for untreated and recurrent esophageal cancer. *Am J Clin Oncol* 2003;26:46–9.
12. Sekine I, Nokihara H, Horiike A, Yamamoto N, Kunitoh H, Ohe Y, et al. Phase I study of cisplatin analogue nedaplatin (254-S) and paclitaxel in patients with unresectable squamous cell carcinoma. *Br J Cancer* 2004;90:1125–8.
13. Graham MV, Purdy JA, Emami B, Harms W, Bosch W, Lockett MA, et al. Clinical dose–volume histogram analysis for pneumonitis after 3D treatment for non-small cell lung cancer (NSCLC). *Int J Radiat Oncol Biol Phys* 1999;45:323–9.
14. Oken MM, Creech RH, Tormey DC, Horton J, Davis TE, McFadden ET, et al. Toxicity and response criteria of the Eastern Cooperative Oncology Group. *Am J Clin Oncol* 1982;5:649–55.
15. Therasse P, Arbuck SG, Eisenhauer EA, Wanders J, Kaplan RS, Rubinstein L, et al. New guidelines to evaluate the response to treatment in solid tumors. European Organization for Research and Treatment of Cancer, National Cancer Institute of the United States, National Cancer Institute of Canada. *J Natl Cancer Inst* 2000;92:205–16.
16. Armitage P, Berry G, Matthews J. Survival analysis. In: Armitage P, Berry G, Matthews J editors. Statistical Methods in Medical Research, 4th edn. Oxford: Blackwell Science 2002; 568–90.
17. Hasegawa Y, Takanashi S, Okudera K, Aoki M, Basaki K, Kondo H, et al. Weekly paclitaxel and nedaplatin with concurrent radiotherapy for locally advanced non-small-cell lung cancer: a phase I/II study. *Jpn J Clin Oncol* 2004;34:647–53.
18. Nemoto K, Matsushita H, Ogawa Y, Takeda K, Takahashi C, Britton KR, et al. Radiation therapy combined with *cis*-diammine-glycolatoplatinum (Nedaplatin) and 5-fluorouracil for untreated and recurrent esophageal cancer. *Am J Clin Oncol* 2003;26:46–9.
19. Cohen MH, Williams GA, Sridhara R, Chen G, McGuinn WD, Jr, Morse D, et al. United States Food and Drug Administration drug approval summary: Gefitinib (ZD1839; Iressa) tablets. *Clin Cancer Res* 2004;10:1212–8.
20. Edelman MJ, Sekine I, Tamura T, Saijo N. Geographic variation in the second-line treatment of non-small cell lung cancer. *Semin Oncol* 2006;33(1 Suppl 1):39–44.
21. Ando M, Okamoto I, Yamamoto N, Takeda K, Tamura K, Seto T, et al. Predictive factors for interstitial lung disease, antitumor response, and survival in non-small-cell lung cancer patients treated with gefitinib. *J Clin Oncol* 2006;24:2549–56.
22. Sekine I, Takada M, Nokihara H, Yamamoto S, Tamura T. Knowledge of efficacy of treatments in lung cancer is not enough, their clinical effectiveness should also be known. *J Thorac Oncol* 2006;1:398–402.
23. Rodrigues G, Lock M, D'Souza D, Yu E, Van Dyk J. Prediction of radiation pneumonitis by dose – volume histogram parameters in lung cancer – a systematic review. *Radiother Oncol* 2004;71:127–38.
24. Mehta V. Radiation pneumonitis and pulmonary fibrosis in non-small-cell lung cancer: pulmonary function, prediction, and prevention. *Int J Radiat Oncol Biol Phys* 2005;63:5–24.
25. Machtay M. Pulmonary complications of anticancer treatment. In Abeloff MD, Armitage JO, Niederhuber JE, Kastran MB, McKenna WG editors. *Clinical Oncology*, 3rd edn. Philadelphia, PA: Elsevier Churchill Livingstone 2004; 1237–50.
26. Grills IS, Yan D, Martinez AA, Vicini FA, Wong JW, Kestin LL. Potential for reduced toxicity and dose escalation in the treatment of inoperable non-small-cell lung cancer: a comparison of intensity-modulated radiation therapy (IMRT), 3D conformal radiation, and elective nodal irradiation. *Int J Radiat Oncol Biol Phys* 2003; 57:875–90.

ANTICANCER RESEARCH 27: 2657-2666 (2007)

Phase I Study of Combination Therapy with S-1 and Weekly Docetaxel for Advanced Gastric Cancer

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Abstract. *Background: The primary objective of this study was to determine the maximum tolerated dose (MTD), the toxicity profile and the recommended dose (RD) for phase II of a combination of S-1 and weekly administration of docetaxel. Patients and Methods: Patients with histologically diagnosed recurrent or unresectable locally advanced gastric cancer were enrolled. A fixed oral dose of 80 mg/m^2 S-1 was given for 3 weeks. Docetaxel was infused intravenously on day 1, 8 and 15, repeated every 5 weeks. A pharmacokinetic study was also performed. Results: A total of 14 patients were enrolled. One dose-limiting toxicity (DLT) (grade 3 diarrhea with febrile neutropenia) occurred at level 2. DLTs occurred in 3/5 patients at level 3, (grade 3 stomatitis, with febrile neutropenia or continuous grade 4 neutropenia). The pharmacokinetic study suggested no drug interactions. Overall response and disease control rates were 20% and 80%, respectively. The response rate at the RD (level 2) was 50%. Overall survival was 9.4 months. Conclusion: RD was level 2 (80 mg/m^2 of S-1 for 3 weeks and 20 mg/m^2 of docetaxel on day 1, 8 and 15, every 5 weeks). Dose intensities of S-1 and docetaxel were 48 mg/m^2/week and 12 mg/m^2/week, respectively. This regimen showed promising activity for advanced gastric cancer.*

The incidence and mortality of gastric cancer has been declining, however, it remains one of the most common causes of cancer-related death (1). It is often diagnosed in advanced stage or recurrent disease, both of which are incurable, and carries a dismal prognosis with a short median survival. The one year survival rate is approximately 50% in stage III gastric cancer patients, and 25% in stage IV. Although gastric cancer has been regarded as a resistant tumor, several clinical trials have revealed that some chemotherapeutic agents are effective. 5-Fluorouracil (5-FU)-containing regimens are considered as standard chemotherapy because they provide survival benefit and improvement in quality of life compared with best supportive care (2-4). Hence in the 1980's, many combinations of drugs, 5-FU/doxorubicin/mitomycin (FAM) (5), 5-FU/doxorubicin/methotrexate (FAMTX) (6), etoposide/doxorubicin/cisplatin (EAP) (7), epirubicin/cisplatin/5-FU (ECF) (8), 5-FU/doxorubicin/cisplatin (FAP) (9) and 5-FU/cisplatin (FP) (10, 11) were reported in the treatment of gastric cancer. Although response rates were improved by 40-70%, the survival advantage over single agent 5-FU alone was not significant and severe adverse effects were observed (12). To improve efficacy of chemotherapy against gastric cancer, development of novel agents and combinations which have higher antitumor activity with favorable safety profiles is crucial.

S-1, a fourth-generation oral fluoropyrimidine, is a formulation of tegafur (FT), 5-chloro-2,4-dihydroxypyridine (CDHP) and potassium oxonate (Oxo) at a molar ratio of 1:0.4:1 (13). FT is the prodrug for cytotoxic fluorouracil (FU) and CDHP prevents its degradation. CDHP is a potent and competitive inhibitor of dihydropyrimidine dehydrogenase, which reduces the degradation of FU and allows efficacious concentrations to enter the anabolic pathway. The diarrheagenic property of FU is a result of its phosphorylation in the intestine, primarily by orotate phosphoribosyltransferase (OPRT). Oxo is a competitive inhibitor for OPRT. Thus, the protective effect of Oxo is due to its ability to reduce phosphorylation of FU. Thus, one component of S-1, CDHP, reduces the degradation of cytotoxic FU, and another component, Oxo, potentially reduces its GI toxicity. Phase II studies of S-1 monotherapy in patients with advanced gastric cancer showed an overall

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Key Words: Gastric cancer, phase I study, S-1, docetaxel, weekly chemotherapy.

response rate of 26-49% with the most relevant side-effects being fatigue, diarrhea and neutropenia (14-16). Recently, phase II studies of S-1 plus cisplatin (17), or S-1 plus irinotecan (18) have been evaluated and showed promising response rates.

Docetaxel is a semisynthetic taxoid which enhances microtubule assembly and inhibits the depolymerization of tubulin (19); it has broad antitumor activity against malignancies. It demonstrated promising single-agent efficacy in gastric cancer (20-23) and was therefore investigated in different combination regimens. The combinations of docetaxel with 5-FU (24), capecitabine (25, 26), irinotecan (27) and cisplatin (28) have demonstrated high efficacy. The triplet combination of docetaxel/cisplatin and 5-FU has significantly prolonged overall survival compared to cisplatin plus 5-FU (29). Thus, docetaxel is one of the key drugs playing an integral part in routine combination regimens against gastric cancer.

Based on the clinical activity of both docetaxel and S-1, and the fact that there is no cross resistance or synergistic anti-tumor effect between docetaxel and 5-FU (30) or S-1 (31, 32) *in vitro* or *in vivo*, two Japanese investigators combined docetaxel and S-1 in a clinical trial (33-35). The recommended dose of docetaxel was 40 mg/m^2 on day 1, in combination with S-1 80 mg/m^2 on days 1-14, every 3-4 weeks. The total dose of docetaxel was restricted by neutropenia, with around 70% of patients having grade 3 or 4 neutropenia (33). The real dose intensities of S-1 and docetaxel were around 40 mg/m^2/week and 10 mg/m^2/week, respectively. A weekly administration schedule of docetaxel has been reported as a safe and effective treatment for advanced gastric cancer (26, 36, 37). The aims of the present study were to determine the maximum-tolerated dose (MTD) of docetaxel with weekly administration in combination with S-1 in order to achieve higher dose intensities of both drugs with a feasible toxicity profile and to establish the recommended dose (RD) for Phase II trials.

Patients and Methods

Eligibility criteria. Patients, aged 20 to 75 years, with at least one measurable lesion of pathologically proven inoperable or recurrent gastric cancer were enrolled. Inoperability was determined on the basis of clinical evaluation, radiological imaging, laparoscopy or laparotomy with failed resection. Patients who had no more than two previous treatment regimens not including taxanes (docetaxel or paclitaxel) or S-1 were eligible.

Other eligibility criteria were: Eastern Cooperative Oncology Group performance status 0 or 1; estimated life expectancy of at least 3 months; adequate renal function (serum creatinine <1.5x upper limit of the reference range (ULN)), adequate hepatic function (serum bilirubin <1.5x ULN; transaminases <2.5x ULN) and adequate hematological function (hemoglobin >8 g/dl, leukocytes >4,000/μL and thrombocytes >100,000/μL). No other anti-tumor therapy was allowed 28 days prior to treatment.

Table I. *Patient characteristics.*

Characteristics	Number of patients
Number of patients (evaluable)	14
Age, years; median (range)	61 (31-76)
Gender	
Male	11
Female	3
Performance status (ECOG)	
0	2
1	12
Histology	
Not assessable 2	
Well-differentiated	0
Moderately differentiated	3
Poorly differentiated	9
Extent of disease	
Primary site only	2
Primary and metastatic sites	9
Metastasis only	3
Previous treatment	
None	7
Surgery alone	2
Surgery and adjuvant chemotherapy	2
Surgery and intra-peritoneal chemotherapy	1
Systemic chemotherapy alone	1
Intra-peritoneal chemotherapy alone	1

Eligibility also included the ability to reliably tolerate and comply with oral medication. Patient compliance was recorded using chemotherapy diary cards. Pre-treatment evaluation included a complete medical history and physical examination, basic laboratory evaluation and staging of the underlying malignancy with either ultrasound, chest radiograph or computed tomography (CT) scan.

Main exclusion criteria were follows: pregnancy or breast feeding, symptomatic infectious disease, pulmonary fibrosis or interstitial pneumonia, grade 3 or severe hemorrhage/bleeding, grade 2 or severe peripheral neuropathy, symptomatic peripheral effusion or ascites, past history or allergic reaction to polysorbate 80, obstructive bowel disease or severe diarrhea, congestive heart failure, uncontrolled angina pectoris, or arrhythmia, uncontrolled diabetes or hypertension, symptomatic brain metastasis and active concomitant malignancy.

Patient characteristics are given in Table I. This was a phase I study, conducted at the Department of Medical Oncology, Kinki University, Japan. This study was approved by the institutional review board of Kinki University and all patients provided written informed consent.

Drug administration. Patients received a dose of intravenous docetaxel administered as a 60 min infusion on day 1, 8 and 15, and oral S-1 administered at a fixed dose of 80 mg/m^2/day on days 1-21, every 5 weeks (Figure 1). Patients were treated for at least two cycles unless disease progression or unacceptable toxicity was observed. The initial starting dose of docetaxel was 15 mg/m^2 (level 1) (Table II). Dose

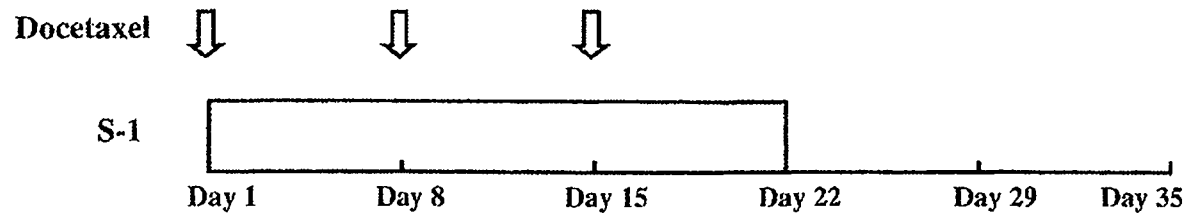

Figure 1. *Treatment schedule of combination therapy with S-1 and docetaxel. Administration of S-1 80 mg/m²/day orally from day 1-21. Administration of docetaxel was given by drip infusion within 60 min. on day 1, 8 and 15. At all dose levels, the administration cycle was repeated every 5 weeks.*

escalation was conducted in increments of 5 mg/m² up to 25 mg/m² (level 3). No intra-individual dose escalation was performed. Docetaxel was only administered on day 8 and 15 if WBC and platelets were >2,000/μl and >75,000/μl, respectively, with non-hematological toxicity <grade 3 and allergic reaction/ AST/ALT/pneumonitis <grade 2. In case of grade 3 neutropenia or thrombocytopenia, or grade 2 diarrhea or mucositis, S-1 administration was interrupted until recovery. Patients were not allowed to escalate or reduce the dose of S-1. If any DLTs were observed, docetaxel was reduced once by one dose level for subsequent courses.

DLTs and MTD. Toxicities were evaluated according to the National Cancer Institute Common Toxicity Criteria (NCI-CTC) version 2 (38). DLTs were defined as follows: (a) grade 4 neutropenia lasting 5 days or longer; (b) febrile neutropenia (grade 3 or 4 neutropenia with fever (≥38.5°C)); (c) grade 4 thrombocytopenia; (d) grade 3 or 4 non-hematological toxicity except for nausea, vomiting, anorexia and general fatigue; (e) failure to administer docetaxel on day 8; (f) failure to administer docetaxel on day 15, even if postponed for one week; and (g) failure to administer S-1 for 14 days continuously during treatment.

Assessment of DLTs was conducted only in the first treatment cycle. Three patients per dose level were planned to be included. In case of one DLT, three further patients were treated at that level. MTD was defined as at least two out of three or three out of six patients with DLT at a given dose level. Throughout this study, the prophylactic administration of granulocyte colony-stimulating factor (G-CSF) was not allowed.

Evaluation during therapy. Hematological and biochemical tests, performance status and clinical assessment of symptoms were monitored at least every week. Tumor response was assessed according to the Response Evaluation Criteria in Solid Tumors (RECIST) (39). All partial or complete responses were confirmed for a minimum of 4 weeks. Patients were considered evaluable for response if they received at least one complete cycle of therapy, unless treatment was stopped due to early toxicity. Time to progression and overall survival were estimated using the Kaplan-Meier method.

Pharmacokinetics. The pharmacokinetics of docetaxel and S-1 were studied during the first cycle of therapy. For docetaxel, 5 ml blood samples were taken from each patient at the following time-points: prior to treatment, 30 min into the drug infusion, at the end of docetaxel infusion, and 30 min, 1 h 2 h, 3 h, 4 h, 7 h and 24 h after the end of the infusion. For S-1, 5 ml blood samples were taken from each patient at the following time-points: prior to dose, and 1 h, 2 h, 4 h, 8 h and 24 h after dose. Initial administration of S-1 was started at 8 h after the end of docetaxel infusion on day 1. To evaluate drug–drug interactions between docetaxel and S-1, the pharmacokinetic analysis of docetaxel was conducted on day 1 and day 8, and that of S-1 was conducted on day 7 and day 8. On day1 only, S-1 was administered in the evening, after the blood correction for pharmacokinetic analysis of docetaxel at 7 h after infusion. All blood samples were centrifuged immediately and the separated plasma samples were frozen at –20°C until analysis. The plasma samples were thawed at ambient temperature, then vortexed and centrifuged for 5 min at 3,000 rpm to remove fibrous materials. Pharmacokinetic analysis for docetaxel was performed according to Yoshida *et al.* (34). Pharmacokinetic analysis for S-1 was carried out as described elsewhere (17).

Table II. *Dose escalation scheme and DLTs in course 1.*

Level	1	2	3
Dose of docetaxel (mg/m²)	15	20	25
Dose of S-1 (mg/m²)	80	80	80
Number of patients	3	6	5
Median number of courses (range)	2 (2-9)	2 (2-5)	1 (1-2)
Number of patients with any DLT/Number of patients	0/3	1/6	3/5
ANC: <500/mm³ for >5 days	0	0	2
Febrile neutropenia	0	1[a]	2
Other grade 3-4 non-hematological toxicity	0	1[a]	3[b]
Inability to receive docetaxel on day 8 or day 15	0	0	1[c]
Inability to receive S-1 more than 14 days	0	0	0

ANC: absolute neutrophil count; [a]Same patient with grade 3 diarrhea with febrile neutropenia; [b]All patients with grade 3 stomatitis; [c]Due to neutropenia.