Impact of *in vitro* chemosensitivity test-guided platinum-based adjuvant chemotherapy on the surgical outcomes of patients with p-stage IIIA non-small cell lung cancer that underwent complete resection

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Abstract. The impact of *in vitro* chemosensitivity test-guided platinum-based adjuvant chemotherapy on the surgical outcomes of patients undergoing complete resection for locally advanced non-small cell lung cancer (NSCLC) has yet to be elucidated. In the present study, the utility of adjuvant chemotherapy based on the collagen gel droplet embedded culture drug sensitivity test (CD-DST) in patients with p (pathology)-stage IIIA NSCLC was retrospectively analyzed. A series of 39 patients that had received platinum-based adjuvant chemotherapy following complete resection between 2007 and 2012 were enrolled. Their surgical specimens were subjected to the CD-DST. The patients were subsequently classified into two groups on the basis of *in vitro* anti-cancer drug sensitivity data obtained using the CD-DST: The sensitive group (25 patients) were treated with regimens including one or two of the anti-cancer drug(s) that were indicated to be effective by the CD-DST, whereas the non-sensitive group (14 patients) were treated with chemotherapy regimens that did not include any CD-DST-selected anti-cancer drugs. There were no significant differences in the background characteristics of the two groups [including in respect of the pathological TN (tumor-lymph node) stage, tumor histology, epidermal growth factor receptor mutation status, the frequency of each chemotherapy regimen, and the number of administered cycles]. The 5-year disease-free survival (DFS) rate of the sensitive group was 32.3%, whereas that of the non-sensitive group was 14.3% (P=0.037). In contrast, no difference in overall survival (OS) was observed (P=0.76). Multivariate analysis revealed that adjuvant chemotherapy based on the CD-DST had a significant favorable effect on the DFS (P=0.01). Therefore, the present study has demonstrated that CD-DST data obtained from surgical specimens aid the selection of effective platinum-based adjuvant chemotherapy regimens for patients undergoing complete resection for p-stage IIIA NSCLC. The use of CD-DST-guided platinum-based regimens may have a beneficial impact on the DFS of such patients.

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Abbreviations: NSCLC, non-small cell lung cancer; CD-DST, collagen gel droplet embedded culture drug sensitivity test; DFS, disease-free survival; OS, overall survival; EGFR, epidermal growth factor receptor; ERCC1, excision repair cross-complementation group 1; RRM1, ribonucleotide reductase regulatory subunit M1; CDDP, cisplatin; CBDCA, carboplatin; DOC, docetaxel; PTX, paclitaxel; VNR, vinorelbine; GEM, gemcitabine; PEM, pemetrexed disodium; HDRA, histoculture drug response assay; ATP-TCA, adenosine triphosphate-based tumor chemosensitivity assay; CT, computed tomography

Key words: adjuvant chemotherapy, stage IIIA, non-small cell lung cancer, collagen gel droplet embedded culture drug sensitivity test, chemosensitivity test, platinum-based regimen, disease-free survival

Introduction

Lung cancer is one of the leading causes of cancer mortality in numerous countries. In non-small cell lung cancer (NSCLC), radical resection is generally recognized to be the most effective treatment, provided that the tumor is resectable. However, ~30-75% of patients with pathological stage IB to IIIA disease who undergo complete resections suffer

postoperative recurrence (local recurrence or distant metastasis) (1,2). Several of these patients may have micro-metastases that are not able to be detected during pre- or intra-operative staging. Therefore, if such micro-metastases could be controlled using adjuvant modalities following surgery, it may be possible to improve the surgical outcomes of patients with stage IB to IIIA NSCLC (1-4). In fact, various large-scale phase III clinical trials have indicated that adjuvant combined platinum-based chemotherapy improved the overall survival (OS) and disease-free survival (DFS) rates of patients with completely resected NSCLC (5-8).

Recently, individualized medication has served an important role in improving chemotherapeutic outcomes, since the effects of anti-cancer drugs are different among individuals. For instance, individualized chemotherapy regimens for NSCLC are often selected on the basis of a number of chemosensitivity-associated biomarkers, including epidermal growth factor receptor (EGFR) mutation status (9), anaplastic lymphoma kinase (ALK) gene rearrangement status (10), excision repair cross-complementation group 1 (ERCC1) status (11), ribonucleotide reductase regulatory subunit M1 (RRM1) (12), class III β-tubulin (13,14), and so on (15). Several recent clinical studies have indicated that such biomarkers may help us to identify subsets of patients that would benefit from adjuvant chemotherapy (15).

In the past 20 years, an *in vitro* chemosensitivity test, the collagen gel droplet embedded culture drug sensitivity test (CD-DST), has been developed on the basis of examinations of various types of malignant tumor at our (16,17) and other (18-20) institutions. Our group has subjected surgically resected samples to this test in order to aid the selection of effective chemotherapy regimens for patients with NSCLC. As a result, it was demonstrated that this test is useful for selecting chemotherapy regimens in patients with NSCLC that suffer postoperative recurrence (17,21). In addition, Kawamura *et al* (22) used this test to select the most appropriate chemotherapy regimens for patients with advanced NSCLC. Of course, there have also been numerous studies in which this test was used to aid the treatment of malignancies other than NSCLC, indicating that the CD-DST may provide useful information that would aid the development of individualized chemotherapy for patients with various types of malignant disease (16-21); for example, it could be used to provide information about chemosensitivity-associated biomarkers such as those described above (9-15).

Nevertheless, there have only been a few reports on the clinical application of this test to aid regimen selection during postoperative adjuvant chemotherapy (23,24). In the present study, in order to determine the impact of *in vitro* chemosensitivity test-guided adjuvant chemotherapy on surgical outcomes, the association between CD-DST findings and the effects of postoperative adjuvant chemotherapy in patients with locally advanced p-stage IIIA NSCLC was retrospectively examined. The results demonstrated that data derived from the CD-DST may aid regimen selection during platinum-based adjuvant chemotherapy for patients with p-stage IIIA NSCLC that undergo complete resection, and that this may improve the surgical outcomes of such patients.

Patients and methods

Patients. Between December 2007 and March 2012, 906 patients underwent surgical resection for lung cancer at our institution (the Osaka Medical Center for Cancer and Cardiovascular Diseases). Of these patients, 107 were diagnosed with p-stage IIIA lung cancer, and potentially curative surgery was performed in 93 patients in spite of the presence of locally advanced disease. Patients that were treated with neo-adjuvant therapy prior to surgery were excluded from the present study, and therefore a total of 39 patients with NSCLC who received platinum-based adjuvant chemotherapy after undergoing complete resection were enrolled. Informed consent for the CD-DST was obtained preoperatively.

The clinicopathological characteristics of the enrolled patients are summarized in Table I. Their mean age was 59 years old (range, 39-76). Twenty-four patients were male and 15 were female. All of the patients underwent a potentially curative lobectomy combined with lymph node dissection. The histological diagnosis was adenocarcinoma in 32 patients, squamous cell carcinoma in 4 patients, and other types in 3 patients (large cell carcinoma in 1 patient, and large cell neuroendocrine carcinoma in 2 patients). As for the pathological (tumor-lymph node) TN stage, 9, 21, 1, 5, and 3 cases were classified as T1N2, T2N2, T3N1, T3N2, and T4N1 respectively, according to the 7th Edition of the TNM classification. The EGFR mutation status of each primary tumor was examined using transbronchial or surgical specimens in 35 cases. Of these, 18 samples were positive for EGFR mutations (del746-750 in 11 samples, L858R in 6 samples, and L861Q in 1 sample). The 17 wild-type EGFR samples included three ALK-positive adenocarcinomas.

CD-DST data acquisition. CD-DST data for each patient's primary tumor were obtained under preoperative informed consent. Note that the use of CD-DST as a highly advanced medical technology was authorized by the Japan Ministry of Health, Labour and Welfare in 2007. In practice, consent was obtained prospectively where possible, which also covered the postoperative treatment in cases in which locally advanced disease was preoperatively predicted.

The CD-DST was performed as previously described by Kobayashi (16) and Higashiyama *et al* (17). In brief, after the primary tumor had been resected, the fresh primary tumor specimen was immediately minced using a scalpel and digested in a cell dispersion enzyme solution (EZ; Kurabo Industries Ltd., Osaka, Japan) for 2 h. The dispersed cancer cells were washed twice and collected by centrifugation at room temperature, 250 x g for 3 min, filtered through an 80-μm nylon mesh, and subsequently incubated in a collagen gel-coated flask (CG-flask; Kurabo Industries Ltd.) in a CO_2 incubator at 37˚C for 24 h. The viable cells that adhered to the collagen gel were collected and suspended in reconstructed type I collagen solution (Cellmatrix Type CD; Kurabo Industries Ltd.) at a final density of $1x10^5$ cells/ml. Three drops of the collagen cell mixture (30 μl/drop) were placed in each well of a 6-well multiplate, and allowed to gel at 37˚C in a CO_2 incubator for 1 h. As a control, this process was repeated using a 60-mm dish. The final cell concentration was ~$3x10^3$ cells/collagen gel droplet. Culture medium [DF medium containing 10% fetal

Table I. Characteristics of NSCLC patients who underwent adjuvant chemotherapy.

Characteristic	Total number of tested patients (n=39)	Sensitive group (n=25)	Non-sensitive group (n=14)	Differences (P-value)
Mean age, years (range)	59 (39-76)	62 (46-76)	55 (39-65)	0.06[a]
Sex (male/female)	24/15	15/10	9 /5	
p-stage				0.08 (T1,T2 vs. T3,T4)
T1N2	9	6	3	
T2N2	21	11	10	
T3N1,T3N2,T4N1	9	8	1	
Histology				0.63 (Sq vs. non-sq)
Sq	4	3	1	
Adeno	32	20	12	
Others	3	2	1	
EGFR status				0.12 (wild-type vs. mutant)
Mutant	18	14	4	
Wild-type	17	9	8	
Unknown	2	4	2	

[a]According to the unpaired t-test. p-stage, pathological stage; Adeno, adenocarcinoma; Sq, squamous cell carcinoma; T,N, tumor/lymph node (status); EGFR, epidermal growth factor receptor.

bovine serum (both from Gibco; Thermo Fisher Scientific, Inc., Waltham, MA, USA)] was overlaid on each well, and the plate was incubated overnight in a CO_2 incubator at 37˚C.

Anti-cancer drugs (the drugs and their dosages are described below) were added, and subsequently the plates were incubated for 1 h (gemcitabine; GEM) or 24 h (other drugs). After the removal of the medium containing the anti-cancer drugs, each well was rinsed twice, overlaid with serum-free culture medium (PCM-2; Kurabo Industries Ltd.), and incubated for 7 days. The medium was changed on the fourth day of the incubation period. At the end of the incubation period, neutral red was added to each well at a final concentration of 50 μg/ml, and the colonies in the collagen gel droplets were stained for 3 h. The collagen droplets in the 60-mm dish were stained immediately prior to exposure (day 1). Thereafter, each collagen droplet was fixed with 10% neutral formalin buffer, washed in water, air-dried, and subjected to image analysis. When the optical density of the control group was >5, the test was regarded a 'success'. *In vitro* sensitivity was expressed as the T/C ratio (%), where T and C are the total cell numbers of the treated and the control group, respectively. For each anti-cancer drug, a T/C ratio (%) of ≤50% was considered to indicate sensitivity.

The anti-cancer drugs and dosages tested in the CD-DST were as follows: 0.2 μg/ml cisplatin (CDDP), 2.0 μg/ml carboplatin (CBDCA), 0.1 μg/ml docetaxel (DOC), 1.0 μg/ml paclitaxel (PTX), 0.05 μg/ml vinorelbine (VNR), 8.0 μg/ml GEM, and 7.0 μg/ml pemetrexed disodium (PEM).

Examined adjuvant chemotherapy regimens. At our institution, adjuvant chemotherapy for locally advanced NSCLC, particularly p-stage IIIA NSCLC, is generally performed using a platinum-based (usually a doublet) regimen within the first 10 weeks following surgery. According to the guidelines used in Japan, a platinum-based chemotherapeutic method, such as CDDP plus VNR (CDDP+VNR) or CBDCA plus PTX (CBDCA+PTX), is usually selected (5,25). Therefore, CDDP+VNR or CBDCA+PTX was selected in cases in which the surgical specimen was found to be sensitive to one or more of these drugs during the CD-DST. When the surgical specimen was judged to be more sensitive to other drugs, regimens containing these drugs were selected, proving that the patient's physical condition allowed it. These patients were defined as the sensitive group. In contrast, when the CD-DST did not identify any appropriate combinations, the CDDP+VNR or CBDCA+PTX regimen was selected. These patients were defined as the non-sensitive group. In addition, one patient in the latter group received the CDDP+DOC regimen (26) at their own request.

The regimens used were as follows: CDDP+VNR in 30 patients, CDDP plus DOC (CDDP+DOC) in 2 patients, CDDP plus GEM (CDDP+GEM) in 1 patient (27,28), CDDP plus PEM (CDDP+PEM) in 1 patient (28), CBDCA+PTX in 3 patients, CBDCA plus GEM (CBDCA+GEM) in 1 patient (27), and CBDCA plus DOC (CBDCA+DOC) in 1 patient (29) (Table II). All regimens were started at the standard doses reported in the guidelines (Table III). Three chemotherapy cycles were generally planned, and, where possible, a fourth was administered. During chemotherapy, toxicities were evaluated using the National Cancer Institute Common Terminology Criteria for Adverse Events (CTC-AE) Version 4.0.

Follow-up and statistical analyses. The follow-up examinations carried out after the adjuvant chemotherapy were generally performed as follows: During the first 36 months after the operation, systemic and local screening examinations were performed using blood tests, chest computed tomography

Table II. Adjuvant chemotherapy regimens performed, and the number of treatment cycles administered.

Treatment	Sensitive group (n=25)	Non-sensitive group (n=14)
Chemotherapy regimen		
CDDP+VNR	19 (76%)	11 (78.6%)
CDDP+DOC	1	1
CDDP+GEM	1	0
CDDP+PEM	1	0
CBDCA+PTX	1	2
CBDCA+GEM	1	0
CBDCA+DOC	1	0
No. of completed chemotherapy cycles		
One	2	0
Two	4	0
Three	1	1
Four	18	13
Tolerability rate (%)[a]	76%	100%

[a]Rate (%)=three or four cycles/total. CDDP, cisplatin; VNR, vinorelbine; DOC, docetaxel; GEM, gemcitabine; PEM, pemetrexed; CBDCA, carboplatin; PTX, paclitaxel.

(CT) scans were routinely obtained every 6 months, and fluoro-2-deoxyglucose positron emission tomography (FDG-PET) scans were generally performed every year. Brain CT or magnetic resonance imaging scans were performed as required. During the first 24 months, such examinations were performed with particular care. From the third postoperative year onwards, such intensive examinations were performed once a year at least.

The time of the initial recurrence was determined based on the onset of clinical symptoms, the detection of blood test abnormalities (e.g., the patient's serum carcinoembryonic antigen level), or the detection of recurrent lesions on imaging, and the DFS period was defined as the period between the operation and the time of the initial recurrence. When a tumor recurred, the initial recurrence site was also evaluated. DFS and OS curves were calculated using the Kaplan-Meier method, and differences were determined using the log-rank test. The Cox proportional hazards regression model was used to perform a multivariate analysis of factors associated with favorable DFS. Statistical analyses were performed using Fisher's exact probability test or the unpaired t-test. $P<0.5$ was considered to indicate a statistically significant difference.

Results

CD-DST data and the types of adjuvant chemotherapy performed. In order to examine the associations between the findings of the CD-DST and the adjuvant chemotherapy regimens performed, the patients were classified into the following two groups as described above: The sensitive group (25 patients), which were treated with regimens including one or two anti-cancer drug(s) that were indicated to be effective by the CD-DST (i.e., CD-DST-selected drugs), and the non-sensitive group (14 patients), who were treated with chemotherapy regimens that did not include any CD-DST-selected anti-cancer drugs.

Table I features the characteristics of the patients in each group. The sensitive group included slightly older patients than the non-sensitive group ($P=0.06$). The sensitive group also exhibited more aggressive T-factors than the non-sensitive group ($P=0.08$). There were no significant differences in sex, histology, or EGFR mutation status between the groups.

Table II shows a summary of the adjuvant chemotherapy regimens performed, and the numbers of cycles administered in each group. The CDDP+VNR regimen was performed in 19 patients (76%) in the sensitive group, and 11 patients (78.6%) in the non-sensitive group, and the ratio of the frequency of the CDDP+VNR regimen to the frequency of other regimens did not differ between the groups. With regard to the number of chemotherapy cycles administered, 19 patients in the sensitive group (76%) and 14 in the non-sensitive group (100%) received ≥3 cycles of platinum-based adjuvant chemotherapy, and there was also a significant difference in the frequency of adjuvant chemotherapy completion between the groups. In the sensitive group, 6 patients received incomplete chemotherapy (only 1 cycle in 2 patients and 2 cycles in 4 patients) because of Grade 3 or 4 hematological toxicities in 2 patients, a severe pulmonary infection in 1 patient, refusal in 2 patients, and cardiac failure in 1 patient. These 6 patients received no additional adjuvant chemotherapy until recurrent disease occurred. It was necessary that appropriate dose reductions of each regimen were performed in certain patients in each group.

DFS and OS. Follow-up examinations were conducted in August 2015. At this time, the patients' follow-up periods ranged from 10.3 to 91.0 months (median: 55.6 months), and recurrent disease occurred in 28 patients. A total of 18 patients had succumbed to cancer, and 1 had died of another disease without suffering recurrence. Among the survivors, the follow-up period ranged from 28.7 to 91.0 months (median: 59.7 months).

The 2-year, 3-year, and 5-year DFS rates for all patients were 40.5, 29.2, and 26.0%, respectively, and the median DFS period was 21.9 months. The DFS curves of the two groups are shown in Fig. 1A. The 2-year, 3-year, and 5-year DFS rates of the sensitive group were 55.7, 37.7, and 32.3%, respectively, and the median DFS period was 25.3 months. The 2-year, 3-year, and 5-year DFS rates of the non-sensitive group were 14.3, 14.3, and 14.3%, respectively, and the median DFS period was 15.4 months. The DFS of the two groups differed significantly ($P=0.037$); i.e., the sensitive group exhibited a significantly improved DFS (Fig. 1A).

The 2-year, 3-year, and 5-year OS rates for all patients were 87.2, 84.5, and 62.1%, respectively, and the median OS period was 67.6 months. The OS curves of the two groups are shown in Fig. 1B. No significant difference in the OS was identified between these groups ($P=0.76$).

Prognostic analysis. Representative candidates were selectively analyzed to identify factors associated with favorable DFS or OS in this series. The analysis included age, sex,

Table III. Univariate analysis of DFS- and OS-associated factors.

Variable			P-value (log-rank test) DFS	OS
Age (years)	≤60 (n=15)	>60 (N=24)	0.33	0.11
Sex	Male (n=24)	Female (N=15)	0.36	0.018
T-stage	T3,T4 (n=9)	T1,T2 (N=30)	0.64	0.69
Histology	Sq (n=4)	Non-Sq (N=35)	0.63	0.054
EGFR status	Mutant (n=18)	Wild-type (N=17)	0.065	0.95
Regimen (CD-DST)	Non-sensitive (n=14)	Sensitive (n=25)	0.037	0.76
Regimen (CDDP/CBDCA)	CDDP-based (n=34)	CBDCA-based (n=5)	0.81	0.56
Chemotherapy completeness	No (n=6)	Yes (n=33)	0.55	0.14

DFS, disease-free survival; OS, overall survival; T (stage), tumor; EGFR, epidermal growth factor receptor; CD-DST, collagen gel droplet embedded culture drug sensitivity test; CDDP, cisplatin; CBDCA, carboplatin; Sq, squamous; non-Sq, non-squamous.

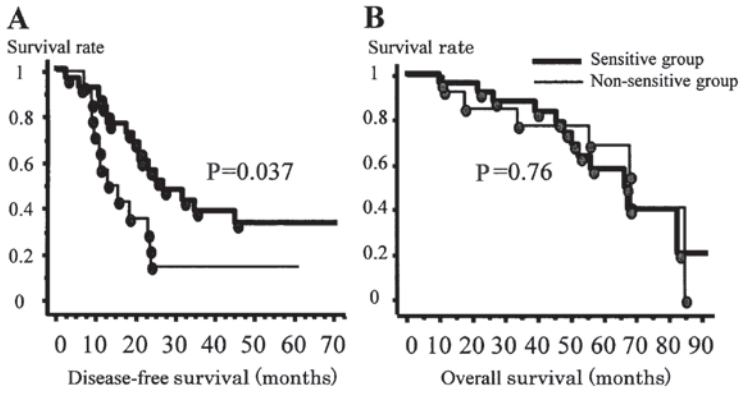

Figure 1. DFS and OS curves of the two groups of enrolled patients. (A) DFS curves of the two groups (i.e., the sensitive and non-sensitive groups). The 2-year, 3-year, and 5-year DFS rates of the sensitive group were 55.7, 37.7, and 32.3%, respectively. The 2-year, 3-year, and 5-year DFS rates of the non-sensitive group were 14.3, 14.3, and 14.3, respectively. The sensitive group exhibited a significantly improved DFS (P=0.037). (B) OS curves of the two groups. No significant difference in the OS was identified between these groups (P=0.76).

T-factor, histology, EGFR status, the administration of regimens involving CD-DST-selected anti-cancer drugs, the administration of CDDP-based or CBDCA-based regimens, and the number of chemotherapy cycles completed. A summary of the results of the univariate analyses is shown in Table III. Regarding DFS, only the administration of regimens involving CD-DST-selected drugs was found to be a prognostic factor (P=0.037), although EGFR status was demonstrated to be a marginally significant factor (P=0.065). By contrast, sex and histology were strongly associated with OS. As described above, the administration of regimens involving CD-DST-selected drugs did not have any effect on OS.

Table IV shows a summary of the results of the multivariate analysis of DFS-associated prognostic factors. According to this analysis, among the 35 patients in the present study (excluding the four patients for whom no information regarding EGFR status was available), regimens involving CD-DST-selected drugs and EGFR status were found to be independent predictors of DFS.

Recurrence pattern following adjuvant chemotherapy. The differences in the initial recurrence patterns of the sensitive and non-sensitive groups were analyzed. Table V shows a summary of the recurrence site data for each group. The two groups exhibited similar recurrence rates (68% vs. 79%). The rate of nodal recurrence was slightly lower in the sensitive group (P=0.08) than in the non-sensitive group, and a similar trend was observed for brain recurrence (P=0.09).

Discussion

Several recent large-scale clinical trials have shown that postoperative platinum-based adjuvant chemotherapy improves the DFS and OS of patients with p-stage IB to IIIA NSCLC (5-8). For instance, the International Adjuvant Lung Cancer Collaborative Trial Group (IALT) demonstrated that the administration of three to four courses of CDDP-based adjuvant chemotherapy after complete resection for p-stage I to IIIA NSCLC improved the 5-year OS rate by 4.1%, and the 5-year DFS rate by 5.1% (5). The JBR.10 study also revealed that CDDP+VNR adjuvant chemotherapy for completely resected stage IB to II NSCLC improved OS by 15% (6). The adjuvant Navelbine International Trialist Association (ANITA) trial reported that adjuvant chemotherapy for stage IB to IIIA

Table IV. Multivariate analysis of DFS-associated prognostic factors among NSCLC patients (n=35) with p-stage IIIA disease who underwent adjuvant chemotherapy.

Variable	Comparison	Odds ratio	95% confidence interval	P-value
EGFR status	Wild-type vs. mutant	0.337	0.139-0.818	0.016
Regimen (CD-DST)	Non-sensitive vs. sensitive	3.152	1.315-7.554	0.010

HSCLC, non-small cell lung cancer; DFS, disease-free survival; EGFR, epidermal growth factor receptor; CD-DST, collagen gel droplet embedded culture drug sensitivity test.

Table V. Sites of recurrence in each group.

	Sensitive group (n=25, %)	Non-sensitive group (n=14, %)
No. of cases of recurrence	n=17 (68)	n=11 (79)
Initial recurrence site		
Intrathoracic		
Node[a]	3 (12)	5 (36)
Pleura	2 (8)	0
Lung	6 (24)	2 (14)
Surgical margin	1 (4)	0
Extrathoracic		
Brain	1 (4)	3 (21)
Bone	1 (4)	0
Spleen	1 (4)	0
Systemic	2 (8)	1 (7)

[a]P=0.08.

NSCLC resulted in an 8.6% increase in the 5-year OS rate, and an 8.7% rise in the 5-year DFS rate (7). In addition, it also resulted in a significant difference in OS in patients with N1 or N2 disease (7). Furthermore, according to a study of the Lung Adjuvant Cisplatin Evaluation (LACE) database, combined CDDP-based adjuvant chemotherapy (CDDP+VNR) improved the OS and DFS rates of completely resected patients, particularly those with stage II or III NSCLC (8). Therefore, adjuvant chemotherapy involving a platinum-based regimen is now the standard treatment for locally advanced NSCLC around the world (4). However, the optimal platinum-based regimen has yet to be elucidated, in terms of the platinum agent itself, as well as the best anti-cancer drug to pair it with. At present, VNR is the most commonly available anti-cancer drug that is suitable for pairing with platinum-based agents (6-8), but, if possible, more effective individualized drugs should be selected. In addition to these anti-cancer drugs, several studies of molecular targeting medicines, such as EGFR inhibitors, have been performed in the adjuvant setting (30-33). In light of the fact that activating mutations in the EGFR gene are strongly correlated with responsiveness to EGFR inhibitors (30,31,33), recent (ongoing) Japanese studies of EGFR inhibitors have only involved patients with tumors expressing EGFR mutations.

In order to improve the efficacy of adjuvant chemotherapy for patients who undergo surgical resection, it is very important to select patients and regimens in an appropriate manner. There are various characteristics that may be taken into account during patient selection, including disease stage, age, tumor histology, risk classification, biomarkers, genetics, and so forth, and it would be useful to be able to accurately identify subgroups of patients with NSCLC who would derive the greatest benefit from individualized chemotherapy regimens (1-4,15). Several studies have reported that individualized adjuvant chemotherapy is possible for patients with NSCLC undergoing surgery. For example, ERCC1, a biomarker that is useful for predicting sensitivity to CDDP, has been reported to aid regimen selection (11,34). Low ERCC1 expression has been suggested to predict increased sensitivity to CDDP-based chemotherapy, as it is results in saturation of the enzyme complex (11). Olaussen *et al* (34) demonstrated that, among ERCC1-negative patients, the chemotherapy group exhibited significantly longer DFS rates compared with the observation group, whereas no significant difference in survival was detected among the ERCC1-positive patients, indicating that CDDP-based chemotherapy should be administered to ERCC1-negative patients. Thus, ERCC-1 could be a useful prognostic and predictive marker; however, a recent report questioned its practical utility (35). Other biomarkers of tumor chemosensitivity to cytotoxic anti-cancer drugs, for example, RRM1, which is a marker of GEM sensitivity, have been identified by experimental and clinical studies, but there are few clinical data regarding the use of chemosensitivity markers to aid regimen selection during adjuvant chemotherapy for NSCLC (12).

On the other hand, *in vitro* chemosensitivity tests for cytotoxic anti-cancer drugs, such as the CD-DS, the histoculture drug response assay (HDRA), the MTT assay, and the adenosine triphosphate-based tumor chemosensitivity assay (ATP-TCA), are promising regimen selection techniques (23,24,36-39). In Japan, the three former tests were used as highly advanced medical technologies between September 2007 and March 2012 (since April 2012, they have been classified as medical services under the Japanese health insurance system). As described in the Introduction, these tests are now widely used in clinical practice during the treatment of lung cancer and other malignancies (17-24). In fact, there have been many reports about the utility of these tests during chemotherapy for various types of advanced and recurrent malignancies (17-24,37-40). Such tests were used to examine surgical specimens, and this revealed that they were clinically useful for regimen

selection during chemotherapy for patients with advanced and recurrent NSCLC (17,21), but scant information is available regarding the clinical application of these tests in the adjuvant chemotherapy setting (23,24). Recently, Tanahashi *et al* (24) reported an interesting observation regarding the use of *in vitro* chemosensitivity tests during adjuvant chemotherapy for patients undergoing surgery for lung cancer: The OS of the patients treated with two HDRA-positive drugs was significantly better (P=0.03) compared with that of patients treated with one HDRA-positive drug or HDRA-negative drugs, indicating that adjuvant chemotherapy based on *in vitro* chemosensitivity test data may have a strong positive influence on the surgical outcomes of patients with NSCLC. However, no significant differences in DFS were detected among the patients in the latter series. By contrast, the present study revealed that adjuvant chemotherapy regimens that included at least one CD-DST-selected drug resulted in significantly more favorable DFS rates in patients with NSCLC with stage IIIA disease (P=0.036) than did regimens that did not involve any CD-DST-selected drugs. However, the use of such regimens did not have any impact on OS. In the present study, multivariate analysis revealed that CD-DST data may be used to improve the DFS rate in patients treated with adjuvant chemotherapy. The present study had a similar design to that performed by Tanahashi *et al* (24), although the *in vitro* chemosensitivity test method differed, as did the patients' disease stages, i.e., the patients enrolled by Tanahashi *et al* (24) had stage II or worse disease, whereas all of the patients in the present study had stage IIIA disease. In addition, the patients were tested at different points in their clinical courses: Since our study included more recent cases, recurrent lesions could have been treated differently (e.g., with molecular targeting agents) compared with the cases observed in the study of Tanahashi *et al* (24). Such factors may have been responsible for the differences in patient outcomes observed between our study and those of Tanahashi's group (24). Although there were differences between the findings of these studies, it was clearly demonstrated that the data obtained with *in vitro* chemosensitivity tests is correlated with surgical outcomes following complete resection in patients with NSCLC.

Maejima *et al* (23) reported that a good correlation exists between *in vitro* sensitivity to S-1 and the outcomes of gastric cancer patients who undergo complete resection followed by adjuvant chemotherapy with S-1. In that study, the CD-DST was used as an *in vitro* sensitivity test to examine the sensitivity of surgical samples to 5-fluorouracil and 5-chloro-2,4-dihydroexpyridine. In their prospective study, the high-sensitivity group exhibited higher 3-year OS and DFS rates compared with the low-sensitivity group. Thus, the CD-DST data exhibited a stronger correlation with DFS. It is noteworthy that, according to most recent report of the Japan multicenter exploratory phase II trial (JACCRO-GC 04) (39), similar results were also observed, indicating that chemosensitivity testing of surgical specimens appears to be a promising approach to selecting adjuvant chemotherapy regimens in patients with gastric cancer. Another similar study of gastric cancer demonstrated that the MTT assay is useful for regimen selection (40). Similarly, Fujita *et al* (41) identified that sensitivity testing is useful for selecting regimens for adjuvant chemotherapy for patients with locally advanced esophageal cancer.

In the present study, the patterns of recurrence that arose in each group after adjuvant chemotherapy for locally advanced NSCLC were also analyzed. An interesting difference was detected between the sensitive and non-sensitive groups: The sensitive group tended to develop fewer recurrent nodal or brain lesions than the non-sensitive group. Our group has previously emphasized the usefulness of CD-DST data for selecting chemotherapy regimens, especially for patients that suffer postoperative nodal recurrence (17). In addition, our group demonstrated how, in the case of certain anti-cancer drugs, few differences were observed between the chemosensitivity of the primary NSCLC tissue and the associated lymph node metastases (42). Several experimental studies have detected unexpected differences in chemosensitivity between primary and metastatic tumors (42,43). These results, and our previous data (17,42), appear to agree with the findings of the present study regarding nodal recurrence. By contrast, it was not possible explain our findings regarding brain recurrence. Regardless, further analyses of this topic are required, since the inter-group differences in recurrence patterns detected in the present study were not statistically significant.

The DFS results obtained in the present study, i.e., that the sensitive group displayed a more favorable prognosis, could be explained by slower tumor growth or a lower grade of tumor malignancy. According to several reports (11,12,24,34), *in vitro* chemosensitivity to certain anti-cancer drugs is strongly associated with the grade of tumor malignancy. As described above, ERCC-1 was found to be a prognostic marker, as well as a predictor, of chemotherapeutic efficacy (12,34). However, our firm opinion is that the differences in DFS between the two groups were due to variations in the efficacy of adjuvant chemotherapy, as there were no apparent differences in the background characteristics of the two groups, and OS was not influenced by the CD-DST status. Therefore, taking into consideration both our previous and present findings (17,21), the CD-DST data are more important as a predictor of the efficacy of anti-cancer drugs, which impacts on DFS, rather than as a prognostic indicator of OS.

The present study had a retrospective design, but was conducted in the clinical setting. It had several limitations. First, it involved a small number of patients, and secondly, all of the patients had stage IIIA disease. The current treatment strategy for patients with locally advanced NSCLC may be about to change. For example, adjuvant therapy using molecular targeting agents could become the standard regimen for patients with locally advanced NSCLC whose tumors are positive for EGFR mutations (30,31,33). In addition, *in vitro* chemosensitivity test-guided platinum-based regimen selection could be used in the clinical setting during the treatment of a limited population of patients with NSCLC, i.e., those who express the wild-type EGFR, in the future. Despite these limitations, at the very least it could be said that CD-DST data provide important information for improving the efficacy of adjuvant chemotherapy in patients with stage IIIA NSCLC.

In conclusion, the present study suggested that CD-DST data obtained from surgical specimens may provide important information for regimen selection during platinum-based adjuvant chemotherapy for patients who undergo complete resection for locally advanced stage IIIA NSCLC. The CD-DST-guided selection of platinum-based regimens could have a favorable

impact on the DFS of such patients. In order to estimate the clinical usefulness of *in vitro* chemosensitivity tests, such as the CD-DST, HDRA, and ATP-TCA, further comparisons of the effects of *in vitro* chemosensitivity test-guided regimens with those of conventional regimens in patients with NSCLC should be performed in a randomized control study.

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