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ORIGINAL REPORT

Phase II Trial of Preoperative Chemoradiotherapy Followed by Surgical Resection in Patients With Superior Sulcus Non–Small-Cell Lung Cancers: Report of Japan Clinical Oncology Group Trial 9806

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A B S T R A C T

Purpose
To evaluate the safety and efficacy of preoperative chemoradiotherapy followed by surgical resection for superior sulcus tumors (SSTs).

Patients and Methods
Patients with pathologically documented non–small-cell lung cancer with invasion of the first rib or more superior chest wall were enrolled as eligible; those with distant metastasis, pleural dissemination, and/or mediastinal node involvement were excluded. Patients received two cycles of chemotherapy every 4 weeks as follows; mitomycin 8 mg/m^2 on day 1, vindesine 3 mg/m^2 on days 1 and 8, and cisplatin 80 mg/m^2 on day 1. Radiotherapy directed at the tumor and the ipsilateral supraclavicular nodes was started on day 2 of each course, at the total dose of 45 Gy in 25 fractions, with a 1-week split. Thoracotomy was undertaken 2 to 4 weeks after completion of the chemoradiotherapy. Those with unresectable disease received boost radiotherapy.

Results
From May 1999 to November 2002, 76 patients were enrolled, of whom 20 had T4 disease; 75 patients were fully assessable. Chemoradiotherapy was generally well tolerated. Fifty-seven patients (76%) underwent surgical resection, and pathologic complete resection was achieved in 51 patients (68%). There were 12 patients with pathologic complete response. Major postoperative morbidity, including chylothorax, empyema, pneumonitis, adult respiratory distress syndrome, and bleeding, was observed in eight patients. There were three treatment-related deaths, including two deaths owing to postsurgical complications and one death owing to sepsis during chemoradiotherapy. The disease-free and overall survival rates at 3 years were 49% and 61%, respectively; at 5 years, they were 45% and 56%, respectively.

Conclusion
This trimodality approach is safe and effective for the treatment of patients with SSTs.

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INTRODUCTION

Superior sulcus tumors (SSTs), involving structures at the thoracic inlet, represent a small subtype of non–small-cell lung carcinoma (NSCLC). These SSTs, first described by Henry Pancoast[1,2] and thus also called Pancoast tumors, have posed a challenging problem for surgeons, radiation oncologists, and medical oncologists alike, ever since they were first described.[3]

Preoperative radiotherapy has long been the community standard in the management of SSTs.[4-17] However, both the complete resection rate (approximately 50%) and long-term survival rate (approximately 30%) have remained poor and unchanged over the last 40 years, since the first treatment strategy was reported in the 1960s. Local control has remained the main problem,[15,17,18] adversely affecting quality of life as well as survival of patients. Presence of mediastinal lymph node metastasis (N2 status) has been reported to be associated with a particularly poor prognosis.[9,18]

However, a series of clinical trials over the last two decades have shown concurrent chemoradiotherapy to be beneficial in the treatment of unresectable stage III NSCLC.[19-21] The addition of chemotherapy to thoracic radiotherapy seems to suppress distant micrometastases,[22,23] and giving

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concurrent chemotherapy with radiotherapy has been shown to yield improved local control[19,24] with survival benefit.

Encouraged by the promising data of concurrent chemoradiotherapy for N2 NSCLC, the Southwest Oncology Group (SWOG) applied this modality as preoperative therapy for patients with SSTs (SWOG 9416, Intergroup Trial 0160), and reported favorable results.[25]

The Japan Clinical Oncology Group (JCOG) launched another trial of this preoperative concurrent chemoradiotherapy, or the trimodality approach, for the treatment of SSTs in 1999, before the first report of SWOG 9416 was published. Our study was initiated to evaluate the safety and efficacy of this treatment strategy in this rare subset of patients with NSCLC. As the induction treatment, we used mitomycin, vindesine, and cisplatin (MVP) combination chemotherapy, which has been demonstrated to be safe and effective for concurrent chemotherapy with thoracic radiotherapy in Japanese trials.[19]

PATIENTS AND METHODS

Eligibility Criteria

Patients with untreated histologically or cytologically documented NSCLC involving the superior sulcus with clinical stage T3 or T4 disease were eligible for entry onto this study. T4 diseases included tumor invasion to the spine (including to a transverse process of vertebra), aorta, or superior vena cava; invasion to the chest wall or subclavian vessels was included in T3 disease. Involvement of the superior sulcus was confirmed by computed tomographic (CT) or magnetic resonance imaging (MRI) evidence of tumor invasion of the first rib or more superior chest wall. Patients with pleural or pericardial dissemination, malignant effusion, and/or distant metastasis (M1) were excluded. Those with clinical N2 disease (mediastinal node involvement) were also excluded; all mediastinal nodes measuring $\geq$ 1.0 cm in size on CT images were required to be biopsied and documented to be negative for metastasis before patient enrollment. However, those with ipsilateral supraclavicular node involvement (N3) were eligible, unless it was accompanied by mediastinal node metastasis. Each patient was required to fulfill the following criteria: 15 to 74 years of age, Eastern Cooperative Oncology Group performance status of 0 to 1; adequate organ function (ie, leukocyte count $\geq$ 4,000/μL, platelet count $\geq 10^5/\mu$L, hemoglobin $\geq$ 11.0 g/dL, serum creatinine less than 1.5 mg/dL, creatinine clearance $\geq$ 60 mL/min, serum bilirubin less than 1.5 mg/dL, serum ALT and AST less than double the upper limit of the institutional normal range, arterial partial pressure of oxygen $\geq$ 70 mmHg, and predicted postoperative forced expiratory volume in 1 second $\geq$ 0.8 L. From July 2001, when the protocol was revised after the death of a patient from septic shock during chemoradiotherapy, those patients with systemic use of corticosteroids were excluded.

Patient eligibility was confirmed by the JCOG Data Center before patient registration. This study was approved by the institutional review boards at each participating center, and written informed consent was obtained from all patients.

Treatment Plan

Induction chemotherapy. Patients received two courses of MVP combination chemotherapy with a 4-week interval in between. Mitomycin was administered at 8 mg/m^2 on chemotherapy day 1, and vindesine was administered at 3 mg/m^2 on days 1 and 8; both were administered as bolus injections. Cisplatin was administered at 80 mg/m^2 as a 2-hour infusion on day 1, with ample hydration and antiemetic administration.

The second cycle of chemotherapy was postponed until all the severe toxicities recovered to grade 1 or 0. If the second cycle could not be started within 2 weeks of the due date, it was canceled, and the patient received only preoperative radiotherapy, if possible.

Induction radiotherapy. Thoracic radiotherapy was started with a linear accelerator ($\geq$ 4 MeV) on chemotherapy day 2. The first session was scheduled to be given with the first chemotherapy cycle at 27 Gy in 15 fractions over 3 weeks. Then the second session was started after a week's interval until day 2 of the second course of chemotherapy. The second session, given with the second cycle of MVP, was administered at 18 Gy in 10 fractions over 2 weeks. The total radiation dose was thus 45 Gy in 25 fractions administered over 6 weeks, including the 1-week split, or interval, between the two sessions; this schedule, including the split, basically followed that of the original method reported by Furuse et al.[19] The radiation field included the primary tumor and the ipsilateral supraclavicular nodes. The mediastinal and hilar nodes were not irradiated, even in cases with hilar node involvement (clinical N1 cases).

Surgery. After the induction chemoradiotherapy, each case was reevaluated to determine the clinical response and resectability. The resectability of the tumor was determined by the multimodality team of each institution, irrespective of the clinical response (tumor shrinkage). Surgical resection of the tumor was performed 2 to 4 weeks after the completion of the induction therapy. The surgical procedures undertaken included lobectomy or pneumonectomy, with systematic node dissection. Standard systematic node dissection, ND2, includes complete removal of the hilar and mediastinal nodes. Less complete dissection includes ND0 (ie, no systematic dissection with or without lymph node sampling) or ND1 (ie, hilar node dissection with or without mediastinal lymph node sampling).

Boost therapy. For unresected or incompletely resected cases, boost radiotherapy of 21.6 Gy in 12 fractions was given. Those who were judged to have undergone complete resection were followed up without additional therapy until clinical evidence of recurrence.

Patient Evaluation and Follow-Up

Before enrollment onto the study, each patient underwent complete medical history taking and physical examination, blood cell count determinations, serum biochemistry testing, arterial blood gas analysis, chest x-ray, ECG, CT scan of the chest, bronchoscopy, CT scan or ultrasound of the upper abdomen, whole-brain CT or MRI, and an isotope bone scan. Chest MRI was recommended for evaluation of the local tumor status but was not mandatory. Blood cell counts, serum biochemistry testing, and chest x-ray were performed weekly during each course of chemotherapy. Chest CT was performed every 3 to 4 weeks during the induction therapy.

Chemotherapy toxicity was evaluated according to the JCOG Toxicity Criteria,[26] modified from the National Cancer Institute Common Toxicity Criteria version 1. Tumor responses were assessed radiographically according to the standard, two-dimensional WHO criteria[27] and were classified into complete response (CR), partial response, no change, progressive disease (PD), and not assessable. Response confirmation at 4 weeks or longer intervals was not necessitated. After curative resection and/or definitive boost radiotherapy, the patients were followed up with periodic re-evaluation, including with chest CT, as well as a systemic survey every 6 months for the first 3 years.

Central Review

Radiographic reviews for eligibility of the enrolled patients and the clinical responses were performed at the time of the JCOG Lung Cancer Surgical Study Group meeting, held every 3 to 4 months. The study coordinator (H.K., a medical oncologist), the group coordinator (M.T., a surgical oncologist), and a few selected investigators of the group reviewed the radiographic films. The clinical response data presented below were all confirmed by this central review.

Statistical Considerations

The primary end point of the study was the survival rate at 3 years. The sample size calculation was performed, as described in Appendix 1 (online only).

Secondary end points included the objective tumor response to chemotherapy, complete resection rate, and postsurgical morbidity/mortality. Both overall survival (OS) and progression-free survival (PFS) were calculated from the date of enrollment by the Kaplan-Meier method. For exploratory analysis to identify prognostic factors, the OS or PFS of subgroups was compared by two-sided log-rank tests. All analyses were performed with the SAS software version 8.2 (SAS Institute, Cary, NC).

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RESULTS

Patient Characteristics

From May 1999 to November 2002, 76 patients from 19 institutions were enrolled onto the study. Three patients were ineligible. One patient was found to have concomitant anemia and did not receive the protocol treatment. Two others were found ineligible by the central review, after completion of the protocol therapy; the tumor was judged not to involve the first rib in one case, and in the other, a mediastinal node was judged to be enlarged on chest CT, without confirmation by mediastinoscopy. These two cases were included in the analysis. Therefore, 75 patients were analyzed to determine the toxicities, response rates, surgical and pathologic results, PFS, and OS. All 76 patients were included in the analysis of the patient characteristics, as shown in Table 1. In each of the T4 cases, the tumor was judged to have involved the spine. Nodal status was clinically determined and was pathologically confirmed in only a few cases.

Induction Therapy Delivery and Toxicity

The study schema with the actual numbers of patients receiving the protocol therapy is shown in Appendix Figure A1 (online only). The induction therapy could be completed in 71 (95%) of the 75 patients. The treatment was terminated in the remaining four patients after only one course of chemotherapy (owing to the development of adverse events in two cases, patient refusal in one case, and early toxicity-related death in one case).

Table 2 lists the major toxicities of the protocol therapy. They were mainly hematologic, and although more than 80% of the patients experienced neutropenia/leukopenia, they were generally transient and not complicated by infection/fever. Overall, toxicities were well tolerated. There was one toxic death on chemoradiotherapy day 6 as a result of severe myelosuppression and subsequent development of septic shock.

Clinical Response to the Induction Therapy

The clinical responses of the 75 eligible patients to induction therapy were judged radiologically and confirmed by the central review. The responses were as follows: CR, 0 patients; partial response, 46 patients; no change, 22 patients; PD, five patients; not assessable, two patients. The overall response rate was 61% (95% CI, 49% to 72%).

Surgical and Pathologic Results

Thoracotomy was performed in 57 (76%) of the 75 patients who received the induction therapy. The surgical procedures undertaken

Table 1. Patient Characteristics (n = 76)

Characteristic	No. of Patients	%
Sex		
Male	67	88
Female	9	12
Age, years		
Median	57.5	
Range	34-74	
ECOG performance status		
0	30	39
1	46	61
Clinical T stage		
T3	56	74
T4	20	26
Clinical N stage		
N0	59	78
N1	9	12
N2*	1	1
N3	7	9
Smoking history		
No	4	5
Yes	72	95
Median smoking history	1.5 packs for 37 years	
Body weight loss within 6 months		
≤ 5%	61	80
5-10%	7	9
> 10%	5	7
Missing	3	4
Histology		
Adenocarcinoma	34	45
Squamous cell carcinoma	27	36
Others/unclassified	15	20
Primary site		
Right	39	51
Left	37	49

Abbreviation: ECOG, Eastern Cooperative Oncology Group.
*Found ineligible by central review but included in the subsequent analyses.

Table 2. Major Toxicities of Induction Therapy (N = 75) and Postsurgical Complications

Toxicity or Complication	No. of Patients: Grade 1/2	Grade 3	Grade 4	% Grade 3/4
Acute toxicity*				
Leukopenia	1/11	37	26†	84
Neutropenia	3/9	26	36†	83
Anemia	19/47	5	0	7
Thrombocytopenia	14/12	9	2†	15
ALT	27/5	2	0	3
Creatinine	18/2	0	0	0
PaO_2	37/6	0	0	0
Emesis	32/25	2	— (not defined)	3
Diarrhea	7/5	1	0	1
Constipation	22/3	1	0	1
Esophagitis	22/9	0	0	0
Infection	10/9	6	1†	9
Neuropathy	8/0	0	— (not defined)	0
Skin toxicity	16/2	1	0	1
Fever	25/19	1	1	3
Postsurgical complications‡				
ARDS	0	1	1 (grade 5)	
Empyema	0	2	0	
Cylothorax	1	1	0	
Pneumonitis	0	1	0	
Late complications‡				
Pneumonitis	0	1	0	
Bleeding	0	0	1 (grade 5)	

Abbreviations: PaO_2, alveolar-arterial difference in partial pressure of oxygen; ARDS, adult respiratory distress syndrome.
*During induction therapy.
†Includes one patient with toxic death owing to septic shock.
‡Report of each complication was evaluated by National Cancer Institute Common Toxicity Criteria version 3.0.

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were as follows: lobectomy, 53 patients; partial resection, three patients; exploratory thoracotomy, one patient; none of the cases required pneumonectomy. Combined resection of the chest wall was undertaken in 51 of the 57 patients. Complete mediastinal lymph node dissection (ND2) was performed in 42 patients, and the remaining 15 patients underwent less extensive dissection or sampling (ND0 or ND1).

The results of thoracotomy were as follows: gross residual tumor (R2 resection, including one with probe thoracotomy), three patients; microscopically residual tumor on pathologic review (R1 resection), three patients; complete surgical and pathologic resection (R0 resection), 51 patients. Pathologic downstaging of the tumor as compared with the clinical stage before induction therapy was achieved in 23 patients (40% of the patients who underwent surgery); this is an inherently inaccurate figure and should be interpreted as such, owing to the lack of pathologic confirmation of the c stage at presentation. Pathologic CR, with no residual viable tumor cells in the resected specimens, was achieved in 12 patients (16% of the 75 treated patients). Table 3 lists the surgical and pathologic results according to the initial clinical T factor.

The major postoperative morbidities included adult respiratory distress syndrome (ARDS) in two patients, empyema in two patients, chylothorax in two patients, and pneumonitis in two patients. One patient died of sudden major bleeding on postoperative day 24. The bleeding was identified at autopsy as being from an intercostal artery. Another patient died of ARDS after off-protocol pneumonectomy. The patient had been judged to have PD in response to the induction therapy as a result of emergence of intrapulmonary metastases. The attending surgeon and the patient agreed to salvage surgery, and the patient developed postoperative ARDS.

Thus the total number of toxic deaths was three, including one caused by septic shock during the induction, one by delayed postoperative bleeding, and one by the development of ARDS after off-protocol, salvage surgery.

Table 3. Surgical and Pathologic Results According to Initial Clinical T Stage

Characteristic	c-T3	c-T4
No. of patients	55	20
No surgery performed		
No.	7	11
%	13	55
Reason for no surgery		
Protocol violation	0	1
Toxic death	0	1
Adverse event	0	1
Progressive disease	2	2
Judged unresectable	0	3
Patient refusal	5	3
Surgical procedures		
Thoracotomy		
No.	48	9
%	87	45
Pneumonectomy	0	0
Lobectomy	45	8
Probe thoracotomy	1	0
Other	2	1
With combined resection	44	7
Rib	38	6
Parietal pleura	4	1
Vertebra	3	3
Major vessel	3	0
Clavicle	1	0
Completeness of resection		
R2 operation	2	1
R1 operation	3	0
R0 operation		
No.	43	8
%	78	40
Pathologic results		
Downstaging	18	5
Pathlogic complete response	9	3

Boost Therapy

Boost radiotherapy was given to 15 patients, including 12 of the 15 patients in whom thoracotomy was not performed after the completion of induction chemoradiotherapy. One patient received boost radiotherapy after grossly incomplete resection, and another received boost radiotherapy after gross complete resection with microscopically residual disease. In 12 of the 15 patients, boost radiotherapy was completed with a total dose of 66.6 Gy.

PFS and OS

Figures 1 and 2 show the PFS and OS curves, updated in November 2006. Forty-one patients were alive, with a median follow-up period of 68 months. The median PFS time was 28 months. The PFS rates at 3 and 5 years were 49% and 45%, respectively. The median OS has not yet been reached. The OS at 3 and 5 years were 61% and 56%, respectively. Subset analysis (Appendix Figs A2 through A5, online only) revealed that clinical T stage was a prognostic factor (Appendix Fig A2). Patients with clinical T3 disease had better outcome than those with clinical T4 disease (the survival rates at 3 and 5 years were 69% and 61%, respectively, versus 40% and 40%, respectively; log-rank $P = .031$). The clinical N stage and histologic type of the tumor did not significantly affect the OS (Appendix Figs A3 and A4) or PFS. As expected, the survival rate was good in patients in whom complete resection could be achieved, with a projected 5-year OS of 70% as compared with 24% in whom complete resection could not be

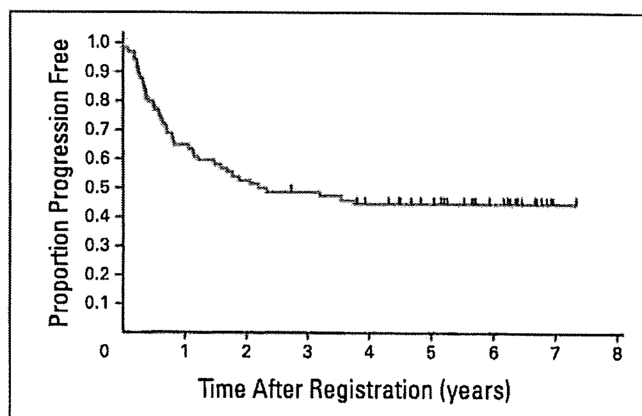

Fig 1. Progression-free survival (PFS) of the 75 eligible patients. PFS at 3 years and 5 years was 49% (95% CI, 38% to 60%) and 45% (95% CI, 34% to 56%), respectively, with a median PFS of 27.7 months.

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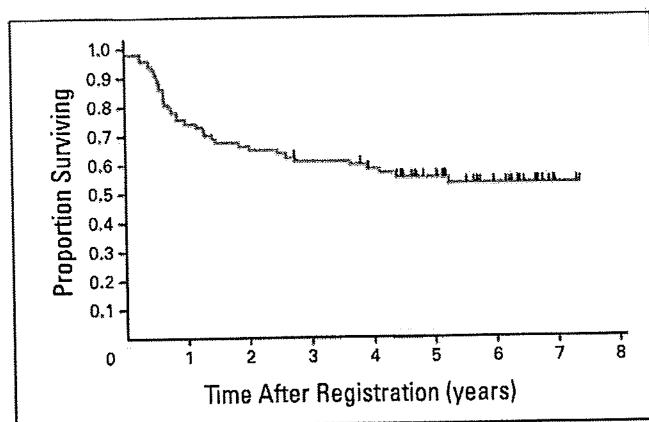

Fig 2. Overall survival (OS) of the 75 eligible patients. OS at 3 years and 5 years was 61% (95% CI, 49% to 71%) and 56% (95% CI, 44% to 66%), respectively. The median OS has not been reached.

achieved (Appendix Fig A5). The survival of the 12 patients with pathologic CR was especially favorable (Appendix Fig A6, online only).

Pattern of Relapse

So far, 39 patients have experienced tumor relapse. Table 4 lists the initial relapse sites, according to the curative extent of the surgical resection. For unresected or incompletely resected cases, locoregional relapse was predominant. To the contrary, for completely resected cases, relapse at distant sites was the most frequent relapse pattern, with some brain-only relapse patients.

DISCUSSION

We conducted a multi-institutional phase II trial of a trimodality approach, namely, preoperative chemoradiotherapy followed by surgical resection, in patients with SSTs. Because of the rarity of this subtype of NSCLC, no randomized trial has been conducted previously.[28] Our report is the second of a large-scale, prospective trial after SWOG 9416/INT 0160 and reproduced its favorable outcomes.[25]

The long-term results of the SWOG 9416/INT 0160 trial were recently published.[29] Although the chemotherapy regimens used were different, a standard classic platinum-based combination was used in both. The preoperative radiotherapy doses were also identical (45 Gy), although a 1-week split (interval between two sessions) was included in our protocol (but not in the SWOG trial). Boost chemotherapy was planned after curative resection in the SWOG trial, but the compliance rate was poor,[25] as in other perioperative therapy reports; we had anticipated that the majority of the patients would not be fit enough for additional toxic therapy after a major thoracic surgery and did not include it in our protocol.

Despite these minor differences, the results of the two trials were strikingly similar (Table A1, online only). The radiologic response rate was higher, whereas the pathologic CR rate was lower in our trial, but the differences were probably not clinically relevant, considering interobserver differences in the response evaluation and the well-known discrepancy between clinical versus pathologic effects. The intensive trimodality approach was found to be feasible in both reports, with a reasonably low toxic death rate of 4%. The resection rate, which had remained unchanged at approximately 50% for almost 40 years with conventional preoperative radiotherapy, was approximately 70% in both studies. Particularly noteworthy was the reproducibility of the favorable survival data, with a 5-year OS rate of 44% in the United States trial and 56% in our trial, which were clearly superior to the historical value of 30%.[3,25]

A shift in the trend of clinical problems also became clear.[25,28,29] The relapse patterns changed from predominantly locoregional[17,18] to mainly distant recurrences in cases with complete resection,[25,28,29] and a significant number of such patients suffered from metastasis in the brain as the initial site of relapse.[29] To the contrary, complete resection could be achieved in less than half of the patients with c-T4 disease, and neither local control nor long-term survival was satisfactory in those in whom it could not be achieved. It seems that there might be room for improvement in radiotherapy.

Several questions remain unresolved. One is that of management of patients with mediastinal node involvement. These clinical N2 cases have been known to have the poorest prognosis[9,18] and were excluded from both the SWOG and JCOG trials. Although trimodality approaches have been reported in cases with clinical N2 stage NSCLC,[30,31] inclusion of the hilar and mediastinal nodes in the irradiation field increased the toxicity risk to an unacceptable level in our prior phase II trial (JCOG 9805).[32]

In addition to the unresolved questions above, our study also had a critical limitation. Although this was a prospective, large-scale, and multi-institutional trial, no definite conclusions could be obtained from the single-arm phase II study. As repeatedly pointed out, however, a phase III trial would be unrealistic due to the rarity of SSTs. Possibly, clinical questions common with other patient subsets could be tested in a phase III trial targeting a broader patient population; for example, patients with SSTs and other stage III NSCLC could be enrolled onto a phase III trial of prophylactic cranial irradiation after definitive induction therapy.[33]

In conclusion, we report a favorable outcome of preoperative chemoradiotherapy in patients with SSTs, confirming the results of the previous SWOG/Intergroup trial. We believe that this strategy may be acceptable as standard for the treatment of this disease and also serves as a reference for future trials.

Table 4. Initial Relapse Sites

Relapse Site	Patients With Complete Resection (n = 51)	Patients Without Complete Resection (n = 24)	Total (N = 75)
Locoregional* only	2	8	10
Distant only	14	6	20
Brain only	4	1	5
Both	4	5	9
Total	20	19	39

*Locoregional = surgical margin, within radiation field, hilar lymph nodes, mediastinal lymph nodes, supraclavicular lymph nodes.

AUTHORS' DISCLOSURES OF POTENTIAL CONFLICTS OF INTEREST

The author(s) indicated no potential conflicts of interest.

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Appendix

The Appendix is included in the full-text version of this article, available online at www.jco.org. It is not included in the PDF version (via Adobe® Reader®).

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Short Communication

Randomised phase II trial of irinotecan plus cisplatin vs irinotecan, cisplatin plus etoposide repeated every 3 weeks in patients with extensive-disease small-cell lung cancer

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Patients with previously untreated extensive-disease small-cell lung cancer were treated with irinotecan 60 mg m^{-2} on days 1 and 8 and cisplatin 60 mg m^{-2} on day 1 with ($n = 55$) or without ($n = 54$) etoposide 50 mg m^{-2} on days 1–3 with granulocyte colony-stimulating factor support repeated every 3 weeks for four cycles. The triplet regimen was too toxic to be considered for further studies.
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Small-cell lung cancer (SCLC), which accounts for approximately 14% of all malignant pulmonary tumours, is an aggressive malignancy with a propensity for rapid growth and early widespread metastases (Jackman and Johnson, 2005). A combination of cisplatin and etoposide (PE) has been the standard treatment, with response rates ranging from 60 to 90% and median survival times (MSTs) from 8 to 11 months in patients with extensive disease (ED)-SCLC (Fukuoka *et al*, 1991; Roth *et al*, 1992). A combination of irinotecan and cisplatin (IP) showed a significant survival benefit over the PE regimen (MST: 12.8 *vs* 9.4 months, $P = 0.002$) in a Japanese phase III trial for ED-SCLC (Noda *et al*, 2002), although another phase III trial comparing these regimens failed to show such a benefit (Hanna *et al*, 2006). Thus, irinotecan, cisplatin and etoposide are the current key agents in the treatment of SCLC. A phase II trial of the three agents, IPE combination, in patients with ED-SCLC showed a promising antitumour activity with a response rate of 77%, complete response (CR) rate of 17% and MST of 12.9 months (Sekine *et al*, 2003).

We have developed these IP and IPE regimens in a 4-week schedule where irinotecan was given on days 1, 8 and 15. The dose of irinotecan on day 15, however, was frequently omitted because of toxicity in both regimens (Noda *et al*, 2002; Sekine *et al*, 2003). The objectives of this study were to evaluate the toxicities and antitumour effects of IP and IPE regimens in the 3-week schedule in patients with ED-SCLC and to select the right arm for subsequent phase III trials.

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PATIENTS AND METHODS

Patient selection

Patients were enrolled in this study if they met the following criteria: (1) a histological or cytological diagnosis of SCLC; (2) no prior treatment; (3) measurable disease; (4) ED, defined as having distant metastasis or contralateral hilar lymph node metastasis; (5) performance status of 0–2 on the Eastern Cooperative Oncology Group (ECOG) scale; (6) predicted life expectancy of 3 months or longer; (7) age between 20 and 70 years; (8) adequate organ function as documented by a white blood cell (WBC) count $\geqslant 4.0 \times 10^3 \mu l^{-1}$, neutrophil count $\geqslant 2.0 \times 10^3 \mu l^{-1}$, haemoglobin $\geqslant 9.5$ g dl^{-1}, platelet count $\geqslant 100 \times 10^3 \mu l^{-1}$, total serum bilirubin $\leqslant 1.5$ mg dl^{-1}, hepatic transaminases $\leqslant 100$ IU l^{-1}, serum creatinine $\leqslant 1.2$ mg dl^{-1}, creatinine clearance $\geqslant 60$ ml min^{-1}, and $PaO_2 \geqslant 60$ torr; and (9) providing written informed consent.

Patients were not eligible for the study if they had any of the following: (1) uncontrollable pleural, pericardial effusion or ascites; (2) symptomatic brain metastasis; (3) active infection; (4) contraindications for the use of irinotecan, including diarrhoea, ileus, interstitial pneumonitis and lung fibrosis; (5) synchronous active malignancies; (6) serious concomitant medical

illness, including severe heart disease, uncontrollable diabetes mellitus or hypertension; or (7) pregnancy or breast feeding.

Treatment schedule

In the IP arm, cisplatin, 60 mg m^{-2}, was administered intravenously over 60 min on day 1 and irinotecan, 60 mg m^{-2}, was administered intravenously over 90 min on days 1 and 8. Prophylactic granulocyte colony-stimulating factor (G-CSF) was not administered in this arm. In the IPE arm, cisplatin and irinotecan were administered at the same dose and schedule as the IP arm. In addition, etoposide, 50 mg m^{-2}, was administered intravenously over 60 min on days 1-3. Filgrastim 50 μg m^{-2} or lenograstim 2 μg kg^{-1} was subcutaneously injected prophylactically from day 5 to the day when the WBC count exceeded $10.0 \times 10^3 \mu l^{-1}$. Hydration (2500 ml) and a $5HT_3$ antagonist were given on day 1, followed by an additional infusion if indicated in both arms. These treatments were repeated every 3 weeks for a total of four cycles.

Toxicity assessment, treatment modification and response evaluation

Toxicity was graded according to the NCI Common Toxicity Criteria version 2.0.

Doses of anticancer agents in the following cycles were modified according to toxicity in the same manner in both arms. Objective tumour response was evaluated according to the Response Evaluation Criteria in Solid Tumors (RECIST) (Therasse *et al*, 2000).

Study design, data management and statistical considerations

This study was designed as a multi-institutional, prospective randomised phase II trial. This study was registered on 6 September 2005 in the University hospital Medical Information Network (UMIN) Clinical Trials Registry in Japan (http://www.umin.ac.jp/ctr/index.htm), which is acceptable to the International Committee of Medical Journal Editors (ICMJE) (http://www.icmje.org/faq.pdf). The protocol and consent form were approved by the Institutional Review Board of each institution. Patient registration and randomisation were conducted at the Registration Center. No stratification for randomisation was performed in this study. The sample size was calculated according to the selection design for pilot studies based on survival (Liu *et al*, 1993). Assuming that (1) the survival curve was exponential for survivals; (2) the MST of the worse arm was 12 months and that of the better arm was 12 months × 1.4; (3) the correct selection probability was 90%; and (4) additional follow-up in years after the end of accrual was 1 year, the estimated required number of patients was 51 for each arm. Accordingly, 55 patients for each arm and their accrual period of 24 months were planned for this study.

The dose intensity of each drug was calculated for each patient using the following formula as previously described:

$$\text{The dose intensity (mg m}^{-2}\text{ week}^{-1}) = \frac{\text{Total milligrams of a drug in all cycles per body surface area}}{\text{Total days of therapy}/7}$$

where total days of therapy is the number of days from day 1 of cycle 1 to day 1 of the last cycle plus 21 days for both arms (Hryniuk and Goodyear, 1990).

Differences in the reason for termination of the treatment and the frequencies of grade 3-4 toxicities were assessed by χ tests. Survival was measured as the date of randomisation to the date of death from any cause or the date of the most recent follow-up for overall survival and to the date of disease progression or the date of death for progression-free survival (PFS). The survival of the arms was estimated by the Kaplan-Meier method and compared in an exploratory manner with log-rank tests (Armitage *et al*, 2002).

RESULTS

Patient characteristics

From March 2003 to May 2005, 55 patients were randomised to IP and 55 patients to IPE. One patient in the IP arm was excluded because the patient was ineligible and did not receive the study treatment. The remaining 109 patients were included in the analyses of toxicity, tumour response and patient survival. There were no differences between the two arms in any demographic characteristics listed (Table 1).

Treatment delivery

Treatment was well tolerated with respect to the number of cycles delivered in both arms (Table 2). Among reasons for termination of the treatment, disease progression was noted in nine (17%)

Table 1 Patient characteristics

	IP (n=54)	IPE (n=55)
Sex		
Female	11	8
Male	43	47
Age (years)		
Median (range)	63 (42–70)	62 (48–70)
PS		
0	11	12
1	42	41
2	1	2
Weight loss		
0–4%	38	43
5–9%	10	10
⩾10%	6	2

Table 2 Treatment delivery

	IP (n=54) No. (%)	IPE (n=55) No. (%)
Number of cycles delivered		
6[a]	—	1 (2)
4	41 (76)	36 (65)
3	6 (11)	6 (11)
2	3 (6)	6 (11)
1	4 (7)	6 (11)
Reasons for termination of the treatment[†]		
Completion	40 (74)	35 (64)
Disease progression	9 (17)	2 (4)
Toxicity	3 (6)	13 (24)
Patient refusal	2 (4)	4 (7)
Others	0 (0)	1 (2)
Total number of cycles delivered	192 (100)	186 (100)
Total number of omission on day 8	35 (18)	37 (17)
Total number of cycles with dose reduction	28 (15)	31 (17)

[†]P=0.013 by χ^2 test. [a]Protocol violation.

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patients in the IP arm and in two (4%) patients in the IPE arm, whereas toxicity was noted in three (6%) patients in the IP arm and 13 (24%) patients in the IPE arm ($P = 0.013$) (Table 2). The dose of irinotecan on day 8 was omitted in 35 (18%) cycles in the IP arm and 37 (17%) cycles in the IPE arm (Table 2). The total dose and dose intensity of cisplatin and etoposide were similar between the IP and IPE arms in the present study (Table 3).

Toxicity

The myelotoxicity was more severe in the IPE arm (Table 4). Grade 3 febrile neutropaenia was noted in 5 (9%) patients in the IP arm and 17 (31%) patients in the IPE arm ($P = 0.005$). Packed red blood cells were transfused in 4 (7%) patients in the IP regimen and 14 (26%) patients in the IPE regimen ($P = 0.011$). Platelet concentrates were needed in none in the IP regimen and 2 (4%) patients in the IPE regimen ($P = 0.16$). Grade 3–4 diarrhoea was observed in 8 (15%) patients in the IP arm and 13 (24%) patients in the IPE arm ($P = 0.262$). Grade 3–4 fatigue was more common in the IPE arm with marginal significance (2 *vs* 11%, $P = 0.054$). The severity of other non-haematological toxicities did not differ significantly between the arms. No treatment-related death was observed in this study.

Table 3 Total dose and dose intensity

	3-week regimens in this study		4-week regimen[a]
	IP ($n = 54$) Median (range)	IPE ($n = 55$) Median (range)	IPE ($n = 30$) Median (range)
Total dose ($mg\,m^{-2}$)			
Cisplatin	240 (60–240)	240 (60–360)	240 (60–240)
Irinotecan	420 (60–480)	390 (60–720)	563 (60–720)
Etoposide	0	600 (150–900)	600 (150–600)
Dose intensity ($mg\,m^{-2}\,week^{-1}$)			
Cisplatin	19 (14–25)	20 (16–34)	15 (12–15)
Irinotecan	33 (14–40)	35 (15–55)	35 (19–45)
Etoposide	0	48 (34–68)	37 (28–38)

[a]From our previous study (Sekine *et al*, 2003).

Table 4 Grade 3–4 toxicities

	IP ($n = 54$)			IPE ($n = 55$)		
	Grade 3	4	3+4 (%)	Grade 3	4	3+4 (%)
Leukocytopaenia	9	1	10 (19)	18	11	29 (53)*
Neutropaenia	17	11	28 (52)	24	28	52 (95)*
Anaemia	18	0	18 (25)	16	9	25 (45)
Thrombocytopaenia	2	0	2 (4)	13	0	13 (13)†
Febrile neutropaenia	5	0	5 (9)	17	0	7 (13)
Diarrhoea	8	0	8 (15)	11	2	13 (24)
Vomiting	4	0	4 (7)	3	0	3 (5)
Fatigue	1	0	1 (2)	5	1	6 (11)‡
Hyponatraemia	9	3	12 (22)	11	2	13 (24)
AST elevation	0	0	0 (0)	3	0	3 (5)
CRN elevation	1	0	1 (2)	0	0	0 (0)

*$P < 0.001$; †$P < 0.01$; and ‡$P = 0.054$ by χ^2 test.

Response, treatment after recurrence and survival

Four CRs and 37 partial responses (PRs) were obtained in the IP arm, resulting in the overall response rate of 76 with 95% confidence interval (CI) of 65–87%, whereas six CRs and 42 PRs were obtained in the IPE arm, and the overall response rate was 87% with a 95% CI of 79–96% ($P = 0.126$). Median PFS was 4.8 months (95% CI, 4.0–5.6) in the IP and 5.4 months (95% CI, 4.8–6.0) in the IPE arm ($P = 0.049$) (Figure 1A). After recurrence, 22 (44%) patients in the IP arm and 8 (16%) patients in the IPE arm received etoposide-containing chemotherapy. The MST and 1-year survival rate were 12.4 months (95% CI, 9.7–15.1) and 54.8% (95% CI, 41.4–68.2%) in the IP and 13.7 months (95% CI, 11.9–15.5) and 61.5% (95% CI, 48.6–74.4%) in the IPE arm ($P = 0.52$), respectively (Figure 1B).

DISCUSSION

This study showed that the IPE regimen in a 3-week schedule with CSF support produced a promising response rate, PFS and overall survival. Haematological toxicity in the IPE arm, however, was very severe in spite of the G-CSF support with the grade 3 febrile neutropaenia noted in 31% of patients.

In comparison between the 3-week IPE regimen in this study and the 4-week IPE regimen in the previous study, the delivery of cisplatin and etoposide was improved in the 3-week IPE regimen when compared with the 4-week IPE regimen at the cost of the irinotecan total dose. The response rate and MST were 87% and 13.7 months, respectively, in the 3-week IPE regimen and 77% and 12.9 months in the previous 4-week schedule, and toxicity profiles were comparable to each other (Sekine *et al*, 2003).

The MST of 12.4 months in the IP arm in this study was comparable to that of the previous phase III study, with an MST of 12.8 months (Noda *et al*, 2002). Thus, this study showed the reproducible excellent survival outcome of patients with ED-SCLC who were treated with the IP combination. In contrast, a recent American phase III study of the PE regimen *vs* IP regimen failed to show the superiority of the IP regimen to the PE regimen; the MST

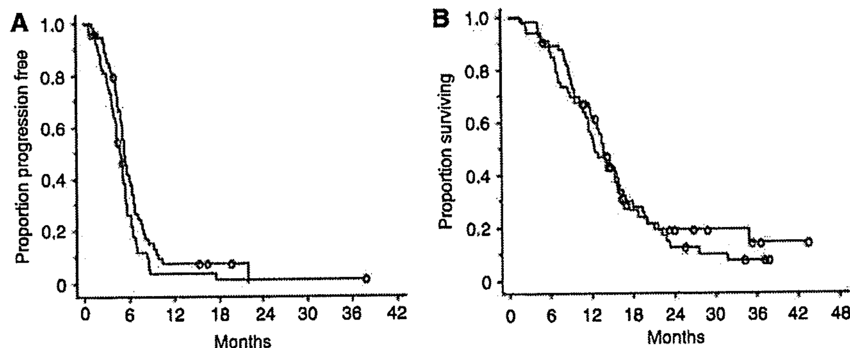

Figure 1 Progression-free survival (**A**) and overall survival (**B**). Thick line indicates the IPE regimen and thin line indicates the IP regimen.

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for the PE regimen was 10.2 months and that for the IP regimen was 9.3 months (Hanna *et al*, 2006). The discrepancy between the Japanese and American trials may be explained by the different cisplatin dose schedules; cisplatin was delivered at a dose of $60\,mg\,m^{-2}$ on day 1 every 3 or 4 weeks in the Japanese trials, whereas cisplatin was delivered at a dose of $30\,mg\,m^{-2}$ on days 1 and 8 every 3 weeks in the American one. A platinum agent administered at divided doses was associated with poor survival in patients with ED-SCLC in our previous randomised phase II study (Sekine *et al*, 2003).

The issue of adding further agents to the standard doublet regimen has been investigated in patients with ED-SCLC. The addition of ifosfamide or cyclophosphamide and epirubicin to the cisplatin and etoposide combination produced a slight survival benefit, but at the expense of greater toxicity (Loehrer *et al*, 1995; Pujol *et al*, 2001). Phase III trials of cisplatin and etoposide with or without paclitaxel showed unacceptable toxicity with 6–13% toxic deaths in the paclitaxel-containing arm (Mavroudis *et al*, 2001; Niell *et al*, 2005). The results in these studies and the current study are consistent in the increased toxicity despite the G-CSF support and no definite survival benefit in the three or four drug combinations over the standard doublet in patients with ED-SCLC.

In conclusion, the IPE regimen was marginally more effective than the IP regimen, but was too toxic despite the administration of prophylactic G-CSF.

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ORIGINAL REPORT

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

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

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A B S T R A C T

Purpose
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.

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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)

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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.

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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	0.756
									63	0.123	
									52	0.102	
									17	0.033	
									13	0.025	
									12	0.023	
									12	0.023	
									11	0.021	
									8	0.016	
									5	0.010	
									4	0.008	
									4	0.008	
									2	0.004	
									1	0.002	
									8	0.016	
									84	0.164	0.207
									11	0.021	
									5	0.010	
									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,

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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	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).

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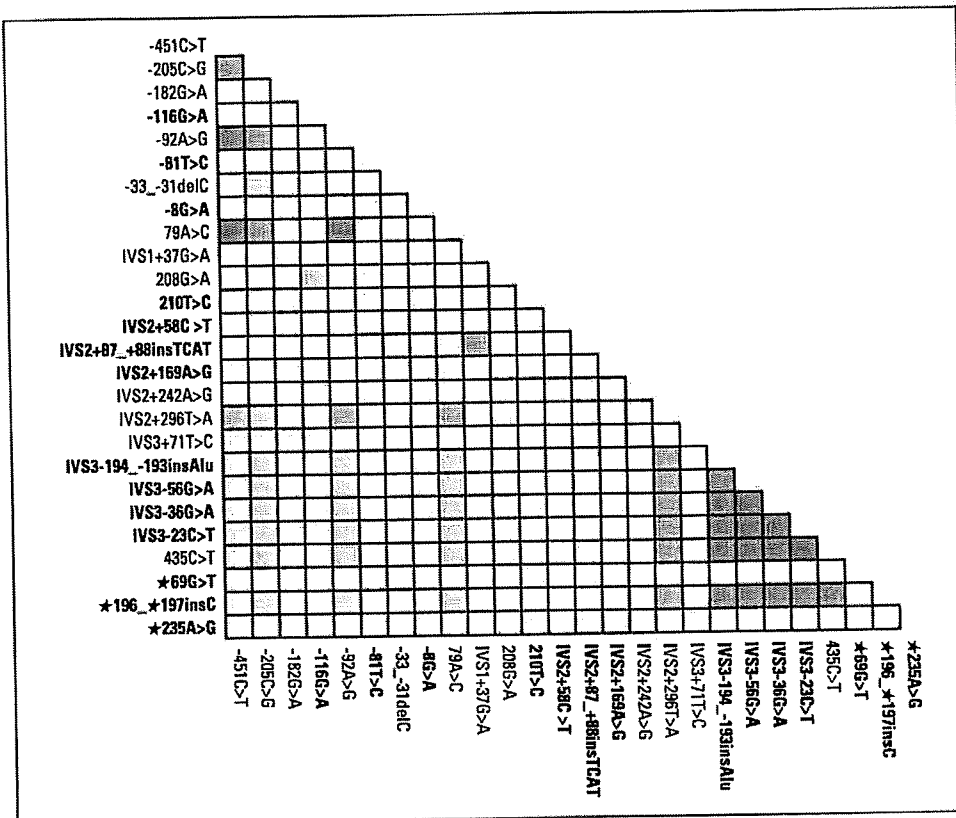

Fig 1. Linkage disequilibrium (LD) among 26 *CDA* variations. Pairwise LD as r^2 (from 0 to 1) is expressed as 10-graded blue color. The density of the blue color increases with higher linkage rates.

*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*.

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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} 1/4-3/4 Quantiles	AUC (hr · μg/mL) Median	AUC 1/4-3/4 Quantiles	CL/m^2 (L/hr/m^2) Median	CL/m^2 1/4-3/4 Quantiles	Vz/m^2 (L/m^2) Median	Vz/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 $P = .0031$).

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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/m² (L/hr/m²)	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/m², 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/m² 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/m²/wk, three of nine at 790 mg/m²/wk, and three of six at 1,000 mg/m²/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

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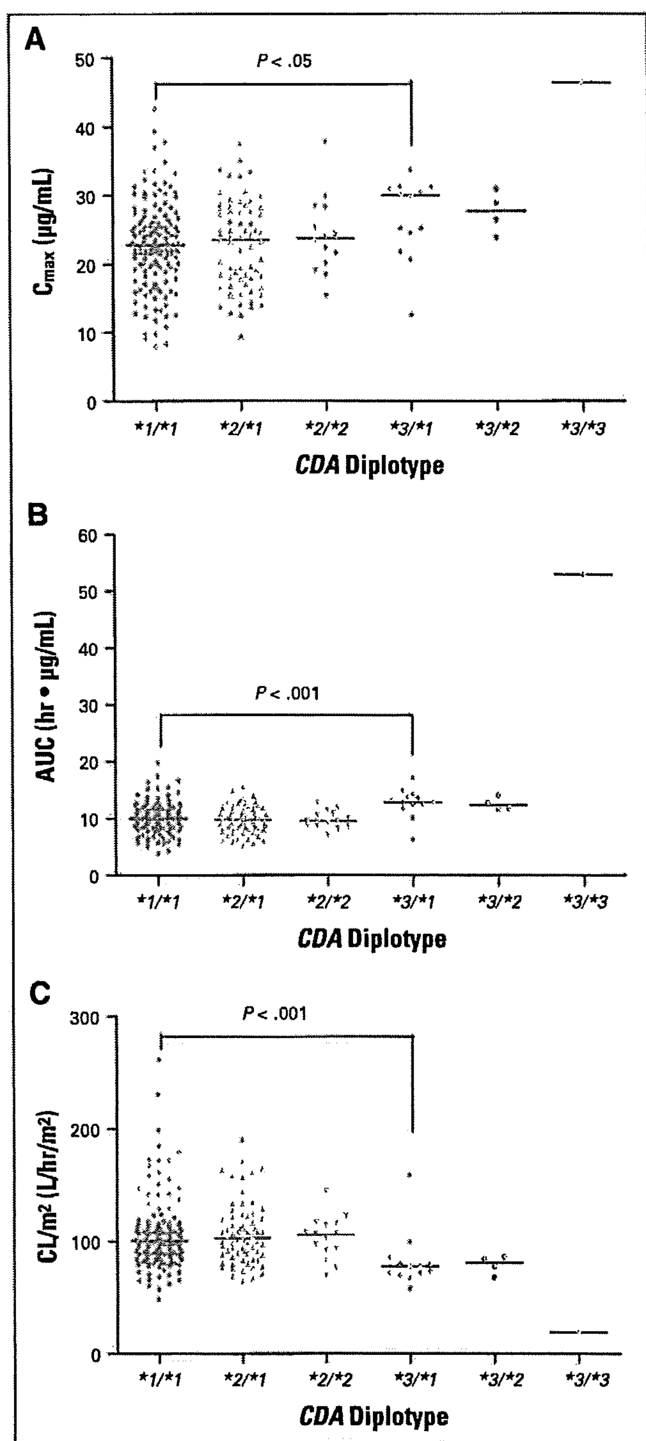

Fig 2. Effects of haplotypes *2 and *3 on the pharmacokinetic parameters of gemcitabine. (A) Peak concentration (C_{max}) and (B) area under the curve (AUC) were corrected assuming that all patients received 1,000 mg/m^2 of gemcitabine. (C) Clearance (CL/m^2). Each point corresponds to an individual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

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

	Median Gemcitabine PK Parameters				Median CDA Activity (units)		
Diplotype	No. of Patients	C_{max} (μg/mL)	AUC (hr·μg/mL)	CL/m² (L/hr/m²)	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.

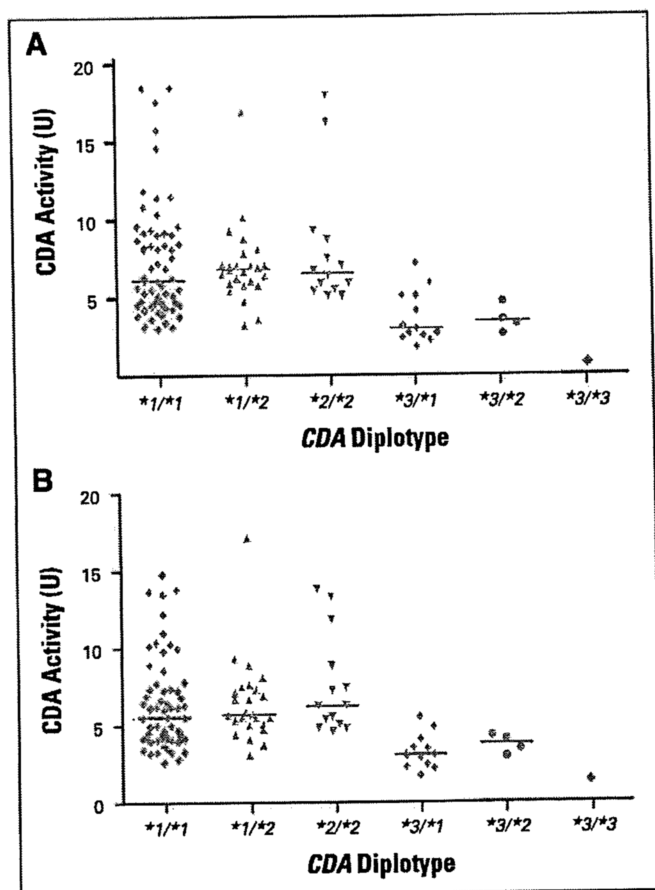

Fig 3. Effects of haplotypes *2 and *3 on plasma cytidine deaminase (CDA) activity toward gemcitabine and cytidine substrates. (A) Gemcitabine was used as a substrate, and (B) cytidine was used as a substrate. Each point corresponds to an individual patient. The bars denote the median values.

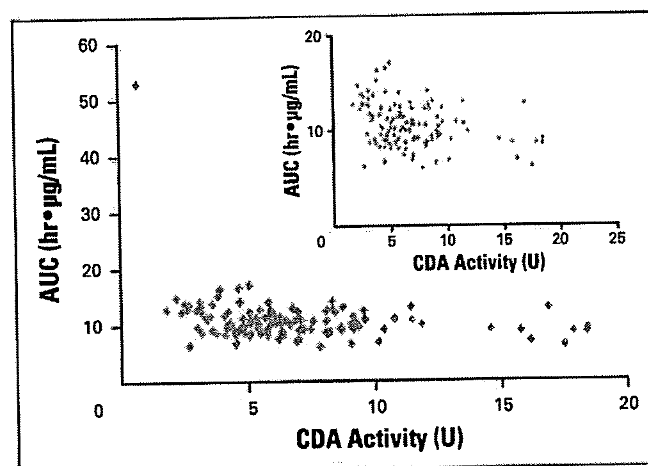

Fig 4. Correlation between plasma area under the curve (AUC) and cytidine deaminase (CDA) activity toward gemcitabine. AUC was corrected assuming that all patients received 1,000 mg/m² of gemcitabine. The inset excludes the data obtained from a homozygous *3 carrier. The correlation coefficient is −0.31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

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