Predominant Infiltration of Macrophages and CD8$^+$ T Cells in Cancer Nests Is a Significant Predictor of Survival in Stage IV Nonsmall Cell Lung Cancer

Osamu Kawai, MD[1,2,3]
Genichiro Ishii, MD, PhD[1]
Kaoru Kubota, MD[2]
Yukinori Murata, MD[4]
Yoichi Naito, MD[1,2]
Tetsuya Mizuno, MD[1,2]
Keiju Aokage, MD[1,2]
Nagahiro Saijo, MD[2]
Yutaka Nishiwaki, MD[2]
Akihiko Gemma, MD[3]
Syoji Kudoh, MD[3]
Atsushi Ochiai, MD, PhD[1]

[1] Pathology Division, Research Center for Innovative Oncology, National Cancer Center Hospital East, Kashiwa, Chiba, Japan.

[2] Thoracic Oncology Division, National Cancer Center Hospital East, Kashiwa, Chiba, Japan.

[3] Department of Internal Medicine, Division of Pulmonary Medicine, Infectious Diseases, and Oncology, Nippon Medical School, Bunkyo, Tokyo, Japan.

[4] Clinical Laboratory Division, National Cancer Center Hospital East, Kashiwa, Chiba, Japan.

BACKGROUND. The purpose of this study was to investigate whether tumor-infiltrating immune cells in biopsy specimens can be used to predict the clinical outcome of stage IV nonsmall cell lung cancer (NSCLC) patients.

METHOD. The authors performed an immunohistochemical study to identify and count the number of CD68$^+$ macrophages, c-kit$^+$ mast cells, and CD8$^+$ T cells in both cancer nests and cancer stroma in pretreatment biopsy specimens obtained from 199 patients with stage IV NSCLC treated by chemotherapy, and then analyzed for correlations between the number of immune cells and clinical outcome, including chemotherapy response and prognosis.

RESULTS. There was no correlation between the number of immune cells in either cancer nests or stroma and chemotherapy response. Patients with more tumor-infiltrating macrophages in cancer nests than in cancer stroma (macrophages, nests > stroma) had significantly better survival than nests < stroma cases median survival time (MST 440 days vs 199 days; $P < .0001$). Patients with more tumor-infiltrating CD8$^+$ T cells in cancer nests than in cancer stroma (CD8$^+$ T cells: nests > stroma) showed significantly better survival than in nests < stroma cases (MST 388 days vs 256 days; $P = .0070$). The proportion of tumor-infiltrating macrophages or CD8$^+$ T cells between cancer nests and stroma became independent prognostic factors in the multivariate analysis. Neither the number of mast cells in nests nor in stroma correlated with the clinical outcome.

CONCLUSIONS. Evaluation of the numbers of macrophages and CD8$^+$ T cells in cancer nests and stroma are useful biomarkers for predicting the prognosis of stage IV NSCLC patients treated with chemotherapy, but could fail to predict chemotherapy response. *Cancer* **2008;113:1387–95.** © *2008 American Cancer Society.*

KEYWORDS: stage IV, nonsmall cell lung cancer, macrophage, CD8$^+$ T cell, mast cell.

We thank Tetsuya Nakatsura for immunological advice and Mai Okumoto and Hiroko Hashimoto for technical assistance.

Address for reprints: Atsushi Ochiai, MD, PhD, Pathology Division, Research Center for Innovative Oncology, National Cancer Center Hospital East, 6-5-1, Kashiwanoha, Kashiwa-City, Chiba, 277-8577, Japan; Fax: (011) 81-7134-6865; E-mail: aochiai@east.ncc.go.jp

Received October 31, 2007; revision received April 10, 2008; accepted April 14, 2008.

Lung cancer is the leading cause of cancer deaths throughout the world, and nonsmall cell lung cancer (NSCLC) accounts for approximately 80% of lung cancer. The prognosis of NSCLC is poor, and patients with advanced NSCLC are candidates for systemic chemotherapy.[1] During the 1990s, 5 new drugs became available for the treatment of metastatic NSCLC: paclitaxel, docetaxel, vinorelbine, gemcitabine, and irinotecan. Each of them has since been evaluated in combination regimens with cisplatin or carboplatin, and the median survival time of patients with metastatic NSCLC treated with such regimens is approximately 8 to 10 months.[2,3] However, some patients with metastatic NSCLC exhibit long-term

© 2008 American Cancer Society
DOI 10.1002/cncr.23712
Published online 31 July 2008 in Wiley InterScience (www.interscience.wiley.com).

survival, and their tumors progress slowly after chemotherapy, or even in the absence of treatment.[4]

Tumor cells are surrounded by infiltrating inflammatory cells, such as lymphocytes, neutrophils, macrophages, and mast cells. Infiltration of $CD8^+$ T cells has been shown to be an important phenomenon for a specific immune response in several tumor systems, and $CD8^+$ T cells have been reported to play an important suppressive role in cancer progression, including ovarian cancer, esophageal cancer, pancreatic cancer, bile duct cancer, gallbladder cancer, and colorectal cancer.[5-12] Immunologists have long considered the presence of tumor-infiltrating macrophages as evidence of a host response against the growing tumor, and the presence of tumor-infiltrating macrophages has been reported to be associated with anticancer immunomechanisms of the tumor-bearing host and a favorable prognosis. However, recently, tumor-associated macrophage infiltration has been found to be correlated with angiogenesis and an unfavorable prognosis in several kinds of cancer, including gastric cancer, endometrial cancer, and breast cancer.[13-15] Furthermore, it has been reported that mast cells produce many angiogenic factors and a variety of cytokines, including transforming growth factor-beta, tumor necrosis factor-α (TNF-α), interleukin-8 (IL-8), fibroblast growth factor-2, and vascular endothelial growth factor, which are implicated in both normal and tumor-associated neoangiogenesis.[16] In fact, mast cell density has been reported to be highly correlated with the extent of both normal and pathologic angiogenesis, such as the angiogenesis observed in chronic inflammatory diseases and tumors, including gastric cancer and endometrial cancer.[17,18]

Tumor-infiltrating immune cells are thought to play important roles in disease progression and therapeutic efficacy. The effect of chemoradiotherapy has been found to be correlated with the presence of $CD8^+$ T cells in esophageal cancer,[11] and cervical cancer patients with T-cell infiltration showed improved local response to radiation therapy.[19]

In the current study, we evaluated the status of tumor-infiltrating immune cells in tumor biopsy specimens obtained from stage IV NSCLC patient and analyzed the numbers of immune cells and clinical outcome, including chemotherapy response and prognosis, for correlations.

MATERIALS AND METHODS

Patients and Tissue Specimens

The tumor specimens analyzed in this study were obtained from a total of 199 patients with stage IV NSCLC who received platinum-based combination chemotherapy (cisplatin plus paclitaxel, docetaxel, gemcitabine, vinorelbine, or irinotecan, or carboplatin plus paclitaxel), which is considered to be the standard regimen[20,21] at the National Cancer Center Hospital East in Kashiwa, Chiba, Japan, between January 1996 and December 2004, with performance status (PS) 0 or 1 on the Eastern Cooperative Oncology Group scale. Of the 199 patients, 184 had died by the time of the analysis. All patients had adequate tumor biopsy specimens obtainable before chemotherapy and were analyzed in this study. The tumor specimens were obtained by bronchoscopy in 152 patients, and by percutaneous needle biopsy in 47 patients. The histological classification was based on a World Health Organization report. Clinical staging was based on an initial evaluation consisting of a clinical assessment, chest radiography, computed tomography of the chest and abdomen, computed tomography or magnetic resonance imaging of the brain, and bone scintigraphy. The current International Staging System was used to stage clinical disease.[22] All target lesions were evaluated for response by computed tomography or magnetic resonance imaging after completion of the first-line chemotherapy, and all patients underwent tumor biopsy and chemotherapy, after obtaining informed consent in accordance with institutional guidelines.

Immunohistochemistry and Cell Counts

All specimens were fixed in 10% formalin and paraffin embedded. Four-micrometer-thick sections were mounted on silanized slides and deparaffinized with xylene and ethanol. To retrieve the antigen for macrophages, sections were pretreated in 0.05% trypsin and incubated for 20 minutes at 37°C in a humidity chamber. Endogenous peroxidase was blocked with 0.3% H_2O_2 in methanol for 15 minutes. We used mouse antihuman CD68 antibody (Dako, Kyoto, Japan) to detect macrophages, mouse antihuman CD8 antibody (Novocastra, Newcastle, UK) to detect T cells, and mouse antihuman c-kit antibody (Dako) to detect mast cells; immunostaining was performed with Envision (Dako). To retrieve the antigen for CD8 and c-kit, sections were immersed in 10 mM citric buffer solution (pH 6.0) and heated to 95°C by exposure to microwave irradiation for 20 minutes.

We performed an immunohistochemical study to identify and count the number of $CD68^+$ macrophages, c-kit$^+$ mast cells, and $CD8^+$ T cells and confirmed that cancer cells and mesenchymal cells such as endothelial cells were not immunostained with these antibodies.

Immunostained cells counts were blinded to the patients' clinical data. Macrophages, $CD8^+$ T cells,

and mast cells in the specimen were counted in 2 locations: in the "cancer nests" and in the "cancer stroma." Cancer nests were defined as "cancer nests without fibroblasts and vasculatures" and cancer stroma as "connective tissues surrounding cancer nests without any cancer cells." Every biopsy specimen had both cancer nest and stroma, and it was possible to distinguish these lesions. We counted $CD68^+$ round cells as macrophages, c-kit^+ round cells as mast cells, and $CD8^+$ round cells as T cells. By using a high-power microscopic field (×400; 0.0625 mm^2), we separately counted the number of macrophages, $CD8^+$ T cells, and mast cells in each 2 most intensive areas. Two pathologists (O.K. and G.I) reviewed all slides and counted the cells.

Statistical Analysis

Statistical analysis was performed using the Scientific Package for Social Sciences (SPSS, Chicago, Ill) software. We used median values to calculate category correlations between macrophages, $CD8^+$ T cells, mast cells, and survival rate by the Kaplan-Meier method, and performed univariate analyses by means of log-rank test. The chi-square test was used to test for relationships between categorical variables. Multivariate analysis was performed by means of the Cox proportional hazards model. Student t test was used to test for correlation between macrophage counts, $CD8^+$ T cell counts, mast cell counts and response to first-line chemotherapy. We evaluated test results as significant if the P value was $P < .05$.

RESULTS

Patient Characteristics

The clinicopathological characteristics of all patients are listed in Table 1. Their median age at the time of diagnosis was 62 years (range, 39 years-79 years), and 139 of the 199 patients were men. There were 134 patients with adenocarcinoma, 41 patients with squamous cell carcinoma, and 24 patients with NSCLC that could not be specified by biopsy specimen.

Macrophages, Mast Cells, and $CD8^+$ T Cells, in Cancer Nests and Cancer Stroma

Macrophages were observed in cancer nests (Fig. 2A) in 194 of the 199 tumors, and the mean number was 18.0 ± 2.4 (median, 13; range, 0-76). Macrophages were also observed in cancer stroma (Fig. 2B) in 195 of the 199 tumors, and the mean number was 15.3 ± 1.9 (median, 12; range, 0-105). Mast cells were observed in cancer nests (Fig. 2C) in 149 tumors and in the cancer stroma (Fig. 2D) in 158 tumors, and the mean number was 4.5 ± 0.8 (median, 2; range, 0-52), and 5.4 ± 0.8 (median, 3; range, 0-28), respectively. $CD8^+$ T cells were observed in cancer nests (Fig. 2E) in 197 tumors, and the mean number was 16.9 ± 2.2 (median, 12; range, 0-89). $CD8^+$ T cells were observed in the cancer stroma (Fig. 2F) in 198 tumors, and the mean number was 15.7 ± 1.8 (median, 13; range, 0-88).

TABLE 1
Patient Characteristics and Response to First-Line Chemotherapy

	Patients (N = 199)	
	No.	%
Age		
Median, y (range)	62 (39-79)	
<70 y	158	79.3
≥70 y	41	20.6
Sex		
Women	60	30.1
Men	139	69.8
Histological diagnosis		
Adenocarcinoma	134	67.3
Squamous cell carcinoma	41	20.6
NSCLC	24	12
ECOG performance status		
0	44	22.1
1	155	77.8
Smoking history		
<20 pack years	73	36.6
≥20 pack years	126	63.3
Median survival time, d (range)	317 (19-1969)	
Response to first-line chemotherapy		
PR	53	26.6
SD	95	47.7
PD	51	25.6

NSCLC indicates nonsmall cell lung cancer; ECOG, Eastern Cooperative Oncology Group; PR, partial response; SD, stable disease; PD, progressive disease.

Relationships between the number of infiltrating macrophages, mast cells, $CD8^+$ T cells, and clinicopathological variables

The numbers of infiltrating macrophages, mast cells, and $CD8^+$ T cells were divided into 2 groups at the median value. The relationships between these groups in cancer nests or stroma and the individual clinicopathological characteristics (sex, age, smoking history, PS, histological type) were examined by the chi-square test. More macrophages were present in cancer nests in the nonadenocarcinomas than in the adenocarcinomas (data not shown).

Correlations between the numbers of macrophages, $CD8^+$ T cells, mast cells, and first-line chemotherapy response

We analyzed the number of macrophages, mast cells, and $CD8^+$ T cells in cancer nests and stroma and

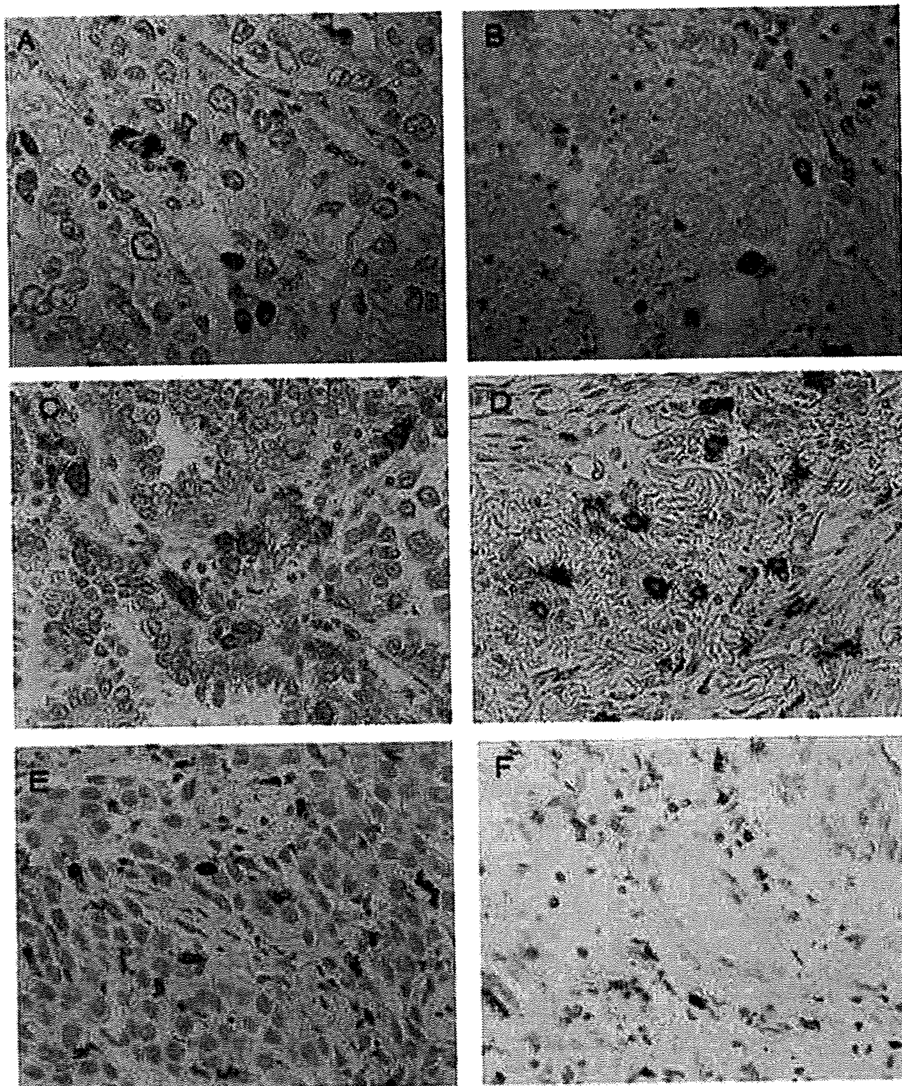

FIGURE 1. Typical photographs are shown of the results of immunostaining for the presence of $CD68^+$ macrophages in (A) cancer nests and in (B) stroma, $C\text{-kit}^+$ mast cells in (C) cancer nests and in (D) stroma, and $CD8^+$ T cells in (E) cancer nests and in (F) stroma.

first-line chemotherapy response for correlations by Student *t* test (Table 2), but the results showed no correlations between numbers of any of the infiltrating immune cells and response to first-line chemotherapy.

Correlations between the numbers of tumor-infiltrating macrophages, mast cells, and $CD8^+$ T cells and patient survival

Kaplan-Meier survival analyses and the log-rank test were performed to compare survival with the number of infiltrating cells (Fig. 2, Table 3). Patients with more macrophages in cancer nests than the median value had the same survival rate as patients with fewer macrophages. By contrast, patients with more macrophages in the cancer stroma had significantly poorer survival than those with fewer macrophages ($P = .0001$). The median survival time was 243 days in the group with higher numbers of macrophages in the stroma, versus 391 days in the group with fewer macrophages in the stroma (1-year survival rate, 33.9% and 55.2%, respectively). Patients were divided into 2 groups, according to the distribution of infiltrating macrophages; a High Nests Macrophage (HNM) group, in which the number of macrophages in the cancer nests was higher than in the cancer stroma (macro-

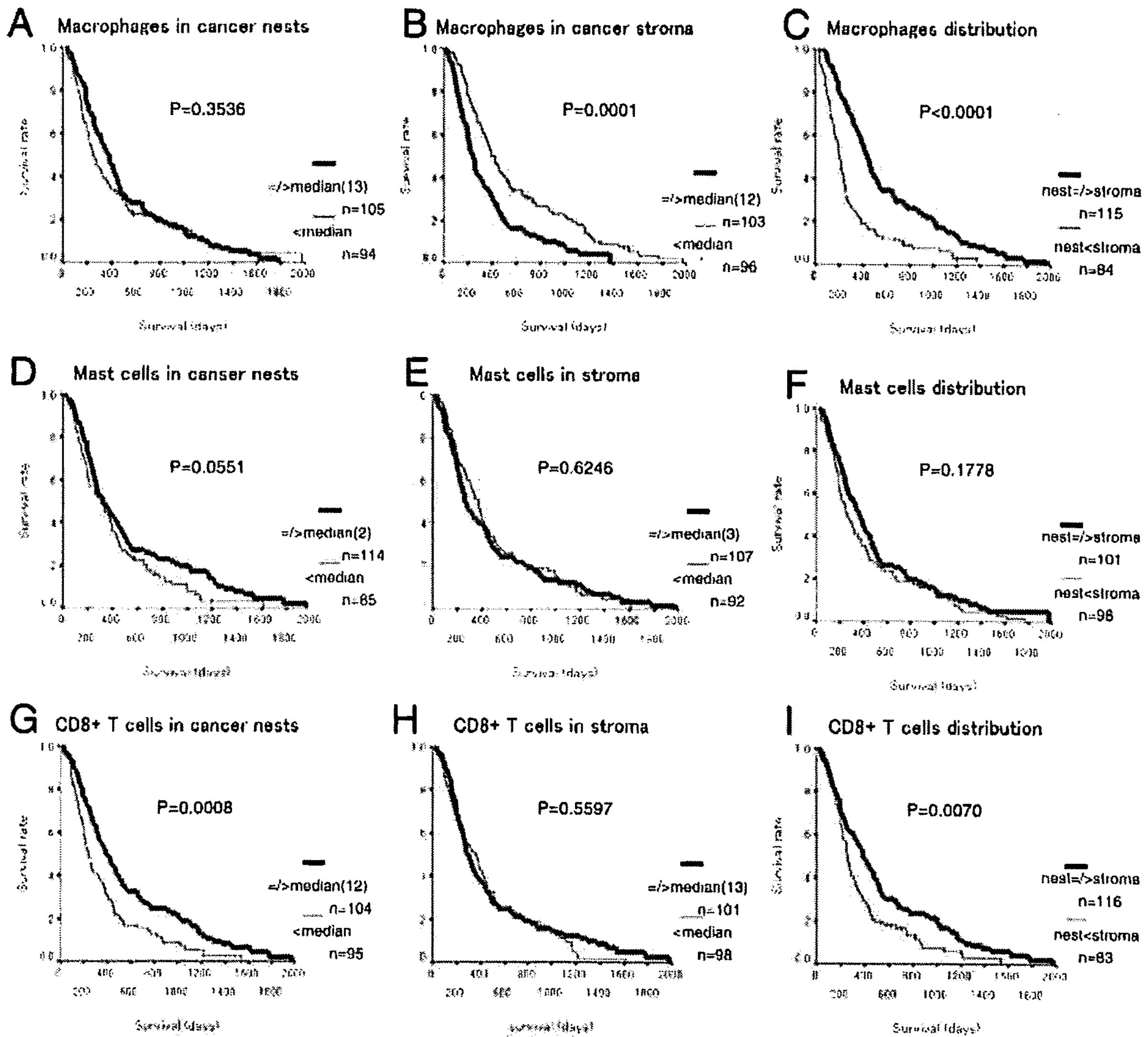

FIGURE 2. Kaplan-Meier analysis of overall survival is shown according to the level of infiltration by macrophages, mast cells, and $CD8^+$ T cells in cancer nests (A), (D), (G) and stroma (B), (E), (H) and their distribution (C), (F), (I). Data were dichotomized at the median value for each parameter (A), (B), (D), (E), (G), (H) and the distribution of infiltrating macrophages, mast cells, and $CD8^+$ T cells (C), (H), (I).

phages, nests > stroma) and a Low Nests Macrophage (LNM) group (nests < stroma). The HNM group had significantly better survival than the LNM group ($P <$ .0001) (Fig. 2C). Median survival time was 440 days in the HNM group versus only 199 days in the LNM group, and the 1-year survival rate was 60.8% and 21.4%, respectively. Although mast cells in the cancer nests have been reported to contribute to a favorable outcome,[23] there was no significant relationship with patient survival in this study (Fig. 2D-F). Figure 2G-I shows the relation between the number of $CD8^+$ T cells and patient survival; there was a positive association between survival and the number of $CD8^+$ T cells in cancer nests (Fig. 2G, $P = .0008$). Median survival was 388 days in the group with the higher number of $CD8^+$ T cells in the cancer nests, versus 242 days in the group with the lower number (1-year survival rate, 52.8% and 34.3%, respectively). According to the distribution of infiltrating $CD8^+$ T cells, patients in the High Nests $CD8^+$ T cell (HNT) group, in which the number of tumor-infiltrating $CD8^+$ T cells was higher in the cancer nests than in the cancer stroma (nests > stroma), had significantly better survival than those in the Low Nests $CD8^+$ T cell (LNT) (nests < stroma) group ($P = .0070$) (Fig. 2I). Median survival time was 440 days in the HNT group, versus

TABLE 2
Correlations Between Immune Cells and Response to First-Line Chemotherapy

Parameter	*t*	95% CI	*P**
Macrophages in cancer nests	−0.577	−7.173-3.946	.556
Macrophages in cancer stroma	0.119	−4.094-4.617	.905
Mast cells in cancer nests	−0.413	−2.310-1.512	.680
Mast cells in cancer stroma	1.476	−0.427-2.929	.143
$CD8^+$ T cells in cancer nests	−1.045	−7.114-2.201	.298
$CD8^+$ T cells in cancer stroma	−0.586	−5.162-2.807	.559

CI indicates confidence interval.
* Student *t* test.

only 199 days in the LNT group, and 1-year survival rate was 53.4% and 31.3%, respectively.

We then classified the patients into 4 groups according to macrophage and $CD8^+$ T cell distribution: 1) the HNM and HNT group (macrophages, nests > stroma; $CD8^+$ T cells, nests > stroma), 2) the HNM and LNT group (macrophages, nests > stroma; $CD8^+$ T cells, nests < stroma), 3) the LNM and HNT group (macrophages, nests < stroma; $CD8^+$ T cells, nests > stroma), and 4) the LNM and LNT group (macrophages, nests < stroma; $CD8^+$ T cells, nests < stroma). Median survival time was 495 days in the HNM and HNT group, versus only 196 days in the LNM and LNT group, and the 1-year survival rate was 68.4% and 12.5%, respectively. Median survival time was 342 days, and 1-year survival rate was 45.0% in the HNM and LNT group; median survival time was 221 days, and the 1-year survival rate was 27.2% in the LNM and HNT group. Patients in the HNM and HNT group had significantly better survival than patients in the other groups (Fig. 3, Table 3)

Multivariate Regression Analysis of Survival in NSCLC Patients

As immune cells in cancer nests and cancer stroma would have different biological activity in regard to tumor progression, it would be meaningful to evaluate immune cell distribution. Considering that the distributions of macrophages in cancer nests and cancer stroma may impact clinical outcome, multivariate analysis of macrophage and $CD8^+$ T cell distribution and clinicopathological predictors of survival was performed by means of the Cox proportional hazards model (Table 4), and both macrophage distribution ($P < .001$) and $CD8^+$ T cell distribution ($P = .040$) emerged as independent favorable prognostic indicators. Smoking status also emerged as an independent prognostic indicator ($P = .033$).

TABLE 3
Overall Survival

	Survival, d				
Groups	No.	Median	95% CI	*P*
Macrophages in cancer nests				.3536
<Median	94	248	192-304	
≥Median	105	376	299-453	
Macrophages in stroma				.0001
<Median	96	391	307-475	
≥Median	103	243	206-280	
Macrophage distribution				<.0001
Nests < stroma	84	199	178-220	
Nests > stroma	115	440	370-505	
Mast cells in cancer nests				.0551
<Median	85	307	201-413	
≥Median	114	317	230-404	
Mast cells in stroma				.6246
<Median	92	366	301-431	
>Median	107	259	200-318	
Mast cell distribution				.1778
Nests < stroma	98	250	188-324	
Nests ≥ stroma	101	370	304-436	
$CD8^+$ T cells in cancer nests				.0008
<Median	95	242	199-285	
≥Median	104	388	290-486	
$CD8^+$ T cells in stroma				.5597
<Median	98	358	237-479	
≥Median	101	297	247-347	
$CD8^+$ T cell distribution				.0070
Nests < stroma	83	256	224-288	
Nests ≥ stroma	116	388	316-460	

CI indicates confidence interval.
*Log-rank test.

DISCUSSION

This is the first study to investigate the relationship between the number of tumor-infiltrating macrophages, mast cells, and $CD8^+$ T cells in tumor biopsy specimens and the clinical outcome of patients with stage IV NSCLC. Patients with higher numbers of tumor-infiltrating macrophages and $CD8^+$ T cells in cancer nests than in cancer stroma had significantly better survival. These factors were also independent prognostic factors in multivariate analysis.

Immunologists have long considered the presence of tumor-infiltrating immune cells as evidence of a host response against the growing tumor. However, recently reports have shown that macrophages and mast cells in cancer stroma secrete several growth factors and proteases involved in angiogenesis, thereby promoting cancer progression. An experimental study has demonstrated that interaction between lung cancer cells and macrophages promotes the invasiveness and matrix-degrading activity of cancer cells,[24] and infiltration by these cells has been reported to be

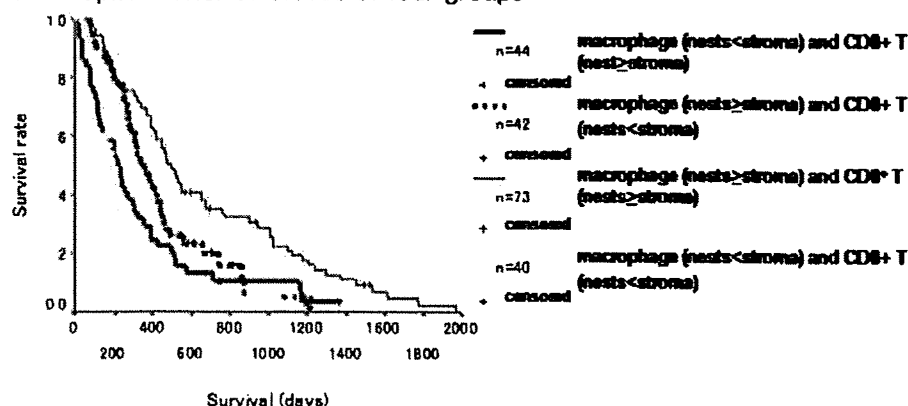

FIGURE 3. Kaplan-Meier analysis of overall survival is shown according to distribution in 4 groups of macrophages and $CD8^+$ T cells. Patients whose tumors contained macrophages in the nest and more $CD8^+$ T cells in the nest had significantly better survival (macrophages, nest > stroma; $CD8^+$ T cells, nest > stroma) than patients with macrophages nest > stroma and $CD8^+$ T cells nest < stroma ($P = .0070$), patients with macrophages nest < stroma and $CD8^+$ T cells nest > stroma ($P = .0010$), and patients with macrophages nest < stroma and $CD8^+$ T cells: nest < stroma ($P < .0001$).

TABLE 4
Multivariate Cox Proportional Hazards Analysis of Overall Survival

Parameter	Hazard Ratio	95% CI	P
Age (<70 y vs ≥70 y)	1.093	0.740-1.613	.655
Sex (men vs women)	1.166	0.772-1.760	.897
PS (0 vs 1)	1.41	0.971-2.049	.071
Smoking (< pack years vs > pack years)	1.561	1.037-2.348	.033
Histology (adeno vs nonadeno)	1.031	0.742-1.432	.856
Macrophage distribution (nests < stroma vs nests > stroma)	0.439	0.320-0.602	<.001
$CD8^+$ T cells distribution (nests < stroma vs nests > stroma)	0.723	0.530-0.985	.040

CI indicates confidence interval; PS, performance status; adeno, adenocarcinoma.

associated with an unfavorable outcome in several kinds of cancers.[25-27] Conversely, macrophages in cancer nests produce cytotoxic cytokines, such as IL-1α, IL-1β, IL-6, and TNF-α, which may protect against tumor progression.[28] Considering the results of this study showing that the distributions of macrophages in cancer nests and cancer stroma impacted outcome of stage IV NSCLC, the macrophages in cancer nests and cancer stroma may have different biological activity in regard to tumor progression. Welsh et al demonstrated that higher numbers of macrophages in cancer stroma and lower numbers of macrophages in cancer nests were unfavorable prognosis factors in surgically resected NSCLC,[23] and their findings are in part consistent with the results of our study. No relationship between the numbers of macrophages in cancer nests and patient survival was found in our study. This can be explained by the difference between the specimens from operable cases of NSCLC (stage I-III) and stage IV cases.

$CD8^+$ T cells with cytotoxic activity play an important role in antitumor immunity. $CD8^+$ T cells can circumvent many of the barriers inherent in cancer-induced stroma, while optimizing T-cell specificity, activation, homing, and antitumor function.[29] The presence of tumor-infiltrating $CD8^+$ T cells has previously been reported to be associated with a favorable outcome, the same as in our own study.[5-12,30] Patients in the HNM and HNT group had significantly better survival (median survival was 495 days; 1-year survival rate was 68.5%) than patients in the HNM group (median survival, 440 days; 1-year survival rate, 60.8%; Fig. 2C) and patients in the HNT group (median survival, 388 days; 1-year survival rate, 53.4%; Fig. 2I). There were also many long-term survivors in the HNM and HNT group, which notably had a 3-year survival rate of 19.1%. Because aggregation of tumor-infiltrating macrophages in cancer nests has been reported to have a beneficial effect by activating cytotoxic T cells,[31] the macrophages and $CD8^+$ T cells in cancer nests should exert synergistic antitumor effects. Infiltration of $CD8^+$ T cells in gastric carcinoma is actually directly correlated with macrophage infiltration, suggesting that macrophages play an important part in the activation of T cells and subsequent tumor cell destruction.[31]

Whether there is any correlation between the presence of tumor-infiltrating mast cells and cancer progression is a matter of controversy. In previous studies, mast cells were found to have antitumor

functions, including serving as natural cytotoxic effectors[32,33] and antitumor compounds,[34] and to be a favorable prognostic factor in surgically resected NSCLC, breast cancer, and colorectal cancer.[35-37] Although mast cells produce histamine, basic fibroblast growth factor, heparin, chymase, and tryptase, which have been shown to promote cancer progression, including in surgically resected NSCLC, gastric cancer, and endometrial cancer,[18,38] no significant relation to survival was found in this study.

Accumulation of immune cells in tumor tissue either before or during chemoimmunotherapy has been reported to be associated with a better clinical response and improved survival.[39-41] The effect of chemoradiotherapy in esophageal cancer is correlated with the number of $CD8^+$ T cells in the tumor of each patient, and the patterns of gene expressions for T cell activation and for tumor vessel formation may become good markers for identifying potential long-term survivors.[11] However, in the present study, no correlations between numbers of macrophages, $CD8^+$ T cells, or mast cells and response to first-line chemotherapy were found (Table 2). The results of our study suggested that patients with a favorable or unfavorable prognosis could be identified by the status of tumor-infiltrating macrophages and $CD8^+$ T cells in tumor biopsy specimens before receiving chemotherapy regardless of chemotherapy response. Cancer patients can mount cellular immune responses against their own tumor cells, and hosts can respond to a large compendium of tumor-associated antigens and epitopes. The natural immune system within the cancer microenvironment may affect its ability to control malignant disease beyond the response to chemotherapy. The only treatment currently available for metastatic NSCLC is chemotherapy, but patients with a poor prognosis, and patients with a predominant distribution of macrophages and $CD8^+$ T cells in the cancer stroma, require some additional therapy to prolong life. For example, elimination of macrophages from the cancer stroma or transfer of $CD8^+$ T cells to cancer nests might be beneficial in prolonging the life of stage IV NSCLC patients in these unfavorable groups.

In conclusion, we found that predominant distribution of macrophages and/or $CD8^+$ T cells in cancer nests as opposed to cancer stroma was correlated with a favorable prognosis in stage IV NSCLC patients. Patients with advanced NSCLC require additional therapy, because the response rate to chemotherapy has been poor (only 30%-40%), and the median survival time of patients with metastatic NSCLC is approximately 8 months to 10 months.[20,21] The results of our study indicate the possibility of using macrophages and $CD8^+$ T cells to treat advanced NSCLC in the future.[42] Decreasing the number of tumor-associated macrophages in the tumor stroma in an animal model of breast cancer effectively altered the tumor microenvironment involved in tumor angiogenesis and progression and markedly suppressed tumor growth and metastasis.[43]

Thus, a more accurate insight into the role of macrophages and $CD8^+$ T cells in tumors and consideration of the local microenvironment in regulating the functions of these cells is needed and has important implications for the design of future clinical trials of adjuvant therapy, as well as for our understanding of the immunopathobiology of stage IV NSCLC.

REFERENCES

1. Nonsmall Cell Lung Cancer Collaborative Group. Chemotherapy in nonsmall cell lung cancer: a meta-analysis using updated data on individual patients from 52 randomized clinical trials. *BMJ.* 1995;311:899-909.
2. Bunn PA Jr, Kelly K. New combinations in the treatment of lung cancer: a time for optimism. *Chest.* 2000;117:138S-143S.
3. Bunn PA Jr. Chemotherapy for advanced nonsmall-cell lung cancer: who, what, when, why? *J Clin Oncol.* 2002;20:23S-33S.
4. Sanchez de Cos Escuin J, Disdier Vicente C, Corral Penafiel J, Riesco Miranda JA, Sojo Gonzalez MA, Masa Jimenez JF. Overall long-term survival in lung cancer analyzed in 610 unselected patients [in Spanish]. *Arch Bronconeumol.* 2004;40:268-274.
5. Fukunaga A, Miyamoto M, Cho Y, et al. CD8+ tumor-infiltrating lymphocytes together with CD4+ tumor-infiltrating lymphocytes and dendritic cells improve the prognosis of patients with pancreatic adenocarcinoma. *Pancreas.* 2004;28:e26-e31.
6. Oshikiri T, Miyamoto M, Shichinohe T, et al. Prognostic value of intratumoral CD8+ T lymphocyte in extrahepatic bile duct carcinoma as essential immune response. *J Surg Oncol.* 2003;84:224-228.
7. Cho Y, Miyamoto M, Kato K, et al. CD4+ and CD8+ T cells cooperate to improve prognosis of patients with esophageal squamous cell carcinoma. *Cancer Res.* 2003;63:1555-1559.
8. Nakakubo Y, Miyamoto M, Cho Y, et al. Clinical significance of immune cell infiltration within gallbladder cancer. *Br J Cancer.* 2003;89:1736-1742.
9. Schumacher K, Haensch W, Roefzaad C, et al. Prognostic significance of activated CD8(+) T cell infiltrations within esophageal carcinomas. *Cancer Res.* 2001;61:3932-3936.
10. Naito Y, Saito K, Shiiba K, et al. CD8+ T cells infiltrated within cancer cell nests as a prognostic factor in human colorectal cancer. *Cancer Res.* 1998;58:3491-3494.
11. Ashida A, Boku N, Aoyagi K, et al. Expression profiling of esophageal squamous cell carcinoma patients treated with definitive chemoradiotherapy: clinical implications. *Int J Oncol.* 2006;28:1345-1352.
12. Zhang L, Conejo-Garcia JR, Katsaros D, et al. Intratumoral T cells, recurrence, and survival in epithelial ovarian cancer. *N Engl J Med.* 2003;348:203-213.

13. Tsutsui S, Yasuda K, Suzuki K, et al. Macrophage infiltration and its prognostic implications in breast cancer: the relationship with VEGF expression and microvessel density. *Oncol Rep.* 2005;14:425-431.
14. Hashimoto I, Kodama J, Seki N, et al: Macrophage infiltration and angiogenesis in endometrial cancer. *Anticancer Res.* 2000;20:4853-4856.
15. Koga J, Kakeji Y, Sumiyoshi Y, et al. Angiogenesis and macrophage infiltration in Borrmann type IV gastric cancer [in Japanese]. *Fukuoka Igaku Zasshi.* 2001;92:334-339.
16. Norrby K. Mast cells and angiogenesis. *APMIS.* 2002;110: 355-371.
17. Ozdemir O. Can a mast cell subtype (MC(T)) play a role in the progression of endometrial cancer through angiogenesis? *Am J Obstet Gynecol.* 2006.
18. Yano H, Kinuta M, Tateishi H, et al. Mast cell infiltration around gastric cancer cells correlates with tumor angiogenesis and metastasis. *Gastric Cancer.* 1999;2:26-32.
19. Nakano T, Oka K, Takahashi T, et al. Roles of Langerhans' cells and T-lymphocytes infiltrating cancer tissues in patients treated by radiation therapy for cervical cancer. *Cancer.* 1992;70:2839-2844.
20. Kelly K, Crowley J, Bunn PA Jr, et al. Randomized phase III trial of paclitaxel plus carboplatin versus vinorelbine plus cisplatin in the treatment of patients with advanced non–small-cell lung cancer: a Southwest Oncology Group trial. *J Clin Oncol.* 2001;19:3210-3218.
21. Schiller JH, Harrington D, Belani CP, et al. Comparison of 4 chemotherapy regimens for advanced nonsmall-cell lung cancer. *N Engl J Med.* 2002;346:92-98.
22. Mountain CF. Revisions in the International System for Staging Lung Cancer. *Chest.* 1997;111:1710-1717.
23. Welsh TJ, Green RH, Richardson D, et al. Macrophage and mast-cell invasion of tumor cell islets confers a marked survival advantage in nonsmall-cell lung cancer. *J Clin Oncol.* 2005;23:8959-8967.
24. Chen JJ, Lin YC, Yao PL, et al. Tumor-associated macrophages: the double-edged sword in cancer progression. *J Clin Oncol.* 2005;23:953-964.
25. Leek RD, Hunt NC, Landers RJ, et al. Macrophage infiltration is associated with VEGF and EGFR expression in breast cancer. *J Pathol.* 2000;190:430-436.
26. Ueno T, Toi M, Saji H, et al. Significance of macrophage chemoattractant protein-1 in macrophage recruitment, angiogenesis, and survival in human breast cancer. *Clin Cancer Res.* 2000;6:3282-3289.
27. Shi H, Xu JM, Hu NZ, et al. Transfection of mouse macrophage metalloelastase gene into murine CT-26 colon cancer cells suppresses orthotopic tumor growth, angiogenesis and vascular endothelial growth factor expression. *Cancer Lett.* 2006;233:139-150.
28. Kataki A, Scheid P, Piet M, et al. Tumor infiltrating lymphocytes and macrophages have a potential dual role in lung cancer by supporting both host-defense and tumor progression. *J Lab Clin Med.* 2002;140:320-328.
29. Kershaw MH, Teng MW, Smyth MJ, et al. Supernatural T cells: genetic modification of T cells for cancer therapy. *Nat Rev Immunol.* 2005;5:928-940.
30. Hiraoka K, Miyamoto M, Cho Y, et al. Concurrent infiltration by CD8+ T cells and CD4+ T cells is a favorable prognostic factor in nonsmall-cell lung carcinoma. *Br J Cancer.* 2006;94:275-280.
31. Ohno S, Inagawa H, Dhar DK, et al. The degree of macrophage infiltration into the cancer cell nest is a significant predictor of survival in gastric cancer patients. *Anticancer Res.* 2003;23:5015-5022.
32. Farram E, Nelson DS. Mouse mast cells as anti-tumor effector cells. *Cell Immunol.* 1980;55:294-301.
33. Ghiara P, Boraschi D, Villa L, et al. In vitro generated mast cells express natural cytotoxicity against tumor cells. *Immunology.* 1985;55:317-324.
34. Henderson WR, Chi EY, Jong EC, et al. Mast cell-mediated tumor-cell cytotoxicity. Role of the peroxidase system. *J Exp Med.* 1981;153:520-533.
35. Fisher ER, Paik SM, Rockette H, et al. Prognostic significance of eosinophils and mast cells in rectal cancer: findings from the National Surgical Adjuvant Breast and Bowel Project (protocol R-01). *Hum Pathol.* 1989;20:159-163.
36. Aaltomaa S, Lipponen P, Papinaho S, et al. Mast cells in breast cancer. *Anticancer Res.* 1993;13:785-788.
37. Tomita M, Matsuzaki Y, Onitsuka T. Correlation between mast cells and survival rates in patients with pulmonary adenocarcinoma. *Lung Cancer.* 1999;26:103-108.
38. Ribatti D, Finato N, Crivellato E, et al. Neovascularization and mast cells with tryptase activity increase simultaneously with pathologic progression in human endometrial cancer. *Am J Obstet Gynecol.* 2005;193:1961-1965.
39. Donskov F, Bennedsgaard KM, Hokland M, et al. Leukocyte orchestration in blood and tumor tissue following interleukin-2 based immunotherapy in metastatic renal cell carcinoma. *Cancer Immunol Immunother.* 2004;53:729-739.
40. Bernengo MG, Quaglino P, Cappello N, et al. Macrophage-mediated immunostimulation modulates therapeutic efficacy of interleukin-2 based chemoimmunotherapy in advanced metastatic melanoma patients. *Melanoma Res.* 2000;10:55-65.
41. Hakansson A, Gustafsson B, Krysander L, et al. Biochemotherapy of metastatic malignant melanoma. Predictive value of tumor-infiltrating lymphocytes. *Br J Cancer.* 2001; 85:1871-1877.
42. Bingle L, Brown NJ, Lewis CE. The role of tumor-associated macrophages in tumor progression: implications for new anticancer therapies. *J Pathol.* 2002;196:254-265.
43. Luo Y, Zhou H, Krueger J, et al. Targeting tumor-associated macrophages as a novel strategy against breast cancer. *J Clin Invest.* 2006;116:2132-2141.

Cancer Chemother Pharmacol (2008) 62:529–537
DOI 10.1007/s00280-007-0634-1

ORIGINAL ARTICLE

Impact of *CYP3A4* haplotypes on irinotecan pharmacokinetics in Japanese cancer patients

Kimie Sai · Yoshiro Saito · Hiromi Fukushima-Uesaka · Koichi Kurose · Nahoko Kaniwa · Naoyuki Kamatani · Kuniaki Shirao · Noboru Yamamoto · Tetsuya Hamaguchi · Hideo Kunitoh · Yuichiro Ohe · Tomohide Tamura · Yasuhide Yamada · Hironobu Minami · Atsushi Ohtsu · Teruhiko Yoshida · Nagahiro Saijo · Jun-ichi Sawada

Received: 6 July 2007 / Accepted: 22 October 2007 / Published online: 8 November 2007
© Springer-Verlag 2007

Abstract
Background and purpose Cytochrome P450 3A4 (CYP3A4) converts an anticancer prodrug, irinotecan, to inactive metabolites such as APC. However, the contribution of *CYP3A4* genetic polymorphisms to irinotecan pharmacokinetics (PK) and pharmacodynamics (PD) is not fully elucidated. In paclitaxel-administered cancer patients, an association of *CYP3A4*16B* harboring the low activity allele **16* [554C > G (Thr185Ser)] has been shown with altered metabolite/paclitaxel area under the plasma concentration–time curve (AUC) ratios, suggesting a possible impact of **16B* on the PK of other drugs. In this study, the effects of *CYP3A4* haplotypes including **16B* on irinotecan PK/PD were investigated in irinotecan-administered patients.
Methods The *CYP3A4* genotypes for 177 Japanese cancer patients who received irinotecan were defined in terms of

K. Sai (✉)
Division of Biosignaling, National Institute of Health Sciences,
1-18-1 Kamiyoga, Setagaya-ku, Tokyo 158-8501, Japan
e-mail: sai@nihs.go.jp

Y. Saito · J.-i. Sawada
Division of Biochemistry and Immunochemistry,
National Institute of Health Sciences,
1-18-1 Kamiyoga, Setagaya-ku, Tokyo 158-8501, Japan

H. Fukushima-Uesaka
Project Team for Pharmacogenetics, National Institute of Health Sciences, 1-18-1 Kamiyoga, Setagaya-ku, Tokyo 158-8501, Japan

K. Kurose · N. Kaniwa
Division of Medical Safety Science,
National Institute of Health Sciences,
1-18-1 Kamiyoga, Setagaya-ku, Tokyo 158-8501, Japan

N. Kamatani
Division of Genomic Medicine,
Department of Advanced Biomedical Engineering and Science,
Tokyo Women's Medical University,
8-1 Kawada-cho, Shinjuku-ku, Tokyo 162-8666, Japan

K. Shirao · N. Yamamoto · T. Hamaguchi · H. Kunitoh ·
Y. Ohe · T. Tamura · Y. Yamada
Division of Internal Medicine,
National Cancer Center Hospital,
5-1-5 Tsukiji, Chuo-ku, Tokyo 104-0045, Japan

Present Address:
K. Shirao
Department of Medical Oncology,
Oita University Faculty of Medicine,
1-1 Idaigaoka, Hasama-machi, Yufu 879-5593, Japan

H. Minami
Division of Oncology/Hematology,
National Cancer Center Hospital East,
6-5-1 Kashiwanoha, Kashiwa, Chiba 277-8577, Japan

Present Address:
H. Minami
Medical Oncology, Department of Medicine,
Kobe University Hospital and Graduate School of Medicine,
7-5-2 Kusunoki-cho, Chuo-ku, Kobe 650-0017, Japan

A. Ohtsu
Division of Gastrointestinal Oncology/Digestive Endoscopy,
National Cancer Center Hospital East,
6-5-1 Kashiwanoha, Kashiwa, Chiba 277-8577, Japan

T. Yoshida
Genetics Division, National Cancer Center Research Institute,
5-1-5 Tsukiji, Chuo-ku, Tokyo 104-0045, Japan

N. Saijo
National Cancer Center Hospital East,
6-5-1 Kashiwanoha, Kashiwa, Chiba 277-8577, Japan

4 major haplotypes, i.e., *1A (wild type), *1G (IVS10 + 12G > A), *16B [554C > G (Thr185Ser) and IVS10 + 12G > A], and *18B [878T > C (Leu293Pro) and IVS10 + 12G > A]. Associations of *CYP3A4* genotypes with irinotecan PK and severe toxicities (grade 3 diarrhea and grade 3 or 4 neutropenia) were investigated.

Results Area under the concentration–time curve ratios of APC/irinotecan, an in vivo parameter for CYP3A4 activity, were significantly higher in females than in males. The male patients with *16B showed significantly decreased AUC ratios (APC/irinotecan) with 50% of the median value of the *non-*16B* male patients (no *16B-bearing female patients in this study), whereas no significant alteration in the AUC ratios was observed in the patients with *18B. A slight trend toward increasing AUC ratios (20%) was detected in both male and female patients bearing *1G. Multivariate analysis confirmed contributions of *CYP3A4*16B* (coefficient ± SE = −0.18 ± 0.077, $P = 0.021$) and *1G (0.047 ± 0.021, $P = 0.029$) to the AUC ratio. However, no significant association was observed between the *CYP3A4* genotypes and total clearance of irinotecan or toxicities (severe diarrhea and neutropenia).

Conclusion This study suggested that *CYP3A4*16B* was associated with decreased metabolism of irinotecan to APC. However, the clinical impact of *CYP3A4* genotypes on total clearance and irinotecan toxicities was not significant.

Keywords CYP3A4 · Haplotype · Irinotecan · Pharmacogenetics

Introduction

Human cytochrome P450 3A4 (CYP3A4) is a major CYP enzyme, abundant in the liver and intestine, and is involved in the metabolism of endogenous substances, including steroid hormones, and a variety of exogenous compounds such as environmental chemicals and pharmaceuticals. Large inter-individual differences in liver and intestinal CYP3A4 expression levels are known and thought to be caused by multiple factors including genetic variations, disease status, and modulation by exogenous stimuli, such as smoking, diet, and drugs [5, 18, 31]. The tissue-specific CYP3A4 expression is regulated by constitutive and inducible mechanisms via activation of the nuclear receptors, pregnane X receptor (PXR), constitutive androstane receptor (CAR), and vitamin D receptor (VDR) [5, 18]. Since approximately half of clinical drugs currently in use are metabolized by CYP3A4 [5, 33], it is important to find suitable biomarkers, including genetic polymorphisms, which can reflect in vivo CYP3A4 activity and predict individual responses to CYP3A4-metabolized drugs. Recent progress in pharmacogenetic research has led to the accumulation of knowledge about *CYP3A4* genetic variations responsible for altered expression or function. To date, more than 30 *CYP3A4* variations have been identified (http://www.cypalleles.ki.se/cyp3a4.htm), and large ethnic differences in their frequencies have been recognized. *CYP3A4*1B* (−392A > G), a single nucleotide polymorphism (SNP) in the 5′-flanking region, is found in Caucasians (2–9.6%) and African-Americans (35–67%), but not in Asians [16]. As relatively frequent coding SNPs, *2 [664T > C (Ser222Pro)] (2.7%) and *17 [566T > C (Phe189Ser)] (2%) were detected in Caucasians; *10 [520G > C (Asp174His)] in Caucasians (0.24–2%) and Mexicans (5%); *15 [485G > A (Arg162 Gln)] (2–4%) in African–Americans; *16 [554C > G (Thr185Ser)] in East Asians (1.4–5%) and Mexicans (5%); *18 [878T > C (Leu293Pro)] (2.3–10%) in East Asians [2, 4, 17, 24]. We previously identified 25 *CYP3A4* haplotypes in a Japanese population [4]. The haplotypes *6 [including 830_831insA (Glu277fsX8)] (0.1%), *11 [including 1088C > T (Thr363Met)] (0.2%), *16B [including 554C > G (Thr185Ser)] (1.4%), and *18B [including 878T > C (Leu293Pro)] (2.8%) were identified, but *1B (−392A > G) was not found. These findings indicate that ethnic-specific *CYP3A4* haplotypes must be taken into consideration in pharmacogenetic studies.

Irinotecan, an anticancer prodrug, is used for treatment of various cancers including lung and colon, and metabolized by CYP3A4 to produce inactive compounds such as APC (a major CYP3A4-mediated product) and NPC (a minor product) [6, 7]. An active metabolite SN-38 (a topoisomerase I inhibitor) is produced from the parent compound by carboxylesterases (CES) [28] and subsequently glucuronidated by UDP-glucuronosyltransferase 1As (UGT1As) to form inactive compound SN-38G [12] (Fig. 1). The parent compound and its metabolites are mainly excreted into the bile [29], where several ABC transporters, such as P-glycoprotein (P-gp), breast cancer resistance protein (BCRP), and multidrug resistance-associated protein 2 (MRP2) are involved in excretion [30]. The dose-limiting toxicities of irinotecan are severe diarrhea and neutropenia, and high plasma concentrations of SN-38 and/or its accumulation in tissues are thought to cause these toxicities [3, 30]. Recent extensive pharmacogenetic studies on irinotecan, mostly focusing on the *UGT1A1* genotypes, have revealed important roles for *UGT1A1*28* and *6 in reduced in vivo UGT activity and enhanced toxicities [1, 8, 9, 11, 13, 22, 26]. On the other hand, CYP3A4 can modulate irinotecan pharmacokinetics (PK). Co-administration of ketoconazole, a CYP3A4 inhibitor and also a potent UGT1A1 inhibitor [34], with irinotecan resulted in a decreased value of the area under the concentration–time curve (AUC) for APC and also increased AUC for SN-38 [14]; and vice versa, co-administration of St. John's Wort,

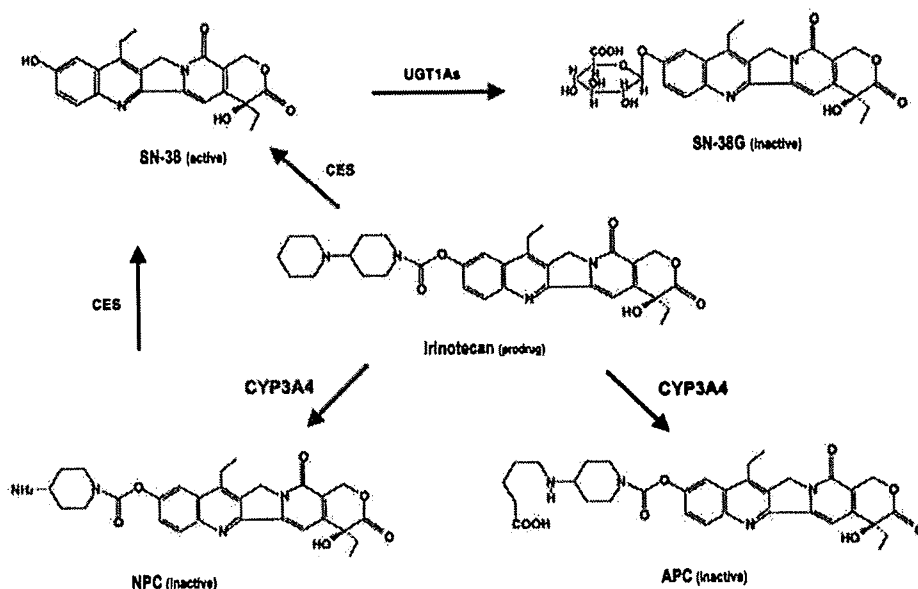

Fig. 1 Irintoecan metabolism in human liver. CYP3A4 mediates oxidation of irinotecan to produce inactive compounds, such as APC (a major CYP3A4-mediated product) and NPC (a minor product)

a CYP3A4 inducer, decreased the AUC of SN-38 [19]. A close association was also reported between in vivo CYP3A4 phenotypes and irinotecan clearance [21]. To date, however, no clinical impact by *CYP3A4* polymorphisms, such as **1B* (−392A > G) and **3* [1334T > C (Met445Thr)], has been demonstrated on irinotecan PK in Caucasians [20]. We previously found that **16* [554C > G (Thr185Ser)] caused decreased in vitro CYP3A4 activities [23]. Furthermore, a significant association of **16B* [harboring 554C > G (Thr185Ser)] was demonstrated with decreased AUC ratios of metabolite/paclitaxel, an in vivo parameter of CYP3A4 activity, in paclitaxel-administered Japanese patients [24].

In this study, to determine the clinical impact of the *CYP3A4* polymorphisms on irinotecan therapy, we identified the *CYP3A4* diplotypes of 177 Japanese cancer patients who received irinotecan and analyzed associations of the *CYP3A4* genotypes with irinotecan PK and toxicities.

Materials and methods

Patients and irinotecan treatment

One hundred seventy-seven patients with cancers who started irinotecan-containing therapy from 2002 to 2004 at two National Cancer Center Hospitals (Tokyo and Kashiwa, Japan) were enrolled for this pharmacogenetic study on irinotecan. This study was approved by the ethics committees of the National Cancer Center and the National Institute of Health Sciences, and written informed consent was obtained from all participants. No participant received irinotecan previously, and other eligibility criteria included: bilirubin < 2 mg/dl, aspartate aminotransferase (GOT) < 105 IU/l, alanine aminotransferase (GPT) < 120 IU/l, creatinine < 1.5 mg/dl, white blood cell count > 3000/μl, performance status of 0–2, and an interval of at least 4 weeks after the last session of chemotherapy (2 weeks after the last session of radiotherapy). Exclusion criteria were diarrhea, active infection, intestinal paralysis or obstruction, and interstitial pneumonitis. Irinotecan was administered as a single agent or in combination chemotherapy at the discretion of attending physicians. Doses and schedules were applied according to the approved treatment recommendations in Japan: intravenous 90-min infusion at a dose of 100 mg/m^2 weekly or 150 mg/m^2 biweekly for irinotecan-monotherapy, and 60 mg/m^2 weekly for combination therapy with cisplatin. Profiles of the patients and irinotecan regimens are summarized in Table 1.

Genotyping of *UGT1A1* and *CYP3A4*

DNA was extracted from pretreatment whole-blood samples taken from 177 patients who received irinotecan. Data on *UGT1A1* genetic polymorphisms obtained from the same set of DNA samples have been published elsewhere [22]. The *CYP3A4* genotypes for 88 patients were previously determined [4]. Additional *CYP3A4* genotyping for the remaining 89 patients was conducted using the pyrosequencing method described previously [24], and the *CYP3A4* diplotypes/haplotypes [4] were inferred using an expectation-maximization-based program, LDSUPPORT [15].

Pharmacokinetics and toxicities

Pharmacokinetic analysis for irinotecan in 176 patients (data on one patient was unavailable) was performed as

Table 1 Profiles of Japanese cancer patients in this study

			No. of patients
Patients for genotyping			177
(Male/female)			(135/42)
Age			
Mean/range	60.5/26–78		
Performance status	0/1/2		84/89/4
Combination therapy, tumor type and initial dose of irinotecan[a]			
Irinotecan monotherapy	Lung	100 (60–100)/w	21
	Colon	150 (120–150)/2w	28
	Others	100 (100–150)/w	7
With platinum-containing drug[b]	Lung	60 (50–90)/w	58
	Stomach	70/2w	9
	Others	60/w	5
With 5-fluorouracil (5-FU)/leucovorin (LV)[c] or tegafur/gimeracil/oteracil potassium[d]	Colon	100 (90–180)/w or 150/2w	34
	Others	90/w or 100/w	2
With mitomycin C (MMC)[e]	Stomach	150/2w	10
	Colon	150/2w	1
With amrubicin[f]	Lung	60/w	2

[a] The median value and range in the parentheses are shown. "/w" and "/2w" represent weekly and biweekly, respectively

[b] Mostly, cisplatin (60 or 80 mg/m^2) was administered after irinotecan treatment

[c] LV (10 mg/m^2) was administered right after irinotecan treatment and then followed by 5-FU treatment. (500 mg/m^2 injection); or LV (200 mg/m^2) was administered simultaneously with irinotecan and followed by 5-FU treatment (400 mg/m^2 bolus injection and 2.0–2.4 g/m^2 infusion)

[d] Tegafur (80 mg/m^2 per day)/gimeracil/oteracil potassium was administered twice (before irinotecan treatment and on the next day)

[e] MMC (5 mg/m^2) was administered just before irinotecan treatment

[f] Amrubicin (30 or 35 mg/m^2) was administered 24 h after irinotecan treatment

previously described [26]. Briefly, heparinized blood was collected before administration of irinotecan, and 0, 0.3, 1, 2, 4, 8, and 24 h after termination of the first infusion of irinotecan. Plasma concentrations of irinotecan and APC were determined by HPLC [25], and AUC_{inf} and other PK parameters were calculated using the trapezoidal method of the 202 non-compartmental model for a constant infusion in WinNonlin ver. 4.01 (Pharsight Corporation, Mountain View, CA, USA). As for the co-administered anti-cancer and other drugs which were administered within 1 week before irinotecan-treatment, no drugs significantly affected the PK parameters related to CYP3A4 activity. Information on foods and drinks taken by the patients which might induce or inhibit CYP3A4 activity was not available.

A complete medical history and data on physical examinations were recorded prior to irinotecan therapy. Complete blood cell counts with differentials and platelet counts, as well as blood chemistry, were measured once a week during the first 2 months of irinotecan treatment. Toxicities were graded according to the Common Toxicity Criteria of National Cancer Institute version 2. Association of genetic factors with irinotecan toxicities was analyzed primarily in patients who received irinotecan as a single agent.

Statistical analysis

Statistical analysis on the differences in PK parameters between sexes and among *CYP3A4* genotypes was performed using the Mann–Whitney test or Kruskal–Wallis test, and associations of *CYP3A4* genotypes with the irinotecan toxicities were assessed by the Chi-square test, using Prism version 4.0 (GraphPad Prism Software Inc. San Diego, CA, USA). $P = 0.05$ (two-tailed) was set as a significant level of difference. Multivariate analysis for the log-transformed AUC ratio (APC/irinotecan) was performed using age, sex, body surface area, dosage of irinotecan, history of smoking or drinking, performance status, co-administered drugs, serum biochemistry parameters at baseline, and genetic factors (including *CYP3A4* haplotypes and the *UGT1A1*6 or *28* haplotype obtained in our previous study [22]) as independent variables. Multivariate analysis on toxicities (grade 3 diarrhea or nadir of absolute neutrophil counts) was conducted for the patients who received irinotecan monotherapy, where the variables included dosing interval and the absolute neutrophil count at baseline, in addition to the other patient background and genetic factors described above. The variables in the final

models for both AUC ratio and toxicities were chosen by the forward and backward stepwise procedure at the significance level of 0.1 using JMP version 6.0.0 software (SAS Institute, Inc., Cary, NC, USA).

Results

Sex difference in PK parameters

Since hepatic CYP3A4 levels were reported to be significantly higher in females than in males [24, 32], we first analyzed the sex differences in the major PK parameters for irinotecan and APC, a major CYP3A4 metabolite (Table 2). As for irinotecan, lower total clearance and MRT, and higher AUC/dose were observed in females, but the differences (3, 5 and 3%, respectively) were not significant. A small but significant increase in C_{max}/dose for irinotecan was observed in females. This is attributable to the smaller distribution volume of females. On the other hand, the median values of AUC/dose and C_{max}/dose for APC of the females were significantly higher than those of the males (1.29- and 1.33-fold, respectively). The AUC ratio (APC/irinotecan), a parameter of in vivo CYP3A4 activity, was significantly higher (1.28-fold) in females than in males. These findings suggest that these differences may reflect the higher CYP3A4 activity in the females.

CYP3A4 genotypes

CYP3A4 diplotypes/haplotypes in 177 Japanese cancer patients were determined according to the previous definition [4]. The *CYP3A4* haplotypes found in this population were **1A* (wild type), **1G* (IVS10 + 12G > A alone), **16B* [554C > G (Thr185Ser) and IVS10 + 12G > A], and **18B* [878T > C (Leu293Pro) and IVS10 + 12G > A]. In the current study, neither **6* [830_831insA (Glu277fsX8)] nor **11* [1088C > T (Thr363Met)] were found. The frequencies of **1G*, **16B*, and **18B* were 0.215, 0.014, and 0.020 (Table 3), and they were comparable to those obtained in previous reports [4, 24]. Note that the haplotypes **16B* and **18B* were detected only in male patients.

Associations of *CYP3A4* genotypes with PK parameters

Considering the significant sex difference in APC levels, associations between the *CYP3A4* genotypes and PK parameters were analyzed for each sex separately. In male patients, no significant differences among the *CYP3A4* genotypes were observed for total clearance and MRT of irinotecan (Fig. 2a, b). In females, a slightly but significantly lower (10%) median value for MRT of irinotecan was observed in patients bearing **1G* compared with those carrying the wild type (**1A*/**1A*) ($P = 0.022$, Mann–Whitney test) (Fig. 2b), whereas no significant **1G*-dependency was observed for total clearance (Fig. 2a). No significant

Table 3 Frequencies of *CYP3A4* haplotypes (A) and diplotypes (B) for Japanese cancer patients in this study

(A) Haplotype group[a]	No. of chromosomes ($N = 354$)	Frequency
*1A	266	0.751
*1G	76	0.215
*16B	5	0.014
*18B	7	0.020
(B) Diplotype	**No. of patients ($N = 177$)**	**Frequency**
*1A/*1A	100	0.565
*1G/*1A	55	0.311
*1G/*1G	10	0.056
*16B/*1A	4	0.023
*16B/*1G	1	0.006
*18B/*1A	7	0.040

[a] Groups based on tagging SNPs of major haplotypes previously defined [4]; **1A* wild type, **1G* IVS10 + 12G > A; **16B* 554C > G (Thr185Ser) and IVS10 + 12G > A; **18B* 878T > C (Leu293Pro) and IVS10 + 12G > A

Table 2 Pharmacokinetic parameters for irinotecan-administered Japanese patients and sex differences

Parameters	Male ($N = 134$) Median (25–75‰)	Female ($N = 42$) Median (25–75‰)	P value[a]
Irinotecan			
Total CL (l/h per m^2)	22.6 (18.5–26.9)	21.8 (17.8–25.1)	0.242
AUC/dose (10^{-3} h m^2 per l)	44.4 (37.3–54.1)	45.8 (39.8–55.8)	0.242
C_{max}/dose (10^{-3} m^2 per l)	10.0 (8.96–11.3)	11.4 (10.4–12.4)	0.0003
MRT (h)	6.61 (6.01–7.40)	6.29 (5.78–7.12)	0.202
APC			
AUC/dose (10 h m^2 per l)	6.72 (5.23–9.49)	8.66 (6.57–13.1)	0.0071
C_{max}/dose (10^{-3} m^2 per l)	0.560 (0.430–0.805)	0.745 (0.610–1.14)	0.0007
AUC ratio (APC/irinotecan)	0.151 (0.114–0.210)	0.194 (0.132–0.266)	0.0179

CL clearance; *MRT* mean residence time

[a] Mann–Whitney test

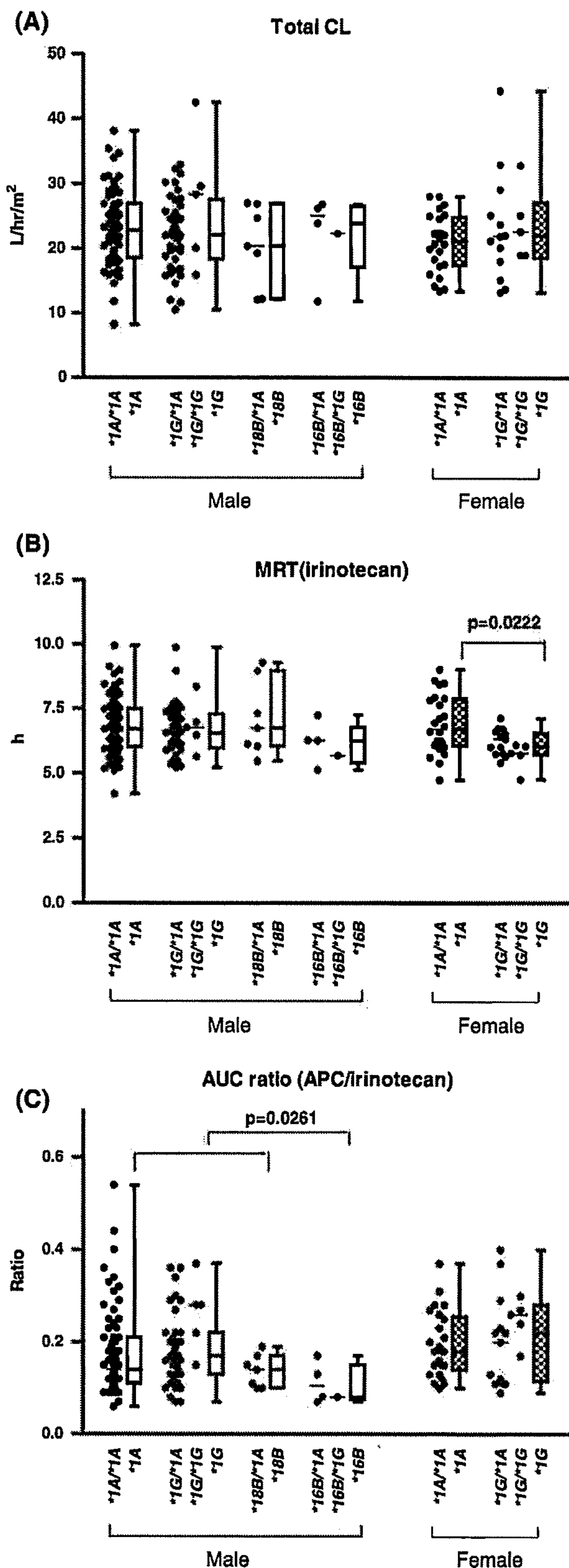

Fig. 2 Association of *CYP3A4* genotypes with irinotecan pharmacokinetics in Japanese cancer patients. The values of mean residence time (MRT) of irinotecan in female patients were significantly lower in those with **1G* than those with the wild-type (**1A/*1A*) ($P = 0.0222$, Mann–Whitney test). The levels of the AUC ratio (APC/irinotecan), a parameter of CYP3A4 activity, in male patients were significantly lower in those with **16B* than those without **16B* ($P = 0.0261$, Mann–Whitney test)

differences in C_{max}/dose for irinotecan among the genotypes were observed in both males and females (data not shown). Regarding the AUC ratio (APC/irinotecan) in males, a significantly lower median value (50%) was observed in patients with **16B* than patients without **16B* (i.e., *non-*16B* patients) ($P = 0.0261$, Mann–Whitney test) (Fig. 2c). In contrast, no significant changes in the AUC ratio (APC/irinotecan) were detected in the male **18B* heterozygotes. In both males and females, a higher median AUC ratio (20%), without statistical significance, was observed in **1G*-bearing patients (**1G/*1A* and **1G/*1G*) than wild-type patients (**1A/*1A*). As for C_{max}/dose of APC, similar trends were observed (without statistical significance): 35% decrease in the median value for **16B* compared with *non-*16B*; 10 and 20% increases in males and females, respectively, for **1G* compared with the wild type (data not shown).

Multivariate analysis of PK parameters

To further clarify contributions of the *CYP3A4* polymorphisms to APC generation, multivariate analysis was conducted on the AUC ratio (APC/irinotecan) data, where variables included patient backgrounds, irinotecan regimens, and *CYP3A4* (**1G, *16B* and **18B*) and *UGT1A1* (**6 or *28*) haplotypes. Significant contributions of *CYP3A4*16B* (coefficient ± SE = -0.18 ± 0.077, $P = 0.021$) and **1G* (0.047 ± 0.021, $P = 0.029$) to the AUC ratio (APC/irinotecan) were confirmed, in addition to the contributions of two patient background factors, sex (female) and hepatic function (serum GOT and ALP) (Table 4). No significant associations were observed between the *CYP3A4* polymorphisms and total clearance or MRT of irinotecan (data not shown).

Associations of *CYP3A4* genotypes with toxicities

Severe irinotecan toxicities, grade 3 diarrhea and grade 3 or 4 neutropenia, were monitored in 176 patients during 2 months after starting irinotecan therapy. Since incidences of severe toxicities depended on the irinotecan regimens used and a higher incidence of severe neutropenia with co-medication was evident [22], associations of the *CYP3A4*

Table 4 Multivariate analyasis of AUC ratio (APC/irinotecan)

Variable	Coefficient	SE	P value
Female	0.040	0.016	0.0132
Serum GOT and ALP[a]	0.110	0.021	<0.0001
Serum creatinine[b]	0.132	0.071	0.0651
*CYP3A4*16B*	−0.180	0.077	0.0213
*CYP3A4*1G*	0.047	0.021	0.0291

The values after logarithmic conversion were used

R^2 0.225; Intercept −0.794; *N* 176

[a] Grade 1 or greater scores in both serum GOT and ALP before irinotecan treatment

[b] The absolute value (mg/dl) before irinotecan treatment

haplotypes with toxicities were evaluated in patients who received irinotecan monotherapy. Because there was no sex difference in the incidences of severe toxicities, the patients with irinotecan monotherapy were not stratified by sex. Furthermore, significant contributions of *UGT1A1*6* and *28 to neutropenia were previously demonstrated [22]. Therefore, the incidence of severe neutropenia was also evaluated among the wild-type patients without *UGT1A1*6 or *28* (*UGT −/−*). No significant differences in the incidences of severe diarrhea and neutropenia were observed among the *CYP3A4* diplotypes of all or *UGT −/−* patients with irinotecan monotherapy (Table 5). It must be noted that the **16B*-bearing patient (*N* = 1) treated with irinotecan monotherapy did not experience either toxicity. Similarly, for **1G* and **18B*, no statistically significant change in the neutropenia or diarrhea incidence was observed. Multivariate analysis also revealed no significant contribution of the *CYP3A4* polymorphisms to severe diarrhea (logistic model) or absolute neutrophil count nadir (data not shown).

Table 5 Association of *CYP3A4* genotypes with severe toxicities in irinotecan monotherapy

Diplotype	Diarrhea[a]/total (%)	Neutropenia[b]/total (%)	
	All	All	UGT-/-[c]
*1A/*1A	3/27 (11.1)	5/27 (18.5)	2/11 (18.2)
*1G/*1A	2/20 (10.0)	5/20 (25.0)	1/9 (11.1)
*1G/*1G	0/3 (0.0)	2/3 (66.7)	0/0 (–)
*16B/*1A	0/1 (0.0)	0/1 (0.0)	0/0 (–)
*18B/*1A	1/4 (25.0)	2/4 (50.0)	0/1 (0.0)
P value[d]	0.8571	0.289	

[a] Grade 3

[b] Grade 3 or 4

[c] Wild type without *UGT1A1 *6 or *28*

[d] Chi-square test

Discussion

In the current study, the higher in vivo CYP3A4 activity in females than in males [24, 32] was suggested from the CYP3A4-mediated APC formation. Since correlations between in vivo CYP3A4 activity and irinotecan PK parameters have been reported [14, 19, 21], clinical impact of *CYP3A4* polymorphisms on irinotecan PK has been presumed. In this study, we demonstrated for the first time a role of *CYP3A4*16B* [554C > G (Thr185Ser) and IVS10 + 12G > A] in reduced APC generation (Fig. 2; Table 4). This finding is concordant with the findings of our previous studies showing a reduced in vitro activity of CYP3A4 by **16* [23] and altered AUC ratios of metabolite/paclitaxel in paclitaxel-administered Japanese patients bearing **16B* [24]. These findings indicate that *CYP3A4*16* could modulate pharmacokinetics of other drugs which are metabolized by CYP3A4. On the contrary, **18B* [878T > C (Leu293Pro) and IVS10 + 12G > A] did not alter the AUC ratios (APC/irinotecan) in irinotecan-administered patients. This also coincides with our previous finding that showed no clinical impact of **18B* on the metabolite/paclitaxel AUC ratio [24].

In the current study, an increasing trend in the AUC ratios (APC/irinotecan) by **1G* (IVS10 + 12G > A) was detected in both males and females, although their increases were small (20% in the median values). In accordance with this tendency, significant reduction in MRT of irinotecan by **1G* was observed in females, whereas this was not significant in males. At present, the reason of this sex-difference in MRT is not clear. Our previous haplotype analysis of the *CYP3A4* and *CYP3A5* regions revealed that *CYP3A4*1G* is mostly linked to *CYP3A5*1* but rarely to *CYP3A5*3* [3] which is a defective allele [10, 16, 17, 33]. Therefore, there is a possibility that *CYP3A5* polymorphisms rather than *CYP3A4*1G* contribute to irinotecan PK. However, this speculation is unlikely because CYP3A5 produces only a very minor metabolite of irinotecan, a de-ethylated product [27]. Since the effect of **1G* was relatively small and was not shown in case of paclitaxel [23], the clinical importance of **1G* should be further evaluated in pharmacogenetic studies on other drugs.

Contrary to the clear reduction in APC production, changes in the PK parameters for the parent compound, i.e., total clearance and Cmax of irinotecan, were not affected by the *CYP3A4* haplotypes. Furthermore, multivariate analysis revealed no associations of the *CYP3A4* haplotypes with the AUC ratio of (SN-38 + SN-38G)/irinotecan, an in vivo parameter for CES activity, and with the AUC ratio of SN-38 (SN-38/irinotecan) (data not shown). We previously observed that the total clearance of irinotecan was affected by other non-genetic factors, such as age, smoking, hepatic and renal functions, and co-administered drugs

(unpublished data), and that the plasma level of SN-38 was largely influenced by *UGT1A1*6* and **28* [22]. Therefore, it is likely that the contribution of CYP3A4 to irinotecan clearance is rather small as compared with other genetic and non-genetic factors.

In accordance with the above observations, no significant associations were observed between the *CYP3A4* haplotypes and severe toxicities (grade 3 diarrhea and grade 3 or 4 neutropenia) in the patients with irinotecan monotherapy (Table 5). Similarly, we observed no significant effect of the *CYP3A4* haplotypes on incidence of the severe toxicities in the patients treated with both irinotecan and cisplatin (data not shown), although the numbers of patients bearing **16B* and **18B* were small. Taken together, the current study indicates that the influence of the *CYP3A4* genotypes on the activation pathway of irinotecan (generation of SN-38) might be small.

In conclusion, the current study suggested that *CYP3A4*16B* was associated with decreased metabolism of irinotecan to APC. However, impact of the *CYP3A4* haplotypes on total clearance of irinotecan and severe toxicities was not significant.

Acknowledgments This study was supported in part by the Program for the Promotion of Fundamental Studies in Health Sciences of the National Institute of Biomedical Innovation and by the Program for the Promotion of Studies in Health Sciences of the Ministry of Health, Labor, and Welfare of Japan. Analytical standards of irinotecan and its metabolites were kindly supplied by Yakult Honsha Co. Ltd. (Tokyo, Japan). We thank Ms. Chie Sudo for her secretarial assistance.

References

1. Ando Y, Saka H, Ando M, Sawa T, Muro K, Ueoka H, Yokoyama A, Saitoh S, Shimokata K, Hasegawa Y (2000) Polymorphisms of UDP-glucuronosyltransferase gene and irinotecan toxicity: a pharmacogenetic analysis. Cancer Res 60:6921–6916
2. Dai D, Tang J, Rose R, Hodgson E, Bienstock RJ, Mohrenweiser HW, Goldstein JA (2001) Identification of variants of CYP3A4 and characterization of their abilities to metabolize testosterone and chlorpyrifos. J Pharmacol Exp Ther 299:825–831
3. de Forni M, Bugat R, Chabot GG, Culine S, Extra JM, Gouyette A, Madelaine I, Marty ME, Mathieu-Boue A (1994) Phase I and pharmacokinetic study of the camptothecin derivative irinotecan, administered on a weekly schedule in cancer patients. Cancer Res 54:4347–4354
4. Fukushima-Uesaka H, Saito Y, Watanabe H, Shiseki K, Saeki M, Nakamura T, Kurose K, Sai K, Komamura K, Ueno K, Kamakura S, Kitakaze M, Hanai S, Nakajima T, Matsumoto K, Saito H, Goto Y, Kimura H, Katoh M, Sugai K, Minami N, Shirao K, Tamura T, Yamamoto N, Minami H, Ohtsu A, Yoshida T, Saijo N, Kitamura Y, Kamatani N, Ozawa S, Sawada J (2004) Haplotypes of CYP3A4 and their close linkage with CYP3A5 haplotypes in a Japanese population. Hum Mutat 23:100
5. Guengerich FP (1999) Cytochrome P-450 3A4: regulation and role in drug metabolism. Annu Rev Pharmacol Toxicol 139:1–17
6. Haaz MC, Rivory L, Riche C, Vernillet L, Robert J (1998) Metabolism of irinotecan (CPT-11) by human hepatic microsomes: participation of cytochrome P-450 3A and drug interactions. Cancer Res 58:468–472
7. Haaz MC, Riche C, Rivory LP, Robert J (1998) Biosynthesis of an aminopiperidino metabolite of irinotecan [7-ethyl-10-[4-(1-piperidino)-1-piperidino]carbonyloxycamptothecine] by human hepatic microsomes. Drug Metab Dispos 26:769–774
8. Han JY, Lim HS, Shin ES, Yoo YK, Park YH, Lee JE, Jang IJ, Lee DH, Lee JS (2006) Comprehensive analysis of UGT1A polymorphisms predictive for pharmacokinetics and treatment outcome in patients with non-small-cell lung cancer treated with irinotecan and cisplatin. J Clin Oncol 24:2237–2244
9. Hoskins JM, Goldberg RM, Qu P, Ibrahim JG, McLeod HL (2007) UGT1A1*28 genotype and irinotecan-induced neutropenia: dose matters. J Natl Cancer Inst 99:1290–1295
10. Hustert E, Haberl M, Burk O, Wolbold R, He YQ, Klein K, Nuessler AC, Neuhaus P, Klattig J, Eiselt R, Koch I, Zibat A, Brockmoller J, Halpert JR, Zanger UM, Wojnowski L (2001) The genetic determinants of the CYP3A5 polymorphism. Pharmacogenetics 11:773–779
11. Innocenti F, Undevia SD, Iyer L, Chen PX, Das S, Kocherginsky M, Karrison T, Janisch L, Ramirez J, Rudin CM, Vokes EE, Ratain MJ (2004) Genetic variants in the UDP-glucuronosyltransferase 1A1 gene predict the risk of severe neutropenia of irinotecan. Clin Oncol 22:1382–1388
12. Iyer L, King CD, Whitington PF, Green MD, Roy SK, Tephly TR, Coffman BL, Ratain MJ (1998) Genetic predisposition to the metabolism of irinotecan (CPT-11). Role of uridine diphosphate glucuronosyltransferase isoform 1A1 in the glucuronidation of its active metabolite (SN-38) in human liver microsomes. J Clin Invest 15:847–854
13. Iyer L, Das S, Janisch L, Wen M, Ramirez J, Karrison T, Fleming GF, Vokes EE, Schilsky RL, Ratain MJ (2002) UGT1A1*28 polymorphism as a determinant of irinotecan disposition and toxicity. Pharmacogenomics J 2:43–47
14. Kehrer DF, Mathijssen RH, Verweij J, de Bruijn P, Sparreboom A (2002) Modulation of irinotecan metabolism by ketoconazole. J Clin Oncol 20:3122–3129
15. Kitamura Y, Moriguchi M, Kaneko H, Morisaki H, Morisaki T, Toyama K, Kamatani N (2002) 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
16. Kuehl P, Zhang J, Lin Y, Lamba J, Assem M, Schuetz J, Watkins PB, Daly A, Wrighton SA, Hall SD, Maurel P, Relling M, Brimer C, Yasuda K (2001) Venkataramanan R, Strom S, Thummel K, Boguski MS, Schuetz E. Sequence diversity in CYP3A promoters and characterization of the genetic basis of polymorphic CYP3A5 expression. Nat Genet 27:383–391
17. Lamba JK, Lin YS, Schuetz EG, Thummel KE (2002) Genetic contribution to variable human CYP3A-mediated metabolism. Adv Drug Deliv Rev 54:1271–1294
18. Martinez-Jimenez CP, Jover R, Donato MT, Castell JV, Gomez-Lechon MJ (2007) Transcriptional regulation and expression of CYP3A4 in hepatocytes. Curr Drug Metab 8:185–194
19. Mathijssen RH, Verweij J, de Bruijn P, Loos WJ, Sparreboom A (2002) Effects of St. John's wort on irinotecan metabolism. J Natl Cancer Inst 94:1247–1249
20. Mathijssen RH, Marsh S, Karlsson MO, Xie R, Baker SD, Verweij J, Sparreboom A, McLeod HL (2003) Irinotecan pathway genotype analysis to predict pharmacokinetics. Clin Cancer Res 9:3246–3253
21. Mathijssen RH, de Jong FA, van Schaik RH, Lepper ER, Friberg LE, Rietveld T, de Bruijn P, Graveland WJ, Figg WD, Verweij J, Sparreboom A (2004) Prediction of irinotecan pharmacokinetics by use of cytochrome P450 3A4 phenotyping probes. J Natl Cancer Inst 96:1585–1592

22. Minami H, Sai K, Saeki M, Saito Y, Ozawa S, Suzuki K, Kaniwa N, Sawada J, Hamaguchi T, Yamamoto N, Shirao K, Yamada Y, Ohmatsu H, Kubota K, Yoshida T, Ohtsu A, Saijo N (2007) Irinotecan pharmacokinetics/pharmacodynamics and UGT1A genetic polymorphisms in Japanese: Roles of UGT1A1*6 and *28. Pharmacogenet Genomics 17:497–504
23. Murayama N, Nakamura T, Saeki M, Soyama A, Saito Y, Sai K, Ishida S, Nakajima O, Itoda M, Ohno Y, Ozawa S, Sawada J (2002) CYP3A4 gene polymorphisms influence testosterone 6-beta-hydroxylation. Drug Metab Pharmacokinet 7:150–156
24. Nakajima Y, Yoshitani T, Fukushima-Uesaka H, Saito Y, Kaniwa N, Kurose K, Ozawa S, Aoyagi N, Kamatani N, Yamamoto N, Kunitoh H, Ohe Y, Tamura T, Yoshida T, Minami H, Saijo N, Katori N, Sawada J (2006) Impact of the haplotype CYP3A4*16B harboring the Thr185Ser substitution on paclitaxel metabolism in Japanese patients with cancer. Clin Pharmacol Ther 80:179–191
25. Sai K, Kaniwa N, Ozawa S, Sawada J (2002) An analytical method for irinotecan (CPT-11) and its metabolites using a high-performance liquid chromatography: parallel detection with fluorescence and mass spectrometry. Biomed Chromatogr 16:209–218
26. Sai K, Saeki M, Saito Y, Ozawa S, Katori N, Jinno H, Hasegawa R, Kaniwa N, Sawada J, Komamura K, Ueno K, Kamakura S, Kitakaze M, Kitamura Y, Kamatani N, Minami H, Ohtsu A, Shirao K, Yoshida T, Saijo N (2004) UGT1A1 haplotypes associated with reduced glucuronidation and increased serum bilirubin in irinotecan-administered Japanese patients with cancer. Clin Pharmacol Ther 75:501–515
27. Santos A, Zanetta S, Cresteil T, Deroussent A, Pein F, Raymond E, Vernillet L, Risse ML, Boige V, Gouyette A, Vassal G (2000) Metabolism of irinotecan (CPT-11) by CYP3A4 and CYP3A5 in humans. Clin Cancer Res 6:2012–2020
28. Slatter JG, Su P, Sams JP, Schaaf LJ, Wienkers LC (1997) Bioactivation of the anticancer agent CPT-11 to SN-38 by human hepatic microsomal carboxylesterases and the in vitro assessment of potential drug interactions. Drug Metab Dispos 25:1157–1164
29. Slatter JG, Schaaf LJ, Sams JP, Feenstra KL, Johnson MG, Bombardt PA, Cathcart KS, Verburg MT, Pearson LK, Compton LD, Miller LL, Baker DS, Persheck CV, Lord RS III (2000) Pharmacokinetics, metabolism, and excretion of irinotecan (CPT-11) following I.V. infusion of [14C]CPT-11 in cancer patients. Drug Metab Dispos 28:423–433
30. Smith NF, Figg WD, Sparreboom A (2006) Pharmacogenetics of irinotecan metabolism and transport: an update. Toxicol In Vitro 20:163–175
31. Wrighton SA, VandenBranden M, Ring BJ (1996) The human drug metabolizing cytochromes P450. J Pharmacokinet Biopharm 24:461–473
32. Wolbold R, Klein K, Burk O, Nussler AK, Neuhaus P, Eichelbaum M, Schwab M, Zanger UM (2003) Sex is a major determinant of CYP3A4 expression in human liver. Hepatology 38:978–988
33. Wojnowski L, Kamdem LK (2006) Clinical I mplications of CYP3A polymorphisms. Expert Opin Drug Metab Toxicol 2:171–182
34. Yong WP, Ramirez J, Innocenti F, Ratain MJ (2005) Effects of ketoconazole on glucuronidation by UDP-glucuronosyltransferase enzymes. Clin Cancer Res 11:6699–6704

Clinical Outcome of Chemoradiation Therapy in Patients with Limited-Disease Small Cell Lung Cancer with Ipsilateral Pleural Effusion

Seiji Niho, MD, Kaoru Kubota, MD,* Kiyotaka Yoh, MD,* Koichi Goto, MD,* Hironobu Ohmatsu, MD,* Keiji Nihei, MD,† Nagahiro Saijo, MD,* and Yutaka Nishiwaki, MD**

Background: The indications for definitive thoracic radiotherapy (TRT) in limited-disease small cell lung cancer (LD-SCLC) and ipsilateral pleural effusion have not been thoroughly investigated. We retrospectively investigated the clinical outcome of LD-SCLC patients with ipsilateral pleural effusion.

Methods: The medical records of SCLC patients who received treatment at the National Cancer Center Hospital East between July 1992 and December 2006 were reviewed. Sixty-three of the 373 LD-SCLC patients (17%) had ipsilateral pleural effusion. Of these, 62 patients received chemotherapy as an initial treatment, and were included in this study. Since about 1998, definitive TRT was routinely performed if the patient's pleural effusion disappeared after induction chemotherapy. The 62 patients were divided into three subgroups: group A included patients who received chemotherapy and TRT ($n = 26$), group B included patients who did not receive TRT in spite of the disappearance of pleural effusion after first-line chemotherapy ($n = 8$), and group C included patients who did not receive TRT and whose pleural effusion persisted after first-line chemotherapy ($n = 28$).

Results: The response rate for first-line chemotherapy was 74%. Ipsilateral pleural effusion disappeared after first-line chemotherapy in 34 patients (55%). The median overall survival time was 11.8 months, and the 2 and 3-year survival rates were 21 and 10%, respectively. In groups A, B, and C, the median survival times were 19.2, 10.5, and 9.2 months, respectively, and the 2-year survival rates were 38, 25, and 7%, respectively.

Conclusion: Long-term survival was achieved by LD-SCLC patients with ipsilateral pleural effusion who successfully underwent chemoradiotherapy.

Key Words: Small cell lung cancer, Limited-disease, Pleural effusion, Chemoradiation.

(*J Thorac Oncol.* 2008;3: 723–727)

Lung cancer is the leading cause of cancer-related deaths worldwide. In Japan, over 56,000 people died of lung cancer in 2003. Small cell lung cancer (SCLC) accounts for about 15% of all forms of lung cancer. SCLC has a more aggressive biologic behavior than non-small cell lung cancer. At the time of presentation, two-thirds of patients exhibit disseminated disease. SCLC is sensitive to chemotherapy, with a response rate of 70 to 80%. A clinical two-stage system proposed by the Veterans Administration Lung Study Group distinguishes limited-disease (LD) and extensive-disease (ED) in SCLC.[1] LD is defined as being limited to one hemithorax, including mediastinal, contralateral hilar, and ipsilateral supraclavicular lymph nodes, whereas ED represents tumor spread beyond these regions. The current standard care for LD-SCLC is a combination of chemotherapy and thoracic radiotherapy (TRT). On the other hand, ED-SCLC is treated with chemotherapy alone. The original definition of LD was a tumor volume that could be encompassed by a reasonable radiotherapy plan. According to the International Association for the Study of Lung Cancer (IASLC)'s consensus report, on the other hand, the classification of LD-SCLC includes bilateral hilar and/or supraclavicular nodal involvement and ipsilateral pleural effusion.[2] However, the indication for definitive TRT in patients with LD-SCLC and ipsilateral pleural effusion have not been thoroughly investigated. Recently, the IASLC proposed the seventh edition of the tumor, node, metastasis (TNM) classification for lung cancer. In the proposals, the presence of a pleural effusion is considered as M1 disease.[3–6]

Definitive TRT is contraindicated in lung cancer patients with malignant pleural effusion. We have sometimes treated SCLC cases in which the ipsilateral pleural effusion disappeared after induction chemotherapy. Should definitive TRT be indicated in SCLC patients if the ipsilateral pleural effusion disappears after induction chemotherapy? Since about 1998, we have routinely performed definitive TRT if the patient's pleural effusion disappeared after induction chemotherapy. In this retrospective study, we investigated the clinical course and outcome of LD-SCLC patients with ipsilateral pleural effusion and exam-

*Division of Thoracic Oncology; and †Division of Radiation Oncology, National Cancer Center Hospital East, Chiba, Japan.

Supported by the Ministry of Health, Labour, and Welfare for the 3rd term Comprehensive Strategy for Cancer Control and a Grant-in-Aid for Cancer Research from the Ministry of Health, Labour, and Welfare, Japan.

Disclosure: The authors declare no potential conflict of interest.

Address for correspondence: Seiji Niho, MD, Division of Thoracic Oncology, National Cancer Center Hospital East, Kashiwanoha 6-5-1, Kashiwa, Chiba 277-8577, Japan. E-mail: siniho@east.ncc.go.jp

Copyright © 2008 by the International Association for the Study of Lung Cancer

ISSN: 1556-0864/08/0307-0723

ined the overall survival in patients who received chemotherapy and TRT, comparing with that of ED-SCLC or LD-SCLC patients without ipsilateral pleural effusion. We also applied the proposed seventh edition of the TNM stage to our cohort.

PATIENTS AND METHODS

We retrospectively reviewed the medical records of lung cancer patients who received treatment at the National Cancer Center Hospital East between July 1992 and December 2006. During this period 699 patients were newly diagnosed as having SCLC. Three-hundred and seventy-three patients were diagnosed as having LD-SCLC, and 326 were diagnosed as having ED-SCLC using conventional staging procedures, including a medical history and physical examination, chest radiography, computed tomography (CT) scan of the chest, CT scan or ultrasound of the abdomen, bone scan, and CT scan or magnetic resonance imaging of the brain. In this study, LD-SCLC was defined as disease limited to one hemithorax, including mediastinal, contralateral hilar, and supraclavicular lymph nodes, ipsilateral pleural effusion, and pericardial effusion; ED-SCLC was defined as tumor spread beyond these manifestations.[2] Sixty-three of the 373 LD-SCLC patients (17, 95% confidence interval (CI): 13–21%) had ipsilateral pleural effusion. Thirty-seven SCLC patients underwent surgical resection as an initial treatment, and 13 patients received only TRT and/or best supportive care. Remaining 649 patients received chemotherapy as an initial treatment. Of these, 62 LD-SCLC patients had ipsilateral pleural effusion, and were included in this study. The patient characteristics are shown in Table 1. The breadth of the pleural effusion was measured using a CT scan of the chest (Figure 1). Cytologic examination of the pleural effusion prior to treatment was performed in 26 patients. Eleven patients had cytologically positive effusion. Ten patients also had pericardial effusion. Three patients had solid pleural tumor and pleural effusion detected on CT scan. Twenty-six patients had atelectasis. Of these, 14 patients received cytologic examination of the pleural effusion, and four patients had cytologically positive effusion.

We collected clinical data on the patients from their medical records; this data included the chemotherapy regimen that was received, the response to first-line chemotherapy, whether pleural effusion disappeared after first-line chemotherapy, and whether the patient underwent definitive TRT. The World Health Organization's response criteria were used.[7]

Overall survival was defined as the interval between the start of treatment and death or the final follow-up visit. Median overall survival was estimated using the Kaplan-Meier analysis method.[8] Survival data was compared among groups using a log-rank test. The breadth of pleural effusion was compared using the Mann-Whitney U test. All reported p values are two-sided.

RESULTS

The induction chemotherapy regimens were shown in Table 2. Most common regimen was cisplatin or carboplatin plus etoposide. In LD patients with ipsilateral pleural effusion, there were three complete responses, 43 partial responses, seven no changes, and six progressive diseases. Response was not evaluated in three patients because of early death. The response rate was 74% (95% CI: 62–84%). Ipsilateral pleural effusion disappeared after first-line chemotherapy in 34 patients (55, 95% CI: 42–68%).

TABLE 1. Patient Characteristics

	LD-SCLC without Ipsilateral Pleural Effusion	LD-SCLC with Ipsilateral Pleural Effusion	ED-SCLC
No. of patients	270	62	317
Sex			
Male	226	50	262
Female	44	12	55
Age, yr			
Median	66	67	66
Range	38–87	46–79	28–85
Performance status			
0	71	2	20
1	178	45	203
2	14	10	59
3	6	5	28
4	1	0	7
Breadth of pleural effusion on CT scan, cm			
Median		2.3	
Range		0.5–9.4	
Cytology of pleural effusion			
Positive		11	
Negative		15	
Not examined		36	

Patients who received chemotherapy as an initial treatment were included.
LD, limited-disease; SCLC, small cell lung cancer; ED, extensive-disease; CT, computed tomography.

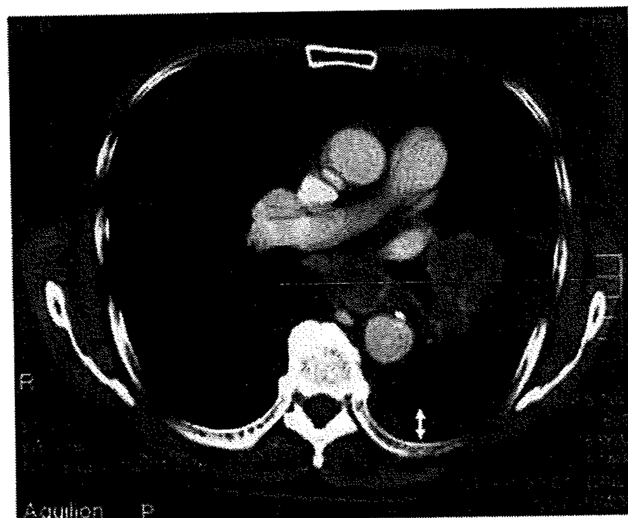

FIGURE 1. Ipsilateral pleural effusion. The arrow indicates the breadth of pleural effusion.

Copyright © 2008 by the International Association for the Study of Lung Cancer