Table 5. Essential clincopathologic information and EMAST among cases with multiple malignant neoplasms

					alternation in the ten selected tetra-nucleotide-repeating regions												
No	Sex	Age	Histology	EMAST level	D8S321	D20S82	UT5037	D8S348	D2S443	D21S1436	D9S747	D9S303	D9S304	MYCL1	Overlapped neoplasms	Outcome	Cause of death
1	M	66	SQC	H	-	Ins	-	-	-	-	-	Ins	-	-	GC+RCC	Dead	RCC
2	M	68	SQC	H	-	-	-	-	Ins	-	-	Ins	-	-	LC+RC	Dead	unknown
3	M	79	SQC	H	-	-	-	-	Ins	Ins	-	-	LOH	-	GC	Dead	AMI
4	M	74	ADC	H	-	Ins	-	-	Ins	-	-	-	-	-	PC	Dead	pneumonia
5	M	60	SQC	H	-	-	-	Ins	-	-	Ins	LOH	-	LOH	RC	Dead	RC
6	M	58	SQC	H	Ins	-	-	LOH	-	Ins	-	-	LOH	-	BC	Dead	BC
7	F	77	ADC	H	Ins	Ins	-	-	LOH	-	-	Ins	-	LOH	MSC	Alive	-
8	M	53	ADC	H	Ins	-	-	-	Ins	-	-	-	-	LOH	GC	Alive	-
9	M	64	ADC	H	Ins	Ins	-	-	-	-	-	-	-	LOH	GC	Alive	-
10	M	82	SQC	L	-	-	-	-	-	LOH	Ins	-	-	-	GC	Dead	NSCLC
11	F	76	ADC	L	-	-	-	-	-	-	-	-	-	-	UC	Alive	-
12	M	55	ADC	L	-	LOH	-	NA	-	Ins	-	-	-	LOH	ML	Dead	ML
13	M	75	ADC	L	-	-	-	-	-	-	-	-	LOH	-	SS	Alive	-
14	M	78	ADC	L	-	-	-	-	-	-	-	-	-	-	PC	Alive	-
15	M	77	SQC	L	LOH	-	-	NA	Ins	-	-	-	NA	-	SCLC	Dead	SCLC
16	M	73	SQC	L	-	-	-	-	-	-	-	-	-	-	GC	Alive	-

EMAST, elevated alterations of selected tetra-nucleotide; SQC, squamous cell carcinoma; ADC, adenocarcinoma; M, male; F, female; H, high; L, low; Ins, instable; LOH, loss of heterozygosity; NA, Not available; -, no alteration; NSCLC, Non-small cell lung carcioma; SCLC, small cell lung carcinoma; GC, gastric cancer; RCC, renal cell cancer; LC, laryngeal cancer; RC, rectal cancer; UC, uteirne cancer; ML, malignant lymphoma; PC, prostate cancer; SS, synovial sarcoma; BC, bladder cancer; MSC, maxillary sinus cancer; AMI, acute myocardial infarction.

MSI and the clinicopathologic parameters examined (data not shown).

Association between LOH and clinicopathologic parameters

Tumors exhibiting LOH in two or more tetra-nucleotide-repeating regions were defined as LOH/selected tetra-nucleotide (ST)-high (20/65, 30.8%), and all other tumors were defined as LOH/ST-low (45/65, 69.2%). There were no significant correlations between the level of LOH/ST and the clinicopathologic parameters (**Table 4**).

Similarly, tumors exhibiting LOH in two or more regions of the Bethesda panel were defined as LOH/Bethesda panel (BP)-high (2/58, 3.4%), and all other tumors were defined as LOH/BP-low (56/58, 96.6%). There were no significant correlations between the level of LOH/BP and the clinicopathologic parameters (data not shown).

Association between EMAST and clinical outcome

The EMAST-high group showed a poorer postoperative overall survival than the EMAST-low group (mean survival time was 1394 days for the EMAST-high group and 2396 days for the EMAST-low group; log-rank test, $P = 0.0018$) (**Figure 6A**). Of the 22 patients with EMAST-high tumors, 12 died; 3 died of NSCLCs (the primary cause), 3 other malignant neoplasms (i.e., renal cell cancer, rectal cancer, bladder cancer), and 6 non-neoplastic diseases (i.e., acute myocardial infarction, cardiac failure, and pneumonia). Of the 43 patients with EMAST-low tumors, 10 died; 6 died of NSCLCs (the primary cause), 2 other malignant neoplasms (i.e., small cell lung cancer and malignant lymphoma), and 2 non-neoplastic diseases (i.e., cardiac failure and decrepitude).

There was no significant difference in the disease-free survival (mean survival time was 1844 days for the EMAST-high group and 1947 days for the EMAST-low group; log-rank test, $P = 0.9146$) (**Figure 6B**), and no association between the level of EMAST and disease recurrence (recurrent rate, 5/22 in the EMAST-high group versus 11/43 in the EMAST-low group, Chi-square test, $P = 0.962$). Moreover, no significant associations were found between the level of LOH/ST, LOH/BP, or MSI and any of the clinicopathologic parameters examined (data not shown).

Discussion

The present study demonstrated that a considerable fraction of NSCLCs was unstable in the ten tetra-nucleotide-repeating regions and that the incidence of EMAST is unequivocally higher than that of traditional MSI. These findings are comparable to those of previous studies which showed an incidence of EMAST in NSCLC of 35-51% [21-23]. The incidence of EMAST differs among the types of malignant neoplasms, 5% in prostate cancer [6], 13% in ovarian cancer [29], 75% in skin cancer [27], and 43.9-45% in bladder cancer [27, 28]. These findings suggest a potential molecular basis for the unique properties in different types of malignant neoplasms.

The most interesting finding of the present study is that patients with EMAST-high NSCLC were affected by additional malignant neoplasms including gastric cancer and renal cell cancer at a significantly higher incidence (42.9% [9/21] in the EMAST-high group versus 16.3% [7/43] in the EMAST-low group). For the 16 patients who were affected by multiple neoplasms, essential information of their clinicopathologic characteristics and alterations in the tetra-nucleotide-repeating markers are described in **Table 5**. Similarly, patients with HNPCC (Lynch syndrome) are also often affected by additional neoplasms, such as endometrial and gastric cancer [14, 15, 31, 32]. HNPCC is an autosomal dominant disease with germ line mutations in the mismatch repair genes (i.e., *h*MSH2, *h*MLH1, and *h*MSH6) [10, 11, 14, 15, 32]. Defects in DNA mismatch repair due to mutations cause traditional MSI and manifest as frame-shift mutations in mono- or di-nucleotide-repeating regions [10, 11, 14, 15, 31, 32]. Traditional MSI has been found in 85-95% of HNPCC (and in 10-15% of sporadic colorectal cancers, in which the mismatch repair genes are silenced by the acquired epigenetic modification) and is well accepted to be an important molecular basis for promoting carcinogenesis of certain types of malignant neoplasms [7-12]. On the other hand, EMAST, distinct from traditional MSI, is not associated with defects in mismatch repair [23, 28]. Although the actual

molecular mechanism of EMAST remains unclear, previous studies of some AAAG-type tetra-nucleotide repeating regions suggest that p53 alterations could be involved [22]. One recent study demonstrated an association between the heterogeneous nuclear expression of hMSH3 and EMAST in colorectal cancer cells, suggesting that an acquired hMSH3 alteration could be its molecular mechanism [36]. The present study investigated the involvement of p53 (LOH of p53 gene and its potential mutations evaluated by immunohistochemistry) in EMAST, but failed to obtain a result that supports previous findings [16, 21-24, 27]. The difference in the tetra-nucleotide repeating regions examined might be responsible for this discrepancy. Thus, establishment of universal markers to evaluate EMAST, like the Bethesda panel, is necessary to verify its clinicopathologic significance. Moreover, a comprehensive search for potential alterations of DNA replication/repair molecules like hMSH3 may lead to elucidation of the molecular mechanism of EMAST.

As for the clinical outcome, a pronounced difference in the overall survival was found between the EMAST high- and low-groups. However, no significant difference was found in the disease-free survival and the recurrent rate, or in histological grade and proliferating activity of neoplastic cells. Notably, 3 of 22 (13.6%) patients with EMAST-high tumor died of other malignant neoplasms, while 2 of 43 (4.7%) patients with EMAST-low tumor died of other neoplasms. These findings suggest that the poorer overall survival in the EMAST-high group might be due to a high susceptibility to malignant neoplasm, and EMAST itself does not promote the progression process of their carcinogenesis (that is, it does not promote the acquisition of highly malignant activity of neoplastic cells).

In conclusion, impairment of molecular machinery that maintains stable replication of the tetra-nucleotide-repeated regions may elevate susceptibility to NSCLCs and certain neoplastic diseases. Elucidation of the potential molecular mechanism of EMAST could lead to discovery of a novel genetic background determining susceptibility to NSCLCs and establishment of a novel disease susceptible for multiple neoplasms including NSCLCs.

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Declaration of Conflicts of interest

None declared.

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ORIGINAL ARTICLE

The role of βIII-tubulin in non-small cell lung cancer patients treated by taxane-based chemotherapy

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Abstract
Background The aim of this study is to evaluate whether class III β-tubulin (TUBB3) expression could predict progression-free survival or overall survival in relapsed non-small cell lung cancer (NSCLC) patients treated with taxene-based chemotherapy.
Methods Immunohistochemistal staining was used to examine the expression of TUBB3 in resected lung tumor specimens obtained from 56 patients treated with platinum-based chemotherapy against recurrent tumors after curative resections. Excision repair cross-complementation group 1, breast cancer susceptibility gene 1, vascular endothelial growth factor, Ki-67, CD34, and p53 were also correlated with clinical features and outcome after treatment.
Results Of the 56 patients enrolled in the study, 29 were treated by carboplatin plus paclitaxel as first-line treatment, and 24 patients received docetaxel monotherapy as second- or third-line treatment. A positive TUBB3 expression is closely associated with a poor response to taxane-based chemotherapy. TUBB3 expression was an independent prognostic factor for predicting poor progression-free survival after docetaxel administration. However, TUBB3 expression could not predict outcome after carboplatin plus paclitaxel treatment. The other biomarkers tested were not independent prognostic factors for predicting outcome after taxane-based chemotherapy.
Conclusion TUBB3 expression is associated with resistance to taxane-based chemotherapy and is an independent prognostic factor for predicting poor progression-free survival after docetaxel treatment alone. TUBB3 expression may be a predictive marker for chemoresistance to docetaxel in NSCLC with postoperative recurrent disease.

Keywords Class III β-tubulin · Taxane · Chemotherapy · NSCLC · Chemoresistance · Prognosis

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Introduction

Lung cancer is the leading cause of cancer death worldwide, and it has a dismal prognosis. Non-small-cell lung cancer (NSCLC) accounts for approximately 80% of all lung cancer cases. One strategy to improve the outcome of patients is to identify biomarkers that may predict the prognosis and chemotherapeutic response of patients to treatment. In advanced disease, performance status has been consistently shown to be the most powerful prognostic tool for survival rates [1]. Investigators have recently documented several promising markers for

chemotherapeutic response and overall survival in advanced NSCLC [2]. However, there has been no established clinical marker that correlates with response to the treatment andprognosis in patients with advanced NSCLC.

Microtubules are complex polymers consisting of tubulin dimers (containing one α-tubulin and one β-tubulin molecule) and a variety of microtubule-associated proteins [3]. The isotype composition of β-tubulins has been related to taxane-based chemotherapy responsiveness [3, 4]. A recent review has documented the correlation between class III β-tubulin (TUBB3) expression and response to anti-microtubule agents in advanced cases of NSCLC [5]. Several studies have demonstrated that high βIII-tubulin expression predicts a poorer outcome in patients with advanced NSCLC treated with paclitaxel-based or vinorelbine-based regimens [6–10]. Hayashi et al. [11] analyzed the in vitro drug (docetaxel and gemcitabine) sensitivity of individual tumor samples obtained from patients with completely resected NSCLC and reported that high βIII-tubulin expression was associated with resistance to docetaxel ($p = 0.0025$) but not to gemcitabine ($p = 0.1465$). However, no data are yet available on the expression of βIII-tubulin in patients with advanced NSCLC treated with docetaxel. Therefore, it is unclear whether the expression of βIII-tubulin is able to predict response and outcome in patients with advanced NSCLC treated by docetaxel or paclitaxel as a single agent.

Nucleotide excision repair (NER) has been described to be involved in the repair of platinum-induced DNA damage [12], and several groups of researchers have investigated the prognostic and predictive significance of NER pathway biomarkers [13–15]. Excision repair cross-complementation group 1 (ERCC1) and breast cancer susceptibility gene 1 (BRCA1) are involved in the NER system, and thesè proteins are also known to be associated with resistance to platinum-based chemotherapy [2, 13–15].

At the present time, paraffin-embedded specimens obtained by bronchial biopsy are the usual materials available for immunohistochemical analysis in advanced NSCLC. However, these tumor samples are occasionally too small for the detection of molecular markers in heterogenous tumor tissue by immunohistochemical analysis. In advanced NSCLC, an adequate amount of specimen is often not available for immunohistochemical staining, and specimen biopsy may bias the immunohistochemical analysis of molecular biomarkers. Therefore, we used tumor samples obtained by the curative resection of primary lung tumors and studied whether the expression of TUBB3 could predict response and outcome in relapsed NSCLC patients receiving docetaxel or paclitaxel plus carboplatin. ERCC1, BRCA, vascular endothelial growth factor (VEGF), CD34, Ki-67, and p53 were also examined with respect to their correlation with response and outcome after chemotherapy.

Materials and methods

Patients

Between March 2003 and May 2008, 973 consecutive patients with resectable NSCLC underwent curative resection at Shizuoka Cancer Center. Of these, 146 patients had a postoperative recurrence. Among the latter group, 60 NSCLC patients had received platinum-based chemotherapy against recurrent disease after curative surgical resection of primary lung tumors. However, specimens for four patients were not available. Therefore, a total of 56 patients were enrolled in this immunohistochemical study. The age of the patients ranged from 45 to 77 years (mean 66 years). None of the patients had received neo-adjuvant or adjuvant chemotherapy. Tumor recurrence was confirmed by bronchoscopy and/or radiological imaging in addition to clinical course. The tumor histology was classified on the basis of the World Health Organization (WHO) criteria. Pathologic tumor-node-metastasis (TNM) stages were established using the International System for Staging Lung Cancer adopted by the American Joint Committee on Cancer and the Union Internationale Centre le Cancer [16]. Patient's demographics are listed in Table 1. The study protocol was approved by the Institutional Review Board.

All patients were treated by platinum doublet chemotherapy. Twenty (36%) patients received gefitinib as epidermal growth factor receptor tyrosine kinase inhibitor (EGFR-TKI) therapy. We used the Response Evaluation Criteria in Solid Tumors (RECIST) to assess response to chemotherapy [17]. Response based on target (and non-target lesions) was defined as: complete response (CR), with the disappearance of all target (non-target) lesions; partial response (PR), with a ≥30% reduction in size (or disappearance of one or more non-target lesions); stable disease (SD), with a >30% decrease and <20% increase in size (or the persistence of one or more non-target lesions); progressive disease (PD), with a >20% increase in size (or the appearance of new non-target lesions and/or progression of existing non-target lesions). The overall response was defined as the best response recorded from the start of treatment until disease progression or recurrence, confirmed by repeated assessments performed no less than 4 weeks after the criteria for response were first met.

Immunohistochemical staining

Immunohistochemical staining was performed according to the procedure described in previous reports [14, 18–21].

Table 1 Patient's demographics

Characteristics	Number of patients (n = 56)
Age (years)	
≤65/>65 years	26/30
Gender	
Male/female	39/17
Performance status	
0/1/2	43/13/0
Smoking history	
Yes/no	43/13
Histology	
AC/non-AC	37/19
Recurrence pattern	
Local relapse	13
Distant metastasis	43
Pathological stage	
IA/IB/IIA/IIB/IIIA/IIIB	10/9/6/7/12/11
Metastatic site	
Lung	21
Bone	10
Brain	9
Liver	4
Lymph node	15
Other	7
Pleural effusion	7
First-line regimen	
Carboplatin + paclitaxel	29
Cisplatin + gemcitabine	18
Cisplatin + docetaxel	5
Cisplatin + vinorelbine	2
Cisplatin + amrubicin	1
Cisplatin + S-1	1
Timing of docetaxel use	
First line	0
Second line	20
Third line	4
Fourth line	0
EGFR-TKI	
Gefitinib	8
Erlotinib	4

AC Adenocarcinoma, *EGFR-TKI* epidermal growth factor receptor-tyrosine kinase inhibitor

The following antibodies were used: a mouse monoclonal antibody against ERCC1 (ABI2356; Abcam, Tokyo, Japan; 1:200 dilution); a mouse monoclonal antibody against BRCA1 (ABI16780; Abcam; 1:50 dilution); a mouse monoclonal antibody against TUBB3 (MMS-435P; Convance, San Diego, CA; 1:500 dilution); a mouse monoclonal antibody against CD34 (Nichirei, Tokyo, Japan,;1:800 dilution); a mouse monoclonal antibody against p53 (D07; DAKO, Glostrup, Denmark; 1:50 dilution); a murine monoclonal antibody against MIB-1 (Dako; 1:40 dilution), specific for human nuclear antigen Ki-67; a monoclonal antibody against VEGF (Immuno-Biological Laboratories, Fujioka, Japan; 1:300 dilution).

ERCC1 and BRCA1 were assessed semiquantitatively by estimating the percentage of tumor cells with positive nuclear and/or cytoplasmic staining on whole tumor slides, (0, no staining; 0.1, positive staining in 1–9% of the tumor cells; 0.5, positive staining in 10–49% of the tumor cells; 1, positive staining in >50% of the tumor cells). The staining intensity was also evaluated in a semiquantitative method representing the average intensity of the stained tumor cells (0, no staining; 1, weak staining; 2, moderate staining; 3, strong staining). The proportion and intensity scores were then multiplied to obtain a total score, which ranged from 0 to 3 (H-score).

TUBB3 was also assessed using the semiquantitative H-score. βIII-tubulin tumor staining (cytoplasm) intensity was graded on a scale of 0–2 using adjacent nonmalignant cells as a reference. The percentage of positive tumor cells was evaluated, and a area proportion score was determined (0 if 0%, 0.5 if 1–9%, 1 if 10–24%, 2 if 25–49%, 3 if 50–74%, and 4 if ≥ 75%). This proportion score was then multiplied by the staining intensity to obtain a final semiquantitative H-score with a range of 0–8 [15].

The detailed protocol for Ki-67 immunostaining has been published elsewhere [22]. A highly cellular area of the immunostained sections was evaluated. All epithelial cells with nuclear staining of any intensity were defined as positive. Approximately 1,000 nuclei were counted on each slide. Proliferative activity was assessed as the percentage of MIB-1-stained nuclei (Ki-67 labeling index) in the sample. The expression of VEGF was quantitatively assessed according to the percentage of immunoreactive cells in a total of 1,000 neoplastic cells. The number of CD34-positive vessels was counted in four selected hot spots in a 400× field (0.26-mm^2 field area). Microvessel density (MVD) was defined as the mean count of microvessels per 0.26-mm^2 field area.

For p53, microscopic examination for the nuclear reaction product was performed and scored. In accordance with a previous report [20], p53 expression in >10% of tumor cells was defined as high expression. Sections were assessed using a light microscopic in a blinded fashion by at least two of the authors.

Statistical analysis

Probability values of <0.05 indicated a statistically significant difference. Fisher's exact test was used to examine the association of two categorical variables. The correlation between different variables was analyzed using the

nonparametric Spearman's rank test. Follow-up for these 56 patients was conducted through patient medical records. The Kaplan–Meier method was used to estimate survival as a function of time, and survival difference was analyzed by the log-rank test. Multivariate analyses were performed using the stepwise Cox proportional hazards model to identify independent prognostic factors. Statistical analysis was performed using JMP 8 (SAS Institute, Cary, NC) for Windows.

Results

Immunohistochemical analysis

Immunohistochemical staining for ERCC1, BRCA1, TUBB3, VEGF, Ki-67, CD34, and p53 was evaluated for the surgically resected 56 primary lesions.

The median H-scores of ERCC1, BRCA1, and TUBB3 were 0.10, 0.10, and 1.00, respectively, and their respective cutoff values were 0.10, 0.10, and 1.00, respectively. A positive rate of ERCC1, BRCA1, and TUBB3 was recognized in 48 (27/56), 41 (23/56), and 32% (18/56), respectively. Figure 1 shows the immunohistochemical staining of TUBB3 and ERCC1.

The median value of the Ki-67 labeling index was 30% (range 2–80%) and the cutoff point value was 30. The staining pattern of VEGF was uniformly localized in the cytoplasm and/or membrane of neoplastic cells. The median rate of VEGF positivity was 40.0% (range 5–0%) and the cutoff point was 40%. The median number of CD34 cells was 29 (range 5–65 cells) and the cutoff point was 29 cells. The positive rate of Ki-67, VEGF, and CD34 was recognized in 50% (28/56), 46% (26/56) and 50% (28/56) of specimens, respectively. p53 had a positive rate of 50% (28/56).

Relationship between TUBB3 and taxane-based chemotherapy as first-line chemotherapy

Chemotherapy regimens as first-line treatment consisted of carboplatin plus paclitaxel in 29 patients, cisplatin plus gemcitabine in 18 patients, cisplatin plus docetaxel in five patients, and other cisplatin-based regimens in four patients. The median number of chemotherapy cycles was four (range 1–6). An overall response (CR + PR) was observed in 18 (32%) patients, and a SD and PD were observed in 28 (50%) and ten (18%) patients respectively. In all patients ($n = 56$), there was no statistically significant difference observed in the mean scores of ERCC1, BRCA1 and TUBB3, Ki-67 labeling index, VEGF positivity, MVD, and the positive rate of p53 between responders (CR or PR) and non-responders (SD or PD).

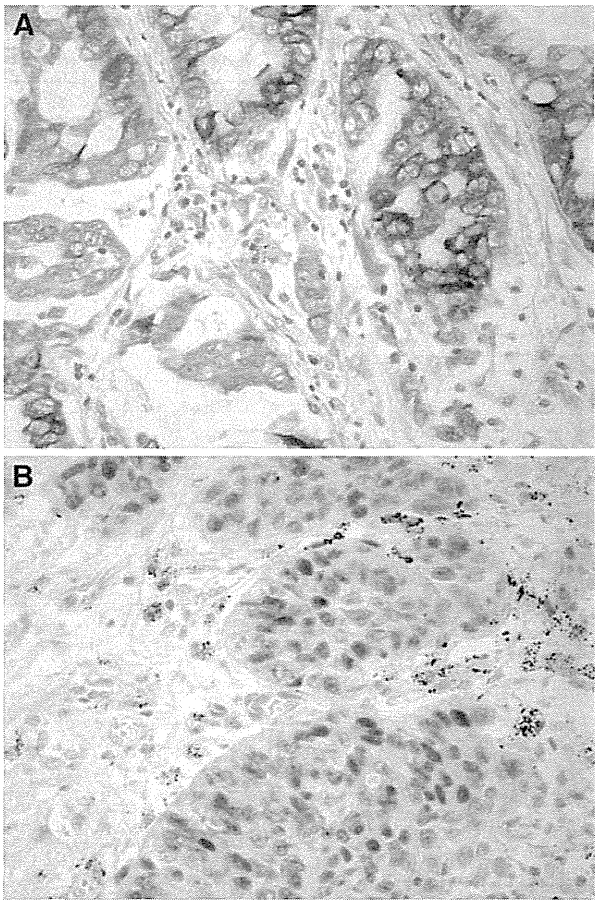

Fig. 1 Immunohistochemical staining of class III β-tubulin (TUBB3) and excision repair cross-complementation group 1 (ERCC1) in non-small cell lung cancer (NSCLC). **a** TUBB3 is stained in cytoplasm (H-score 6), **b** ERCC1 is stained in nuclei (H-score 3)

We analyzed the chemotherapeutic response and outcome in 29 patients treated by carboplatin and paclitaxel. Table 2 shows the comparison between therapeutic regimens with ($n = 29$) or without ($n = 27$) paclitaxel. No statistically significant difference was observed between taxane and non-taxane regimens. Of the 29 patients who received paclitaxel, eight patients (28%) had a PR and 21 had a response of SD or PD. A statistically significant difference in the mean scoring of TUBB3 expression was recognized between responder (PR) and non-responder (non-PR) ($p = 0.048$) (Fig. 2a). However, no statistically significant difference in the other biomarkers was recognized between responder and non-responder.

Relationship between TUBB3 and docetaxel as second- or third-line chemotherapy

Of the 56 patients, 24 were treated by docetaxel alone as second- or third-line chemotherapy. Twenty (83%) and

four (17%) patients received docetaxel as second-line and third-line chemotherapy, respectively. Table 3 shows the comparison between regimens with ($n = 24$) or without ($n = 32$) docetaxel. The incidence of smokers was significantly higher among patients treated with docetaxel than among those treated with regimens without docetaxel ($p = 0.027$). No statistically significant difference was observed for the other variables. Of the 24 patients receiving docetaxel, two patients (8.3%) had a PR, 12 patients showed a response of SD, and ten patients showed a response of PD. Patients with a PR or SD had a median of five treatment cycles with docetaxel (range 2–15), whereas those who had a response of PD had a median of two treatment cycles (range 1–2), which was a significant difference ($p = 0.002$). We then compared the expression of these biomarkers according to disease control [Non-PD (PR or SD) vs. PD]. Only TUBB3 expression was significantly higher in patients with a non-PD response compared with those with a response of PD ($p = 0.043$) (Fig. 2b). No statistically significant difference in the other biomarkers was observed between non-PD and PD responses.

Table 2 Comparison of different variables between patients treated or not treated with carboplatin plus paclitaxel as first-line chemotherapy

Variables	Total ($n = 56$)	TXL ($n = 29$)	Non-TXL ($n = 27$)	p value
Age				
≤65/>65 years	26/30	15/14	11/16	0.435
Gender				
Male/female	39/17	19/10	20/7	0.567
Performance status				
0/1	43/13	22/7	21/6	1.000
Smoking history				
Yes/no	43/13	21/8	22/5	0.532
Histology				
AC/non-AC	34/22	19/10	15/12	0.585
TUBB3				
Positive/negative	18/38	7/22	11/16	0.254
ERCC1				
Positive/negative	27/29	15/14	12/15	0.605
BRCA1				
Positive/negative	23/33	11/18	12/15	0.786
Ki-67				
Positive/negative	28/28	12/17	16/11	0.284
VEGF				
Positive/negative	26/30	16/13	10/17	0.193
CD34				
Positive/negative	28/28	14/15	14/13	1.000
P53				
Positive/negative	28/28	15/14	13/14	1.000

TXL Paclitaxel, *AC* adenocarcinoma, *TUBB3* class III β-tubulin, *BRCA1* breast cancer susceptibility gene 1, *VEGF* vascular endothelial growth factor

Survival analysis after carboplatin plus paclitaxel treatment as first-line chemotherapy

The survival analysis after first-line chemotherapy in all patients ($n = 56$) demonstrated that the median progression-free survival (PFS) and overall survival (OS) were 7.5 and 30.8 months, respectively. In the 29 patients receiving carboplatin and paclitaxel, the median PFS and OS were 7.6 and 31.5 months, respectively. Table 4 shows the PFS and OS according to different variables. The univariate analysis revealed that smoker, a histology of non-adenocarcinoma (AC), and a positive Ki-67 labeling index were significantly associated with poor PFS. However, the multivariate analysis found that none of the variables analyzed was an independent prognostic factor for predicting poor PFS. In the OS analysis, smoker, non-AC, and the positive expression of the Ki-67 labeling index, VEGF,

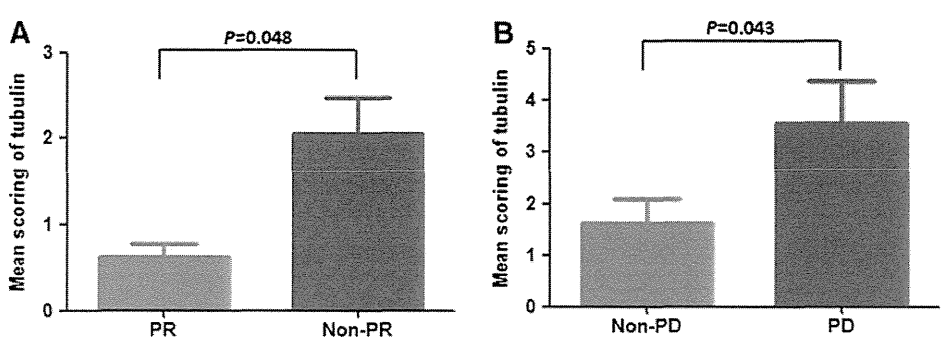

Fig. 2 Relationship between tumor response and the expression of TUBB3 in patients treated by taxane-based chemotherapy. a Analysis of the 29 patients treated by carboplatin plus paclitaxel revealed a statistically significant difference in the mean scoring of TUBB3 between responder (*PR*) and non-responder (*Non-PR*) ($p = 0.048$). *PR* Partial response. b Analysis of the 24 patients treated by docetaxel demonstrated that statistically significant difference in the mean scoring of TUBB3 was recognized between non-progressive disease (*Non-PD*) and progressive disease (*PD*) ($p = 0.043$)

Table 3 Comparison of different variables between patients treated or not treated with docetaxel as second- and third-line chemotherapy

Variables	Total ($n = 56$)	DTX ($n = 24$)	Non-DTX ($n = 32$)	p value
Age				
≤65/>65 years	26/30	9/15	17/15	0.287
Gender				
Male/female	39/17	19/5	20/7	0.749
Performance status				
0/1	43/13	19/5	24/8	0.760
Smoking history				
Yes/no	43/13	22/2	21/11	0.027
Histology				
AC/non-AC	34/22	16/8	18/14	0.581
TUBB3				
Positive/negative	18/38	11/13	7/25	0.083
ERCC1				
Positive/negative	27/29	12/12	15/17	1.000
BRCA1				
Positive/negative	23/33	9/15	14/18	0.784
Ki-67				
Positive/negative	28/28	13/11	15/17	0.284
VEGF				
Positive/negative	26/30	13/11	13/19	0.418
CD34				
Positive/negative	28/28	14/10	14/18	0.418
P53				
Positive/negative	28/28	11/13	17/15	0.787

DTX docetaxel, *AC* adenocarcinoma, *TUBB3* class III β-tubulin, *ERCC1* excision repair cross-complementation group 1, *BRCA1* breast cancer susceptibility gene 1, *VEGF* vascular endothelial growth factor

Table 4 Survival analysis in patients treated with carboplatin plus paclitaxel as first-line chemotherapy

Variables	Progression-free survival		Overall survival	
	MST (months)	p value	MST (months)	p value
Age				
≤65/>65 years	18.9/31.5	0.971	18.9/60.7	0.096
Gender				
Male/female	22.7/34.9	0.079	31.5/34.3	0.166
Performance status				
0/1	26.6/23.9	0.139	34.0/23.9	0.076
Smoking history				
Yes/no	19.5/36.3	0.031	19.5/65.3	<0.001
Histology				
AC/non-AC	34.3/11.7	<0.001	34.8/15.8	<0.001
TUBB3				
Positive/negative	9.2/7.6	0.521	22.7/32.9	0.741
ERCC1				
Positive/negative	9.2/6.1	0.064	26.9/34.8	0.112
BRCA1				
Positive/negative	7.1/9.6	0.169	18.7/34.3	0.776
Ki-67				
Positive/negative	6.1/11.6	0.029	18.6/60.7	<0.001
VEGF				
Positive/negative	7.5/9.3	0.931	18.9/60.7	<0.001
CD34				
Positive/negative	7.5/11.4	0.994	20.9/60.7	0.007
P53				
Positive/negative	7.3/9.6	0.403	18.9/34.8	0.177

MST median survival time, *PTX* paclitaxel, *AC* adenocarcinoma, *TUBB3* class III β-tubulin, *ERCC1* excision repair cross-complementation group 1, *BRCA1* breast cancer susceptibility gene 1, *VEGF* vascular endothelial growth factor

and CD34 were significantly associated with poor outcome; however, multivariate analysis demonstrated that none of these variables was a statistically significant prognostic factor.

Survival analysis following treatment with docetaxel as second- or third-line chemotherapy

The median PFS and OS were 4.5 and 12.0 months, respectively, in the 24 patients treated with docetaxel. Table 5 shows the PFS and OS according to the different variables. The univariate analysis revealed that a positive TUBB3 expression was significantly associated with prognostic factors for predicting poor PFS. The multivariate analysis demonstrated that the positive expression of TUBB3 was an independent prognostic factor for predicting poor PFS following docetaxel treatment. No statistically significant prognostic factor in the OS was observed among these variables. Figure 3 shows the Kaplan–Meier survival curves in patients with a positive and negative response for TUBB3 expression. Of these 24 patients, ten were treated by EGFR-TKI.

Discussion

To the best of our knowledge, this is the first study to evaluate the prognostic significance of TUBB3 expression in patients with relapsed NSCLC who received docetaxel as a single chemotherapeutic agent. Positive TUBB3 expression was closely associated with resistance to taxane-based chemotherapy (docetaxel or carboplatin plus paclitaxel). Our results demonstrate that a positive TUBB3 expression was an independent prognostic factor among our patients for predicting poor PFS following docetaxel administration. However, TUBB3 expression could not

predict outcome after the administration of carboplatin plus paclitaxel chemotherapy.

Platinum-based chemotherapy remains the mainstay of treatment for advanced NSCLC. However, patients do not respond equally after treatment, demonstrating various degrees of chemoresistance or chemosensitivity. Thus, treatment selection is considered to be a critical problem in clinical practice. Several researchers have recently documented biomarkers for predicting chemoresistance in patients with advanced NSCLC [2]. TUBB3 expression has been reported to be valuable biomarker of taxane resistance in both advanced NSCLC disease and early disease [5]. A number of studies have shown that higher TUBB3 expression predicted poorer outcome in patients with advanced NSCLC treated by carboplatin plus paclitaxel and that low TUBB3 expression was closely associated with a better response to paclitaxel-based chemotherapy [6–10]. However, the results of our study indicate that while high TUBB3 expression predicted a poor response to carboplatin plus paclitaxel chemotherapy, it was not closely associated with a poor outcome after chemotherapy. Although our small sample size may bias the relationship between TUBB3 expression and outcome after treatment, our results suggest that TUBB3 expression may be a useful predictive marker for resistance to docetaxel as compared with paclitaxel. Docetaxel has a twofold higher affinity for the target site tubulin than paclitaxel. Moreover, docetaxel is associated to incomplete cross-resistance as individuals previously treated with paclitaxel may benefit from docetaxel [23]. Docetaxel is one of the standard treatments given to previously treated NSCLC patients [24], but pemetrexed or erlotinib have also been selected as second- or third-line chemotherapy [24]. In patients with *EGFR* mutations, erlotinib or gefitinib should be administered for the refractory disease, whereas it is difficult to select the most effective agent from among docetaxel, pemetrexed, or erlotinib in patients with the *EGFR* wild type. The development of predictive markers to guide personalized treatment is important for improving the outcome for patients with previously treated NSCLC. Moreover, docetaxel or pemetrexed as second-line treatment have a response of <10%, indicating that it is important to increase disease control with these agents [24]. Therefore, we compared the tumor response between non-PD and PD in patients with NSCLC treated by docetaxel. As this is a retrospective study, a further prospective study is warranted to determine

Table 5 Survival analysis in patients treated with docetaxel as second- or third-line chemotherapy

Variables	Progression-free survival		Overall survival	
	MST (months)	*p* value	MST (months)	*p* value
Age				
≤65/>65 years	4.0/5.0	0.287	10.0/24.0	0.288
Gender				
Male/female	5.0/4.0	0.102	13.0/20.0	0.461
Performance status				
0/1	4.0/6.0	0.661	18.0/10.0	0.224
Smoking history				
Yes/no	4.5/3.5	0.402	12.0/25.0	0.177
Histology				
AC/non-AC	4.5/4.5	0.325	21.0/10.0	0.094
TUBB3				
Positive/negative	2.0/6.0	0.002	24.0/12.0	0.733
ERCC1				
Positive/negative	4.0/5.5	0.158	10.0/26.0	0.112
BRCA1				
Positive/negative	4.0/5.0	0.715	11.0/21.0	0.544
Ki-67				
Positive/negative	5.0/4.0	0.593	10.0/24.0	0.057
VEGF				
Positive/negative	4.0/6.0	0.107	10.0/21.0	0.199
CD34				
Positive/negative	4.0/5.5	0.702	11.0/13.0	0.682
P53				
Positive/negative	4.0/5.0	0.278	10.0/24.0	0.083

MST median survival time, *DTX* docetaxel, *AC* adenocarcinoma, *TUBB3* class III β-tubulin, *ERCC1* excision repair cross-complementation group 1, *BRCA1* breast cancer susceptibility gene 1, *VEGF* vascular endothelial growth factor

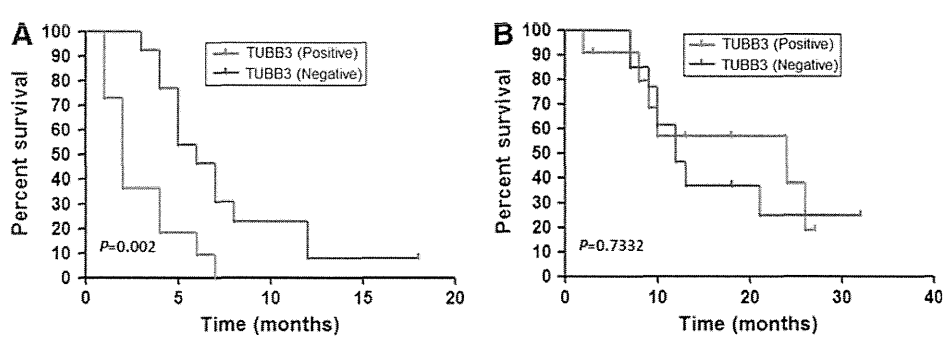

Fig. 3 Kaplan–Meier survival analysis for TUBB3 expression. Difference in progression-free survival (**a**) and overall survival (**b**) between subgroups was analyzed using the log-rank test

the prognostic significance of TUBB3 expression in advanced NSCLC patients who received a docetaxel monotherapy.

In our study, positive ERCC1 and BRCA1 expression was not closely associated with poor outcome and response to platinum-based chemotherapy. However, Azuma et al. [25] reported that ERCC1 expression may be useful for predicting outcome in NSCLC patients treated by platinum-based chemotherapy against relapsed tumors after curative resection. Their retrospective study demonstrated that negative ERCC1 expression was a significantly favorable factor for outcome after chemotherapy. However, the results of large-scale studies have revealed that resected NSCLC patients with high ERCC1 expression have a better survival than those with low ERCC1 expression [13] and that platinum-based adjuvant chemotherapy significantly prolongs outcome among patients with ERCC1-negative tumors but not among patients with ERCC1-positive tumors [14]. Moreover, recent work suggests that ERCC1 expression is predictive in adenocarcinoma, but not in other types of lung cancer [26]. Consequently, it remains unclear whether ERCC1 expression is able to predict outcome after platinum-based chemotherapy in NSCLC patients with postoperative recurrent disease.

Our study has a number of limitations. Firstly, the study population was small, which may have resulted in bias regarding the close relationship between TUBB3 expression and resistance to taxane-based chemotherapy. Secondly, we analyzed the outcome after chemotherapy in patients with advanced NSCLC with postoperative recurrence. However, OS has been documented to be better in patients with postoperative recurrence than in those with stage IV disease (OS: 21.3 vs. 13.3, respectively; $p < 0.001$) [27]. The survival time of our patients also seemed to be than that of patients with stage IV. As NSCLC patients with postoperative recurrence have characteristics that differ from those with stage IV disease, the results of our study may not be always be consistent with those of patients with stage IV disease. Moreover, the tumor cells are molecularly very different between the primary resected stage IA tumor and the local/metastatic relapse in a third-line regimen; therefore, our results have a biological limitation.

In conclusion, TUBB3 expression estimated by immunohistochemistry is an independent prognostic factor for progression-free survival in relapsed NSCLC patients treated with docetaxel monotherapy. Moreover, positive TUBB3 expression is closely associated with a poor response to taxane-based chemotherapy. TUBB3 expression may be a predictive marker for chemoresistance to docetaxel—but not docetaxel—in NSCLC patients with postoperative recurrent disease. However, a large prospective study is warranted for the confirmation of our results.

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Conflict of interest The authors declare that they have no financial or personal relationships with other individuals or organizations that could inappropriately influence this research.

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Original article

Feasibility of postoperative adjuvant chemotherapy of cisplatin plus vinorelbine for completely resected non-small-cell lung cancer: A retrospective study in Japan

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ABSTRACT

Background: The efficacy of postoperative adjuvant cisplatin (CDDP)-based chemotherapy, such as the combination of CDDP and vinorelbine (VNR), has been established for surgically resected non-small-cell lung cancer (NSCLC). However, the optimal treatment schedule and dosage for CDDP and VNR are unknown. We evaluated patient compliance with and the safety of adjuvant chemotherapy of CDDP at 80 mg/m^2 administered on day 1 plus VNR at 25 mg/m^2 administered on days 1 and 8, every 3 weeks.

Methods: Medical records of 100 surgically resected NSCLC patients, treated with a combination of CDDP and VNR at the Shizuoka Cancer Center between February 2006 and October 2011, were retrospectively reviewed.

Results: Eighty-three (83%) patients completed the planned 4 cycles of CDDP plus VNR and 59 (59%) received the planned doses. Sixty-eight percent of the patients experienced a decreased neutrophil count (grade 3/4 toxicity); 1%, a decreased platelet count; and 4%, febrile neutropenia. No treatment-related deaths were noted in this study. Univariate analysis of the factors influencing patient compliance with this adjuvant chemotherapy showed that neither patient characteristics nor surgical procedure was significantly associated.

Conclusions: Our results indicated that adjuvant chemotherapy with CDDP at 80 mg/m^2 administered on day 1 plus VNR at 25 mg/m^2 administered on days 1 and 8, every 3 weeks, was feasible for surgically resected NSCLC cases.

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1. Introduction

Non-small-cell lung cancer (NSCLC) accounts for approximately 80–85% of lung cancer cases, and the 5-year survival rate of lung cancer resection patients is reported to be approximately 60%. The postoperative 5-year survival rate of Stage II–IIIA patients, in particular, is unsatisfactory at 30–60% [1]. The efficacy of postoperative adjuvant chemotherapy has been documented since 2004 [2,3]. According to a meta-analysis of 4584 patients enrolled in a recent large-scale comparative study for cisplatin (CDDP)-based postoperative chemotherapy (Lung Adjuvant Cisplatin Evaluation [LACE]), the hazard ratio (HR) against death in all patients was 0.89 (95% confidence interval [CI], 0.82–0.96), corresponding to an absolute survival benefit of 5.4% at 5 years. Subgroup analysis of the LACE study indicated that among the various drugs co-administered with CDDP, only vinorelbine (VNR) significantly prolonged survival ($p=0.005$). With regard to disease stage, postoperative chemotherapy significantly improved the survival time in Stages II and III, and an improvement trend was shown in Stage IB, whereas a deteriorating trend was found in Stage IA [4]. However, the treatment schedule and dosage for CDDP and VNR varied [2,3,5,6]. We have previously reported on the safety of and patient compliance with adjuvant chemotherapy with CDDP (80 mg/m^2 at day 1) and VNR (25 mg/m^2 at days 1 and 8) repeated every 3 weeks [7]. This schedule and dosage was found to be safe and effective for Japanese patients with advanced NSCLC [8]. The rate of completion of the planned 4 cycles was 92% in 25 resected NSCLC patients. However, this study involved only a small number of patients. Therefore, we retrospectively evaluated patient compliance with and the safety of adjuvant chemotherapy with CDDP at 80 mg/m^2 administered on day 1 plus VNR at 25 mg/m^2 administered on days 1 and 8, every 3 weeks, in surgically resected NSCLC patients.

2. Material and methods

The medical records of surgically resected NSCLC patients treated with adjuvant chemotherapy of CDDP plus VNR at the Shizuoka Cancer Center between February 2006 and October 2011 were retrospectively reviewed. As a rule at our institution, the inclusion of adjuvant chemotherapy patients in this study was based on the following criteria: (i) age less than 75 years; (ii) pathological Stage II–IIIA; and (iii) performance status (PS) 0 or 1. CDDP at 80 mg/m^2 was administered on day 1, and VNR at 25 mg/m^2 was administered on days 1 and 8. The combination of these drugs was repeated every 3 weeks, and each 3-week treatment schedule was counted as 1 cycle. We defined the completion of adjuvant chemotherapy as the administration of CDDP plus VNR on day 1 of 4 cycles. Treatment change, such as reducing, skipping, or delaying a dose, was based on the physician's decision. Chemotherapy-related toxicities were graded according to the National Cancer Institute Common Terminology Criteria version 4.0 (NCI-CTC v4.0). This study included the patients mentioned in our previous report [7]. Univariate analyses were performed to identify risk factors for not completing adjuvant chemotherapy of CDDP plus VNR. All categorical variables were analyzed using the chi-square test or Fisher's exact test, as appropriate. Clinical evaluation of relapse-free survival (RFS) after surgical resection was conducted using the Kaplan–Meier method to assess the time to relapse or death. All p values were reported as 2-sided, and values less than 0.05 were considered statistically significant. This study was approved by the Institutional Review Board (23-J69-23-1-3, October 24, 2011).

3. Results

3.1. *Patient characteristics*

One hundred NSCLC patients were treated with postoperative adjuvant chemotherapy of CDDP plus VNR. The characteristics of the patients are shown in Table 1. The median age was 63 years (range, 36–74 years) and 34% of the patients were female. Twenty percent of the patients had never smoked. Histologically, adenocarcinoma and squamous cell carcinoma were observed in 67% and 27% of patients, respectively. Eighty-seven percent of the patients had undergone a lobectomy, and 13% a pneumonectomy. Pathological Stages IIA, IIB, and IIIA were observed in 31%, 22%, and 47% of patients, respectively. The median time from surgical resection to the start of adjuvant chemotherapy was 45 days (range, 29–79 days).

Table 1 – Patient characteristics.

	Number of patients	(%)
Gender		
Male	66	(66)
Female	34	(34)
Age, years		
Median	63	
(Range)	(36–74)	
Smoking status		
Never-smoked	20	(20)
Prior or current smoker	80	(80)
Performance status (ECOG)		
0	62	(62)
1	38	(38)
Histology		
Adenocarcinoma	67	(67)
Squamous cell carcinoma	27	(27)
Others	6	(6)
Pathological stage		
IIA	31	(31)
IIB	22	(22)
IIIA	47	(47)
Surgical procedure		
Lobectomy	81	(81)
Lobectomy with chest wall resection	6	(6)
Pneumonectomy	13	(13)
Time from surgical resection to start of adjuvant chemotherapy, days		
Median	45	
(Range)	(29–79)	

($n=100$).

3.2. *Compliance with adjuvant chemotherapy and observed toxicities*

Of the 100 NSCLC patients treated with adjuvant chemotherapy, 83 (83%) completed the planned 4 cycles of CDDP plus VNR and 59 (59%) received the planned doses (Table 2). The reasons for discontinuation of chemotherapy were toxicity in 8 patients (8%) and patient refusal in 8 patients (8%). The median doses received were 320 mg/m^2 for CDDP and 200 mg/m^2 for VNR. In addition, the mean doses received were 283 mg/m^2 for CDDP and 173 mg/m^2 for VNR (Table 3). This study included only 12 patients who were 70 years or older, but 11 of these patients (92%) completed the planned 4 cycles of CDDP plus VNR.

The incidence of toxicity at grade 2 or worse is shown in Table 4. Sixty-eight percent of patients experienced a decreased neutrophil count (grade 3/4 toxicity); 34%, a decreased white blood cell count; 15%, anemia; and 1%, a decreased platelet count. Febrile neutropenia was reported in 4% of the patients. Although the use of oral antibiotics and granulocyte-colony stimulating factor (G-CSF) against afebrile neutropenia was based on the physician's decision, most patients were not treated with prophylactic oral antibiotics and G-CSF. Frequently observed non-hematological toxicities

Table 2 – Compliance with adjuvant chemotherapy of cisplatin and vinorelbine.

	Number of patients	(%)
Planned 4 cycles completed	83	(83)
Planned doses received		
(Cisplatin 320 mg/m^2 and vinorelbine 200 mg/m^2)	59	(59)
Discontinuation of adjuvant chemotherapy	17	(17)
Reason		
Toxicity	8	(8)
Patient refusal	8	(8)
Other	1	(1)

Table 3 – Dosage of cisplatin and vinorelbine received in adjuvant chemotherapy.

Cycle	1	2	3	4	Total
Cisplatin (*n*)	100	91	88	83	100
Dose (mg/m^2)					
Planned	80	80	80	80	320
Received (median)	80	80	80	80	320
(mean)	80	71	68	63	283
Vinorelbine (*n*)	100	91	88	83	100
Dose (mg/m^2)					
Planned	50	50	50	50	200
Received (median)	50	50	50	50	200
(mean)	48	44	42	40	173

Table 4 – Toxicities related to adjuvant chemotherapy of cisplatin and vinorelbine.

	Grade 2		Grade 3		Grade 4	
	No. of patients	(%)	No. of patients	(%)	No. of patients	(%)
Anemia	32	(32)	15	(15)	0	
Febrile neutropenia	–		4	(4)	0	
Constipation	16	(16)	1	(1)	0	
Nausea	38	(38)	8	(8)	0	
Vomiting	8	(8)	2	(2)	0	
Fatigue	20	(20)	1	(1)	0	
Infection	6	(6)	4	(4)	0	
ALT increase	0		2	(2)	0	
AST increase	1	(1)	2	(2)	0	
Blood bilirubin increase	3	(3)	0		0	
Creatinine increase	9	(9)	0		0	
Neutrophil count decrease	15	(15)	26	(26)	42	(42)
Platelet count decrease	1	(1)	0		1	(1)
WBC decrease	35	(35)	31	(31)	3	(3)
Anorexia	54	(54)	7	(7)		

ALT: Alanine aminotransferase; AST: Aspartate aminotransferase and WBC: White blood cell.

Table 5 – Univariate analysis of factors influencing patient compliance to adjuvant chemotherapy of cisplatin and vinorelbine.

	Completion of 4 cycles		Discontinuation		*p*-value
	No. of patients	(%)	No. of patients	(%)	
Gender					0.305
Female	30	(36)	4	(24)	
Male	53	(64)	13	(76)	
Age, years					0.505
Median	64		63		
(Range)	(36–74)		(39–72)		
Smoking status					0.327
Never-smoker	18	(22)	2	(12)	
Previous or current smoker	65	(78)	15	(88)	
Performance status (ECOG)					0.403
0	53	(64)	9	(53)	
1	30	(36)	8	(47)	
Pathological stage					0.249
IIA	28	(34)	3	(18)	
IIB	16	(19)	6	(35)	
IIIA	39	(47)	8	(47)	
Surgical procedure					0.154
Lobectomy	69	(83)	12	(70)	
Lobectomy with chest wall resection	3	(4)	3	(18)	
Pneumonectomy	11	(13)	2	(12)	
Time from surgical resection to start of adjuvant chemotherapy, days					0.557
Median	45		44		
(Range)	(29–79)		(32–65)		

of grade 2 or worse included anorexia (61%) and nausea (46%). No treatment-related deaths were noted in this study.

The results of univariate analysis of the factors influencing adjuvant chemotherapy compliance are shown in Table 5. Patient characteristics were not significantly associated with compliance to adjuvant chemotherapy. In addition, surgical procedure and time from surgical resection to the start of adjuvant chemotherapy were not significantly associated with patient compliance. Lobectomy with chest wall resection tended to be associated with poor compliance to adjuvant chemotherapy.

3.3. *Relapse-free survival*

In this analysis, the median follow-up duration was 13.3 months. The Kaplan–Meier curve for relapse-free survival (RFS) from the time of surgical resection is shown in Fig. 1. The 1- and 2-year RFS rates were 75% and 62%, respectively.

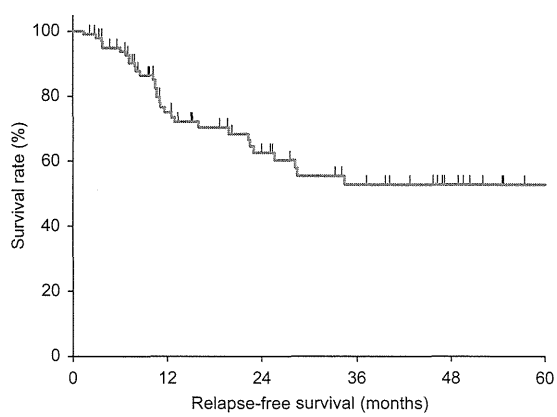

Fig. 1 – Relapse-free survival (RFS) curve for surgically resected NSCLC patients treated with adjuvant chemotherapy of cisplatin plus vinorelbine. Total number of patients = 100. The curve was plotted using Kaplan–Meier analysis. The 1- and 2-year RFS rates were 75% and 62%, respectively.

4. Discussion

This study demonstrated the feasibility of postoperative adjuvant chemotherapy of CDDP plus VNR (CDDP at 80 mg/m^2 was administered on day 1 and VNR at 25 mg/m^2 was administered on days 1 and 8, every 3 weeks) for surgically resected NSCLC patients. LACE meta-analysis and randomized trials have demonstrated the efficacy of CDDP-based adjuvant chemotherapy, in particular, CDDP plus VNR [3,4,6]. However, the treatment schedule and dosage were varied. In the trial conducted by the International Adjuvant Lung Cancer Trial Collaborative Group, CDDP at 80–120 mg/m^2 was administered every 3 or 4 weeks, and VNR at 30 mg/m^2 was administered weekly [2]. In the JBR.10 trial, CDDP at 50 mg/m^2 was administered on days 1 and 8 every 4 weeks, and VNR at 25 mg/m^2 was administered weekly [3]. In the Adjuvant Navelbine International Trialist Association (ANITA) study, CDDP at 100 mg/m^2 was administered every 4 weeks, and VNR at 30 mg/m^2 was administered weekly [6]. However, only 45% of the patients randomized to receive adjuvant chemotherapy completed the planned 4 cycles in the JBR.10 trial, and only 50% of the patients completed the cycles in the ANITA study. This

compliance with adjuvant chemotherapy of CDDP plus VNR is unsatisfactory, and the optimal treatment schedule and dosage for CDDP and VNR needs to be determined. The median dose received for CDDP was 320 mg/m^2, which is comparable to the results of the LACE meta-analysis (median dose of CDDP was 303 mg/m^2) [9]. The median dose of VNR is also comparable (this study, 200 mg/m^2 vs. LACE, 236 mg/m^2). Therefore, CDDP at 80 mg/m^2 on day 1 and VNR at 25 mg/m^2 on days 1 and 8 (every 3 weeks), as evaluated in this study, might be the optimal treatment schedule and dosage.

There have been few reports concerning factors influencing adjuvant chemotherapy compliance [10–12]. In the JBR.10 trial, the extent of the surgery and the patient's age and gender were reported to be related to compliance with adjuvant chemotherapy. In particular, patients who had undergone pneumonectomy were more likely to discontinue therapy because of toxicity than patients who had undergone less extensive resections [10]. In addition, a trend towards better compliance with adjuvant chemotherapy was reported in patients who underwent a thoracoscopic lobectomy than in those who underwent a thoracotomy [13]. On the other hand, some reports showed no effect of age and gender on compliance with adjuvant chemotherapy [12–14]. Our study did not identify any factors that influenced adjuvant chemotherapy compliance. This might have been because most of the patients in this study were younger than 75 years and showed good performance status.

A major limitation of this retrospective analysis was that the evaluation of toxicities, such as non-hematological toxicities, might be underestimated. The incidence of hematological toxicities was similar to that in previous reports [3,6,8]. Reducing, skipping, or delaying a dose in the planned chemotherapy was based on the physician's decision and might have been influenced by the physician's bias. However, we followed the chemotherapy regimen of CDDP at 80 mg/m^2 on day 1 and VNR at 25 mg/m^2 on days 1 and 8, every 3 weeks, and none of the patients received a modified dosage of CDDP or VNR from the first cycle of adjuvant chemotherapy. Although the RFS data was immature, the 2-year RFS rate was comparable to that in the trial conducted by the International Adjuvant Lung Cancer Trial Collaborative Group including Stage I patients (the 2-year disease-free survival rate was 61.0%) [2].

5. Conclusion

Our results indicated that adjuvant chemotherapy with CDDP at 80 mg/m^2 administered on day 1 plus VNR at 25 mg/m^2 administered on days 1 and 8, every 3 weeks, was feasible for surgically resected NSCLC patients. This treatment schedule and dosage for CDDP and VNR might be a good candidate for the reference arm of future phase III studies.

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This study was not supported by any funding.

Conflict of interest

The authors have no conflict of interest.

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Depolarized MUC1 Expression Is Closely Associated With Hypoxic Markers and Poor Outcome in Resected Non –Small Cell Lung Cancer

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