Adenosine Triphosphate-based Chemotherapy Response Assay (ATP-CRA)-guided *versus* Empirical Chemotherapy in Unresectable Non-small Cell Lung Cancer*

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Abstract. *Background: We retrospectively compared adenosine triphosphate-based chemotherapy response assay (ATP-CRA)-guided and empirical chemotherapies for unresectable non-small cell lung cancer (NSCLC) in this case-control study. Patients and Methods: Unresectable NSCLC patients receiving ATP-CRA-guided platinum-based doublets as first-line therapy were enrolled as cases (n=27; 14 platinum-sensitive and 13 platinum-resistant patients). Performance status, stage, and chemotherapeutic regimen-matched patients receiving empirical chemotherapy were selected from the retrospective database as controls (n=93) in a case to control ratio of ~1:3. Results: Response rate and survival (progression-free; overall) in both groups were not significantly different. However, the platinum-sensitive subgroup by ATP-CRA showed a higher response rate than the empirical group (71 versus 38%; p=0.023) with a trend toward longer progression-free survival (8.7 versus 4.8 months for platinum-sensitive versus empirical; p=0.223) and overall survival (not reached versus 12.6 months for platinum-sensitive versus empirical for p=0.134). Conclusion: ATP-CRA may be helpful in selecting platinum-responsive patients in unresectable NSCLC. We consider that nonplatinum doublets in platinum-resistant patients by ATP-CRA may be a more adapted approach than platinum-based doublets in future clinical trials.*

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Non-small cell lung cancer (NSCLC) is the leading cause of cancer-related death around the world (1). Most patients with NSCLC eventually succumb to distant metastasis. Despite advances in palliative chemotherapy, however, the prognosis of advanced NSCLC is still very poor. To overcome this limitation, attention is turning to developing of techniques that could provide predictive information regarding a particular tumors chemosensitivity, as a means of enhancing patient selection for a specific chemotherapy option. This is based on the concept developed 30 years ago of selecting chemotherapeutic agents for an individual patient by *in vitro* drug sensitivity testing (2).

With this background, we have already reported the outcomes of an *in vitro* chemosensitivity test, adenosine triphosphate-based chemotherapy response assay (ATP-CRA)-guided platinum-based two-drug chemotherapy for unresectable NSCLC (3). The methodology of ATP-CRA was feasible as an *in vitro* chemosensitivity and resistance assay before chemotherapy. This assay had the advantages of a short test turnaround time of 7 days and a high assay success rate of 89% , despite using a limited volume of tumor sample from bronchoscopic biopsies (3, 4). The study showed more favorable responses and survival in the chemosensitive subgroup than in the chemoresistant subgroup within the assay-guided chemotherapy group. However, our previous study was not randomized in order to compare *in vitro* assay-guided chemotherapy to empirical chemotherapy. As a result, the clinical benefit of *in vitro* assay-guided chemotherapy remained unanswered. This motivated us to perform the current study that compares ATP-CRA-guided chemotherapy to empirical chemotherapy in unresectable NSCLC. In the current study, prospective data of patients extracted from the previous reported trial with ATP-CRA-guided chemotherapy (3) were compared to retrospective data of patients receiving empirical chemotherapy in unresectable NSCLC.

Patients and Methods

Patients. Between January 2004 and December 2005, we prospectively performed ATP-CRA-guided platinum-based doublet chemotherapy for chemonaïve, unresectable NSCLC under a clinical trial setting (3). Medical records of unresectable NSCLC patients diagnosed at the same period and receiving platinum-based empirical chemotherapy were retrospectively reviewed. Through retrospective review, patients who met the following criteria were eligible: i) histologically or cytologically proven NSCLC, ii) stage IIIB or IV disease [American Joint Cancer Committee (AJCC) staging 2002] (5), iii) receiving at least one cycle of platinum-based doublet chemotherapy, iv) Eastern Cooperative Oncology Group performance status (ECOG PS) ≤2, v) adequate organ function, vi) no previous chemo- or radiotherapy, and vii) no history of other malignancies (excluding nonmelanoma skin cancer or carcinoma *in-situ* of the uterine cervix) within 5 years. From 341 eligible patients by retrospective review, ECOG PS (0-1 *versus* 2)-, stage (IIIB *versus* IV)-, and chemotherapy regimen (platinum plus gemcitabine *versus* paclitaxel *versus* vinorelbine)-matched patients were randomly selected at a case to control ratio of ~1:3 between the assay-guided (cases) and empirical chemotherapy (controls) groups (Figure 1).

Chemotherapy response and survival were compared between the assay-guided and empirical chemotherapy groups. This study was approved by the Institutional Review Board of Severance Hospital, Yonsei University Health System.

ATP-CRA methodology. ATP-CRA was performed as described elsewhere (3, 4). Briefly, cancer cells were isolated from tumor tissues and normal cells were specifically eliminated. Separated tumor cells were diluted using Iscove's Modified Dulbecco's Medium (IMDM) (GIBCO BRL, Rockville, MD, USA), including 10% fetal bovine serum (GIBCO BRL, Rockville, MD, USA), and seeded on an ultra-low attachment plate (Costar, Cambridge, MA, USA). In the treated groups, chemotherapeutic agents were added to the seeded cell cultures which were then incubated. In the untreated control groups, IMDM without chemotherapeutic agents was added. Cells from the untreated control and treated groups were lysed and the amount of ATP in the cell lysates was measured as previously described. The cell death rate for each drug was defined as the rate of ATP luminescence reduction in the treated group compared to the untreated control.

Chemotherapy. In the assay-guided treatment group, a drug sensitivity was defined as a drug producing a 30% or more reduction in ATP compared to untreated controls (3, 6). All the patients received platinum-based two-drug chemotherapy regardless of their *in vitro* platinum-sensitivity. One of the following nonplatinum drugs combined with platinum was chosen based on the ATP-CRA results as recently reported (3): gemcitabine (1,000 mg/m^2, days 1 and 8, every 3 weeks), paclitaxel (175 mg/m^2, day 1, every 3 weeks), or vinorelbine (30 mg/m^2, days 1 and 8, every 3 weeks). In cases sensitive to no drug *in vitro*, the drug with the highest cell death rate was chosen. In the empirical group, a nonplatinum agent was chosen depending on physicians' discretion and administered in the same dosing schedules as the assay group. Platinum choice [cisplatin (75 mg/m^2, every 3 weeks) or carboplatin (area under the curve of 5, every 3 weeks)] was determined by renal function and ECOG PS similarly in both groups; carboplatin was preferred in cases of creatinine clearance ≤60 ml/min or PS of 2. Chemotherapy was delivered up to a maximum of 6 cycles or until the appearance of progressive disease.

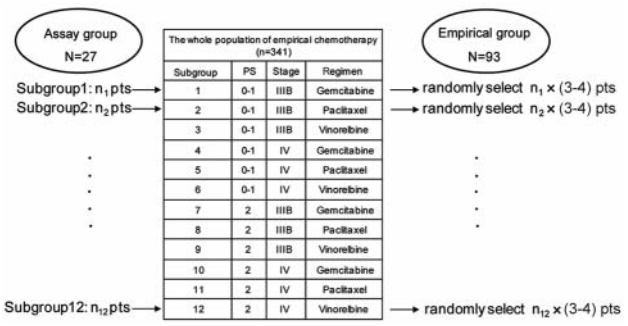

Figure 1. *Method of patient selection. PS, Performance status; pts, patients. The assay group (n=27) and whole population of empirical chemotherapy (n=341) were divided into subgroups 1 to 12 according to PS, stage and chemotherapy regimen. From subgroups 1 to 12 of the whole population of empirical chemotherapy, patients were randomly selected at a ratio of 1:3 to 1:4 between the assay (cases) and empirical chemotherapy groups (controls).*

Analysis of end points and statistical considerations. The primary end point was the clinical response rate (RR). Response was assessed every 2 cycles. Tumor response was evaluated according to the Response Evaluation Criteria in Solid Tumors (7). The secondary end points were progression-free survival (PFS) and overall survival (OS). PFS was defined as the time from commencement of chemotherapy until progression or death. OS was defined as the time from chemotherapy to death from all causes.

All statistical calculations were carried out using SPSS for Windows, version 12.0 (SPSS Inc., USA). All *p*-values were two-sided and the α-value was set at 0.05. Chi-square or Fisher exact test was used to compare categorical variables. Survival was calculated using the Kaplan-Meier method. A log-rank test was used to compare survival between groups. Multivariate analysis for prognosticators was performed by Cox's proportional hazard regression model.

Results

Patients characteristics. In the assay group, 27 patients were included (Table I). Ninety-three corresponding patients with matched prognosticators were included in the empirical group (Table I). All matched ratios were ~1:3, meeting appropriately targeted ratio except ECOG PS of 2 (1:2.5 for assay:empirical). In spite of this, matched prognosticators, namely, ECOG PS, stage and chemotherapeutic regimen were well-balanced statistically between the two groups. ECOG PS was 0-1 in 87.1-89.2% and 2 in 10.8-12.9%. The stages of patients were IIIB in 30.1-33.3% and IV in 66.7-69.9%. The nonplatinum agents administered were paclitaxel in 40.7-42.0%, gemcitabine in 33.3%, and vinorelbine in 24.7-26.0%. Variables other than matched prognosticators were not different between the two groups, either. However, in the assay group, carboplatin was used slightly more frequently (29.6 *versus* 17.2%; p=0.155) and brain metastasis was a little more common (18.5 *versus* 10.8%; p=0.283).

Table I. *Patient characteristics*.

	Assay group (n=27)		Matched ratio	Empirical group (n=93)		
	N	(%)	Ass:Emp	N	(%)	*P*-value[a]
Median age (years) (range)	62	(33-76)	-	59	(29-74)	0.529
Gender						
Male	16	(59.3)	-	59	(63.4)	0.693
Female	11	(40.7)	-	34	(36.6)	
ECOG performance status						
0-1	23	(89.2)	1:3.6	83	(87.1)	0.797
2	4	(10.8)	1:2.5	10	(12.9)	
Histology						
Adenocarcinoma	16	(59.3)	-	46	(49.5)	0.456
Squamous cell carcinoma	10	(37.0)	-	36	(38.7)	
Other	1	(3.7)	-	11	(11.8)	
Stage						
IIIB	9	(33.3)	1:3.1	28	(30.1)	0.749
IV	18	(66.7)	1:3.6	65	(69.9)	
Brain metastasis						
Absent	22	(81.5)	-	83	(89.2)	0.283
Present	5	(18.5)	-	10	(10.8)	
Chemotherapy regimen						
Gemcitabine+platinum	9	(33.3)	1:3.4	31	(33.3)	0.990
Paclitaxel+platinum	11	(40.7)	1:3.5	39	(42.0)	
Vinorelbine+platinum	7	(26.0)	1:3.3	23	(24.7)	
Platinum						
Cisplatin	19	(70.4)	-	77	(82.8)	0.155
Carboplatin	8	(29.6)	-	16	(17.2)	
Assay results within assay group						
Platinum-sensitive	14	(51.9)	-		-	-
Sensitive to both drugs	8					
Sensitive to platinum alone	6					
Platinum-resistant	13	(48.1)	-		-	-
Sensitive to nonplatinum drug	6					
Sensitive to neither drug	7					

Ass: Assay group, Emp: empirical group. [a]*p*-value was calculated by Chi-square test or Fisher's exact test except for age (Mann-Whitney test).

Within the assay group, ATP-CRA results were as follows: 8 patients were sensitive to both platinum and nonplatinum agents, 6 patients were sensitive to platinum alone, 6 patients were sensitive to nonplatinum agents alone, and 7 patients were sensitive to neither drug. When the former 2 subgroups were categorized as the 'platinum-sensitive group' (n=14) and the latter 2 subgroups as the 'platinum-resistant group' (n=13), there were no significant differences in pretreatment parameters such as ECOG PS, histology and stage between these two groups (data not shown).

Comparison of response between assay-guided versus empirical groups. Median number of cycles administered were 3 (range, 1-6) and 4 (range, 1-9), with median relative dose intensities of 85.7% and 81.8% for the assay-guided and empirical groups, respectively. Chemoradiotherapy was administered to 2 patients (7.4%) in the assay-guided group and 11 (11.8%) in the empirical group. Two patients (7.4%) received curative-intent surgery in the assay-guided group after chemotherapy and 3 patients (3.2%) in the empirical group.

Only 1 patient from the empirical group was not assessable for response due to loss of follow-up after the 1st cycle of chemotherapy. Within the assay group, the RR was significantly higher in the platinum-sensitive group (71.4%) than that in the platinum-resistant group (23.1%; p=0.021). However, when the platinum-sensitive and -resistant groups were combined in the assay group, the assay group only had a trend toward a slightly higher RR than the empirical group (48.1 *versus* 38.0% for the assay *versus* empirical group; p=0.347; Table II) under the intent-to-treat analysis. In spite of this, the platinum-sensitive group still showed a higher response rate than the empirical group (Figure 2; p=0.023). In the subgroup analysis according to nonplatinum agents, the paclitaxel group had a trend toward a higher response rate that favored assay-guided chemotherapy

Table II. *Comparison of treatment outcomes based on regimens between assay-guided versus empirical groups.*

	Assay group	Empirical group	*P*-value
Gemcitabine+platinum	(n=9)	(n=31)	
Sensitive to both/pl/nonpl/neither (%)	33/0/33/34	-	-
Response rate (%)	55.6	45.2	0.712
PFS (mo), median (95% CI)	3.8 (0.3-7.3)	5.3 (3.9-6.7)	0.967
OS (mo), median (95% CI)	9.0 (2.9-15.1)	12.6 (6.5-18.7)	0.860
Paclitaxel+platinum	(n=11)	(n=39)	
Sensitive to both/pl/nonpl/neither (%)	27/37/9/27	-	-
Response rate (%)	54.5	30.8	0.147
PFS (mo), median (95% CI)	5.8 (0.1-12.3)	4.2 (2.7-5.7)	0.285
OS (mo), median (95% CI)	24.1 (7.5-40.7)	11.9 (8.7-15.1)	0.332
Vinorelbine+platinum	(n=7)	(n=23)	
Sensitive to both/pl/nonpl/neither (%)	29/29/29/13	-	-
Response rate (%)	28.6	40.9	0.677
PFS (mo), median (95% CI)	3.6 (0.5-6.7)	5.1 (0.1-13.0)	0.367
OS (mo), median (95% CI)	11.2 (NA)	NR	0.892
Total patients	(n=27)	(n=93)	
Response rate (%)	48.1	38.0	0.347
PFS (mo), median (95% CI)	4.4 (2.7-6.1)	4.8 (3.5-6.1)	0.918
OS (mo), median (95% CI)	15.7 (0.1-32.7)	12.6 (9.0-16.2)	0.642

Both/pl/nonpl/neither: Sensitive to both drugs/platinum/nonplatinum/neither; PFS: progression-free survival; mo: month; CI: confidence interval; OS: overall survival; NA: not available; NR: not reached.

(p=0.147; Table II). In the gemcitabine and vinorelbine group, there were no differences in response between assay-guided and empirical chemotherapy (Table II).

Comparison of progression-free and overall survival between assay-guided versus empirical groups. Eight patients were excluded from the PFS analysis: 1 (an early drop-out because of the patient's refusal) from the assay group and 7 (an unclear date of progression) from the empirical group. At the median follow-up duration of 11.2 months, 23 (88.5%) out of the 26 patients in the assay group and 80 (93.0%) out of the 86 patients in the empirical group experienced disease progression. Within the assay group, the platinum-sensitive subgroup showed a significantly longer PFS than the platinum-resistant subgroup (p=0.047). There was also a trend toward the longest PFS in the platinum-sensitive group [median, 8.7 months, 95% confidence interval (CI) 2.6-14.8 months], followed by the empirical group (median, 4.8 months, 95% CI 3.5-6.1 months) and then the platinum-resistant group (median, 2.4 months, 95% CI 1.9-2.9 months) although these differences were not statistically significant (Figure 3A). However, no difference in median PFS was observed between the assay and empirical groups (4.4 *versus* 4.8 months for assay *versus* empirical; p=0.918; Figure 3C; Table II).

Sixteen (69.6%) out of 23 progressive patients from the assay group and 60 (75.0%) out of 80 progressed patients from the empirical group received 2nd-line chemotherapy.

All the patients were included in the OS analysis. Fifteen (55.6%) out of the 27 patients in the assay group and 56 (60.2%) out of the 93 patients in the empirical group died. All deaths were caused by cancer progression except 2 (1 pneumonia in the assay group, 1 traffic accident in the empirical group). Although within the assay group median OS was longer in the platinum-sensitive subgroup (not reached) than that in the platinum-resistant subgroup (11.0 months; p=0.068), there was no difference in median OS between the assay-guided (15.7 months, 95% CI 0.1-32.7 months) and empirical groups (12.6 months, 95% CI 9.0-16.2; p=0.642; Figure 3D; Table II). However, the platinum-sensitive subgroup by the assay showed a trend toward favorable OS compared to the empirical group (median, not reached *versus* 12.6 months for the platinum-sensitive *versus* empirical group; p=0.134; Figure 3B).

In addition, no differences in PFS or OS between the assay-guided and empirical groups were observed in the subgroup analysis according to nonplatinum agents (Table II).

Prognosticators for PFS and OS. Significant factors by univariate analysis, matched variables (ECOG PS, stage, chemotherapeutic agents), and choice of chemotherapy (assay-guided *versus* empirical) were put into the multivariate analysis (Table III). ECOG PS was the only prognosticator for both PFS (hazard ratio, 3.33) and OS (hazard ratio, 2.74). Assay-guided *versus* empirical chemotherapy was not a prognosticator.

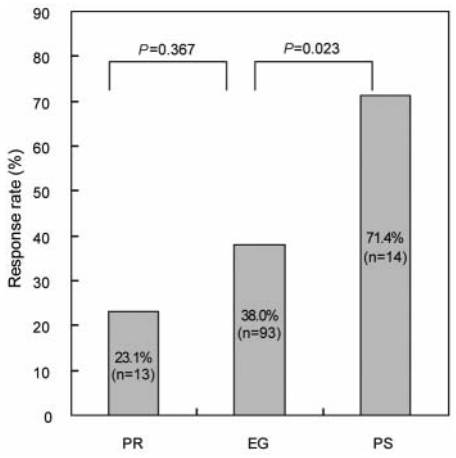

Figure 2. *Comparison of response rates: platinum-resistant by ATP-CRA (PR), platinum-sensitive by ATP-CRA (PS) and empirical group (EG).*

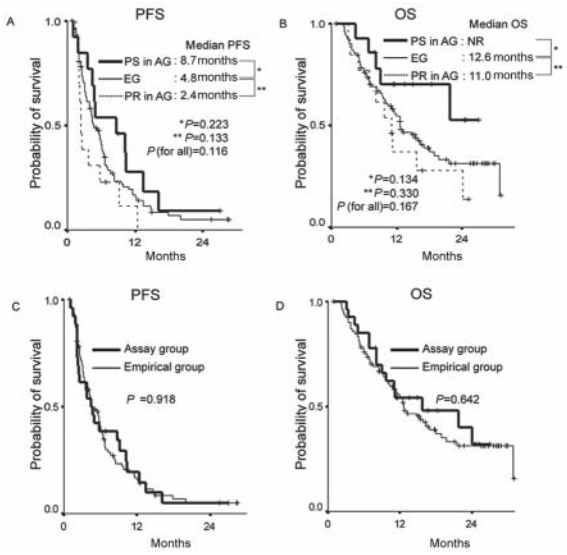

Figure 3. *Progression-free survival (PFS; A, C) and overall survival (OS; B, D). PS, Platinum-sensitive; PR, platinum-resistant; AG, assay group; EG, empirical group; NR, not reached.*

Discussion

Palliative chemotherapy with doublet regimens using platinum or third-generation agents such as taxanes and gemcitabine is the standard of care in advanced NSCLC (8-10). However, recent studies continue to indicate that we have reached an efficacy plateau with chemotherapy combination with or without a platinum agent. As one of various strategies to overcome this problem, chemosensitivity and resistance assays have been studied in NSCLC (11-14). Until now, studies of this type have been limited in number (15). Moreover, no randomized trials comparing assay-guided chemotherapy to empirical chemotherapy have been performed on NSCLC. In the current study, based on our previously reported data on ATP-CRA-guided platinum-based chemotherapy in unresectable NSCLC, we set a control arm consisting of individuals with empirical platinum-based chemotherapy. Patients were not randomly assigned to either of the groups. However, all patients were treated at the same institute within the same period using identical therapeutic standards. Both treatment directives and follow-up for the controls were identical compared to the ATP-CRA group. Patients from both groups were well matched for all criteria known to influence outcome. Therefore, the two arms in our study are comparable even though this study was not designed prospectively.

In our study on platinum-based chemotherapy in unresectable NSCLC, RRs according to regimens were not statistically different, which was in accordance with previous reports (16-18). The platinum-sensitive group by ATP-CRA showed a higher response rate than the empirical group as *in vitro* assay-guided chemotherapy has been reported to show higher RRs than empirical chemotherapy in various types of cancer (19, 20). This result encourages the further testing of ATP-CRA in clinical trials. However, when the platinum-sensitive and resistant groups were combined in the assay group, the assay group only had a trend toward a slightly higher RR than the empirical group. Moreover, *in vitro* assay-guided chemotherapy was not more beneficial than empirical chemotherapy in terms of survival (PFS or OS) in the current study.

Theoretically, ATP-CRA-guided chemotherapy should be superior to empirical chemotherapy. In reality, it is not. There are several possible explanations for the non-superiority of assay-guided chemotherapy in our study. Firstly, the proportion of patients who are going to experience benefits from platinum-based chemotherapy may be predetermined from the pool of NSCLC patients. This hypothesis is supported by the previous reports that in advanced NSCLC, none of platinum-based doublets as a first-line chemotherapy was superior to others (16-18). Under this assumption, the efficacy of platinum-based chemotherapy cannot be improved, even with assay-guided chemotherapy.

Secondly, there was not enough variety of chemotherapeutic regimens in the assay-guided group, in which nonplatinum doublets were not allowed. In designing this trial, a chemotherapeutic regimen was set as a matching variable between the assay-guided and empirical groups to augment comparability. On the contrary, this point may have offset potential differences in efficacy between the assay-guided and empirical chemotherapies. For example, even patients with platinum resistance received platinum-based chemotherapy. Alternatively, they could have received nonplatinum doublets as another option in platinum-resistant

Table III. *Prognosticators for progression-free and overall survival.*

		Progression-free survival		Overall survival	
		Univariate	Multivariate	Univariate	Multivariate
Variable		*P*-value	HR (95% CI)	*P*-value	HR (95% CI)
Age	(<65 *vs.* ≥65 years)	0.355		0.217	
Gender	(male *vs.* female)	0.059		0.718	
ECOG PS	(0-1 *vs.* 2)	<0.001	3.33 (1.85-5.96)	<0.001	2.74 (1.45-5.19)
Histology	(adenocarcinoma *vs.* squamous)	0.261		0.689	
Stage	(IIIB *vs.* IV)	0.028	1.60 (0.98-2.61)	0.051	1.30 (0.69-2.43)
Brain metastasis	(absent *vs.* present)	0.024	2.03 (1.08-3.80)	0.186	1.46 (0.73-2.94)
Nonplatinum		0.739		0.317	
	Gemcitabine		1		1
	Paclitaxel		1.35 (0.84-2.18)		0.95 (0.54-1.67)
	Vinorelbine		1.83 (1.03-3.26)		0.69 (0.31-1.50)
Platinum	(cisplatin *vs.* carboplatin)	0.483		0.654	
Choice of CTx	(assay *vs.* empirical)	0.918	1.10 (0.69-1.76)	0.642	1.24 (0.69-2.21)

All the variables except nonplatinum were dichotomized ones. The former in these dichotomized variables was considered a reference value for multivariate analyses. HR: Hazard ratio; CI: confidence interval; ECOG PS; Eastern Cooperative Oncology Group performance status; CTx: chemotherapy.

cases. Third-generation-based nonplatinum combinations have been reported to be possible alternatives to platinum-based doublets (8). In addition, Figure 3A and 3B show that the survival curves of the empirical group are located between platinum-sensitive and -resistant groups, suggesting patients receiving drugs to which they were *in vitro* sensitive had the best outcomes and those receiving drugs to which they were *in vitro* resistant had room for improved survival with alternative drugs. Our ATP-CRA could predict chemoresistance rather than chemosensitivity as we previously reported (3). From the perspective of this chemoresistance assay, further randomized trials should be designed.

Thirdly, biopsied tissue used in ATP-CRA may have not been representative of individual patients' whole tumor (21-23). This has always been a big issue for *in vitro* chemosensitivity tests. Although this is not the case in NSCLC, Cho and colleagues reported that ATP-CRA results changed according to depth of invasion of the tested tissue in advanced colorectal cancer (24). Therefore, in further clinical trials, specimens for chemosensitivity tests should be taken from various parts of the whole tumor.

When patients are sensitive to both platinum and nonplatinum agents, drugs selected by *in vitro* tests are the most ideal choice. In this study, however, both types of drugs were administered in only 30% of patients because only 30% of patients had tumors with sensitivity to both drugs. Moreover, platinum sensitivity was not inferior to doublet sensitivity in predicting efficacy of platinum-based doublets in our study (data not shown). Therefore, we recommend that only platinum be tested with ATP-CRA and platinum-resistant tumors be treated with nonplatinum combinations in clinical trial settings. Low expression of the excision repair cross-complementing 1 (*ERCC1*) gene has been reported to predict platinum-sensitivity in NSCLC (25, 26). Therefore, platinum sensitivity with ATP-CRA is worth comparing to *ERCC1* testing in NSCLC.

In conclusion, although ATP-CRA-guided chemotherapy did not show superior efficacy compared to empirical chemotherapy in unresectable NSCLC, ATP-CRA may be helpful in selecting platinum-responsive patients. We consider that nonplatinum doublets in platinum-resistant patients by ATP-CRA may be a more adapted approach than platinum-based doublets in future clinical trials.

Conflict of Interest Statement

Sung Ho Choi is an employee of ISU ABXIS CO., LTD, where ATP-CRA was performed. There is no conflict of interest for other authors.

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