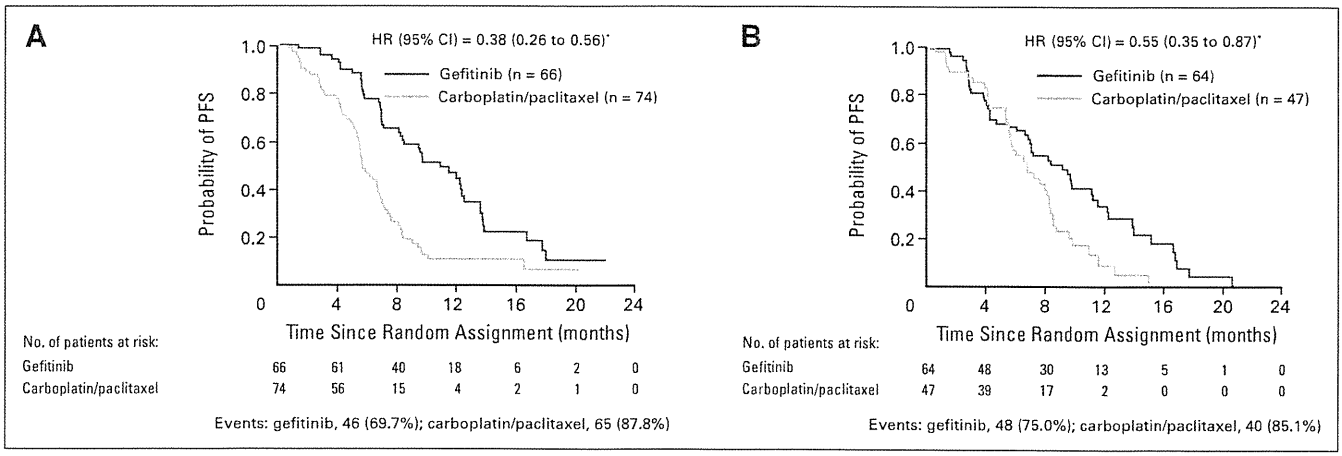

Fig 5. Kaplan-Meier curves for progression-free survival (PFS) by epidermal growth factor receptor (*EGFR*) mutation type (intent-to-treat population). Hazard ratio (HR) < 1 implies a lower risk of progression/death for patients treated with gefitinib. (A) Exon 19 deletion. (B) L858R. (*) Cox analysis with covariates (performance status [0-1, 2], smoking history [never, light ex-smoker], and sex).

versus Chemotherapy (EURTAC) study is ongoing. Therefore to date, including IPASS, five randomized studies have shown that EGFR TKIs offer significant benefits over standard chemotherapy in patients with *EGFR* mutation–positive tumors.

In IPASS, high *EGFR* gene copy number was predictive for the effect of gefitinib versus carboplatin/paclitaxel on PFS. The significantly longer PFS with gefitinib in patients with both high *EGFR* gene copy number and *EGFR* mutation–positive tumors was not observed in patients with high *EGFR* gene copy number without an accompanying mutation, suggesting that the apparent PFS benefit was driven by overlap with a coexisting *EGFR* mutation (77.6% of patients with high *EGFR* gene copy number also had *EGFR* mutation–positive tumors). Patients with *EGFR* mutation–positive tumors without accompanying high *EGFR* gene copy number showed longer PFS with gefitinib than with carboplatin/paclitaxel, suggesting that *EGFR* mutations determine the treatment outcomes independent of the status of *EGFR* gene copy number.

Post hoc analyses of PFS by *EGFR* mutation type showed that PFS was significantly longer for gefitinib than for carboplatin/paclitaxel in both the exon 19 deletions and exon 21 L858R subgroups, with a slightly greater advantage in the exon 19 deletions subgroup. First-line, single-arm studies[35,36] have reported an increased response to EGFR TKIs in patients with exon 19 deletions *v* exon 21 L858R mutation. However, IPASS (HR, 0.78; 95% CI, 0.51 to 1.19), WJTOG3405 (HR, 1.13; 95% CI, 0.63 to 2.03; $P = .681$), and NEJ002 (11.5 *v* 10.8 months; $P = .90$) randomized phase III studies and the prospective phase II iTARGET study ($P = .600$) showed no significant difference in PFS for gefitinib between the exon 19 deletions and exon 21 L858R mutation subgroups.[24,25,33]

In summary, *EGFR* mutation was the strongest predictive biomarker for benefit of gefitinib over carboplatin/paclitaxel on PFS and ORR. Post hoc analyses suggested that the predictive value of *EGFR* gene copy number for PFS benefit with gefitinib was driven by the overlap of high *EGFR* gene copy number with a positive *EGFR* mutation status. Treatment-related differences for PFS seen in patients with a positive *EGFR* mutation status were not apparent for OS. The OS results were likely confounded by the high proportion of patients receiving different types of subsequent therapies and, in particular, crossing over to the alternative treatment.

AUTHORS' DISCLOSURES OF POTENTIAL CONFLICTS OF INTEREST

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Thymidylate synthase and dihydropyrimidine dehydrogenase expression levels are associated with response to S-1 plus carboplatin in advanced non-small cell lung cancer

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ABSTRACT

S-1 is an oral fluoropyrimidine derivative that is active against non-small cell lung cancer (NSCLC). Development of S-1 combination chemotherapy for advanced NSCLC is under way. Given the importance of designing therapeutic strategies based on specific tumor biology, we have evaluated the relation between immunohistochemical expression levels of thymidylate synthase (TS), orotate phosphoribosyltransferase (OPRT), or dihydropyrimidine dehydrogenase (DPD) and the response to treatment with S-1 plus carboplatin in patients with advanced NSCLC. Chemotherapy-naïve patients with advanced (stage IIIB or IV) NSCLC, an Eastern Cooperative Oncology Group performance status of 0 or 1, adequate organ function, and archival tumor tissue were assigned to receive S-1–carboplatin ($n = 22$). The predictive or prognostic relevance of the molecular markers was also examined by their evaluation in patients treated with paclitaxel plus carboplatin ($n = 25$). Expression levels of TS, OPRT, or DPD in tumor specimens did not differ significantly between patients treated with S-1–carboplatin and those treated with paclitaxel–carboplatin. A low expression level of TS or of DPD was associated with a better response and longer survival in patients treated with S-1–carboplatin but not in those treated with paclitaxel–carboplatin. Tumor expression levels of TS and DPD are predictive of response to S-1–carboplatin chemotherapy in patients with advanced NSCLC.

1. Introduction

Lung cancer is the most common cause of cancer-related death worldwide, with non-small cell lung cancer (NSCLC) accounting for ~75% of all lung cancer cases [1]. Platinum-based chemotherapy regimens are the standard first-line treatment for individuals with advanced NSCLC, but the efficacy of such regimens has reached a plateau [2]. Both experimental and clinical studies have revealed that many molecules contribute to the various biological behaviors of malignant tumors including NSCLC. New strategies based on a better understanding of tumor biology are thus needed to maximize the efficacy of current treatments. Indeed, certain molecular markers, such as excision-repair cross-complementation type 1 (ERCC1), ribonucleotide reductase subunit M1 (RRM1), and breast cancer 1 (BRCA1), have been associated with the sensitivity of NSCLC tumors to cisplatin-based regimens, although there is currently insufficient evidence to recommend their routine clinical use [3–5].

5-Fluorouracil (5-FU), a pyrimidine analog that is metabolized by pyrimidine metabolic pathways, has been used worldwide for chemotherapy in individuals with various solid organ malignancies. Encouraging clinical results have recently led to the development of a new generation of oral fluoropyrimidines, commonly referred to as dihydropyrimidine dehydrogenase (DPD)-inhibitory fluoropyrimidines (DIFs). S-1 is one anticancer agent developed on the basis of the DIF concept and contains the 5-FU prodrug tegafur, potassium oxonate, and 5-chloro-2,4-dihydroxypyridine (CDHP), an inhibitor of DPD. S-1 is active against a wide range of solid tumors including NSCLC, and the development of S-1 combination chemotherapy for advanced NSCLC is under way [6–10]. Phase I or II studies have shown that combination therapy with S-1 and platinum compounds (cisplatin or carboplatin) is feasible and well tolerated in patients with advanced NSCLC, with efficacy results similar to those obtained with other platinum doublets [7–9].

Several enzymes participate in the metabolic pathways of 5-FU or folate, including thymidylate synthase (TS), a target enzyme of 5-FU; DPD, which catalyzes the degradation of 5-FU; and orotate phosphoribosyltransferase (OPRT). Previous studies have demonstrated a correlation between the expression levels of TS, DPD, and

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OPRT in solid tumors and 5-FU sensitivity [11]. However, the clinical relevance of these enzymes has not been established for NSCLC patients treated with S-1 or S-1 combination chemotherapy. We have now investigated the predictive value of TS, DPD, or OPRT expression in individuals with NSCLC treated with S-1 plus carboplatin (CBDCA). These molecular markers were also examined by their evaluation in patients treated with paclitaxel plus carboplatin.

2. Patients and methods

2.1. Patient characteristics

The present retrospective study recruited consecutive patients with advanced NSCLC who received chemotherapy at Kinki University Hospital between June 2003 and October 2009. Patients met all of the following criteria: a histological diagnosis of NSCLC with at least one measurable lesion; a clinical stage of IIIB or IV; an Eastern Cooperative Oncology Group (ECOG) performance status of 0 or 1; an age of 75 years or younger; adequate hematologic, hepatic, and renal function; treatment with CBDCA at an area under the curve (AUC) of 6 on day 1 and paclitaxel (PTX) at 200 mg/m^2 on day 1 or with CBDCA at an AUC of 5 on day 1 and S-1 at 80 mg/m^2 on days 1–14 every 3 weeks as first-line chemotherapy; and sufficient tissue available in paraffin blocks for assessment by immunohistochemistry. Tumor response was examined by computed tomography and was evaluated according to the Response Evaluation Criteria in Solid Tumors (RECISTs). Many patients had already died before the initiation of immunohistochemical analysis, preventing us from obtaining informed consent. The institutional review board therefore approved our study protocol with the conditions that samples would be processed anonymously and analyzed for protein expression and that the study would be disclosed publicly, according to the Ethical Guidelines for Human Genome Research published by the Ministry of Education, Culture, Sports, Science, and Technology, the Ministry of Health, Labor, and Welfare, and the Ministry of Economy, Trade, and Industry of Japan. The present study also conforms to the provisions of the Declaration of Helsinki.

2.2. Immunohistochemistry and scoring of protein expression

Sections (thickness, 4 μm) were depleted of paraffin with xylene and then rehydrated, and endogenous peroxidase activity was quenched by incubation with 0.3% hydrogen peroxide in methanol. The antigen retrieval was carried out by microwaving in citrate buffer, pH 6.0 (TS, OPRT) or in 1 mM EDTA, pH 8.0 (DPD) for 10 min. After washing in phosphate buffered saline, the sections were then incubated with polyclonal antibodies (Taiho Pharmaceutical Co., Saitama, Japan) to either TS (dilution of 1:100), OPRT (dilution of 1:1000), or DPD (dilution of 1:1350) overnight at room temperature. Biotinylated goat anti-rabbit IgG was applied as a secondary antibody for 30 min, followed by streptavidin–biotinylated peroxidase complex for 30 min at room temperature. Peroxidase activity was visualized with diaminobenzidine tetrahydrochloride (DAB) solution (DAKO Co. Ltd., Santa Barbara, CA), and counter staining was performed with hematoxylin. The human colon cancer cell line DLD-1/FrUrd, human breast cancer cell line MDA-MB-435S, and human pancreatic cancer cell line MIAPaCa-2 were used as positive controls for the staining of TS, OPRT, and DPD, respectively. All of the immunostained sections were reviewed by two observers (N.H. and K.N.) without knowledge of the patients' characteristics. Sections with discrepant results were jointly re-evaluated until a consensus was reached. Cytoplasmic staining for TS, OPRT, and DPD was scored in a semiquantitative manner reflecting both the intensity of staining and the percentage of cells with staining at each intensity. Staining intensity was classified as 0 (no staining), +1 (weak staining), +2 (distinct staining), or +3 (strong staining). A value designated the HSCORE was obtained as $\Sigma(I \times PC)$, where I and PC represent staining intensity and the percentage of cells that stain at each intensity, respectively. The selection of clinically important cutoff scores for TS, OPRT, or DPD expression was based on receiver operating characteristic (ROC) curve analysis.

Table 1
Patient characteristics.

Characteristic	S-1 plus CBDCA*n* (%)	PTX plus CBDCA*n* (%)
Sex		
Male	15 (68)	17 (68)
Female	7 (32)	8 (32)
Age (years)[a]	63 (39–73)	65 (48–74)
Smoking history		
Never-smoker	3 (14)	7 (28)
Smoker	19 (86)	18 (72)
Tumor histology		
Adenocarcinoma	16 (73)	16 (64)
Squamous cell	1 (4)	5 (20)
Other	5 (23)	4 (16)
Disease stage		
IIIB	3 (14)	4 (16)
IV	19 (86)	21 (84)
Tumor response[b]		
ORR (CR + PR)	9 (41)	9 (36)
SD	6 (27)	10 (40)
PD	7 (32)	6 (24)

[a] Data are presented as median (range).
[b] ORR, overall response rate; CR, complete response; PR, partial response; SD, stable disease; PD, progressive disease.

2.3. Statistical analysis

Expression levels of TS, OPRT, and DPD were compared between groups with the Mann–Whitney U test, and the relations between these variables were evaluated with Pearson's correlation test. Differences between the two treatment groups for demographic characteristics and the relation between treatment response and the expression of TS or DPD were evaluated with the two-sided Fisher's exact test. Overall survival and progression free survival were assessed from the first day of chemotherapy administration to the data of death from any cause and the date of objective disease progression, respectively. Patients without documented death at the time of the final analysis were evaluated at the date they were last known to be alive or of their last objective tumor assessment. The Kaplan–Meier method was used to estimate the probability of survival as a function of time, and differences in the survival of subgroups of patients were evaluated with the log-rank test. All P values were based on a two-tailed statistical analysis, and a P value of <0.05 was considered statistically significant. All statistical analysis was performed with GraphPad prism software (version 5.0; GraphPad Software, San Diego, CA).

3. Results

3.1. Patient characteristics

A total of 47 patients met the eligibility criteria (Table 1). Twenty-two patients were treated with S-1 plus CBDCA (S-1 arm) and 25 patients were treated with PTX plus CBDCA (PTX arm). Most (68%) patients were male in both groups. The median age of the patients was 63 years (range, 39–73) in the S-1 arm and 65 years (range, 48–74) in the PTX arm. Adenocarcinoma was the predominant histological type of NSCLC, accounting for 73% of patients

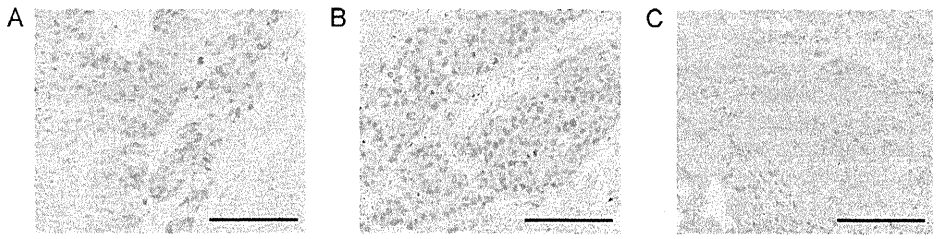

Fig. 1. Immunohistochemical staining of human NSCLC tissue. Representative sections of carcinomas with high levels of expression of TS (A), OPRT (B), or DPD (C) are shown. Scale bars, 125 μm.

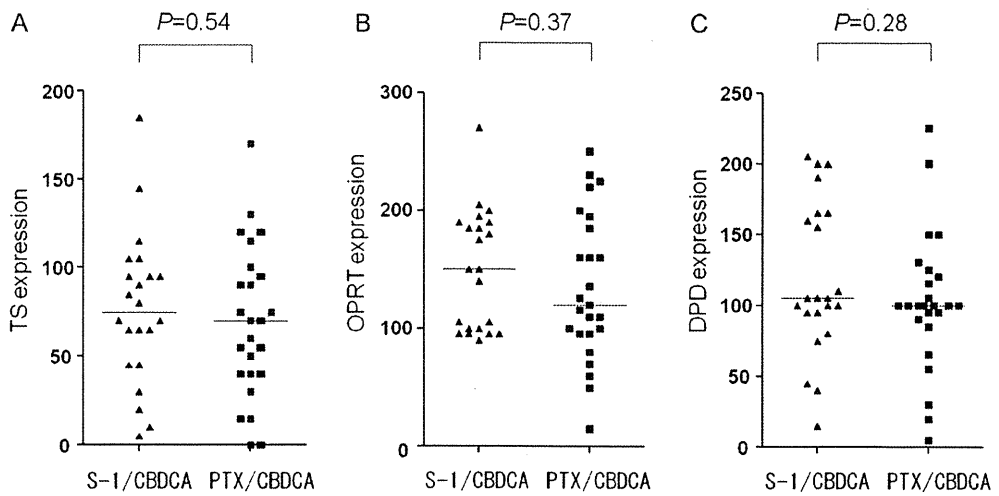

Fig. 2. Expression levels of TS (A), OPRT (B), and DPD (C) in NSCLC specimens of patients treated with S-1 plus CBDCA or with PTX plus CBDCA. Median values for expression level (HSCORE) are indicated by the horizontal lines. *P* values were determined with the Mann–Whitney *U* test.

in the S-1 arm and 64% in the PTX arm. The S-1 and PTX arms included 19 (86%) and 21 (84%) patients, respectively, with stage IV disease. There were no significant differences in sex distribution, age, smoking history, tumor histology, or disease stage between the S-1 and PTX arms. The median number of treatment cycles was 4 (range, 1–6) and 3 (range, 1–6) in the S-1 and PTX arms, respectively. The overall response rate (ORR = complete response [CR] + partial response [PR]) was 41% in the S-1 arm and 36% in the PTX arm (Table 1). The median follow-up time was 14.2 months, and the median overall survival was 15.5 months in the S-1 arm and 13.3 months in the PTX arm, with no significant difference in this parameter between the two arms ($P = 0.52$, log-rank test; data not shown).

3.2. Expression levels of TS, OPRT, and DPD in tumor specimens

We examined the expression levels of TS, OPRT, and DPD in tumor sections by immunohistochemistry (Fig. 1). In the S-1 arm, intratumoral TS, OPRT, and DPD expression levels (HSCOREs) varied from 5 to 185 (median, 75), from 90 to 270 (median, 150), and from 15 to 205 (median, 105), respectively (Fig. 2). In the PTX arm, these values ranged from 0 to 170 (median, 70), from 15 to 250 (median, 120), and from 5 to 225 (median, 100), respectively. No significant difference in TS ($P = 0.54$), OPRT ($P = 0.37$), or DPD ($P = 0.28$) expression levels was apparent between the two arms. The expression level of DPD was not correlated with that of TS ($R^2 = 0.0090$, data not shown), and the expression level of OPRT was not correlated with that of TS or DPD ($R^2 = 0.0074$ and 0.11, respectively; data not shown).

We next evaluated the relation between the expression of these enzymes and the tumor response to treatment. Tumors were categorized as either responding (CR or PR) or nonresponding (stable disease [SD] or progressive disease [PD]). In the S-1 arm, the TS expression level for the responding groups (range, 5–105) was significantly ($P = 0.006$) lower than that for the nonresponding groups (range, 65–185) (Fig. 3A). In contrast, the level of TS expression did not differ significantly ($P = 0.63$) between responders and nonresponders in the PTX arm (Fig. 3A). The expression levels of OPRT (Fig. 3B) and DPD (Fig. 3C) were not significantly associated with tumor response in the S-1 arm or the PTX arm, although the expression level of DPD tended to be lower in responders than in nonresponders of the S-1 arm ($P = 0.055$).

3.3. Predictive relevance of TS and DPD expression levels in NSCLC

We performed ROC curve analysis to establish the optimal cutoff values for the HSCORE of enzyme expression level for differentiation of responders from nonresponders. Values of 55, 97.5, and 162.5 for TS, OPRT, and DPD, respectively, were obtained for the S-1 arm (Fig. 4). In patients treated with S-1 plus CBDCA, response rates were 100% (6 out of 6) and 19% (3 out of 16) for tumors with low (<55) or high (≥55) levels of TS expression ($P = 0.001$), respectively (Table 2). In contrast, there was no significant ($P = 1.0$) difference in response rate between tumors with high or low levels of TS expression in the PTX arm. In the S-1 arm, the response rate for tumors with a high level (≥162.5) of DPD expression was significantly lower than that for those with a low level (<162.5) of DPD expression (0 versus 56%, $P = 0.046$) (Table 2), whereas no such difference was observed in the PTX arm ($P = 0.52$). In the S-1 arm, the expression level of DPD was not correlated with that of TS ($R^2 = 0.046$); however, the responder in low DPD levels ($n = 9$) included all the responder in low TS levels ($n = 6$) (Fig. 5). No signif-

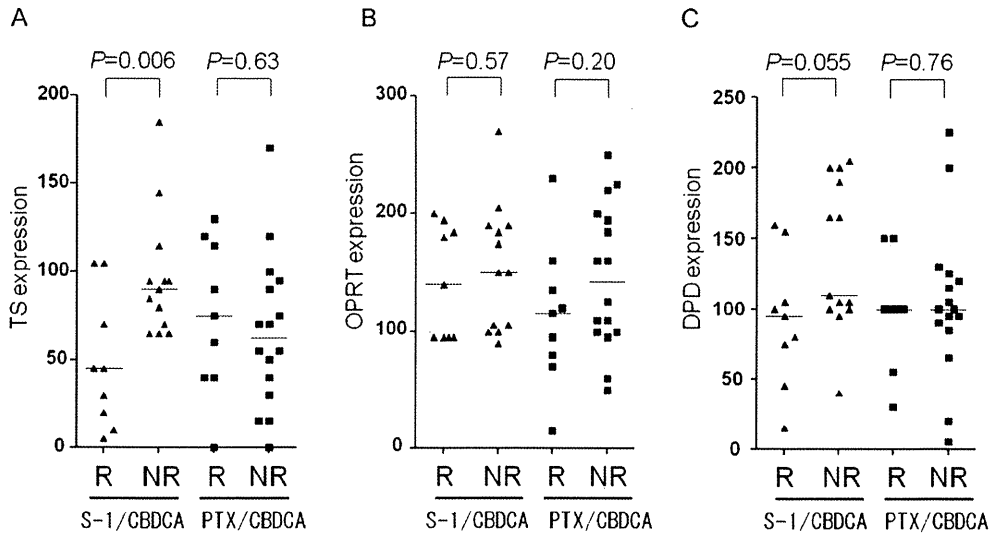

Fig. 3. Relation of expression levels of TS (A), OPRT (B), or DPD (C) in NSCLC specimens of patients treated with S-1 plus CBDCA or with PTX plus CBDCA to treatment response. NR and R represent nonresponders and responders, respectively, and median values for expression level (HSCORE) are indicated by the horizontal lines. *P* values were determined with the Mann–Whitney *U* test.

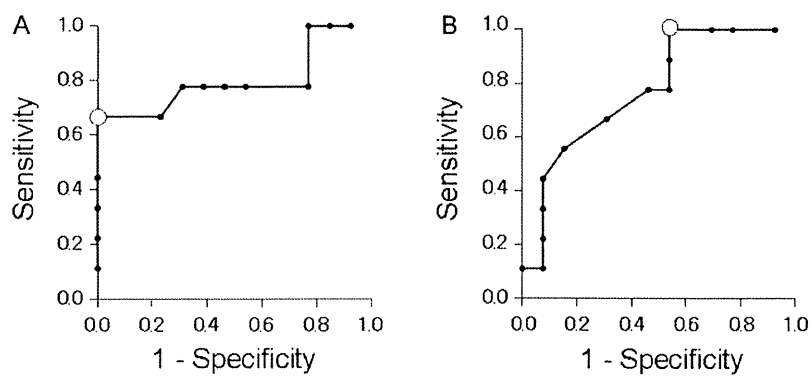

Fig. 4. Receiver operating characteristic (ROC) analysis based on intratumoral TS (A) and DPD (B) expression levels with response to S-1/CBDCA therapy. The optimal cut-off point (open circle) was 55 and 162.5 for TS and DPD, respectively, which yielded the maximum sensitivity plus specificity.

icant association between high or low OPRT expression level and response rate was apparent in either arm.

Finally, for patients treated with S-1 plus CBDCA, the progression-free survival in low TS group tended to be longer than that in high TS group, although the difference was not statistically significant ($P=0.11$) (Fig. 6A). Patients with a low level of TS expression had a significantly ($P=0.02$) longer overall survival than did those with a high level (Fig. 6B). In contrast, there was no significant difference in progression-free survival ($P=0.62$) and overall survival ($P=0.83$) between patients with a high or low level of TS expression in the PTX arm (Fig. 6C and D). Progression-free survival and overall survival for patients with a low level of DPD expression was significantly ($P=0.013$ and 0.009, respectively; data not shown) longer than that for those with a high level in the S-1 arm, whereas no such difference ($P=0.57$ and 0.27, respectively) was apparent in the PTX arm (data not shown). As shown in Table 3, 73% of patients with recurrence disease in the S-1 arm and 84% in the PTX arm received subsequent treatment. No bias for subsequent treatments between the two arms was observed.

4. Discussion

We have investigated the relation between intratumoral expression levels of TS, OPRT, or DPD and clinical outcome for NSCLC patients treated with S-1 plus CBDCA (S-1 arm) or with PTX plus CBDCA (PTX arm). The expression level of these proteins was assessed by immunohistochemical analysis in a semiquantitative manner by scoring the proportions of tumor cells with defined staining intensities relative to the total number of tumor cells. ROC curves are commonly used to determine biologically or clinically

Table 2
Tumor response to treatment according to TS or DPD expression level. All *P* values were determined with Fisher's exact test.

Relative expression level	S-1 plus CBDCA ($n=22$)			PTX plus CBDCA ($n=25$)		
	Responders*n* (%)	Nonresponders*n* (%)	*P*	Responders*n* (%)	Nonresponders*n* (%)	*P*
High TS	3 (19)	13 (81)	0.001	6 (38)	10 (63)	1.0
Low TS	6 (100)	0 (0)		3 (33)	6 (67)	
High DPD	0 (0)	6 (100)	0.046	0 (0)	2 (100)	0.52
Low DPD	9 (56)	7 (44)		9 (39)	14 (61)	

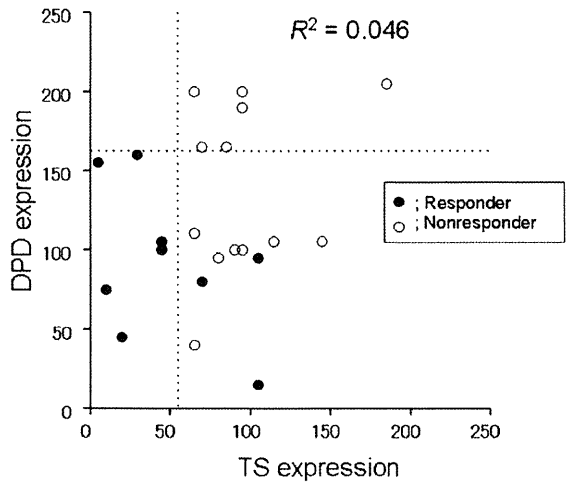

Fig. 5. Correlation between TS and DPD expression levels in patients treated with S-1 plus CBDCA. The dotted lines indicate the optimal cutoff values for the HSCORE of each expression level. The closed circles represent the responder, and the open circles represent the nonresponder.

relevant cutoff scores in such analysis [12,13], and we therefore used ROC curves to define the optimal cutoff values of TS or DPD expression level (HSCORE) for discrimination of responders from nonresponders in the S-1 arm. We found that low levels of TS and DPD expression were associated with a better treatment response and a longer survival time in NSCLC patients in the S-1 arm. To examine the predictive or prognostic relevance of the cutoff values for TS or DPD expression level determined in the S-1 arm, we applied these values to the results obtained for patients treated with PTX plus CBDCA, which is a standard first-line chemotherapy regimen for advanced NSCLC. Neither the expression of TS nor that of DPD showed a significant association with treatment response or survival in the PTX arm. These results thus indicate that the expression levels of TS and DPD are independent predictive markers, rather than prognostic markers, in patients with advanced NSCLC receiving S-1-based chemotherapy.

Table 3
Treatment after recurrence in each arm.

Variable	Treatment arm		*P*
	S-1 plus CBDCA *n* (%)	PTX plus CBDCA *n* (%)	
Any treatment	16 (73)	21 (84)	0.48
Radiotherapy	3 (14)	4 (16)	1.00
Any chemotherapy	11 (50)	17 (68)	0.25
Docetaxel	5 (23)	9 (36)	0.36
Gefitinib	3 (14)	8 (32)	0.18

TS is an essential enzyme that catalyzes the transfer of a methyl group from methylenetetrahydrofolate to dUMP in order to generate dTMP [14,15]. The subsequent phosphorylation of dTMP to dTTP provides a direct precursor for DNA synthesis. Several in vitro studies with tumor cell lines have implicated up-regulation of TS expression as a mechanism of resistance to 5-FU that develops after exposure to the drug [16–19]. Previous clinical studies have also shown that a low level of TS expression was associated with high sensitivity to 5-FU, to 5-FU plus cisplatin, or to 5-FU plus methotrexate in colorectal or gastric cancer [11]. A low level of TS expression in NSCLC tumors has also been associated with longer survival in patients treated with oral 5-FU–based agents after curative resection [20–22]. S-1 is an oral fluoropyrimidine derivative

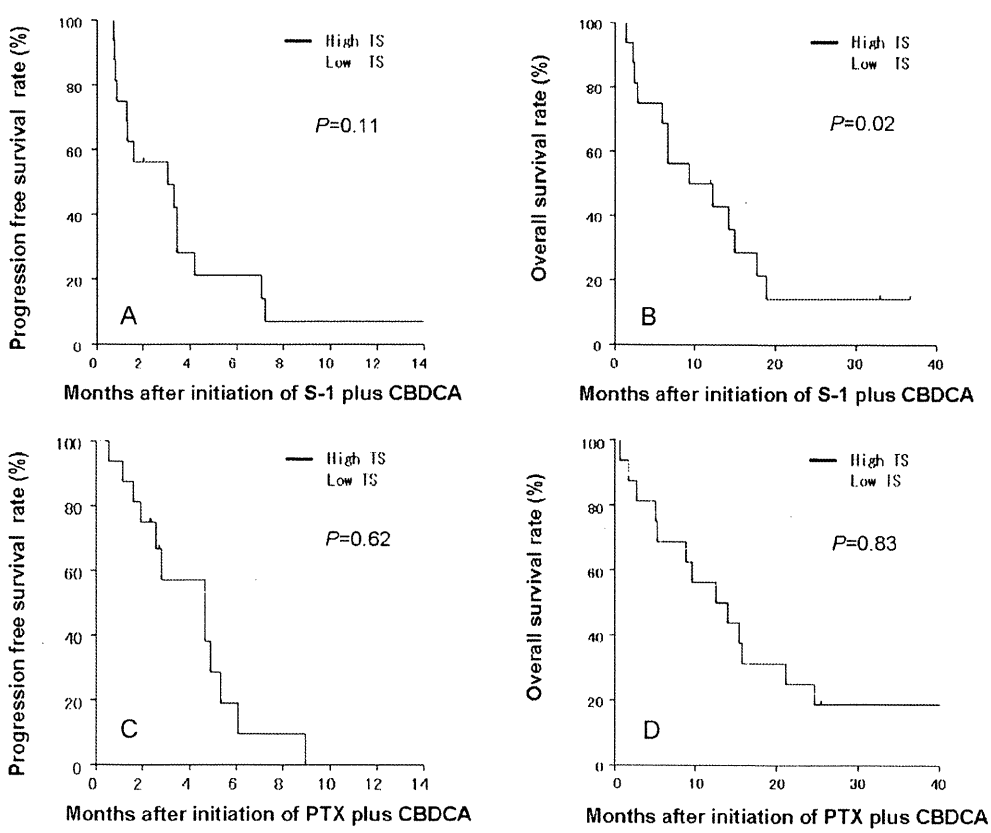

Fig. 6. Progression-free survival and overall survival according to expression level of TS in NSCLC tumors of patients treated with S-1 plus CBDCA (A and B) or with PTX plus CBDCA (C and D). *P* values were determined with the log-rank test.

that contains the 5-FU prodrug tegafur, and it is therefore expected to have an antitumor effect in patients with tumors sensitive to 5-FU. Indeed, low levels of TS expression in gastric cancer have been linked to a favorable clinical outcome after S-1 treatment [23–25]. However, the relation between TS status and tumor response to S-1 or to S-1 combination therapy has not previously been examined for NSCLC. We have now shown that a low level of TS expression was significantly associated with response to treatment with S-1 plus CBDCA in patients with advanced NSCLC.

DPD is an initial and rate-limiting enzyme in the catabolism of 5-FU, with >80% of 5-FU being degraded to inactive metabolites by this enzyme in human tissues, and DPD activity therefore modulates the antitumor effects of 5-FU. In vitro studies have shown that overexpression of DPD in cancer cell lines confers resistance to 5-FU [16,26]. Several clinical studies have also shown that a high level of DPD expression in tumors was associated with poor survival in NSCLC patients treated with oral 5-FU-based agents after curative surgery [20,21,27,28], whereas a relation between DPD expression level and clinical outcome after 5-FU treatment has not been definitively demonstrated for colorectal or gastric cancer [11]. S-1 contains CDHP, an inhibitor of DPD, and an antitumor effect of S-1 is therefore expected even in tumors with a high level of DPD activity. Indeed, patients with gastric cancer expressing DPD at high levels were found to benefit from S-1 treatment [23–25]. No previous studies have evaluated the relation between DPD expression and S-1 sensitivity in NSCLC, however. We have now shown that a high level of DPD expression in NSCLC predicts resistance to S-1-based chemotherapy. DPD activity levels have been shown to be higher in NSCLC tissue than in other solid tumors including gastric, colorectal, and breast cancer [29]. The apparent discrepancy between the demonstrated clinical efficacy of S-1 in patients with gastric cancer expressing DPD at high levels [23–25] and our finding that no NSCLC patients with a high level of DPD expression responded to treatment with S-1 plus CBDCA may be attributable to the fact that DPD activity levels in NSCLC tissue are about twice those in gastric cancer [29]. In cancers with a high level of DPD expression, such as NSCLC, the amount of the free enzyme may be maintained in excess of that of the CDHP-bound enzyme.

Molecular targeting therapies have been developed as a new strategy for the treatment of advanced NSCLC, and somatic mutations in the epidermal growth factor receptor (*EGFR*) gene are the most robust biomarker for EGFR tyrosine kinase inhibitor (TKI) therapy in NSCLC. A recent study has reported that *EGFR* mutant tumors have a lower sensitivity to another oral 5-FU derivative, uracil-tegafur, than that of *EGFR* wild-type tumors [30]. We have previously shown that EGFR-TKI-induced downregulation of TS is responsible for the enhanced antitumor effect of combined treatment with S-1 [31–33]. Based on these results, further studies are warranted to investigate the relationship between the presence of *EGFR* mutation and TS/DPD expression levels in NSCLC.

In conclusion, we have shown that the tumor expression levels of TS and DPD were predictive of tumor response to S-1-based chemotherapy in patients with advanced NSCLC. S-1 in combination with platinum compounds (cisplatin or CBDCA) is currently under evaluation as a first-line treatment for advanced NSCLC in randomized phase III studies. It will be necessary to confirm that the expression levels of TS and DPD can predict clinical outcome in these clinical trials, given that our findings derive from a limited retrospective study of a relatively small number of patients. Further prospective studies of these biomarkers are also needed to address the issue of reproducibility in a large series of patients.

Conflict of interest statement

The authors declare no conflict of interest.

Acknowledgments

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ORIGINAL ARTICLE

Patterns of recurrence and outcome in patients with surgically resected small cell lung cancer

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Abstract
Background Although prophylactic cranial irradiation (PCI) in limited-stage (LS) small cell lung cancer (SCLC) patients who are surgically resected and treated with adjuvant chemotherapy is considered to be a reasonable treatment option, the efficacy of PCI for those patients remains unclear.
Methods The records of 28 patients with SCLC undergoing curative surgery at the Aichi Cancer Center Hospital between 1995 and March 2008 were retrospectively reviewed to assess patterns of relapse and overall survival.
Results The patients were 27 men and 1 woman. Eight patients underwent induction chemotherapy. Fourteen patients (50%) had pathologic stage (p-stage) I disease, 7 patients (25%) had p-stage II, and 7 patients (25%) had p-stage III. Nineteen patients underwent adjuvant chemotherapy and one patient received adjuvant chemoradiotherapy. There were a total of 13 deaths and 8 were disease-related. Most patients developed hematogenous distant metastases before their death. The 5-year overall probability of survival was 47%. Ten (36%) of the 28 patients had a relapse. Two had a local relapse alone, one patient had combined local and distant relapses, and seven patients had distant metastases alone as their first site of failure. Four patients with p-stage II/III disease developed brain metastases with a cumulative incidence at 1 and 2 years of 25 and 36%, respectively.
Conclusions Our retrospective study suggested that PCI might have a role in surgically resected patients with p-stage II/III SCLC because of their relatively high frequency of brain metastasis.

Keywords Cranial irradiation · Metastasis · Small cell lung cancer · Thoracic surgery

Introduction

Lung cancer is a leading cause of cancer mortality in the United States and in Japan [1, 2]. Lung cancer consists of two main histologic types; small cell lung cancer (SCLC) accounting for about 15% of lung cancer and non-SCLC (NSCLC) [3]. Concurrent chemoradiation therapy and prophylactic cranial irradiation (PCI) for limited stage (LS)-SCLC results in 5-year survival for approximately 25% of patients [4], and chemotherapy with platinum and etoposide or with CPT-11 only results in 5-year survival for fewer than 1% of patients with extensive stage (ES)-SCLC [5]. Because of its aggressive nature, for example rapid growth and early dissemination in lymph nodes, bones, adrenal glands, liver, and brain, the efficacy of surgery for treatment of SCLC is regarded as very limited [6], although it has been established by publications in the 1970s and early 1980s which showed long-term survival in

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surgically treated early stage patients [7]. At present, surgery plus adjuvant chemotherapy is standard of care for patients with clinical stage (c-stage) I SCLC [8].

At the time of initial diagnosis, 10–14% of patients with SCLC have detectable brain metastases and at the time of death [9] approximately one-third of patients harbor clinically recognized brain metastases, and over 50% of patients have brain metastases at postmortem examination [10]. The risk of central nervous system metastasis developing 2 years after successful treatment of SCLC has been reported to be approximately 35–65% [11]. A meta-analysis of the efficacy of PCI in 987 SCLC patients revealed a 25.3% decrease in cumulative incidence of brain metastasis at 3 years after PCI and an absolute increase in overall survival of 5.4% at 3 years [12]. Although this study included 140 patients with ES-SCLC, PCI has traditionally been limited to patients with LS-SCLC after meaningful response from combined-modality treatment has been achieved. However, recent results from a randomized study provide evidence that PCI not only reduces the incidence of symptomatic brain metastases but also prolongs disease-free and overall survival in patients with ES-SCLC [13]. Consequently, PCI is recommended for patients with limited-stage disease and extensive-stage disease who achieve a complete or near complete response to treatment, and can be considered for patients with a partial response to initial therapy even in ES-SCLC.

However, there is limited information about the frequency of brain failure in patients with early LS-SCLC who underwent surgery with adjuvant chemotherapy. The issue whether all patients with LS-SCLC undergoing surgery should receive PCI remains unclear. So, in this study, we reviewed our surgical results for 28 patients with LS-SCLC in our institution to see the relapse patterns, the frequency of brain metastasis and overall survival.

Patients and methods

Patients

Approval for this study was obtained from, and the need for individual patient consent was waived by, the institutional review board. Between 1995 and March 2008, twenty-eight patients with SCLC underwent surgery with nodal resection at the Department of Thoracic Surgery of Aichi Cancer Center Hospital. We collected complete clinical data for all patients, none of whom was lost to follow up.

Histological diagnosis

For histological diagnosis patients were subjected to bronchoscopic biopsy or cytology and/or CT-guided biopsy. For 5 of 28 patients (18%), histological or cytological diagnosis was not obtained preoperatively. Preoperative diagnosis of SCLC was achieved for 10 patients only. For the remaining 13 patients, the preoperative diagnosis was large-cell neuroendocrine carcinoma (LCNEC) in three cases, adenocarcinoma in three cases, squamous cell carcinoma in three cases, carcinoma in two cases, NSCLC in one case, and large-cell carcinoma in one case. The histology of all the surgical resection specimens was reviewed. In all cases, diagnosis by light microscopy was confirmed by immunohistochemical methods. Histologic classification was performed according to the World Health Organization classification [14]. The postoperative diagnosis was SCLC in 15 cases and combined SCLC in 13 cases.

Diagnostic workup

Standard diagnostic workup for all patients consisted in X-ray of the chest and thoracic and abdominal computed tomography (CT), bronchoscopy, brain magnetic resonance imaging (MRI) or CT, and bone scintigraphy or positron emission tomography (PET). Mediastinoscopy was not done. We used the TNM classification system of the International Union Against Cancer in this study [15], because precise staging and discrimination between choices of different options of treatment were required for surgical approach for these selected LS-SCLC patients. Pretreatment c-stages were IA, 15 patients; IB, 6 patients; IIA, 2 patients; IIB, 3 patients; IIIA, 2 patients.

Treatment

Eight patients underwent induction chemotherapy. Among these, three patients with c-stage II/III disease who were preoperatively diagnosed as NSCLC on biopsy, received induction chemotherapy consisting of platinum (CDDP or carboplatin) and taxane (paclitaxel or docetaxel). Four patients with c-stage I SCLC and one patient with c-stage II SCLC consented to our in-house clinical procedure and received induction chemotherapy consisting of platinum and etoposide. Twenty-one patients received adjuvant treatment, and seven patients were not treated with adjuvant chemotherapy because of poor general condition ($n = 1$), refusal to consent ($n = 3$), and old age ($n = 3$). Nineteen patients underwent adjuvant chemotherapy consisting of platinum and etoposide or CPT-11. One double-cancer patient with pT1N1 SCLC and advanced hypopharynx cancer simultaneously received chemoradiotherapy consisting of CDDP plus 5-FU and intensity-modulated radiation therapy (IMRT: 66 Gy). One patient underwent adjuvant chemoradiotherapy consisting of CDDP plus etoposide and concurrent thoracic RT (42 Gy).

Survival was determined by use of the institutional database, which is updated with an annual institutional census or with each patient visit.

Statistical analysis

Statistical analysis was carried out using SPSS software (SPSS, Chicago, IL, USA). Overall survival of patients from the time of operation was estimated by means of the Kaplan–Meier method. The cumulative incidence of brain metastasis was also calculated. Patients suffering progression at non-central nervous system sites and/or death from any cause were considered censored.

Results

Patient characteristics

From January 1995 to March 2008, a total of 28 patients underwent complete resection for SCLC. Baseline characteristics of patients according to the postoperative diagnosis are listed in Table 1. Twenty-seven were men and 1 was a woman. The median age of patients was 64.5 years (range 41–77). All patients had a history of cigarette smoking. No patient had a central tumor. Preoperative diagnosis of SCLC was made in 36% ($n = 10$) of 28 patients; postoperative diagnosis of combined SCLC was made in 15% ($n = 2$) of 13 patients (Table 1). Among 7 patients with c-stage II/III disease, 6 cases were preoperatively diagnosed as NSCLC on biopsy. Eight patients underwent induction chemotherapy. Three patients with c-stage II/III disease whose preoperative diagnosis was NSCLC received chemotherapy with platinum and taxane. One patient with c-stage II SCLC and four patients with c-stage I SCLC received induction chemotherapy consisting of platinum and etoposide. All patients underwent lobectomy with mediastinal lymph node dissection.

There was no perioperative death. Regarding pathologic stage, 13 patients had IA disease, 1 patient had IB, 4 patients had IIA, 3 patients had IIB, 5 patients had IIIA, and 2 patients had IIIB. Postoperatively, 21 patients received adjuvant treatment and 7 patients were not treated with adjuvant chemotherapy because of poor general condition ($n = 1$), patient refusal ($n = 3$), or old age ($n = 3$). Nineteen patients underwent adjuvant chemotherapy and one patient received chemoradiotherapy (Table 1). One patient with pT1N1SCLC and advanced hypopharynx cancer simultaneously received chemoradiation therapy consisting of CDDP plus 5-FU and intensity-modulated radiation therapy (66 Gy). Relationship between pretreatment clinical stages and postoperative pathologic stages is shown in Table 2. Because of inaccuracy of clinical staging, clinical understaging rate was approximately 36% (10/28), although 8 patients received induction therapy.

Table 1 Demographics, clinical characteristics, and perioperative treatment of patients

	Postoperative diagnosis		
	Total ($n = 28$)	SCLC ($n = 15$)	Combined SCLC ($n = 13$)
Age (years)			
Median	64.5	64	65
Range	41–77	54–77	41–77
Sex			
Male	27	14	13
Female	1	1	0
Clinical stage			
I	21	12	9
II	5[a,b]	3	2
III	2[a]	0	2
Pathologic stage			
I	14	8	6
II	7	4	3
III	7	3	4
Preoperative diagnosis			
SCLC	10	8	2
LCNEC	3	1	2
Sq	3	2	1
Ad	3	1	2
La	1	0	1
NSCLC	1	0	1
Carcinoma	2	1	1
Tumor not diagnosed	5	2	3
Induction therapy			
Platinum + etoposide	5	5	0
Platinum + taxane	3	0	3
Adjuvant therapy			
Platinum + etoposide	12	10	2
CDDP + etoposide + RT	1	1	0
Platinum + CPT-11	7	1	6
CDDP + 5-FU + RT	1[c]	1	0

[a] Six cases of seven patients with clinical stage II/III disease were preoperatively diagnosed as NSCLC on biopsy

[b] One patient who was preoperatively diagnosed as SCLC received induction CDDP plus etoposide chemotherapy followed by surgery and two course of adjuvant chemotherapy

[c] One patient with advanced hypopharynx cancer and pT1N1 SCLC underwent chemoradiotherapy with CDDP + 5-FU + RT

Patient outcome

The median follow-up was 41.6 months (interquartile range 25.6–57.3) for all patients and 57.3 months (interquartile range 28.9–79.3) for those still alive. No patients

dropped out of the follow-up during the study period. The median survival for all patients was 59.2 months and the 5-year overall probability of survival was 47% (Fig. 1). Five-year survival for patients with c-stage I ($n = 21$), c-stage II ($n = 5$), and c-stage III disease ($n = 2$) were 53, 0, and 100%, respectively (Fig. 2). Five-year survival for patients with p-stage I ($n = 14$), p-stage II ($n = 7$), and p-stage III disease ($n = 7$) were 64, 25, and 43%, respectively (Fig. 3). There was no significant difference in survival among c-stages ($p = 0.08$, log-rank test) and between patients with p-stage I disease and those with p-stage II disease ($p = 0.35$, log-rank test). Despite small sample size, there were significant differences in survival between patients with p-stage I disease and those with p-stage III disease ($p = 0.04$, log-rank test). Overall survival did not differ significantly between patients with combined SCLC and those with SCLC ($p = 0.91$, log-rank test) (Fig. 4).

Ten (36%) of the 28 patients had a relapse (Table 3). Among these patients, two had a local relapse alone, one patient had combined local and distant relapses, and the other seven patients had distant metastases alone as their first site of failure. Nine patients relapsed within 2 years after surgery. Median relapse-free survival for all patients was 52.5 months (95%CI 16.6, N/A). There was no obvious difference in relapse pattern between patients with combined SCLC and those with SCLC. Four patients with p-stage II/III disease developed brain metastases with a cumulative incidence at 1 and 2 years of 25 and 36%, respectively (Fig. 5). One patient with p-stage III disease developed brain metastasis concurrently with liver and bone metastases. The other patient with p-stage III disease and two patients with p-stage II disease had brain metastases as the only site of first recurrence. There were a total of 13 deaths and 8 were disease-related. Most patients

Table 2 Relationship between clinical and pathologic stages

Clinical stage	Pathologic stage		
	I	II	III
I	12 (3)	4 (1)	5
II	1 (1)	3 (1)	1 (1)
III	1 (1)	0	1

Numbers in parentheses are the numbers of patients who received induction therapy

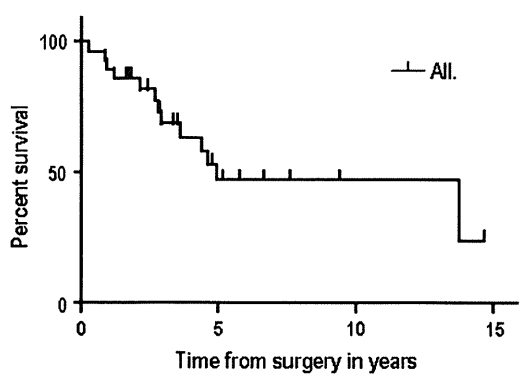

Fig. 1 Survival curve for patients with resected SCLC

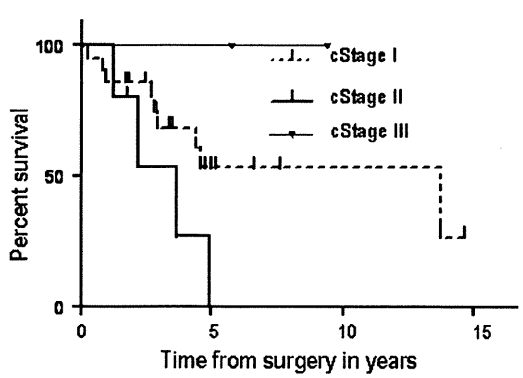

Fig. 2 Survival curves for patients with resected SCLC by clinical stages

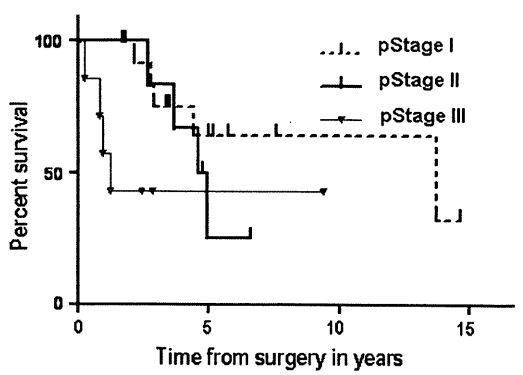

Fig. 3 Survival curves for patients with resected SCLC by pathologic stages

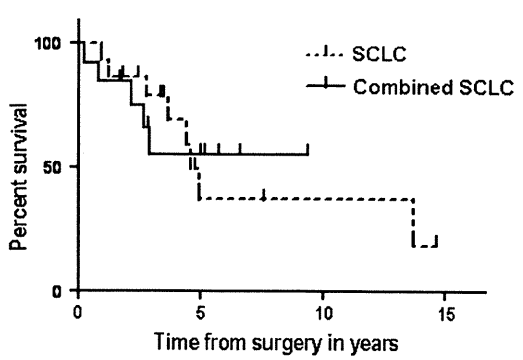

Fig. 4 Survival curves for patients with resected SCLC according to histologic subtypes

Table 3 Site of the first relapse by pathologic and clinical stages

Variables	Overall	p-Stage I	p-Stage II	p-Stage III	c-Stage I	c-Stage II	c-Stage III
No. of patients	28 (15)	14 (8)	7 (4)	7 (3)	21 (12)	5 (3)	2 (0)
No. of recurrences	10 (6)	3 (1)[a]	4 (3)	3 (2)[b]	5 (3)[a,b]	5 (3)	0
Recurrence							
Local							
Mediastinum	3 (1)	2 (1)[a]	0	1	2 (1)[a]	1	0
Distant							
Brain	4 (3)	0	3 (2)	1 (1)[b]	2 (2)[b]	2 (1)	0
Bone	3 (3)	1 (1)[a]	0	2 (2)[b]	2 (2)[a,b]	1 (1)	0
Liver	2 (2)	1 (1)[a]	0	1 (1)[b]	2 (2)[a,b]	0	0
Lung	1	1	0	0	1	0	0
Adrenal gland	1 (1)	0	1 (1)	0	0	1 (1)	0

Numbers in parentheses are the number of patients with postoperative diagnosis of SCLC

[a] One patient with clinical and pathological stage I disease developed local relapse concurrently with liver and bone metastases

[b] One patient with clinical stage I and pathological stage III disease developed brain metastasis simultaneously with liver and bone metastases

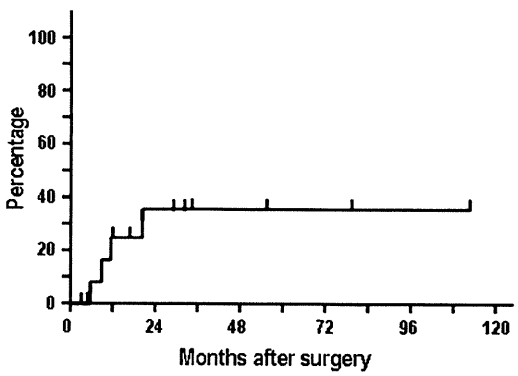

Fig. 5 Cumulative incidence of brain metastasis in patients with p-stages II/III disease

developed hematogenous distant metastases before their deaths.

Discussion

The prognosis of resected SCLC is considered to be poorer than that of surgically treated NSCLC. Vallieres et al. [16] has reported that 5-year survival of surgically treated LS-SCLC patients was approximately 50% for p-stage I disease, approximately 40% for p-stage II, and approximately 15% for p-stage III. A Japanese large-scale registry study reported that 5-year survival of resected SCLC patients was approximately 60% for p-stage I disease, approximately 40% for p-stage II, and approximately 30% for p-stage III [17]. In our study, the 5-year probability of survival was 64% for p-stage I disease, 25% for p-stage II, 43% for p-stage III. Our results are almost similar to those of the Japanese study.

Because of its aggressive nature, for example rapid growth and early dissemination in lymph nodes, bones, adrenal glands, liver, and brain, the role of surgery in treatment of SCLC is considered to be very limited [6]. SCLC usually occurs centrally, and typical initial radiographic images show a larger hilar mass with bulky mediastinal lymphadenopathy. Thus, SCLC for indication of surgical resection which often arises peripherally is relatively rare [7]. In fact, a large-scale registry study from Japan reported that there were a few SCLC patients (3%) among 13010 lung cancer patients who underwent surgery at the certified teaching hospitals in 1999 [17]. Although patients with very limited SCLC (cT1-2N0) basically proceed to surgical resection, there are several other situations in which surgery can be useful [18]. One situation is surgery for confirmation of diagnosis. Another situation is surgery for patients with preoperative diagnosis of resectable NSCLC. A third situation is improvement of local control in the combination treatment with chemotherapy and radiotherapy, because for patients with combined histology tumors, for example combined SCLC, the NSCLC component is less sensitive to chemotherapy and radiotherapy. Surgery for these situations may contribute to prolonged survival for undiagnosed lung cancer and T1-3N1-2 SCLC. In our study, histological or cytological diagnosis was not achieved preoperatively for 5 of 28 patients, and preoperative diagnosis of SCLC was achieved for 10 patients only. Furthermore, approximately half (13) of 28 patients had combined SCLC histology.

Recurrence in the brain is associated with substantial morbidity and mortality in SCLC [13]. Because of high risk of brain metastasis after diagnosis of SCLC, PCI has been studied in an attempt to treat and control metastatic brain tumors before clinical manifestation. The benefit of PCI is

greatest for patients with LS-SCLC and ES-SCLC who have complete or near complete response to treatment [12]. However, use of PCI after combined-modality treatment with surgery for resectable LS-SCLC has not yet been investigated sufficiently. As far as we are aware, only 2 Japanese studies have reported the frequency of brain relapse after surgery for LS-SCLC [19, 20]. One Japanese multi-institutional phase II study (JCOG9101) has reported that recurrence after surgery occurred in 43% (26/61) of patients overall, in 29% (10/35) of patients with p-stage I disease, in 50% (4/8) of patients with p-stage II, and in 67% (12/18) of patients with p-stage III, and that the incidence of brain metastasis was 15% (9/61) in patients overall, 11% (4/35) in patients with p-stage I disease, 38% (3/8) in patients with p-stage II, and 11% (2/18) in patients with p-stage III [20]. Another Japanese study showed that relapse after surgery occurred in 34 of 69 (49%) patients who underwent complete resection of SCLC and in 27% (8/30) of patients with p-stage I disease, 58% (7/12) of patients with p-stage II, 69% (18/26) of patients with p-stage III, and 100% (1/1) of patients with p-stage IV [19]. In this report, the frequency of brain relapse as a first relapse site was reported to be 7% (2/30) for patients with p-stage I disease, 25% (3/12) for patients with p-stage II, 27% (7/26) for patients with p-stage III, and 100% (1/1) for patients with p-stage IV. Combined results from our study and from two other Japanese studies revealed that brain metastases as first site of failure developed in 26 (16%) of the total of 158 patients who underwent surgery for LS-SCLC, in 6 (8%) of 79 patients with p-stage I disease, 8 (30%) of 27 patients with p-stage II, and 11 (22%) of 51 patients with p-stage III. For LS-SCLC patients treated with chemoradiation therapy, the frequency of brain metastasis as the first recurrence site has been reported to be 37% [21]. In addition, as for the role of PCI for treatment of NSCLC, the risk of brain metastasis has been reported to be 17% (71/422) for patients with stage III NSCLC treated with chemoradiation therapy [22]. Thus, although it is unclear whether PCI after combined modality treatment with surgery for resectable LS-SCLC could improve survival, PCI may be beneficial at least for patients with p-stage II/III disease to reduce the incidence of brain metastasis, although a randomized study is necessary.

The principal role of PCI is to prevent brain failure and to reduce its frequency, and to improve survival [13]. Surgically treated patients with p-stage I SCLC are the most favorable subset in SCLC. Combined results from our study and two other Japanese studies revealed that brain metastases as first site of failure developed in only 8% (6/79) of patients with p-stage I SCLC. It has been reported that in adjuvant trasuzumab studies of breast cancer PCI would not be justified by a frequency of less than 5% in incidence of brain metastasis [23]. In this regard, very early LS-SCLC, for example p-stage I disease, would be excluded from candidates for PCI, because of low frequency of brain relapse.

Our retrospective study suggested that PCI might be suitable for surgically resected patients with p-stage II/III SCLC to reduce the incidence of brain metastasis, although a randomized study is necessary. It is likely that very early LS-SCLC, for example p-stage I disease would be excluded from candidates for PCI.

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Conflict of interest The authors declare no conflicts of interest.

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Clinical Investigation

A Phase I Study of Chemoradiotherapy with Use of Involved-Field Conformal Radiotherapy and Accelerated Hyperfractionation for Stage III Non–Small-Cell Lung Cancer: WJTOG 3305

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Summary

This phase I study of chemoradiotherapy used involved-field conformal radiotherapy with accelerated Twice-daily hyperfractionation in patients with stage III non-small cell lung cancer. Although the dose of radiation was escalated to 72 Gy in 48 fractions, the maximum tolerated dose was not reached.

Purpose: A Phase I study to determine a recommended dose of thoracic radiotherapy using accelerated hyperfractionation for unresectable non–small-cell lung cancer was conducted.
Methods and Materials: Patients with unresectable Stage III non–small-cell lung cancer were treated intravenously with carboplatin (area under the concentration curve 2) and paclitaxel (40 mg/m^2) on Days 1, 8, 15, and 22 with concurrent twice-daily thoracic radiotherapy (1.5 Gy per fraction) beginning on Day 1 followed by two cycles of consolidation chemotherapy using carboplatin (area under the concentration curve 5) and paclitaxel (200 mg/m^2). Total doses were 54 Gy in 36 fractions, 60 Gy in 40 fractions, 66 Gy in 44 fractions, and 72 Gy in 48 fractions at Levels 1 to 4. The dose-limiting toxicity, defined as Grade $\geq$4 esophagitis and neutropenic fever and Grade $\geq$3 other nonhematologic toxicities, was monitored for 90 days.
Results: Of 26 patients enrolled, 22 patients were assessable for response and toxicity. When 4 patients entered Level 4, enrollment was closed to avoid severe late toxicities. Dose-limiting toxicities occurred in 3 patients. They were Grade 3 neuropathy at Level 1 and Level 3 and Grade 3 infection at Level 1. However, the maximum tolerated dose was not reached. The median survival time was 28.6 months for all patients.

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Conclusions: The maximum tolerated dose was not reached, although the dose of radiation was escalated to 72 Gy in 48 fractions. However, a dose of 66 Gy in 44 fractions was adopted for this study because late toxicity data were insufficient. © 2011 Elsevier Inc.

Keywords: Non–small-cell lung cancer, Chemoradiation, Three-dimensional conformal radiotherapy, Accelerated hyperfractionation, Dose escalation

Introduction

For the treatment of locally advanced inoperable non–small-cell lung cancer (NSCLC), concurrent chemoradiotherapy has shown significantly better survival than sequential therapy (1–4). Even in concurrent chemoradiotherapy, however, locoregional control is unsatisfactory at a standard dose of 56 to 60 Gy (3, 5–7). To improve locoregional control, several dose escalation trials have been performed using three-dimensional (3D) planning techniques, and it has been suggested that 74 Gy is tolerable with concurrent or sequential chemotherapy (8–11).

In conventional fractionation, the benefits of dose escalation are considered limited. Irradiation at a dose of 74 Gy in conventional fractionation requires more than 7 weeks. Even at standard doses, accelerated repopulation is induced during the later part of radiation therapy and is a cause of radiation failure (12). When the treatment time is prolonged, the influence of accelerated repopulation becomes more evident.

Therefore, dose escalation without prolonged treatment time is supposed to bring better outcome, and accelerated hyperfractionation seems to be an effective strategy for shortening treatment time. As the first step to verify the hypothesis, the West Japan Thoracic Oncology Group designed a Phase I trial to define the maximum tolerated dose (MTD) of 3D conformal radiotherapy (CRT) using accelerated hyperfractionation in NSCLC patients.

Methods and Materials

Eligibility

The patient eligibility was as follows: histologic or cytologic diagnosis of NSCLC, unresectable Stage IIIA or IIIB disease, age less than 75 years, Eastern Cooperative Oncology Group performance score of 0 to 1, and function as shown by laboratory determinations including leukocyte count of at least 4,000/mm^3, hemoglobin concentration of at least 9.5 g/dL, platelet count of at least 100,000/mm^3, aspartate aminotransferase and alanine aminotransferase of 2.0 times the upper limit of normal range or less, serum total bilirubin of 1.5 mg/dL or less, serum creatinine of 1.5 mg/dL or less, and PaO_2 at rest of at least 70 mm Hg.

The patients were ineligible if they met any of the following criteria: supraclavicular nodal metastases, interstitial pneumonitis or pulmonary fibrosis, prior thoracic radiation therapy, malignant pleural effusion or malignant pericardial effusion, active concomitant malignancy or recent (<3 years) history of any malignancy, or other serious concomitant medical conditions. The study protocol was approved by each institutional review board for clinical use. All patients gave written informed consent before enrollment.

Patient assessment

All patients underwent a complete medical history and physical examination. Imaging studies, including chest X-ray, computed tomography of the chest and upper abdomen, computed tomography or magnetic resonance imaging of the brain, and positron emission tomography, were required.

Treatment schedule

The patients received concurrent chemoradiotherapy using accelerated hyperfractionation. On Days 1, 8, 15, and 22, carboplatin (area under the concentration curve 2 using the Calvert equation) and paclitaxel (40 mg/m^2) were administered intravenously.

After the concurrent chemoradiotherapy, the patients received two cycles of consolidation chemotherapy consisting of carboplatin (area under the concentration curve 5) and paclitaxel (200 mg/m^2) with an interval of 3 weeks. The first cycle of consolidation chemotherapy was begun 4 weeks after the concurrent chemoradiotherapy, if leukocyte count was at least 4,000/mm^3, platelet count at least 100,000/mm^3, aspartate aminotransferase and alanine aminotransferase 2.0 times the upper limit of normal range or less, serum total bilirubin 1.5 mg/dL or less, serum creatinine 1.5 mg/dL or less, and Eastern Cooperative Oncology Group performance score of 0 to 2. The subsequent cycle of consolidation chemotherapy was repeated if leukocyte count was at least 3,000/mm^3, neutrophil count at least 1,500/mm^3, platelet count at least 100,000/mm^3, serum creatinine 1.5 mg/dL or less, and body temperature not exceeding 38°C.

The 3D CRT began on Day 1. Irradiation was performed with 4-MV or higher photons from a linear accelerator. Patients received 1.5 Gy per fraction twice daily with at least a 6-hour interval between each fraction.

Target volume definitions

Elective nodal irradiation was not performed. The gross tumor volume (GTV) was defined as the volume occupied by visible disease. The GTV included the primary tumor and involved lymph nodes measuring larger than 1.0 cm (short axis measurement) or lymph nodes with a diameter of 5 mm or more as shown by positron emission tomography. The clinical target volume (CTV) was defined as the GTV of the primary tumor plus a margin of 5 mm for all borders and GTV of the lymph nodes without a margin. The planning target volume (PTV) was the CTV plus an adequate margin added to compensate for variability in treatment setup, breathing, or motion during treatment. In

general, the PTV included the CTV plus 1.0 cm of expansion at all borders.

Tissue inhomogeneity corrections were used. The volume of both lungs that received more than 20 Gy should not exceed 35% of the total lung, and the maximum dose to the spinal cord could not exceed 45 Gy. It was desirable but not required that the PTV receive more than 93% but less than 107% of its prescribed dose. After the dose of 36 Gy was reached, the PTV could be reduced after shrinkage of the GTV.

Dose escalation

The MTD was defined as the dose at which 3 or more of 6 patients experienced a dose-limiting toxicity (DLT). The DLT was defined as Grade 4 or more esophagitis, neutropenic fever, dermatitis, or nausea/vomiting and other Grade 3 or more nonhematologic toxicity. Furthermore, interruption of irradiation for more than 2 weeks was also defined as a DLT. The DLT was monitored for 90 days.

Irradiation was performed for 5 days per week. The prescribed doses were 54 Gy in 36 fractions over 3.6 weeks, 60 Gy in 40 fractions over 4.0 weeks, 66 Gy in 44 fractions over 4.4 weeks, and 72 Gy in 48 fractions over 4.8 weeks (Levels 1–4). When the DLT was observed in 0 of 4 patients, in $\leq$1 of 5 patients, or in $\leq$2 of 6 patients at each level, the radiation dose was to be escalated.

Evaluation

The Response Evaluation Criteria in Solid Tumors were used for response assessment (13). Toxicity was evaluated according to the National Cancer Institute Common Toxicity Criteria (version 3.0). An extramural review was conducted to validate the eligibility of the patients and staging.

The duration of survival was counted from the day of entry to the study, and the overall survival was calculated according to the Kaplan-Meier method (14).

Results

Patients' characteristics

Between April 2006 and April 2008, 26 patients were enrolled in this study. Four patients were excluded because of allergic reactions to paclitaxel on Day 1 (n = 1), cerebral infarction on Day 2 (n = 1), and supraclavicular nodal metastases (n = 2). The remaining 22 patients were included in the analysis. They were 6, 7, 5, and 4 patients at Levels 1 through 4, respectively. Although, as a rule, 4 to 6 patients were enrolled in each level, 1 patient was increased at Level 2 because the sixth and seventh patients enrolled at the same time. The baseline characteristics of the 22 patients are summarized in Table 1.

When 4 patients entered Level 4, enrollment was closed to avoid severe late toxicities in the esophagus and the bronchia.

Treatment administration

All patients received full doses of radiation therapy, and interruption of radiation therapy was required in only 4 patients.

Table 1 Patient characteristics (n = 22)

Characteristics	n	%
Age (y)		
Median		63
Range		45–70
Sex		
M	19	86
F	3	14
ECOG performance status		
0	7	32
1	15	68
Histology		
Squamous cell carcinoma	10	45
Adeno carcinoma	10	45
Large cell carcinoma	0	0
Others	2	10
Stage		
IIIA	11	50
IIIB	11	50

Abbreviation: ECOG = Eastern Cooperative Oncology Group.

Interruptions ranged from 1 day to 7 days. All patients received four cycles of concurrent chemotherapy, and 19 patients (86%) received two cycles of consolidation chemotherapy.

Toxicity

The major toxicities are summarized in Table 2.

The DLTs occurred in 3 patients. Two cases of Grade 3 neuropathy were observed, one at Level 1 and the other at Level 3, and one case of Grade 3 infection occurred at Level 1. Furthermore, Grade 5 radiation pneumonitis was observed at Level 1; however, it was not treated as a DLT because the event occurred after the observation period of 90 days. Grade 3 esophagitis was observed in 3 patients, 1 at Level 3 and the others at Level 4. Grade 3 esophagitis and nausea were not defined as DLTs.

At Level 4, no DLT occurred in the 4 patients. Therefore, the MTD was not reached in the present study.

Response and survival

The figure shows the overall survival. The median survival time was 28.6 months for all patients and 30.2 months for patients who received more than 60 Gy. The response rate was 77% for all patients.

Patterns of relapse

Table 3 shows the first sites of relapse. Of 11 patients with locoregional relapse, 1 had upper mediastinal lymph node metastasis, which was located out of the radiation portal. Of 5 patients with distant metastasis, 3 had lung metastasis.

Discussion

With the use of 3D planning techniques, several dose escalation trials have been performed. Kong *et al.* reported that doses of CRT

Table 2 Major toxicities (n = 22)

	Grade 3		Grade 4		Grade 5	
Toxicity	n	%	n	%	n	%
Hematologic						
Leukopenia	16	73	1	5		
Neutropenia	5	23	12	55		
Anemia	2	9	0	0		
Thrombocytopenia	0	0	0	0		
Nonhematologic						
Neuropathy	2	9	0	0	0	0
Infection	1	5	0	0	0	0
Pneumonitis	0	0	0	0	1	5
Esophagitis	3	14	0	0	0	0
Nausea	1	5	0	0	0	0

could be escalated up to 103 Gy for smaller tumors (15). However, the 5-year local control rates were only 49% even at 92 to 103 Gy and 35% at 74 to 84 Gy. The insufficient local control indicated limitation of dose escalation in conventional fractionation and warranted further exploration for different strategies.

A risk of severe late toxicities, such as esophageal stenosis and bronchial occlusion, was predicted from the beginning of the study. After that, experience with Levels 1 through 3 indicated that prescription of high doses in the esophagus or the main bronchi was inevitable in most patients. Therefore, enrollment was closed in the middle of Level 4 to avoid severe late toxicities. Emami *et al.* reported that in treatment of the esophagus, the tolerance dose that would result in a 50% probability of complications within 5 years of treatment was 72 Gy (16). However, data on tolerance doses by accelerated hyperfractionation are lacking. Therefore, careful long-term follow-up of the present study is required. Recently, Atsumi *et al.* reported that the severity and frequency of esophageal stenosis after radiation therapy were greater in patients with esophageal cancer with full circumference involvement and increased with esophageal wall thickness (17). The tolerance dose for the esophagus might be higher in patients without esophageal cancer than in those with esophageal cancer.

In radiation therapy using accelerated hyperfractionation, acute esophagitis is a toxicity of particular concern. In the present study, 3 patients experienced Grade 3 esophagitis: 2 of 4 patients at Level 4, but only 1 patient at Levels 1 through 3 (n = 18). The

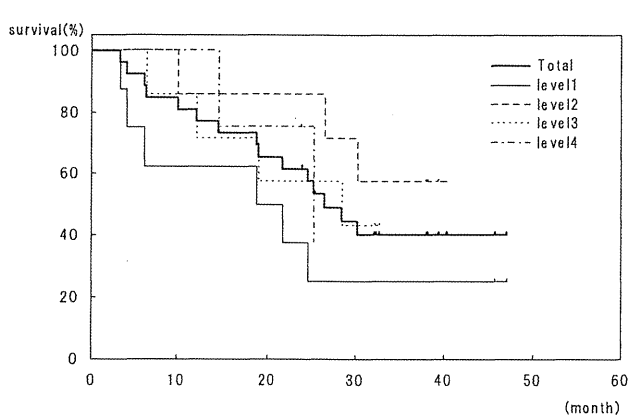

Fig. Kaplan-Meier survival curves for all patients and for patients in Levels 1–4. The median survival time was 28.6 months for all patients.

Table 3 Site of first failure (n = 22)

Site	n	%
Progression free	9	41
Locoregional alone	8	36
Locoregional and distant	3	14
Distant*	5	23
Lung	3	14
Brain	1	5
Small intestine	1	5

* Distant includes locoregional and distant, and distant alone.

low frequency of esophagitis has often been observed in other Japanese trials (18, 19). The causes of this phenomenon are not well known. One possible explanation is differences in ethnic background. Twice-daily CRT with a dose of 1.3 to 1.45 Gy per fraction could be recommended in patients with other ethnic backgrounds, if this regimen is shown to bring a better outcome.

Another toxicity of concern is radiation pneumonitis. In the present study, Grade 5 radiation pneumonitis was observed in 1 patient. In other patients, however, Grade 3 or more radiation pneumonitis was not observed. Some radiation pneumonitis is inevitable to some degree, and the frequency of Grade 3 or more pneumonitis was rather low in the present study. Tsujino *et al.* reported a decreased incidence of radiation pneumonitis by accelerated hyperfractionation in the treatment of limited-stage small-cell lung cancer, although initially they expected that accelerated hyperfractionation would increase the incidence and severity of radiation pneumonitis (20). The results in the present study are consistent with those reported by Tsujino *et al.*

The DLTs observed in the current study were Grade 3 neuropathy and Grade 3 infection. They were mainly caused by chemotherapy. By contrast, Grade 5 radiation pneumonitis was not treated as a DLT because it occurred after the observation period of 90 days. The definition of DLT used in this study was probably inadequate for a radiation dose escalation study. Chemotherapy-induced toxicities should be given less consideration, and those caused by radiation therapy should be more strictly weighed.

The cutoff of 90 days for the observation period of DLTs was considered not sufficient. However, the observation period could not be more prolonged because the Phase I study had to be completed within a suitable time. Toxicities were evaluated after the observation period. In recent Phase I studies, the observation period was defined similarly (21–23).

Although the number of patients was relatively small in the present study, the method of assigning 6 patients to each dose bin is an option in a Phase I study (21). However, the data about survival were not reliable because of the small sample size. The data are to be verified in the following Phase II study.

In the present study, the dose of CRT was escalated to 72 Gy in 48 fractions, and MTD was not reached. In principle, 72 Gy should be the recommended dose. However, late toxicity data are insufficient, and enrollment was closed in the middle of Level 4. Furthermore, on the basis of our experience with the treatment of small-cell lung cancer, CRT with a dose of 66 Gy in accelerated hyperfractionation brings better local control than 74 Gy in conventional fractionation, which was defined as a recommended dose in several trials. The favorable median survival time of 30.2 months for patients who received 60 to 72 Gy in the present study is consistent with our experience. Therefore, a dose of 66 Gy in 44 fractions was adopted in the present study. On the basis