Table 2
Immunohistochemistry results of non-small-cell lung cancer with elevated serum progastrin-releasing peptide concentrations.

	Serum proGRP (pg/dL)	Histological subtype at diagnosis	Specimen	Stage	IHC					
					TTF-1	CEA	CGA	Synapto-phisin	NCAM	proGRP
1	973	Large	TBLB	IV	–	++	++	++	+	–
2	363	LCNEC	TBLB	IV	++	–	++	++	++	++
3	269	LCNEC	TBLB	IV	++	++	++	++	++	+
4	229	Large	TBLB	IV	–	–	–	–	–	–
5	78.6	Large	TBLB	IV	–	–	–	–	–	–
6	60.3	Adeno	TBLB	IV	++	–	–	–	–	–
7	56.7	Sq	Surgery	Rec	–	+	–	+	++	–
8	56.0	Adeno	Surgery	Rec	++	–	–	+	–	–
9	55.3	Adeno	TBLB	IIIB	++	–	–	+	+	–
10	54.7	Adeno	TBLB	IV	++	–	–	+	–	–
11	53.1	Adeno	Surgery	IB	++	–		+	–	–
12	51.8	Adeno	TBLB	IV	++	+	++	++	++	+
13	51.4	Adeno	Surgery	IB	++	++	++	++	++	++
14	48.3	Sq	TBLB	IB	–	++	–	–	–	–
15	47.4	Sq	Surgery	IIIA	–	++	–	–	–	–
16	47.3	Sq	TBLB	IIIA	–	–	–	+	–	–
17	46	Sq	TBLB	IIIB	–	++	–	–	–	–

IHC: immunohistochemistry, proGRP: progastrin-releasing peptide, TTF-1: thyroid transcription factor 1, CEA: carcinoembryonic antigen, CGA: chromogranin A, NCAM: neural cell adhesion molecule, Adeno: adenocarcinoma, Sq: squamous cell carcinoma, Large: large cell carcinoma, LCNEC: large cell neuroendocrine carcinoma, TBLB: transbronchial lung biopsy, –: negative, +: weakly positive, ++: strongly positive.

3.2. Cytological and histological examination

Cytological specimens corresponding to 27 of the 34 serum proGRP-positive NSCLCs were reevaluated. All 5 cytology specimens diagnosed as LCNEC contained typical neuroendocrine features such as rosette-like and palisading patterns. A rosette-like formation was found in only 1 cytology specimen diagnosed as squamous cell carcinoma, and the other 21 specimens did not contain the typical cytological features of neuroendocrine differentiation.

IHC staining was performed on 17 histological specimens of the 34 serum proGRP-positive NSCLCs (7 adenocarcinomas, 5 squamous cell carcinomas, 3 large cell carcinomas, and 2 LCNECs) to examine neuroendocrine differentiation (Table 2). Four of 17 specimens (24%) showed positive staining (2 weakly and 2 strongly positive) for proGRP, and some neuroendocrine markers were positive in 11 of 17 specimens (64.7%). In particular, 2 of 7 adenocarcinomas, 1 of 3 large cell carcinomas, and 2 of 2 LCNECs showed strongly positive staining for at least 2 out of the 3 neuroendocrine markers CGA, synaptophysin, and NCAM. None of the squamous cell carcinomas showed strongly positive staining for at least 2 out of the 3 neuroendocrine markers CGA, synaptophysin, and NCAM. One of 5 squamous cell carcinomas showed strongly positive staining for NCAM. One of 5 squamous cell carcinomas showed strongly positive staining for NCAM. There was no significant relationship between serum proGRP concentrations and proGRP immunoreactivity.

3.3. Response to chemotherapy

Twenty of 34 serum proGRP-positive NSCLC patients received platinum-based chemotherapy (Table 3). There were 11 partial responses, 4 stable diseases, and no responses observed in the 5 patients. The objective response rate was 55.0%. The median survival of the 20 patients was 11 months, and the 1-year survival rate was 48%.

On the other hand, 232 of 387 serum proGRP-negative NSCLC patients received platinum-based chemotherapy. There were 82 partial responses, 97 stable diseases, and no complete responses observed in the 53 patients. The objective response rate was 35.0%, the median survival was 11.5 months, and the 1-year survival rate was 49.1%.

Table 3
Platinum doublet regimens administered to serum proGRP-positive and -negative patients.

Regimens	ProGRP-positive NSCLC patients ($n=20$)		ProGRP-negative NSCLC patients ($n=232$)	
	n	%	n	%
CBDCA/PTX	9	45	141	60.7
CBDCA/GEM	0	0	16	7
CBDCA/VP16	1	5	5	2
CDDP/DOC	3	15	53	23
CDDP/S-1	2	10	4	1.8
CDDP/VNR	2	10	1	0.5
CDDP/VP16	1	5	0	0
CDDP/CPT11	2	10	2	1
CDDP/GEM	0	0	10	4

CBDCA: carboplatin, CDDP: cisplatin, PTX: paclitaxel, GEM: gemcitabine, VP16: etoposide, DOC: docetaxel, VNR: vinorelbine, CPT11: irinotecan, ProGRP: progastrin-releasing peptide.

4. Discussion

In the present study, the positive rate of serum proGRP concentration in NSCLC patients was 8.1% (34/421), and histological neuroendocrine features were detected in 11 of 17 (64.7%) serum proGRP-positive NSCLC specimens. Several studies have examined serum proGRP concentrations in lung cancer, and all of these studies reported that serum proGRP was a specific tumor marker for SCLC [6,9]. The sensitivity and specificity for SCLCs were around 70% and 99%, respectively, and serum proGRP was superior to NSE [10].

Although increases in serum ProGRP concentration have been observed in some NSCLC patients in previous studies, the reported positive rates of serum proGRP in NSCLCs demonstrated a wide range of variability (3–30%) [6,11]. The 8% positive rate found in the present study was relatively higher than in previous studies, with the exception of 2 reports (the Takada study used a lower cutoff for positive, at 34 pg/mL, and the Molina study included a higher proportion of renal failure patients) [11,12]. Although several studies have reported that serum proGRP values were elevated in NSCLC patients, there have been few studies examining the clinicopathological characteristics of serum proGRP-positive NSCLC patients [13]. Only 1 study examined the clinicopathological

characteristics of 24 NSCLC patients with elevated serum proGRP concentrations. Positive IHC staining for neuroendocrine differentiation was reported in only 4 of 24 serum proGRP-positive NSCLCs, and a small-cell component or neuroendocrine differentiation was detected in all 4 of those patients [13].

In the present study, 27 cytological specimens were reevaluated and IHC staining was performed on 17 histological specimens out of 34 serum proGRP-positive NSCLCs. The cytology results showed the presence of neuroendocrine features such as rosette-like formations in the analysis of 1 squamous cell carcinoma and 5 LCNECs. Histologic examination also identified 2 cases with neuroendocrine morphology (i.e., LCNEC). In addition, weakly or strongly positive staining for some neuroendocrine markers was observed in 12 of 17 histological specimens.

Previous reports have suggested that renal failure can be a source of false-positive proGRP results [11,14], and serum proGRP concentrations were elevated in patients who had serum creatinine levels greater than 1.6 mg/dL [7,13,15]. Therefore, the elevation of serum proGRP detected in present study could be associated with renal dysfunction in the patients analyzed. However, the serum creatinine levels were less than 1.2 mg/dL (median, 0.7 mg/dL) in all 34 serum proGRP-positive NSCLC patients Furthermore, no correlation was observed between serum creatinine and serum proGRP concentrations (data not shown). These results indicate that renal dysfunction could not have accounted for the serum proGRP elevations seen in the present study.

Recently, several reports have suggested that the clinical features of NSCLC with neuroendocrine differentiation, such as LCNEC, may be different from other types of NSCLC [3,16]. The clinical characteristics of serum proGRP-positive NSCLCs were similar to those of SCLCs, as suggested by data showing that the majority of patients were male and heavy smokers. The response rate to platinum-doublet chemotherapy in the present study was 55.0%. This response rate seems higher than the response rates to platinum-doublet chemotherapy reported for nonselected NSCLC [17,18] and serum ProGRP-negative NSCLC in this study, but they were similar to the previously reported response rates to platinum-doublet chemotherapy in LCNEC [16,19]. These results suggest that the sensitivity to chemotherapy in serum proGRP-positive NSCLC may be different than that of other types of NSCLC.

In the present study, IHC staining and a morphologic description were not performed in NSCLC patients with non-elevated proGRP. Therefore, the number of false-negative results and the real performance of the proGRP assay in the detection of neuroendocrine differentiation remain unknown. However, the difficulty in obtaining sufficient tissue by biopsy, and limited tumor tissue sampling make the accurate diagnosis of neuroendocrine differentiation in NSCLC complicated. Sensitive and simple methods for the detection of neuroendocrine differentiation of NSCLC are greatly desired.

In conclusion, serum proGRP-positive NSCLCs may contain manifest neuroendocrine differentiation. In addition, serum proGRP-positive NSCLC patients may have different clinical characteristics compared to other NSCLC patients

Conflict of interest statement

The authors declare no conflicts of interest.

Acknowledgements

The study was conducted by the Cancer Institute Hospital, Japanese Foundation for Cancer Research and Cancer Institute. We thank all investigators in our group.

References

[1] The editorial board of the cancer statistics in Japan. Foundation for Promotion of Cancer Research; October 1, 2007. p. 11–49.
[2] Travis WD, Corrin B, Shimosato Y, Brambilla E.Histological typing of lung and pleural tumors. 3rd ed. Berlin, Heildelberg, Germany: Springer-Verlg; 1999.
[3] Iyoda A, Hiroshima K, Toyozaki T, Haga Y, Fujisawa T, Ohwada H. Clinical characterization of pulmonary large cell neuroendocrine carcinoma and large cell carcinoma with neuroendocrine morphology. Cancer 2001;91: 1992–2000.
[4] Takei H, Asamura H, Maeshima A, Suzuki K, Kondo H, Niki T, et al. Large cell neuroendocrine carcinoma of the lung: a clinicopathologic study of eighty-seven cases. J Thorac Cardiovasc Surg 2002;124:285–92.
[5] Travis WD, Linnoila RI, Tsokos MG, Hitchcock CL, Cutler Jr GB, Nieman L, et al. Neuroendocrine tumors of the lung with proposed criteria for large-cell neuroendocrine carcinoma. An ultrastructural, immunohistochemical, and flow cytometric study of 35 cases. Am J Surg Pathol 1991;15:529–53.
[6] Miyake Y, Kodama T, Yamaguchi K. Pro-gastrin-releasing peptide(31–98) is a specific tumor marker in patients with small cell lung carcinoma. Cancer Res 1994;54:2136–40.
[7] Nakahama H, Tanaka Y, Fujita Y, Fujii M, Sugita M. CYFRA 21-1 and ProGRP, tumor markers of lung cancer, are elevated in chronic renal failure patients. Respirology 1998;3:207–10.
[8] Kodama T, Abe S, Yamaguchi K, Eguchi K, Saigenji K, Kameya T, et al. Clinical significance for diagnosis of serum ProGRP by enzyme-linked immunosorbent assay (ELISA) [in Japanese]. Igaku To Yakugaku 1994;32:87–97.
[9] Holst JJ, Hansen M, Bork E, Schwartz TW. Elevated plasma concentrations of C-flanking gastrin-releasing peptide in small-cell lung cancer. J Clin Oncol 1989;7:1831–8.
[10] Yamaguchi K, Aoyagi K, Urakami K, Fukutani T, Maki N, Yamamoto S, et al. Enzyme-linked immunosorbent assay of pro-gastrin-releasing peptide for small cell lung cancer patients in comparison with neuron-specific enolase measurement. Jpn J Cancer Res 1995;86:698–705.
[11] Molina R, Auge JM, Alicarte J, Filella X, Vinolas N, Ballesta AM. Pro-gastrin-releasing peptide in patients with benign and malignant diseases. Tumour Biol 2004;25:56–61.
[12] Takada M, Kusunoki Y, Masuda N, Matui K, Yana T, Ushijima S, et al. Pro-gastrin-releasing peptide (31–98) as a tumour marker of small-cell lung cancer: comparative evaluation with neuron-specific enolase. Br J Cancer 1996;73:1227–32.
[13] Goto K, Kodama T, Hojo F, Kubota K, Kakinuma R, Matsumoto T, et al. Clinicopathologic characteristics of patients with nonsmall cell lung carcinoma with elevated serum progastrin-releasing peptide levels. Cancer 1998;82: 1056–61.
[14] Casesa A, Filellab X, Molinab R, Ballestab AM, Lopez-Pedreta J, Reverta L. Tumor markers in chronic renal failure and hemodialysis patients. Nephron 1991;57:183–6.
[15] Kamata K, Uchida M, Takeuchi Y, Takahashi E, Sato N, Miyake Y, et al. Increased serum concentrations of pro-gastrin-releasing peptide in patients with renal dysfunction. Nephrol Dial Transplant 1996;11:1267–70.
[16] Fujiwara Y, Sekine I, Tsuta K, Ohe Y, Kunitoh H, Yamamoto N, et al. Effect of platinum combined with irinotecan or paclitaxel against large cell neuroendocrine carcinoma of the lung. Jpn J Clin Oncol 2007;37:482–6.
[17] Ohe Y, Ohashi Y, Kubota K, Tamura T, Nakagawa K, Negoro S, et al. Randomized phase III study of cisplatin plus irinotecan versus carboplatin plus paclitaxel, cisplatin plus gemcitabine, and cisplatin plus vinorelbine for advanced non-small-cell lung cancer: four-arm cooperative study in japan. Ann Oncol 2007;18:317–23.
[18] Schiller JH, Harrington D, Belani CP, Langer C, Sandler A, Krook J, et al. Comparison of four chemotherapy regimens for advanced non-small-cell lung cancer. N Engl J Med 2002;346:92–8.
[19] Yamazaki S, Sekine I, Matsuno Y, Takei H, Yamamoto N, Kunitoh H, et al. Clinical responses of large cell neuroendocrine carcinoma of the lung to cisplatin-based chemotherapy. Lung Cancer 2005;49:217–23.

Cancer Chemother Pharmacol
DOI 10.1007/s00280-011-1768-8

ORIGINAL ARTICLE

Pharmacokinetic and pharmacodynamic study on amrubicin and amrubicinol in Japanese patients with lung cancer

Yoshinori Makino · Noboru Yamamoto · Hitoshi Sato · Reiko Ando · Yasushi Goto · Chiharu Tanai · Hajime Asahina · Hiroshi Nokihara · Ikuo Sekine · Hideo Kunitoh · Yuichiro Ohe · Erika Sugiyama · Nobuaki Yokote · Tomohide Tamura · Hiroshi Yamamoto

Received: 2 June 2011 / Accepted: 14 October 2011

Abstract
Purpose The pharmacokinetic (PK)–pharmacodynamic (PD) relationship of amrubicin and its active metabolite, amrubicinol, has only been evaluated using trough levels of these agents since the full PK profiles not yet been clarified so far. This study was performed to analyze the full PK profiles of amrubicin and amrubicinol and to evaluate their toxicity–PK relationships in Japanese patients.
Methods Amrubicin (35–40 mg/m^2) was administered to 21 lung cancer patients on days 1–3 every 3–4 weeks. Fourteen blood samples were obtained per patient over the course of 3 administration days. The plasma concentrations of amrubicin and amrubicinol were quantitated by HPLC, and the relationships between PK parameters of these compounds and hematological toxicities were evaluated.
Results The overall PK profiles of amrubicin and amrubicinol were well characterized using a 3-compartment model and a 1-compartment model with a first-order metabolic process, respectively. The major toxicities were hematological. The clearance of amrubicinol was significantly correlated with grade 4 neutropenia ($P = 0.01$). The percentage decreases in the neutrophil count, hemoglobin level and platelet count were well correlated with the amrubicinol AUC.
Conclusion The pharmacokinetic profiles of amrubicin and amrubicinol were clarified, and the subsequent PK–PD analyses indicate that the clearance of amrubicinol is the major determinant of neutropenia.

Keywords Amrubicin · Amrubicinol · PK–PD study · Myelosuppression · Blood cell destruction

Y. Makino (✉) · R. Ando · N. Yokote · H. Yamamoto
Department of Pharmacy, National Cancer Center Hospital, 5-1-1 Tsukiji, Chuo-ku, Tokyo 104-0045, Japan
e-mail: ymakino@ncc.go.jp

N. Yamamoto · Y. Goto · C. Tanai · H. Asahina · H. Nokihara · I. Sekine · H. Kunitoh · Y. Ohe · T. Tamura
Division of Internal Medicine and Thoracic Oncology, National Cancer Center Hospital, 5-1-1 Tsukiji, Chuo-ku, Tokyo 104-0045, Japan

H. Sato · E. Sugiyama
Clinical and Molecular Pharmacokinetics/Pharmacodynamics, School of Pharmaceutical Sciences, Showa University, 1-5-8 Hatanodai, Shinagawa-ku, Tokyo 142-8555, Japan

Introduction

Amrubicin (AMR) and its active metabolite, amrubicinol (AMR-OH), markedly inhibit topoisomerase II activity and are effective against lung cancer [1]. AMR is approved in Japan for the treatment of small-cell lung cancer (SCLC) and non-small-cell lung cancer (NSCLC). In AMR monotherapy, AMR is administered at a dose of 35–45 mg/m^2/day on three consecutive days every 3–4 weeks. Six phase II studies for second-line or third-line AMR monotherapy for the treatment of SCLC have demonstrated overall response rates (ORRs) of 21–53% and a median survival period of 6–12 months [2–7]. Two phase II studies of previously treated NSCLC have been reported, with ORRs of 11.5 and 13.5%, respectively [5, 8]. In these phase II studies, the incidences of grade 3 or 4 myelosuppression were 82% (39–97%) [Median (Range)] for neutropenia, 28% (8–38%) for thrombocytopenia and 27% (5–41%) for anemia, respectively. Furthermore, the incidence of febrile neutropenia was 12% (2–35%).

The pharmacokinetic (PK)–pharmacodynamic (PD) profiles of AMR and AMR-OH have not yet been fully clarified so far. In a previous report by Matsunaga et al. [9],

Published online: 01 November 2011

full-sampling data were obtained from only one subject treated with 30 mg/m^2 of amrubicin and other data were obtained spars-sampling points from 15 patients (30–45 mg/m^2). On the other hand, significant relationships were observed between the plasma trough level of AMR-OH on day 4 and neutropenia and anemia [10]. The trough level of AMR-OH was correlated with the percent change in the neutrophil count [10]. However, the previous studies did not obtain plasma sampling points capable of fully characterizing the PK profiles of AMR and AMR-OH for consecutive days. Generally, the use of plasma drug concentration at only one particular time point should cause some uncertainty for establishing efficacy–PK or toxicity–PK relationships, and full PK profiling of a drug and subsequent modeling approach are highly preferable.

We, therefore, conducted a PK–PD study on AMR and AMR-OH in which we determined the PK model parameters of these agents throughout 3 days of administration and evaluated the toxicity–PK relationships in Japanese lung cancer patients based on the full PK profiles.

Materials and methods

Patients and treatments

This study was conducted at the National Cancer Center Hospital, Tokyo, Japan. Patients were eligible for participation in this study if they were 20 years or older and had been diagnosed as having lung cancer and had received AMR monotherapy. Patients with hepatitis B or C virus or human immunodeficiency virus infections and those who were considered by their physician to be ineligible as a trial candidate were excluded. Written informed consent was provided by each patient before study enrollment. The study was approved by the ethical review boards of the National Cancer Center Hospital and Showa University. It was conducted in accordance with the Declaration of Helsinki and all applicable laws and regulations.

AMR (Calsed^{TM}; Dainippon Sumitomo Pharmaceuticals Co., Ltd., Osaka, Japan) was dissolved in 50 mL of physiological saline and was administered intravenously as a 5-min infusion at a dose of 35–40 mg/m^2/day on days 1–3 every 3–4 weeks. For second- or later-line treatment of small or non-small-cell lung cancer with AMR, the recommended dose of AMR is 40 mg/m^2, in general, with some dose reduction (e.g., 35 mg/m^2) as needed based on the judgment of the attending physician. Prophylactic antiemetics (granisetron and dexamethasone) were used only as required and according to the physician's discretion. Other medications for underlying diseases, complications and pain control were allowed.

Before treatment, all patients underwent a medical history survey, physical and hematological examinations and serum biochemistry tests. The physical examination and biochemistry tests were repeated as part of normal clinical practice. The toxicities were graded according to the National Cancer Institute Common Toxicity Criteria, Version 3.0. Response was assessed according to the Response Evaluation Criteria in Solid Tumors [11].

Pharmacokinetic sampling and drug assays

PK evaluations were performed in all patients during the initial cycle of treatment. Heparinized venous blood samples (4 mL) were taken before infusion, at the end of the AMR infusion (0 min), as well as at 5, 15 and 30 min and 1, 2, 4, 8 and 24 h after the end of the infusion and at 0 min and 8 h after the infusions on days 2 and 3.

The plasma samples were stored at −80°C until analysis. The plasma concentrations of AMR and AMR-OH were measured using a previously reported high-performance liquid chromatography (HPLC) method [12]. The components were separated using HPLC on two reverse-phase columns linked with a connector (Onyx Monolithic C18, 100 × 4.6 mm) using 4 mM sodium 1-octanesulfonate, 2.3 mM acetic acid:tetrahydrofuran:dioxane (15:2:6, v/v/v) as an eluent. AMR and AMR-OH were measured using a fluorescence detector set at an excitation wavelength of 480 nm and a detection wavelength of 550 nm. Standard AMR and AMR-OH powders with purities >99% were supplied by Dainippon Sumitomo Pharmaceuticals Co., Ltd. (Osaka, Japan). The assay was validated according to the guidelines recommended by the U.S. Food and Drug Administration. The limit of quantitation was 2.5 ng/mL for both AMR and AMR-OH. The percentage recovery from the plasma proved to be higher than 88.1%. Intraday accuracy ranged from −4.1 to 0.8% for AMR and −9.8 to −2.1% for AMR-OH. The interday accuracy ranged from −3.1 to 3.0% for AMR and −4.0 to 2.3% for AMR-OH. The intraday precision ranged from 1.4 to 8.8% for AMR and 1.3 to 4.2% for AMR-OH. The interday precision ranged from 2.7 to 8.8% for AMR and 5.3 to 5.5% for AMR-OH.

Pharmacokinetic analysis

The PK parameters were estimated using a non-linear least-squares regression analysis (WinNonlin, Version 5.0.1; Pharsight, Cary, NC, USA) with a weighting factor of $1/Y^2$, where Y represents the observed data. The individual plasma concentration–time data were fitted to one-, two- or three-exponential equations using a constant infusion input for AMR and a one- or two-compartment model with a first-order metabolic process from AMR to AMR-OH

(parameterized by k_{in}). The PK model was optimized on the basis of Akaike's information criteria (AIC). Fitted parameters were permitted in the computation of the following PK parameters: AUC, peak plasma concentration of day 1 (C_{max}), total body clearance (CL) and volume of distribution at steady state (Vd_{ss}).

Pharmacodynamic analysis

The relationships between PK parameters (AUC and C_{max}) of AMR-OH and the hematologic toxicity were evaluated. The percentage decrease in the hematologic count or level (neutrophils and hemoglobin) was calculated as follows:

$$\%\ \text{Decrease in hematologic count or level} = \frac{\text{pretreatment count or level} - \text{nadir count or level}}{\text{pretreatment count or level}} \times 100$$

The results were plotted as a function of the AUC and C_{max} of AMR-OH, respectively. Relationships between adverse effects and PK exposure (AUC) or C_{max} were fitted using a non-linear least-squares regression and a weighting factor of unity according to a sigmoid model, as follows:

$$\text{Adverse effect } (\%) = \frac{E_{max} \cdot \text{AUC}^{\gamma}}{\text{EC}_{50}{}^{\gamma} + \text{AUC}^{\gamma}} \times 100$$

or

$$\text{Adverse effect } (\%) = \frac{E_{max} \cdot C_{max}{}^{\gamma}}{\text{EC}_{50}{}^{\gamma} + C_{max}{}^{\gamma}} \times 100$$

where E_{max} represents the maximum effect and EC_{50} is the AUC (or C_{max}) of AMR-OH at which the effect was 50% of the maximum effect. A non-linear least-squares regression was conducted using WinNonlin to estimate E_{max}, EC_{50} and the sigmoidicity coefficient (γ). The strength of the relationship between the percent decrease in the hemoglobin level and the AUC (or C_{max}) of AMR-OH was assessed using a least-squares linear regression analysis.

In the PD analysis, the patient characteristics as well as the PK parameters were compared among patients who experienced grade 4 neutropenia (<500/μL). The patient characteristics that were evaluated for possible association with grade 4 neutropenia were age, sex, performance status (0 vs. ≥1), type of disease (SCLC vs. NSCLC), smoking index, prior surgery, prior thoracic or brain irradiation, the number of prior chemotherapy regimens (1 vs. ≥2), albumin, total bilirubin, aspartate aminotransferase (AST), alanine aminotransferase (ALT), lactate dehydrogenase (LDH), serum α_1-acid glycoprotein (AGP) and pretreatment neutrophil counts. PK parameters, including C_{max}, AUC and CL of AMR and AMR-OH, were also compared between patients who experienced and those who did not experience grade 4 neutropenia.

Statistical analyses

The obtained data were presented as mean ± standard deviation (SD). To identify factors associated with grade 4 neutropenia, continuous variables were compared between patients with and those without grade 4 neutropenia using the Mann–Whitney *U*-test, and differences in the distribution of dichotomized variables were evaluated using the χ^2-test or the Fisher exact test, as appropriate. $P < 0.05$ was considered statistically significant, and all *P*-values were two-tailed. To identify variables significantly associated with grade 4 neutropenia, multivariate logistic regression analyses were performed. All statistical analyses were performed using the statistical software JMP 4.0 (SAS Institute, Cary, NC, USA).

Results

Patient characteristics

Twenty-one patients were enrolled in this study from May 2007 to June 2009. The patient characteristics are listed in Table 1. Seventeen patients had SCLC, and 4 patients had NSCLC. Seventeen patients were men, and 4 were women; all patients had a good performance status, and the median age was 64 years. All 21 patients had previously undergone at least one chemotherapy regimen. All patients had received a platinum agent (cisplatin or carboplatin), and 20 patients had received some form of topoisomerase inhibitor (irinotecan, etoposide or topotecan). Only one patient who had been diagnosed as having squamous cell carcinoma had not been treated with a topoisomerase inhibitor. All patients were included in the PK and toxicity evaluations. Eighteen patients were assessed for response and survival. Two patients were not assessed for response because of the occurrence of interstitial pneumonia or a cardiac event after the second cycle; chemotherapy was discontinued in these patients. Another patient developed disseminated intravascular coagulation (DIC), and chemotherapy was ceased because of the presence of grade 4 thrombocytopenia despite an insufficient response during the first cycle. Seven patients had received granulocyte colony-stimulating factor during the first cycle. All 21 patients had received prophylactic antiemetics. Among these patients, 8 were treated with granisetron and dexamethasone and 13 were treated with granisetron only prior to treatment with AMR on 3 consecutive days.

Pharmacokinetics

Patients received AMR at a dose of 40 mg/m^2 except for one patient with squamous cell carcinoma who received a

Table 1 Patient characteristics

$n = 21$	n	Median	Range
Sex			
Male/female	17/4		
Age (y.o.)		64	39–81
Disease			
SCLC (LD/ED)	5/12		
NSCLC (LCNEC/SQ)	3/1		
PS			
0/1/2	10/10/1		
Smoking history			
±	2/19		
Smoking index		1,165	0–3,200
Pretreatment			
Surgery			
±	15/6		
Radiation			
±	11/10		
Thoracic	5		
Whole brain	6		
Other	1		
Chemotherapy			
0/1/2≤	0/16/5		
CDDP/CPT	11		
CBDCA/ETOP	8		
Others	12		
Characteristics			
Height (cm)		163.8	155–177.5
Body weight (kg)		57	36–74.95
Body surface area (m^2)		1.62	1.28–1.89
Serum creatinine (mg/dL)		0.8	0.6–1.5
Aspartate amino transferase, AST (IU/L)		24	15–111
Alanine transaminase, ALT (IU/L)		18	7–61
Total bilirubin (mg/dL)		0.4	0.3–1.5
Lactate dehydrogenase, LDH (U/L)		233	133–1,286
Serum albumin (g/dL)		3.9	2.5–4.6
α_1-Acid glycoprotein, AGP (mg/dL)[a]		103.5	50–292
White blood cell (×1,000/μL)		5.4	2.5–15.2
Hemoglobin (g/dL)		11.9	7.2–15.3
Platelet (×10,000/μL)		22.2	12.2–37.3
Absolute neutrophil count, ANC (×1,000/μL)		3.6	1.4–11.9

SCLC small-cell lung cancer, *LD* limited disease, *ED* extensive disease, *NSCLC* non-small-cell lung cancer, *LCNEC* large-cell neuroendocrine carcinoma, *SQ* squamous cell carcinoma, *PS* performance status, *CDDP* cisplatin, *CPT* irinotecan, *CBDCA* carboplatin, *ETOP* etoposide

[a] α_1-Acid glycoprotein (AGP) data were obtained from 18 patients

dose of 35 mg/m^2 based on the judgment of the attending physician. Together, the patients received a total of 71 cycles (median of 4 cycles [range, 1–7]) of therapy. A total of 294 plasma samples were obtained for the PK analyses. The PK profiles for AMR and AMR-OH were well characterized using a 3-compartment model and a 1-compartment model with a first-order metabolic process from AMR to AMR-OH, respectively (Fig. 1). The plasma dispositions of AMR and AMR-OH, to which the PK models were fitted, are shown in Fig. 2, and the pharmacokinetic parameters are listed in Table 2.

The plasma concentrations of AMR decreased sharply shortly after the drug infusion and then slowly declined as a result of drug elimination and distribution into the peripheral and blood compartments. The infusion of AMR was followed 2 h later (t_{max}) by the peak plasma concentration of AMR-OH (C_{max}: 23 ± 7 μg/L; Fig. 2; Table 2). In two patients, AMR was metabolized rapidly to AMR-OH, leading to the generation of a large variation in the metabolic rate constant (k_{in}).

Toxicities

Grade 3/4 hematological toxicities consisted of neutropenia (81%), thrombocytopenia (33%) and anemia (19%). The most frequent grade 4 hematological toxicity, observed in 13 patients (62%), was neutropenia. The mean percentages of the decrease in the white blood cell count, absolute neutrophil count, platelet count and hemoglobin level for all 21 patients were 71 ± 20%, 85 ± 21%, 56 ± 29% and 16 ± 7%, respectively. All non-hematological toxicities were mild (≤grade 2). Three patients (14%) experienced febrile neutropenia. Dose reduction was required in 32% (6/19) of the patients, and a treatment delay (4 weeks or more) was needed in 12 patients because of prolonged hematological toxicity during the second cycle.

Responses

One patient with SCLC had a complete response, 8 patients had partial responses (SCLC 6, large-cell neuroendocrine carcinoma 2), 4 patients had stable disease (SCLC) and 5 patients had progressive disease (SCLC 4, squamous 1).

PK–PD relationship for hematologic toxicity

The present PK–PD analyses demonstrated that the percentage decrease in the absolute neutrophil count was related to the AUC and C_{max} of AMR-OH, as described by the sigmoid maximum effect (E_{max}) model. The plot in Fig. 3a and b depicts the E_{max} relationship using the data obtained from all patients. On the basis of the E_{max} model

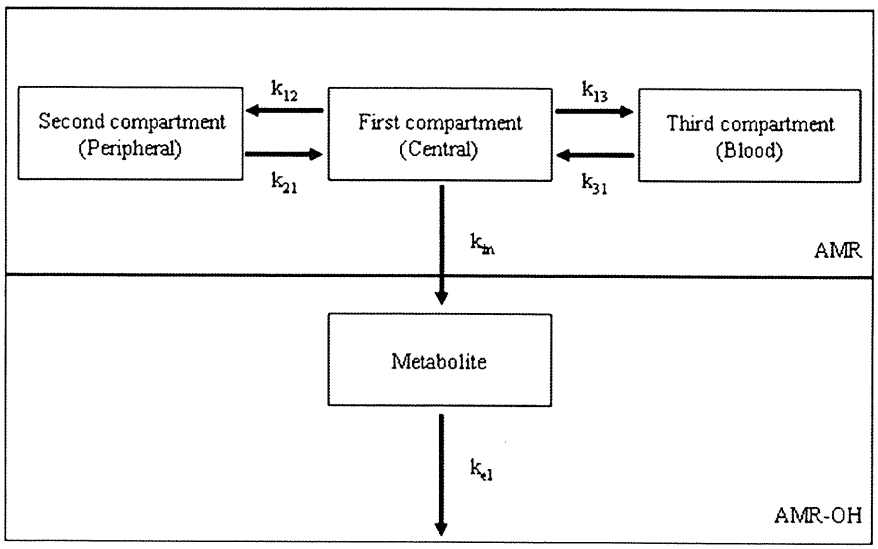


Fig. 1 Schematic illustration of pharmacokinetic model for amrubicin and amrubicinol. The model includes three compartments for amrubicin and one for amrubicinol: k_{12}, k_{21}, k_{13} and k_{31} represent the rate constants for the intercompartmental transfers of AMR. k_{in} represents the metabolic conversion rate constant from AMR to AMR-OH. k_{el} represents the elimination rate constant for AMR-OH

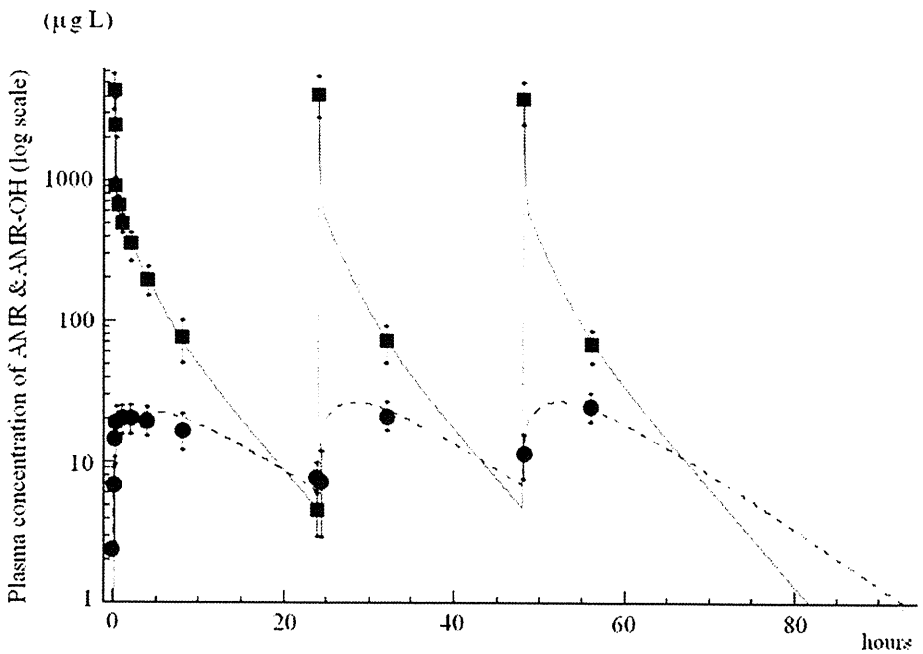


Fig. 2 Concentrations in plasma versus time curves of AMR and AMR-OH. Plasma concentration–time profiles for AMR (*filled square*) and AMR-OH (*filled circle*). *Squares* or *circles* and vertical bars, mean measured concentration ± SD; lines, best-fit lines from the pharmacokinetic analysis (*solid lines* for AMR and *dashed lines* for AMR-OH)

fitting, the AUC and C_{max} that yielded a 50% decrease in the absolute neutrophil count were predicted to be 306.1 h μg/L and 13.2 μg/L, respectively. The shapes of the curves were steep ($\gamma = 8.4$ for AUC; $\gamma = 7.7$ for C_{max}), and these models provided a correlation between the PK of AMR-OH and neutropenia ($r = 0.8296$ for AUC; $r = 0.8035$ for C_{max}). A least-squares linear regression analysis showed that the percent decrease in the hemoglobin level could be estimated by the AUC and C_{max} of AMR-OH ($r = 0.6554$ for AUC; $r = 0.7267$ for C_{max}) (Fig. 3c, d). The AUC and C_{max} that yielded a 50% decrease in the platelet count were predicted to be 549.4 h μg/L and 22.1 μg/L, respectively. The shapes of the curves were gentle ($\gamma = 2.0$ for AUC; $\gamma = 1.9$ for C_{max}), and these models provided the correlation between the PK of AMR-OH and thrombocytopenia ($r = 0.679$ for AUC; $r = 0.6301$ for C_{max}) (Fig. 3e, f). When the characteristics of patients who experienced or did not experience grade 4 neutropenia were compared, the distribution of the performance status was significantly different, and the pretreatment body weight in patients with grade 4 neutropenia was significantly lower than in those without

Table 2 Pharmacokinetic parameters of AMR and AMR-OH

PK parameter	NCA		Model (single dose)[a]		Model (multiple dose)[b]	
	Mean	SD	Mean	SD	Mean	SD
AMR (NCA; constant infusion, Model; 3-compartment infusion)						
AUC (h μg/L)	3,218	684.6	3,175	730.7	3091	579
CL (L/h)	20.6	5.1	20.1	5	21.0	4.4
C_{max} (μg/L)	4,355	1,308	5,500	3,734	4,200	1,149
Vd_{ss} (L)	71.5	15.9	73	17.1	74.0	16.8
k_{12} (1/hr)	–	–	8.0	3.0	6.4	3.5
k_{13} (1/hr)	–	–	2.0	2.7	1.0	2.1
k_{21} (1/hr)	–	–	2.4	1.8	2.4	2.5
k_{31} (1/h)	–	–	0.4	0.2	0.4	0.2
AMR-OH (NCA; extravascular, model; 1-compartment with first-order metabolism)						
k_{in} (1/h)	–	–	12.7	32.3	2.3	2.0
AUC (h μg/L)	515	146	506.9	140.1	459.5	122.3
CL (L/h)	134.3	48.2	135.3	44.9	150.0	53.9
C_{max} (μg/L)	22.5	6.7	22.2	6.6	23.1	6.7
Vd_{ss} (L)	2,899	1,083	2,935	1,223	2,708	1,042

NCA non-compartmental analysis

[a] Model analysis using 9 plasma sampling points (0, 5, 15 and 30 min, and 1, 2, 4, 8 and 24 h after the end of infusion) after AMR infusion on day 1

[b] Model analysis using all plasma sampling points (0, 5, 15 and 30 min and 1, 2, 4, 8 and 24 h after the end of infusion on day 1 and 0 min and 8 h after the end of infusion on days 2 and 3) after AMR infusion on days 1–3

(Table 3). Among the PK parameters, the CL of AMR-OH and the Vd_{ss} of AMR were significantly lower in patients with grade 4 neutropenia.

On the other hand, in the present multivariate analysis, none of the parameters were identified as significant variables of grade 4 neutropenia.

Discussion

The intrinsic activity of the metabolite of AMR, AMR-OH, has been known to be 10–100 times higher than that of AMR [13].

The aims of the present study were to determine the PK parameters of AMR and AMR-OH and to clarify the relationships between PK parameters and toxicity associated with AMR therapy. For this purpose, we carried out an extensive PK–PD study on AMR and AMR-OH in 21 patients with lung cancer, where a total of 14 blood sampling per patient was accomplished over the course of 3 intravenous AMR administration days.

The PK profiles of AMR and AMR-OH were well characterized using a 3-compartment model with a short infusion and a 1-compartment model with a first-order metabolic process from AMR to AMR-OH, respectively. All PK model parameters of AMR and AMR-OH over the 3 administration days could be well extrapolated using the compartment model parameters obtained from a 24-h single-dose and non-compartmental analysis. The PK profiles for AMR and AMR-OH did not show non-linearity or accumulation. Therefore, the 3-day PK profile can be simulated using the plasma trough level observed on the first administration day, enabling the doses on days 2 and 3 to be adjusted, if necessary.

Using the compartment analysis, we were able to perform a kinetic approach to identifying the mechanisms responsible for the metabolism of AMR to AMR-OH and the subsequent metabolic pathway, thereby enabling a quantitative correlation between the PK and the hematological toxicities arising from AMR therapy. The AMR-OH clearance is an apparent clearance, since the percentage of AMR metabolized into AMR-OH is unknown and subject to interindividual variability.

In the present PK–PD study, it was found that a higher C_{max} and AUC of AMR-OH in the plasma was associated with a risk of grade 4 neutropenia and the percentage decrease in the absolute neutrophil count, as well as with the decrease in the platelet count. On the other hand, both parameters were well correlated with a linear model of the percentage decrease in the hemoglobin level. AMR is a quinone-containing anthracycline agent. Doxorubicin has a similar quinone structure and is known to reduce its respective semiquinone-free radicals in the presence of flavoenzymes. Free radicals can also be formed from the

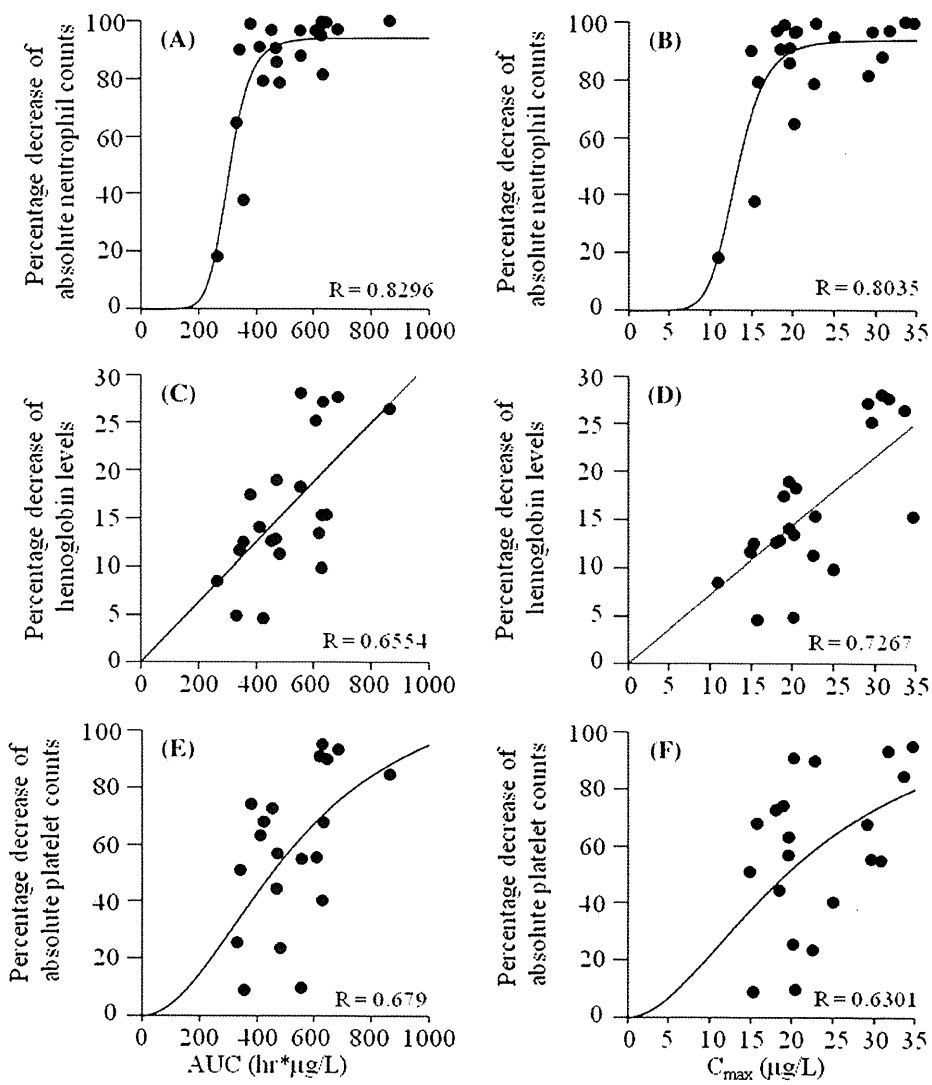


Fig. 3 PK–PD correlation between hematological toxicity and AMR-OH PK parameters. Relationship between the percent decrease in the neutrophil, hemoglobin or platelet count and the AUC or C_{max} of AMR-OH; The solid lines indicate the best fit of a sigmoid E_{max} pharmacodynamic model to the data [neutrophil, AUC: (**a**), C_{max}: (**b**), platelet, AUC: (**e**), C_{max}: (**f**)]. The *solid line* is the linear regression line, and the *dashed line* is the 95% CI for individual estimates [hemoglobin, AUC: (**c**), C_{max}: (**d**)]

interaction of doxorubicin with iron to form a doxorubicin-iron III complex. The in vitro data indicated that dexrazoxane, which prevents anthracycline-mediated cardiotoxicity, inhibited the binding of doxorubicin to red blood cells but had no effect on the association of doxorubicin with erythrocyte ghosts [14]. These findings and a previous report describing an interaction between anthracyclines and iron or hemoglobin support our notion that the third compartment of the parental compound corresponds to the blood cells and that AMR-OH was converted from AMR in the blood, forming an AMR-OH-iron III complex that may directly destroy blood cells. Therefore, the cause of the severe hematological toxicities of AMR may be related not only to myelosuppression but also to the destruction of blood cells.

In multivariate analysis, we could not find the pretreatment factors or PK parameters to predict the severe neutropenia, possibly because the sample size was too small. On the other hand, body weight was found to be positively correlated with the Vd_{ss} of AMR. Moreover, the low Vd_{ss} of AMR was significantly correlated with the high AUC of AMR-OH, suggesting that patients with the low Vd_{ss} of AMR rapidly metabolized AMR to AMR-OH; as a result, hematological toxicities tended to be serious to such patients.

AMR was metabolized rapidly to AMR-OH in 2 patients, leading to the generation of large variations in the metabolic rate constant (k_{in}). The AMR-OH concentrations at the end of infusion in these patients were 29.9 and 11.0 ng/mL, far above the average AMR-OH concentration at the corresponding time point. Other PK parameters and their variations were similar between the single-dose and multiple-dose studies. When these data were excluded, the k_{in} in the single-dose study was determined to be 2.8 ± 0.5 (mean ± SD), which is close to the k_{in} value determined in the multiple-dose study.

The major pathway of AMR metabolism involves the reduction in the C-13 carbonyl group to a hydroxyl group

Table 3 Characteristics and pharmacokinetics of AMR and AMR-OH in patients with or without grade 4 neutropenia

Neutropenia	Neutrophils ≥ 500/μL	Neutrophils < 500/μL	*P* value
Patients (*n*)	8	13	
Sex (*n*)			0.13
Female	0	4	
Male	8	9	
Performance status (n)			0.001
0	8	3	
≥1	0	10	
Number of prior chemotherapy regimens (*n*)			0.33
1	5	11	
≥2	3	2	
Age (years)			1.00
Median	65	64	
Range	39–76	42–81	
Body weight			0.02
Median	65	53	
Range	55–75	36–72	
Serum creatinine			0.11
Median	0.9	0.8	
Range	0.7–1.5	0.0–1.1	
Total bilirubin			0.19
Median	0.4	0.6	
Range	0.3–0.6	0.3–1.5	
Aspartate amino transferase, AST			0.25
Median	23	26	
Range	15–32	16–111	
Serum albumin			0.08
Median	4.1	3.8	
Range	3.5–4.4	2.5–4.6	
AMR clearance, AMR-CL (L/h)			0.06
Median	21.9	18.5	
Range	18.8–33.8	11.7–24.4	
AMR distribution, Volume at steady state, Vd_{ss} (L)			0.02
Median	79.2	63.2	
Range	64.5–116.9	45.7–87.6	
AMR-OH clearance, AMR-OH-CL (L/h)			0.01
Median	167.0	105.9	
Range	86.8–278.4	77.6–149.8	

Sex, performance status and number of prior chemotherapy regimens were analyzed using the χ^2-test or the Fisher exact test. Others were analyzed using the Mann–Whitney *U*-test

by carbonyl reductase (CBR). Then, AMR and AMR-OH are inactivated by NAD (P) H: quinone oxide reductase (NQO) and NADPH P-450 reductase [15].

Genetic polymorphisms of these metabolic enzymes are reportedly related to the PK of several anticancer agents [16–18]. CBR-1 D2 diplotypes tagged by at least one variant allele at the CBR-1 c.627C > T and +967G > A loci are correlated with significantly higher exposure levels of doxorubicin, suggesting the possibility that the intracellular conversion to doxorubicinol is reduced in Asian patients with breast cancer [15]. In another investigation, the NQO-1 609 C > T polymorphism resulted in a significantly reduced tumor NQO-1 activity and a reduced survival in subsets of patients receiving intraperitoneal hyperthermic mitomycin C [18]. However, the exact reason for interindividual variations in the PK of AMR remains unknown; thus, the relationship between genetic polymorphisms of metabolic enzymes and transporters for AMR and AMR-OH should be evaluated in the future.

In this study, several types of lung cancer patients were enrolled (including 17 patients with SCLC, 3 with large-cell neuroendocrine carcinomas and 1 with squamous cell carcinoma). Furthermore, among 17 patients with SCLC, five subjects had limited disease and 12 had extensive disease. These variations of the tumor properties inevitably led to a limitation of this study, i.e., the difficulty in clarifying the PK–PD relationship between the AUC of AMR/AMR-OH and the tumor response.

In conclusion, we clarified the full PK profiles of AMR and AMR-OH and found that the CL of AMR-OH is the major determinant of neutropenia. PK–PD evidence has not yet been reported for this compound on a global basis so far, since AMR is approved only in Japan. Thus, the present findings should be of great importance for avoiding or reducing severe hematological toxicities associated with AMR therapy. In order to confirm our findings and to identify factors influencing the interindividual variabilities in the PK–PD parameters for AMR, further population PK studies and pharmacogenetic studies on a larger number of patients are highly warranted.

Acknowledgments This work was supported in part by the Foundation for the Promotion of Cancer Research in Japan.

References

1. Hanada M, Mizuno S, Fukushima A, Saito Y, Noguchi T, Yamaoka T (1998) A new antitumor agent amrubicin induces cell growth inhibition by stabilizing topoisomerase II-DNA complex. Jpn J Cancer Res 89:1229–1238
2. Onoda S, Masuda N, Seto T, Eguchi K, Takiguchi Y, Isobe H, Okamoto H, Ogura T, Yokoyama A, Seki N, Asaka-Amano Y, Harada M, Tagawa A, Kunikane H, Yokoba M, Uematsu K, Kuriyama T, Kuroiwa Y, Watanabe K (2006) Phase II trial of amrubicin for treatment of refractory or relapsed small-cell lung cancer: Thoracic Oncology Research Group Study 0301. J Clin Oncol 24:5448–5453
3. Kato T, Nokihara H, Ohe Y, Yamamoto N, Sekine I, Kunitoh H, Kubota K, Nishiwaki Y, Saijo N, Tamura T (2006) Phase II trial of amrubicin in patients with previously treated small cell lung cancer (SCLC) J Clin Oncol, ASCO Annual Meeting Proceedings Part I, (18S):7061

4. Inoue A, Sugawara S, Yamazaki K, Maemondo M, Suzuki T, Gomi K, Takanashi S, Inoue C, Inage M, Yokouchi H, Watanabe H, Tsukamoto T, Saijo Y, Ishimoto O, Hommura F, Nukiwa T (2008) Randomized phase II trial comparing amrubicin with topotecan in patients with previously treated small-cell lung cancer: North Japan Lung Cancer Study Group Trial 0402. J Clin Oncol 26:5401–5406
5. Kaira K, Sunaga N, Tomizawa Y, Yanagitani N, Shimizu K, Imai H, Utsugi M, Iwasaki Y, Iijima H, Tsurumaki H, Yoshii A, Fueki N, Hisada T, Ishizuka T, Saito R, Mori M (2010) A phase II study of amrubicin, a synthetic 9-aminoanthracycline, in patients with previously treated lung cancer. Lung Cancer 69:99–104
6. Ettinger DS, Jotte R, Lorigan P, Gupta V, Garbo L, Alemany C, Conkling P, Spigel DR, Dudek AZ, Shah C, Salgia R, McNally R, Renschler MF, Oliver JW (2010) Phase II study of Amrubicin as second-line therapy in patients with platinum-refractory small-cell lung cancer. J Clin Oncol 28:2598–2603
7. Jotte R, Conkling P, Reynolds C, Galsky MD, Klein L, Fitzgibbons JF, McNally R, Renschler MF, Oliver JW (2011) Randomized phase II trial of single-agent amrubicin or topotecan as second-line treatment in patients with small-cell lung cancer sensitive to first-line platinum-based chemotherapy. J Clin Oncol 29:287–293
8. Kaneda H, Okamoto I, Hayashi H, Yoshioka H, Miyazaki M, Kudoh S, Kimura T, Sugiura T, Sawa T, Takeda K, Iwamoto Y, Satouchi M, Akita K, Saito H, Goto I, Shibata K, Fukuoka M, Nakagawa K (2010) Phase II trial of amrubicin for second-line treatment of advanced non-small cell lung cancer: results of the West Japan Thoracic Oncology Group trial (WJTOG0401). J Thorac Oncol 5:105–109
9. Matsunaga Y, Hamada A, Okamoto I, Sasaki J, Moriyama E, Kishi H, Matsumoto M, Hira A, Watanabe H, Saito H (2006) Pharmacokinetics of amrubicin and its active metabolite amrubicinol in lung cancer patients. Ther Drug Monit 28:76–82
10. Kimura T, Kudoh S, Mitsuoka S, Yoshimura N, Tanaka H, Asai K, Kyoh S, Tochino Y, Umekawa K, Hirata K (2009) Plasma concentration of amrubicinol in plateau phase in patients treated for 3 days with amrubicin is correlated with hematological toxicities. Anticancer Drugs 20:513–518
11. Therasse P, Arbuck SG, Eisenhauer EA, Wanders J, Kaplan RS, Rubinstein L, Verweij J, Van Glabbeke M, van Oosterom AT, Christian MC, Gwyther SG (2000) New guidelines to evaluate the response to treatment in solid tumors. J Natl Cancer Inst 92:205–216
12. Ando R, Makino Y, Tamura T, Yamamoto N, Nishigaki R, Kimura T, Yokote N, Yamamoto H (2010) Simple and sensitive HPLC method for determination of amrubicin and amrubicinol in human plasma: application to a clinical pharmacokinetic study. Biomed Chromatogr 24:301–306
13. Yamaoka T, Hanada M, Ichii S, Morisada S, Noguchi T, Yanagi Y (1998) Cytotoxicity of amrubicin, a novel 9-aminoanthracycline, and its active metabolite amrubicinol on human tumor cells. Jpn J Cancer Res 89:1067–1073
14. Vaidyanathan S, Boroujerdi M (2000) Interaction of dexrazoxane with red blood cells and hemoglobin alters pharmacokinetics of doxorubicin. Cancer Chemother Pharmacol 46:93–100
15. Tani N, Yabuki M, Komuro S, Kanamaru H (2005) Characterization of the enzymes involved in the in vitro metabolism of amrubicin hydrochloride. Xenobiotica 35:1121–1133
16. Lal S, Sandanaraj E, Wong ZW, Ang PC, Wong NS, Lee EJ, Chowbay B (2008) CBR1 and CBR3 pharmacogenetics and their influence on doxorubicin disposition in Asian breast cancer patients. Cancer Sci 99:2045–2054
17. Fan L, Goh BC, Wong CI, Sukri N, Lim SE, Tan SH, Guo JY, Lim R, Yap HL, Khoo YM, Iau P, Lee HS, Lee SC (2008) Genotype of human carbonyl reductase CBR3 correlates with doxorubicin disposition and toxicity. Pharmacogenet Genomics 18:621–631
18. Fleming RA, Drees J, Loggie BW, Russell GB, Geisinger KR, Morris RT, Sachs D, McQuellon RP (2002) Clinical significance of a NAD(P)H: quinone oxidoreductase 1 polymorphism in patients with disseminated peritoneal cancer receiving intraperitoneal hyperthermic chemotherapy with mitomycin C. Pharmacogenetics 12:31–37

Gefitinib as First-line Treatment in Elderly Epidermal Growth Factor Receptor-mutated Patients With Advanced Lung Adenocarcinoma: Results of a Nagano Lung Cancer Research Group Study

Kazuhiro Asami,[1] Tomonobu Koizumi,[2] Kazuya Hirai,[3] Shingo Ameshima,[4] Akihiro Tsukadaira,[5] Nobutoshi Morozumi,[6] Akio Morikawa,[7] Shinji Atagi,[1] Masaaki Kawahara[8]

Abstract

Efficacy of first-line gefitinib for elderly epidermal growth factor receptor mutated patients with lung adenocarcinoma is uncertain. This study was aimed to investigate efficacy of gefitinib for such population. The primary endpoint was response rate (RR) and at least 12 cases were needed. Overall RR was 59% (95% confidence interval, 33%-81%) and first-line gefitinib was effective for elderly patients.

Introduction: Feasibility of gefitinib therapy in elderly patients with non–small-cell lung cancer is uncertain. This phase II study aimed to investigate the efficacy and usefulness of gefitinib therapy as a first-line treatment for elderly patients who have advanced lung adenocarcinoma with epidermal growth factor receptor (*EGFR*) mutations. **Patients and Methods:** We enrolled chemotherapy-naïve advanced lung adenocarcinoma patients aged 75 years or older. Patients were administered gefitinib (250 mg) once daily until progression or unacceptable toxicity. The primary endpoint was response rate (RR), and secondary endpoints were disease control rate (DCR; defined as complete response [CR] plus partial response [PR] plus stable disease [SD]), progression-free survival (PFS), overall survival (OS), and toxicity profile. **Results:** Between April 2008 and November 2009, 17 lung adenocarcinoma patients were enrolled. Overall RR was 59% (95% confidence interval [CI]: 33% to 81%), with 2 patients achieving CR and 8 PR. SD was noted in 5 patients, and DCR was 88% (95% CI: 62% to 98%). Median PFS was 12.9 months (95% CI: 2.2 to 23.6 months), and median OS had not yet been reached. Major grade 3 toxicities were skin rash (12%) and increased levels of aspartate aminotransferase or alanine aminotransferase (18%). **Conclusion:** First-line treatment with gefitinib was effective and well-tolerated in elderly patients with *EGFR* mutations.

Clinical Lung Cancer, Vol. 12, No. 6, 387-92
Keywords: EGFR mutation, Elderly patients, First-line treatment, Gefitinib, Lung adenocarcinoma

Introduction

Lung cancer is one of the most common cancers in the world.[1] Non–small-cell lung cancer (NSCLC) constitutes nearly 80% of all lung cancer cases, and approximately one-third of patients with advanced NSCLC are 75 years of age or older, a number which is rapidly increasing.[2]

Studies have indicated that both monochemotherapy and platinum-based combination chemotherapy can be exercised as first-line

[1]Department of Internal Medicine, National Hospital Organization Kinki-chuo Chest Medical Center, Sakai, Japan
[2]Comprehensive Cancer Center, Shinshu University Hospital, Matsumoto, Japan
[3]Department of Pulmonary Diseases, Nagano Municipal Hospital, Nagano, Japan
[4]Department of Respiratory Medicine, Fukui University School of Medicine, Fukui, Japan
[5]Department of Pulmonary Diseases, Iida Municipal Hospital, Iida, Japan
[6]Department of Pulmonary Diseases, Saku Central Hospital, Saku, Japan
[7]Department of Respiratory Surgery, Showa Inan Hospital, Komagane, Japan
[8]Department of Internal Medicine, Federation of National Public Service Personnel Mutual Aid Associations, Otemae Hospital, Osaka, Japan

Submitted: Jan 2, 2011; Revised: Jan 20, 2011; Accepted: Feb 22, 2011

Address for correspondence: Kazuhiro Asami, MD, Department of Internal Medicine, National Hospital Organization Kinki-chuo Chest Medical Center, 1180 Nagasone-cho, Kita-ku, Sakai, Osaka 591-8555, Japan.
Tel: +81-72-252-3021; Fax: +81-72-251-1372;
e-mail contact: kazu.taizo@nifty.com

1525-7304/$ - see frontmatter
doi: 10.1016/j.cllc.2011.02.004

therapies in non-selected elderly NSCLC patients.[3-11] However, markedly few NSCLC patients are ultimately eligible for participation in clinical trials, with only 30% of advanced NSCLC patients aged 65 years or older eligible for enrollment.[12] As such, concerted efforts are needed to develop more effective and more feasible treatment plans for elderly patients who have advanced NSCLC.

Gefitinib (IRESSA; AstraZeneca, Wilmington, DE) targets the tyrosine kinase domain of the epidermal growth factor receptor (*EGFR*). In a subset analysis of data from a multi-institutional randomized phase II trial of gefitinib involving Japanese patients with NSCLC who had previously received other treatments (IDEAL 1), the overall response rate (RR) for non-selected Japanese patients was 28%, with no significant difference noted in terms of RR between patient age groups ($\leq$ 65 years or $>$ 65 years old).[13] Being a never smoker, female, and having adenocarcinoma were considered the most relevant clinical predictors for a positive response to gefitinib, and the presence of sensitive *EGFR* mutations was posited as perhaps the most relevant biologic factor associated with improved response to gefitinib and disease survival.[14] Further, several phase II studies in patients with NSCLC who had sensitive *EGFR* mutations demonstrated overall RRs of 75% to 91% and progression-free survival (PFS) ranging from 7.7 to 9.7 months.[15-17]

In a recent prospective study involving first-line gefitinib therapy in patients who have advanced NSCLC with *EGFR* mutations who had poor performance status (PS), investigators reported a high RR (66%) and relatively long overall survival (OS; 17.8 months).[18] However, at present, no prospective trials have investigated the efficacy of first-line gefitinib therapy in elderly *EGFR*-mutated patients with advanced NSCLC. Given that several investigators have cited response to gefitinib as a prognostic factor in patients with NSCLC,[19, 20] we hypothesized that evaluation of response to gefitinib may be beneficial in elderly advanced NSCLC patients to more quickly assess the usefulness of continuing gefitinib therapy.

Here, we conducted a prospective study aimed at assessing efficacy and feasibility of gefitinib as first-line therapy in elderly patients (aged $\geq$ 75 years) with advanced NSCLC and gefitinib-sensitive *EGFR* mutations.

Patients and Methods

Eligibility

Chemotherapy-naïve patients with *EGFR* mutations and stage IIIB or IV lung adenocarcinoma were eligible to participate in the present study. Other criteria included being aged 75 years or more, having an Eastern Cooperative Oncology Group PS of 0 to 2, and having a neutrophil count $\geq$ 1,500, hemoglobin $\geq$ 9.0 g/dL, platelet count $\geq$ 75,000, aspartate aminotransferase (AST) and alanine aminotransferase (ALT) $\leq$ 2.5 times the upper limit of normal (ULN), bilirubin $\leq$ 1.25 times the ULN, serum creatinine $\leq$ 1.5 times the ULN, arterial oxygen pressure $\geq$ 60 mm Hg, and an estimated life expectancy of more than 12 weeks.

Exclusion criteria were presence of symptomatic brain metastases, active infection, interstitial pneumonia or pulmonary fibrosis as determined by computed tomography (CT) scan, active gastric ulcer, uncontrolled diabetes mellitus, uncontrolled cardiovascular disease, chronic liver disease, active concomitant cancer, or severe allergy. All patients provided written informed consent before enrollment, and the institutional review board of each participating institution approved this protocol. The study was undertaken in accordance with Declaration of Helsinki.

Egfr Gene Analysis

We examined *EGFR* mutation status using the peptide nucleic acid—locked nucleic acid (PNA-LNA, Mitsubishi Chemical Medience, Tokyo, Japan) polymerase chain reaction (PCR) clamp method with paraffin sections of biopsy samples or cytological specimens from patients diagnosed with lung adenocarcinoma. PNA-LNA PCR clamp is designed to detect 11 different *EGFR* mutations, with respective values for sensitivity and specificity of 89% and 100%, respectively.

Studytreatment

Gefitinib (250 mg) was administered orally once daily, with therapy continued until disease progression, intolerable toxicity, or consent withdrawal. Alternate-day administration of gefitinib for reduction of toxicity was permitted. Protocol treatment could be temporarily interrupted for a maximum of 14 days to alleviate gefitinib-related adverse events (AEs). After the study period, patients were permitted any subsequent treatments desired, including continuation of gefitinib treatment.

Treatment Assessment

Response to gefitinib therapy was evaluated by CT scan every 4 weeks for the first 3 months of therapy, and every 12 weeks subsequently until disease progression, assessed using the response evaluation criteria in solid tumors (RECIST) version 1.0. Disease control rate (DCR) was defined as the rate of complete response (CR) plus partial response (PR) plus stable disease (SD). PFS was defined as the period from initiation of gefitinib therapy to the date of progressed disease (PD) confirmation or death from any cause. OS was defined as the interval between the date of therapy initiation and the date of death from any cause.

Toxicities were graded according to the National Cancer Institute Common Toxicity Criteria version 3.0. Duration of response was defined as the time from the first objective assessment of CR or PR to the first instance of progression or death.

Statistical Consideration

With regard to our primary endpoint, a Simon two-stage optimal design was used to determine the total number of patients required for the present study. We set an RR of 75% in enrolled patients and a rate of 30% as the lower limit of interest, with $\alpha = 0.05$ and $\beta = 0.1$. The estimated accrual number was at least 12 cases or more. PFS and OS were estimated via the Kaplan-Meier method, and for differences between curves the *P* value was calculated by the log-rank test. A *P* value of less than .05 was accepted as statistically significant. Feasibility was evaluated based on the ratio of patients able to complete the protocol therapy without suffering any serious toxicity preventing treatment continuation; if 80% or more of patients were able to complete the protocol therapy without serious toxicity within 3 months of starting the therapy, barring temporary interruptions, this protocol treatment was to be determined feasible. Statistical analyses were performed using SPSS software version 11.0 (IBM, Chicago, IL).

Table 1 Patient Characteristics (n = 17)

Characteristic	Number of Patients (%)
Median Age, Years (Range)	81 (75-88)
Gender	
Male	4 (24)
Female	13 (76)
Smoking Status	
Never	11 (65)
Former/current	6 (35)
Performance Status (ECOG)	
0-1	14 (83)
2	3 (17)
Stage	
IIIB	3 (18)
IV	14 (82)
EGFR Mutation	
Exon 19 deletion mutation	7 (41)
L858R	10 (59)

Abbreviations: ECOG = Eastern Cooperative Oncology Group; EGFR = epidermal growth factor receptor.

Table 2 Response to Gefitinib Therapy (n = 17)

Response	Number of Patients	Response Rate (%)	95% CI
CR	2	12	
PR	8	47	
SD[a]	5	29	
PD	2	12	
Overall Response Rate	10	59	33-81
Disease Control Rate[b]	15	88	62-98

Abbreviations: CR = complete response; PD = progressive disease; PR = partial response; SD = stable disease;
[a] Stable disease was confirmed and sustained for 8 weeks or longer.
[b] Disease control rate is defined as CR + PR + SD.

Figure 1 Kaplan-Meier Plot of Progression-free Survival (PFS) With Gefitinib (n = 17)

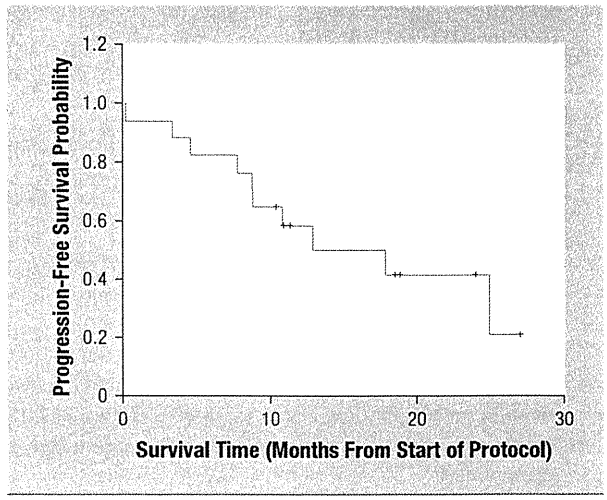
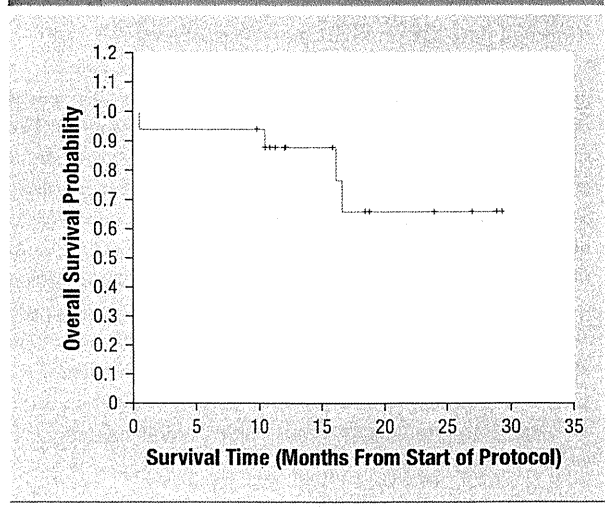


Figure 2 Kaplan-Meier Plot of Overall Survival (OS) With Gefitinib (n = 17)



Results

Patient Characteristics

From April 2008 to November 2009, 17 lung adenocarcinoma patients who had *EGFR* mutations were enrolled in the study, with all proving eligible to participate. Patient characteristics are described in detail in Table 1. All patients had histopathologically diagnosed lung adenocarcinoma. *EGFR* mutations were detected using the same biopsy sample or cytological specimen that had been diagnosed as lung adenocarcinoma.

Response and Survival

Objective tumor RRs are described in Table 2. Overall RR and DCR were 59% and 88%, respectively. Two patients showed dramatic response to gefitinib therapy and were evaluated as having achieved CR; of these two, one had an exon 19 deletion mutation and the other an L858R point mutation.

The median follow-up time was 15.9 months (range, 0.5 to 29.3 months). As of November 2010, seven patients were still receiving protocol treatment with gefitinib, two of whom were receiving alternate-day administration. Ten patients had discontinued protocol therapy at the time of writing this paper. One patient died of respiratory failure because of an exacerbation of primary lung cancer 12 days after initiation of gefitinib therapy, and nine were confirmed to have PD. Of these nine patients with PD, four died of tumor progression, two others were treated with the best supportive care available, and subsequent therapy was provided after disease progression in the remaining three patients, with two receiving second-line therapy with erlotinib and the other continuous gefitinib therapy.

Table 3 Adverse Events Within 3 Months of Starting Gefitinib (n = 17)

Toxicity	Grade 1 (n)	Grade 2 (n)	Grade 3 (n)	Grade 4 (n)	Number of Toxicities ≥ Grade 1 (%)	Number of Toxicities ≥ Grade 3 (%)
Skin Rash	4	2	2	0	8 (47)	2 (12)
Dry Skin	10	1	0	0	11 (65)	0 (0)
Itching	4	0	0	0	4 (24)	0 (0)
Diarrhea	2	0	0	0	2 (12)	0 (0)
AST or ALT[a]	0	4	3	0	7 (41)	3 (18)
Stomatitis	0	1	0	0	6 (1)	0 (0)

[a] Increased levels of aspartate aminotransferase or alanine aminotransferase.

Median PFS was 12.9 months (95% confidence interval [CI]: 2.2-23.6 months; Fig 1). Median survival time had not been determined at the time of printing. The 1-year survival rate was 88% (Fig 2), and the median duration of response was 8.7 months (range, 2.8 to 24.1 months).

Feasibility

All patients were evaluated for toxicity (Table 3). The most common AE associated with gefitinib was skin toxicity, with dry skin observed in 65% of patients. Although no AEs of grade 4 or more or interstitial lung disease (ILD) were observed within 3 months of starting gefitinib therapy, serious AEs of grade 3 were noted in four patients (increased levels of AST or ALT in two, skin rash in one, and increased levels of AST or ALT as well as skin rash in a fourth). These serious AEs were improved after temporary withdrawal of gefitinib, and protocol treatment was resumed with daily or alternate administration of gefitinib. Most other toxicities observed were mild, and no patients were unable to continue protocol treatment due to AEs that developed within 3 months of starting gefitinib, nor were any treatment-related deaths noted in the same period. All patients examined were able to undergo protocol treatment without discontinuation due to gefitinib-related toxicities. In total, 15 patients were able to continue protocol treatment beyond the initial 3 months, with only 2 discontinuing treatment within 3 months of starting, due to PD or cancer death.

Discussion

In the present study, we observed a high RR and extended survival time, results which were far superior to those achieved in any previous prospective trials using cytotoxic agents in elderly patients with advanced NSCLC. Our results confirmed the tolerability and usefulness of gefitinib for elderly NSCLC patients. However, our study is the first to examine the usefulness of gefitinib as first-line treatment in elderly *EGFR*-mutated patients. In the future, clinical trials should be performed to clarify its efficacy and usefulness compared with other treatments involving cytotoxic agents.

Several prospective clinical trials involving cytotoxic agents in elderly patients with advanced NSCLC cited an RR, median OS, and 1-year survival rate of approximately 10% to 35%, 5 to 14 months, and 15% to 60%, respectively.[4,5,21-25] In two recent clinical phase III trials involving *EGFR*-mutated patients with NSCLC, Mitsudomi et al and Maemondo et al demonstrated significantly higher objective RR and longer PFS in patients on gefitinib than in those receiving platinum-based combination chemotherapy (gefitinib RR and OS of 62% to 74% and 9.2 to 10.8 months, respectively).[26-27] Although both of these trials targeted patients aged 75 years or younger, our findings were similar to these previous results in terms of RR and PFS.

We found that toxicity with gefitinib therapy in elderly patients was generally acceptable, indicating suitable feasibility. No patients discontinued protocol therapy due to gefitinib-related AEs in this study, and none developed ILD. A history of smoking is a risk factor of development of ILD for patients receiving any treatment with cytotoxic agent, not limited to any treatment including gefitinib. *EGFR* mutations are frequently detected in patients who either never smoked or are light smokers.[28] In fact, more than half of the patients enrolled in the present study had never smoked. As such, non-smoking status may relatively reduce the risk of development of ILD during treatment of *EGFR*-mutated patients. Unfortunately, we were unable to measure quality of life sufficiently in this study, necessitating further investigations to evaluate how this protocol treatment with gefitinib influences quality of life in elderly patients.

Despite the findings of Mitsudomi et al and Maemondo et al described above,[26,27]which noted significantly longer PFS in patients receiving gefitinib treatment, these studies were unable to definitively demonstrate the superiority of gefitinib in terms of OS in non-elderly NSCLC patients, possibly because a majority of patients who had received platinum-based chemotherapy crossed over to gefitinib only after failure of first-line treatment. Standard second-line treatments are typically established in non-elderly NSCLC patients, and second-line or later therapies strongly influence OS.[29] Further, non-elderly patients can also receive platinum-based combination chemotherapy as second-line treatments. In fact, approximately half of patients receiving gefitinib treatment in IRESSA Pan-Asia Study (IPASS), which compared gefitinib with carboplatin/paclitaxel as a first-line treatment for patients with lung adenocarcinoma, received chemotherapy with carboplatin/paclitaxel as second-line treatment,[30] and more than 70% of patients receiving gefitinib in a phase III study comparing gefitinib with carboplatin/paclitaxel as a first-line treatment for advanced NSCLC with sensitive *EGFR* mutations (NEJ 0002) received carboplatin/paclitaxel as second-line treatment.[27] Whether or not *EGFR* mutation can be used to predict survival benefit of gefitinib treatment in non-elderly patients is unclear. In contrast to non-elderly patients, treatment in general is limited in elderly NSCLC patients. Consequently, we hypothesize

that gefitinib may indeed help prolong OS in elderly *EGFR*-mutated patients.

In the present study, after first-line therapy failed in elderly NSCLC patients, only a few patients received second-line therapy. Three of nine patients (33%) received subsequent therapy after failure of protocol therapy with gefitinib, one of whom continued gefitinib therapy beyond disease progression. Most patients enrolled eventually died of tumor progression or selected treatment with the best supportive care available. At present, data on the efficacy of gefitinib therapy in elderly *EGFR*-mutated patients is lacking. As for chemotherapy with cytotoxic agents in elderly patients, in the Multicenter Italian Lung Cancer in the Elderly Study (MILES),[5] which compared three groups, only 6% to 13% of patients received second-line treatment with cytotoxic agent chemotherapy, possibly because some patients became unable to receive second-line therapy due to toxicities resulting from prior chemotherapy regimens and aggravation of their condition due to rapid tumor progression. Further, no standard second-line therapy has been established for use in elderly NSCLC patients. Therefore, we suspect that an effective agent with high acceptability as a first-line treatment will help prolong OS in elderly patients who have NSCLC.

Our study demonstrated remarkable efficacy and tolerance of gefitinib in elderly *EGFR*-mutated patients. Further, our results support the notion that gefitinib is most effective when administered as a first-line treatment in elderly *EGFR*-mutated patients. We believe that patients, particularly elderly patients, who have sensitive *EGFR* mutations should not miss out on the chance to receive gefitinib therapy simply due to badly timed administration, but nevertheless understand the need for clinical trials to validate our hypothesis.

Conclusion

First-line gefitinib monotherapy had a positive effect on *EGFR*-mutated elderly lung adenocarcinoma patients, improving response rate and survival time. A large-scale phase III study of first-line gefitinib therapy in this same class of patients will be required to clarify whether or not gefitinib therapy can significantly prolong OS.

Clinical Practice Points

Single or platinum-based doublet chemotherapy as a first-line treatment is standard treatment for elderly patients (75 years or older) with advanced NSCLC. In previous clinical trials, RR, median OS, and 1-year survival rate was approximately 30%, 10 months, and 50%, respectively.

EGFR-mutated patients have a significantly longer survival time and higher RR than those with wild-type *EGFR* when treated with gefitinib. Two recent phase III trials demonstrated significantly higher RR and longer PFS in patients on gefitinib than in patients receiving platinum-based doublet chemotherapy. American Society of Clinical Oncology (ASCO) guideline described that the first-line use of gefitinib may be recommended for patients with activating *EGFR*-mutated patients with advanced NSCLC. However, data on the feasibility of gefitinib therapy as first-line treatment in elderly *EGFR*-mutated patients is lacking.It is not clear whether *EGFR* mutation would predict survival benefit by gefitinib treatment in elderly *EGFR*-mutated patients.

This study is the first phase II trial that confirmed usefulness and tolerability of gefitinib therapy as first-line treatment for elderly *EGFR*-mutated patients. These results were far superior to those of any other previous trials with cytotoxic agent in terms of RR and survival time.

These results suggest that gefitinib therapy is a reasonable option and that may be able to prolong survival time when administered as a first-line treatment in elderly *EGFR*-mutated patients with advanced NSCLC.

Acknowledgments

We thank the patients and their families for their support and participation in this study. We also thank Sanae Maruyama of Shinshu University and all of the members of Nagano Lung Cancer Research Group for their helpful support.

Disclosure

There is no conflict of interest in relation to this study. This study is registered with UMIN-CTR [http://www.umin.ac.jp/ctr/index.htm, identification number UMIN000002783], November 19, 2009.

Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.cllc.2011.02.004.

References

1. Parkin DM. Global cancer statistics in the year 2000. *Lancet Oncol* 2001; 2:533-43.
2. Travis WD, Travis LB, Devesa SS. Lung cancer. *Cancer* 1995; 75:191-202.
3. Azzoli CG, Baker S Jr, Temin S, et al. American Society of Clinical Oncology Clinical Practice Guideline update on chemotherapy for stage IV non-small-cell lung cancer. *J Clin Oncol* 2009; 27:6251-66.
4. Gridelli C, Perrone F, Gallo C, et al. Effects of vinorelbine on quality of life and survival of elderly patients with advanced non-small-cell lung cancer. The Elderly Lung Cancer Vinorelbine Italian Study Group. *J Natl Cancer Inst* 1999; 91:66-72.
5. Gridelli C, Perrone F, Gallo C, et al. Chemotherapy for elderly patients with advanced non-small-cell lung cancer: the Multicenter Italian Lung Cancer in the Elderly Study (MILES) phase III randomized trial. *J Natl Cancer Inst* 2003; 95:362-72.
6. Ricci S, Antonuzzo A, Galli L, et al. Gemcitabine monotherapy in elderly patients with advanced non-small cell lung cancer: a multicenter phase II study. *Lung Cancer* 2000; 27:75-80.
7. Fidias P, Supko JG, Martins R, et al. A phase II study of weekly paclitaxel in elderly patients with advanced non-small cell lung cancer. *Clin Cancer Res* 2001; 7:3942-49.
8. Ohe Y, Ohashi Y, Kubota K, et al. Randomized phase III study of cisplatin plus irinotecan versus carboplatin plus paclitaxel, cisplatin plus gemcitabine, and cisplatin plus vinorelbine for advanced non-small-cell lung cancer: Four-Arm Cooperative Study in Japan. *Ann Oncol* 2007; 18:317-23.
9. Belani CP, Fossella F. Elderly subgroup analysis of a randomized phase III study of docetaxel plus platinum combinations versus vinorelbine plus cisplatin for first-line treatment of advanced nonsmall cell lung carcinoma (TAX 326). *Cancer* 2005; 104:2766-74.
10. Ohe Y, Niho S, Kakinuma R, et al. A phase II study of cisplatin and docetaxel administered as three consecutive weekly infusions for advanced non-small-cell lung cancer in elderly patients. *Ann Oncol* 2004; 15:45-50.
11. Buffoni L, Dongiovanni D, Barone C, et al. Fractionated dose of cisplatin (CDDP) and vinorelbine (VNB) chemotherapy for elderly patients with advanced non-small cell lung cancer: phase II trial. *Lung Cancer* 2006; 54:353-7.
12. Gridelli C, Rossi A, Galetta D, et al. Italian clinical research in non-small-cell lung cancer. *Ann Oncol* 2005; 16(suppl 4):iv110-115.
13. Nishiwaki Y, Yano S, Tamura T, et al. Subset analysis of data in the Japanese patients with NSCLC from IDEAL 1 study on gefitinib [in Japanese]. *Gan To Kagaku Ryoho* 2004; 31:567-73.
14. Cappuzzo F, Hirsch FR, Rossi E, et al. Epidermal growth factor receptor gene and protein and gefitinib sensitivity in non-small-cell lung cancer. *J Natl Cancer Inst* 2005; 97:643-55.
15. Inoue A, Suzuki T, Fukuhara T, et al. Prospective phase II study of gefitinib for chemotherapy-naive patients with advanced non-small-cell lung cancer with epidermal growth factor receptor gene mutations. *J Clin Oncol* 2006; 24:3340-46.
16. Asahina H, Yamazaki K, Kinoshita I, et al. A phase II trial of gefitinib as first-line therapy for advanced non-small cell lung cancer with epidermal growth factor receptor mutations. *Br J Cancer* 2006; 95:998-1004.
17. Yoshida K, Yatabe Y, Park JY, et al. Prospective validation for prediction of gefitinib sensitivity by epidermal growth factor receptor gene mutation in patients with non-small cell lung cancer. *J Thorac Oncol* 2007; 2:22-8.

18. Inoue A, Kobayashi K, Usui K, et al. First-line gefitinib for patients with advanced non-small-cell lung cancer harboring epidermal growth factor receptor mutations without indication for chemotherapy. *J Clin Oncol* 2009; 27:1394-1400.
19. Wataya H, Okamoto T, Maruyama R, et al. Prognostic factors in previously treated non-small cell lung cancer patients with and without a positive response to the subsequent treatment with gefitinib. *Lung Cancer* 2009; 64:341-5.
20. Tsujino K, Kawaguchi T, Kubo A, et al. Response rate is associated with prolonged survival in patients with advanced non-small cell lung cancer treated with gefitinib or erlotinib. *J Thorac Oncol* 2009; 4:994-1001.
21. Kudoh S, Takeda K, Nakagawa K, et al. Phase III study of docetaxel compared with vinorelbine in elderly patients with advanced non-small-cell lung cancer: results of the West Japan Thoracic Oncology Group Trial (WJTOG 9904). *J Clin Oncol* 2006; 24:3657-63.
22. Berardi R, Porfiri E, Scartozzi M, et al. Elderly patients with advanced non-small cell lung cancer. a pase II study with weekly cisplatin and gemcitabine. *Oncology* 2003; 65:198-203.
23. Feliu J, Martin G, Madronal C, et al. Combination of low-dose cisplatin and gemcitabine for treatment of elderly patients with advanced non-small-cell lung cancer. *Cancer Chemother Pharmacol* 2003; 52:247-52.
24. Buffoni L, Dongiovanni D, Barone C, et al. Fractionated dose of cisplatin (CDDP) and vinorelbine (VNB) chemotherapy for elderly patients with advanced non-small cell lung cancer: phase II trial. *Lung Cancer* 2006; 54:353-7.
25. Maestu I, Gomez-Aldaravi L, Torregrosa MD, et al. Gemcitabine and low dose carboplatin in the treatment of elderly patients with advanced non-small cell lung cancer. *Lung Cancer* 2003; 42:345-54.
26. Mitsudomi T, Morita S, Yatabe Y, et al. Gefitinib versus cisplatin plus docetaxel in patients with non-small-cell lung cancer harbouring mutations of the epidermal growth factor receptor (WJTOG3405): an open label, randomised phase 3 trial. *Lancet Oncol* 2010; 11:121-8.
27. Maemondo M, Inoue A, Kobayashi K, et al. Gefitinib or chemotherapy for non-small-cell lung cancer with mutated EGFR. *N Engl J Med* 2010; 362:2380-88.
28. Ando M, Okamoto I, Yamamoto N, et al. Predictive factors for interstitial lung disease, antitumor response, and survival in non-small-cell lung cancer patients treated with gefitinib. *J Clin Oncol* 2006; 24:2549-56.
29. Stinchcombe TE, Socinski MA. Considerations for second-line therapy of non-small cell lung cancer. *Oncologist* 2008; 13(suppl 1):28-36.
30. Mok TS, Wu YL, Thongprasert S, et al. Gefitinib or carboplatin-paclitaxel in pulmonary adenocarcinoma. *N Engl J Med* 2009; 361:947-57.

Surg Today (2011) 41:1492–1497
DOI 10.1007/s00595-011-4509-z

SURGERY TODAY

Original Article

Concurrent Chemoradiotherapy Using Cisplatin Plus S-1, an Oral Fluoropyrimidine, Followed by Surgery for Selected Patients with Stage III Non-Small Cell Lung Cancer: A Single-Center Feasibility Study

RIICHIROH MARUYAMA[1,2], FUMIHIKO HIRAI[1], KAORU ONDO[1], TAKURO KOMETANI[1], MOTOHARU HAMATAKE[1], TAKASHI SETO[1], KENJI SUGIO[1], and YUKITO ICHINOSE[1]

[1]Department of Thoracic Oncology, Kyushu Cancer Center, 3-1-1 Notame, Minami-ku, Fukuoka 811-1395, Japan
[2]Department of Surgery and Science, Graduate School of Medical Sciences, Kyushu University, Fukuoka, Japan

Abstract

Purpose. This single-institutional study was designed to determine whether S-1, an oral fluoropyrimidine, plus cisplatin with concurrent radiotherapy is feasible as an induction treatment for locally advanced non-small cell lung cancer (NSCLC).

Methods. Eighteen patients were analyzed in this study from July 2005 to March 2008. The patients received 40 mg/m^2 S-1 orally twice per day on days 1 through 14 and 22 through 35, and cisplatin (60 mg/m^2) was injected intravenously on days 8 and 29. The patients also underwent radiotherapy, and received a total dose of 40 Gy in 20 fractions beginning on day 1. Surgical resection was performed from 3 to 6 weeks after completing the induction treatment.

Results. Nine (50%) of the 18 patients who received the induction treatment achieved a partial response. One patient refused to undergo surgery. The remaining 17 patients underwent a complete surgical resection. There were no deaths nor any major morbidities during the perioperative period. The recurrence-free survival and overall survival rate at 2 years for the patients who underwent resection were 63.3% and 88.2%, respectively.

Conclusion. Induction treatment using S-1 plus cisplatin and concurrent radiotherapy and surgical resection for selected patients with stage III NSCLC is a feasible and promising new treatment modality.

Key words Non-small cell lung cancer · Stage III · Induction chemoradiotherapy · S-1 · Cisplatin · Surgical resection

Reprint requests to: K. Sugio
Received: December 2, 2010 / Accepted: January 12, 2011

Introduction

The standard treatment for unresectable stage III non-small cell lung cancer (NSCLC) is concurrent chemoradiotherapy. Two large multicenter randomized phase III trials have explored the role of surgery versus radiotherapy after induction treatment for stage III-N2 NSCLC.[1,2] No data have so far shown that neoadjuvant treatment followed by surgery results in prolonged survival in comparison with adequate chemoradiation alone. Surgery after chemo-radiotherapy therefore remains controversial for patients with this stage of disease.

Both cisplatin and 5-fluorouracil (5-FU) have a radiosensitizing effect for NSCLC.[3,4] UFT contains tegafur (a prodrug that is converted to 5-FU by cells) and uracil at a 1:4 molar ratio concentration. Ichinose et al. reported the results of a multi-institutional phase II trial, which demonstrated that UFT plus cisplatin with concurrent radiotherapy is a feasible and effective treatment for locally advanced NSCLC. The response rate and median survival of locally advanced unresectable stage III (IIIA 20%, IIIB 80%) patients in that study were 81% and 16.5 months, respectively, with low-grade toxicities.[5] Ichinose et al. also reported a phase II trial using UFT plus cisplatin with concurrent radiotherapy as induction treatment for marginally resectable stage IIIB NSCLC. The surgical morbidity and mortality rates were 36% and 4%, respectively. The calculated 1- and 3-year survival rates in the resected patients were 82% (95% confidence interval [CI]: 66%–98%) and 67% (95% CI 47%–87%), respectively.[6] S-1 (Taiho Pharmaceutical, Tokyo, Japan) is an orally active combination of tegafur, gimeracil (an inhibitor of dihydropyrimidine dehydrogenase, which degrades fluorouracil), and oteracil (which inhibits the phosphorylation of fluorouracil in the gastrointestinal tract, thereby reducing the

gastrointestinal toxicity of fluorouracil) in a molar ratio of 1:0.4:1.[7] S-1 was developed to improve the tumor-selective cytotoxicity of 5-FU, while reducing gastrointestinal toxicity through the addition of these modulators. Ohyanagi et al. reported a phase II trial, which demonstrated that S-1 plus cisplatin with concurrent radiotherapy was a promising treatment for unresectable stage III NSCLC. The response rate and median survival were 87.5% and 33.1 months, respectively, with mild toxicities. The 1- and 2-year survival rates were 89.5% and 56%, respectively.[8]

We designed a single-institutional feasibility study of the combination chemotherapy using S-1 plus cisplatin with concurrent radiotherapy followed by surgical resection for selected patients with stage III NSCLC.

Patients and Methods

Patients

Eighteen patients were registered from July 2005 to March 2008. All patients provided their written informed consent according to institutional guidelines. All patients were required to be at least 20 years of age, with an Eastern Cooperative Oncology Group (ECOG) performance status (PS) of 0 to 1, with no uncontrolled cardiac or hepatic disease. All had adequate bone marrow function (defined as a total leukocyte count $\geq 3.5 \times 10^9$/l, absolute neutrophil count [ANC] $\geq 2 \times 10^9$/l, platelet count $\geq 100 \times 10^9$/l, and hemoglobin level ≥9.0 g/dl); adequate renal function (defined as a serum creatinine level <1.5 mg/dl); adequate hepatic function (defined as a total bilirubin level <1.5 mg/dl and serum aspartate aminotransferase and/or alanine aminotransferase levels <2 times the upper normal limit for the laboratory); no severe cardiac disease or arrhythmia on an electrocardiogram; and a room air oxygen partial pressure >60 mmHg. The clinical or pathological stage of the disease was based on the General Rules for Clinical and Pathological Records of Lung Cancer (6th edition) edited by the Japan Lung Cancer Society.[9] A contralateral mediastinal lymph node was defined as being metastatic when both the ipsilateral and contralateral mediastinal lymph nodes were swollen, with a short diameter of more than 1.5 cm determined by high-resolution thin-section computed tomography (CT) scans.

Mediastinoscopy was not routinely performed, although it is sometimes considered a gold-standard criterion for the evaluation of N2 disease. In addition, the presence of a region of focal low density (other than fat) suggesting necrosis, or surrounding fat infiltration suggesting extrafascial extension, were used as malignant criteria for the evaluation of mediastinal lymph nodes on CT. Most of the cases were suspected to have invasive and extranodal expansion. The disease was defined to be potentially and technically resectable if a tumor appeared to be removable by the replacement of the superior vena cava or great vessels, carinal resection, a partial resection of vertebrae, a resection of the involved bilateral mediastinal or supraclavicular lymph nodes, and that concurrent chemoradiotherapy was preferable to a definitive resection to render their disease resectable. The histological analysis of the tumor was based on the World Health Organization classification for cell types.[10] The clinicopathological characteristics of the patients are shown in Table 1. An "unclassified carcinoma" indicates a malignant epithelial neoplasm that cannot be placed into one of the categories.

Table 1. Clinicopathological characteristics of the patients

Parameter	No.
Age (years), median (range)	59 (47–77)
Sex	
Male	15
Female	3
Performance status (ECOG)	
0	12
1	6
Histological type	
Adenocarcinoma	9
Squamous cell carcinoma	5
Large cell carcinoma	1
Pulmonary blastoma	1
Unclassified carcinoma	2
Clinical stage	
IIIA	10
IIIB	8

ECOG, Eastern Cooperative Oncology Group

Treatment Schedule

S-1 was administered orally at 40 mg/m^2 twice per day on days 1 through 14 and 22 through 35. The actual dose of S-1 was 40 mg twice a day in a patient with a body surface area (BSA) <1.25 m^2, 50 mg twice a day for those with a BSA of 1.25 m^2 but <1.5 m^2, and 60 mg twice a day for those with a BSA >1.5 m^2. Cisplatin (60 mg/m^2) was administered during a 90-min infusion on days 8 and 29 when patients were hydrated with at least 2500 ml of saline infusion. Radiotherapy was administered in five fractions per week from a megavolt linear accelerator at a daily dose of 2 Gy from day 1 up to a total dose of 40 Gy (20 fractions). The target volume included the primary disease site with a 2-cm margin around the mass, and the ipsilateral hilum. The entire width of the mediastinum was included with a 2-cm margin around the radiographically visible area of involvement (as determined by a pretreatment CT scan). The inferior margin extended 4 cm below the carina or 2 cm below the radiographically visible tumor mass. The supraclavicular fossa was not

irradiated when no tumor was detected in that tissue by either a physical or radiographic examination. The blood cell counts and chemistries were examined at least once a week. Patients were not to receive prophylactic granulocyte-colony stimulating factor (G-CSF) during any cycle. The use of G-CSF was allowed only for patients who had an ANC $<0.5 \times 10^9$/l, neutropenic fever, or documented infections while neutropenic.

Evaluations

The response was evaluated according the RECIST (Response Evaluation Criteria in Solid Tumors),[11] and toxicity criteria were based on the Common Terminology Criteria for Adverse Events (CTCAE), version 3.0.

Statistical Analysis

The duration of the recurrence-free survival (RFS) was calculated from the starting date of induction chemoradiotherapy until either the first evidence of recurrence or death due to any cause, or until the last follow-up (censored). The overall survival (OS) was calculated from the starting date of induction chemoradiotherapy until death due to any cause, or the last follow-up (censored). The survival curve was made using the Kaplan–Meier method.[12] All data were analyzed using the IBM SPSS Statistics 18 software package (SPSS Japan, an IBM company, Tokyo, Japan).

Results

Induction Treatment and Toxicity

All patients received the planned dose of radiotherapy, and 15 patients (83%) underwent two cycles of chemotherapy as induction treatment. Nine (50%) of the 18 patients achieved a partial response after receiving the induction treatment (95% CI, 21%–72%). The hematological and nonhematological toxicities of grade 2 or worse that were observed during the induction chemoradiotherapy are listed in Table 2. Leukopenia was the most frequently observed adverse event; however, the incidence of grade 3 or 4 was only 22.2%. The incidence of the other adverse events of grade 3 or 4 toxicities was 11.1% for neutropenia and anemia. This regimen was therefore associated with manageable toxicity. No toxic deaths occurred. Severe nonhematological toxicity was uncommon.

Surgery

One patient refused to undergo surgery. The remaining 17 patients underwent a complete surgical resection (complex pneumonectomy in four patients including 3 intrapericardial pneumonectomies, 3 simple pneumonectomies, and 6 complex lobectomies including 3 sleeve lobectomies and 4 simple lobectomies). There were no deaths or any major morbidity during the perioperative period other than a rethoracotomy, which was performed for bleeding or chylothorax in one patient each.

Table 2. Hematological and nonhematological toxicities observed during induction chemoradiotherapy

	No. of patients (%)		
	Grade 2	Grade 3	Grade 4
WBC	7 (39)	4 (22)	0
ANC	8 (44)	2 (11)	0
Hb	1 (6)	2 (11)	0
Plt	0	0	0
Febrile neutropenia	0	1 (6)	0
AST	1 (6)	0	0
ALT	0	0	0
Hyperbilirubinemia	0	0	0
Hypocalcemia	1 (6)	0	0
Hyperkalemia	1 (6)	0	0
Fatigue	1 (6)	0	0
Nausea/vomiting	1 (6)	0	0
Mucositis	1 (6)	0	0
Dermatitis	3 (17)	0	0
Esophagitis	1 (6)	0	0

WBC, white blood cell count; ANC, absolute neutrophil count; Hb, hemoglobin; Plt, platelets; AST, aspartate aminotransferase; ALT, alanine aminotransferase

Pathological Findings

There were three pathological complete responses (Ef3: no viable tumor cells in resected specimens), and some pathological response was recognized in all of the remaining patients (Ef1a: ≥2/3 viable tumor cells, in 3 patients; Ef1b: 1/3 ≤ viable tumor cells < 2/3, in 2 patients, Ef2: <1/3 viable tumor cells, in 9 patients).

Survival and Recurrence

The median observation time was 29 months. The calculated RFS (Fig. 1) and OS (Fig. 2) rates at 2 years for resected patients were 63.3% and 88.2%, respectively. Recurrence developed in 6 of the 17 resected patients. The recurrence site was distant in 5 patients (brain in 2 patients, contralateral lung in 2, and ipsilateral neck lymph nodes in 1), and 1 patient had a local recurrence in the ipsilateral mediastinal lymph nodes.

Discussion

S-1 plus cisplatin with concurrent radiotherapy followed by surgery for selected stage III NSCLC is feasible and