because a certain number of patients per hospital are needed to maintain the quality of trials by training doctors in the application of a new drug. Thus, enhancing patient recruitment in each hospital participating in the trial is the most important consideration.

A high standard of oncology clinical practice as the basis for clinical trials

Since a high standard of clinical practice is the basis for all clinical trials, the infrastructure for oncological clinical practice should be promptly advanced. The shortage of human resources including medical oncologists and oncology nurse practitioners in Japan is serious and acute. In the United States, medical oncology was established as a separate discipline by the American Board of Internal Medicine in 1971, and approximately 8,000 certified internists as of 2003 have been further certified by the Board in the subspecialty of medical oncology (Holland et al. 2003). In contrast, medical oncology has not been established as an academic unit or a regular university course in many medical schools in Japan. The Japanese Society of Medical Oncology was launched as an association in 1993, and framed the system of cancer medical specialists in 2003. A total of 1,479 doctors were certified as a tentative medical oncology supervisor between 2003 and 2005, and 47 doctors as a medical oncology specialist in 2005 (Table 3) (Japanese Society of Medical Oncology 2005).

To deal with complex cancer care, oncology nurse practitioners in the United States have become an integral part of the multidisciplinary team in the care of patients. As of 2002, more than 19,000 oncology nurse practitioners have been certified by the Oncology Nursing Society in the United States (Rieger 2003). In contrast, the number of oncology nurse practitioners registered in the Japanese Nursing Association was only 44 as of 2005 (Table 3) (Japanese Nursing Association 2005). Introduction of oncology nurse practitioners in clinical practice should lessen the burden on oncologists significantly and help them to have the incentive to take part in registration-directed clinical trials.

The infrastructure and human resources to support clinical trials

The infrastructure to support in-house clinical trials remains insufficient and even lacking in almost all institutes in Japan, while it has been advanced systematically in the United States. In the 1960s, General Clinical Research Centers were founded with the support of National Institutes of Health in 80 universities and academic institutions to provide the primary resources and optimal environment necessary for investigators to conduct clinical research. They include experienced nursing, laboratory, computer system, and biostatistical staff (Robertson and Tung 2001; General Clinical Research Centers 2005). To carry out a multicenter trial, a central data center

TABLE 3. Medical oncology professionals in Japan and the USA.

Professionals	*n* of medical oncology professionals	
	Japan	USA
Medical oncologists	47 [1]	8,000 [2]
Oncology nurse practitioners	44 [3]	19,000 < [4]
Clinical research coordinators	335 [5]	10,723 [6]

[1] Certified by the Japanese Society of Medical Oncology in 2005.
[2] Certified by the American Board of Internal Medicine as of 2003.
[3] Certified by the Japanese Nursing Association as of 2005.
[4] Certified by the Oncology Nursing Society as of 2002.
[5] Certified by the Japanese Society of Clinical Pharmacology and Therapeutics as of 2005.
[6] Certified by the Association of Clinical Research Professionals as of 2005.

is needed to deal with the increased administrative difficulties and quality assurance problems associated with this type of trial (Pollock 1994). The quality control and quality assurance system of the Japan Clinical Oncology Group has been significantly developed during the last two decades (Japan Clinical Oncology Group 2005). Using Internet resources may facilitate developing national and regional networks for clinical trials by reducing the burden associated with the extensive research time and considerable cost of all these processes (Paul et al. 2005).

The new GCP demands more of the clinical researchers in time, resources and money to enhance the science, credibility, and ethics of clinical trials for approval (Sweatman 2003). The clinical research coordinator (CRC) plays a key role in the clinical trial process by supporting investigators. The CRCs are involved in every aspect of registration-directed clinical trials, including protocol development, checking eligibility criteria, informed consent, organizing study schedules, checking clinical tests, filling in case report forms, and providing support for monitoring and auditing the trials (Rico-Villademoros et al. 2004; Sakamoto 2004). Association of Clinical Research Professionals in the USA has offered the CRC certification since 1992, and there are 10,723 CRCs to date (Association of Clinical Research Professionals 2006). The Japanese Society of Clinical Pharmacology and Therapeutics launched the certified CRC system in 2003, and there were 335 certified CRCs as of 2005 (Table 3) (The Japanese Society of Clinical Pharmacology and Therapeutics 2005).

In conclusion, clinical trials of anticancer agents for approval have been developing significantly, but still remain at an unsatisfactory level. Development of the infrastructure and human resources for clinical trials is an urgent task to complete good quality clinical trials for approval without delay.

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Epidermal Growth Factor Receptor Mutation Detection Using High-Resolution Melting Analysis Predicts Outcomes in Patients with Advanced Non–Small Cell Lung Cancer Treated with Gefitinib

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Abstract

Purpose: Epidermal growth factor receptor (*EGFR*) mutations, especially deletional mutations in exon 19 (DEL) and L858R, predict gefitinib sensitivity in patients with non–small cell lung cancer (NSCLC). In this study, we validated *EGFR* mutation detection using high-resolution melting analysis (HRMA) and evaluated the associations between *EGFR* mutations and clinical outcomes in advanced NSCLC patients treated with gefitinib on a larger scale.

Experimental Design: The presence of DEL or L858R was evaluated using HRMA and paraffin-embedded tissues and/or cytologic slides from 212 patients. In 66 patients, the results were compared with direct sequencing data.

Results: HRMA using formalin-fixed tissues had a 92% sensitivity and a 100% specificity. The analysis was successfully completed in 207 patients, and DEL or L858R mutations were detected in 85 (41%) patients. The response rate (78% versus 8%), time-to-progression (median, 9.2 versus 1.6 months), and overall survival (median, 21.7 versus 8.7 months) were significantly better in patients with *EGFR* mutations ($P < 0.001$). Even among the 34 patients with stable diseases, the time-to-progression was significantly longer in patients with *EGFR* mutations. Patients with DEL ($n = 49$) tended to have better outcomes than those with L858R ($n = 36$); the response rates were 86% and 67%, respectively ($P = 0.037$), and the median time-to-progression was 10.5 and 7.4 months, respectively ($P = 0.11$).

Conclusions: HRMA is a precise method for detecting DEL and L858R mutations and is useful for predicting clinical outcomes in patients with advanced NSCLC treated with gefitinib.

Gefitinib (Iressa; AstraZeneca) is an orally active, selective epidermal growth factor receptor (EGFR) tyrosine kinase inhibitor. Phase II studies have shown gefitinib antitumor activity in patients with advanced non–small cell lung cancer (NSCLC; refs. 1, 2). Several studies have shown that the response rate to gefitinib is higher in women, patients with adenocarcinoma, never smokers, and Japanese or East Asians (1–3); subsequently, somatic mutations in the kinase domain of *EGFR* were suggested to be a determinant of gefitinib sensitivity (4, 5). Since then, many retrospective studies have consistently revealed that *EGFR* mutations, mainly in-frame deletions including amino acids at codons 747 to 749 in exon 19 (DEL) and a missense mutation at codon 858 (L858R) in exon 21, are associated with tumor response, time-to-progression, and overall survival in NSCLC patients treated with gefitinib (6–8).

In our previous study, which clearly showed a correlation between *EGFR* mutations and gefitinib sensitivity in patients with recurrent NSCLC after surgical resection of the primary tumor (6), we used methanol-fixed, paraffin-embedded surgical specimens and did laser capture microdissection and direct sequencing, which we considered to be the most precise methods available for identifying mutations at that time. However, these methods are not useful in clinical practice for the treatment of advanced NSCLC for two reasons. First, the diagnostic samples of advanced NSCLC tumors, unlike surgical specimens, contain a small amount of tumor cells and are highly contaminated with normal cells. Second, laser capture microdissection and direct sequencing require special

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Table 1. Patient characteristics (N = 212)

	n (%)
Age (y)	
Median (range)	62 (29-84)
Sex	
Women	92 (43)
Men	120 (57)
Smoking history*	
Never smokers	96 (45)
Former smokers	38 (18)
Current smokers	78 (37)
Histology	
Adenocarcinoma	193 (91)
Others	19 (9)
Performance status †	
0	59 (28)
1	123 (58)
2	22 (10)
3	8 (4)
Stage	
III	42 (20)
IV	75 (35)
Recurrence after surgery	95 (45)
Gefitinib therapy	
First line	89 (42)
Second line	66 (31)
Third or more line	57 (27)

*Never smokers were defined as patients who have never had a smoking habit and former smokers were defined as patients who had stopped smoking at least 1 y before diagnosis.
† At the beginning of gefitinib therapy.

instruments and cost time and money. Recently, high-resolution melting analysis (HRMA) using the dye LCGreen I (Idaho Technology) was introduced as an easy, quick, and precise method for mutation screening (9), and we established a method for detecting DEL and L858R mutations using HRMA. Our cell line study revealed that DEL and L858R mutations could be detected using HRMA in the presence of 10% and 0.1% mutant cells, respectively (10). We also showed that the two major mutations could be identified by HRMA using DNA extracted from archived Papanicolaou-stained cytologic slides with 88% sensitivity and 100% specificity (10).

In this study, we validated *EGFR* mutation detection by HRMA using DNA extracted from archived paraffin-embedded tissues. We also did the HRMA in advanced NSCLC patients treated with gefitinib on a larger scale using archived tissues and/or cytologic slides.

Patients and Methods

Patients. Among 364 consecutive patients with NSCLC who began receiving gefitinib monotherapy (250 mg/d) at the National Cancer Center Hospital between July 2002 and December 2004, 212 patients were retrospectively analyzed using HRMA. One hundred fifty-two patients were excluded from the analysis because tumor samples were not available (n = 126) or their informed consent to the genetic analysis was not obtained (n = 26).

High-resolution melting analysis. On a protocol approved by the Institutional Review Board of the National Cancer Center Hospital, we did the following genetic analyses. Formalin-fixed, paraffin-embedded tissues and/or Papanicolaou-stained cytologic slides containing sufficient tumor cells (at least 1% of nucleated cells) were selected after microscopic examination by a pathologist (K.T.). The detailed analysis method has been described previously (10). Briefly, DNA was extracted from the tissues and/or cytologic slides using a QIAamp DNA Micro kit (Qiagen). PCR was done using dye LCGreen I and primers designed to amplify a region containing E746-I759 of *EGFR* [DEL-specific primer, AAAATTCCCGTCGCTATC (forward) and AAGCAGAAACTCACATCG (reverse)] or L858 of *EGFR* [L858R-specific primer, AGATCACA-GATTTTGGGC (forward) and ATTCTTTCTCTTCCGCAC (reverse)] on a LightCycler (Roche Diagnostics). The PCR products were denatured at 95°C for 5 min and cooled to 40°C to form heteroduplexes. The LightCycler capillary was then transferred to an HR-1 (Idaho Technology), a HRMA instrument, and heated at a transition rate of 0.3°C per second. Data were acquired and analyzed using the accompanying software (Idaho Technology). After normalization and temperature adjustment steps, melting curve shapes from 78.5°C to 85.5°C were compared between samples and control samples. Human Genomic DNA (Roche Diagnostics) was used as a control sample with wild-type (WT) *EGFR*. Samples revealing skewed or left-shifted curves from those of control samples were judged to have mutations. All analyses were done in a blinded fashion.

Table 2. Clinical validation of HRMA and direct sequencing without laser capture microdissection

	HRMA without LCM			Direct sequencing without LCM (6)
	Formalin-fixed tissues	Methanol-fixed tissues	Cytologic slides (10)	
n	66	66	29	66
Successfully analyzed, n (%)	63 (95)	66 (100)	28 (97)	66 (100)
True positive	34	36	14	28
True negative	26	29	12	29
False positive	0	0	0	0
False negative	3	1	2	9
Sensitivity (%)	92	97	88	76
Specificity (%)	100	100	100	100
Positive predictive value (%)	100	100	100	100
Negative predictive value (%)	90	97	86	76

NOTE: The results of these analyses were compared with those of direct sequencing with LCM (used as the "gold standard" method). True positive is defined as the correct detection of deletional mutations in exon 19 or L858R.
Abbreviation: LCM, laser capture microdissection.

Table 3. *EGFR* mutations among patient subgroups

	n	*EGFR* mutations				*P*
		DEL	L858R	Total	%	
Total	207	49	36	85	41	
Sex						
Women	89	31	17	48	54	0.001
Men	118	18	19	37	31	
Smoking history						
Never smokers	93	30	19	49	53	0.002*
Former smokers	38	12	10	22	58	
Current smokers	76	7	7	14	18	
Histology						
Adenocarcinoma	189	48	35	83	44	0.007
Others	18	1†	1‡	2	11	

*Comparison between never smokers and others.
†Pleomorphic carcinoma.
‡Adenosquamous carcinoma.

Clinical validation of HRMA. Direct sequencing with and without laser capture microdissection had been done in 66 patients with recurrent NSCLC after surgery in the previous study (6). In these patients, HRMA was done using both formalin-fixed and methanol-fixed surgical specimens without laser capture microdissection, and the results were compared with the results of direct sequencing with laser capture microdissection, which we considered to be the gold standard method.

Radiologic evaluation. One board-certified radiologist (U.T.) who was unaware of the patients' mutational statuses reviewed the baseline, the first follow-up, and confirmatory imaging studies and classified the tumor responses into complete response (CR), partial response (PR), stable disease (SD), and progressive disease (PD) using standard bidimensional measurements (11). In patients without measurable lesions, significant clinical benefit and disease progression were defined as clinical PR and clinical PD, respectively. Patients who died before the follow-up imaging studies were classified as PD. SD was subdivided into minor response (MR), long SD, and short SD. MR was defined as a ≥25% decrease in the sum of the products of the perpendicular diameters of all measurable lesions, and long SD meant that SD lasted for >6 months. Responders were defined as patients with CR, PR, or clinical PR.

Statistical analysis. The associations among *EGFR* mutations, patient characteristics, and tumor responses to gefitinib were assessed using a χ^2 test. The differences in time-to-progression and overall survival according to the patient subgroups were compared using Kaplan-Meier curves and log-rank tests. The starting point of the time-to-progression and overall survival was the first administration of gefitinib. Multivariate analyses using logistic regression models and Cox proportional hazard models were done to assess the association between the clinical outcomes and the following factors: age (<70 versus ≥70 years), sex, smoking history (never smokers versus others), histology (adenocarcinoma versus others), performance status (0/1 versus 2/3), stage (recurrence after surgery versus III/IV), prior chemotherapy (yes versus no), and the mutational status of *EGFR* (mutant versus WT). All analyses were done using the SPSS statistical package (SPSS version 11.0 for Windows; SPSS, Inc.).

Results

Patient characteristics. The patient characteristics are listed in Table 1. All the patients were East Asians: 210 Japanese, 1 Korean, and 1 Chinese. The median follow-up time for the survivors was 29.7 months (range, 10.7-49.8 months).

Clinical validation of HRMA. The clinical validation of the HRMA results using various samples is shown in Table 2. The sensitivity of HRMA using DNA extracted from formalin-fixed tissues was 92%, significantly higher than that of direct sequencing without laser capture microdissection but lower than that of HRMA using methanol-fixed tissues. The specificity and positive predictive values were 100% in all the analyses.

Mutational analysis. HRMA was completed in 207 patients. Five patients could not be successfully analyzed because of incomplete PCR. Of the 207 patients, 130 were analyzed using tissue samples (96 samples were obtained by thoracotomy, 17 by mediastinoscopic lymph node biopsy, 9 by thoracoscopic lung or pleural biopsy, 5 by resection or biopsy of distant metastases, and 3 by transbronchial lung biopsy), and 117 were analyzed using cytology samples (43 samples were obtained by bronchial brushing or washing, 40 from pleural effusion, 9 by transbronchial needle aspiration, 8 from pericardial effusion, 7 by needle aspiration of superficial lymph nodes, 6 by percutaneous needle aspiration of lung tumors, and 4 from sputum). In 40 patients who were analyzed using both tissue and cytology samples, 4 had inconsistent results; mutations were detected only in tissue samples and not in cytology samples (3 patients) or vice versa (1 patient). These four patients were judged to have mutations because false-negative results were more common than false-positive results in the validation of HRMA. Consequently, DEL and L858R mutations were detected in 49 (24%) and 36 (17%) patients, respectively, and these mutations were mutually exclusive. The other 122 (59%) patients were classified as having WT *EGFR* in this study, although some of them may have had minor mutations. As

Table 4. *EGFR* mutations and response to gefitinib

	Responders		SD			PD	Response rate (%)	*P*
	CR	PR	MR	Long SD	Short SD			
WT	0	10	2	4	17	89	10/122 (8)	$<10^{-23}$
Mutant	2	64*	6	4	1	8†	66/85 (78)	
DEL	0	42	2	2	1	2	42/49 (86)	0.037
L858R	2	22	4	2	0	6	24/36 (67)	
Total	2	74	8	8	18	97	76/207 (37)	

*Including four clinical responders without measurable lesions.
†Including a patient who had no measurable lesions at baseline.

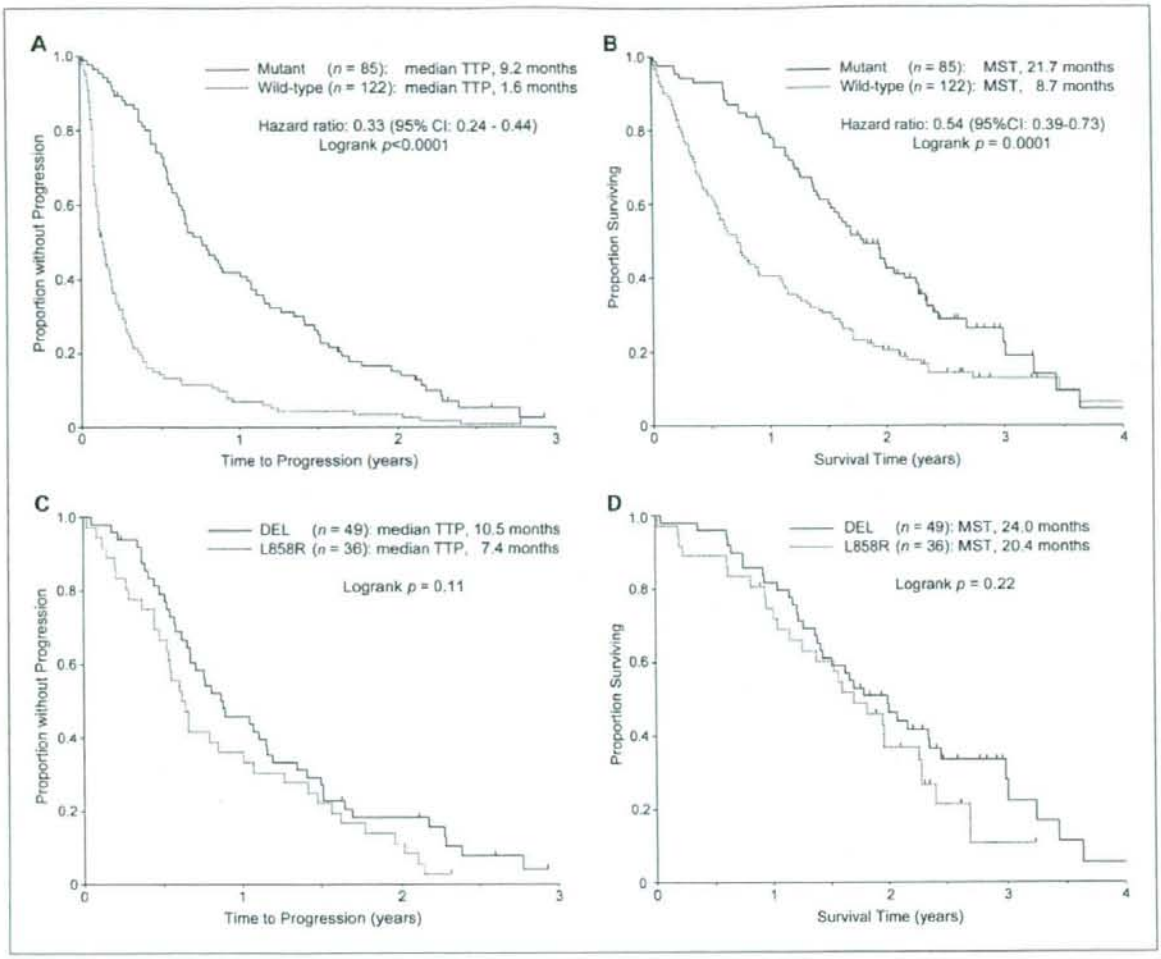

Fig. 1. Kaplan-Meier plot of time-to-progression (*A*) and overall survival (*B*) for patients with or without *EGFR* mutations. Kaplan-Meier plot of time-to-progression (*C*) and overall survival (*D*) for patients with DEL or L858R mutations. TTP, time-to-progression; MST, median survival time.

shown in Table 3, *EGFR* mutations were detected more frequently in women, never smokers, and patients with adenocarcinoma. Patient characteristics were not significantly different between patients with DEL mutations and those with an L858R mutation.

EGFR mutations and clinical outcomes. The association of the mutational status of *EGFR* and the response to gefitinib is shown in Table 4. The response rate was significantly higher in patients with *EGFR* mutations than in those with WT *EGFR* (78% versus 8%; $P < 10^{-23}$). Among patients with *EGFR* mutations, those with DEL mutations had a higher response rate than those with an L858R mutation (86% versus 67%; $P = 0.037$). Tumor responses were classified as SD in 11 patients with *EGFR* mutations and in 23 patients with WT *EGFR*. Among the patients with SD, a MR and/or a long SD (>6 months) were observed more frequently (91% versus 26%; $P = 0.0004$) and the time-to-progression was significantly longer (median, 6.9 versus 4.4 months; $P = 0.019$) in the patients with *EGFR* mutations than in the patients with WT *EGFR*.

As shown in Fig. 1, the time-to-progression (median, 9.2 versus 1.6 months; $P < 0.0001$) and overall survival (median, 21.7 versus 8.7 months; $P = 0.0001$) were significantly longer in patients with *EGFR* mutations than in those with WT *EGFR*. Patients with DEL mutations tended to have a longer time-to-progression (median, 10.5 versus 7.4 months; $P = 0.11$) and overall survival (median, 24.0 versus 20.4 months; $P = 0.22$) than those with an L858R mutation, although the difference did not reach statistical significance.

Clinical outcomes among subgroups of patients are shown in Table 5. In the univariate analysis, sex, smoking history, and histology were significant predictive factors for gefitinib sensitivity.

In the multivariate analyses, the mutational status of *EGFR* was an independent predictive factor of response [odds ratio, 38.9; 95% confidence interval (95% CI), 15.7-96.5; $P < 0.001$], time-to-progression (hazard ratio, 0.33; 95% CI, 0.24-0.45; $P < 0.001$), and overall survival (hazard ratio, 0.48; 95% CI, 0.34-0.67; $P < 0.001$). A poor performance status (2/3) was an

independent predictor of a shorter time-to-progression (hazard ratio, 1.80; 95% CI, 1.19-2.72; $P = 0.006$) and overall survival (hazard ratio, 3.97; 95% CI, 2.56-6.16; $P < 0.001$), and a history of prior chemotherapy was another independent predictor of a shorter overall survival (hazard ratio, 1.59; 95% CI, 1.14-2.23; $P = 0.006$). However, other clinical characteristics, including sex, smoking history, and histology, were not independent predictive factors for any clinical outcomes.

Discussion

In the current study, we showed the practicality of our new HRMA method for detecting two major *EGFR* mutations, DEL and L858R. The sensitivity and specificity of the analysis were 92% and 100%, respectively, when archived formalin-fixed, paraffin-embedded tissues were used without laser capture microdissection. Given the similar results that were obtained when Papanicolaou-stained cytologic slides were used (10), DEL and L858R mutations can likely be detected from such archived samples with about a 90% sensitivity and 100% specificity. Because the mutations were detected by HRMA even when only a small proportion (0.1% or 10%) of mutant cells existed (10), laser capture microdissection or other enrichment procedures are not needed in most cases. This is a major advantage of HRMA over direct sequencing because direct sequencing requires laser capture microdissection for accurate evaluation (6). However, there remained some risk of indeterminate or false-negative results because the DNA might have degenerated during sampling or the preservation of the archived samples. In fact, an analysis using methanol-fixed tissues, which are known to preserve DNA better than formalin-fixed tissues (12), was stable with no indeterminate and fewer false-negative results. Thus, an even higher sensitivity can be expected when fresh tumor samples are used. In any event, HRMA was successfully used to identify *EGFR* mutations and, more importantly, predict the clinical outcomes of gefitinib-treated patients with a high sensitivity and specificity.

Although the detection of *EGFR* mutations can provide patients with NSCLC and their physicians with critical information for optimal decision making, such tests are not common in clinical settings mainly because of the difficulty and impracticality of direct sequencing. Recently, highly sensitive nonsequencing methods to detect *EGFR* mutations in small tumor samples contaminated with normal cells have been reported (10, 13-21). Among them, HRMA has the advantages of being able to identify the mutations with less labor, time, and expense; PCR and the melting analysis can be done in the same capillary tube within a few hours, and the running cost is only about 1 U.S. dollar per sample. HRMA is expected to be one of the most practical methods for detecting *EGFR* mutations in clinical settings.

We analyzed consecutive gefitinib-treated patients in a single institution on a larger scale than any other previous report. The mutational analysis by HRMA was successful in 207 patients and confirmed strong and independent associations between the two major *EGFR* mutations and clinical outcomes. Clinical predictors, such as sex, smoking history, and histology, added little predictive information to that provided by the mutational analysis. We believe that the mutational status of *EGFR* is the most important predictor of clinical outcomes in gefitinib-treated patients.

Among the patients without the two major mutations, 8% were responders. This result may be due to false-negative HRMA results, other *EGFR* mutations, or other determinants of gefitinib sensitivity. As for other *EGFR* mutations, the direct sequencing of exons 18 to 24 was done in four responders without DEL or L858R mutations, and one of them had G719C and S768I mutations. Although missense mutations at codon 719 of *EGFR* (G719C, G719S, or G719A) may be associated with gefitinib sensitivity, the predictive significance of these mutations is unclear because the number of reported patients is small (6). At present, we consider the accurate detection of the two major *EGFR* mutations to be sufficient for optimal decision making.

Recently, the *EGFR* copy number was reported to be another predictor of gefitinib sensitivity (6, 22, 23), and Cappuzzo et al. (22) suggested that this factor was a stronger predictor of overall survival than *EGFR* mutations. Our previous study also showed that the *EGFR* copy number evaluated by quantitative

Table 5. Clinical outcomes among subgroups of patients

	n	Response rate (%)	*P*	Median TTP (mo)	*P*	MST (mo)	*P*
Total	207	37		3.7		14.5	
Sex							
Women	89	51	<0.001	5.6	0.17	18.3	0.15
Men	118	26		2.3		9.6	
Smoking history							
Never smokers	93	51	<0.001*	6.2	0.073*	16.9	0.22*
Former smokers	38	47		5.2		14.5	
Current smokers	76	14		2.2		9.1	
Histology							
Adenocarcinoma	189	40	0.004	4.3	0.060	15.1	0.10
Others	18	6		1.6		4.9	
EGFR mutations							
DEL/L858R	85	78	<0.001	9.2	<0.001	21.7	<0.001
WT	122	8		1.6		8.7	

Abbreviations: TTP, time-to-progression; MST, median survival time.
*Comparison between never smokers and others.

PCR was associated with response; however, an increased *EGFR* copy number was concentrated in patients with *EGFR* mutations and was not an independent predictor of response and overall survival (6). In the current study, we showed that *EGFR* mutations were associated with better outcomes even among patients with SD. The interpretation of this result is difficult because a long SD might be caused by intrinsic characteristics independent of treatment; however, this result suggested that *EGFR* mutations predicted not only "super responders" but also "non-super responders" who gained a clinical benefit. Contrary to these findings, Cappuzzo et al. (22) showed that *EGFR* mutations predicted only responders and were not associated with overall survival, whereas *EGFR* copy number was associated with both response and SD and was an independent predictor of overall survival. Although the reason of these discrepancies is unclear, we consider that if *EGFR* mutations are accurately identified, *EGFR* copy number adds little information for patient selection, at least in Japanese patients.

About the outcomes of patients with DEL or L858R mutations, our larger scale study produced results similar to those of some previous studies, which indicated that DEL mutations were associated with better outcomes after EGFR tyrosine kinase inhibitor treatment than an L858R mutation (24–27). Further investigations are needed to clarify the difference in the biological characteristics of the two mutations. However, in the current study, the difference was small and even patients with an L858R mutation had favorable outcomes: the response rate was 67%, the median time-to-progression was 7.4 months, and the median survival time was 20.4 months. We now think that both DEL and L858R mutations should be treated equally in clinical decision-making.

In conclusion, the detection of DEL and L858R mutations using HRMA is accurate and practical. Using HRMA, we confirmed a strong association between the two major *EGFR* mutations and clinical outcomes in patients with advanced NSCLC treated with gefitinib.

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Effect of Platinum Combined with Irinotecan or Paclitaxel against Large Cell Neuroendocrine Carcinoma of the Lung

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Background: The efficacy of chemotherapy in patients with large cell neuroendocrine carcinoma of the lung (LCNEC) remains unclear.
Methods: Of 42 consecutive patients with LCNEC, 22 with measurable disease receiving chemotherapy were enrolled as the subjects of this study. The clinical characteristics and objective responses to chemotherapy in these patients were analysed retrospectively.
Results: The distribution of the disease stage in the patients consisting of 21 males and one female (median age: 67 years; range: 47–78 years) was as follows: stage IIB ($n = 1$), stage IIIA ($n = 1$), stage IIIB ($n = 5$), stage IV ($n = 8$), and post-operative recurrence ($n = 7$). Chemotherapy consisted of cisplatin and irinotecan ($n = 9$), a platinum agent and paclitaxel ($n = 6$), paclitaxel alone ($n = 1$), cisplatin and vinorelbine ($n = 1$), cisplatin and docetaxel ($n = 1$), and a platinum and etoposide ($n = 4$). The objective response rate in the 22 patients was 59.1% (95% CI, 38.1–80.1). An objective response was obtained in five of the nine patients receiving irinotecan and five of the seven patients receiving paclitaxel. The progression-free survival, median overall survival and 1-year survival rates were 4.1 months (95% CI, 3.1–5.1), 10.3 months (95% CI, 5.8–14.8) and 43.0% (95% CI, 20.7–65.3), respectively. The median overall survival of the patients treated with irinotecan or paclitaxel was 10.3 months (95% CI, 0–21.8), and the 1-year survival rate of these patients was 47.6% (95% CI, 20.4–74.8).
Conclusion: Our results suggest that irinotecan and paclitaxel may be active against LCNEC.

Key words: lung cancer – large cell neuroendocrine carcinoma – chemotherapy – irinotecan – paclitaxel

INTRODUCTION

Neuroendocrine tumors of the lung can be placed in the biological spectrum ranging from typical to atypical carcinoid, which are tumors of low to intermediate grade malignancy, to large cell neuroendocrine carcinomas (LCNEC) and small-cell lung carcinomas (SCLC), which are high-grade malignant tumors. LCNEC was proposed as a separate category by Travis et al. in 1991, who recognized a type of poorly differentiated high-grade carcinoma exhibiting features of neuroendocrine appearance on light microscopy, immunohistochemistry, and/or electron microscopy (1). Several different terminologies and classifications have been proposed to date, and this class of tumors is likely to become widely recognized and included in the updated histological classification of the World Health Organization (2).

The clinical features of LCNEC have not yet been completely clarified. The prognosis of patients with surgically resected LCNEC is intermediate between that of an atypical carcinoid and SCLC, and is the same as that of resected non-small-cell lung carcinoma (NSCLC), except for stage I LCNEC, which has a poorer prognosis than that of stage I NSCLC (3–6). In a multi-institutional study in Japan, it was found that both LCNEC and SCLC were similarly aggressive and that there was no survival difference between the two types of lung cancer (7). In a small case series of LCNEC, we reviewed the records of patients with surgically resected,

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and patients treated medically who were autopsied before 1995, and determined that the chemosensitivity of LCNEC to cisplatin-based regimens may be intermediate between that of NSCLC and SCLC (8). Third generation cytotoxic agents developed in the 1990s, such as paclitaxel, docetaxel, gemcitabine, vinorelbine and irinotecan, have been shown to be active agents against advanced lung cancer, and combinations of platinum and one of the third generation cytotoxic agents have been shown to be superior in terms of prolonging the survival to the existing platinum-based combinations in both patients with NSCLC and those with SCLC (9–14). In the present study, we conducted a retrospective review of the records of our patients with LCNEC who had been treated with chemotherapy, and analysed the efficacy of the chemotherapy regimens.

PATIENTS AND METHODS

From April 1999 to January 2006, 42 patients were diagnosed as having LCNEC at our institution. Of these, one patient underwent surgery, four were treated with radiation therapy alone, and three received only supportive care. Of the 34 patients who had received chemotherapy, four who had also received concurrent radiotherapy and two without evaluable lesions were excluded from this study. In addition, six patients who entered a phase II trial of cisplatin and irinotecan combination for LCNEC were also excluded from this study, because their results will be published elsewhere. Thus, 22 patients were finally enrolled as the subjects of this study.

The histological confirmation of the diagnosis of LCNEC in the medically treated patients was based on examination of biopsy and/or cytology specimens. The histological or cytological diagnosis was reviewed by one of the authors (K.T.). We classified LCNEC according to the histopathological criteria proposed in the WHO classification. Immunohistochemical analysis was performed to confirm the neuroendocrine differentiation of the tumor cells (2).

Clinical information about the cases was obtained from medical records. All patients underwent a chest and abdominal computed tomography, a head computed tomography or magnetic resonance imaging and a bone scintigraphy in clinical disease staging before chemotherapy. The clinical disease staging was reassessed according to the latest International Union Against Cancer (UICC) staging criteria (15). The response to chemotherapy and the survival were assessed retrospectively. The objective tumor response was evaluated according to the Response Evaluation Criteria in Solid Tumor guidelines (16). The survival distributions for overall survival (OS) and progression-free survival (PFS) were estimated according to the Kaplan–Meier method (17). The OS was measured from the date of start of chemotherapy to the date of death or the last follow-up. For PFS, documented disease recurrence was scored as an event. All analyses were performed using the SPSS statistical software (SPSS version 11.0 for Windows; SPSS Inc, Chicago, IL).

RESULTS

The clinical characteristics of the 22 patients are summarized in Table 1. Surgical resected primary tumor, incisional biopsy of metastatic lesion, exploratatory thoracotomy, transbronchial or percutaneous biopsy and cytological examination were positive in seven, five, two, six and two patients, respectively. Thus, the histological diagnosis was made based on examination of a large tumor sample in 14 (63.6%) of the 22 patients. The marked predominance of men and smokers in this study was consistent with the demographic features of our previous LCNEC studies (6–8). One patient with stage IIB received chemotherapy and was enrolled to this study, because surgical resection and definitive radiotherapy were not indicated in this patient because of his poor pulmonary function. Abnormally high serum levels of CEA, NSE and proGRP at the start of chemotherapy were found in 52.4% (11/21), 72.7% (16/22) and 52.4% (11/21) of the patients, respectively.

Table 1. Patient characteristics

Characteristics		*n*	%
Gender	Male	21	95
	Female	1	5
Age	Median (range)	67 (47–78)	
Smoking history	Yes	21	95
	No	1	5
Performance status	0	7	32
	1	14	64
	2	1	5
Clinical stage	IIB	1	4
	IIIA	1	5
	IIIB	5	23
	IV	8	36
	Post-operative recurrence	7	32
Prior treatment	None	14	64
	Surgery	7	32
	Surgery for brain metastasis	1	5
	Radiotherapy	3	14
Site of metastasis	None	7	32
	Brain	2	9
	Lung	3	14
	Liver	5	23
	Bone	4	18
	Lymph node	6	27
	Others	3	14

The chemotherapy regimens used were as follows: cisplatin (80 mg/m^2, day 1) and irinotecan (60 mg/m^2, days 1 and 8) ($n = 6$); cisplatin (60 mg/m^2, day 1) and irinotecan (60 mg/m^2, days 1, 8 and 15) ($n = 3$); carboplatin (AUC = 6, day 1) and paclitaxel (200 mg/m^2, day 1) ($n = 5$); cisplatin (80 mg/m^2, day 1) and paclitaxel (175 mg/m^2, day 1) ($n = 1$); paclitaxel alone (80 mg/m^2, weekly) ($n = 1$); cisplatin (80 mg/m^2, day 1) and vinorelbine (20 mg/m^2, days 1, 8 and 15) ($n = 1$); cisplatin (25 mg/m^2, days 1, 8 and 15) and docetaxel (20 mg/m^2, days 1, 8 and 15) ($n = 1$); carboplatin (AUC = 5, day 1) and etoposide (100 mg/m^2, days 1–3) ($n = 3$); cisplatin (80 mg/m^2, day 1) and etoposide (100 mg/m^2, days 1–3) ($n = 1$). The median number of chemotherapy cycles was three (range, 1–5). One complete response and 12 partial responses were noted in the 22 patients, yielding an overall response rate of 59.1% (95% CI, 38.1–80.1) (Table 2). An objective response was obtained in five of the nine patients (55.6%) receiving irinotecan and five of the seven patients (71.4%) receiving paclitaxel. The toxicities related to these treatments were, in general, acceptable. Two patients received gefitinib after failure of the first-line chemotherapy, but none of them achieved an objective response. The overall PFS, median OS and 1-year survival rate of all the patients were 4.1 months (95% CI, 3.1–5.1), 10.3 months (95% CI, 5.8–14.8) and 43.3% (95% CI, 21.0–65.6), respectively (Fig. 1). The median OS of the patients treated with irinotecan or paclitaxel was 10.3 months (95% CI, 0–21.8), and the 1-year survival rate of these patients was 47.6% (95% CI, 20.4–74.8).

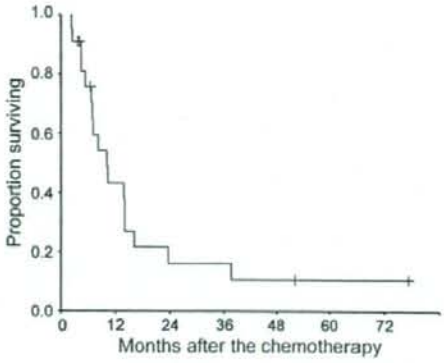

Figure 1. Kaplan–Meier curve for overall survival ($n = 22$). The median survival time was 10.3 months, and the 1- and 2-year survival rates were 43.3 and 16.2%, respectively.

DISCUSSION

In this study, the histological diagnosis of LCNEC was based on examination of a large tumor sample in 14 (63.6%) of the 22 patients, based on biopsies or cytological specimens in the remaining patients (36.4%). Numerous studies have demonstrated that the diagnosis of LCNEC is possible from biopsies or cytological specimens if a sufficient number of tumor cells can be obtained (8,18–21). To establish the pathological diagnosis of LCNEC in this series, we performed a pathological review of the biopsy and cytology specimens, because it was difficult to obtain large specimens of the tumor in these patients with advanced cancer treated medically.

We previously reported a response rate of 64% in 14 chemo-naïve patients with LCNEC who received cisplatin plus mitomycin, vindesine, or etoposide (8). In that study, however, patients with a diagnosis of poorly differentiated adenocarcinoma, poorly differentiated squamous cell carcinoma, large cell carcinoma and small cell carcinoma were selected, and then a diagnosis of LCNEC was made retrospectively by reviewing autopsy or surgically resected specimens. Thus, they were not consecutive, but highly selected patients. This explains, at least partly, the high response rate in the previous study. On the other hand, in the current study we analysed consecutive patients with a diagnosis of LCNEC that is established before treatment.

Rossi et al. showed that objective responses were observed in six (50%) of 12 patients with metastatic LCNEC who received a platinum and etoposide regimen, while no response was obtained in 15 patients receiving regimens for NSCLC treatment (cisplatin and gemcitabine in 10 patients, gemcitabine alone in two patients, and carboplatin and paclitaxel in three patients) (22). In addition, the patients receiving the platinum and etoposide regimen had a significantly better survival than the patients who received the other regimens (median survival time, 51 months versus 21 months). These survival data, however, sound too good for lung cancer patients with a metastatic disease. Neither patient characteristics nor explanation for

Table 2. Chemotherapy regimens and responses

Regimens		No. of patients	CR/PR/SD/PD	Response rate (%)
CPT-11-based	CDDP + CPT-11	9	0 / 5 / 3 / 1	55.6
PTX-based	CBDCA + PTX	5	0 / 3 / 2 / 0	60.0
	CDDP + PTX	1	1 / 0 / 0 / 0	–
	PTX	1	0 / 1 / 0 / 0	–
VNR-based	CDDP + VNR	1	0 / 1 / 0 / 0	–
DTX-based	CDDP + DTX	1	0 / 1 / 0 / 0	–
ETP-based	CBDCA + ETP	3	0 / 0 / 3 / 0	0
	CDDP + ETP	1	0 / 1 / 0 / 0	–
Total		22		59.1

CPT-11, irinotecan; PTX, paclitaxel; VNR, vinorelbine; DTX, docetaxel; ETP, etoposide; CDDP, cisplatin; CBDCA, carboplatin; CR, complete response; PR, partial response; SD, stable disease; PD, progressive disease.

such a long survival was presented in this report (22). Another case series of LCNEC showed that three patients with a stage IV disease received platinum-based chemotherapy (cisplatin and etoposide, carboplatin and gemcitabine, and cisplatin, docetaxel and gemcitabine) but none of them achieved an objective response. Of five patients who received gefitinib as salvage therapy, one achieved a partial response (23).

In this study, the clinical response rates of LCNEC to chemotherapy regimens containing irinotecan or paclitaxel were as high as 70%. The published response rates of NSCLC and SCLC to these regimens are 30–33% and 68–84%, respectively (10–14). The PFS of 4.1 months and median OS of 10.3 months were comparable to the results of previous randomized phase III trials that have reported PFS values of 4.1–6.9 months and median OS values of 9.3–12.8 months in extensive-stage disease SCLC (14). Thus, the response rate and survival of LCNEC were comparable with those of SCLC. Although our retrospective review of clinical data revealed heterogeneous approaches in treatment regimens, our results suggested that irinotecan and paclitaxel may be active agents against LCNEC. LCNEC exhibit both features of NSCLC and SCLC in terms of the morphology and immnohistochemistry, and these anti-cancer agents are effective against both of these types of lung cancer. Considered together, the combinations of cisplatin and irinotecan, and carboplatin and paclitaxel may be promising regimens for LCNEC.

To evaluate the efficacy of irinotecan- or paclitaxel-based combined chemotherapy for LCNEC, it is necessary to perform prospective phase II trials. However, such trials for LCNEC may be difficult to perform for the following reasons. First, patient accrual is problematic because LCNEC is a relatively rare tumor and accounts for only about 3% of lung cancer patients treated by surgical resection (6). It took us 7 years to accumulate 22 patients with LCNEC treated with chemotherapy. Besides, some studies have revealed the efficacy of adjuvant chemotherapy for both SCLC and NSCLC (24–26). Thus, when patients treated with platinum-based adjuvant chemotherapy regimens are excluded, few subjects with LCNEC with the diagnosis confirmed based on examination of large tumor specimens may remain. Therefore, these trials may only be possible as multi-institutional studies. Second, because it can sometimes be difficult to define the histology of LCNEC without examination of specimens large enough to appreciate the histological architecture and obtain reproducibility, pathological review by experts panel would be needed in these trials.

In conclusion, our results showed that irinotecan- or paclitaxel-based regimens may be as active against LCNEC as that against SCLC. A phase II multi-institutional trial is under way in Japan to elucidate the efficacy of cisplatin- and irinotecan-based therapy regimens against LCNEC.

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Conflict of interest statement

None declared.

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Bodyweight change during the first 5 days of chemotherapy as an indicator of cisplatin renal toxicity

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To determine whether bodyweight (BW) loss, daily urine volume (UV) or furosemide use are associated with cisplatin nephrotoxicity, performance status, serum chemistries before treatment, average daily UV, maximum BW loss and use of furosemide on days 1–5 of chemotherapy were evaluated retrospectively in chemotherapy-naive patients with thoracic malignancies who had received 80 mg/m² cisplatin. Associations between these parameters and the worst serum creatinine levels (group 1, grade 0–1; and group 2, grade 2–3) during the first cycle were evaluated. Of the 417 patients (327 men and 90 women; median age, 59 years), 390 were categorized into group 1 and 27 were categorized into group 2. More women and older patients were observed in group 2 than in group 1 (11.1 *vs* 5.2%, $P = 0.044$, and 65 *vs* 59 years, $P = 0.041$, respectively). The median average daily UV was 3902 mL in group 1 and 3600 mL in group 2 ($P = 0.021$). A maximum BW loss ≥2.1 kg was noted in 4.4% of patients in group 1 and 18.5% of patients in group 2 ($P = 0.006$). Furosemide was used in 206 (49.4%) patients. The median total dose of furosemide in groups 1 and 2 were 0 mg and 26 mg, respectively ($P = 0.024$). A multivariate analysis showed that a maximum BW loss ≥2.1 kg and the total furosemide dose were significantly associated with group category. In conclusion, BW loss and total furosemide dose were associated with cisplatin nephrotoxicity. (*Cancer Sci* 2007; 98: 1408–1412)

Cisplatin alone or in combination with other chemotherapeutic agents has been the most frequently used chemotherapy regimen against a variety of solid tumors for 30 years because of its significant therapeutic effects.[1] In spite of intensive efforts to devise platinum analogs and the successful development of carboplatin, cisplatin remains a key agent in the treatment of germ cell tumors, head and neck cancer and bladder cancer, as shown in several randomized controlled trials comparing the two platinum agents.[2] In addition, cisplatin has a significant role in the treatment of lung and ovarian cancers, although carboplatin is becoming increasingly used against these cancers as an alternative chemotherapeutic agent.[3,4]

Cisplatin nephrotoxicity has been a major dose-limiting toxicity for this drug in most drug administration schedules.[5] Although the exact mechanism is unclear, high concentrations of platinum and widespread necrosis were observed in the proximal tubules of the kidney. This tubular impairment secondarily leads to a reduction in renal blood flow and glomerular filtration rate, potentiating primary tubular damage. This vicious circle causes a delayed deterioration in renal function, as an increase in the serum creatinine level typically appears 6–7 days after cisplatin administration in humans.[5,6] The standard prophylaxis for cisplatin nephrotoxicity is a normal saline infusion of 1–4 L with osmotic diuresis on the day of cisplatin administration.[5] Although this vigorous hydration diminishes life-threatening renal toxicity, 7–40% of patients still develop a mild to moderate increase in their serum creatinine levels, which influences subsequent cisplatin therapy.[7,8] For the prevention of cisplatin nephrotoxicity, the maintenance of good renal hemodynamics may be necessary for a week or longer after cisplatin administration, although indicators of hydration management on day 2 of chemotherapy and thereafter have not been reported. The purpose of this retrospective study was to evaluate bodyweight (BW) changes, daily urine volumes (UV) and use of furosemide on days 1–5 of chemotherapy as well as pretreatment patient characteristics in the hope of finding an association between these factors and nephrotoxicity during the first cycle of cisplatin-based chemotherapy.

Patients and Methods

Patient selection. Patients were selected retrospectively for the present study according to the following criteria: (1) a histological or cytological diagnosis of thoracic malignancy; (2) no prior chemotherapy; (3) a chemotherapy treatment regimen that included 80 mg/m² of cisplatin; and (4) treatment as an in-patient at the National Cancer Center Hospital. Patients were excluded if: (1) their pretreatment serum creatinine level was abnormal; or (2) no record of BW or daily UV on days 1–5 of chemotherapy was available.

Treatment. Cisplatin at a dose of 80 mg/m² was administered intravenously over 60 min on day 1 in combination with other chemotherapeutic agents. Hydration just before cisplatin administration consisted of 500 mL normal saline, 500 mL 5% glucose and 10 mL KCl over 4 h. Hydration just after cisplatin infusion consisted of 500 mL normal saline with 40 g mannitol over 2 h, followed by 500 mL normal saline, 1000 mL 5% glucose and 15 mL KCl over 6 h. On days 2–5, 1000 mL normal saline, 1000 mL 5% glucose and 20 mL KCl were administered over 8 h. Antiemetic prophylaxis consisted of a $5HT_3$ antagonist and 16 mg dexamethasone on day 1 followed by 8 mg dexamethasone on days 2 and 3, 4 mg on day 4 and 2 mg on day 5. Furosemide was given orally or intravenously if fluid retention was suspected based on an increased BW or a decreased UV. These treatments were repeated every 3–4 weeks.

Data collection and statistical analyses. The patients' baseline characteristics, including age, sex and performance status as well as serum albumin, Na, K, Ca and fasting blood sugar levels were analyzed. The modified Ca level was calculated using the following formula:

$$\text{modified Ca (mg/dL)} = \text{serum Ca (mg/dL)} + 4 - \text{serum albumin (g/dL)}.$$

The daily UV and BW at 0800 hours (before breakfast) and at 1600 hours (before dinner) were measured once a day on days

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Table 1. Patient demographics and pretreatment blood chemistry tests in groups categorized according to worst creatinine grade

		Group 1 (n = 390)		Group 2 (n = 27)		P-value
		n	%	n	%	
Sex	Male	310	94.8	17	5.2	0.044
	Female	80	88.9	10	11.1	
Age (years)	Median	59	(Range 18–77)	65	(Range 38–74)	0.041
Performance status	0	169	92.3	14	7.7	0.82
	1	218	94.3	13	5.6	
	2–3	3	100	0	0	
Serum albumin	≥3.7 g/dL	319	94.1	20	5.9	0.32
	≤3.6 g/dL	71	91.0	7	9.0	
Serum Na	≥138 mEq/L	341	93.2	25	6.8	0.43
	≤137 mEq/L	49	96.1	2	3.9	
Serum K	≤4.9 mEq/L	373	93.7	25	6.3	0.46
	≥5.0 mEq/L	17	89.5	2	10.5	
Modified Ca†	≤10.4 mg/dL	376	93.3	27	6.7	0.31
	≥10.5 mg/dL	14	100	0	0	
Fasting blood sugar	≤125 mg/dL	322	92.8	25	7.2	0.36
	≥126 mg/dL	54	96.4	2	3.6	
	Not done	14	100	0	0	

†Calculated using the equation: modified Ca (mg/dL) = serum Ca (mg/dL) + 4 – serum albumin (g/dL). Groups 1 and 2 were patients with worst creatinine grades of 0–1 and 2–3, respectively.

1–5 of the chemotherapy regimens. The BW at 0800 hours on day 1 was used as the baseline BW. During the chemotherapy course, blood chemistry was analyzed at least once a week. Data on furosemide use and the BW gain just before furosemide use during the first course of chemotherapy were obtained from medical charts.

The worst serum creatinine level during the first course of chemotherapy was graded (WCG) according to the National Cancer Institute (NCI) Common Toxicity Criteria, version 2.0. The patients were categorized into two groups according to their WCG: patients with WCG_{0-1} (group 1) and patients with WCG_{2-3} (group 2). The daily UV and BW changes, compared with the baseline BW, on days 2–5 of the chemotherapy regimens were noted, and differences in the averages of these measures between groups 1 and 2 were evaluated using repeated measures analyses of variance. Correlations between daily UV and BW changes were assessed using scatter diagrams and Pearson correlation coefficients.

The daily UV on days 1–5 and the maximum BW loss during days 1–5 of the first chemotherapy course were calculated for each patient. These parameters, the pretreatment parameters, the use of furosemide, and their associations with the two group categories were evaluated using χ-tests for categorical variables, Mann–Whitney tests for continuous variables, and logistic regression analyses for both types of variables. The total furosemide dose was calculated using the following formula:[9]

$$\text{total furosemide dose (mg)} = \text{intravenous dose (mg)} + 0.65 \times \text{oral dose (mg)}.$$

The Dr SPSS II 11.0 for Windows software package (SPSS Japan, Tokyo, Japan) was used for the statistical analyses.

Results

Between November 2000 and May 2006, 427 patients met the four inclusion criteria. Of these, six patients were excluded because their pretreatment serum creatinine levels were elevated, and four patients were excluded because no data on their daily UV or BW were available. Thus, a total of 417 patients were analyzed in the present study. The subjects comprised 327 men and 90 women, with a median age of 59 years (range 18–78 years) (Table 1). Non-small cell lung cancer was the most common tumor type, noted in 338 patients, followed by small cell lung cancer in 71 patients, thymic cancer in four patients, malignant mesothelioma in three patients, and tracheal cancer in one patient. Thirty-two patients with stage I–II diseases received chemotherapy as an adjuvant therapy after surgery. The remaining 385 patients with stage III–IV diseases or postoperative recurrent diseases received chemotherapy for the treatment of locally advanced or metastatic diseases.

All of the patients received cisplatin at a dose of 80 mg/m^2 in combination with other agents. The chemotherapy regimens were cisplatin and vinorelbine (n = 200), cisplatin and etoposide (n = 77), cisplatin, vindesine and mitomycin (n = 48), cisplatin and irinotecan (n = 41), cisplatin and gemcitabine (n = 41), and cisplatin and docetaxel (n = 10). The WCG was evaluated in all of the patients, with 390 patients categorized into group 1 and 27 patients categorized into group 2.

The average daily UV during days 1–5 of the chemotherapy regimens showed that the UV on day 1 did not differ between groups 1 and 2, but the daily UV on days 2–5 in group 2 were lower than those in group 1 (Fig. 1A, P = 0.042). The average changes in BW on days 2–5 showed that patients gained BW on days 2–3 and lost BW on days 4–5 (Fig. 1B). The line plotting the changes in BW in group 2 was always below that for group 1 (P = 0.036). Thus, the patients in group 2 retained less water than the patients in group 1. Furthermore, the patients in group 2 may have developed dehydration on day 5, as their average BW dropped to below the baseline level (Fig. 1B). Scatter diagrams comparing the average UV on days 1–2 and the BW change on day 3, and the average UV on days 1–4 and the BW change on day 5 showed no correlation between the UV and BW changes (data not shown), suggesting that the reduction in fluid intake may have caused the BW loss.

The development of renal toxicity was associated with some patient demographics. The percentage of women was higher in group 2 than in group 1 (11.1 *vs* 5.2%, P = 0.04). The median age of the patients in group 1 was 59 years (range 18–77 years), whereas that for group 2 was 65 years (range 38–74 years) (P = 0.041). None of the pretreatment chemistry parameters differed between the groups (Table 1). The frequency of renal toxicity did not differ according to chemotherapy regimen but was associated with a decreased average daily UV during days

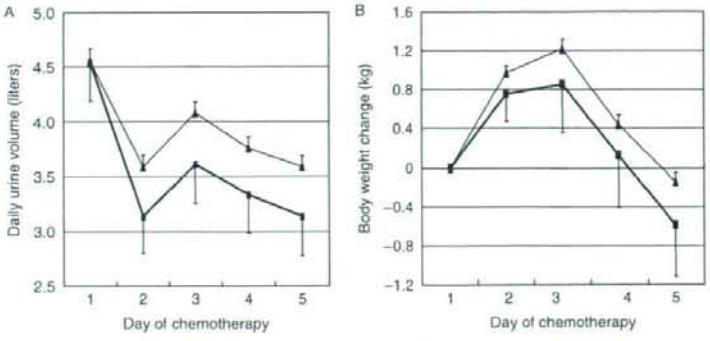

Fig. 1. (A) Average daily urine volumes during days 1–5 of chemotherapy. The differences were statistically significant ($P = 0.042$, repeated measures analysis of variance). (B) Average bodyweight changes on days 1–5 of chemotherapy. The differences were statistically significant ($P = 0.036$, repeated measures analysis of variance). Thin line with closed triangles: group 1, patients with a worst creatinine grade of 0–1 ($n = 390$); thick line with closed squares: group 2, patients with a worst creatinine grade of 2–3 ($n = 27$). Error bars show the 95% confidence intervals.

Table 2. Treatment-related parameters and groups categorized according to worst creatinine grade

		Group 1 ($n = 390$)		Group 2 ($n = 27$)		*P*-value
		n	%	*n*	%	
Agents combined with cisplatin	Vinorelbine	184	92.0	16	8.0	0.83
	Etoposide	74	96.1	3	3.9	
	Vindesine + mitomycin	45	93.8	3	6.2	
	Gemcitabine	39	95.1	2	4.9	
	Irinotecan	39	95.1	2	4.9	
	Docetaxel	9	90.0	1	10.0	
Average daily urine volume (mL)[†]	Median	3902	(Range 2058–6680)	3600	(Range 1700–5020)	0.021
	≤3000	41	87.2	6	12.8	0.054
	3001–4000	185	92.5	15	7.5	
	≥4001	164	96.5	6	3.5	
Maximum bodyweight loss (kg)[‡]	Median	0.2	(Range 0–3.9)	0.4	(Range 0–4.6)	0.11
	0	172	95.0	9	5.0	0.006
	0.1–2.0	201	93.9	13	6.1	
	≥2.1	17	77.3	5	22.7	
Total furosemide dose[§]	Median	0	(Range 0–160)	26	(Range 0–360)	0.024
	0	201	95.2	10	4.7	0.015
	1–30	87	94.6	5	5.4	
	31–60	70	93.3	5	6.7	
	61–90	11	91.7	1	8.3	
	≥91	21	77.8	6	22.2	

[†]The average daily urine volume on days 1–5 of chemotherapy. [‡]Maximum body weight loss during days 1–5 of chemotherapy. [§]Total furosemide dose (mg) = intravenous dose (mg) + 0.65 × oral dose (mg). Groups 1 and 2 were patients with worst creatinine grades of 0–1 and 2–3, respectively.

1–5 of the chemotherapy regimens (Table 2). In addition, only 5–6% of the patients with a maximum BW loss of 2 kg or less were classified as WCG_{2-3}, whereas 23% of the patients with a maximum BW loss of more than 2 kg were classified as WCG_{2-3} ($P = 0.006$). Furosemide was administered to 206 of the 417 patients (49.4%). Of these patients, 198 did not complain of any symptoms whereas eight developed mild edema in the lower extremities or face, which disappeared after a few days. The difference in the frequencies of renal toxicity among patients who received furosemide and those who did not (8.3 *vs* 4.7%, respectively; $P = 0.14$) was not large enough to be statistically significant. Administration route (intravenous or oral), day of use (day 1, day 2 or days 3–8), or BW gain just before use of furosemide (0–1.4, 1.5–2.9 or ≥3.0 kg) did not influence the frequency of renal toxicity. The total dose of furosemide, however, differed between groups 1 and 2 (median, 0 mg; range, 0–160 mg *vs* median, 26 mg; range, 0–360 mg, respectively; $P = 0.024$). In particular, 22% of the patients who received more than 90 mg of furosemide were classified as WCG_{2-3} (Table 2).

A multivariate analysis showed that the maximum BW loss (odds ratio, 1.77; 95% confidence interval, 1.08–2.90) and the total furosemide dose (odds ratio, 1.21; 95% confidence interval, 1.11–1.33) were significantly associated with the WCG_{2-3} category. Associations with sex and the daily UV were marginally significant (Table 3).

Discussion

The present study showed that the maximum BW loss during days 1–5 of chemotherapy was associated with the development of cisplatin renal toxicity. In particular, 23% of patients with a maximum BW loss of more than 2 kg were classified as WCG_{2-3}. Because dehydration amounting to as little as a 2% loss in BW results in impaired physiological and performance responses,[10] the BW loss and dehydration observed in the present study may be enough to aggravate cisplatin nephrotoxicity. No correlation was noted between the UV and BW changes, suggesting that the dehydration was attributable to a reduced oral intake by patients as a result of cisplatin-induced emesis. BW measurements are

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Table 3. Multivariate analysis of pretreatment and treatment-related parameters and groups categorized according to worst creatinine grade

Parameter		Odds ratio (95% confidence interval†)	*P*-value
Sex	Male	1	0.082
	Female	2.34 (0.90–6.10)	
Age	10-year increments	1.55 (0.91–2.64)	0.11
Average daily urine volume†	100-mL increments	0.94 (0.88–1.00)	0.073
Body weight loss	1-kg decrements	1.77 (1.08–2.90)	0.024
Total furosemide dose	10-mg increments	1.21 (1.11–1.33)	<0.001

†The average daily urine volume on days 1–5 of chemotherapy.

a simple and useful indicator of the hydration status of these patients.

The current study also showed that the total furosemide dose was associated with the development of renal toxicity. Vigorous fluid infusion and diuresis with mannitol or furosemide have been used widely for the prevention of cisplatin nephrotoxicity.[11,12] These interventions are thought to reduce the cisplatin concentration in the renal tubules and the time during which this drug and the tubular epithelial cells are in contact.[5] However, numerous experimental studies have provided conflicting results regarding the renal protective effects of these diuretics; cisplatin nephrotoxicity was reduced in some studies but was enhanced in others.[5] A randomized trial of cisplatin at a dose of 100 mg/m^2 and hydration with or without mannitol in patients with malignant melanoma showed that this regimen prevented nephrotoxicity during the first treatment course.[13] Another randomized trial of cisplatin hydration with mannitol or furosemide in patients with advanced solid tumors showed that a serum creatinine elevation of more than 2 mg/dL was observed in 28% of the courses in the mannitol-treated group and 19% of the courses in the furosemide-treated group.[14] A third randomized trial of cisplatin at a dose of 75 mg/m^2 and hydration alone, hydration with mannitol, or hydration with furosemide showed that creatinine clearance did not change before or after cisplatin treatment in the hydration alone and the furosemide-treated groups, but decreased in the mannitol-treated group.[15] However, these randomized trials included only small numbers of patients and therefore are not conclusive. Thus, no reports have convincingly shown any advantage of diuretics in preventing cisplatin nephrotoxicity. These studies differed from the current study, in which furosemide was administered only when fluid retention was suspected based on an increased BW or a decreased UV. Although an association between renal toxicity and the total furosemide dose was observed in this study, patients with fluid retention may be more prone to develop renal toxicity. Another explanation is that furosemide may have a direct toxic effect on the kidney. Thus, the administration of furosemide may be inevitable in some cases to prevent fluid overload during aggressive hydration, but its frequent use should be avoided.

Because renal function decreases physiologically with aging,[16] cisplatin use in elderly patients remains controversial. Some authors of clinical studies for patients aged 70 years or older have concluded that the use of cisplatin at moderate doses (60–100 mg/m^2) should be encouraged in these patients, just as it is in younger patients.[17–19] Studies that evaluated risk factors for cisplatin nephrotoxicity in more than 400 patients showed that an older age was a significant risk factor in two studies[7,20] but not in a third study.[8] In the current study, age was not a risk factor for renal toxicity according to a multivariate analysis, probably because 80 mg/m^2 of cisplatin was administered only to selected elderly patients. In our practice, many elderly patients are treated with cisplatin at a dose of 25 mg/m^2 on three consecutive days or weekly; these patients were excluded from the present study.

In the present study women were more likely to suffer from cisplatin nephrotoxicity than men. Another study also showed that women had a twofold increased risk for renal toxicity compared with men.[7] Although the reason for this difference is not definitely known, it may be explained, at least in part, by a 15% lower unbound cisplatin clearance in women than men,[7,21] because pharmacokinetics of unbound cisplatin have been repeatedly shown to be correlated with cisplatin nephrotoxicity.[22–24]

Although intravenous fluid infusion on the day of cisplatin administration is a well established treatment for preventing nephrotoxicity, the use of subsequent fluid infusions has not been reported. Because the present study showed that dehydration progressed on day 5 in many cases and an elevated serum creatinine level appeared thereafter, maintaining the total body water level during days 1–5 of chemotherapy seems to be important for the prophylaxis of cisplatin nephrotoxicity. For this purpose, a BW measurement carried out before breakfast would be a simple and useful indicator; if oral intake is found to be insufficient, vigorous infusion therapy on days 2–5 may be effective.

In conclusion, the maximum BW loss during days 1–5 of chemotherapy and the total furosemide dose were associated with the development of cisplatin renal toxicity. Maintaining total body water levels during this period seems to be important, and measuring BW would be a simple and useful indicator for this purpose.

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Serum Total Bilirubin as a Predictive Factor for Severe Neutropenia in Lung Cancer Patients Treated with Cisplatin and Irinotecan

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Objective: To clarify the association between pre-treatment total bilirubin (PTB) level and severe toxicity in patients receiving cisplatin and irinotecan.
Methods: We analyzed retrospectively the relationships of grade 4 neutropenia or grade 3–4 diarrhea and clinical variables including PTB and pre-treatment neutrophil counts (PNC) using a logistic regression model.
Results: One hundred and twenty-seven patients (93 men, 34 women; median age: 61 years; range: 24–74 years) received cisplatin (60 or 80 mg/m^2) on day 1 and irinotecan (60 mg/m^2) on days 1 and 8 every 3 weeks or on days 1, 8 and 15 every 4 weeks. Grade 4 neutropenia occurred in 29 patients (23%) and grade 3–4 diarrhea occurred in 13 patients (10%). Grade 4 neutropenia was associated with a higher PTB level (odds ratio: 4.9; 95% confidence interval: 1.4–17.7), a higher cisplatin dose (2.8, 1.0–7.8) and a lower PNC (1.5, 1.0–2.3). Grade 3–4 diarrhea was associated with liver metastasis (11.2, 2.2–57.4), a higher cisplatin dose (5.0, 1.2–21.3) and a lower PNC (2.0, 1.1–3.6).
Conclusions: PTB level was associated with the severity of neutropenia caused by cisplatin and irinotecan.

Key words: irinotecan – toxicity – lung cancer

INTRODUCTION

Although irinotecan is an active agent against several solid tumors, it sometimes exhibits serious adverse effects, the most common being bone marrow toxicity, in particular leucopenia and neutropenia, and ileocolitis, which leads to diarrhea (1–4). The severity of these toxicities varies greatly between individuals, and thus identifying pre-treatment factors that predict an increased risk for severe toxicities is a critical issue in the treatment of cancer patients undergoing chemotherapy.

Irinotecan needs to be activated by systemic carboxylesterases to SN-38 to exert its anti-tumor activity, which is mediated by the inhibition of topoisomerase I (5). Glucuronidation of SN-38 (SN-38G) by UDP-glucuronosyltransferase (UGT) 1A1 during biliary excretion is the primary route of detoxification and elimination. A higher ratio of plasma SN-38 to SN38-G has been correlated with severe diarrhea, suggesting that the efficiency of SN-38 glucuronidation is an important determinant of toxicity (6–8).

Genetic polymorphisms of the UGT 1A1 gene, such as the number of TA repeats in the TATA box that are associated with reduced transcriptional efficiency and functional activity, have been reported previously (7). Some studies have demonstrated an association between UGT1A1 polymorphisms and the risk for severe toxicity from irinotecan (6, 8–11).

The UGT1A1 enzyme is also responsible for hepatic bilirubin glucuronidation. Serum bilirubin levels, therefore, may reflect UGT1A1 activity and may also be associated with irinotecan activity and toxicity. The pre-treatment serum total bilirubin (PTB) level has been shown to be related to

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