Continued.

Haplotypes[a,c]	Nucleotide change[a]	Intron 11		Intron 12						Exon 13				Intron 13	Exon 14			Intron 14		Exon 16	3'-UTR			Number	Frequency	
		IVS11 +11_14 delTCAG CCCCG	IVS11 +12 C>T	IVS11 +20 G>A	IVS12 +42 G>A	IVS12 −173 A>G	IVS12 −120 G>A	IVS12 −108 C>G	IVS12-34 C>T	IVS12-33T>G	1453 G>A	1455 C>T	1506 C>T	1510 G>A	1648 C>T	IVS13 +33 C>T	1708 C>T	1716 G>A	1738 G>C	IVS14 +8 G>A	IVS14 +50 C>G	1975 G>A	*150_*152 delCT C	2250 (*307) C>G	2349 (*306) G>A	2445 (*372) G>A		
Amino Acid Change											A485T	A485T	A303V	G504R	R390W		R570C	S572S	E580Q			A659T						
*1	*1a																										158	0.336	0.545
	*1b																										55	0.117	
	*1c																										12	0.026	
	*1d																										6	0.013	
	*1e																										5	0.011	
	*1f																										3	0.006	
	*1g																										1	0.006	
	*1h																										2	0.004	
	*1i																										2	0.004	
	*1 other[d]																										10	0.021	
*28	*28a																										83	0.177	0.430
	*28b																										42	0.089	
	*28c																										21	0.045	
	*28d																										20	0.043	
	*28e																										12	0.026	
	*28f																										5	0.011	
	*28g																										4	0.009	
	*28h																										3	0.006	
	*28i																										2	0.004	
	*28j																										2	0.004	
	*28k																										2	0.004	
	*28 other[d]																										6	0.013	
*35	*35a																										1	0.002	0.002
*36	*36a[b]																										1	0.002	0.002
*42	*42a																										3	0.006	0.009
	*42b[b]																										1	0.002	
*43	*43a																										1	0.002	0.002
*9	*9a[b]																										1	0.002	0.002
*11	*11a[c]																										1	0.002	0.002
*111	*111a[c]																										1	0.002	0.002
*117	*117[c]																										1	0.002	0.002
*47	*47[c]																										1	0.002	0.002
																											470	1.000	1.000

IV and *V*) were inferred. The *28*, *35*, *36* (known haplotypes), *42*, *43* (novel unambiguous haplotypes), *I*, *II*, *III*, *IV* and *V* (novel ambiguous haplotypes) harbor one of the nonsynonymous variations, 1508C>T (A503V), 1738G>C (E580Q), 683C>T (P228L), 1237G>A (G413S), 1975G>A (A659T), 1453G>A (A485T), 1510G>A (G504R), 1648C>T (R550W), 1708C>T (R570C), and 86C>T (T29M). Despite the finding from one subject, the *43a* haplotype was unambiguous since this subject had a heterozygous 1975G>A (A659T) as sole heterozygous site on the basis of homozygous *1a* haplotypes. The most common haplotype was *1a* (frequency: 0.336), followed by *28a* (0.177), *1b* (0.117), *28b* (0.089), *28c* (0.045) and *28d* (0.043). These 6 common haplotypes accounted for 81% of all the inferred haplotypes. Gomez *et al.* reported the haplotype frequencies of *POR* (from exon 2 to 3′-UTR) in the Caucasian populations, in which the group haplotype frequencies were 0.690 for *1*, 0.292 for *28*, 0.001 for *36* and 0.006 for *37* (1508C>T [A503V] and 1891G>A [V631I].[6] The *28* group haplotype was more prevalent in Japanese (frequency = 0.430) than in Caucasians, and the *37* haplotype was not detected in the Japanese (**Table 3B**).

In conclusion, we identified 75 genetic variations in the *POR* gene including 26 novel ones from 235 Japanese subjects. Four novel variations resulted in amino acid substitutions. Based on the LD profile, the analyzed region was divided into 2 blocks and their haplotype structures were inferred. This is the first report to comprehensively analyze the *POR* gene and to estimate its haplotype structure in a Japanese population. This information is useful for pharmacogenetic studies investigating the relationship between the interindividual differences in drug metabolism by CYPs and *POR* haplotypes.

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Tumor *KRAS* Status Predicts Responsiveness to Panitumumab in Japanese Patients with Metastatic Colorectal Cancer

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Objective: Mutation status of the *KRAS* gene in tumors has been shown to be a predictive biomarker of response to anti-epidermal growth factor receptor antibody therapy in patients with metastatic colorectal cancer. This retrospective analysis examined the association between efficacy and safety of the fully human anti-epidermal growth factor receptor antibody panitumumab and *KRAS* mutation status in Japanese metastatic colorectal cancer patients using data from two clinical trials with adherence to good clinical practices.

Methods: An exploratory, integrated analysis of data from *KRAS* evaluable patients enrolled in a Phase 1 study (Study 20040192) and a Phase 2 study (Study 20050216) was performed. Paraffin-embedded tumor samples were analyzed for *KRAS* status. Primary efficacy endpoint of this analysis was objective tumor response per modified response evaluation criteria in solid tumors; a key secondary endpoint was progression-free survival. Safety endpoints included incidence of adverse events.

Results: Tumor samples with known *KRAS* status were available from 8 of 13 (62%) metastatic colorectal cancer patients in the Phase 1 study and 16 of 53 patients (30%) in the Phase 2 study. Overall, 14 (58%) patients had wild-type *KRAS* tumors and 10 (42%) patients had mutated *KRAS* tumors. Four (17%) patients had a partial response; all responders had tumors with wild-type *KRAS*. Results of all secondary efficacy endpoints also favored patients with wild-type *KRAS*. Treatment-related adverse events were predominantly mild to moderate and skin related, and were similar between patients with tumors with wild-type and mutated *KRAS* in this small patient population.

Conclusions: Mutated *KRAS* status in tumors of Japanese patients with metastatic colorectal cancer is associated with lack of response to panitumumab therapy.

Key words: panitumumab – epidermal growth factor receptor – colorectal cancer – KRAS

INTRODUCTION

Expression of the epidermal growth factor receptor (EGFR) is frequently associated with malignant transformation in human cancers (1). This observation led to the development of anti-EGFR therapies for the treatment of EGFR-expressing tumors (2). While anti-EGFR antibody therapies have demonstrated efficacy in patients with metastatic colorectal cancer (mCRC) (3), the level of EGFR expression has not been shown to be associated with response to anti-EGFR antibodies (4,5). Biomarkers that can identify patients who are likely to respond to anti-EGFR therapy are needed.

Downstream signaling pathways are activated when EGFR binds to its ligand. The *KRAS* gene codes for a protein that is a member of the ras family of small G-proteins involved in intracellular signaling. When EGFR binds to ligand, its tyrosine kinase function is activated, ultimately resulting in the activation of the Ras-Raf-MAP kinase signaling cascade (6). Ras is activated by binding to GTP and deactivation of

ras in normal cells is accomplished by hydrolysis of GTP. Mutations in the *KRAS* gene that abolish the intrinsic GTPase activity result in constitutively active ras proteins that are oncogenic (7). It is possible that downstream signaling pathways are therefore constitutively activated and can become EGFR independent. In support of this hypothesis, mutations in *KRAS* have been shown to predict non-responsiveness to anti-EGFR antibody therapies in patients with mCRC (8–11).

KRAS mutations occur in ~30–50% of all patients with colorectal cancers (8–10,12,13). *KRAS* mutations have been reported in ~30% of Japanese patients with colorectal cancers (14,15). Anti-EGFR antibody therapies are therefore likely to be ineffective in at least one-third of Japanese patients with mCRC, highlighting the importance of screening for these mutations in tumors.

Panitumumab is a fully human monoclonal antibody against EGFR that is indicated as monotherapy for treatment of EGFR-expressing mCRC in the USA and EGFR-expressing plus wild-type *KRAS*-expressing mCRC in the European Union (EU) (16,17). In retrospective analyses of data from panitumumab clinical trials, including a Phase 3 trial comparing panitumumab monotherapy with best supportive care, the presence of a mutated *KRAS* gene in tumors was associated with lack of response (18,19). This retrospective integrated analysis of data from two clinical trials (20,21) is the first study to examine the efficacy and safety of panitumumab monotherapy according to tumor *KRAS* status in Japanese patients with mCRC.

PATIENTS AND METHODS

Study Design

This was a retrospective, exploratory integrated analysis of data from two clinical trials (20,21) in Japanese patients with mCRC to examine the efficacy and safety of panitumumab monotherapy according to tumor *KRAS* status.

Study 20040192 was a Phase 1 clinical trial of panitumumab monotherapy in Japanese patients with advanced solid tumors (20). Key objectives of this study were to evaluate the safety, pharmacokinetics, immunogenicity and clinical efficacy of panitumumab at various dose/dosing schedules in Japanese patients with advanced solid tumors. Patients with documented, advanced solid tumors that were refractory to standard chemotherapy or for which no standard therapy was available were eligible. Patients were sequentially enrolled into one of three panitumumab dosing cohorts: 2.5 mg/kg once weekly (QW), 6.0 mg/kg once every 2 weeks (Q2W) and 9.0 mg/kg once every 3 weeks (Q3W). These doses are all considered to reach clinically active panitumumab exposures. Objective responses were determined by the investigators using modified response evaluation criteria in solid tumors (RECIST) (22). Eighteen patients (six per cohort) were enrolled in the study. Only patients with mCRC were included in this analysis.

Study 20050216 was an open-label, single-arm, Phase 2 clinical trial of panitumumab monotherapy in Japanese patients with EGFR-expressing mCRC, who had developed disease progression while on or after prior fluoropyrimidine, irinotecan and oxaliplatin therapy (21), which were the same eligibility criteria as those used in the global Phase 3 trial (23). Key objectives of this study were to assess the effect of treatment with panitumumab on best overall objective response rate, progression-free survival, overall survival, safety and pharmacokinetics of Japanese patients with mCRC. Patients received panitumumab 6.0 mg/kg Q2W until disease progression or intolerability. Objective responses were determined by central radiographic review and by the investigators using modified RECIST, as defined in the pivotal trial (23). Fifty-three patients enrolled in the study and received at least one dose of panitumumab.

Participation in the biomarker analysis was optional in the two studies and additional informed consent was required to participate. Therefore, only the subset of patients with the additional informed consent enrolled in the studies were included in the analysis reported here. Overall, tumor samples from 28 of the total 66 patients with mCRC enrolled in the studies were available for biomarker analyses.

Study Endpoints

The primary efficacy endpoint was the objective tumor response rate. A key secondary efficacy endpoint was progression-free survival time. An *ad hoc* analysis of change in target lesions (sum of target lesion diameters) from baseline was also performed. Safety endpoints included the incidence of treatment-emergent adverse events. Pharmacokinetics of panitumumab were characterized for selected patients.

Tumor *KRAS* Assessments

A retrospective analysis of *KRAS* mutation status (wild-type or mutated) was conducted using existing paraffin-embedded tumor tissues. Most specimens were from the primary tumor; three specimens were from metastatic sites. Samples were tested using the K-RAS Mutation kit (RUO KR-02) from DxS (Manchester, UK), which was the convenient, commercially available method used to detect *KRAS* mutations in the pivotal panitumumab trial. DNA was extracted from paraffin-embedded tumor samples using the QIAamp® DNeasy kit (QIAGEN, Inc., Valencia, CA, USA). All testing was performed by a central laboratory (HistoGeneX, Antwerp, Belgium); personnel performing the assays were blinded to the clinical outcomes.

The K-RAS Kit utilizes the amplification refractory mutation system (ARMS®) (24) for mutation-specific amplification and Scorpions® (25,26) technology to detect the mutations. ARMS technology is based on the observation that *Taq* DNA polymerase is ineffective at amplifying oligonucleotides with a mismatched 3′ residue. Primers for seven specific mutations in the *KRAS* gene (with the mutations appearing at the 3′ end of the primers) are used to amplify

mutated *KRAS* sequences in PCR reactions: Gly12Ala, Gly12Asp, Gly12Arg, Gly12Cys, Gly12Ser, Gly12Val and Gly13Asp. Scorpions, bifunctional molecules comprising a PCR primer covalently linked to a probe, are included in the PCR reaction. The Scorpion probe consists of a fluorophore and a quencher that are separated by the specific probe sequence. Complementary stem sequences flanking the specific probe sequence cause the Scorpion probe to form a hairpin structure in which the fluorophore and quencher are brought together, resulting in loss of fluorescence from the fluorophore. When the Scorpion probe is heat-denatured and then allowed to cool and bind to its target amplicon (a mutated *KRAS* sequence that has been amplified by an ARMS probe), the fluorophore and quencher are separated and fluorescence is increased. The fluorescence is measured in a LightCycler® 480 Instrument (Roche Applied Science, Indianapolis, IN, USA) using software version LCS480 1.2.9.11. This kit has the ability to detect ~1% mutated DNA in a background of wild-type genomic DNA. The failure rate of the *KRAS* assay was 4% in a large data set of patients with mCRC in a prior panitumumab clinical study (18).

Statistical Analyses

All analyses were descriptive evaluations to assess the relationship between clinical outcome and tumor *KRAS* mutation status in Japanese patients with mCRC who had measurable disease at baseline. Because of the small number of patients with samples available for *KRAS* testing, data from the two studies were pooled for these analyses, ignoring the potential heterogeneity in the analysis sets between the studies. All efficacy and safety analyses were performed on the *KRAS* analysis sets (enrolled patients who had: given consent for biomarker analysis; measurable disease at baseline; evaluable *KRAS* status and received at least one dose of panitumumab). Analyses were stratified by *KRAS* status; no other covariates were considered. No hypothesis testing was performed to compare endpoints between wild-type *KRAS* and mutated *KRAS* strata.

For continuous endpoints, the mean and standard deviation (SD) values are provided. The frequency and percentage distributions are provided for discrete data. The objective response rate and its two-sided 95% confidence interval (CI) were calculated; the 95% CI was based on the *F* distribution method (27). Kaplan–Meier estimates for progression-free survival time and 95% CIs were calculated; the 95% CI was based on a sign test (28). No imputation for missing or incomplete data was performed. All analyses were performed using SAS version 8.2 or higher (SAS Institute Inc., Cary, NC, USA) on the Sun/UNIX platform (Sun Microsystems, Inc; Santa Clara, CA, USA).

RESULTS

Patients

Participation in the biomarker analyses was optional in these studies and required additional written consent. Of the 66 patients with mCRC enrolled in the two studies, consent to participate in the study was obtained from 28 patients. Of these, 24 had known *KRAS* status. Patient demographics and disease characteristics at baseline are shown in Table 1.

At the time of data cut-off (12 April 2007), all patients had ended treatment because of disease progression ($n = 28$). All patients completed the protocol-specified safety follow-up. No patient included in this analysis withdrew from the studies. All patients had baseline measurable disease.

KRAS Status

KRAS status was determined in 24 (34%) patients, including 8 patients from Study 20040192 and 16 patients from Study 20050216. Of these, 14 (58%) had tumors with wild-type *KRAS* and 10 (42%) had tumors with mutated *KRAS*. The *KRAS* test failed for four patients: there was insufficient DNA in the tumor samples for three patients, and the tissue failed pathology review (i.e. no tumor sample) for one patient.

Table 1. Patient demographics, disease characteristics and dose assignments at baseline

	Panitumumab		
	Wild-type *KRAS* ($n = 14$)	Mutated *KRAS* ($n = 10$)	All patients ($n = 24$)
Sex, *n* (%)			
Men	9 (64)	4 (40)	13 (54)
Women	5 (36)	6 (60)	11 (46)
Age, years			
Mean (SD)	55.4 (12.3)	63.6 (10.1)	58.8 (11.9)
Range	32–71	45–77	32–77
Primary diagnosis, *n* (%)			
Colon cancer	6 (43)	5 (50)	11 (46)
Rectal cancer	2 (14)	3 (30)	5 (21)
Colorectal cancer[a]	6 (43)	2 (20)	8 (33)
ECOG performance status, *n* (%)			
0	11 (79)	8 (80)	19 (79)
1	3 (21)	2 (20)	5 (21)
Assigned dose cohort in Study 20040192, *n*			
N[b]	6	2	8
2.5 mg/kg QW	3	0	3
6.0 mg/kg Q2W	1	2	3
9.0 mg/kg Q3W	2	0	2

ECOG, Eastern Cooperative Oncology Group; SD, standard deviation; QW, once weekly; Q2W, every 2 weeks; Q3W, every 3 weeks.
[a]Specification of cancer site (colon vs. rectum) was not required in Study 20040192.
[b]Number of patients in Study 20040192 only.

Efficacy Outcomes

In the *KRAS* analysis set, four patients had a partial response to panitumumab therapy; all four responders had tumors expressing wild-type *KRAS* (Table 2). Six patients with tumors with wild-type *KRAS* had stable disease (median duration of 13.2 weeks; 95% CI: 11.1, 15.1). Of patients with tumors expressing mutated *KRAS*, none had a partial response and one had stable disease for 11.4 weeks. Patients with wild-type *KRAS*-expressing tumors had longer progression-free survival (median 13.2 weeks) than patients with mutated *KRAS* (median 7.3 weeks) (Fig. 1).

Table 2. Best objective response and progression-free survival

	Panitumumab		
	Wild-type *KRAS* ($n = 14$)	Mutated *KRAS* ($n = 10$)	All patients ($n = 24$)
Best objective response, *n* (%)			
Partial response	4 (29)	0	4 (17)
Stable disease	6 (43)	1 (10)	7 (29)
Progressive disease	4 (29)	9 (90)	13 (54)
Objective response rate			
Patients with partial response, *n*	4	0	4
Rate, %	28.6	0	16.7
95% CI	8.4–58.1	0–30.9	4.7–37.4
Progression-free survival			
Median weeks	13.2	7.3	7.8
95% CI	8.0–23.1	7.1–7.6	7.4–11.4

CI, confidence interval.

In an *ad hoc* analysis, the maximum decrease in the sum of target lesion diameters was determined for all patients. Of the 14 patients with tumors expressing wild-type *KRAS*, the sum of target lesion diameters was decreased in 10 patients; 4 of these patients had a partial response and 6 patients had stable disease (Fig. 2). The remaining four patients with wild-type *KRAS* in their tumors had an increase in the sum of target lesion diameters and had progressive disease. All patients with mutated *KRAS* in their tumors had an increase in the sum of their target lesion diameters.

Safety Outcomes

All 24 patients in the *KRAS* analysis set experienced an adverse event (related or unrelated to panitumumab therapy) during the study. Six (25%) patients had an adverse event with the worst grade of 3; 3 (13%) patients had an event with the worst grade of 4 and 7 (29%) patients had a serious adverse event. Treatment-related adverse events also occurred in all patients, including two (8%) patients with an adverse event with a worst grade of 3 and one (4%) patient with a serious adverse event. All skin-related events were Grade 1 or 2 in severity. Hypomagnesemia was reported in five (36%) patients with tumors expressing wild-type *KRAS* and in four (40%) patients with tumors expressing mutated *KRAS*. Adverse events occurring in 20% or more of the patients are shown in Table 3. No patient had an investigator-reported adverse event with a preferred term indicative of an infusion reaction or a reaction to panitumumab. No deaths or withdrawals due to adverse events were reported in the *KRAS* analysis set. No marked difference in adverse events was observed based on *KRAS* mutation status.

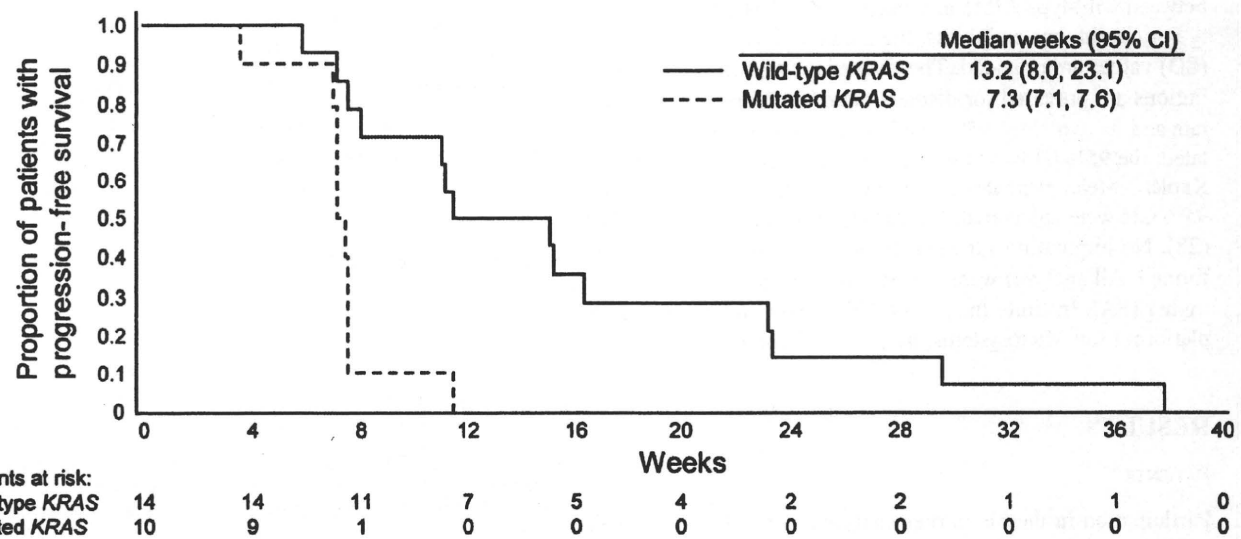

Figure 1. Progression-free survival. The proportion of patients with progression-free survival over time (weeks) is shown.

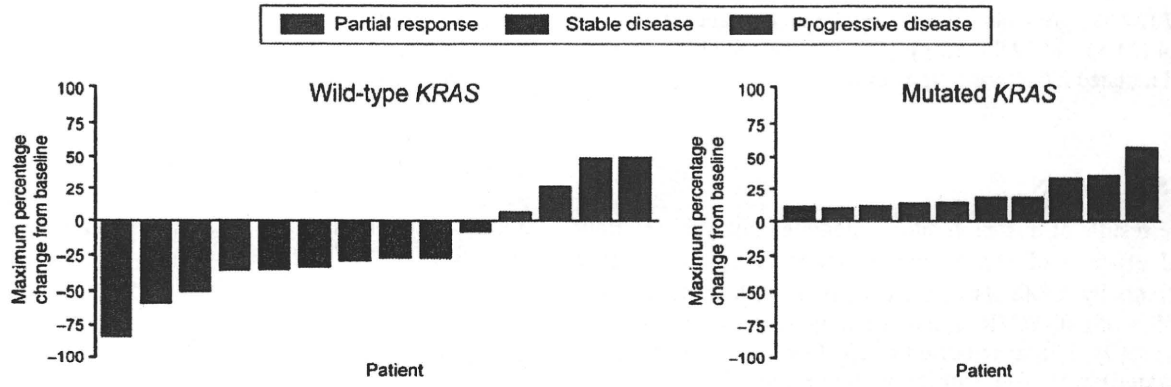

Figure 2. Changes in target lesions by *KRAS* status. The maximum percentage changes in the sum of target lesions for individual patients are shown for patients with tumors expressing wild-type *KRAS* (left panel) and mutated *KRAS* (right panel). The best overall response is designated by green bars for a partial response, blue bars for stable disease and red bars for progressive disease.

Table 3. Summary of adverse events and adverse events occurring in 20% or more of the patients[a]

Adverse event, *n* (%)	Panitumumab					
	Wild-type *KRAS* ($n = 14$)		Mutated *KRAS* ($n = 10$)		All patients ($n = 24$)	
	Any grade	Grade ≥3	Any grade	Grade ≥3	Any grade	Grade ≥3
Patients with any adverse event	14 (100)	6 (43)	10 (100)	3 (30)	24 (100)	9 (38)
Anorexia	7 (50)	3 (21)	8 (80)	0	15 (63)	3 (13)
Dry skin	8 (57)	0	7 (70)	0	15 (63)	0
Fatigue	9 (64)	3 (21)	6 (60)	0	15 (63)	3 (13)
Rash	7 (50)	0	7 (70)	0	14 (58)	0
Paronychia	9 (64)	0	4 (40)	0	13 (54)	0
Acne	6 (43)	0	3 (30)	0	9 (38)	0
Hypomagnesemia	5 (36)	0	4 (40)	0	9 (38)	0
Diarrhea	5 (36)	0	3 (30)	0	8 (33)	0
Pruritus	6 (43)	0	2 (20)	0	8 (33)	0
Back pain	3 (21)	0	3 (30)	0	6 (25)	0
Constipation	6 (43)	0	0	0	6 (25)	0
Acneiform dermatitis	6 (43)	0	0	0	6 (25)	0
Nausea	4 (29)	0	2 (20)	0	6 (25)	0
Abdominal pain	4 (29)	0	1 (10)	0	5 (21)	0
Pyrexia	3 (21)	0	2 (20)	1 (10)	5 (21)	1 (4)

[a]Includes events that were related or unrelated to treatment with panitumumab; adverse events were coded using the Medical Dictionary for Regulatory Activities (MedDRA) version 9.0; severity was graded according to the National Cancer Institute Common Toxicity Criteria (NCI CTC) version 2.0 with the exception of skin toxicities, which were graded using the modified Common Terminology Criteria for Adverse Events (CTCAE) version 3.0.

Pharmacokinetic Evaluation

This pharmacokinetic analysis only included patients who received panitumumab at 6 mg/kg Q2W. Panitumumab pharmacokinetic data were available for 10 patients with tumors expressing mutated *KRAS* (from 2 patients in Study 20040192 and 8 patients in Study 20050216) and 9 patients with tumors expressing wild-type *KRAS* (from 1 patient in Study 20040192 and 8 patients in Study 20050216). The pharmacokinetic profiles of panitumumab were similar between patients with wild-type and mutated *KRAS* status. On the basis of the population pharmacokinetic model (29), the mean (SD) areas under the curves at steady-state were 1110 (385) and 863 (240) μg·day/ml, the mean maximum concentrations were 168 (38.8) and

137 (27.0) μg/ml and the mean minimum concentrations were 38.4 (21.8) and 27.9 (13.2) μg/ml for patients with wild-type and mutated *KRAS* status, respectively.

DISCUSSION

The results of this exploratory integrated analysis of safety and efficacy of panitumumab monotherapy in Japanese patients by *KRAS* status are consistent with those of other studies of anti-EGFR antibodies in patients with mCRC (9–11,18,19). The distribution of *KRAS* status (wild-type vs. mutated) was also similar to those seen in these other studies. Results of efficacy endpoints were favorable in patients with tumors expressing wild-type *KRAS*, and mutated tumor status was associated with lack of response to anti-EGFR therapy. In this limited analysis, response to panitumumab therapy was seen only in patients with tumors expressing wild-type *KRAS*. Patients with tumors with wild-type *KRAS* showed a trend toward longer progression-free survival.

Technical issues in sample processing resulted in the loss of four samples for testing, including lack of sufficient material for testing ($n = 3$) and *KRAS* assay failure ($n = 1$). Assay failure can be caused by inappropriate tissue fixation at the time of tissue collection. It is important, therefore, for investigators enrolling patients in clinical trials to ensure availability of proper materials and procedures for tissue collection. These precautions should enhance the quality and quantity of data obtained in clinical trials for these types of analyses.

The safety profile of panitumumab in this study was also consistent with prior studies of panitumumab monotherapy in patients with mCRC (23,30,31). Skin toxicities and hypomagnesemia are known effects of EGFR inhibition but are generally manageable. Because of the small sample size and the varying doses of panitumumab received by patients in the 20040192 study, it is not possible to draw meaningful conclusions regarding potential differences in the incidence or severity of adverse events between patients with tumors expressing wild-type or mutated *KRAS*.

Panitumumab exhibits pharmacokinetics that are consistent with target-mediated drug disposition, involving saturable binding to EGFR and subsequent internalization and degradation inside the cells (32). In addition, panitumumab is cleared by the reticuloendothelial system, similar to other endogenous immunoglobulins. As it is unlikely that *KRAS* is involved in the clearance of panitumumab, it was not unexpected that pharmacokinetic profiles of panitumumab were similar between patients with tumors expressing mutated *KRAS* and patients with tumors expressing wild-type *KRAS*.

The results of this study also support the need for *KRAS* testing to assist in identification of patients who are unlikely to respond to panitumumab therapy. Expression of EGFR on tumors is a requirement of both the USA (16) and EU (17) labels, although EGFR expression has now been shown to have no predictive value with respect to response to anti-EGFR therapy (4,5). Data from our analysis support the suggestion that panitumumab therapy should be restricted to patients whose tumors express wild-type *KRAS*.

In conclusion, the efficacy of panitumumab in the treatment of mCRC is similar in Japanese patients and Western patients. Additionally, panitumumab efficacy according to *KRAS* status is similar in Japanese and non-Japanese patients.

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Conflict of interest statement

Toshihiko Doi, Makoto Tahara, Takayuki Yoshino and Tomohide Tamura have no disclosures. Kentaro Yamazaki has been granted a contribution or research support from Taiho, Pfizer, Takeda and Chugai. Yasuhide Yamada has been a lecturer and received fees at lectures sponsored by Taiho, Chugai, Novartis, Bayer, Pfizer Japan and Yakult Honsha. Bing-Bing Yang and Kelly Smith Oliner are compensated employees and shareholders of Amgen Inc. Satoru Otani and Daisuke Asahi are compensated employees of Takeda Bio Development Center Limited.

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Phase II study of nedaplatin and docetaxel in patients with advanced squamous cell carcinoma of the lung

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Background: The treatment of squamous cell carcinoma of the lung has not advanced sufficiently. Nedaplatin is a second-generation platinum compound that is active against squamous cell carcinoma of the lung, with a response rate of ~40%.

Patients and methods: Eligible patients with advanced squamous cell carcinoma of the lung were treated with docetaxel (60 mg/m^2) and nedaplatin (100 mg/m^2) administered i.v. on day 1; these doses were determined in an earlier phase I study. The treatment cycles were repeated every 3 weeks. The primary end point was the response rate, and the secondary end points were overall survival, progression-free survival, and toxicity.

Results: Twenty-one patients were enrolled. Eighteen of the patients were male, and the median age was 67 years. The objective response rate was 62%. The median progression-free survival time was 7.4 months. The median survival time was 16.1 months, and the 1-year survival rate was 66.7% (95% confidence interval 46.5% to 86.8%). The most common adverse event was neutropenia (grade 3/4, 86%). Non-hematological toxic effects were relatively mild. One patient died of sepsis.

Conclusions: Combination chemotherapy with nedaplatin and docetaxel is highly active and has an acceptable toxicity. Further investigation of nedaplatin and docetaxel is warranted.

Key words: chemotherapy, docetaxel, nedaplatin, non-small-cell lung cancer, squamous cell carcinoma

introduction

Lung cancer is the leading cause of cancer-related deaths worldwide [1]. More than half of patients with non-small-cell lung cancer (NSCLC) have advanced disease at the time of their diagnoses, and these patients are candidates for treatment with platinum-based combination chemotherapy [2–4]. A recent meta-analysis showed a significant but modest survival benefit of chemotherapy over supportive care alone, with a 9% increase in overall survival at 1 year [5]. Therefore, advances in treatment of NSCLC are urgently needed.

Historically, histological subtypes have not been used to select appropriate treatments for advanced NSCLC. NSCLC consists mainly of adenocarcinoma, squamous cell carcinoma, and large-cell carcinoma, and these classifications are grouped together as a single entity for therapy. However, recent advances in molecular-targeted agents have changed this paradigm [6–8]. Epidermal growth factor receptor (EGFR) tyrosine kinase inhibitors, such as erlotinib and gefitinib, produce dramatic and sustainable responses when used against adenocarcinoma, especially in the presence of an EGFR mutation [9–11]. Bevacizumab, an mAb for vascular endothelial growth factor, produces an additional survival benefit when combined with carboplatin and paclitaxel [12]. However, fatal hemoptysis has been reported in patients with hilar squamous cell carcinoma; therefore, the use of bevacizumab is now restricted to non-squamous NSCLC [13]. Moreover, the novel multitargeted antifolate pemetrexed shows a greater antitumor activity against adenocarcinoma than against squamous cell carcinoma [14]. As a result of these developments, the survival of patients with adenocarcinoma has been improving; however, that of patients with squamous cell carcinoma has remained the same [15].

Nedaplatin is a second-generation platinum compound that is active against NSCLC, especially squamous cell carcinoma, with a response rate of 40% [16, 17]. An earlier phase I study demonstrated that nedaplatin (100 mg/m^2) could be safely administered in combination with docetaxel (60 mg/m^2) [18]. Based on the results of this previous study, we conducted a phase II study to evaluate the efficacy and tolerability of nedaplatin and docetaxel.

patients and methods

patients and evaluations

The eligibility criteria were as follows: histologically or cytologically proven squamous cell carcinoma of the lung; stage III (unresectable and unfit for

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definitive radiotherapy), stage IV, or recurrent disease after surgery; aged 20–75 years; Eastern Cooperative Oncology Group (ECOG) performance status (PS) of zero or one; chemotherapy naive; measurable lesion according to the RECIST version 1.0; and preserved organ function (white blood cell count ≥ 4.0 × 10^9/l, absolute neutrophil count ≥ 2.0 × 10^9/l, platelet count ≥ 100 × 10^9/l, hemoglobin ≥ 9.5 g/dl, total bilirubin ≤ 1.5 mg/dl (0.3–1.2), aspartate aminotransferase ≤ 100 IU/l (13–33), alanine aminotransferase ≤ 100 IU/l (8–42 for male and 6–27 for female), serum creatinine ≤ 1.5 mg/dl (0.6–1.1 for male and 0.4–0.7 for female), and PaO_2 ≥ 60 Torr or oxygen saturation ≥ 94% at ambient air). Patients were excluded if pulmonary fibrosis was visible on a chest radiograph (pulmonary emphysema was allowed), uncontrolled pericardial or pleural effusion that needed immediate drainage was present, symptomatic brain metastasis had occurred, or a concomitant serious illness contraindicating systemic chemotherapy was present. Patients who were pregnant or breast-feeding were also excluded. All the patients provided their written informed consent before enrollment, and the study was approved by the institutional ethics boards of the National Cancer Center.

Clinical status, hematology, biochemistry and chest radiographs were assessed at least every 2 weeks. Disease status was assessed every 2 months. Toxicity was graded using the Common Terminology Criteria for Adverse Events version 3.0.

protocol treatment

Nedaplatin and docetaxel were administered on day 1 every 3 weeks for up to four cycles. Docetaxel diluted in 250 ml of 5% glucose was administered as a 1-h infusion. Nedaplatin was diluted in >300 ml of normal saline or 5% xylitol and was administered as a 90-min i.v. infusion. Dexamethasone (8 mg), granisetron, and fluids (1000 ml) were also administered i.v.

Dose reduction was required if patients experienced the following toxic effects: grade 3 or more non-hematological toxicity (except for nausea, vomiting, anorexia, hyperglycemia, hyponatremia, mucositis, constipation, rash), grade 4 leukopenia or neutropenia lasting for >4 days, grade 4 thrombocytopenia, febrile neutropenia, >14 days delay of the next chemotherapy cycle.

design and statistics

The primary objective was the response rate. The secondary objectives were progression-free survival, overall survival, and toxicity. The tumor responses were evaluated by radiologists. The planned sample size was 21, based on an alpha of 0.05, 90% power, H0 = 20% and H1 = 50% [19]. After the accrual of 12 patients, an interim analysis was planned. If only two or fewer responses had occurred, then the study would be stopped early. If seven or fewer responses were observed by the end of the trial, then no further investigation of this combination was deemed as being warranted. This study was registered with University Hospital Medical Information Network-Clinical Trials Registry, available at http://www.umin.ac.jp/ctr/index-j.htm (ID: UMIN000001227).

results

From August 2006 to January 2009, 21 patients were enrolled. The patient demographics and disease characteristics are summarized in Table 1. All the patients had confirmed squamous cell carcinoma; the median age was 67 years, 18 patients were male, and 16 patients had an ECOG PS of one. Nedaplatin and docetaxel were administered to all the patients. The median number of treatment cycles was 4 (range 1–4). Relative dose intensity for planned cycles of chemotherapy was 77.9% for both docetaxel and nedaplatin.

efficacy

At the interim analysis, 7 of 13 patients had achieved a partial response. Therefore, the accrual continued up to 21 patients. At the end of the study, the objective response rate was 62% (Figure 1 and Table 2). Figure 2 shows the progression-free survival. The median progression-free survival time was 7.4 months [95% confidence interval (CI) 3.5–11.4 months], and the progression-free survival rate at 1 year was 24.8% (95% CI 4.6% to 44.9%). Figure 3 shows the overall survival. The median overall survival time was 16.1 months (the median follow-up time was 20.9 months for the censored cases), and the overall survival rate at 1 year was 66.7% (95% CI 46.5% to 86.8%). Post-study treatment included radiotherapy (nine for

Table 1. Patient characteristics

	Patients (n = 21)	
	n	%
Age, median (range) (years)	67 (40–78)	
Gender		
Female	3	14
Male	18	86
ECOG performance status		
0	5	24
1	16	76
Smoking, median (range) (pack-years)	49 (0–100)	
Never smoker	1	5
Former smoker	6	29
Current smoker	14	67
Stage		
III	11	52
IV	8	38
Recurrence	2	10

ECOG, Eastern Cooperative Oncology Group.

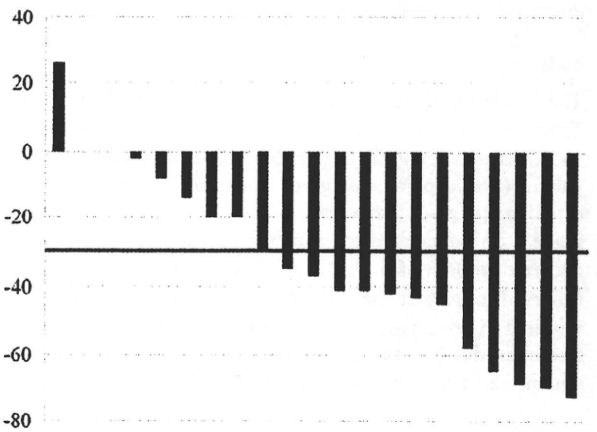

Figure 1. Waterfall plots for the degree of tumor shrinkage. Most patients achieved tumor shrinkage. Partial response was observed in 13 patients and the response rate was 62%.

Table 2. Objective response (RECIST version 1.0)

	n	%
Number of patients evaluated	21	
CR	0	0
PR	13	62
SD	6	29
PD	1	5
NE	1	5
Response rate (95% CI), %		62 (39–85)

CR, complete response; PR, partial response; SD, stable disease; PD, progressive disease; NE, not evaluable; CI, confidence interval.

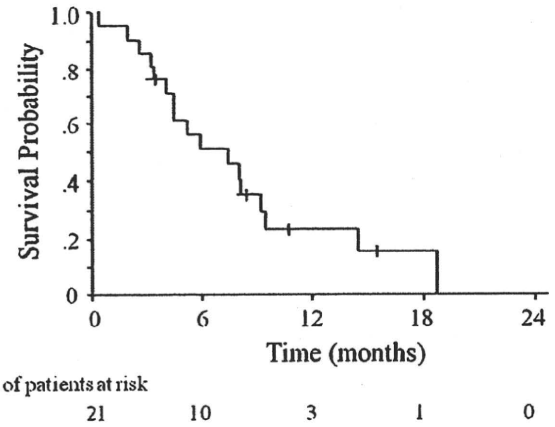

Figure 2. Progression-free survival. The median progression-free survival time was 7.4 months (95% CI 3.5–11.4 months), and the progression-free survival rate at 1 year was 24.8% (95% CI 4.6% to 44.9%). CI, confidence interval.

local tumors, two for lymph node metastases, one for bone metastasis, and one for brain metastasis) and systemic chemotherapy (seven with gemcitabine and vinorelbine, two with gemcitabine monotherapy, two with nedaplatin and docetaxel rechallenge, and four others).

safety

The incidence of treatment-related adverse events is listed in Table 3. Non-hematologic toxicity was generally grade 1 or 2 and consisted primarily of gastrointestinal disorders, with one patient (5%) experiencing grade 3 anorexia and two (10%) experiencing grade 3 nausea. None of the patients experienced grade 3 or higher diarrhea. Grade 3 or higher neutropenia occurred in 86% of the patients, and febrile neutropenia was observed in 24% of the patients. Dose modification was required in seven patients (33%). One patient died of sepsis on treatment day 9 of the first cycle. An autopsy revealed that the patient had previously undiagnosed liver cirrhosis.

discussion

Nedaplatin is a second-generation platinum compound and is comparable in efficacy with cisplatin against NSCLC, with

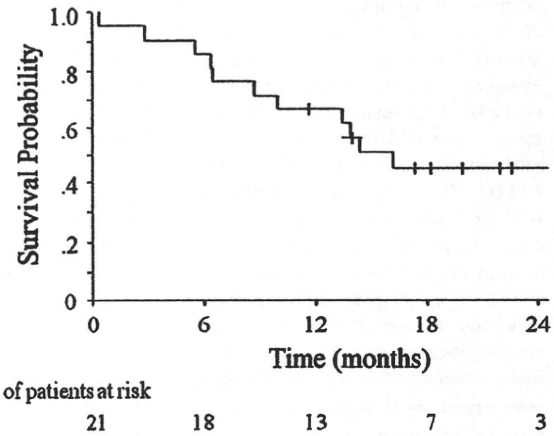

Figure 3. Overall survival. The median overall survival time was 16.1 months (the median follow-up time was 20.9 months for the censored cases), and the overall survival rate at 1 year was 66.7% (95% CI 46.5% to 86.8%). CI, confidence interval.

Table 3. Adverse events (NCI–CTC version 3.0, $n = 21$)

Adverse event	Grade 1–2 (%)	Grade 3 (%)	Grade 4 (%)
Leukocytes	43	48	10
Neutrophils/granulocytes	14	29	57
Hemoglobin	90	5	0
Platelets	86	10	5
AST	43	0	0
ALT	29	0	0
Creatinine	14	0	0
Febrile neutropenia	0	14	10
Anorexia	67	5	0
Nausea	52	10	0
Vomiting	24	0	0
Diarrhea	48[a]	0	0
Alopecia	76	0	0

[a]24% grade 2.

NCI–CTC, National Cancer Institute—Common Toxicity Criteria; AST, aspartate aminotransferase; ALT, alanine aminotransferase.

a reduced renal toxicity. A randomized trial comparing nedaplatin and vindesine with cisplatin and vindesine showed similar survival outcomes, with a median survival time of 8.9 months for nedaplatin–vindesine and 9.1 months for cisplatin–vindesine [20]. However, only 36 of the 136 patients in this series had squamous cell carcinoma of the lung.

Nedaplatin has been combined with gemcitabine, irinotecan, or paclitaxel in patients with NSCLC and with docetaxel mainly in patients with esophageal cancer [21–33].

Nedaplatin is more active against squamous cell carcinoma of the lung than adenocarcinoma as a single agent, based on the results of phase II trials [16, 17]; however, the reason for this difference in the antitumor activity among histological subtypes has not been fully investigated. A preclinical study demonstrated that in squamous carcinoma cells (PC-10), the intracellular concentration of nedaplatin promptly rose and

reached a higher concentration than that in adenocarcinoma cells (PC-3), suggesting a higher antitumor activity of nedaplatin against squamous cell carcinoma [34].

The response rate of 62% in the current study met the criteria for further investigation. Furthermore, the median progression-free survival time of 7.4 months and the median overall survival time of 16.1 months were promising. A selection bias may have caused these survival outcomes. However, the median survival time of 16.1 months is comparable with that of stage III disease treated with definitive chemoradiotherapy and suggested a potential benefit of nedaplatin and docetaxel against squamous cell carcinoma of the lung.

In patients with unresectable squamous cell carcinoma, nedaplatin was also combined with paclitaxel in a phase I trial [35]. The response rate was 55%; however, paclitaxel was reported to be correlated with a shorter progression-free survival time compared with gemcitabine, docetaxel, and vinorelbine based on a meta-analysis [36]. Docetaxel is one of the most promising agents against NSCLC, including squamous cell carcinoma. In the TAX 301 JP trial, the combination of cisplatin and docetaxel showed a statistically significant survival benefit over cisplatin and vindesine [37]. A subgroup analysis showed similar survival benefits for patients with adenocarcinoma and those with squamous cell carcinoma. Therefore, nedaplatin and docetaxel is the most promising combination against squamous cell carcinoma of the lung.

Grade 3 or more neutropenia was observed in 86% of patients in the current study. High incidence of grade 3 or more neutropenia with 84% (74 of 88 patients) treated with docetaxel and cisplatin was also observed in the randomized phase III trial WJOG3405, which compared gefitinib with cisplatin and docetaxel in patients with EGFR-mutant NSCLC in Japan [38]. Moreover, single-agent docetaxel at a dose of 60 mg/m^2 resulted in 84.6% (632 of 747) of patients with grade 3 or more neutropenia [39]. In contrast, in the international phase III trial TAX 326 comparing docetaxel plus platinum with vinorelbine plus cisplatin, grade 3 or more neutropenia was observed in 74.5% of patients treated with cisplatin and docetaxel [40]. The reason for this difference is not known. Ethnic difference between the Japanese and the Caucasian is one of the explanations. However, in TAX 301 JP conducted in Japan, grade 3 or more neutropenia in patients treated with cisplatin and docetaxel is 74.1% (112 of 151), which is similar with TAX 326. Neutropenia should be carefully managed and the risk factor of serious neutropenia should be further examined.

In the current study, one patient died of sepsis. The autopsy revealed that the patient was affected by liver cirrhosis, which was been unaware of before treatment. Full dose of docetaxel is not appropriate for patients with impaired liver function and the administration of docetaxel in patients with liver cirrhosis should be avoided [41]. Other toxic effects were relatively mild.

In conclusion, the combination of nedaplatin and docetaxel showed a promising response rate and overall survival time, with acceptable toxic effects. A multicenter phase III trial comparing nedaplatin and docetaxel with cisplatin and docetaxel is currently under way (WJOG 5208L).

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disclosure

The authors declare no conflict of interest.

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CLINICAL INVESTIGATION

PHASE I STUDY OF CONCURRENT HIGH-DOSE THREE-DIMENSIONAL CONFORMAL RADIOTHERAPY WITH CHEMOTHERAPY USING CISPLATIN AND VINORELBINE FOR UNRESECTABLE STAGE III NON–SMALL-CELL LUNG CANCER

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Purpose: To determine the maximum tolerated dose in concurrent three-dimensional conformal radiotherapy (3D-CRT) with chemotherapy for unresectable Stage III non–small-cell lung cancer (NSCLC).

Patients and Methods: Eligible patients with unresectable Stage III NSCLC, age ≥20 years, performance status 0–1, percent of volume of normal lung receiving 20 GY or more (V_{20}) ≤30% received three to four cycles of cisplatin (80 mg/m^2 Day 1) and vinorelbine (20 mg/m^2 Days 1 and 8) repeated every 4 weeks. The doses of 3D-CRT were 66 Gy, 72 Gy, and 78 Gy at dose levels 1 to 3, respectively.

Results: Of the 17, 16, and 24 patients assessed for eligibility, 13 (76%), 12 (75%), and 6 (25%) were enrolled at dose levels 1 to 3, respectively. The main reasons for exclusion were V_{20} >30% (n = 10) and overdose to the esophagus (n = 8) and brachial plexus (n = 2). There were 26 men and 5 women, with a median age of 60 years (range, 41–75). The full planned dose of radiotherapy could be administered to all the patients. Grade 3–4 neutropenia and febrile neutropenia were noted in 24 (77%) and 5 (16%) of the 31 patients, respectively. Grade 4 infection, Grade 3 esophagitis, and Grade 3 pulmonary toxicity were noted in 1 patient, 2 patients, and 1 patient, respectively. The dose-limiting toxicity was noted in 17% of the patients at each dose level. The median survival and 3-year and 4-year survival rates were 41.9 months, 72.3%, and 49.2%, respectively.

Conclusions: 72 Gy was the maximum dose that could be achieved in most patients, given the predetermined normal tissue constraints. © 2011 Elsevier Inc.

Lung cancer, Chemotherapy, Radiotherapy, High dose, Conformal.

INTRODUCTION

Approximately one third of patients with non–small-cell lung cancer (NSCLC) present with locally advanced Stage III disease at the initial diagnosis (1). Of this category, Stage IIIA disease with bulky N2 and Stage IIIB disease without pleural effusion are characterized by a large primary lesion and/or involvement of the mediastinal or supraclavicular lymph nodes. In addition, the majority of these patients have occult systemic micrometastases. Concurrent thoracic radiotherapy and chemotherapy has been the standard care for these patients with unresectable disease (2, 3). A platinum doublet with a third-generation anticancer agent combined with thoracic radiotherapy was reported to yield a median overall survival time (OS) of more than 2 years and long-term survivors (4–6), but the effect of platinum-based chemotherapy has reached a plateau.

The failure pattern in patients with Stage III NSCLC treated by concurrent chemoradiotherapy was roughly local recurrence alone in one third of the patients, both local and distant recurrence in another third of patients, and distant metastasis without local failure in the remaining third of patients (2, 5).

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Thus, improvement of local control and suppression of distant metastasis are essential for prolongation of patient survival.

The conventional total dose of thoracic radiotherapy in patients with inoperable NSCLC has been 60 Gy administered in 30 fractions. This dose was established in 1987 by randomized Radiation Therapy Oncology Group trials that demonstrated better 3-year survival with a radiation dose of 60 Gy than with lower doses (7). In these trials, two-dimensional treatment planning was used, wherein the tumor volume was defined on kilovoltage radiographs (7). Thereafter, the standard initial target volume included the primary tumor, metastatic lymph nodes, and adjacent uninvolved ipsilateral hilar and mediastinal regions (elective nodal irradiation: ENI). Except for selected patients, excessive toxicity hampered an increase of the total dose to over 60 Gy in patients with locally advanced NSCLC.

It is, however, time now to reconsider the optimal dose of thoracic radiotherapy using new techniques in patients with locally advanced NSCLC, for the following reasons. First, positron emission tomography (PET) provides more accurate diagnosis of mediastinal lymph node metastases (8) and more accurate quantification of the tumor volumes, especially when atelectasis is present (9). Second, three-dimensional conformal radiation therapy (3D-CRT) enables radiation oncologists to delineate the tumor and adjacent normal tissue more sharply and to choose beam angles to maximize tumor coverage with minimum irradiation of normal tissues (10). Third, omission of the ENI resulted in improvement of radiation-associated toxicity without worsening the local control rate of the tumor (11, 12). Thus, by use of these new techniques, the optimal dose of thoracic radiation could exceed the conventional 60 Gy.

Two dose escalation studies in patients with locally advanced NSCLC showed that the total dose of thoracic radiotherapy could be increased up to 90 Gy in concurrent chemoradiotherapy using the 3D-CRT technique combined with weekly carboplatin and paclitaxel chemotherapy (13, 14). In these trials, chemoradiotherapy was administered after induction chemotherapy. However, it remained unclear whether these doses could be delivered safely to the majority of patients with locally advanced NSCLC, because it is not known how many patients were screened for the trials and how many of them were actually registered, and because some of the registered patients were excluded from the chemoradiotherapy phase after induction chemotherapy. The total number of patients evaluated in the two trials was also limited. Furthermore, chemotherapy other than weekly carboplatin and paclitaxel has not been evaluated in the setting of combined chemotherapy with high-dose thoracic radiotherapy, to our knowledge. The objectives of the current study were (1) to evaluate the toxicity of concurrent high-dose 3D-CRT without ENI with cisplatin and vinorelbine for unresectable Stage III NSCLC, (2) to determine the maximum tolerated dose (MTD) of thoracic radiotherapy, and (3) to observe the antitumor effects of this regimen.

PATIENTS AND METHODS

Study design

This study was designed as a Phase I study at the National Cancer Center Hospital. The protocol and consent form were approved by the Institutional Review Board of the National Cancer Center on July 28, 2005. We planned to treat 12 patients at a dose level and follow them up at least 6 months, and then escalate to the next level if 67% of the patients did not experience dose-limiting toxicity (DLT). We followed widely accepted normal tissue dose constraints. Patients with percent volume of the normal lung receiving 20 Gy or more (V_{20}) of greater than 30% were excluded and treated outside the study. Other dosimetric constraints were applied at the discretion of the treating radiation oncologist. Maximum doses exceeding 50 Gy to the spinal cord, 66 Gy to the esophagus, or 66 Gy to the brachial plexus were generally excluded.

Patient selection

Previously untreated patients with locally advanced NSCLC without effusion were screened for entry into this study. The eligibility criteria were (1) histologically or cytologically proven NSCLC, (2) unresectable Stage IIIA or IIIB disease confirmed by both computed tomography (CT) and PET, (3) no previous treatment, (4) measurable disease, (5) $V_{20} \leq 30\%$, (6) age ≥ 20 years, (7) Eastern Cooperative Oncology Group performance status (PS) of 0 or 1, and (8) adequate bone marrow function (white blood cell [WBC] count $\geq 4.0 \times 10^9$/L, hemoglobin ≥ 9.5 g/dL, and platelet count $\geq 100 \times 10^9$/L), liver function (total bilirubin ≤ 1.5 mg/dL and transaminase ≤ 80 IU/L), renal function (serum creatinine ≤ 1.5 mg/dL), and pulmonary function ($PaO_2 \geq 70$ Torr under room air). Patients were excluded if (1) they had malignant pleural or pericardial effusion or (2) they had a concomitant serious illness such as uncontrolled angina pectoris, myocardial infarction in the previous 3 months, heart failure, uncontrolled diabetes mellitus, uncontrolled hypertension, interstitial pneumonitis or lung fibrosis identified by a chest x-ray, infection, or other diseases contraindicating chemotherapy or radiotherapy, or (3) they were pregnant or breast feeding. All patients gave their written informed consent.

Pretreatment evaluation

The pretreatment assessment included a complete blood cell count and differential count, routine chemistry determinations, creatinine clearance, blood gas analysis, electrocardiogram, lung function testing, chest x-rays, chest CT scan, brain CT scan or magnetic resonance imaging, abdominal CT, and PET.

Treatment schedule

Chemotherapy consisted of cisplatin 80 mg/m^2 on Day 1 and vinorelbine 20 mg/m^2 on Days 1 and 8, repeated every 4 weeks for three to four cycles. Cisplatin was administered by intravenous infusion for 60 minutes with 2,500 to 3,000 mL of intravenous fluid for hydration and prophylactic antiemetic therapy consisting of a 5-hydroxytriptamine-3 antagonist on Day 1 and a corticosteroid on Days 1 to 5. Vinorelbine, diluted in 50 mL of normal saline, was administered intravenously.

Radiation therapy started on Day 1 of the first cycle of chemotherapy and was delivered with megavoltage equipment (6–10 MV) once daily for 5 days a week. The total dose was 66 Gy in 33 fractions at level 1, 72 Gy in 36 fractions at level 2, and 78 Gy in 39 fractions at level 3. All patients underwent a 3D treatment planning CT 3 to 7 days before the start of the treatment, and the eligibility was finally confirmed based on evaluation using the

dose–volume histogram (DVH). The gross tumor volume (GTV) was defined as the primary tumor delineated on pulmonary windows of the chest CT or on the diagnostic PET scans. Atelectasis or secondary changes in the peripheral lung region of the primary tumor were not included. Metastatic lymph nodes defined as nodes of 1 cm or larger visualized on mediastinal windows of the CT images or PET-positive lymph nodes were also included in the GTV. The clinical target volume (CTV) was equivalent to the GTV. Uninvolved mediastinum or supraclavicular fossae were not included in the CTV. The planning target volume (PTV) was determined as the CTV plus 1.0 cm for the anterior, posterior, medial, and lateral margins and a 1.0 to 2.0 cm for the superior and inferior margins, taking account of setup variations and internal organ motion. The spinal cord dose was typically limited to 44 Gy, but a maximum of 50 Gy was allowed. The lung V_{20} was limited to 30% in all patients. The maximum dose to the brachial plexus and esophagus did not exceed 66 Gy. The 100% dose was prescribed to the reference point located in the central part of the PTV, and the entire PTV was covered with 95–107% of the prescribed dose principally, but variation of ±10% was allowed. Lung heterogeneity corrections using the equivalent path length algorithm were applied in all patients.

Toxicity assessment and treatment modification

Complete blood cell counts and differential counts, routine chemistry determinations, and a chest x-ray were performed once a week during the course of treatment. Toxicity was graded according to the Common Terminology Criteria for Adverse Events (CTCAE v3.0). The lung toxicity grade was defined as the highest grade among cough, dyspnea, obstruction/stenosis of airways, pneumonitis/pulmonary infiltrates, and pulmonary fibrosis in the pulmonary/upper respiratory section (15).

Vinorelbine administration on Day 8 was omitted if any of the following were noted: WBC count $<3.0 \times 10^9$/L, neutrophil count $<1.5 \times 10^9$/L, platelet count $<100 \times 10^9$/L, Grade 2–3 elevation of the serum hepatic transaminase level or total serum bilirubin levels, Grade 2–3 infection, Grade 2–3 pneumonitis, other ≥Grade 3 nonhematologic toxicity, body temperature ≥38°C, or PS of 2–3. Subsequent cycles of cisplatin and vinorelbine chemotherapy were delayed if any of the following toxicities were noted on Day 1: WBC count $<3.0 \times 10^9$/L, neutrophil count $<1.5 \times 10^9$/L, platelet count $<100 \times 10^9$/L, serum creatinine level ≥1.6 mg/dL, Grade 2–3 elevation of the serum hepatic transaminase level or total serum bilirubin levels, Grade 2–3 infection, Grade 2–3 pneumonitis, other ≥Grade 3 nonhematologic toxicity, body temperature ≥38°C, or PS of 2–3. If these toxicities did not recover within 6 weeks from Day 1 of the previous cycle of chemotherapy, subsequent cycles of chemotherapy were stopped. The dose of cisplatin was reduced by 25% in all subsequent cycles if the serum creatinine level rose to 2.0 mg/dL or higher. The dose of vinorelbine was reduced by 25% in all subsequent cycles if any of the following toxicities were noted: WBC count $<1.0 \times 10^9$/L, platelet count $<25 \times 10^9$/L, or Grade 3 infection or liver dysfunction. Thoracic radiotherapy was suspended if any of the following were noted: body temperature ≥38°C, Grade 3 esophagitis, PS of 3, or suspected radiation pneumonitis. Thoracic radiotherapy was terminated if any of the following were noted: Grade 4 esophagitis, Grade 3 or 4 pneumonitis, PS of 4, or duration of radiotherapy of over 62 days (level 1), 67 days (level 2), or 70 days (level 3). Any protocol-defined treatments were terminated if Grade 4 nonhematologic toxicities other than transient electrolyte disturbances or a PS of 4 was noted.

Dose-limiting toxicity and maximum tolerated dose

The DLT was defined as the following toxicities observed during a 6-month period from the start of treatment: (1) Grade 3 esophagitis, lung toxicity, myelitis, dermatitis associated with radiation, and cardiac toxicity associated with radiation, (2) Grade 4 nonhematologic toxicity, or (3) treatment termination due to prolonged toxicity. Twelve patients were enrolled at each dose level. All patients were followed up for at least 6 months to evaluate DLT. During the period, if none to 4 of the 12 patients experienced DLT, the next cohort of patients was treated at the next higher dose level. If 5 or more of the 12 patients experienced DLT, that level was considered to be the MTD. The recommended dose for Phase II trials was defined as the dose preceding the MTD.

Response evaluation

Objective tumor response was evaluated according to the Response Evaluation Criteria in Solid Tumors (RECIST) ver. 1.0 (16).

Follow-up

Patients who completed the protocol therapy were followed up to monitor toxicity, response, and recurrence. CT of the chest was performed every 2 to 4 months for 1 year, every 6 months for 2 years, and then yearly for 2 years. The relapse pattern was categorized into (1) local alone, including relapse from the primary site or the hilar, mediastinal, or supraclavicular lymph nodes, (2) distant metastasis alone, including pleural dissemination, pleural and pericardial effusions, and distant metastases, and (3) local and distant.

Statistical analyses

Progression-free survival time (PFS) and OS were estimated by the Kaplan-Meier method. The PFS was measured from the date of registration to the date of disease progression or death resulting from any cause or date of last follow-up. The OS was measured from the date of registration to the date of death resulting from any cause or date of last follow-up. Patients who were lost to follow-up without events were censored at the date of their last known follow-up. A confidence interval (CI) for the response rate was calculated by the method used for exact binomial CIs. The Dr. SPSS II 11.0 software package for Windows (SPSS Japan Inc., Tokyo, Japan) was used for the statistical analyses.

RESULTS

Registration and characteristics of the patients

From August 2005 to September 2008, 57 patients were deemed to initially be eligible. Of these, 3 patients were excluded because idiopathic interstitial pneumonitis ($n = 1$) and anemia ($n = 2$) developed. Explanation of the study using the consent form was given to 54 patients, and informed consent was obtained in 51 patients. The 51 patients underwent 3D treatment planning, and eligibility was finally confirmed in 31 patients. Those 31 were enrolled into this study. A total of 20 patients were excluded as a result of the DVH evaluation: because of V_{20} higher than 30% in 10 patients, overdose to the esophagus in 8 patients, and overdose to the brachial plexus in 2 patients. Eventually, of 17 patients assessed as to their eligibility for dose level 1, 16 patients for dose level 2, and 24 patients to dose level 3, 13 (76%), 12 (75%), and 6 (25%) patients were actually enrolled into levels 1 to 3, respectively (Fig. 1).

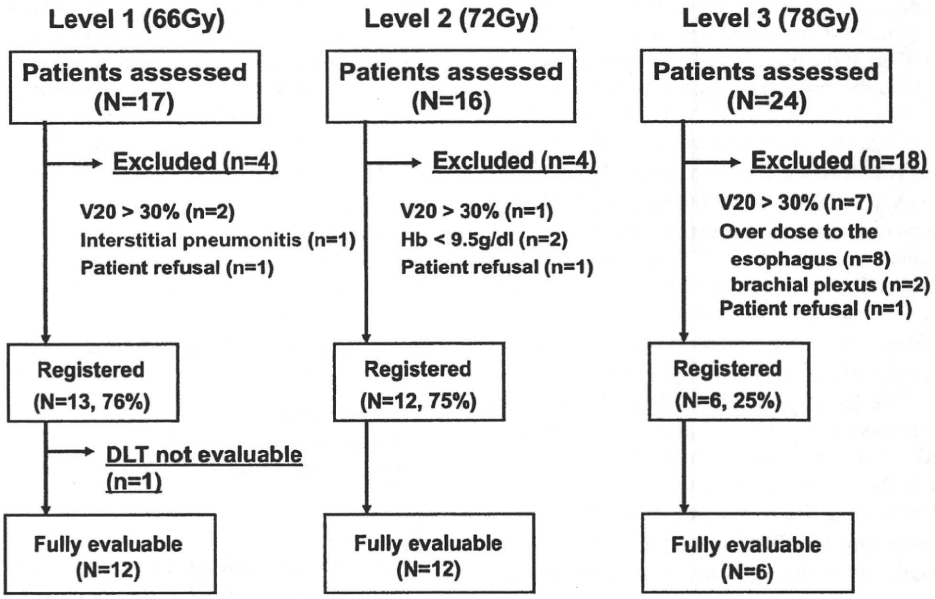

Fig. 1. Algorithm illustrating the flow of the patients. Of the 17, 16, and 24 patients assessed for eligibility, 13 (76%), 12 (75%), and 6 (25%) were actually enrolled at dose levels 1, 2, and 3, respectively.

The pretreatment characteristics of the patients enrolled in this trial are shown in Table 1. The majority of the patients were in good general condition, with a PS of 0 in 25 (81%) and no weight loss in 26 (84%) patients. Adenocarcinoma was the predominantly encountered histological characteristic, seen in 23 (74%) patients.

Treatment delivery

The treatment delivery to the patients was fairly good (Table 2). The planned dose of radiotherapy was administered to all patients of all the three dose levels. More than 80% of the patients received three to four cycles of chemotherapy without or with only one omission of vinorelbine on Day 8, regardless of the dose levels.

Toxicity and DLTs

The hematologic toxicity was comparable to that of other concurrent chemoradiotherapy (Table 3). Grade 4 septic shock was encountered during the fourth cycle of chemotherapy in 1 patient enrolled at dose level 1, but it was manageable by standard care with antibiotics. Other nonhematologic toxicities were mild and acceptable.

Table 1. Patient characteristics

Characteristic	*n*	(%)
Sex		
M	26	(84)
F	5	(16)
Age (y)		
Median (range)	60	(41–75)
Performance status		
0	25	(81)
1	6	(19)
Body weight loss (%)		
0	26	(84)
0.1–5.0	2	(6)
≤5.0	3	(10)
Histology		
Adenocarcinoma	23	(74)
Squamous cell carcinoma	4	(13)
NSCLC, not otherwise specified	4	(13)
Stage		
IIIA	20	(65)
IIIB	11	(35)

Abbreviation: NSCLC = non–small-cell lung cancer.

Table 2. Treatment delivery

	Level 1 (n = 13)	Level 2 (n = 12)	Level 3 (n = 6)
Radiotherapy			
Total dose (Gy)			
66	13 (100)	–	–
72	–	12 (100)	–
78	–	–	6 (100)
Delay (days)			
≤5	11 (85)	5 (42)	5 (83)
6–10	2 (15)	6 (50)	0
11–15	0	1 (8)	1 (17)
Chemotherapy			
No. of cycles			
4	6 (46)	6 (50)	4 (67)
3	6 (46)	4 (33)	2 (33)
2	0	1 (8)	0
1	1 (8)	1 (8)	0
No. of VNR omissions			
0	10 (77)	7 (58)	2 (33)
1	2 (15)	4 (33)	3 (50)
2	0	0	1 (17)
3	1 (8)	1 (8)	0

Abbreviation: VNR = vinorelbine administered on Day 8.

Table 3. Toxicity

	Grade										
	Level 1			(n = 13)	Level 2			(n = 12)	Level 3			(n = 6)
Toxicity	2	3	4	(3+4 %)	2	3	4	(3+4 %)	2	3	4	(3+4 %)
Leukopenia	4	6	2	(62)	1	3	8	(92)	1	3	2	(83)
Neutropenia	4	4	4	(62)	0	1	10	(92)	1	3	2	(83)
Anemia	8	2	2	(31)	7	3	1	(33)	2	2	0	(50)
Thrombocytopenia	0	0	0	(0)	1	1	0	(8)	0	0	0	(0)
Febrile neutropenia	–	1	0	(8)	–	3	0	(25)	–	1	0	(17)
Infection	0	0	1	(8)	0	1	0	(8)	2	0	0	(0)
Esophagitis	1	1	0	(8)	2	1	0	(8)	0	0	0	(0)
Lung toxicity	2	0	0	(0)	0	0	0	(0)	0	1	0	(17)
Anorexia	3	0	0	(0)	2	2	0	(17)	0	0	0	(0)
Nausea	3	0	0	(0)	3	0	0	(0)	0	0	0	(0)
ALT elevation	1	1	0	(8)	0	0	0	(0)	1	0	0	(0)
CRN elevation	7	0	0	(0)	4	0	0	(0)	0	0	0	(0)

Abbreviations: ALT = alanine aminotransferase; CRN = creatinine.

Of the 13 patients at dose level 1, one was excluded from the analysis of the DLT because he received only one cycle of chemotherapy as a result of the development of cisplatin-induced renal toxicity. Two (17%) of the remaining 12 patients at this dose level developed DLT: Grade 3 esophagitis in 1 patient and Grade 4 septic shock in the other. At dose level 2, two (17%) DLTs were noted: Grade 3 esophagitis in 1 patient and treatment delay by more than 15 days in the other. One (17%) of the 6 patients at dose level 3 developed Grade 3 bronchial stenosis without local recurrence of the disease. This was considered to be a Grade 3 lung toxicity and was counted as DLT. No other DLTs were noted. Thus, inasmuch as the incidence of DLT was below 33% at all dose levels, MTD was not reached.

Preliminary efficacy results

Objective responses and survival were evaluated in the 31 patients. Two patients showed complete responses and 27 showed partial responses, which represented a response rate (95% CI) of 94% (79–99). Disease progression was noted in 23 patients, and the median PFS was 11.6 months with a median duration of follow-up of 30.5 (range, 9.0–49.5) (Fig. 2). The first relapse sites aresummarized in Table 4. Brain metastasis alone as the first relapse site was noted in 7 (23%) patients. The median OS was 41.9 months, and the 2-, 3-, and 4-year survival rates (95% CI) were 83.6% (65.0–92.8), 72.3% (51.9–85.2), and 49.2% (26.2–68.7), respectively (Fig. 3).

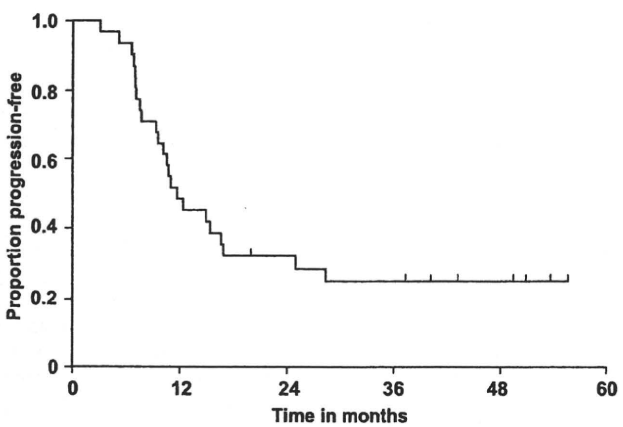

Fig. 2. Progression-free survival (n = 31). The median progression-free survival was 11.6 months, with a median duration of follow-up of 30.5 months (range, 9.0–49.5 months).

DISCUSSION

This study showed that concurrent 3D-CRT to the thorax with cisplatin plus vinorelbine chemotherapy was safe even up to 78 Gy in patients with unresectable Stage III NSCLC. This does not mean, however, that doses as high as 78 Gy can be given to all patients with this disease, because the safety in this study was shown only in highly selected patients by a PET/CT and DVH evaluation and by the standard staging procedure. Twenty-five of the 33 patients met the eligibility criteria for enrollment at dose levels 1 and 2, whereas only 6 of the 24 patients could be enrolled at dose level 3 in this study—that is, only one fourth of the patients could be treated with 78 Gy. Thus, this study showed that 72 Gy was the maximum dose that could be achieved in most patients given the predetermined normal tissue constraints, which forced three quarters of the enrolled patients at the 78-Gy level to not be eligible on the basis of those normal

Table 4. First relapse sites (n = 31)

Sites	n	(%)
Local recurrence alone	6	(19)
Local and distant metastasis	6	(19)
Distant metastasis alone	11	(35)
Brain alone	7	(23)
No relapse	8	(26)

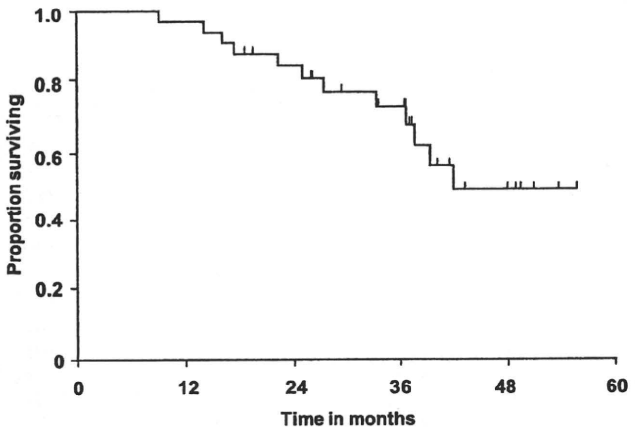

Fig. 3. The median overall survival was 41.9 months, and the 2-, 3-, and 4-year survival rates (95% CI) were 83.6% (65.0–92.8), 72.3% (51.9–85.2), and 49.2% (26.2–68.7), respectively.

tissue constraints, and that the maximum tolerated dose was not determined because of this issue.

One obstacle to enrolling patients at dose level 3 was that the lung V_{20} often exceeded 30% when the total dose was increased to 78 Gy. This lung V_{20} dose constraint might have been too strict. According to a recent review, it is prudent to limit V_{20} to ≤30–35% with conventional fractionation, but there is no sharp dose threshold below which there is no risk for severe radiation pneumonitis (17). This is partly because DVH-based parameters will change at specific phases of the respiratory cycle when CT images for DVH evaluation have been obtained, there is uncertainty regarding how much of the bronchus should be defined as lung, and the lung edges may vary with the CT window level setting. In addition, patient-associated factors such as age, smoking status, lung function, and preexisting lung damage may influence the incidence and severity of radiation pneumonitis (18). If the threshold of V_{20} were set at higher than 30% (*e.g.*, 35%), then more patients would meet the eligibility criteria, but safety might not be guaranteed. Given that the definite threshold cannot be determined, a strict constraint should be introduced. This study showed that the lung toxicity was acceptable when the V_{20} was kept within 30%; therefore, we decided to use this eligibility criterion for concurrent chemotherapy and high-dose radiotherapy for a subsequent Phase II study.

Another obstacle was overdose to the esophagus and brachial plexus, which were close to the subcarinal (No. 7) and supraclavicular lymph nodes, respectively, that were frequently involved in patients with advanced NSCLC; therefore, the volume of these serial organs were included, in part, in the PTV in many patients with Stage III disease. The radiation tolerance doses of these organs have been defined as no higher than 72 Gy when one third of the organs are included in the irradiation volume (19). However, few data are available on the radiation tolerance doses of normal organs in humans; therefore, whether or not radiation doses above 72 Gy may be tolerated is unknown, especially when only small percentages of the organs are actually included in the irradiation volume. Notwithstanding, we do not agree that the radiation dose can be increased close to the intolerable level, because serious radiation toxicity to these serial organs could be irreversible, frequently leaves severe sequelae, and is fatal in some cases.

The toxicity observed in this trial was comparable to that in our previous study of concurrent chemoradiotherapy with vinorelbine and cisplatin chemotherapy plus thoracic radiation at a total dose of 60 Gy administered in 30 fractions: Grade 3–4 neutropenia in 77% and 67% of patients, Grade 3–4 esophagitis in 6% and 12% of patients, and Grade 3–5 lung toxicity in 3% and 7% in the current and previous studies, respectively (5). This suggests that patient selection using PET/CT and DVH evaluation may be useful to keep the toxicity associated with high-dose thoracic radiation within the range of toxicity induced by conventional-dose thoracic radiation.

In this study, a remarkably high proportion (74%) of subjects had adenocarcinoma, which may provide an explanation for the high rate of subsequent brain metastases. Patient selection also affects the treatment efficacy considerably; therefore, it is difficult to compare it between the current and previous studies. However, the median PFS of 11.6 months and median OS of 41.9 months sound promising. We are conducting a Phase II study of concurrent 3D-CRT at a total dose of 72 Gy and chemotherapy with cisplatin and vinorelbine.

In conclusion, concurrent 3D-CRT with cisplatin and vinorelbine chemotherapy was feasible up to 72 Gy, in patients with unresectable Stage III NSCLC. At the level of 78 Gy, however, only 25% of the patients assessed for eligibility were found to be actually eligible. Thus, 72 Gy in 36 fractions was the maximum dose that could be achieved in most patients given the predetermined normal tissue constraints when administered concurrently with cisplatin and vinorelbine.

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