| 発表者氏名 | 論文タイトル名 | 発表誌名 | 巻号 | ページ | 出版年 |
|---|---|---|---|---|---|
| 古川洋一，吉田輝彦，中村祐輔，森谷宜皓. | HNPCC の登録と遺伝子解析プロジェクト —大腸癌研究会 HNPCC の登録と遺伝子解析プロジェクト— | 家族性腫瘍 | 7 | 2-7 | 2007 |
| 山本聖一郎，藤田 伸，赤須孝之，森谷宜皓，渡邉昌彦. | 腹腔鏡下前方切除術は安全な術式か？ | 外科治療 | 96 | No2 | 2007 |
| 山田敬教，山本聖一郎，藤田 伸，赤須孝之，石黒成治，森谷宜皓. | 術前に虫垂粘液嚢腫と診断し、腹腔鏡下虫垂切除を施行した虫垂仮性憩室の１例. | 日本大腸肛門病学会雑誌 | 60 | 161-166 | 2007 |
| 森谷宜皓. | 大腸癌- 標準手術手技の up to date- ，生涯教育コース Continuing Education Cours, | 第 107 回日本外科学会定期学術集会 | | 70-74 | 2007 |
| 桐山真典，山本聖一郎，藤田 伸，赤須孝之，石黒成治，森谷宜皓. | 切除可能であった腹腔鏡下腹会陰式直腸切断術後局所再発の１例 | 臨床外科 | 62(8) | 1139-1142 | 2007 |
| 山本聖一郎，藤田 伸，赤須孝之，小林豊，山口智弘、森谷宜皓. | 直腸、肛門管癌に対する腹腔鏡手術の治療成績 | 癌の臨床 | 53 | 753-757 | 2007 |
| Nakagohri, T., Kinoshita, T., Konishi, M., Takahashi, S., Tanizawa, Y. | Clinical Results of Extended Lymphadenectomy and Intraoperative Radiotherapy for Pancreatic Adenocarcinoma. | Hepato-Gastroenterology | 54(74) | 564-569 | 2007 |
| Nakagohri, T., Kinoshita, T., Konishi, M., Takahashi, S., and Gotohda, N. | Surgical outcome of intraductal papillary mucinous neoplasms of the pancreas. | Ann Surg Oncol | 14(11) | 3174-3180 | 2007 |
| Mitsunaga, S., Hasebe, T., Kinoshita, T., Konishi, M., Takahashi, S., Gotohda, N., Nakagohri, T., and Ochiai, A. | Detail Histologic Analysis of Nerve Plexus Invasion in Invasive Ductal Carcinoma of the Pancreas and Its Prognostic Impact. | Am J Surg Pathol | 11(31) | 1636-1644 | 2007 |
| Kajiwara, M., Fujii, S., Takahashi, S., Konishi, M., Nakagohri, T., Gotohda, N., and Kinoshita, T. | Adenocarcinoma of the minor duodenal papilla with intraepithelial spread to the pancreatic duct. | Virchows Arch | 451 | 1075-1081 | 2007 |
| Kajiwara, M., Gotohda, N., Konishi, M., Nakagohri, T., Takahashi, S., Kojima, M., Hasebe, T., and Kinoshita, T. | Cystic endocrine tumor of the pancreas with an atypical multilocular appearance. | J Hepatobiliary Pancreat Surg | 14 | 586-589 | 2007 |

| 発表者氏名 | 論文タイトル名 | 発表誌名 | 巻号 | ページ | 出版年 |
|---|---|---|---|---|---|
| Yokomine K, Nakatsura T, Senju S, Nakagata N, Minohara M, Kira JI, Motomura Y, Kubo T, Sasaki Y, and Nishimura Y. | Regression of intestinal adenomas by vaccination with heat shock protein 105-pulsed bone marrow-derived dendritic cells in $Apc^{Min/+}$ mice. | Cancer Sci. | 98(12) | 1930-1935 | 2007 |
| Fuji S, Kim SW, Mori S, Fukuda T, Kamiya S, Yamasaki S, Morita-Hoshi Y, Ohara-Waki F, Honda O, Kuwahara S, Tanosaki R, Heike Y, Tobinai K, and Takaue Y. | Hyperglycemia during the neutropenic period is associated with a poor outcome in patients undergoing myeloablative allogeneic hematopoietic stem cell transplantation | Transplantation | 84 | 814-820 | 2007 |
| Kusumoto S, Mori S, Nosaka K, Morita-Hoshi Y, Onishi Y, Kim SW, Watanabe T, Heike Y, Tanosaki R, Takaue Y, and Tobinai K. | T-cell large granular lymphocyte leukemia of donor origin after cord blood transplantation. | Clin. Lymphoma Myeloma | 7 | 475-479 | 2007 |
| Maruyama D, Fukuda T, Kato R, Yamasaki S, Usui E, Morita-Hoshi Y, Kim SW, Mori S, Heike Y, Makimoto A, Tajima K, Tanosaki R, Tobinai K, and Takaue Y. | Comparable antileukemia/lymphoma effects in nonremission patients undergoing allogeneic hematopoietic cell transplantation with a conventional cytoreductive or reduced-intensity regimen. | Biol. Blood Marrow Transplant | 13 | 932-941 | 2007 |
| Onishi Y, Mori S, Kusumoto S, Sugimoto K, Akahane D, Morita-Hoshi Y, Kim SW, Fukuda T, Heike Y, Tanosaki R, Tobinai K, and Takaue Y. | Unrelated-donor bone marrow transplantation with a conditioning regimen including fludarabine, busulfan, and 4 Gy total body irradiation. | Int. J. Hematol. | 85 | 256-263 | 2007 |
| Iizuka A, Ikarashi Y, Yoshida M, Heike Y, Takeda K, Quinn G, Wakasugi H, Kitagawa M, and Takaue Y. | Interleukin (IL)-4 promotes T helper type 2-biased natural killer T (NKT) cell expansion, which is regulated by NKT cell-derived interferon-gamma and IL-4. | Immunology | 123 | 100-107 | 2007 |
| Morita-Hoshi Y, Heike Y, Kawakami M, Sugita T, Miura O, Kim SW, Mori SI, Fukuda T, Tanosaki R, Tobinai K, and Takaue Y. | Functional analysis of cytomegalovirus-specific T lymphocytes compared to tetramer assay in patients undergoing hematopoietic stem cell transplantation. | Bone Marrow Transplant | | *in press* | |

| 発表者氏名 | 論文タイトル名 | 発表誌名 | 巻号 | ページ | 出版年 |
|---|---|---|---|---|---|
| Yamasaki S, Heike Y, Mori S, Fukuda T, Maruyama D, Kato R, Usui E, Koido K, Kim SW, Tanosaki R, Tobinai K, Teshima T, and Takaue Y. | Infectious complications in chronic graft-versus-host disease: a retrospective study of 145 recipients of allogeneic hematopoietic stem cell transplantation with reduced- and conventional-intensity conditioning regimens. | Transpl. Infect. Dis. | | *in press* | |
| Endo, Y., Sakai, R., Ouchi, M., Onimatsu, H., Hioki, M., Kagawa, S., Uno, F., Watanabe, Y., Urata, Y., Tanaka, N., Fujiwara, T. | Virus-mediated oncolysis induces danger signal and stimulates cytotoxic-T-lymphocyte activity via proteasome activator upregulation. | Oncogene | | *in press* | |
| Hashimoto, Y., Watanabe, Y., Shirakiya, Y., Uno, F., Kagawa, S., Kawamura, H., Nagai, K., Tanaka, N., Kumon, H., Urata, Y., Fujiwara, T. | Establishment of Biological and Pharmacokinetic Assays of Telomerase-Specific Replication-Selective Adenovirus (TRAD). | Cancer Science | | *in press* | |
| Fujiwara, T., Urata, Y., Tanaka, N. | Telomerase-specific oncolytic virotherapy for human cancer with the hTERT promoter. | Curr. Cancer Drug Targets | 7 | 191-201 | 2007 |
| 藤原俊義、田中紀章 | GFP 発現ウイルス製剤を用いた消化器癌微小転移の *in vivo* イメージングシステム. | 医学のあゆみ | 220 | 659-661 | 2007 |
| 藤原俊義、田中紀章 | 癌のウイルス療法. | 外科治療 | 96 | 368-373 | 2007 |
| 藤原俊義、田中紀章 | テロメラーゼ特異的ウイルス製剤の癌診断・治療への応用. | ゲノム医学 | 7 | 129-134 | 2007 |
| 藤原俊義、田中紀章 | テロメラーゼ依存性腫瘍融解ウイルス製剤のがん診断・治療への応用. | 日本臨床 | 65 | 1913-1922 | 2007 |

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

*Emiko Sugiyama, Nahoko Kaniwa, Su-Ryang Kim, Ruri Kikura-Hanajiri, Ryuichi Hasegawa, Keiko Maekawa, Yoshiro Saito, Shogo Ozawa, Jun-ichi Sawada, Naoyuki Kamatani, Junji Furuse, Hiroshi Ishii, Teruhiko Yoshida, Hideki Ueno, Takuji Okusaka, and Nagahiro Saijo*

From the Project Team for Pharmacogenetics; Divisions of Medicinal Safety Sciences, Pharmacognosy and Phytochemistry, Biochemistry and Immunochemistry, and Pharmacology, National Institute of Health Sciences; Division of Genomic Medicine, Department of Advanced Biomedical Engineering and Science, Tokyo Women's Medical University; Genetics Division, Research Institute, National Cancer Center, Tokyo; and Hepatobiliary and Pancreatic Oncology Division, National Cancer Center Hospital East, Kashiwa, Japan.

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.

Address reprint requests to Nahoko Kaniwa, PhD, Division of Medicinal Safety Sciences, National Institute of Health Sciences, 1-18-1 Kamiyoga, Setagaya-ku, Tokyo 158-8501, Japan; e-mail: nkaniwa@nihs.go.jp.



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 | IVSI+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 | No. | Frequency | |
| 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 | | | | | | |
| | | | | | | | | | 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 $\chi^2$ 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‡ | GCTCCTGTTTCCC**/-**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 | CCCAGCCCAGCA**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 | GCCAACATCTTC**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 | GTCCTACAAGAT**T/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).

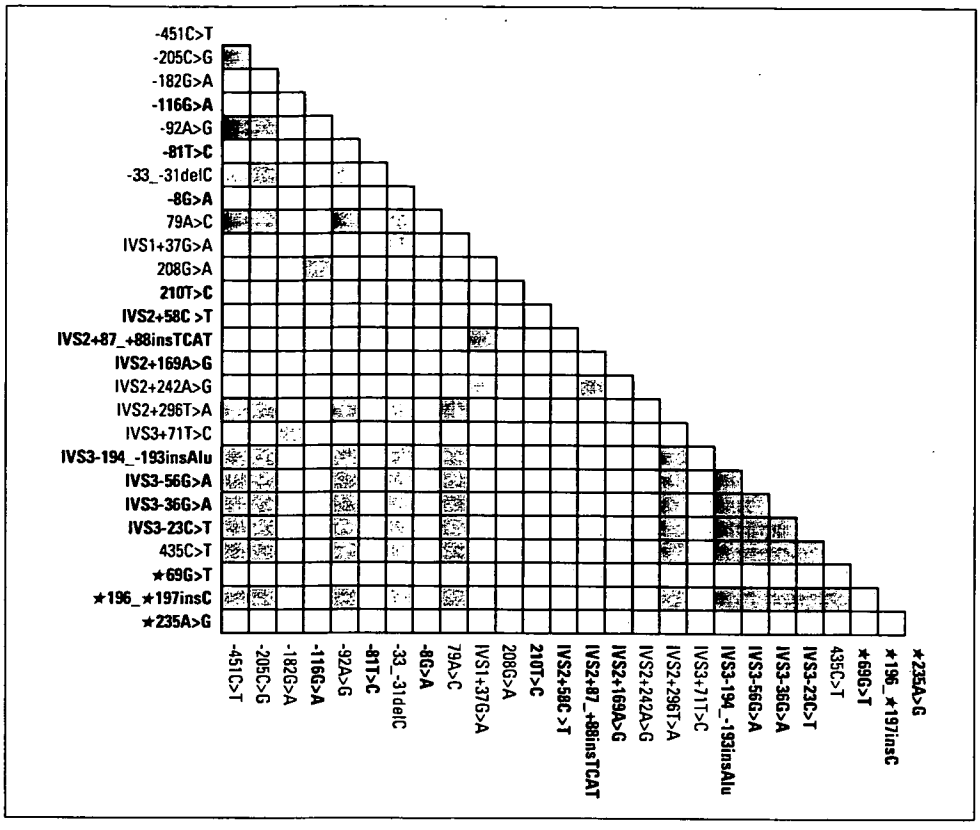


**Fig 1.** Linkage disequilibrium (LD) among 26 *CDA* variations. Pairwise LD as $r^2$ (from 0 to 1) is expressed as 10-graded blue color. The density of the blue color increases with higher linkage rates.

*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 ($\chi^2$ 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) | | AUC (hr · μg/mL) | | $CL/m^2$ (L/hr/$m^2$) | | $Vz/m^2$ (L/$m^2$) | |
|---|---|---|---|---|---|---|---|---|
| | Median | 1/4-3/4 Quantiles | Median | 1/4-3/4 Quantiles | Median | 1/4-3/4 Quantiles | Median | 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 | | | | |
|---|---|---|---|---|---|---|
| | | $C_{max}$ ($\mu$g/mL) | AUC (hr · $\mu$g/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 |
| **1d/*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

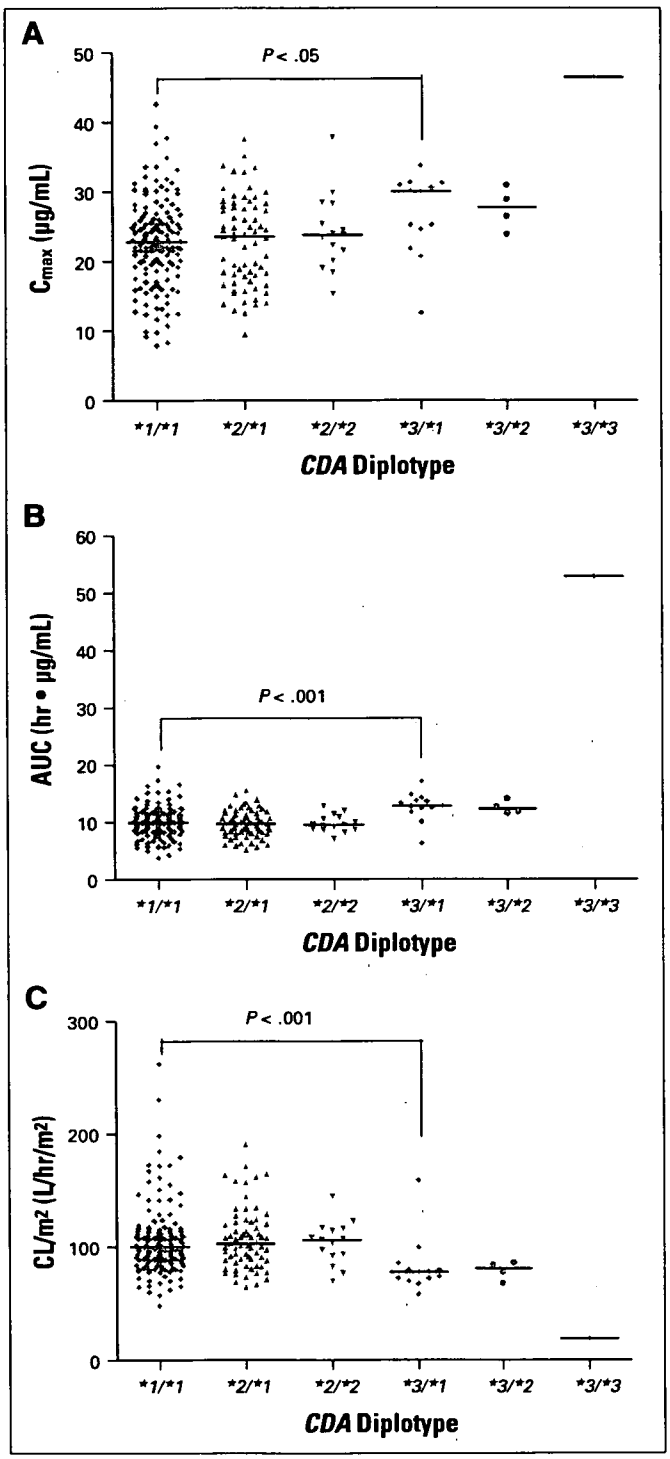


**Fig 2.** Effects of haplotypes *2 and *3 on the pharmacokinetic parameters of gemcitabine. (A) Peak concentration ($C_{max}$) and (B) area under the curve (AUC) were corrected assuming that all patients received 1,000 mg/m$^2$ of gemcitabine. (C) Clearance (CL/m$^2$). Each point corresponds to an individual 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.

## AUTHORS' 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

## AUTHOR CONTRIBUTIONS

**Conception and design:** Nahoko Kaniwa, Shogo Ozawa, Jun-ichi Sawada, Naoyuki Kamatani, Hideki Ueno, Takuji Okusaka, Nagahiro Saijo
**Financial support:** Jun-ichi Sawada, Teruhiko Yoshida, Nagahiro Saijo
**Administrative support:** Nahoko Kaniwa, Ryuichi Hasegawa, Yoshiro Saito, Shogo Ozawa, Jun-ichi Sawada, Teruhiko Yoshida, Nagahiro Saijo
**Provision of study materials or patients:** Keiko Maekawa, Yoshiro Saito, Shogo Ozawa, Junji Furuse, Hiroshi Ishii, Hideki Ueno, Takuji Okusaka
**Collection and assembly of data:** Emiko Sugiyama, Su-Ryang Kim, Ruri Kikura-Hanajiri, Keiko Maekawa
**Data analysis and interpretation:** Emiko Sugiyama, Nahoko Kaniwa, Su-Ryang Kim, Yoshiro Saito, Junji Furuse, Hiroshi Ishii, Hideki Ueno, Takuji Okusaka
**Manuscript writing:** Emiko Sugiyama, Nahoko Kaniwa, Su-Ryang Kim, Hideki Ueno
**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 | | | | Median CDA Activity (units) | | |
|---|---|---|---|---|---|---|---|
| | No. of Patients | $C_{max}$ (μg/mL) | AUC (hr·μg/mL) | $CL/m^2$ (L/hr/$m^2$) | 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; $CL/m^2$, 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.

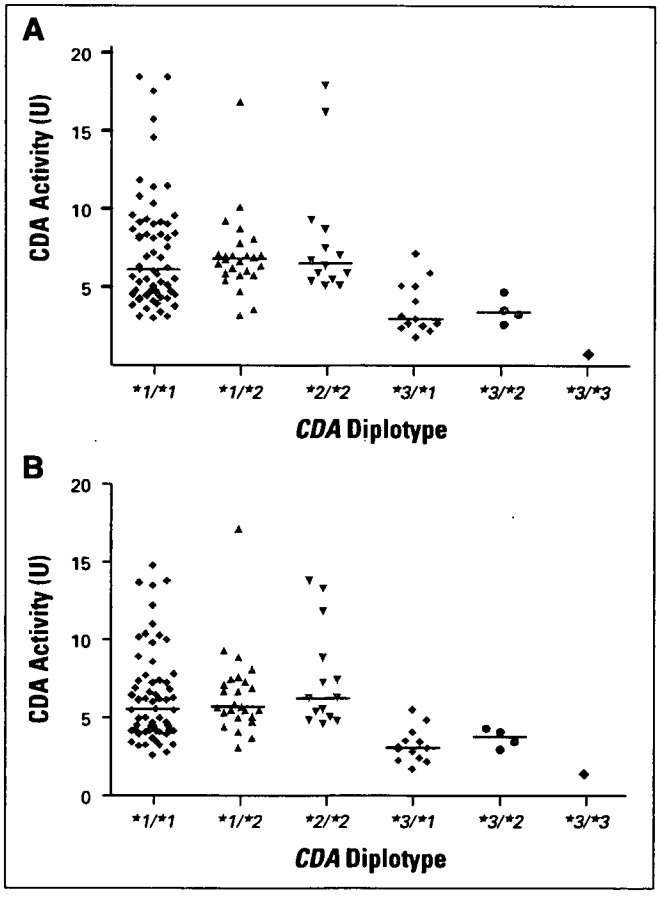


**Fig 3.** Effects of haplotypes **2* and **3* on plasma cytidine deaminase (CDA) activity toward gemcitabine and cytidine substrates. (A) Gemcitabine was used as a substrate, and (B) cytidine was used as a substrate. Each point corresponds to an individual patient. The bars denote the median values.

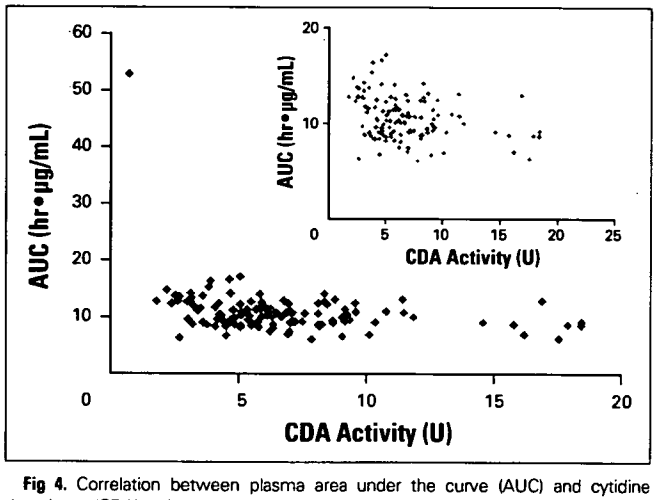


**Fig 4.** Correlation between plasma area under the curve (AUC) and cytidine deaminase (CDA) activity toward gemcitabine. AUC was corrected assuming that all patients received 1,000 mg/$m^2$ of gemcitabine. The inset excludes the data obtained from a homozygous **3* carrier. The correlation coefficient 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 ($P < .05$).
*$\chi^2$-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 mCNT3) 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.

original article

*Annals of Oncology* 18: 317–323, 2007
doi:10.1093/annonc/mdl377
Published online 1 November 2006

# Randomized phase III study of cisplatin plus irinotecan versus carboplatin plus paclitaxel, cisplatin plus gemcitabine, and cisplatin plus vinorelbine for advanced non-small-cell lung cancer: Four-Arm Cooperative Study in Japan

Y. Ohe[1]*, Y. Ohashi[2], K. Kubota[3], T. Tamura[1], K. Nakagawa[4], S. Negoro[5], Y. Nishiwaki[3], N. Saijo[3], Y. Ariyoshi[6] & M. Fukuoka[4]
For the FACS Cooperative Group

[1]*Department of Internal Medicine, National Cancer Center Hospital, Tokyo;* [2]*Department of Biostatistics/Epidemiology and Preventive Health Sciences, School of Health Sciences and Nursing, The University of Tokyo, Tokyo;* [3]*Thoracic Oncology Division, National Cancer Center Hospital East, Kashiwa, Chiba;* [4]*Department of Medical Oncology, Kinki University School of Medicine, Osakasayama, Osaka;* [5]*Department of Thoracic Oncology, Hyogo Medical Center for Adults, Akashi, Hyogo;* [6]*Aichi Cancer Center Aichi Hospital, Okazaki, Aichi, Japan*

*Received 16 May 2006; revised 13 August 2006; accepted 30 August 2006*

**Background:** To compare the efficacy and toxicity of three platinum-based combination regimens against cisplatin plus irinotecan (IP) in patients with untreated advanced non-small-cell lung cancer (NSCLC) by a non-inferiority design.
**Patients and methods:** A total of 602 patients were randomly assigned to one of four regimens: cisplatin 80 mg/m$^2$ on day 1 plus irinotecan 60 mg/m$^2$ on days 1, 8, 15 every 4 weeks (IP) carboplatin AUC 6.0 min × mg/mL (area under the concentration–time curve) on day 1 plus paclitaxel 200 mg/m$^2$ on day 1 every 3 weeks (TC); cisplatin 80 mg/m$^2$ on day 1 plus gemcitabine 1000 mg/m$^2$ on days 1, 8 every 3 weeks (GP); and cisplatin 80 mg/m$^2$ on day 1 plus vinorelbine 25 mg/m$^2$ on days 1, 8 every 3 weeks (NP).
**Results:** The response rate, median survival time, and 1-year survival rate were 31.0%, 13.9 months, 59.2%, respectively, in IP; 32.4%, 12.3 months, 51.0% in TC; 30.1%, 14.0 months, 59.6% in GP; and 33.1%, 11.4 months, 48.3% in NP. No statistically significant differences were found in response rate or overall survival, but the non-inferiority of none of the experimental regimens could be confirmed. All the four regimens were well tolerated.
**Conclusion:** The four regimens have similar efficacy and different toxicity profiles, and they can be used to treat advanced NSCLC patients.
**Key words:** carboplatin, cisplatin, gemcitabine, irinotecan, non-small-cell lung cancer, paclitaxel, randomized phase III study, vinorelbine



## introduction

Nearly 60 000 patients in Japan died of lung cancer in 2004, and the mortality rate is still increasing [1]. Even old-generation cisplatin-based chemotherapy provides a survival benefit and symptom relief in patients with inoperable non-small-cell lung cancer (NSCLC) [2]. Several anticancer agents including irinotecan, paclitaxel, docetaxel, gemcitabine, and vinorelbine, were developed in the 1990s and most of them have mechanisms of action that differ from those of the old-generation agents [3–7]. The combinations of platinum and these new agents developed in the 1990s are more useful against advanced NSCLC than old-generation combination chemotherapy, and doublets of platinum and new-generation anticancer agents are considered standard chemotherapy regimens for advanced NSCLC, although no consistent standard regimens have yet been established [8–17].

Two phase III studies comparing cisplatin plus irinotecan (IP) with cisplatin plus vindesine for advanced NSCLC have been conducted in Japan [18, 19]. Fukuoka et al. [20] reported the results of a combined analysis of the 358 eligible stage IV patients in these studies. They carried out a multivariate analysis using the Cox regression model with adjustment for well-known prognostic factors, and the Cox regression analysis demonstrated that treatment with IP was one of significant independent favorable factor. Based on their data, we selected IP for the reference arm in our study.

The Ministry of Health, Labour and Welfare of Japan approved the prescription of paclitaxel, gemcitabine, and

*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: x+81-3-3542-7006; E-mail: yohe@ncc.go.jp

vinorelbine for NSCLC in 1999 and requested a phase III study to confirm the efficacy and safety of these agents. The Japanese investigators and the pharmaceutical companies decided to conduct a four-arm randomized phase III study for NSCLC, the so-called FACS, Four-Arm Cooperative Study. The purpose of the study was to compare the efficacy and toxicity of three platinum-based combination regimens, carboplatin plus paclitaxel (TC), cisplatin plus gemcitabine (GP), cisplatin plus vinorelbine (NP), with IP as the reference arm.

## patients and methods

### patient selection

Patients with histologically and/or cytologically documented NSCLC were eligible for participation in the study. Each patient had to meet the following criteria: clinical stage IV or IIIB (including only patients with no indications for curative radiotherapy, such as malignant pleural effusion, pleural dissemination, malignant pericardiac effusion, or metastatic lesion in the same lobe), at least one target lesion >2 cm, no prior chemotherapy, no prior surgery and/or radiotherapy for the primary site, age 20–74 years, Eastern Cooperative Oncology Group performance status (PS) of 0 or 1, adequate hematological, hepatic and renal functions, partial pressure of arterial oxygen ($paO_2$) ≥60 torr, expected survival >3 months, able to undergo first course treatment in an inpatient setting, and written informed consent. The study was approved by the Institutional Review Board at each hospital. Written informed consent was obtained from every patient.

### treatment schedule

All patients were randomly assigned to one of the four treatment groups by the central registration office by means of the minimization method. Stage, PS, gender, lactate dehydrogenase (LDH) and albumin values, and institution were used as adjustment variables. The first group received the reference treatment, 80 mg/m$^2$ of cisplatin on day 1 and 60 mg/m$^2$ of irinotecan on days 1, 8, and 15, and the cycle was repeated every 4 weeks. The second group received 200 mg/m$^2$ of paclitaxel (Bristol-Myers K.K., Tokyo, Japan) over a 3-h period followed by carboplatin at a dose calculated to produce an area under the concentration–time curve of 6.0 min × mg/mL on day 1 and the cycle was repeated every 3 weeks. The third group received 80 mg/m$^2$ of cisplatin on day 1 and 1000 mg/m$^2$ of gemcitabine (Eli Lilly Japan K.K., Kobe, Japan) on days 1, 8 and the cycle was repeated every 3 weeks. The fourth group received 80 mg/m$^2$ of cisplatin on day 1 and 25 mg/m$^2$ of vinorelbine (Kyowa Hakko Kogyo Co. Ltd., Tokyo, Japan) on days 1, 8 and the cycle was repeated every 3 weeks. Each treatment was repeated for three or more cycles unless the patient met the criteria for progressive disease or experienced unacceptable toxicity.

### response and toxicity evaluation

Response was evaluated according to the Response Evaluation Criteria in Solid Tumors, and tumor markers were excluded from the criteria [21]. Objective tumor response in all responding patients was evaluated by an external review committee with no information on the treatment group. Toxicity grading criteria in National Cancer Institute Common Toxicity Criteria Ver 2.0 were used to evaluate toxicity.

### quality of life assessment

Quality of life (QoL) was evaluated by means of the Functional Assessment of Cancer Therapy—Lung (FACT-L) Japanese version and the QoL Questionnaire for Cancer Patients Treated with Anticancer Drugs (QoL-ACD), before treatment, immediately before the second cycles of chemotherapy, and 3 and 6 months after the start of treatment [22–24].

### statistical analysis and monitoring

The primary end point of this study was overall survival (OS), and the secondary end points were response rate, response duration, time to progressive disease (TTP), time to treatment failure (TTTF), adverse event, and QoL. The 1-year survival rate of the control group in this study was estimated to be 43% based on the data in published papers, and the 1-year survival rate in the other treatment group was expected to be 50%. The lower equivalence limit for 1-year survival rate was set as '−10%'. The criterion for the non-inferiority of each treatment was a lower limit of the two-sided 95% confidence interval (CI) of the 1-year survival rate of treatment minus that of control larger than the lower equivalence limit. Because the non-inferiority of each treatment versus the control was to be evaluated independently, a separate null hypothesis was stated for each treatment, and for that reason no multiple comparison adjustment was included in the study. Based on the above conditions and binomial distribution, 135 patients were needed per arm for a one-sided Type I error of 2.5% and 80.0% power. In view of the possibility of variance inflation due to censoring, the sample size was set at 600 (150 per arm).

Central registration with randomization, monitoring, data collection, and the statistical analyses were independently carried out by a contract research organization (EPS Co., Ltd, Tokyo, Japan).

## results

### patient characteristics

From October 2000 to June 2002, a total of 602 patients were registered by 44 hospitals in Japan. All patients had been followed up for >2 years, and 447 patients had died as of June 2004. Of the 602 patients registered, 151 were allocated to the reference treatment, IP, and 150, 151, and 150 patients were allocated to TC, GP, and NP, respectively. Since 10 patients did not receive chemotherapy and 11 patients were subsequently found to be ineligible, 592 patients were assessable for toxicity and 581 patients were assessable for efficacy. Four patients did not receive chemotherapy due to electrolytic disorder, fever, symptomatic brain metastases, and rapid tumor progression in IP, two patients due to refusal and pneumonia in TC, four patients due to lower WBC counts (two patients), rapid tumor progression, and nephritic syndrome in NP. Two patents were ineligible due to wrong stage in IP, two patients were wrong stage and one patient had double cancer in TC, two patients were wrong diagnosis, one patient had massive pleural effusion, one patient received prior chemotherapy in GP, one patient had no target lesions in NP. Age, gender, PS, stage, and LDH and albumin values were well balanced in each arm (Table 1). Fewer patients with adenocarcinoma and more patients with squamous cell carcinona were, however, entered in three experimental arms than in IP.

### objective tumor response and response duration

Objective tumor response is shown in Table 2. Forty-five partial responses occurred in the 145 assessable patients in the reference arm, IP, for an objective response rate of 31.0% with a median response duration of 4.8 months. The response rate and median response duration were 32.4% and 4.0 months in TC, 30.1% and 3.5 months in GP, and 33.1% and 3.4 months in NP. The response rates in TC, GP, and NP were not statistically different from the rate in IP according to the results of the $\chi^2$ test.

**Table 1.** Patient characteristics and treatment delivery

| | Cisplatin + irinotecan | Carboplatin + paclitaxel | Cisplatin + gemcitabine | Cisplatin + vinorelbine |
|---|---|---|---|---|
| Assessable patients | 145 | 145 | 146 | 145 |
| Gender (male/female) | 97/48 | 99/46 | 101/45 | 101/44 |
| Age, median (range) | 62 (30–74) | 63 (33–74) | 61 (34–74) | 61 (28–74) |
| PS (0/1) | 44/101 | 44/101 | 45/101 | 45/100 |
| Histology | | | | |
| Adenocarcinoma | 121 | 104 | 108 | 109 |
| Squamous cell carcinoma | 16 | 31 | 29 | 29 |
| Others | 8 | 10 | 9 | 7 |
| Stage (IIIB/IV) | 31/114 | 28/117 | 30/116 | 26/119 |
| No. of cycles | | | | |
| Mean ± SD | 3.0 ± 1.3 | 3.5 ± 1.5 | 3.2 ± 1.2 | 3.1 ± 1.3 |
| Median | 3 | 3 | 3 | 3 |
| Range | 1–7 | 1–10 | 1–7 | 1–8 |

PS, performance status; SD, standard deviation.

**Table 2.** Survival, TTP, TTTF, response rate, and response duration

| | N | Median survival, months | 1-year survival (%) | Difference in 1-year survival from IP | 2-year survival (%) | TTP (median), months | TTTF (median), months | Response rate (%) | Response duration (median), months |
|---|---|---|---|---|---|---|---|---|---|
| Cisplatin + irinotecan | 145 | 13.9 | 59.2 | – | 26.5 | 4.7 | 3.3 | 31.0 | 4.8 ($n$ = 45) |
| Carboplatin + paclitaxel | 145 | 12.3 | 51.0 | −8.2% (95% CI −19.6% to 3.3%) | 25.5 | 4.5 ($P$ = 0.355)[a] | 3.2 ($P$ = 0.282)[a] | 32.4 ($P$ = 0.801)[b] | 4.0 ($n$ = 47) |
| Cisplatin + gemcitabine | 146 | 14.0 | 59.6 | 0.4% (95% CI −10.9% to 11.7%) | 31.5 | 4.0 ($P$ = 0.170)[a] | 3.2 ($P$ = 0.567)[a] | 30.1 ($P$ = 0.868)[b] | 3.5 ($n$ = 44) |
| Cisplatin + vinorelbine | 145 | 11.4 | 48.3 | −10.9% (95% CI −22.3% to 0.5%) | 21.4 | 4.1 ($P$ = 0.133)[a] | 3.0 ($P$ = 0.091)[a] | 33.1 ($P$ = 0.706)[b] | 3.4 ($n$ = 48) |

[a]Compared with IP by the generalized Wilcoxon test.

[b]Compared with IP by the $\chi^2$ test.

CI, confidence interval; IP, cisplatin plus irinotecan; TTP, time to progressive disease; TTTF, time to treatment failure.

## OS, TTP disease, and TTTF

OS and TTP are shown in Figure 1. Median survival time (MST), the 1-year, and 2-year survival rate in IP were 13.9 months, 59.2%, and 26.5%, respectively. The MSTs, 1-year, and 2-year survival rates were, respectively, 12.3 months, 51.0%, and 25.5% in TC; 14.0 months, 59.6%, and 31.5% in GP; and 11.4 months, 48.3%, and 21.4% in NP. The lower limits of the 95% CI of the difference in 1-year survival rate between IP and TC (−19.6%), GP (−10.9%), and NP (−22.3%) were below −10%, which was considered the lower equivalence limit (Table 2). Thus, the results did not show non-inferiority in three experimental regimens compared with reference treatment. Median TTP and median TTTF were 4.7 and 3.3 months, respectively in IP. Median TTP and TTTF were, respectively, 4.5 and 3.2 months in TC, 4.0 and 3.2 months in GP, and 4.1 and 3.0 months in NP. There were no statistical differences in either TTP or TTTF in TC, GP, or NP, compared with IP according to the results of the generalized Wilcoxon test (Table 2).

## hematologic and non-hematologic toxicity

In IP, 47.6% and 83.7% of patients developed grade 3 or worse leukopenia and neutropenia, respectively (Table 3). The incidences of grade 3 or worse leukopenia (33.1%, $P$ = 0.010) and neutropenia (62.9%, $P$ < 0.001) were significantly lower in GP than in IP. The incidence of grade 3 or worse leukopenia (67.1%, $P$ < 0.001) was significantly higher in NP than in IP. Grade 3 or worse thrombocytopenia developed in 5.4% of the patients in IP, and the incidence was significantly higher in GP (35.1%, $P$ < 0.001). The incidence of febril neutropenia in IP was 14.3%, and was significantly lower in GP (2.0%, $P$ < 0.001).

Grade 2 or worse nausea, vomiting, anorexia, and fatigue occurred in 60.5%, 51.0%, 65.3%, and 38.8%, respectively, of the patients in IP. The incidences of grade 2 or worse nausea (TC: 25.0%, $P$ < 0.001, NP: 47.3%, $P$ = 0.022), vomiting (TC: 22.3%, $P$ < 0.001, NP: 36.3%, $P$ = 0.011), and anorexia (TC: 32.4%, $P$ < 0.001, NP: 49.3%, $P$ = 0.005) were significantly lower in TC and NP than in IP. Grade 2 or worse diarrhea was

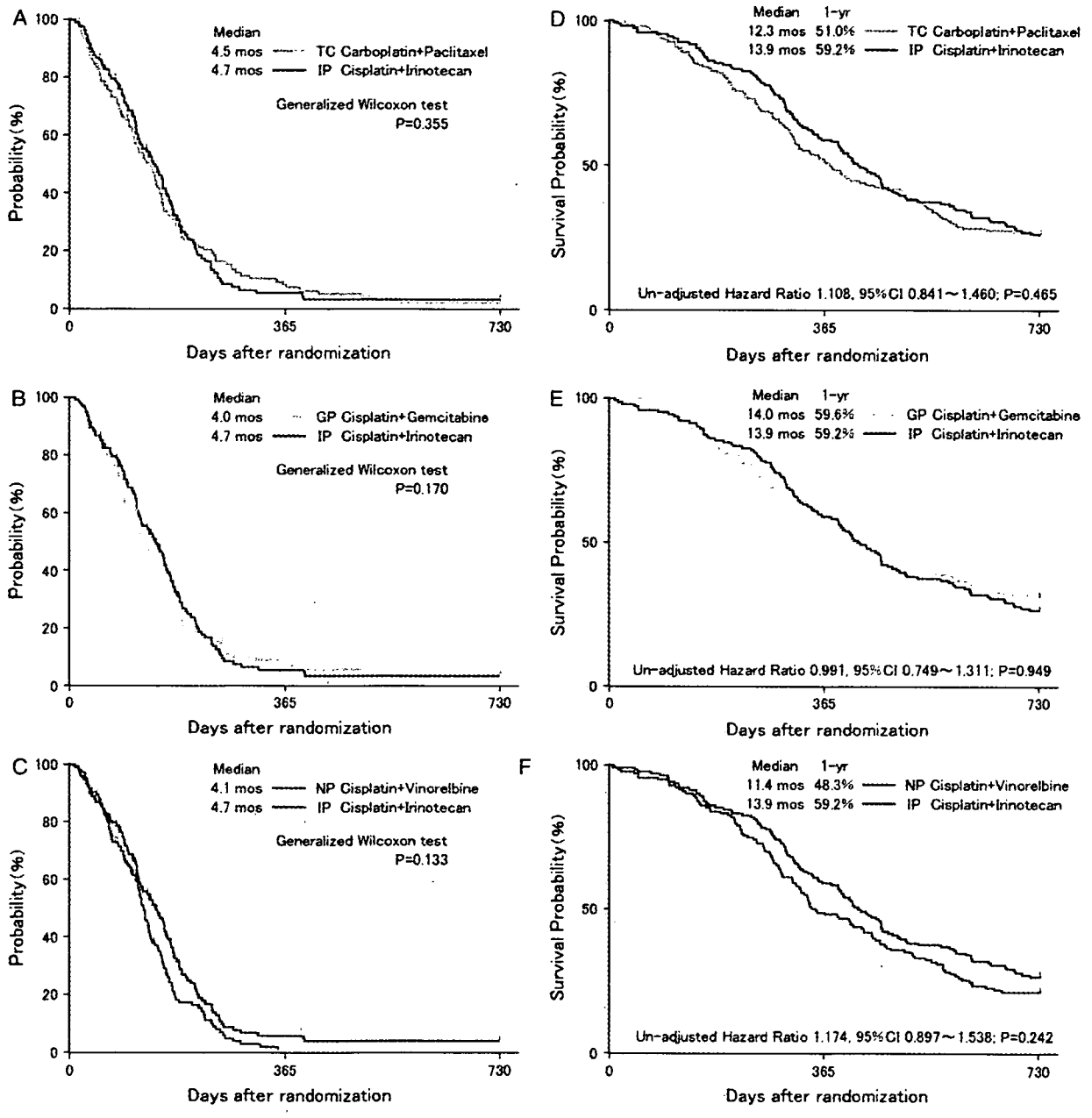


**Figure 1.** Overall survival (OS) and time to progressive (TTP) disease. TTP and OS in the carboplatin plus paclitaxel (TC) (A, D), cisplatin plus gemcitabine (GP) (B, E), and cisplatin plus vinorelbine (NP) (C, F) were not statistically significantly different from the values in the cisplatin plus irinotecan.

significantly less frequent in TC (6.8%), GP (8.6%), and NP (11.6%) than in IP (48.3%, $P < 0.001$). The incidences of grade 2 or worse sensory neuropathy (16.9%, $P < 0.001$), arthralgia (21.6%, $P < 0.001$), and myalgia (17.6%, $P < 0.001$) were significantly higher in TC than in IP. Grade 2 alopecia occurred in 30.6% of the patients in IP, and its incidence was significantly higher in TC (44.6%, $P = 0.013$) and significantly lower in GP (15.2%, $P = 0.001$) and NP (8.9%, $P < 0.001$). Grade 2 injection site reactions were more frequent in NP (26.7%) than in IP (4.8%, $P < 0.001$).

A total of five patients died of treatment-related toxicity: three in IP (cerebral hemorrhage, interstitial pneumonia, acute circulatory failure/disseminated intravascular coagulation: 2.0%), one in TC (acute renal failure: 0.7%), and one in NP (pulmonary embolism: 0.7%).

## second-line treatment

Data on second-line treatment, but not third-line or later treatment, was available in this study, and they showed that

**Table 3.** Toxicity

| | IP ($n = 147$) | | | TC ($n = 148$) | | | GP ($n = 151$) | | | NP ($n = 146$) | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Grade (%) | | | Grade (%) | | | Grade (%) | | | Grade (%) | | |
| | 2 | 3 | 4 | 2 | 3 | 4 | 2 | 3 | 4 | 2 | 3 | 4 |
| Leukocytes | 42 | 43 | 5 | 39 | 42 | 3 | 40 | 31[a] | 2[a] | 25 | 51[b] | 16[b] |
| Neutrophils | 11 | 39 | 45 | 5 | 19 | 69 | 21 | 40 | 23[a] | 5 | 16 | 72 |
| Hemoglobin | 42 | 24 | 7 | 42 | 13[a] | 2[a] | 44 | 22 | 5 | 43 | 25 | 5 |
| Platelets | 6 | 5 | 1 | 9 | 11 | 0 | 22 | 35[b] | 0[b] | 3 | 1[a] | 0[a] |
| Febrile neutropenia | – | 14 | 0 | – | 18 | 0 | – | 2[a] | 0[a] | – | 18 | 0 |
| Nausea | 32 | 29 | – | 14[c] | 11[c] | – | 35 | 23 | – | 33[c] | 14[c] | – |
| Vomiting | 38 | 13 | 0 | 17[c] | 5[c] | 0[c] | 34 | 14 | 0 | 29[c] | 7[c] | 0[c] |
| Anorexia | 30 | 33 | 2 | 15[c] | 17[c] | 1[c] | 31 | 26 | 1 | 29[c] | 20c | 1[c] |
| Fatigue | 27 | 12 | 1 | 26 | 2 | 1 | 17[c] | 3[c] | 0[c] | 23[c] | 3[c] | 0[c] |
| Diarrhea | 33 | 15 | 1 | 4[c] | 3[c] | 0[c] | 7[c] | 2[c] | 0[c] | 8[c] | 4[c] | 0[c] |
| Constipation | 27 | 7 | 0 | 30 | 8 | 0 | 33 | 9 | 0 | 40[d] | 14[d] | 0[d] |
| Neuropathy, motor | 1 | 0 | 0 | 1 | 1 | 1 | 0 | 0 | 0 | 0 | 0 | 0 |
| Neuropathy, sensory | 1 | 0 | 0 | 14[d] | 3[d] | 0[d] | 0 | 0 | 0 | 0 | 0 | 0 |
| Alopecia | 31 | – | – | 45[d] | – | – | 15[c] | – | – | 9[c] | – | – |
| Arthralgia | 2 | 0 | 0 | 20[d] | 2[d] | 0[d] | 0 | 0 | 0 | 1 | 0 | 0 |
| Myalgia | 1 | 0 | 0 | 16[d] | 2[d] | 0[d] | 0 | 0 | 0 | 1 | 1 | 0 |
| Injection site reaction | 5 | 0 | – | 5 | 0 | – | 5 | 0 | – | 27[d] | 0[d] | – |
| Pneumonitis | 0 | 1 | 1 | 0 | 1 | 0 | 0 | 0 | 0 | 0 | 1 | 0 |
| Creatinine | 8 | 1 | 0 | 2[c] | 0[c] | 0[c] | 7 | 0 | 0 | 8 | 1 | 0 |
| AST | 7 | 1 | 1 | 5 | 1 | 0 | 6 | 3 | 0 | 1 | 3 | 0 |
| Fever | 2 | 0 | 0 | 5 | 1 | 0 | 1 | 0 | 0 | 1 | 0 | 0 |
| Treatment-related death | 3 (2.0%) | | | 1 (0.7%) | | | 0 | | | 1 (0.7%) | | |

[a]Incidence of grade 3 or 4 toxicity significantly ($P < 0.05$) lower than that with IP.
[b]Incidence of grade 3 or 4 toxicity significantly ($P < 0.05$) higher than that with IP.
[c]Incidence of grade 2 or worse toxicity is significantly ($P < 0.05$) lower than that with IP.
[d]Incidence of grade 2 or worse toxicity significantly ($P < 0.05$) higher than that with IP.
GP, cisplatin plus gemcitabine; IP, cisplatin plus irinotecan; NP, cisplatin plus vinorelbine; TC, carboplatin plus paclitaxel.
AST, aspartate aminotransferase; –, no category in the criteria.

60%–74% of the patients received chemotherapy and 6%–9% received thoracic irradiation as second-line treatment (Table 4). The percentages of patients in each treatment group who received second-line chemotherapy were not significantly different ($P = 0.081$).

### quality of life

The details of the QoL analysis will be reported elsewhere. No statistically significant difference in global QoL was observed among the four treatment groups based on either the FACT-L Japanese version or the QoL-ACD. Only the physical domain evaluated by QoL-ACD was significantly better in TC, GP, and NP than in IP.

## discussion

Many randomized phase III studies have compared platinum-plus-new-agent doublets in NSCLC, but, this is the first to evaluate the efficacy of an irinotecan-containing regimen in comparison with other platinum-plus-new-agent doublets in NSCLC [14–17]. Although non-platinum-containing chemotherapy regimens are used as alternatives, doublets of platinum and a new-generation anticancer agent, such as TC, GP, and NP, are considered standard chemotherapy regimens for advanced NSCLC worldwide [13–17, 25]. Although the non-inferiority of none of the three experimental regimens could be confirmed in this study, no statistically significant differences in response rate, OS, TTP, or TTTF were observed between the reference regimen and the experimental regimens. All four platinum-based doublets have similar efficacy against advanced NSCLC but different toxicity profiles. Nevertheless, IP was still regarded as the reference regimen in this study because the non-inferiority of none of the three experimental regimens could be confirmed.

OS in this study was relatively longer than previously reported. The estimated 1-year survival rate in the reference arm was 43%, but the actual 1-year survival rate was 59.2%, much higher than expected. The MSTs reported for patients treated with TC, GP, and NP in recent phase III studies have ranged from 8 to 10 months, and in the present study they were 12.3, 14.0, and 11.4 months, respectively [14–17]. One reason for the good OS in this study was the difference in patient selection criteria, for example exclusion of PS2 patients. Ethnic differences in pharmacogenomics have also been indicated as a possible reason for the good OS in this study [26]. The OS in IP in this study, however, was better than in previous Japanese studies [18, 19]. TTP in this study ranged from 4.0 to 4.7 months, and was similar to the TTP of 3.1–5.5 months reported in the literature [15, 16]. OS not TTP was longer in this study

**Table 4.** Second-line treatment

| | Cisplatin + irinotecan | Carboplatin + paclitaxel | Cisplatin + gemcitabine | Cisplatin + vinorelbine | |
|---|---|---|---|---|---|
| Number of patients | 145 | 145 | 146 | 145 | |
| Chemotherapy | 107 (74%) | 87 (60%) | 101 (69%) | 95 (66%) | $P = 0.081$ |
| Docetaxel | 39 | 25 | 50 | 51 | |
| Gefitinib | 11 | 9 | 18 | 12 | |
| Paclitaxel | 15 | 14 | 7 | 11 | |
| Gemcitabine | 24 | 28 | 17 | 28 | |
| Vinorelbine | 9 | 12 | 2 | 9 | |
| Irinotecan | 15 | 4 | 3 | 3 | |
| Thoracic irradiation | 8 | 10 | 13 | 10 | |

than previously reported, and higher 2-year survival rates, 21.4%–31.5%, were observed in the minimum 2-year follow-up in this study. Second-line or later treatments may affect survival, because docetaxel has been established as standard second-line chemotherapy for advanced NSCLC [27, 28]. Gefitinib is also effective as second-line or later chemotherapy for advanced NSCLC, especially in Asian patients, never smokers and patients with adenocarcinoma [29–32].

The toxicity profile of each treatment differed and the toxicity of all four regimens was well tolerated. Overall QoL was similar in the four platinum-based doublets. Only physical domain QoL evaluated by the QoL-ACD was statistically better in TC, GP, and NP than in IP. This finding is presumably attributable to the fact that diarrhea is a statistically less frequent adverse effect of TC, GP, and NP than of IP.

In conclusion, all four platinum-based doublets had similar efficacy for advanced NSCLC but different toxicity profiles. All the four regimens can be used to treat advanced NSCLC patients in clinical practice.

## appendix

Institutions of the FACS Cooperative Group: National Hospital Organization (NHO) Hokkaido Cancer Center, Tohoku University Hospital, Yamagata Prefectural Central Hospital, Niigata Cancer Center Hospital, Tochigi Cancer Center, NHO Nishigunma National Hospital, Saitama Cancer Center, National Cancer Center Hospital East, Chiba University Hospital, National Cancer Center Hospital, Tokyo Medical University Hospital, Japanese Foundation for Cancer Research, Kanagawa Cancer Center, Yokohama Municipal Citizen's Hospital, Kanagawa Cardiovascular and Respiratory Center, Aichi Cancer Center Hospital, Prefectural Aichi Hospital, Nagoya City University Hospital, NHO Nagoya Medical Center, Nagoya University Hospital, Gifu Municipal Hospital, NHO Kyoto Medical Center, Osaka City General Hospital, Osaka City University Hospital, Osaka Medical Center for Cancer and Cardiovascular Diseases, NHO Toneyama Hospital, Osaka Prefectural Medical Center for Respiratory and Allergic Diseases, Kinki University School of Medicine, Rinku General Medical Center Izumisano Municipal Hospital, Kobe Central General Hospital, The Hospital of Hyogo College of Medicine, Hyogo Medical Center for Adults, Tokushima University Hospital, Kagawa Prefectural Central Hospital, NHO Shikoku Cancer Center Hospital, Hiroshima University Medical Hospital, NHO Kyushu Cancer Center Hospital, Kyushu University Hospital, National Nagasaki Medical Center, Nagasaki Municipal Hospital, Nagasaki University Hospital of Medicine and Dentistry, Kumamoto Chuo Hospital, Kumamoto Regional Medical Center, NTT West Osaka Hospital.

## acknowledgements

This study was supported by Bristol-Myers K.K., Tokyo; Eli Lilly Japan K.K., Kobe; and Kyowa Hakko Kogyo Co. Ltd, Tokyo, Japan.

## references

1. Cancer Statistics in Japan 2005: The Editorial Board of the Cancer Statistics in Japan. Tokyo, Japan: Foundation for Promotion of Cancer Research 2005.
2. Non-Small Cell Lung Cancer Collaborative Group. Chemotherapy in non-small cell lung cancer: a meta-analysis using updated data on individual patients from 52 randomised clinical trials. BMJ 1995; 311: 899–909.
3. Fukuoka M, Niitani H, Suzuki A et al. A phase II study of CPT-11, a new derivative of camptothecin, for previously untreated non-small-cell lung cancer. J Clin Oncol 1992; 10: 16–20.
4. Rowinsky EK, Donehower RC. Paclitaxel (taxol). N Engl J Med 1995; 332: 1004–1014.
5. Gelmon K. The taxoids: paclitaxel and docetaxel. Lancet 1994; 344: 1267–1272.
6. Hertel LW, Border GB, Kroin JS et al. Evaluation of the antitumor activity of gemcitabine (2′,2′-difluoro-2′-deoxycytidine). Cancer Res 1990; 50: 4417–4422.
7. Binet S, Fellous A, Lataste H et al. Biochemical effects of navelbine on tubulin and associated proteins. Semin Oncol 1989; 16 (2 Suppl 4): 9–14.
8. Kubota K, Watanabe K, Kunitoh H et al. Phase III randomized trial of docetaxel plus cisplatin versus vindesine plus cisplatin in patients with stage IV non-small-cell lung cancer: the Japanese Taxotere Lung Cancer Study Group. J Clin Oncol 2004; 22: 254–261.
9. Le Chevalier T, Brisgand D, Douillard JY et al. Randomized study of vinorelbine and cisplatin versus vindesine and cisplatin versus vinorelbine alone in advanced non-small cell lung cancer: results of a European multicenter trial including 612 patients. J Clin Oncol 1994; 12: 360–367.
10. Belani CP, Lee JS, Socinski MA et al. Randomized phase III trial comparing cisplatin-etoposide to carboplatin-paclitaxel in advanced or metastatic non-small cell lung cancer. Ann Oncol 2005; 16: 1069–1075.
11. Yana T, Takada M, Origasa H et al. New chemotherapy agent plus platinum for advanced non-small cell lung cancer: a meta-analysis. Proc Am Soc Clin Oncol 2002; 21: 328a.
12. Baggstrom MQ, Socinski MA, Hensing TA et al. Third generation chemotherapy regimens (3GR) improve survival over second generation regimens (2GR) in stage IIIB/IV non-small cell lung cancer (NSCLC): a meta-analysis of the published literature. Proc Am Soc Clin Oncol 2002; 21: 306a.