Preoperative CYFRA 21-1 Levels As a Prognostic Factor in c-Stage I Non-Small Cell Lung Cancer

Hisashi Suzuki[1], Shigemi Ishikawa[1], Hiroaki Satoh[2], Hiroichi Ishikawa[3], Mitsuaki Sakai[1], Tatsuo Yamamoto[1], Masataka Onizuka[1], Yuzuru Sakakibara[1]

[1]Department of Surgery and [2]Internal Medicine, Graduate School of Comprehensive Human Science, University of Tsukuba

[3]Department of Internal Medicine, Tsukuba Medical Center Hospital

[1-2] 1-1-1 Tennoudai, Tsukuba, Ibaraki, 305-8575, Japan

[3] 1-3-1 Amakubo, Tsukuba, Ibaraki, 305-8558, Japan

Word Count: 2628 words

Corresponding Author: Shigemi Ishikawa, MD

Department of Surgery, Graduate School of Comprehensive Human Science, University of Tsukuba

1-1-1 Tennoudai, Tsukuba, Ibaraki, 305-8575, Japan

E-mail: ishikawa@md.tsukuba.ac.jp Tel: +81-29-853-3210 Fax: +81-29-853-3097

Abstract

Objective. The clinical importance of preoperative CYFRA 21-1 measurement in early stage non-small cell lung cancer (NSCLC) is still unclear. The aim of this study is to clarify the prognostic value of preoperative CYFRA 21-1 levels in clinical stage (c-stage) I NSCLC.

Methods. The records of 101 c-stage I NSCLC patients who had undergone complete resection were analyzed to correlate preoperative CYFRA 21-1 levels to both the pathologic factors of resected specimens and postoperative outcomes. The cut-off value was set at 3.5 ng/mL.

Results. Six cases (5.9%) showed high CYFRA 21-1 (≥3.5 ng/ml). The 5-year survival of normal and high CYFRA 21-1 groups were 83.3% and 50.0%, respectively. Patients with high CYFRA 21-1 had significantly poor outcomes (P=0.006). In univariate analysis, preoperative serum CYFRA 21-1 level, pT, pN and p-stage were significantly associated with prognosis. Multivariate analysis showed that only CYFRA 21-1 level retained as an independent prognostic factor (relative risk=9.79, P=0.002).

Conclusions. CYFRA 21-1 is an independent predictor of poor outcome for c-stage I NSCLC. Elevated preoperative CYFRA 21-1 levels in early stage NSCLC may indicate a subgroup at high risk of early death, which has the potential for better survival with additional systemic chemotherapy. (196 words)

Keywords: CYFRA 21-1, Early stage diseases, Non-small cell lung cancer, Prognostic factors, Thoracic surgery, Tumor markers

Introduction

Cytokeratin 19 is expressed in simple epithelium such as bronchial epithelium and is particularly abundant in carcinoma of the lung [1]. A fragment of cytokeratin 19, CYFRA 21-1 can be measured in serum by an immunoradiometric assay using two mouse monoclonal antibodies KS 19-1 and BM 19-21 [2]. The CYFRA 21-1 serum level has been reported and accepted as a useful tumor marker in patients with non-small cell lung cancer (NSCLC) [2-6]. However, the clinical importance of CYFRA 21-1 level in early lung cancer is not clear. Meanwhile, the prognosis for early stage NSCLC patients who undergo curative operations remains unsatisfactory, as the 5-year postoperative survival rate is about 70% even in c-stage IA [7]. Therefore, it would be beneficial to identify some preoperative factors which could help with the prognosis of early stage patients. This study was performed to clarify the prognostic value of preoperative CYFRA 21-1 levels in completely resected c-stage I NSCLC patients.

Materials and Methods

Patients

Between August 1994 and May 2001, 309 consecutive primary NSCLC patients underwent surgical resection after measurement of serum CYFRA 21-1 at the Department of Thoracic Surgery,

Tsukuba University Hospital, Japan. Of these patients, 101 cases diagnosed as c-stage I were analyzed in this study, and all cases had formal lobectomy with systemic lymphadenectomy and no induction chemotherapy. The characteristics of the 101 patients included in the study are shown in Table 1. The study population consisted of 61 male and 40 female patients. The median age was 67 years (ranging from 33 to 81 years). Surgical procedures consisted of lobectomy in 97 patients and bilobectomy in 4 patients. The study protocol was approved by the Institutional Review Board of University of Tsukuba.

TNM Evaluation and Histology

The staging of each case was determined according to the TNM classification of the revised International System for the Staging of Lung Cancer [8] and the histological classification of the tumors was based on the World Health Organization's criteria [9]. All patients were preoperatively diagnosed as T1-2N0 status by chest computer assisted tomography (CT) and as M0 by abdominal ultrasonography, brain CT or magnetic resonance imaging, and bone scintigraphy. After pathological examination of resected surgical specimens, 66 patients were categorized as pT1 and 20 as pT2. Seven patients were diagnosed as pT3 because of tumor invasion to the chest wall or mediastinal pleura and 8 were diagnosed as pT4 because of satellite tumor nodules within the

primary tumor lobe. Lymph node involvement was observed postoperatively in 27 patients, of which 17 were pN1 and 10 were pN2. Surgical pathologic stagings (p-stage) were stage I in 65 patients, p-stage II in 18, p-stage IIIA in 8 because of mediastinal nodal involvement, p-stage IIIB in 8 because of the presence of satellite nodules within the primary tumor lobe, and p-stage IV in 2 because of the presence of pulmonary metastasis beyond the primary tumor lobe. These metastatic nodules which co-existed either in the same lobe as the primary lesion or beyond it were so small that only histological examination of the surgical specimen revealed the lesions. The histologic types of the primary lesions were mainly adenocarcinoma (n=72), as shown in Table 1.

Serum Assay

The serum levels of CYFRA 21-1 were determined using a chemiluminescent enzyme immunoassay (Lumipulse I CYFRA, Fujirebio Inc., Tokyo, Japan). We used 3.5 ng/ml as the cut-off value of serum CYFRA 21-1, as established by the Japan CYFRA research group [10].

Statistical Analyses and Survival Rates

Data is shown as mean ± SE. The significance of the differences between two independent groups was determined by unpaired t-test, and that among more than two groups was determined by

analysis of variance and Tukey's test. The length of survival was defined as the interval in months between the day of surgical resection and the date of death or last follow-up visit. The median follow-up for surviving patients was 73.0 months. The survival rates were calculated by the Kaplan-Meier method and univariate analyses were performed by means of the log-rank test. Multivariate analyses were performed by means of the Cox proportional hazards model. A 0.10 level of probability value was the significance level used for adding and deleting a co-variable from the model. All statistical analyses were performed using SPSS 10.1J for Windows (SPSS Inc., Chicago, IL) and a probability value less than 0.05 was considered to be significant.

Results

The CYFRA 21-1 levels for groups divided into different categories are shown in Table 2. The preoperative CYFRA 21-1 level of all 101 patients was 1.66 ± 0.09 ng/ml (ranging from 0.5 to 4.3 ng/ml). Six out of the 101 patients (5.9%) had high CYFRA 21-1 levels. These 6 patients with high CYFRA 21-1 were older than 64 years old: 5 were male and 1 was female, consisting of 1 squamous cell carcinoma and 5 non-squamous cell carcinoma, and 5 p-stage I and 1 p-stage III cases.

Patient Profile and CYFRA 21-1 Level

The CYFRA 21-1 level was observed to be higher in males and in those older than 64 years old. The differences, however, were not statistically significant.

Histology and CYFRA 21-1 Level

As for histological types, the CYFRA 21-1 levels were 1.54 ± 0.10 ng/ml for adenocarcinoma, 1.92 ± 0.19 ng/ml for squamous cell carcinoma, and 1.97 ± 0.41 ng/ml for large cell carcinoma. There were no significant differences in the CYFRA 21-1 levels among these groups. Comparison of the CYFRA 21-1 levels between squamous cell carcinoma patients and the others showed an upward trend in this factor in the squamous cell carcinoma group (P=0.06).

pTNM and CYFRA21-1 Level

The serum levels of CYFRA 21-1 were 1.44 ± 0.10 ng/ml for pT1, 2.16 ± 0.21 ng/ml for pT2, 1.66 ± 0.29 ng/ml for pT3, and 2.19 ± 0.37 ng/ml for pT4 patients. A significant difference was found between the pT1 and pT2 groups (P=0.006). No significant differences were observed between other individual groups. The CYFRA 21-1 level for a group of pT2-4 patients was 2.06 ± 0.16 ng/ml and the difference between the pT1 and pT2-4 groups was also significant (P<0.001).

Although we performed a linear regression analysis to confirm the correlation between tumor size and CYFRA 21-1 level, no correlation was observed (r=0.162, *P*=0.102).

The CYFRA 21-1 levels were 1.69 ± 0.11 ng/ml for the pN0 patients and 1.56 ± 0.14 ng/ml for the pN1-2 patients. The difference in the CYFRA 21-1 serum levels was not significant between the groups with and without lymph node involvement.

The levels of CYFRA 21-1 were 1.66 ± 0.12 ng/ml for p-stage I, 1.72 ± 0.15 ng/ml for p-stage II, 1.20 ± 0.11 ng/ml for p-stage IIIA, 2.19 ± 0.37 ng/ml for p-stage IIIB, and 0.75 ± 0.25 ng/ml for p-stage IV. Among these groups, there were no significant differences in the levels of CYFRA 21-1. A comparison between the groups of p-stage I and of p-stages II through IV showed no significant differences either.

CYFRA 21-1 Level and Survival

The 3- and 5-year survival rates after surgery for all patients were 86.6% and 81.1%, respectively. Figure 1 shows survival curves for patients with preoperatively normal serum CYFRA 21-1 level and patients with preoperatively high (≥3.5 ng/ml) serum CYFRA 21-1 level. The 5-year survival rate was 84.8% for normal CYFRA 21-1 patients and 50.0% for high CYFRA 21-1 patients, and the difference between these two groups was statistically significant (*P*=0.006). Six patients had high

CYFRA 21-1 levels, three of whom died of distant metastases of NSCLC, and their preoperative CYFRA 21-1 levels were 3.6 ng/ml with p-stage IB squamous cell carcinoma, 3.8 ng/ml with p-stage IB adenocarcinoma, and 4.1 ng/ml with p-stage IIIB adenocarcinoma.

The potential prognostic factors were subjected to a univariate survival analysis (Table 3). CYFRA 21-1 levels (P=0.006), pT factor (P=0.019), pN factor (P=0.016), and pathological stage (P=0.004) were significantly associated with a worse outcome. To determine independent prognostic factors, we performed multivariate analyses according to the Cox proportional hazards model. Table 4 shows the results of the multivariate analyses. The CYFRA 21-1 serum level was the only factor to retain a significant independent prognostic impact on overall survival (relative risk=9.79, P=0.002).

Discussion

The most important findings of this study are that the c-stage I NSCLC patients with preoperative serum levels of CYFRA 21-1 higher than 3.5 ng/ml had significantly worse outcomes after surgical resection and multivariate analysis showed CYFRA 21-1 to be an independent prognostic factor.

CYFRA 21-1 is measured by a sandwich enzyme-linked immunosorbent assay developed to detect a soluble cytokeratin 19 fragment which is expressed in bronchial epithelium and malignant lung

tumors. In 1993, Pujol et al. [2] demonstrated the reliability of this method as a new tumor marker for lung cancer, especially NSCLC. Since then, many studies on the relationship between CYFRA 21-1 levels and NSCLC have been reported, and CYFRA 21-1 is now known as a useful tumor marker for NSCLC with high positive rates [3-5]. Variation in CYFRA 21-1 levels among different histological groups in NSCLC have also been reported, showing higher sensitivities in squamous cell carcinoma than in adenocarcinoma [5, 11]. In regards to squamous cell carcinoma cases, Reinmuth et al. [5] demonstrated that CYFRA 21-1 is the marker with the highest sensitivity compared to other markers, such as carcinoembryonic antigen and squamous cell carcinoma-antigen. In our study, squamous cell carcinoma group had an upward trend of CYFRA 21-1 levels as compared with other histological types, although the difference was not statistically significant (P=0.06). The low proportion of squamous cell carcinoma in our subjects (21/101 patients or 20.8%) may be a reason that the difference did not reach statistical significance.

According to the pathologic T status, we observed a significantly lower CYFRA 21-1 level in pT1 patients as compared to pT2 and pT2-4 patients. Lai et al. [3] classified the patients into three groups according to tumor size in squamous cell carcinoma with p-stages I, II, and partial IIIA, and showed significantly higher serum CYFRA 21-1 levels in those with larger tumor size. However, a linear regression analysis performed on our cases did not show the correlation between tumor size

and CYFRA 21-1 level. In c-stage I NSCLC, tumor size may not be a main cause of CYFRA 21-1 level elevation.

As for lymph node involvement, Satoh et al. [6] showed that the increased preoperative levels of CYFRA 21-1 suggested the presence of N2 disease. Pujol et al. [2, 12] also stated that patients who presented lymph node involvement (N2 or N3) demonstrated higher serum CYFRA 21-1 levels, when compared with patients without mediastinal lymph node involvement (N0 or N1). In our study, however, there were no significant differences in the serum levels of CYFRA 21-1 according to nodal status. The different characteristics of the subjects might be the reason for the discrepancy between our results and other reports. Our subjects consisted only of c-stage I patients, all of whom have no lymph node enlargement. These findings imply that lymph node involvement with lymphadenopathy may tend to elevate CYFRA 21-1 levels. This may be one of the reasons why the CYFRA 21-1 levels in our study did not differ significantly in different pathologic stage groups as opposed to other reports showing higher CYFRA 21-1 levels in more advanced NSCLC patients [3, 5]. Most cases diagnosed as advanced stages in our study were those with involved lymph nodes in normal size or those with small pulmonary metastases.

Although many studies have shown that CYFRA 21-1 is a good marker for NSCLC for diagnosis, CYFRA 21-1 does not seem to be a good marker for the early detection and screening of lung

cancer because of its low positive rate in early stage lung cancer [3, 5]. In our study of 101 c-stage I NSCLC cases, there were only six cases (5.9%) with the CYFRA 21-1 serum levels higher than the cut-off value of 3.5 ng/ml. Therefore, the importance of measuring CYFRA 21-1 level in early stage lung cancer has been unclear. Recently, Muley et al. reported the prognostic value of CYFRA 21-1 level in completely resected p-stage I cases [13, 14]. The proportion of high CYFRA 21-1 in their study was 21.2% (30 out of 141 cases), which is higher as compared to our result (5.9%). We speculate that this discrepancy was due to the difference in the proportion of histological types. CYFRA 21-1 is generally known to be sensitive to squamous cell carcinoma as compared to adenocarcinoma [3, 5, 11]. The proportion of squamous cell carcinoma and adenocarcinoma in Muley's study was 38.6% (59/141) and 49% (75/141), respectively, whereas our study was 20.8% (21/101) and 71.3% (72/101). The large amount of adenocarcinoma cases in our subjects might be the reason for the lower proportion of high CYFRA 21-1 cases. This finding implies that CYFRA 21-1 might not be a good marker for detecting small adenocarcinoma. However, our results are very similar to Muley's findings in terms of the significance of CYFRA 21-1 as a prognostic predictor. Therefore, measurement of CYFRA 21-1 in early NSCLC is useful and valuable as a prognostic indicator. A meta-analysis performed by Pujol et al. [15] concerning the prognostic effect of serum CYFRA 21-1 levels in NSCLC at any stage showed a shorter survival

rate of patients with high pretreatment CYFRA 21-1 levels as compared to patients with a normal value, and multivariate analysis demonstrated that CYFRA 21-1 was a prognostic determinant. Recent presented data of randomized trials has shown an absolute survival benefit for patients with completely resected early stage NSCLC receiving adjuvant chemotherapy as compared to observation alone [16-18]. CYFRA 21-1, a prognostic factor which is easily assessable preoperatively using a commercial kit, is considered to be very useful in developing a treatment plan for c-stage I NSCLC patients and might be used as an indicator to select a subset which has the potential for better prognosis with additional systemic chemotherapy, either preoperatively or postoperatively. In addition, many molecular markers such as p53, K-ras, and erbB-2 have been investigated in resected specimens, and some were shown to be significant prognostic factors for early stage NSCLC [19, 20]. Further studies on CYFRA 21-1 in combination with these molecular markers might establish helpful and reliable guidance for developing treatment plans that provide more favorable outcomes for early stage NSCLC patients.

In conclusion, we have demonstrated the prognostic value of preoperative CYFRA 21-1 levels in identifying cases with poor prognosis among c-stage I NSCLC. Preoperative factors that can predict prognosis are very useful in planning a treatment for lung cancer. The high-risk c-stage I NSCLC patients identified by elevated CYFRA 21-1 level may need further evaluation and require

multi-modality treatment for better postoperative survival.

Table 1. Patient Characteristics

Categories	No. of Patients
All patients	101
Sex	
Male	61
Female	40
Age	
< 65 years old	45
≥ 65 years old	56
CYFRA 21-1	
< 3.5 ng/ml	95
≥ 3.5 ng/ml	6
Histological type	
Adenocarcinoma	72
Squamous cell carcinoma	21
Large cell carcinoma	7
Mucoepidermoid carcinoma	1

Pathological stage	
I	65
II	18
IIIA	8
IIIB	8
IV	2
Surgical procedure	
Lobectomy	97
Bilobectomy	4

Median age: 67 years old, range 33 - 81 years old.

Table 2. Serum Levels of CYFRA 21-1

Categories	No. of Patients		CYFRA 21-1, ng/ml	*P* Value
	All	≥3.5 ng/ml		
All patients	101	6	1.66 ± 0.09	
Sex				
Male	61	5	1.78 ± 0.11	0.078
Female	40	1	1.47 ± 0.14	
Age				
< 65 years old	45	0	1.49 ± 0.10	0.092
≥ 65 years old	56	6	1.79 ± 0.13	
Histological type				
Squamous cell carcinoma	21	1	1.92 ± 0.19	0.060
Others	80	5	1.59 ± 0.10	
Pathological T factor				
pT1	66	2	1.44 ± 0.10	0.0006*
pT2-4	35	4	2.06 ± 0.16	
Lymph node involvement				

pN0	74	5	1.69 ± 0.11	0.491
pN1-2	27	1	1.56 ± 0.14	
Pathological stage				
I	65	5	1.66 ± 0.12	0.994
II + III + IV	36	1	1.66 ± 0.13	

Values are shown as mean ± SE. *P<0.05

Table 3. Univariate Analyses of Patient Characteristics and Survival After Surgery

Factors	No. of Patients	5-Year Survival (%)	*P* Value
Overall	101	81.1	
Sex			
Male	61	75.8	0.15
Female	40	88.6	
Age			
<65 years old	45	83.5	0.61
≥65 years old	56	78.5	
Histological type			
Squamous cell carcinoma	21	82.6	0.36
Others	80	77.5	
CYFRA 21-1			
<3.5 ng/ml	95	84.8	0.006*
≥3.5 ng/ml	6	50.0	
Pathological T factor			
pT1	66	89.3	0.019*

pT2-4	35	64.0	
Lymph node involvement			
pN0	74	88.5	0.016*
pN1-2	27	60.0	
Pathological stage			
I	65	90.5	0.004*
II + III + IV	36	63.2	

*P<0.05

Table 4. Multivariate Analyses Using the Cox Proportional Hazards Model

Factors	Relative Risk	95% CI	*P* Value
CYFRA 21-1 (<3.5 vs ≥3.5 ng/ml)	9.79	2.24-42.8	0.002*
pT factor (pT1 vs pT2-4)	2.28	0.85-6.17	0.10
pN factor (pN0 vs pN1-2)	1.68	0.41-6.83	0.47
Pathological stage (I vs II-IV)	3.22	0.66-15.6	0.15

CI, confidence interval. *P<0.05

Figure 1.

The cumulative survival curves for patients with preoperatively normal serum CYFRA 21-1 level and patients with preoperatively high (≥3.5 ng/ml) serum CYFRA 21-1 level. The 5-year survival rate was 84.8% for normal CYFRA 21-1 patients and 50.0% for high CYFRA 21-1 patients, and the difference between these two groups was statistically significant (*P*=0.006).

References

1. Broers JL, Ramaekers FC, Rot MK, Oostendorp T, Huysmans A, van Muijen GN, Wagenaar SS, Vooijs GP. Cytokeratins in different types of human lung cancer as monitored by chain-specific monoclonal antibodies. Cancer Res. 1988;48:3221-3229.

2. Pujol JL, Grenier J, Daures JP, Daver A, Pujol H, Michel FB. Serum fragment of cytokeratin subunit 19 measured by CYFRA 21-1 immunoradiometric assay as a marker of lung cancer. Cancer Res. 1993;53:61-66.

3. Lai RS, Hsu HK, Lu JY, Ger LP, Lai NS. CYFRA 21-1 enzyme-linked immunosorbent assay. Evaluation as a tumor marker in non-small cell lung cancer. Chest. 1996;109:995-1000.

4. Nisman B, Lafair J, Heching N, Lyass O, Baras M, Peretz T, Barak V. Evaluation of tissue polypeptide specific antigen, CYFRA 21-1, and carcinoembryonic antigen in nonsmall cell lung carcinoma: does the combined use of cytokeratin markers give any additional information? Cancer. 1998;82:1850-1859.

5. Reinmuth N, Brandt B, Semik M, Kunze WP, Achatzy R, Scheld HH, Broermann P, Berdel WE, Macha HN, Thomas M. Prognostic impact of Cyfra21-1 and other serum markers in completely resected non-small cell lung cancer. Lung Cancer. 2002;36:265-270.

6. Satoh H, Ishikawa S, Kamma H, Ohtsuka M, Hasegawa S. Pre-operative CYFRA 21-1

levels in patients with lung cancer: correlation with mediastinal lymph node involvement. Eur J Cancer. 1998;34:1469-1470.

7. Goya T, Asamura H, Yoshimura H, Kato H, Shimokata K, Tsuchiya R, Sohara Y, Miya T, Miyaoka E. Prognosis of 6644 resected non-small cell lung cancers in Japan: a Japanese lung cancer registry study. Lung Cancer. 2005;50:227-234.

8. Sobin LH, Fleming ID. TNM Classification of Malignant Tumors, fifth edition (1997). Union Internationale Contre le Cancer and the American Joint Committee on Cancer. Cancer. 1997;80:1803-1804.

9. The World Health Organization histological typing of lung tumours. Second edition. Am J Clin Pathol. 1982;77:123-136.

10. Kawai T, Ohkubo A, Hasegawa S, Kuriyama T, Kato H, Fukuoka M, Ohkawa J, Yotsumoto H, Sugama Y, Kawate N, Takada M, Tatsumi K, Satoh H, Kitamura S. [Study of standard level, cut off level, diagnostic specificity and sensitivity for a new tumor marker CYFRA in lung cancer measured by EIA]. Kiki Shiyaku Jpn. 1993;16:1232-1238.

11. Takei Y, Minato K, Tsuchiya S, Takise A, Nakano H, Ezawa K, Fueki N, Hoshino H, Naruse I, Nomoto T, Makimoto T, Ishihara S, Saito R, Mori M. CYFRA 21-1: an indicator of survival and therapeutic effect in lung cancer. Oncology. 1997;54:43-47.

12. Pujol JL, Boher JM, Grenier J, Quantin X. Cyfra 21-1, neuron specific enolase and prognosis of non-small cell lung cancer: prospective study in 621 patients. Lung Cancer. 2001;31:221-231.

13. Muley T, Dienemann H, Ebert W. Increased CYFRA 21-1 and CEA levels are negative predictors of outcome in p-stage I NSCLC. Anticancer Res. 2003;23:4085-4093.

14. Muley T, Dienemann H, Ebert W. CYFRA 21-1 and CEA are independent prognostic factors in 153 operated stage I NSCLC patients. Anticancer Res. 2004;24:1953-1956.

15. Pujol JL, Molinier O, Ebert W, Daures JP, Barlesi F, Buccheri G, Paesmans M, Quoix E, Moro-Sibilot D, Szturmowicz M, Brechot JM, Muley T, Grenier J. CYFRA 21-1 is a prognostic determinant in non-small-cell lung cancer: results of a meta-analysis in 2063 patients. Br J Cancer. 2004;90:2097-2105.

16. Winton T, Livingston R, Johnson D, Rigas J, Johnston M, Butts C, Cormier Y, Goss G, Inculet R, Vallieres E, Fry W, Bethune D, Ayoub J, Ding K, Seymour L, Graham B, Tsao MS, Gandara D, Kesler K, Demmy T, Shepherd F. Vinorelbine plus cisplatin vs. observation in resected non-small-cell lung cancer. N Engl J Med. 2005;352:2589-2597.

17. Kato H, Ichinose Y, Ohta M, Hata E, Tsubota N, Tada H, Watanabe Y, Wada H, Tsuboi M, Hamajima N, Ohta M. A randomized trial of adjuvant chemotherapy with uracil-tegafur for

adenocarcinoma of the lung. N Engl J Med. 2004;350:1713-1721.

18. Wada H, Miyahara R, Tanaka F, Hitomi S. Postoperative adjuvant chemotherapy with PVM (Cisplatin + Vindesine + Mitomycin C) and UFT (Uracil + Tegaful) in resected stage I-II NSCLC (non-small cell lung cancer): a randomized clinical trial. West Japan Study Group for lung cancer surgery (WJSG). Eur J Cardiothorac Surg. 1999;15:438-443.

19. D'Amico TA, Massey M, Herndon JE, 2nd, Moore MB, Harpole DH, Jr. A biologic risk model for stage I lung cancer: immunohistochemical analysis of 408 patients with the use of ten molecular markers. J Thorac Cardiovasc Surg. 1999;117:736-743.

20. Fukuyama Y, Mitsudomi T, Sugio K, Ishida T, Akazawa K, Sugimachi K. K-ras and p53 mutations are an independent unfavourable prognostic indicator in patients with non-small-cell lung cancer. Br J Cancer. 1997;75:1125-1130.