Table 1. The surgical outcome for resected non-small cell lung cancer: 5-year survival rate (%)

Stage	Clinical staging		Pathological staging	
	Mountain	Japan	Mountain	Japan
IA	61	72.1	67	79.5
IB	38	49.9	57	60.1
IIA	34	48.7	55	59.9
IIB	24	40.6	39	42.2
IIIA	22	35.8	38	29.8
IIIB	9	28.0	3–7	19.3
IV	13	20.9	1	20.0

The new paradigm shift for the adjuvant treatment after surgery is demonstrated here by the Japanese and international trials that have been reported since 2003.

Japanese trials

A large-scale randomized phase III study of postoperative adjuvant chemotherapy with UFT for p-stage I adenocarcinoma: the JLCRG (Japan Lung Cancer Research Group) trial (presented in ASCO 2003[13])

The oral antimetabolite UFT is composed of tegafur and uracil mixed at the ratio of 1:4. This drug has been developed by Taiho Pharmaceutical Corporation, Tokyo, Japan. UFT produced higher levels of 5-FU without the toxic level of 5-FU.

Concerning adjuvant treatment using UFT, the West Japan Study Group for Lung Cancer Surgery reported that postoperative adjuvant treatment with UFT in patients with completely resected stage I–III disease prolonged survival significantly longer than observation alone. The 5-year and 10-year survival rates were 64% and 48% in the UFT group, and 49% and 32% in the control group, respectively (P = 0.02).[11] In a subgroup analysis, no statistically significant difference in the overall survival of patients with squamous cell carcinoma between the two groups was observed (P = 0.24). In contrast, the patients with adenocarcinoma in the UFT group had a significantly better survival than those in the control group (P = 0.009).[12] In addition, most patients with adenocarcinoma had stage I disease. This trial demonstrated that UFT was useful in postoperative adjuvant chemotherapy against the earlier stage of non-small cell lung cancer. However, this study involved issues with respect to study design, because enrolled subjects varied from stage I to III, with a broad range of outcome. Those results prompted us to conduct a prospective randomized trial of UFT as a postoperative adjuvant treatment for patients whose stage I adenocarcinoma was completely resected. In the confirmatory study conducted by the Japan Lung Cancer Research Group (JLCRG), patients with completely resected pathological stage I adenocarcinoma of the lung were randomized with stratification according to their pathological T status (T1 versus T2), gender, and age, which were separated between less than 65 years old and 65 years old or over, to either receive the oral administration of UFT (tegafur 250 mg/m^2/day) for 2 years or no treatment. The patients with limited resection, such as wedge resection, were excluded. A follow-up examination was performed every 3 months for the first 2 years after the patient's operation and every 6 months thereafter. The primary endpoint was overall survival.

From January 1994, through March 1997, 999 patients were entered into the study. Twenty patients withdrew their informed consent or were found to be ineligible before the start of treatment. The number of eligible randomized patients was 491 in the UFT group and 488 in the nontreatment control group. Main patient characteristics were as follows: men, 48.7%; more than 65 years old, 43.9%; pathological T1, 73.1%. There were no significant differences in the baseline characteristics of the patients. The median duration of follow-up for all 979 patients was 73 months, with range 61–94 months.

Few severe adverse reactions were associated with UFT administration. There was no grade 4 adverse reaction. In total, 10 (2%) of 482 patients developed a grade 3 adverse reaction. The percentage of compliance for UFT administration was calculated based on the number of patients who actually took UFT and the number of patients without recurrence, second cancer, or death who were expected to take UFT. The percentage of compliance was 80% [95% confidence interval (CI), 77%–84%) at 6 months, 74% (95% CI, 70%–78%) at 12 months, 69% (95% CI, 65%–73%) at 18 months, and 61% (95% CI, 77%–84%) at 24 months. The main reasons for discontinuation of UFT administration were as follows: an adverse reaction in 123 patients, patient refusal in 52, and the doctor's judgment in 34.

Overall survival between the two groups showed a statistically significant difference in favor of the UFT group based on a Kaplan–Meier analysis (P = 0.04). The 5-year survival rate (5YS) was 87.9% in the UFT group and 85.4% in the control group, respectively. Treatment failure was documented in 22.6% of the patients in the UFT group and 26.4% in the control group, respectively. The most frequent failure pattern was distant metastasis in both groups. The 5-year cancer-free survival rate was 82.8% in the UFT group and 80.4% in the control group. There is no significant difference between the two groups at P = 0.25.

Concerning subset analysis of pathological T factors, although there was no statistical difference in the T1 population, in the T2 subset, the 5-year survival rate was 84.9% in the UFT group and 73.5% in the control group. The hazard ratio was 0.0842 in the UFT group with a clear statistical difference (P = 0.0051). Concerning interaction in relation to treatment effect, treatment with UFT tended to improve the survival rate among the patients with tumors that were 2–3 cm in diameter and provided 30% survival benefit for patients with tumor that was more than 3 cm in diameter. These findings indicated that the effect of UFT might be related to certain biological factors.

In conclusion, oral demonstration with UFT in the postoperative adjuvant setting yielded a significant improvement in survival in patients with pathological stage I adenocarcinoma of the lung, particularly in stage 1B, T2 N0 M0. These results of this study may be able to confirm the previous UFT adjuvant trial.

Meta-analysis of six randomized adjuvant trials with UFT (presented at ASCO 2004[14])

Clinical trials assessing the response of non-small cell lung cancer to postoperative adjuvant chemotherapy should use survival as the primary endpoint. Response should be evaluated by means of randomized controlled studies using surgical therapy alone as control. Single studies usually do not provide clear-cut conclusions because of limited sample size. A meta-analysis of all properly randomized clinical trials comparing long-term adjuvant chemotherapy with UFT, an oral fluorinated pyrimidine derivative, with surgery alone in patients with completely resected non-small cell lung cancer was demonstrated.

Six randomized trials have been conducted that compare surgery alone with adjuvant chemotherapy with UFT. The analysis was based on individual patient data provided by the principal investigator of each trial. In data from 2003, eligible patients were analyzed on an intention-to-treat bias. The endpoint of interest was overall survival at 5 years after surgery. Major prognostic factors were well balanced between the UFT group and surgery-alone group. Most patients had early-stage non-small cell lung cancer. The distribution of pathological T1 and T2 stages among this population was 65% and 34%, respectively.

The 5-year overall survival rate and 7-year overall survival rate were 81.8% and 76.5% and 77.2% in the control group; 7-year overall survival rates were 81.8% and 76.5% and 77.2% in the control group, and 7-year overall survival was 69.5% for the surgery-alone group. The result of meta-analysis demonstrated that adjuvant chemotherapy with UFT significantly improved the overall 5-year survival rate, with hazard ratio (HR) 0.77 (95% CI, 0.63–0.94; $P = 0.011$). Heterogeneity of effect among the six studies was not significant ($P = 0.76$).

The subset analysis of this meta-analysis indicated that UFT treatment provided a definitive survival benefit in most of the subset. This meta-analysis of the T1 subset population demonstrated that treatment with UFT provided a definitive survival benefit for patients with tumor that was 2–3 cm in diameter. Therefore, on the basis of our meta-analysis, postoperative adjuvant chemotherapy with UFT has a beneficial effect on outcome in patient with curatively resected non-small cell lung cancer more than 2 cm in size. Recently, Dr. Hotta from Okayama University has also demonstrated the benefit of UFT in the postoperative adjuvant setting based on the meta-analysis of five abstracts regarding UFT adjuvant trials (HR, 0.799; 95% CI, 0.668–0.957, $P = 0.015$).[15] These results seem to confirm the previous Hamada data.

A randomized phase III study for Bestatin (Ubenimex) as postoperative adjuvant treatment in patients with stage I squamous cell lung cancer (presented at ASCO 2001[16])

In a placebo-controlled phase III trial sponsored by the Japanese NK421 Lung Cancer Study Group, the more derived immunomodulator Bestatin (Ubenimex) was used as adjuvant therapy for patients with stage I squamous cell carcinoma following completed resection.

Confirmation of the patient eligibility and the randomization were performed within 4 weeks after each operation. The oral administration started within 1 week after their randomization. One capsule of either Bestatin or placebo was administered orally after breakfast every day for 2 years postoperatively. No additional treatment was allowed until definitive recurrence or appearance of second cancer was diagnosed.

A follow-up examination was performed every 3 months for 2 years after operation and every 6 months thereafter. The primary endpoint of the study was overall survival, and the second endpoint was disease-free survival and safe assessment. The number of patients was 202 in the Bestatin group and 198 in the placebo group. There is no significant difference in baseline characteristic of patients; 97.6% and 96.3% of the projected dose of Bestatin and placebo were administered, respectively.

The median duration follow-up for 400 patients was 77 months. Overall 5-year survival rate was significantly increased for patients receiving Bestatin compared with those receiving placebo. Disease-free survival was also significantly higher in the Bestatin group compared with placebo group, 71% versus 62%. According to multivariate analysis for survival, significant prognostic factors were performance status (PS), blood transfusion, and treatment arm.

Short summary and consideration of Japanese adjuvant trials

A couple of randomized clinical trials have demonstrated survival advantage in patients predominantly with no lymph metastasis. The effectiveness of UFT in N0 patients was confirmed. The patients with completely resected stage I non-small cell lung cancer, especially T2N0 adenocarcinoma, will benefit from adjuvant chemotherapy with UFT. UFT provides 2.5% (T2, 11.4%) benefit for absolute 5-year survival rate. HR for death in patients with stage I and T2 was 0.706 and 0.48, respectively.

Future issues for UFT adjuvant chemotherapy are to be considered as follows: (1) Do patients with stage II and/or stage III disease benefit from UFT adjuvant therapy? (2) Which regimen is better, UFT or platinum-based doublet chemotherapy in the patient with stage IB and II or III? (3) Is treatment for 1 year equivalent to treatment for 2 years? (4) What is the mechanism of UFT effectiveness in the adjuvant setting? and (5) There is need for confirmatory studies in other countries. As for Bestatin, it is necessary to do another confirmatory clinical trial.

Table 2. Potential functional mechanism of UFT and bestatin

	UFT	Bestatin
Production	5-FU derivative; tegafur and uracil	Culture filtrate of *Streptomyces olivoleticuli*
Basic concept	Antimetabolic drug	Immunomodulator
Anticancer effect (possibility)	Biochemical moduration, incidence of apotosis, inhibition of angiogenesis	Inhibition of angiogenesis Introduction of apotopsis

Table 3. The efficacy of the postoperative adjuvant chemotherapy for non-small cell lung cancer based on the pathological stage

	IALT	JBR 10	CALGB 9633	JLCRG/UFT
p-stage I	Negative	Positive (IB)	Positive (IB)	Positive
p-stage II	Negative	Positive		
p-stage IIIA	Positive			
Survival benefit[a]	4.1%	15%	12% (4-year survival)	2.5% IB 11%
HR	0.86	0.70	0.62	0.71 IB 0.48
95% CI	0.76–0.98	0.52–0.92	0.41–0.95	0.51–0.98 IB 0.29–0.81

Positive, 10% improvement for the hazard ratio
HR, hazard ratio; 95% CI, 95% confidential interval
[a]Absolute difference of the 5-year survival rate between the adjuvant group and the surgery-alone group

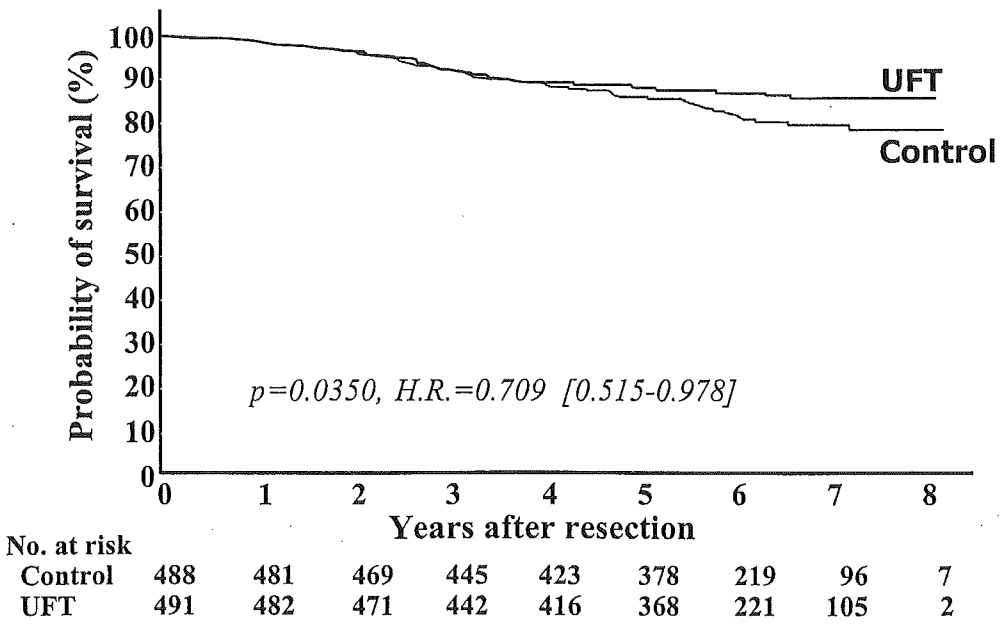

Fig. 1. Overall survival among all 979 eligible patients in the Japan Lung Cancer Research Group (JLCRG) trial. The hazard ratios indicate the risk of death in the UFT group as compared with the control group; 95% confidential intervals are shown in *brackets*. UFT, uracil-tegafur (From ref. 13 with permission)

On the basis of the comparison of mechanism between UFT and Bestatin, both drugs have been shown to inhibit angiogenesis and induce apoptosis in vivo and in vitro (Table 2). Although these data should be confirmed in future, the administration of a less cytotoxic agent and/or cytostatic drug in the adjuvant setting may improve survival for patients with early-stage non-small cell lung cancer.

Brief results of international trials

International adjuvant lung trial (IALT) (presented at ASCO 2003[17])

On the basis of a previous meta-analysis, an international adjuvant lung cancer trial was designed to evaluate the effect of cisplatin-based adjuvant chemotherapy on survival after completely resection of non-small cell lung cancer. Patients were randomly assigned either to three or four

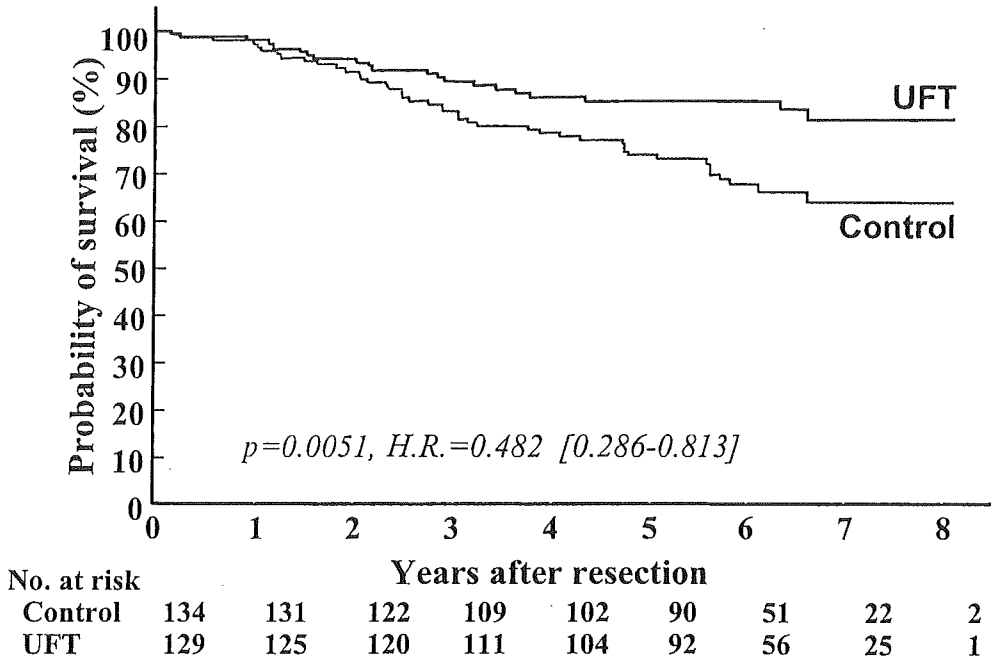

Fig. 2. Surgical outcome of T2 subset population in the JLCRG trial. UFT, uracil-tegafur (From ref. 13 with permission)

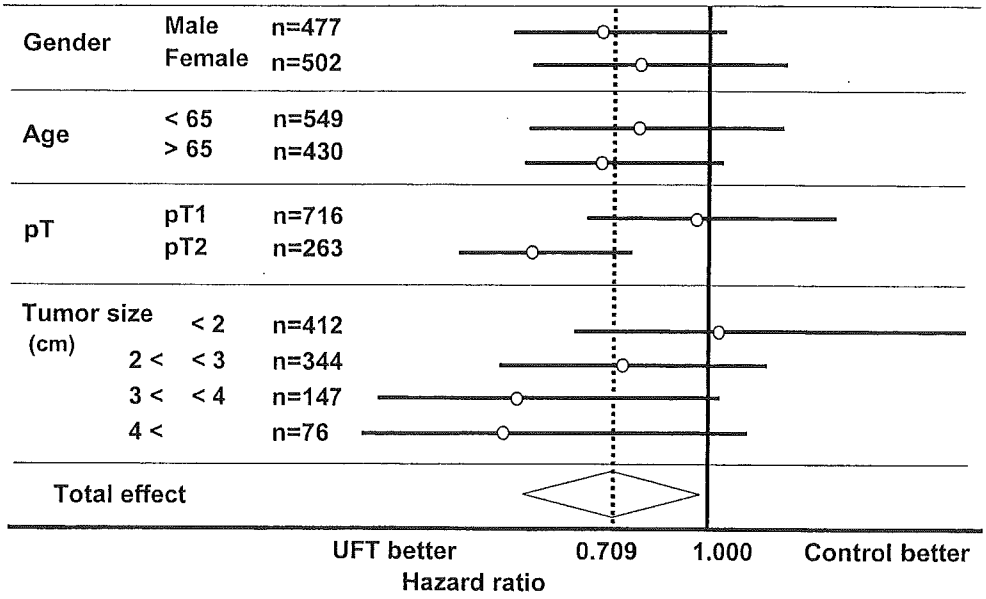

Fig. 3. Interaction in relation to treatment effect. Each *square* represents the estimated treatment effect, *horizontal lines* represent the 95% confidential intervals (CI), and the *diamond* corresponds to the 95% CI for the entire group of patients (From ref. 13 with permission)

cycles of cisplatin-based chemotherapy or to observation (without chemotherapy). Before randomization, in each center, time in the pathological stage to be included in its policy for chemotherapy and postoperative radiotherapy policy were determined. The main endpoint was overall survival.

A total of 1867 patients underwent randomization; 36.5% had pathological stage I disease, 24.2% stage II, and 39.3% stage III. The drug allocated with cisplatin was etoposide in 56.5% of patients, vindesine in 26.8%, vinblastine in 11%, and vindesine in 5.58%. Of the 932 patients assigned to chemotherapy, 73.8% received at least 240 mg cisplatin per square meter of body surface area. In total, 23% of 932 patients developed a grade 4 adverse reaction. Seven patients (0.8%) died of chemotherapy-induced toxic effects. The median duration of follow-up was 56 months. Patients assigned to chemotherapy had a significant higher survival rate than those without chemotherapy (44.5% vs. 40.4% at 5 years; HR for death, 0.86; 95% CI, 0.76–0.93; $P < 0.003$). Disease-free survival rate was also significantly different between the two group (39.4% vs. 34.3% at 5 years; HR, 0.83; 95% CI, 0.74–0.94; $P < 0.003$). Seven patients (0.8%) died of chemotherapy-related toxic events. A total of 22.6% of the patients had at least one episode of

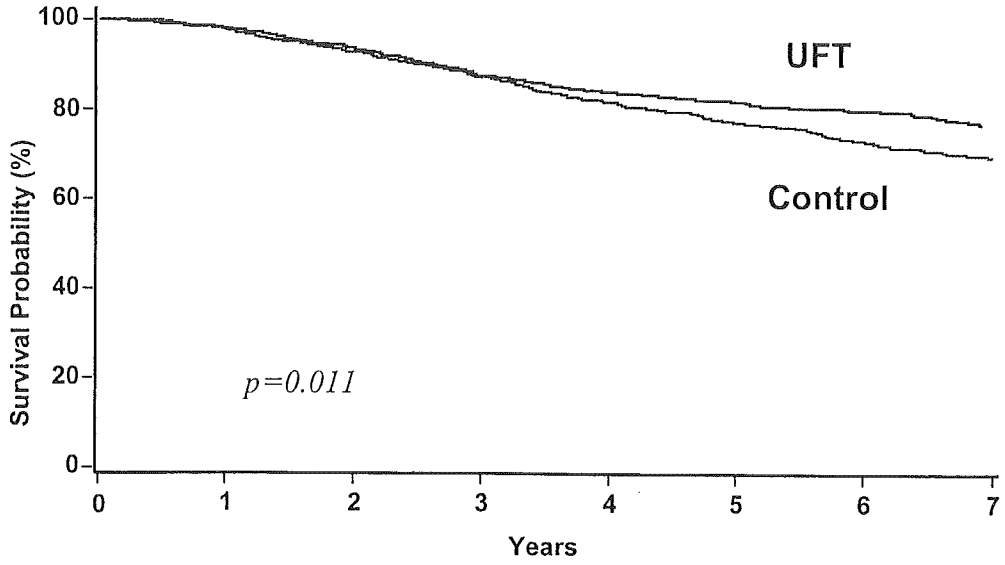

Fig. 4. Overall survival among all 2003 eligible patients in meta-analysis of six UFT trials. *P* values were calculated with stratified log-rank test (From ref. 14 with permission)

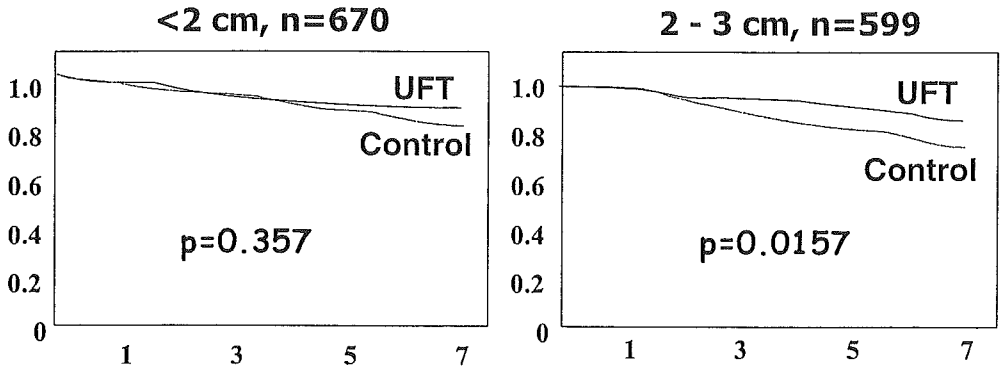

Fig. 5. Overall survival for exploratory analysis of T1 population (n = 1269) in UFT meta-analysis. *P* values were calculated with stratified log rank test (From ref. 14 with permission)

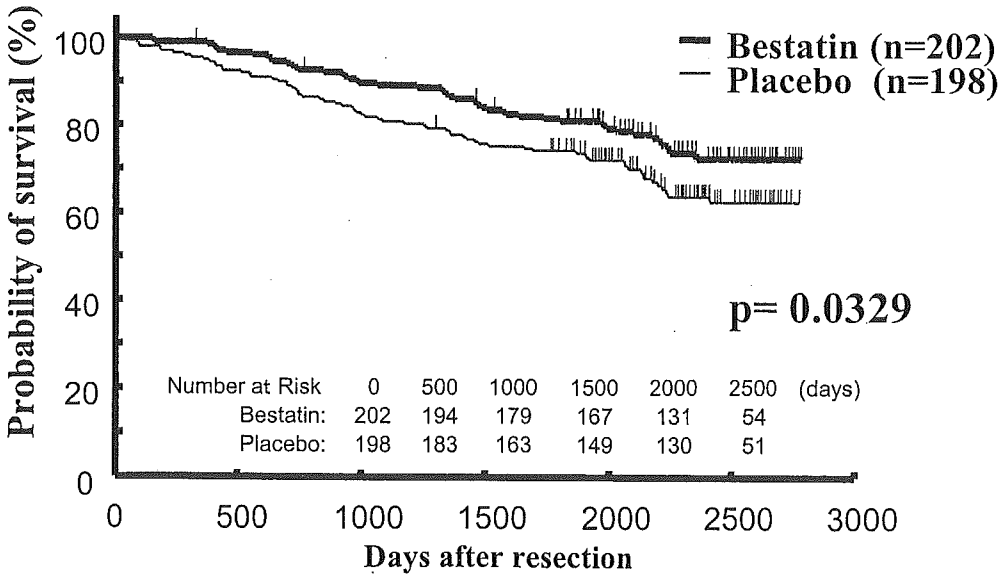

Fig. 6. Overall survival among all 400 eligible patients in Bestatin trial. *P* values were calculated with stratified log-rank test. (From ref. 16 with permission)

grade 4 toxicity, mainly neutropenia (17.5%), thrombocytopenia (2.6%), and vomiting (3.3%). These results have confirmed the meta-analysis in 1995.

NCI-Canada trial, JBR10 (presented at ASCO 2004[18])

Patients with p-stage IB and II except T3N0 were randomly assigned either to three or four cycles of cisplatin-based chemotherapy with cisplatin (50 mg/m^2, days 1, 8, every 4 weeks) or vinorelbine (25 mg/m^2, weekly to 16 weeks), or observation. A total of 344 patient underwent randomization. Stratified factors were the status of lymph node and *ras* gene. In overall survival in this study, patients with chemotherapy had a significantly higher survival rate than those with observation (69% vs. 54%, $P = 0.012$), at HR of 0.696 (95% CI, 0.524–0.923).

U.S. trial, Cancer and Leukemia Group B (CALGB) 9633 (presented in ASCO 2004[19])

Patients with p-stage IB were randomly assigned to either three or four cycles of the chemotherapy with carboplatin (AUC = 6, day 1, every 3 weeks) and paclitaxel (200 mg/m^2, day 1, every 3 weeks), or observation. A total of 482 patients underwent randomization. Stratified factors were histology, differentiation, and the status of mediastinoscopy. The median duration to follow-up was 34 months; patients assigned to chemotherapy had a significantly higher survival rate than those assigned to observation (71% vs. 59% at 4-year survival rate, $P = 0.028$). HR for this trial was 0.62 (95% CI, 0.41–0.95).

Short summary of international trials

The NCI-C and CALGB studies confirmed positive IALT results of the benefit for postoperative platinum-based chemotherapy in completely resected non-small cell lung cancer. The good results of NCI-C and CALGB trials might be due to patient selection, such as earlier-stage disease (IB and II), uniform patient population, more frequent incidence of women than ILT, and the therapeutic strategy of chemotherapy, such as a two-drug regimen with third-generation agent, better compliance, and no radiotherapy in patients without lymph node metastasis.

The summary was based on the international trial; consistent reductions in the risk of death have been observed in recent adjuvant platin-based trials and the 1995 meta-analysis. Adjuvant platinum-based chemotherapy should be recommended to completely resected non-small cell lung cancer patients with good performance status.

Consideration: future perspective

Even if completely resected stage I non-small cell lung cancer is due to recurrent disease in the majority of patients, adjuvant therapy had aimed at eradication of micrometastasis. Recent development of molecular biological techniques permits us to predict the chemotherapeutic response. In the adjuvant setting, the selection of anticancer drugs should depend on the analysis of molecular biological makers for resected materials in addition to pathological stage. In addition to cooperation with new chemotherapeutic agents, such as taxane, camptothecin, and gemcitabine, there are even newer classes of antineoplastic therapy, such as antiangiogenic inhibitor and tyrosine kinase inhibitor, that should be defined. The role of newer classes of some biological therapies with anticancer effect will be defined in coming years. The clinical benefit of platinum-based adjuvant therapy was confirmed. This paradigm is strongly recommended at stage IB and II non-small cell lung cancer. In stage IIIA, further subset analysis is necessary in the new meta-analysis, including IALT (Table 3). On the other hand, platinum-based chemotherapy has some potential of severe adverse events. Although there was no treatment-related death by carboplatin with paclitaxel in the CALGB trials, the feasibility of the platinum-based regimen in the adjuvant setting has not been confirmed yet in Japan. Careful observation after platinum-based chemotherapy is necessary.

Conclusion

Adjuvant chemotherapy for pathological stage IB to II, completely resected non-small cell lung cancer is standard care based on clinical trials. UFT showed the strongest evidence for IB in Japan. Platinum doublet chemotherapy with a third-generation anticancer agent is also recommended. Although there is no evidence of the feasibility of a platinum-based regimen in the adjuvant setting in Japan, adjuvant chemotherapy should be offered as standard care to patients after completely resected early-stage non-small cell lung cancer.

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Expression pattern of the scaffold protein IQGAP1 in lung cancer

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Abstract. IQGAP1 is a scaffold protein whose function relates to signal transduction, cell adhesion, local invasion, and distant metastasis of cancer cells. We examined the expression patterns of this protein and clinicopathologic features of lung cancer, and the antibody against IQGAP1 was used for immunohistochemical analysis. Of the 70 surgical specimens examined, there were 40 adenocarcinomas, 19 squamous cell carcinomas, 5 large cell carcinomas, 3 small cell carcinomas, 2 carcinoid tumors, and 1 mucoepidermoid carcinoma. The localization of IQGAP1 was classified into three types: 1) cytoplasmic, 2) membranous, and 3) reduced expression. In adenocarcinoma, the 3 types were observed equally, and differentiation grade was related to the expression pattern. The cytoplasmic type was common in well-differentiated adenocarcinomas, and membranous or reduced expression was frequently seen in moderately- or poorly-differentiated adenocarcinomas. In squamous cell carcinoma, the membranous type was most common. Although the staining pattern of IQGAP1 did not correlate with the positivity of regional lymph nodes, survival in those patients with a cytoplasmic type was significantly better than others with adenocarcinoma (p=0.0144). Expression typing of IQGAP1 in lung cancer was associated with histologic type and can be used to predict survival in patients with adenocarcinoma of the lung.

Introduction

In cancer cells, abnormal protein expression affects signal transduction, cell adhesion, local invasion, and distant metastasis. IQGAP proteins are multidomain molecules that contain several protein-interacting motifs, and IQGAP1 is a component of signaling networks that are integral to maintaining cytoskeletal architecture and cell adhesion (1). These functions include modulating the actin cytoskeleton (2), mediating signaling by the Rho family GTPases (3) and calmodulin (4), and regulating the E-cadherin and ß-catenin function (5,6).

Recent microarray analysis has revealed that highly-metastatic mouse melanoma cells have gene expression of IQGAP1 increased by >2.5-fold (7). In human clinical cases, IQGAP1 was overexpressed in colon cancer compared with normal tissue, and IQGAP1 tended to be expressed more at the invasive front (8). These reports imply that IQGAP1 may play an important role in tumor development and malignant behavior.

We performed an immunohistochemical analysis using a newly-developed specific antibody against IQGAP1 to elucidate the relationship between expression patterns of IQGAP1 and clinicopathologic features of patients with lung cancer.

Patients and methods

Patients. The patients included 45 men and 25 women with an average age of 63 years. Lobectomy and mediastinal lymph node dissection were performed in all cases, and no pre-surgical chemotherapy or radiotherapy was administered. The diagnosis of lung cancer was established by histologic examination of the surgical specimens (9), and TNM staging was performed using the latest criteria (10) with results showing pathologic stage IA in 19, IB in 15, IIA in 2, IIB in 12, IIIA in 13, IIIB in 5, and IV in 4 cases. Informed consent for immunohistochemical analysis of the primary lung cancer was obtained from all patients, and the median follow-up period of the censored cases was 60 months.

Tissue samples. The tissue samples were fixed in buffered formaldehyde and stored as paraffin-embedded blocks until use. Distribution of the IQGAP1 antigen in normal human tissues was examined using NormalGrid™ Multi-Tissue control slides (Biomeda Corp., Foster City, CA).

Generation of specific antibody against IQGAP1. We selected a cDNA clone (KIAA0051) coding IQGAP1 from the Kazusa cDNA library to generate a specific antibody for immunohistochemistry. We screened the HUGE database, which contains human novel large cDNAs identified in the Kazusa cDNA sequencing project (11) and is available at

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Key words: adenocarcinoma, carcinogenesis, cytoskeleton, immunohistochemistry, survival

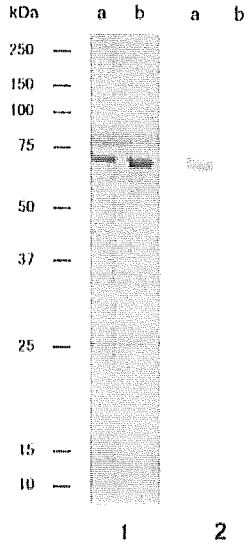

Figure 1. Western blot analysis of the reactivity of affinity-purified anti-IQGAP1 antibody (KD0019). Lane 1 indicates the results of SDS-PAGE stained by Coomassie Brilliant Blue. (a) Purified maltose-binding protein-fusion protein carrying the antigenic region of IQGAP1, and (b) control protein. Lane 2 indicates the results of Western blot analysis reacted with affinity-purified anti-IQGAP1 antibody, KD0019. (a) Purified maltose-binding protein-fusion protein carrying the antigen region of IQGAP1, and (b) control protein.

Table I. The staining pattern of IQGAP1 according to the histologic type of lung cancer.

Staining pattern	Histologic type					Total
	Ad	Sq	La	Sm	Mis	
C-type	15	4	1	3	2	25
M-type	14	13	1	0	0	28
R-type	11	2	3	0	1	17
Total	40	19	5	3	3	70

Ad, adenocarcinoma; Sq, squamous cell carcinoma; La, large cell carcinoma; Sm. small cell carcinoma; Mis, miscellaneous tumors; C-type, cytoplasmic type; M-type, membranous type; R-type, reduced expression type.

http://www.kazusa.or.jp/huge. Using KIAA0051 as a template, a DNA fragment coding 200 amino acids (E201-N400) was amplified by polymerase chain reaction (5' primer, CGC GGATCCGAAGAAATCAACAACATGAAGACTG; and 3' primer, CCCAAGCTTCTAGTTTGCAGCATCCACTCC AGACTGC), fused to the plasmid pDEST15 and propagated in *Escherichia coli*. The protein was purified and used as an immunogen. A rabbit was immunized with the purified IQGAP1, and antiserum was purified with N-hydroxy-succinimide (NHS)-activated Sepharose 4 Fast Flow (Amersham Biosciences, Piscataway, NJ) bound with the same antigen protein. The purified rabbit-specific antibody against IQGAP1 was named KD0019 and used for immunohistochemistry.

Western blot analysis confirms the reactivity of affinity-purified anti-IQGAP1 antibody, KD0019. To verify the specificity of KD0019, 1.2 μg of purified maltose-binding fusion protein carrying the antigen region of IQGAP1 or a control sequence was separated by sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE), followed by transfer onto a polyvinylidene fluoride (PVDF) membrane. The blotted membrane was reacted with KD0019 or affinity-purified control antibody, followed by alkaline phosphatase-conjugated anti-rabbit IgG antibody (7500-fold dilution) (Promega, Madison, WI). The immunoreactive proteins were visualized with the bromo-chloro-iodoryl phosphate (BCIP)/ nitro-blue tetrazolium (NBT) color development substrate (Promega).

Immunohistochemistry. The streptavidin-biotin peroxidase complex (SABC) technique was used for immunohistochemical staining. Sections (4 μm thick) were cut, kept in xylene, rehydrated and washed with water, then treated with 0.3% hydrogen peroxide in methanol for 10 min to inhibit endogenous peroxidase and autoclaved in a citrate buffer solution (10 mM sodium citrate, pH 6.0) at 100°C for 20 min to retrieve antigenicity. After blocking non-specific binding with 5% normal rabbit serum, sections were incubated with primary antibody, KD0019 (500 ng/ml) at 4°C overnight. Slides were then washed and incubated with a second antibody, biotinylated anti-rabbit IgG (LSAB kit) (Dako, Copenhagen, Denmark) for 15 min at room temperature. Finally, the slides were washed and incubated with SABC Elite reagent (Dako) for 15 min at room temperature. Specific staining was developed with diaminobenzidine tetrahydrochloride supplemented with 0.03% hydrogen peroxide and counterstained with hematoxylin, and lung cancers were classified into three types according to the staining pattern of IQGAP1: 1) cytoplasmic (C-type), 2) membranous (M-type), and 3) reduced expression (R-type).

Statistical analysis. Differences between groups were evaluated using the χ^2 test, the survival rate was calculated by the Kaplan-Meier method, and survival differences were compared using the log-rank test as a univariate analysis. $p<0.05$ was considered significant.

Results

Specificity of anti-IQGAP1 antibody. Affinity-purified anti-IQGAP1 antibody, KD0019, strongly reacted with the maltose-binding IQGAP1 fusion protein, but not the control molecule (Fig. 1).

Localization of IQGAP1 in normal human tissues. In normal lung tissues, strong staining was observed in alveolar macrophages, bronchial epithelium, and bronchial glands. In bronchial glands, localization of IQGAP1 was limited to the cytoplasm and cell boundaries of serous glands; no mucous glands were stained (Fig. 2A). Skin, epithelium of the external glands of the prostate, Kupffer cells, and distal urinary tubules in the kidney all showed relatively strong staining (Fig. 2B-E). Skin showed the strongest positive reaction in both the cell membrane and cytoplasm. Only weak staining was observed in the spleen, uterus, placenta,

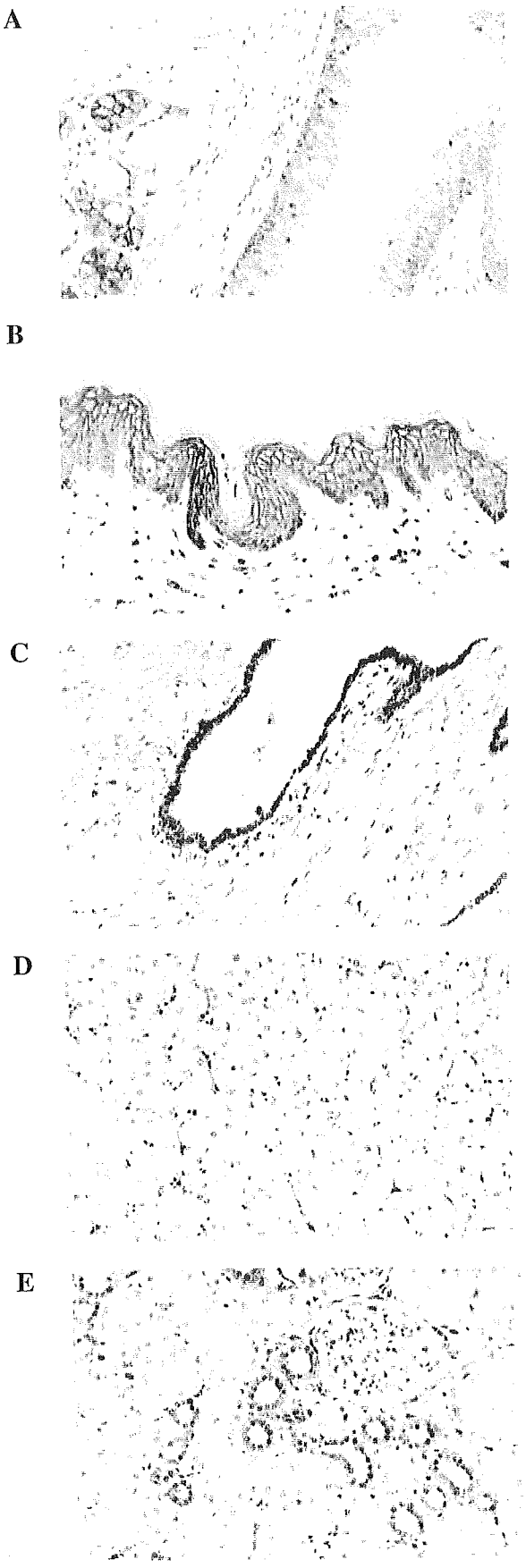

Figure 2. The distribution of IQGAP1 in normal lung. IQGAP1 strongly stained (A) bronchial epithelium and serous glands in normal lung, (B) normal skin, (C) external glands of the prostate, (D) Kupffer cells, and (E) distal urinary tubules in the kidney.

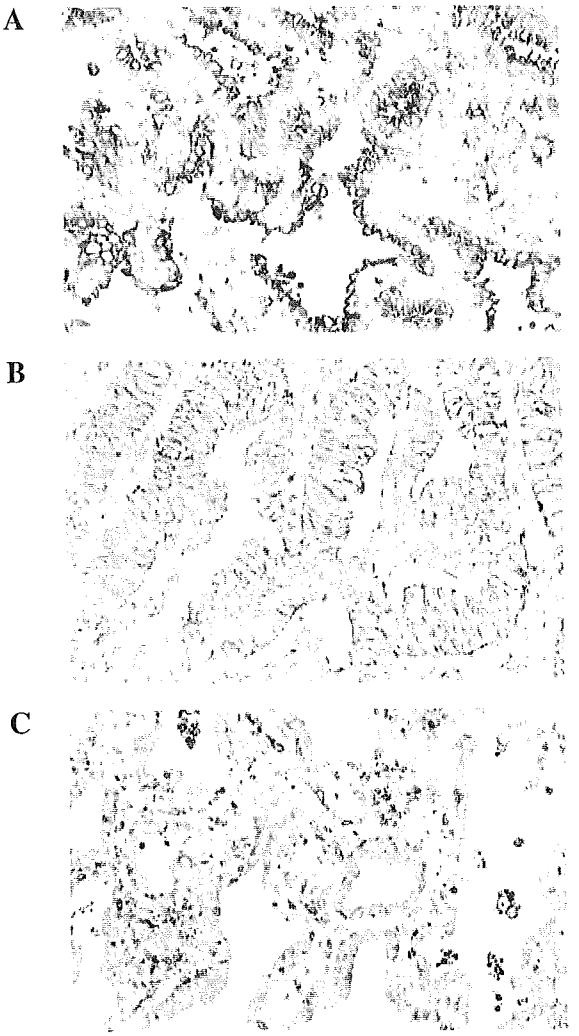

Figure 3. Representative staining patterns of IQGAP1 in lung cancer. Adenocarcinomas showing (A) cytoplasmic, (B) membranous, and (C) reduced expression type.

tonsil, testis, ovary, pancreas, breast, heart, stomach, small and large intestine, brain, pituitary gland, and adrenal gland.

Localization of IQGAP1 in lung cancer

Expression pattern according to histologic type. Of the 70 surgical specimens examined, there were 40 adenocarcinomas, 19 squamous cell carcinomas, 5 large cell carcinomas, 3 small cell carcinomas, 2 carcinoid tumors, and 1 mucoepidermoid carcinoma. A representative staining pattern is shown in Fig. 3A-C. In squamous cell carcinoma, M-type was frequently seen (68.4%, 13/19); in adenocarcinomas, the 3 types were equally observed (Table I). All 3 small cell lung cancers and 2 carcinoids showed weak cytoplasmic staining.

Expression pattern and grade of differentiation of lung cancer. In adenocarcinoma, differentiation grade was related to the expression pattern (Table II). C-type was common in well-differentiated adenocarcinoma, and M- and R- types were frequently seen in moderately- and poorly-differentiated adenocarcinoma (p=0.0004). In squamous cell carcinoma, a difference in staining pattern according to differentiation grade was not observed (p=0.3960).

Table II. The staining pattern of IQGAP1 according to the differentiation grade of lung cancer.

Staining pattern	Ad			Sq			Total
	WD	MD	PD	WD	MD	PD	
C-type	10	5	0	1	1	2	19
M-type	2	9	3	3	9	1	27
R-type	1	6	4	0	0	2	13
Total	13	20	7	4	10	5	59

Ad, adenocarcinoma; Sq, squamous cell carcinoma; La, large cell carcinoma; Sm. small cell carcinoma; Mis, miscellaneous tumors; C-type, cytoplasmic type; M-type, membranous type; R-type, reduced expression type.

Table III. The staining pattern of IQGAP1 according to nodal status.

Staining pattern	Ad		Sq		Total
	N(-)	N(+)	N(-)	N(+)	
C-type	12	3	2	2	19
M-type	7	7	6	7	27
R-type	4	7	0	2	13
Total	23	16	8	11	59

N(-), node negative; N(+), node positive; Ad, adenocarcinoma; Sq, squamous cell carcinoma; C-type, cytoplasmic type; M-type, membranous type; R-type, reduced expression type.

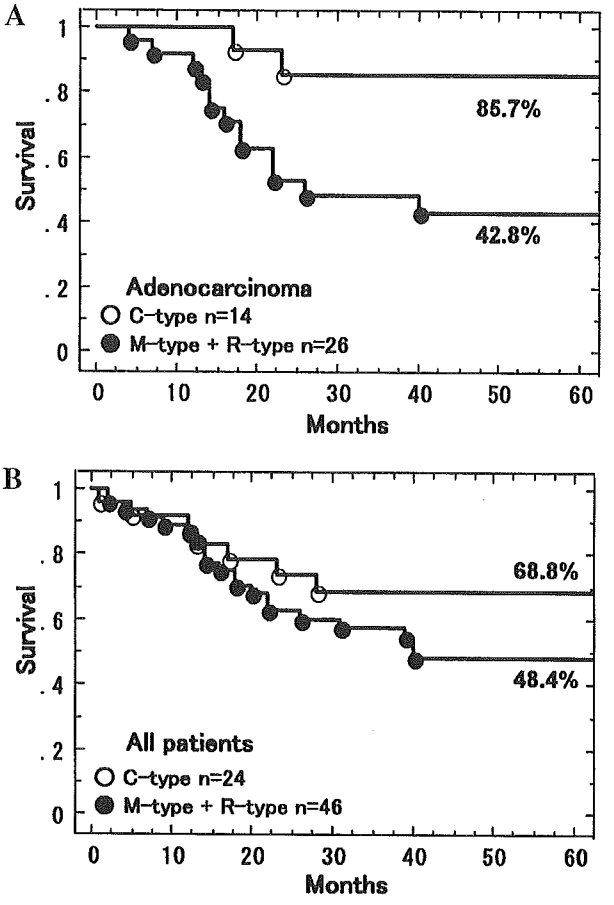

Figure 4. Kaplan-Meier survival curves after resection of lung cancer. (A) Staining pattern of IQGAP1 was significantly related to survival in patients with adenocarcinoma (p=0.0144). (B) Staining pattern of IQGAP1 was not related to survival when all patients were analyzed together (p=0.2363).

Expression pattern and nodal status. Staining patterns of IQGAP1 did not correlate with positivity of the regional lymph nodes (Table III).

Expression pattern and survival of patients with lung cancer. In adenocarcinoma, a survival difference was observed between the C group and the M and R groups (p=0.0144; Fig. 4A). This difference was not observed in all cases of lung cancer or other histologic types (p=0.2363; Fig. 4B).

Discussion

IQGAP1 is a scaffold protein that plays an important role in molding the cytoskeleton, signal transduction, and intercellular adhesion, and co-localizes with actin filaments in the cell cortex. It binds *in vitro* to F-actin and several signaling proteins, including calmodulin, Cdc42, Rac1, and ß-catenin (12,13). F-actin binding activity of IQGAP1 is regulated by its reversible association with these signaling molecules, but the mechanism is unclear (14).

Previously, localization of IQGAP1 in malignant tumors had only been examined in adenocarcinomas, and gastric, colon, and endometrial cancer. In gastric cancer, IQGAP1 was frequently observed diffusely in the cytoplasm in intestinal-type, well-differentiated tumors, but it was expressed at the cell membrane in diffuse-type, poorly-differentiated tumors (15). In that report, E-cadherin was localized to the cell membrane of the well-differentiated type and in the cytoplasm of poorly-differentiated tumors; thus, subcellular localization of IQGAP1 was inversely correlated with E-cadherin localization. Movement of IQGAP1 from the cytoplasm to the cell membrane could be correlated with E-cadherin dysfunction and dedifferentiation in gastric carcinogenesis. In colorectal cancer, IQGAP1 seemed to be expressed more at the invasion front of the tumor, and this expression pattern was most apparent in advanced disease (8). In endometrial cancer, α-catenin and IQGAP1 were absent from cell adhesive sites in well-differentiated adenocarcinomas (16). All of these results suggest that the differential grade of adenocarcinomas is associated with abnormal intracellular localization of IQGAP1.

Lung cancer has four major histologic types: 1) adenocarcinoma, 2) squamous cell carcinoma, 3) small cell carcinoma, and 4) large cell carcinoma, unique from other cancers. Thus, we were interested in IQGAP1 expression according to the histologic type of lung cancer. In normal tissues, we demonstrated that stratified squamous epithelium showed strong staining in both cytoplasm and cell boundaries in skin. Therefore, it is not surprising that squamous cell

carcinoma was positive for IQGAP1. However, squamous cell carcinoma frequently showed membranous staining, whereas normal stratified squamous epithelium showed both cytoplasmic and membranous staining. This altered localization of IQGAP1 may relate to the dysfunction of cell adhesion or signal transduction during carcinogenesis in squamous cell carcinomas.

Concerning glandular cells, the cytoplasm of normal bronchial serous glands were specifically stained, whereas mucous glands were not. Well-differentiated adenocarcinoma frequently showed a cytoplasmic staining pattern, whereas moderately- or poorly-differentiated adenocarcinoma showed membranous staining or reduced expression. Thus, our findings are similar to gastric (15) and endometrial (16) cancer. This might result in a worse prognosis for patients with lung adenocarcinoma showing membranous or reduced staining patterns. An inverse correlation of membranous expression of IQGAP1 and either E-cadherin or α-catenin (15,16) might explain some dedifferentiated features of cancer cells. Since the most common cause of death in this series of patients was distant metastasis, abnormal localization of IQGAP1 might play a role in local invasion and distant metastases.

In summary, the expression pattern of IQGAP1 in lung cancer was different according to histologic type, which may reflect the features of cancer cell origin. In adenocarcinoma, cytoplasmic staining was frequently observed in the well-differentiated type, and survival of patients with this type was better than those with membranous or reduced expression. Therefore, the expression pattern of IQGAP1 can predict survival in patients with lung adenocarcinoma.

Acknowledgements

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Gefitinib in the adjuvant setting: safety results from a phase III study in patients with completely resected non-small cell lung cancer

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Standard therapy for stage I–IIIA non-small cell lung cancer (NSCLC) is surgery, although adjuvant therapies are required to prevent disease recurrence and improve patient survival. This is the first study that planned to administer adjuvant gefitinib (Iressa) 250 mg/day or placebo to randomized patients with completely resected NSCLC (stage IB–IIIA) 4–6 weeks following surgery, for 2 years, until recurrence/withdrawal. However, recruitment was stopped after the randomization of 38 patients, because interstitial lung disease (ILD)-type events were being increasingly reported in Japan in the advanced disease setting. Finally, the trial was halted. Safety data for 38 recruited patients (18 gefitinib and 20 placebo) showed no unexpected adverse drug reactions (ADRs), with the most common being grade 1/2 gastrointestinal and skin disorders in 12 and 16 patients receiving gefitinib and in five and six patients receiving placebo, respectively. Grade 3/4 ADRs occurred in four patients receiving gefitinib and one patient receiving placebo. ILD-type events were reported in one patient receiving gefitinib (concomitantly with other ILD-inducing drugs) who died and two patients receiving placebo. Eight patients receiving gefitinib withdrew due to ADRs compared with three patients receiving placebo. Adverse events associated with surgical complications were reported for six patients receiving gefitinib and four patients receiving placebo. In the adjuvant setting there were no unexpected adverse events observed. Gefitinib had no impact on surgery-related complications when given within 4–6 weeks post-operatively. *Anti-Cancer Drugs* 16:1123–1128 © 2005 Lippincott Williams & Wilkins.

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Keywords: gefitinib, non-small cell lung cancer, phase III, safety

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Sponsorship: This trial was coordinated and supervised by the Study Coordinating Committee (principal investigators plus AstraZeneca personnel), and the Independent Data Monitoring Committee (lung cancer and statistical experts independent of AstraZeneca), with funding and organizational support from the trial sponsor AstraZeneca.

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Introduction

Non-small cell lung cancer (NSCLC) is generally not diagnosed until the disease is symptomatic, by which time more than two-thirds of patients are in the advanced stages of disease and have a poor prognosis [1]. Approximately 25% of patients with NSCLC are diagnosed when their disease is in the early stages; however, as many of these patients frequently have undetectable metastases, disease often recurs in distant sites [2]. Adjuvant therapies are therefore required to help prevent disease recurrence and as they will need to be given to patients post-operatively for a prolonged period, they should be well tolerated.

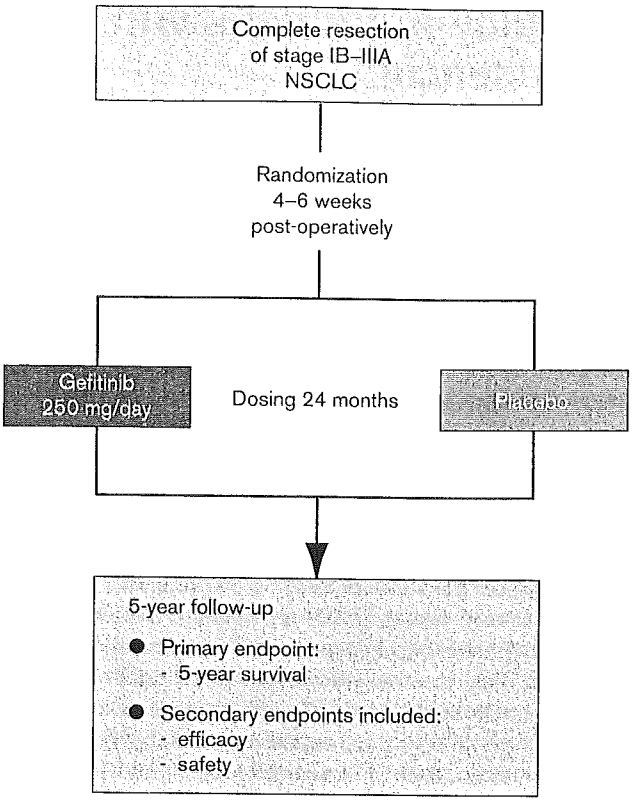
Although some clinical trials in NSCLC have shown a significant survival benefit with adjuvant uracil plus tegafur (UFT) and cisplatin-based chemotherapy [3–7], others have not observed a significant improvement in survival [5,8,9]. At the time of commencing this study, there were no standard adjuvant treatment regimens for NSCLC.

Gefitinib (Iressa), an orally active epidermal growth factor receptor tyrosine kinase inhibitor (EGFR-TKI), was approved in Japan for the treatment of inoperable or recurrent NSCLC in 2002. Two large phase II trials, IDEAL (Iressa Dose Evaluation in Advanced Lung cancer) 1 and 2, observed objective responses and stable disease in more than 40% of pre-treated patients with NSCLC receiving 250 mg/day gefitinib, with the majority of adverse events (AEs) being mild to moderate gastrointestinal and skin disorders [10,11]. Gefitinib was not associated with the well-recognized AEs observed with cytotoxic chemotherapy (e.g. bone marrow depression, neurotoxicity, nephrotoxicity). The tolerability profile of gefitinib has been confirmed by data from the

Expanded Access Programme, through which more than 39 000 patients have received gefitinib 250 mg/day on a compassionate-use basis. Furthermore, a retrospective analysis of 9515 US patients who had received gefitinib for 1 year or more via the Expanded Access Programme showed a 1-year survival rate of 33% [12], which compares with the IDEAL studies [10,11]. Recently, Onn *et al.* observed efficacy (16% with objective responses and 45% with stable disease) and a low incidence of grade 3/4 AEs in Japanese patients with NSCLC, most of whom had been treated with second-line gefitinib or above (99% of patients) [13].

To date, there is no experience of using gefitinib in the post-operative adjuvant setting. This phase III trial was initially undertaken to compare survival rates in patients with completely resected stage IB–IIIA NSCLC who had been treated with adjuvant gefitinib 250 mg/day or placebo. However, in October 2002, recruitment was halted following high-profile media activity around reports of gefitinib-related interstitial lung disease (ILD)-type events in patients with advanced or metastatic NSCLC in Japan. In March 2003, the trial was halted because of an increased withdrawal rate. As enrollment could not be resumed until the prospective investigation into gefitinib-related ILD-type events in Japan was completed, the trial was closed. Consequently survival data are not available, although data from patients recruited to the study have been subsequently analyzed for safety.

Fig. 1



Trial design schema.

Methods

Patients

Patients were eligible for inclusion in the trial if they had histologically confirmed NSCLC (post-operative stage IB–IIIA) that had been completely resected 4–6 weeks before the start of treatment. Patients were required to be 20–75 years of age, with a WHO performance status (PS) 0–1, no previous history of chemotherapy, radiotherapy or immunotherapy for NSCLC and no co-malignancies within the past 5 years. All patients gave written, informed consent to participate in the trial, which was conducted in accordance with the Declaration of Helsinki [14] and Good Clinical Practice guidelines.

Study design

This randomized (1:1), double-blind, placebo-controlled, phase III multicenter survival study planned to recruit 670 patients (335 per group) and randomize them to receive either gefitinib (250 mg) or placebo (Fig. 1). Treatment was to be continued for 2 years, or until recurrence/secondary carcinoma or withdrawal criteria were met. An Independent Data Monitoring Committee (IDMC) was set up to assess the efficacy and safety of gefitinib post-operatively, and would advise whether the study should be continued, changed or discontinued.

Assessments

Efficacy

Disease recurrence or secondary carcinogenesis were assessed using X-rays every 3 months during treatment and every 6 months during the follow-up period. Computed tomography (CT) scans were carried out 8 weeks after the first dose (where necessary, the pre-operative thoracoabdominal CT scan could be used), at week 48 during treatment, at week 104 after withdrawal/completion and every 52 weeks thereafter, unless disease recurrence was observed.

Safety

AEs were to be recorded and coded using MedDRA (Medical Dictionary for Regulatory Activities) version 6.0, graded using National Cancer Institute Common Toxicity Criteria (NCI-CTC) version 2.0 and assigned causality by the investigators. AEs associated with post-operative complications were defined as events occurring within 90 days after surgery and were recorded without regard to causality. Treatment could be interrupted for up to 14 days, although the IDMC later recommended that drug interruption could be allowed for more than 14 days in cases where ILD-type events were suspected, but could not be confirmed, in order to ensure the safety of

patients who remained in the trial after recruitment was halted. Hematology, biochemistry and urinalysis were also measured at baseline and during the study.

Role of the funding source

This trial was coordinated and supervised by the principal investigators, the IDMC and AstraZeneca personnel, with funding and organizational support from the trial sponsor AstraZeneca.

Results

Patients

Between August and October 2002, 38 patients were randomized into the trial – 18 received gefitinib and 20 received placebo. Patient demography was well balanced between the treatment arms, with the majority of patients having adenocarcinoma histology and WHO PS 1 (Table 1). When the trial was stopped, four patients in the gefitinib arm and 11 patients in the placebo arm were still receiving treatment (Fig. 2). Of the 23 patients who withdrew, 13 did so because of AEs (10 in the gefitinib arm and three in the placebo arm), five were unwilling to continue with treatment (three in the gefitinib arm and two in the placebo arm), two had disease recurrence (both in the placebo arm) and three withdrew for other reasons (one patient in the gefitinib arm had incomplete recovery from surgery that was not drug related, and two patients in the placebo arm had pre-existing interstitial pneumonia and were withdrawn at the request of the sponsor).

Table 1 Patient demography

	Gefitinib 250 mg/day ($n=18$)	Placebo ($n=20$)
Sex [*n* (%)]		
male	14 (77.8)	15 (75.0)
female	4 (22.2)	5 (25.0)
Median age [years (range)]	64.0 (49–73)	62.5 (52–73)
WHO PS [*n* (%)]		
0	5 (27.8)	9 (45.0)
1	13 (72.2)	11 (55.0)
Histology [*n* (%)]		
squamous cell carcinoma	4 (22.2)	6 (30.0)
adenocarcinoma	14 (77.8)	14 (70.0)
Stage [*n* (%)]		
IB	7 (38.9)	8 (40.0)
IIA	2 (11.1)	1 (5.0)
IIB	3 (16.7)	5 (25.0)
IIIA	6 (33.3)	6 (30.0)

Efficacy

From the limited efficacy data, disease recurrence was not seen in patients receiving gefitinib at data cutoff. Three patients who received placebo (one with stage IB and two with stage IIB) experienced disease recurrence – two patients recurred during the trial and one patient recurred after the trial had stopped.

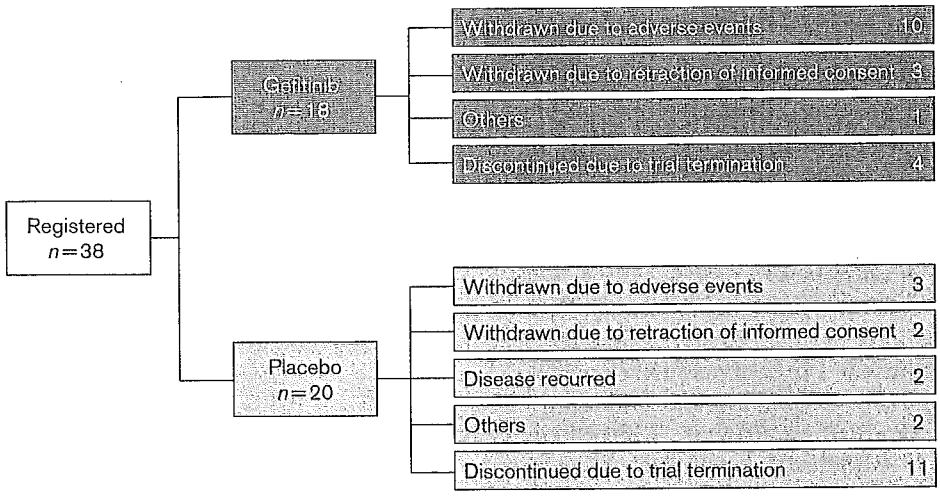
ADRs

No unexpected ADRs were observed and, in general, the frequency of all ADRs was higher for gefitinib versus placebo (Table 2). The most common ADRs were mild to moderate grade 1/2 gastrointestinal and skin disorders. Grade 3/4 ADRs were seen in four patients in the gefitinib arm and one patient in the placebo arm (Table 3), all of whom had treatment withdrawn (the patient with grade 3 eczema had treatment withdrawn due to grade 2 impetigo).

Respiratory ADRs

The majority of respiratory ADRs were grade 1/2 and occurred within 1 month of treatment. In the gefitinib arm, two patients experienced cough (associated with post-operative complications), one patient had dyspnea,

Fig. 2



Trial outcome. .

Table 2 Common ADRs occurring in two or more patients

AE (MedDRA term)[a]	Gefitinib 250 mg/day ($n=18$)	Placebo ($n=20$)
Abnormal hepatic function	4	0
Acne	2	0
Anorexia	5	1
Cough	2[b]	1
Diarrhea	9	2
Dry skin	3	0
Eczema	8	2
Elevated ALT/AST	2	0
Fatigue	2	0
Gastritis	3[b]	0
Loose stools	4	0
Nausea	3	0
Rash	5	3
Sputum	0	2
Stomatitis	2	0

[a]A patient could have more than one AE.
[b]All were associated with post-operative complications.

Table 3 Grade 3/4 ADRs

AE (MedDRA term)	Grade	Gefitinib 250 mg/day ($n=18$)	Placebo ($n=20$)
Abnormal hepatic function	3	1	0
Eczema	3	1	0
Elevated ALT	3	1	0
Neutropenia	3	0	1
Pneumonitis	4	1	0

Table 4 Exposure of patients to gefitinib

	Gefitinib 250 mg/day ($n=18$)	Placebo ($n=20$)
Median duration of treatment [days (range)]	86.5 (4–195)	144.0 (20–197)
Dosing period (n)		
<60 days	6	2
60–120 days	9	4
≥ 120 days	3	14
No. dose interruptions (n)		
1	5	6
2	2	2
≥ 3	2	2

Table 5 ADRs leading to patient withdrawals

Adverse event (MedDRA term)	Grade	Gefitinib 250 mg/day ($n=18$)	Placebo ($n=20$)
Eczema	2	1	0
Elevated ALT/AST	2	1	0
	3	1	0
Hepatic function abnormalities	2	1	0
	3	1	0
ILD	1	0	1
Impetigo	2	1	0
Neutropenia	3	0	1
Paronychia	2	1	0
Pneumonitis	4	1	0
Pulmonary fibrosis	1	0	1

and one patient experienced grade 4 ILD-type events (pneumonitis) 107 days after starting gefitinib and was withdrawn from the study. The patient with pneumonitis had taken concomitant shosaikoto, a Chinese herbal medicine, and loxoprofen, both of which have previously been shown to induce pneumonitis [15,16]. Twenty-one days later bacterial pneumonia related to methylprednisolone therapy was diagnosed, and the patient subsequently died 37 days later due to both pneumonitis and bacterial pneumonia. In the placebo arm, one patient who experienced cough and grade 1 pulmonary fibrosis had had interstitial changes on their chest X-ray at enrollment, and in a second patient, pre-existing non-specific interstitial pneumonia was exacerbated resulting in grade 1 ILD. In both patients, these conditions persisted following withdrawal of study drug.

Interruptions and withdrawals due to ADRs

ADRs requiring interruptions in therapy were similar between patients receiving gefitinib or placebo (Table 4) and were usually for less than 14 days, although four patients in the gefitinib arm required treatment to be interrupted for 14 days (including one patient whose treatment was interrupted for 20 days). The majority of ADRs leading to withdrawal were usually mild-to-moderate grade 1/2 in severity (Table 5). Grade 3 ADRs leading to withdrawal occurred in two patients receiving gefitinib (hepatic function abnormalities, elevated ALT) and in one patient receiving placebo (neutropenia), and grade 4 pneumonitis led to the withdrawal of one patient who was receiving gefitinib. Following withdrawal of gefitinib treatment, grade 3 abnormal hepatic function and elevated ALT resolved, and grade 3 neutropenia persisted.

AEs associated with post-operative complications

As there are no safety data regarding the use of gefitinib in the post-operative setting, AEs associated with the healing process were examined to provide preliminary safety data on the start of the dosing timing in the adjuvant setting for gefitinib. AEs related to post-operative complications were observed in six patients in the gefitinib arm and four patients in the placebo arm. In the gefitinib arm, the most frequent AEs were grade 1/2 cough (four patients) and gastritis (three patients), and in the placebo arm grade 1/2 pain (three patients). Grade 1 cough, grade 1 supraventricular arrhythmia and grade 2 dyspnea were also experienced by three out of four patients receiving placebo.

Discussion

This trial was designed to compare survival rates in patients with completely resected stage IB–IIIA NSCLC who had received adjuvant therapy with gefitinib 250 mg/day or placebo. However, incidences of ADRs of ILD-

type events in the advanced disease setting have been increasingly reported since gefitinib was launched in Japan, and new recruitment was put on hold on 23 October 2002 at the request of the Ministry of Health, Labor and Welfare. In order to evaluate the ILD and ensure the safety of the trial patients, two separate Co-ordination Committee and IDMC meetings (December 2002 and January 2003) were conducted to discuss the feasibility of continuing the study and management of the trial patients. Based on the updated information on ADRs of interstitial pneumonia, the committees concluded that the study could be continued because the possibility of risk did not exceed that of benefit to enrolled patients. The IDMC also suggested that top priority should be given to assure the safety of the patients receiving gefitinib, and that discontinuation should be considered if flu-like symptoms including difficulty in breathing, fever and coughing occurred.

A 'Supplemental Explanation Sheet and Informed Consent Form' was provided four times to enrolled patients, offered updated information and methods to assure and manage any safety issues, and confirmed the patients' willingness to continue participating in the study. In December 2002, AstraZeneca KK gave the principal investigators the option to suspend gefitinib treatment at once. With the extensive monitoring of the trial patients in terms of safety, there were still an increasing number of withdrawals. In addition, enrollment could not be resumed until the prospective investigation on gefitinib-related ILD was completed. Based on these facts, the sponsor finally decided to terminate the trial in March 2003.

The types of AEs reported in this trial were similar to that already reported in the large phase II IDEAL 1 and 2 trials for patients with locally advanced or metastatic NSCLC [10,11]. Three patients experienced ILD-type events – two in the placebo arm and one patient in the gefitinib arm (this patient was also taking two other medications known to induce ILD) [15,17]. It has generally been observed that a higher frequency of ILD-type events are reported in Japanese patients taking gefitinib compared with those in other south-east Asian countries and the rest of the world (1.6, 0.3, and 0.3%, respectively) [18]. The occurrence of ILD in Japanese patients and the reasons for such an ethnic stratification in ILD incidence following gefitinib treatment require further clarification.

The most common reason for withdrawal in both treatment arms was due to toxicity, with the majority of drug-related AEs being grade 1/2 in severity. In the advanced or metastatic disease setting, few patients who experience grade 1/2 drug-related AEs withdraw from treatment with gefitinib, and in IDEAL 1, which recruited Japanese patients, two out of 103 patients who received gefitinib 250 mg/day withdrew from therapy due to ADRs [18]. Several factors may explain the high number of withdrawals (including withdrawal of treatment for less severe ADRs) reported in this trial data compared with previously reported studies. These reasons include the fact that patients with early-stage NSCLC may be less tolerant of AEs compared with patients with advanced NSCLC who have received prior chemotherapy. In contrast to the other studies, the impact of heavy media coverage surrounding gefitinib-related ILD cannot be ignored.

It has been suggested that the dosage and schedule of gefitinib used in this study may not best suit patients with completely resected NSCLC in terms of tolerability and a number of adjustments may need to be taken into consideration when planning an adjuvant study of gefitinib in the future. It is unlikely that the time frame of 4–6 weeks is too short before starting adjuvant treatment, as other adjuvant trials conducted in Japanese patients have used similar time frames [3,4]. It may be possible to lengthen the duration by which gefitinib could be interrupted for toxicity, since 14 days may be too short for patients recovering from AEs such as hepatic enzyme elevation, or to reduce the dose following toxicity to perhaps 250 mg every other day, although this would require further study into the efficacy of such an approach.

With no experience of using gefitinib in post-operative patients there was a concern that EGFR-TKIs might impact on surgery-related complications (especially on the healing process) due to their mode of action. In order to assess this, the trial was designed to allow a safety review of the first 60 patients. Due to the early termination of the study, we have only 38 patients' (18 on gefitinib) data for review; however, there does not seem to be any impact on surgery-related complications when gefitinib was administered within 4–6 weeks after surgery, as evidenced by a similar number of these AEs that occurred in both groups. This indicates that it may be feasible to administer gefitinib in the adjuvant setting within this time frame.

In conclusion, this is the first study to investigate the use of EGFR-TKIs as adjuvant therapy. Despite the absence of survival data, there were no unexpected AEs seen in the adjuvant setting compared with those already reported for patients with locally advanced or metastatic NSCLC. However, it was observed that there were more AEs leading to withdrawal in the gefitinib arm, even though the majority of AEs were grade 1/2 in severity, suggesting that a daily dose of gefitinib 250 mg may not best suit patients with completely resected NSCLC in terms of tolerability.

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Locally Recurrent Central-Type Early Stage Lung Cancer < 1.0 cm in Diameter After Complete Remission by Photodynamic Therapy*

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Background: **It is well known that central-type early stage lung cancer < 1.0 cm in diameter shows almost 100% complete response (CR) to photodynamic therapy (PDT). However, we have encountered cases of local recurrence after CR of tumors with a surface diameter < 1.0 cm.**
Patients and methods: **Ninety-three patients with 114 lesions were followed up, and cases of recurrence after CR has been obtained with initial tumors that had a diameter < 1.0 cm were examined. We compared the cytologic findings of local recurrence after CR to the cytologic findings before PDT. The relationship between the cell features and the depth of bronchial tumor invasion before PDT and on recurrence was evaluated.**
Results: **The CR and 5-year survival rates of patients with lesions < 1.0 cm were 92.8% (77 of 83 patients) and 57.9%, respectively; meanwhile, in the group of patients with lesions ≥ 1.0 cm, CR and 5-year survival rates were 58.1% (18 of 31 patients) and 59.3%. There was a significant difference in efficacy between the two groups ($p < 0.001$). Recurrences after CR were recognized in 9 of 77 lesions (11.7%) < 1.0 cm. When the recurrent tumor cells showed type I-II (low-to-moderate atypia) at the same site initially treated, CR could be obtained by a second PDT. Type III cells (high-grade atypia) showed the characteristics of tumor cells from deeper layers of the bronchial wall. Local recurrence at the same site may be caused by residual tumor cells from deep layers because of inadequate laser irradiation and penetration.**
Conclusions: **To reduce the recurrence rate, it is essential to accurately grasp the tumor extent and the depth of the bronchogenic carcinoma before performing PDT. Analysis of cell features of recurrent lesions after CR appears to be a useful source of information as to the depth of cancer invasion in the bronchial wall.** ***(CHEST 2005; 128:3269–3275)***

Key words: early stage lung cancer; occult lung cancer; photodynamic therapy; porfimer sodium

Abbreviations: AFB = autofluorescence bronchoscopy; CIS = carcinoma *in situ*; CR = complete remission; EBUS = endobronchial ultrasonography; ESLC = early stage lung cancer; PDT = photodynamic therapy; PR = partial remission

Lung cancer has a tendency to develop in older people, with a very poor prognosis. A total of 55,000 Japanese died from lung cancer in 2003, which made it the number-one cause of cancer death. Although diagnostic techniques such as high-resolution CT scan, video bronchoscopy, fluorescence bronchoscopy, and endobronchial ultrasonography (EBUS) have been developed recently, many patients with newly detected lung cancer still have inoperable advanced cancer. Therefore, the detection of early stage lung cancer (ESLC) is considered essential to reduce the mortality rate. Meanwhile, even when ESLC is detected, some cases are inoperable because of cardiopulmonary dysfunction due to age. Endoscopic procedures that are minimally invasive and do not compromise pulmonary function

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are considered useful modalities for centrally located lung cancer. In particular, photodynamic therapy (PDT) is considered a useful and attractive modality for central-type ESLC.[1–7] Its action mechanism is considered to involve singlet oxygen, which is generated through photochemical reactions and causes degenerative necrosis of cells that have taken up the photosensitizer, *ie*, tumor cells.[8]

PDT using red laser light and a tumor-specific photosensitizer was established as a new therapeutic modality for central-type ESLC in 1982.[1] The length of longitudinal tumor extent was the only independent predictive factor for complete remission (CR), and 100% CR in lesions < 1.0 cm in diameter treated by PDT was reported.[5] However, we have encountered local recurrences after CR of tumor even in cases with a surface diameter < 1.0 cm. Therefore, we investigated the characteristics and cytomorphologic features of primary lesions and recurrences after CR in patients with lesions < 1.0 cm in diameter.

Materials and Methods

Patient Selection

A total number of 145 patients with 191 lesions of endoscopic ESLC underwent PDT from February 1980 to April 2001 in the Department of Tokyo Medical University. Of the 145 patients with 191 lesions, 93 patients with 114 lesions were followed up, and cases of recurrence after CR was obtained with initial tumors with a diameter < 1.0 cm were examined.

Procedures of PDT and Follow-up

The depth of tumor invasion was judged by biopsy specimen and CT scan, and was also evaluated by bronchoscopic findings based on the diagnostic criteria of ESLC defined by the Japan Lung Cancer Society.[9] To determine tumor size, bronchoscopic biopsies of the proximal and distal sites of the lesion and bronchoscopic measurements using forceps were performed. PDT procedures were performed with the combination of porfimer sodium (Photofrin; Wyeth Japan K.K.; Tokyo, Japan) that is taken up selectively in tumor, and an argon gas laser system (model 770; Spectra-Physics; Mountain View, CA) or excimer dye laser (EDL-1; Hamamatsu Photonics; Hamamatsu, Japan). Laser irradiation was performed via a quartz fiber inserted through the biopsy channel of the endoscope at 48 h after the IV administration of 2.0 mg/kg of porfimer sodium. The total energy of the laser irradiation was 100 J/cm^2, and energy levels in this range do not cause any heat degeneration or other adverse effects. The duration of irradiation required usually 10 to 20 min. Clean-up bronchoscopies to remove necrotic tissue produced by the PDT reaction were performed at 1, 3, and 7 days after PDT. Both cytologic and histologic examinations via fiberoptic bronchoscopy were performed at 1, 2, and 3 months, and thereafter at 3-month intervals in the first year and 6-month intervals after the second year until 5 years after PDT.

Efficacy Evaluation

The antitumor effect of initial treatment was rated based on endoscopic measurement of tumor size using forceps, morphologic observations, and histopathologic examination by biopsy, according to the general rules of the Japan Lung Cancer Society[9] and the Japan Society of Clinical Oncology.[10] The antitumor effect was rated at 1 month and 2 months after PDT. Antitumor effect was rated as CR (no demonstrable tumor microscopically by brushing and/or biopsy for a period of 4 weeks), partial remission (PR) [≥ 50% reduction in tumor size], no change (< 50% reduction or < 25% increase in tumor size), progressive disease (> 25% increase in tumor size), or not evaluable.

Evaluation of Cytomorphologic Features of Local Recurrences

In the central-type ESLC < 1.0 cm in greatest dimension, we have compared the cytologic findings of local recurrence after CR to the cytologic findings before PDT using bronchial brushing specimen. Cytologic findings were classified into three cytologic morphotypes using the classification of cell features proposed by Konaka and coworkers,[11] which appears to yield information as to the depth of cancer invasion in the bronchial wall. The classification was described as follows: type I cell, low-grade atypia (resembling atypical squamous cell metaplasia); type II cell, moderate-grade atypia (resembling early stage squamous cell carcinoma); and type III cell, high-grade atypia (resembling invasive squamous cell carcinoma). The biopsy specimens before PDT and on recurrence, or resected materials, in cases of resection after recurrence, were examined histopathologically, and the depth of bronchial wall invasion was classified into three groups: grade 1, carcinoma *in situ* (CIS) or microinvasion; grade 2, extramuscular bronchial wall invasion; and grade 3, intracartilaginous to extracartilaginous invasion. The relationship between the cell features and the depth of bronchial tumor invasion before and after PDT was evaluated.

Statistical Analysis

Statistical analysis were done using statistical software (Stat Flex for Windows, version 5.0; Artec; Osaka, Japan). The χ^2 test was used to compare the efficacy of PDT between lesions < 1.0 cm and > 1.0 cm in diameter. Differences between the survival rates of two groups in the Kaplan-Meier survival curves were analyzed using the log-rank test; $p < 0.05$ was considered to indicate a statistically significant difference.

Results

Results of PDT for Central-Type ESLC

A total of 93 patients with 114 lesions of central-type ESLC who underwent PDT were examined. Thirteen synchronous lesions in six cases, 15 metachronous lesions in six cases, and 5 synchronous/metachronous lesions in one case were observed. The evaluation of the efficacy of PDT is shown in Table 1. CRs and PRs were obtained in 75 patients with 95 lesions (83.3%) and in 18 patients with 19 lesions (16.7%) out of 93 patients with 114 lesions. Each lesion with PR was subsequently treated with other modalities, including surgery in 13 cases, chemotherapy in 5 cases, or radiotherapy in 1 case, and finally achieved 100% CR. Recurrences after CR were recognized in 12 of 95 lesions (12.6%). The 114 lesions were classified in two groups according to the