

## Individualized Adjuvant Chemotherapy for Surgically Resected Lung Cancer and the Roles of Biomarkers

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Several prospective randomized trials for patients with completely resected stages II and IIIA nonsmall cell lung cancer have confirmed a survival benefit with cisplatin-based adjuvant chemotherapy. The Lung Adjuvant Cisplatin Evaluation, which is based on pooled analyses of five randomized trials, has demonstrated a 4.2% absolute survival benefit at 5 years. The stage is the benchmark standard used to decide the indication for adjuvant chemotherapy; however, it is important to identify and select the patients who would benefit from adjuvant chemotherapy and to choose the optimal regimen for each case. The translational research was performed using specimens obtained in the above adjuvant trials also to obtain information concerning biomarkers and subsets of patients who would benefit from adjuvant chemotherapy. The extent to which individualized treatment of lung cancer can be provided, especially adjuvant chemotherapy, is discussed in this manuscript. (*Ann Thorac Cardiovasc Surg* 2009; 15: 144–149)

**Key words:** lung cancer, adjuvant chemotherapy, individualized treatment, biomarker

### Introduction

Surgery is considered to be the standard treatment for early-stage nonsmall cell lung cancer (NSCLC). However, distant metastasis occurred in nearly 60% of patients with stages I to IIIA NSCLC after complete resection. Micrometastasis of the tumor is generally regarded as the cause of recurrence; therefore systemic chemotherapy after surgery is a rational strategy to reduce the risk of recurrence and metastasis.

Recent large-scale randomized trials have confirmed a survival benefit of adjuvant cisplatin-based chemotherapy following complete surgical resection of NSCLC (Table 1). The Lung Adjuvant Cisplatin Evaluation

(LACE) study was based on a pooled meta-analysis of individual patient data from 5 trials (Adjuvant Lung Project Italy [ALPI];<sup>1)</sup> Adjuvant Navelbine International Trialist Association [ANITA];<sup>2)</sup> Big Lung Trial [BLT];<sup>3)</sup> International Adjuvant Lung Cancer Trial [IALT];<sup>4)</sup> and JBR.10<sup>5)</sup>). The overall hazard ratio (HR) of death was 0.89 (95% confidence interval [CI]; 0.82–0.96;  $p < 0.005$ ), which corresponds to a 5-year survival benefit of 4.2% with chemotherapy.<sup>6)</sup> The survival benefit varied with stage, and the results showed that the cisplatin-based adjuvant chemotherapy improved survival in patients with completely resected stage II and stage III NSCLC (Table 1). Japanese adjuvant trials showed that a survival benefit was obtained with adjuvant chemotherapy using uracil-tegafur (UFT) in stage I adenocarcinoma.<sup>7)</sup> Meta-analysis revealed that the benefit was limited to those with a tumor size of 2 cm or more.<sup>8)</sup> This suggests that the indications of adjuvant chemotherapy might extend from pathological stage I to stage III, which means that most operated NSCLC cases should be recommended to receive postoperative chemotherapy after surgery.

However, it is a sad scenario when many patients receive toxic agents with few benefits; therefore the

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**Table 1. Results of representative adjuvant trials**

	ALPI	IALT	JBR.10	ANITA01	BLT
Publication year	2003	2004	2005	2006	2004
Stage	I–IIIA	I–IIIA	IB, II	IB–IIIA	I–IIIA
No. of cases	1,209	1,867	482	840	381
Regimen	CDDP + VDS + MMC	CDDP + ETP CDDP + VLB/ VDS/VNR	CDDP + VNR	CDDP + VNR	CDDP + MMC + IFO/VLB CDDP + VDS/ VNR
HR (95% CI)	0.96 (0.81–1.13)	0.86 (0.76–0.98)	0.69 (0.52–0.91)	0.80 (0.66–0.96)	1.02 (0.77–1.35)
P value	0.589	<0.03	0.009	0.017	0.90
Survival benefit at 5 years (%)	3	4.1	15	8.6	–
TRD (%)	0.5	0.8	0.8	1.7	3.1

ALPI, Adjuvant Lung Project Italy; IALT, International Adjuvant Lung Cancer Trial; ANITA, Adjuvant Navelbine International Trialist Association; BLT, Big Lung Trial; HR, hazard ratio; 95% CI, 95% confidence interval; TRD, total radiation dose; CDDP, cisplatin; VDS, vindesine; MMC, mitomycin C; ETP, etoposide; VLB, vinblastine; VNR, vinorelbine; IFO, ifosfamide.

pursuit to identify patients who would really benefit from specific regimens is important. To classify them and to apply the optimal therapy to each subgroup would provide a breakthrough in lung cancer management. The ability to identify responder patients with particular drugs or regimens is a challenge that requires the application of translational research to clinical practice.<sup>9,10</sup> The strong relationship between epidermal growth factor receptor (EGFR) mutation and high response to gefitinib<sup>11,12</sup> is a typical example of the individualized treatment of lung cancer.

A resection of NSCLC usually yields large amounts of tissue for molecular analysis. Rapid advances in technology have led to advanced assays to measure changes in deoxyribonucleic acid (DNA), ribonucleic acid (RNA), and proteins, which help to identify potential molecular biomarkers of clinical outcome. Translational research performed using specimens obtained in some of the adjuvant trials mentioned above has provided some information about biomarkers and identification of the subsets of patients who would benefit from adjuvant chemotherapy. Many biomarkers have been evaluated in the context of the largest positive adjuvant NSCLC trials, such as gene expression signatures, p53 expression, and *K-ras* mutations;<sup>13</sup> DNA-repaired genes;<sup>14</sup> and class III  $\beta$ -tubulin ( $\beta$ TubIII) expression status.<sup>15</sup> The individualized treatment for the determination of adjuvant chemotherapy is extensively discussed in this manuscript.

### Excision Repair Cross-Complementation Group 1<sup>14</sup>

The excision repair cross-complementation group 1 (ERCC1) enzyme plays a role in the nucleotide excision repair pathway that recognizes and removes cisplatin-induced DNA adducts.<sup>16</sup> IALT demonstrated a 5% survival benefit in overall 5-year survival among 1,867 NSCLC patients who received adjuvant cisplatin-based chemotherapy after curative surgery.<sup>4</sup>

ERCC1 expression was evaluated by immunohistochemistry in a total of 761 consecutive tumor samples from IALT. It was found to be positive in 335 (44%) and negative in 426 (56%). Cisplatin-based adjuvant chemotherapy significantly prolonged the survival in ERCC1 negative cases (HR: 0.65; 95% CI: 0.50–0.86), but not in ERCC1 positive cases (HR: 1.14; 95% CI: 0.84–1.55). Among patients who received no adjuvant chemotherapy, those with ERCC1 – positive tumors survived longer than those with ERCC1 – negative tumors (HR: 0.66; 95% CI: 0.49–0.90). The result showed that completely resected ERCC1 – negative NSCLC cases could benefit from cisplatin-based adjuvant chemotherapy (Table 2). The evaluation of ERCC1 expression in NSCLC before chemotherapy predicts the effect of cisplatin-based adjuvant chemotherapy, and this is therefore our promising biomarker for individualized treatment.

**Table 2. Results of IALT and ERCCI**

Group	All patients	Chemotherapy group	Control group	Hazard ratio for death (95% CI)	P value
Patients with ERCCI – negative tumors				0.65 (0.50–0.86)	0.002
Deaths – no./total no. of patients	218/426	105/224	113/202		
Rate of survival at 5 yr – % (95% CI)	44 (38–49)	47 (40–55)	39 (32–47)		
Median survival – months	48	56	42		
Patients with ERCCI – positive tumors				1.14 (0.84–1.55)	0.40
Deaths – no./total no. of patients	172/335	92/165	80/170		
Rate of survival at 5 yr – % (95% CI)	43 (37–49)	40 (32–49)	46 (37–55)		
Median survival – months	52	50	55		

ERCCI, excision repair cross-complementation group 1; 95% CI, 95% confidence interval; yr, year.

**Table 3. Results of JBR.10 and  $\beta$ -tubulin III expression**

Low expression (132 cases)		
	RFS	OS
Observation (60)	1	1
Chemotherapy (72)	0.78	1.00
	95% CI: 0.44–1.37 (p = 0.4)	95% CI: 0.57–1.75 (p = 0.99)
High expression (133 cases)		
Observation (65)	1	1
Chemotherapy (68)	0.45	0.64
	95% CI: 0.27–0.75 (p = 0.002)	95% CI: 0.39–1.04 (p = 0.007)

RFS, relapse-free survival; OS, overall survival; 95% CI, 95% confidence interval.

### Class III $\beta$ -Tubulin<sup>15)</sup>

Tubulins constitute a family of globular proteins that make up microtubules in cells; they are vital for cell structure, movement, mitosis, and metabolism (vesicular transport). High expression  $\beta$ TubIII in advanced NSCLC is known to correlate with both reduced response rates and inferior survival following treatment with antimicrotubule agents.

Winton et al. published the results of a randomized trial of adjuvant vinorelbine and cisplatin compared with observation in completely resected stage IB and stage II NSCLC (National Cancer Institute of Canada Clinical Trials Group [NCIC] JBR.10).<sup>5)</sup> A total of 482 patients were randomly assigned to either an adjuvant group (cisplatin/vinorelbine) or an observation group. The adjuvant group had a statistically significant longer survival than the observation group (69% vs. 54% at 5 years; p = 0.002).<sup>5)</sup> Tumor tissues of resected specimens were collected from 265 out of 482 patients. Immunohistochemical staining

was performed to evaluate the expression of  $\beta$ TubIII. High  $\beta$ TubIII expression is a sign of poor relapse-free survival (RFS) (HR: 1.52; 95% CI: 1.05–2.22; p = 0.03), and a similar trend was observed in overall survival (OS) (HR: 1.39; 95% CI: 0.96–2.01; p = 0.08). However, the high  $\beta$ TubIII expression group (n = 133) cases in the adjuvant group had more significantly favorable RFS (HR: 0.45; 95% CI: 0.27–0.75; p = 0.002) than in the observation group, and similar results were observed in OS (HR: 0.64; 95% CI: 0.39–1.04; p = 0.007). These results showed that adjuvant chemotherapy might prolong the RFS and OS in the high-tubulin expression patients, but the effect was unclear for the low-tubulin expression cases (Table 3).

### KRAS and p53<sup>13)</sup>

Protein expression of p53 and gene mutation of p53 and RAS were retrospectively evaluated using NSCLC samples obtained in the NCIC JBR.10 study. A total of 132 out of 253 cases showed p53 protein overexpression. And

**Table 4. Results of JBR.10 and p53, RAS expression**

Marker	No. of patients	Overall survival			
		Median	Hazard ratio	95% CI	P
p53 wild type					
Observation	136	6.2	1		0.04
Chemotherapy	137	7.8	0.67	0.46 to 0.98	
p53 mutant					
Observation	64	5.4	1		0.35
Chemotherapy	60	NR	0.78	0.46 to 1.32	
RAS wild type					
Observation	169	6.2	1		0.03
Chemotherapy	164	NR	0.69	0.49 to 0.97	
RAS mutant					
Observation	42	6.5	1		0.70
Chemotherapy	46	6.2	0.91	0.47 to 1.78	

95% CI, 95% confidence interval; NR, not reported.

though patients with p53-positive tumors had an overall significantly shorter survival than those with p53-negative tumors (HR: 1.89; 95% CI: 1.07–3.34;  $p = 0.03$ ), p53-positive tumors did show significant benefits from adjuvant chemotherapy (HR: 0.54; 95% CI: 0.32–0.92;  $p = 0.02$ ). Patients with p53-negative tumors, however, had no survival benefit from adjuvant chemotherapy (HR: 1.40; 95% CI: 0.78–2.52;  $p = 0.26$ ).

In 333 patients with wild-type RAS, survival was significantly prolonged by adjuvant chemotherapy, compared with observation-only cases (HR: 0.69; 95% CI: 0.49–0.97;  $p = 0.03$ ). But no survival benefit for adjuvant chemotherapy was recognized in patients with RAS mutant tumor (HR: 0.91; 95% CI: 0.47–1.78;  $p = 0.70$ ) (Table 4).

## Comments

Evidence-based medicine has been increasingly emphasized in medical practice in recent years, and standardized treatment methods have been developed mainly by multicenter randomized control trials. However, since the biological nature of tumors varies with each patient and their physical constitution, individualized treatment that takes both of these aspects into consideration could provide ideal optimal care for each cancer patient. Scientists have made enormous efforts to discover powerful biomarkers to help evaluate the biological behavior of cancer, and this strategy should be beneficial in selecting the most suitable therapy for each patient. Several bio-

markers were evaluated using samples obtained in large adjuvant chemotherapy trials of lung cancer (Table 5), and some hold promise. The increased interest in identifying biomarkers with implications for personalized treatment is reflected in a recent decision by the Food and Drug Administration (FDA) to allow, under certain conditions, retrospective analyses of biomarkers from completed trials.<sup>17</sup> The relationship between the histological type of lung cancer and the sensitivity of pemetrexed has been reported in inoperable lung cancer cases.<sup>18</sup> Pemetrexed is currently approved in the United States in combination with cisplatin for the treatment of malignant mesothelioma and for second-line treatment of advanced NSCLC. A recent phase III trial compared cisplatin and gemcitabine with cisplatin and pemetrexed for the treatment of advanced NSCLC. This noninferiority phase III randomized study compared OS between two groups. The OS for cisplatin/pemetrexed was noninferior to cisplatin/gemcitabine. Statistically, OS was significantly superior for cisplatin/pemetrexed than cisplatin/gemcitabine was in adenocarcinoma patients and large cell carcinoma patients (12.6 vs 10.9 months, 10.4 vs 6.7 months, respectively).<sup>18</sup> Because pemetrexed is an antifolate that inhibits multiple enzymes involved in purine and pyrimidine synthesis, thymidylate synthase (TS) is its main target. Preclinical data indicate that overexpression of TS correlates with lower sensitivity to pemetrexed. The baseline expression of TS gene and TS protein was significantly higher in patients with squamous cell carcinoma than in those with adenocarcinoma. This might be

**Table 5. Drug sensitivity, prognosis, and biomarkers**

Biomarker	ERCC1		RRM1		BRCA1		Class III $\beta$ -tubulin		KRAS	
	High	Low	High	Low	High	Low	High	Low	Wild	Mutant
Expression										
Prognosis	Good		Good		Poor		Poor			
Sensitivity	CDDP	○				○				○
	Taxane				○		○			○
	VNR						○			○
	GEM			○						○

ERCC1, excision repair cross-complementation group 1; RRM1, ribonucleotide reductase subunit M1; BRCA1, breast cancer 1, early onset; CDDP, cisplatin; VNR, vinorelbine; GEM, gemcitabine.

part of the explanation for the higher response of adenocarcinoma to pemetrexed. Further analysis of the relationship between the chemotherapy regimen and TS will be performed in a prospective manner; patients with stage II and stage III completely resected NSCLC are being treated with standard adjuvant chemotherapy or an individualized regimen determined by TS and ERCC1 expression (International Tailored Chemotherapy Adjuvant [ITACA] trial).<sup>19)</sup>

Here is another approach to determine a suitable biomarker using proteomics. Maeda et al. performed a comprehensive protein analysis using surgically resected specimens of stage I adenocarcinoma by liquid chromatography tandem mass spectrometry, followed by bioinformatical investigations to identify protein molecules.<sup>16)</sup> Two kinds of molecules (myosin IIA and vimentin) were identified as being related to prognosis and also to the responsiveness to adjuvant chemotherapy. Patients lacking expression of both myosin IIA and vimentin showed a significantly better outcome, regardless of postoperative adjuvant chemotherapy using UFT.

The nonrelapse survival of these patients at 5 years was 100%, which is better than that of patients positive for both myosin IIA and vimentin. Also, cases lacking expressions of both the two proteins had a good prognosis, irrespective of whether the patients had undergone adjuvant chemotherapy. In cases showing a positive expression of both myosin IIA and vimentin, the 5-year survival benefit was approximately 19% by adjuvant chemotherapy using UFT. Therefore these two proteins appear to be potentially useful biomarkers for the selection of adjuvant chemotherapy.<sup>16)</sup>

The current retrospective data are by no means sufficient to support the routine use of molecular markers to guide adjuvant therapy for NSCLC outside of a clinical

trial.

Before a molecular test can be adopted for routine practice, valid and standardized laboratory techniques must be established. The establishment of the feasibility of molecularly tailored adjuvant therapy for patients with resected NSCLC requires a prospective phase II trial. It is also important not only to select a suitable regimen, but also to develop innovative treatments, such as gene and molecular-targeted therapy.

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