Table 2. EGFR inhibitor activity against an $EGFR^{L858R/T790M}$-containing cell line (H1975)

Compound	Target	Clinical development	IC_{50} (nM)
CI-1033	EGFR	P-II	20
EKB-569	ERBB1,2	P-II	30
CL-387,785	EGFR	Research compound	51
SU-11464	EGFR	Research compound	500
ZD6474	VEGF2, EGFR	P-II	2000
GW572016	ERBB1,2,4	P-III	4000
Gefitinib	EGFR	Approved	7000
PKI-166	EGFR	P-II	8000
Erlotinib	EGFR	Approved	10000

Adapted from Carter et al.[42]

Shibata et al., using comparative genomic hybridizati on experiments, reported that the mutational status of the *EGFR* gene is significantly associated with the specific gain or loss of genetic material, including the amplification of the *EGFR* gene.[33] Mutation and amplification are probably both important in determining TKI sensitivity. To resolve this controversy, both *EGFR* mutations and amplification should be determined prospectively in future clinical trials.

Other parameters, such as the expression of phosphorylated AKT,[34] the amplification of *HER2*,[35] and the expression of EGFR protein,[31] are reported to affect sensitivity to EGFR-TKIs.

Resistance to gefitinib

Pao et al. first reported that lung cancers with *KRAS* mutations are resistant to EGFR-TKIs.[36] None of nine tumors with *KRAS* mutations responded to EGFR-TKIs.[36] As described previously, some tumors without *EGFR* mutations do respond to TKIs, but when these tumors harbor *KRAS* mutations, a tumor response to TKIs cannot be expected.

In contrast to the inherent resistance to gefitinib induced by *KRAS* mutations described above, it is common for patients to show progressive disease after presenting with an initial marked response to gefitinib. The mean duration of the response is about 3–7 months.[4,5] Most of these tumors have *EGFR* mutations that confer sensitivity to TKIs, such as exon 19 deletions and L858R, resulting in a good clinical response. However, the emergence of acquired resistance cannot be explained by the selection of tumor cells with wild-type *EGFR* genes, because their mutational status remains unchanged after they acquire resistance to TKIs. In February 2005, it was reported that a secondary mutation resulting in a threonine-to-methionine change at codon 790 is responsible for at least half the acquired resistance to gefitinib and erlotinib.[37,38] Crystal structure modeling has revealed that position T790 is located in the ATP-binding pocket of the catalytic region and appears to be critical for the binding of erlotinib and gefitinib. Substitution of the threonine at this codon with a bulkier residue, such as methionine, is thought to sterically hinder the binding of these two drugs.

In the case of chronic myeloid leukemia (CML), secondary mutations in the kinase domain of the *ABL1* gene are considered to be one of the mechanisms of acquired drug resistance to imatinib, a tyrosine kinase inhibitor specific for BCR–ABL1, KIT, and PDGFA.[39,40] The structural similarity between ABL1 and EGFR tyrosine kinases is fairly high, and the most common mutation related to acquired resistance is a threonine-to-isoleucine mutation at codon 315 (T315I) of ABL1, corresponding to T790M of EGFR. Reflecting this structural similarity, in 2003, before the discovery of the activating mutations of the *EGFR* gene in lung cancer, it was reported that artificially introduced T790M caused resistance to EGFR-specific 4-anilinoquinazoline inhibitors, including gefitinib and erlotinib[41]. In the case of CML, 20–30 other mutations of the *ABL1* gene, in addition to T315I, have been identified as mechanisms of acquired resistance to imatinib.[39] Although secondary *EGFR* mutations other than T790M are possible, only T790M has so far been detected in clinical samples.

To overcome acquired resistance, a new class of EGFR-TKIs is being developed that can be used as second-generation drugs. Carter et al. found that the EGFR inhibitors EKB-569 and CI-1033, but not GW-572016 and ZD-6474, potently inhibit EGFR (L858R, T790M) kinase (Table 2).[42] EKB-569 and CI-1033 are already in clinical trials.

TKIs and clinical trials

In four randomized trials comparing TKI plus platinum doublet and platinum doublet (i.e., INTACT 1 and 2 using gefitinib, and TALENT and TRIBUTE using erlotinib), the addition of TKI did not yield a survival advantage over platinum doublet. However, subgroup analysis in the TRIBUTE trial showed that the addition of erlotinib to carboplatin plus paclitaxel conferred an advantage in overall survival in patients who were never-smokers (MST 22.5 months vs 10.1 months for others; $P = 0.01$).[43]

In a randomized placebo-controlled trial (BR.21) to determine whether erlotinib prolongs survival in patients with NSCLC after the failure of first- or second-line chemotherapy, erlotinib significantly prolonged survival, with an MST of 6.7 months vs 4.7 months (hazard ratio 0.70; $P < 0.001$).[44] In contrast, a similar placebo-controlled randomized trial using gefitinib instead of erlotinib (ISEL trial)

failed to show an overall survival advantage in the gefitinib treatment group (MST of 5.6 months vs 5.1 months; $P = 0.087$).[45] However, gefitinib prolonged survival in never-smokers (MST 8.9 months vs 6.1 months; $P = 0.012$), as well as in Asian patients (MST 9.5 months vs 5.5 months; $P = 0.010$) in preplanned subset analyses.[45] Following these results, the U.S. Food and Drug Administration limits the indication of gefitinib to cancer patients who are currently benefiting or have previously benefited from gefitinib treatment, or are enrolled in clinical trials as of June 2005.

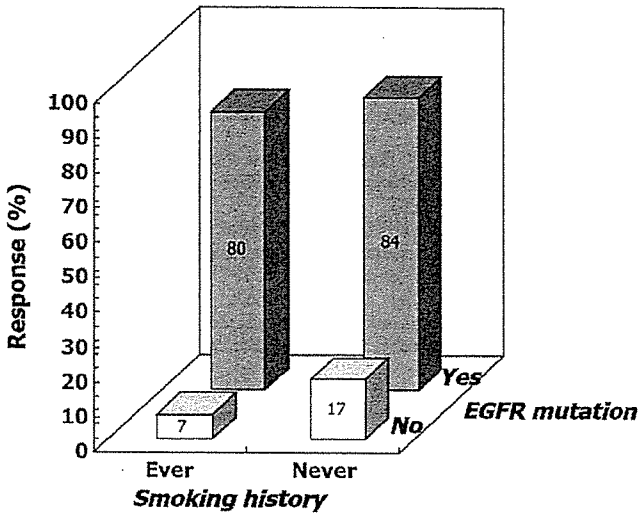

Fig. 7. Smoking history, *EGFR* mutation, and response to gefitinib (data from Mitsudomi et al.[23])

As has been described, EGFR-TKIs are not universally effective for lung cancer, but these drugs are effective in patients who have particular clinical or biological characteristics, e.g., Asian, nonsmoking female patients with adenocarcinomas with *EGFR* mutations. The different outcomes of the BR.21 and ISEL trials are at least partly attributable to differences in the degree of dilution in the two trials of patients with the abovementioned characteristics by those without such characteristics. Therefore, patients who would benefit from gefitinib therapy should be concentrated in future clinical trials. Smoking history and *EGFR* mutations are good predictors of response in patients treated with EGFR-TKIs. Which of these two markers should we use in future clinical trials? In our exploratory subset analysis, tumor response was observed in 16/19 patients with both *EGFR* mutations and no smoking history.[23] Whereas a response was seen in 1/6 never-smokers without *EGFR* mutations, a response was seen in 8/10 smokers with *EGFR* mutations.[23] Therefore, our limited experience indicates that *EGFR* mutations may be superior to smoking history in

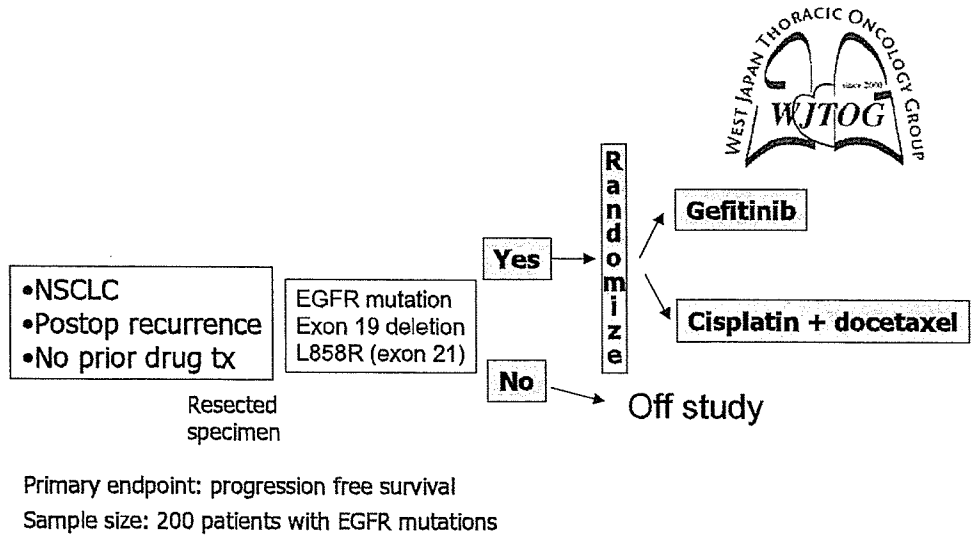

Fig. 8. Ongoing phase III trial comparing gefitinib monotherapy with cisplatin plus docetaxel in patients with recurrent disease after they had undergone pulmonary resection for non-small-cell lung cancer (*NSCLC*) (WJTOG3405)

Table 3. Results of phase III clinical trials involving EGFR-TKIs

TKI	Trial[Ref.]	Design	Result
G	INTACT I[49]	GP + G vs GP	Negative
G	INTACT II[50]	TC + G vs TC	Negative
E	TRIBUTE[43]	TC + E vs TC	Negative
E	TALENT	GP + E vs GP	Negative
E	BR.21[44]	E vs BSC	Positive
G	ISEL[51]	G vs BSC	Negative
G	S0023	PE/TRT → D → G vs PE/TRT → D	Terminated

G, gefitinib; E, erlotinib; GP, gemcitabine+cisplatin; TC, paclitaxel+cisplatin; BSC, best supportive care; PE, cisplatin+etoposide; D, docetaxel; TRT, thoracic radiotherapy

the selection of patients who would benefit from TKI treatment. Obviously, the detection of *EGFR* mutations requires laborious laboratory work. Hence, smoking history can be used in contexts in which *EGFR* gene testing is not readily available. In this way, the survival benefit of EGFR-TKIs, especially gefitinib, should be demonstrated in future clinical trials in a defined subset of patients with lung cancer. We, the West Japan Thoracic Oncology Group (WJTOG), have just launched a phase III clinical trial comparing gefitinib monotherapy with cisplatin plus docetaxel in lung cancer patients with *EGFR* mutations who have had recurrent disease after pulmonary surgery. The primary endpoint is progression-free survival and the sample size is 200 patients with *EGFR* mutations. To assure tumor specimens of good quality to avoid possible false negative results for mutation analyses, we decided to limit patients who had postoperative recurrence. We also limit our mutation search to deletions in exon 19 and L858R, because it would be less laborious and these two are most reliable predictor for response or survival. The primary endpoint is progression-free survival, to avoid confounding by possible crossover between two arms.

Conclusions

The development of EGFR-TKIs and the discovery of *EGFR* gene mutations have provided a great opportunity to develop individualized therapies for lung cancer. In Japan, a considerable fraction of patients undergoing gefitinib treatment suffer from fatal interstitial lung disease (ILD) (approximately 6% by prospective analysis). Pre-existing pulmonary fibrosis and smoking history are regarded as risk factors for ILD.[46] In this regard, it is also necessary to select patients who are likely to benefit from gefitinib therapy.

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PTEN and *PIK3CA* Expression Is Associated with Prolonged Survival after Gefitinib Treatment in *EGFR*-Mutated Lung Cancer Patients

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Background: We and other researchers have previously reported that pulmonary adenocarcinomas with epidermal growth factor receptor (EGFR) mutations are usually sensitive to gefitinib, an EGFR-specific tyrosine kinase inhibitor, although this relationship is not complete. In this study, we searched for mutations or changes in the expression of genes downstream to EGFR and evaluated their relationship with the effectiveness of gefitinib.

Methods: We studied 78 lung cancer patients who had recurrent disease after surgical resection and were treated with gefitinib. We searched for mutations occurring in the *KRAS* and *PIK3CA* gene. We also evaluated the expression level of EGFR, *PIK3CA*, and *PTEN* by real-time reverse transcriptase polymerase chain reaction. Gefitinib effectiveness was evaluated by imaging studies; a survival analysis was also done.

Results: We found seven (9%) somatic mutations in *KRAS* and two (2%) in *PIK3CA*. *EGFR* mutations were present in 44 (56%). *KRAS* mutations were found only in tumors without *EGFR* mutations, whereas *PIK3CA* mutation was found in tumors with *EGFR* mutation. Tumor response was assessable in 52 tumors. None of the six tumors with *KRAS* mutations responded to gefitinib treatment; however, two tumors with *PIK3CA* mutations showed partial response. Survival was significantly longer in patients with *EGFR* mutations or in those without *KRAS* mutations. In tumors with *EGFR* mutations, survival was longer in those with high *PIK3CA* or *PTEN* expression than in those with low expression of these molecules.

Conclusions: An evaluation of the *KRAS* mutation, as well as *PIK3CA* and *PTEN* expression, might help identify lung cancer patients who are most suitable for gefitinib treatment.

Key Words: Non-small cell lung carcinomas, Epidermal growth factor receptor, Gefitinib, Phosphatidylinositol 3′-kinase catalytic alpha, *PTEN*.

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Gefitinib (Iressa) is an orally bioavailable tyrosine kinase inhibitor (TKI) specific for epidermal growth factor receptor (EGFR). Recently, we, as well as others, have reported that activating mutations of EGFR are present in a subset of pulmonary adenocarcinomas and that tumors with EGFR mutations are highly sensitive to gefitinib.[1–7] However, about 10% of patients who exhibited a clear benefit from gefitinib treatment did not have an EGFR mutation, and about 20% of patients with an EGFR mutation did not respond to the gefitinib treatment [1–7].

After ligand binding, EGFR is autophosphorylated at several tyrosine residues. This autophosphorylation then turns on downstream signaling pathways, including the Ras/Raf/mitogen-activated protein kinase (MAPK) pathway and the phosphatidyl inositol 3′-kinase (PI3K)-Akt pathway. The MAPKs have been linked to cell proliferation and transformation in vitro.[8] The Akt pathway plays a critical role in facilitating cell survival.[9] Phosphatidylinositol 3′-kinase catalytic alpha (PIK3CA) positively regulates Akt, whereas PTEN negatively regulates Akt. Recently, PIK3CA has been reported to have somatic mutations in some colorectal, gastric, and lung cancers.[10] If alteration of PIK3CA or PTEN functionally activates Akt, then it is possible that these tumors would have a different response to gefitinib treatment. Indeed, expression of phosphorylated-Akt (p-Akt) has been reported to be predictive of response to gefitinib.[6,11]

In this study, we added 19 more patients to the 59 patients previously analyzed for EGFR mutations,[5] searched for mutations in the *KRAS* and *PIK3CA* genes, and quantitated messages of *EGFR*, *PIK3CA*, and *PTEN* by reverse transcriptase polymerase chain reaction (RT-PCR) in an attempt to confirm and extend our previous findings.[5]

PATIENTS AND METHODS

Patients and Gefitinib Treatment

From July 2002 through November 2004, 79 patients were treated with gefitinib for more than 3 weeks for recurrent disease after they had undergone pulmonary resection between 1997 and 2004. We studied 78 patients whose tumors were available for RNA extraction. There were 44 men and 34 women with a median age at operation of 61.9 (range, 39–80) years, and a median age at starting oral gefitinib treatment of 64.3 (range, 40–85) years. Forty-five

patients were former/current smokers, with a median pack-years (number of cigarette packs per day times years) of 40; the remaining 33 were never smokers. Sixty-eight patients had adenocarcinomas, six had squamous cell carcinomas, three had large cell carcinomas, and one had adenosquamous carcinoma. Twelve patients had stage IA disease, nine had stage IB, three had stage IIA, seven had stage IIB, 32 had stage IIIA, 10 had stage IIIB, and five had stage IV at the time of surgery. Forty-nine patients had received chemotherapy before gefitinib treatment (29, platinum doublet; 18, vinorelbine or gemcitabine monotherapy; two, oral uracil-tegafur [UFT]). Twenty patients did not receive any chemotherapy or radiotherapy before gefitinib treatment. Gefitinib 250 mg/day was started, and the patients were followed until February 2005, with a median duration of 121 (range, 23–939) days. In 61 patients, oral gefitinib was stopped due to progressive disease in 30, no change in four, regrowth after partial response in 16, and adverse reactions in 11. Response to gefitinib treatment was judged according to RECIST, except that confirmation at 4-week intervals was not necessarily mandated. Measurable disease was present in 52 of 78 tumors, and tumor response was evaluated in these subjects.

Mutation Analysis of Lung Cancer Specimens

After obtaining appropriate approval from the institutional review board and written informed consent from the patients, tumor samples were rapidly frozen in liquid nitrogen and stored at −80°C after resection. A surgical pathologist (Y.Y.) grossly dissected the frozen tumor specimens to enrich the tumor cell population as much as possible. Total RNA was isolated using the RNeasy kit (Qiagen, Valencia, CA). After cDNA was converted, an *EGFR* mutation at tyrosine kinase (exon 18-21) and a *KRAS* mutation at codons 12, 13, and 61 were searched for as described previously.[12] RT-PCR products were diluted and directly sequenced using the Big Dye Terminator v3.1/1.1 cycle sequencing kit (Applied Biosystems, Foster City, CA) according to the manufacturer's instructions. Sequencing products were electrophoresed on an ABI PRISM 3100 (Applied Biosystems). Both the forward and reverse sequences obtained were analyzed by BLAST, and chromatograms were manually reviewed.

Mutations of *PIK3CA* occurring in a whole open reading frame were searched. Primer sequences were designed according to GenBank (accession number NM 006218); these are available upon request. The RT-PCR conditions were one cycle of 95°C for 11 minutes, 45 cycles of 95°C for 35 seconds, 62°C for 35 seconds, 72°C for 55 seconds, followed by one cycle of 72°C for 10 minutes.

Expression Analysis of Lung Cancer Specimens

Expression of *EGFR*, *PIK3CA*, and *PTEN* genes was quantitated using the SYBR Green method (QuantiTect SYBR Green PCR Kit; Qiagen) on ABI7900HT (Applied Biosystems). Primer sequences are available upon request. Quantitation was performed in duplicate, and the expression level of 18S ribosomal RNA (rRNA) was used as an internal control. Expression values of each gene were used as relative values compared with control sample (RERF-LC-AI, adenocarcinoma cell line).

Statistical Analysis

To compare proportions, the χ^2, t, or Kruskal-Wallis test was used. The Kaplan-Meier method was used to estimate the probability of survival as a function of time, and survival differences were analyzed by the log-rank test. The two-sided significance level was set at $p < 0.05$. The Cox proportional hazards modeling technique was applied for univariate or multivariate analysis of the overall survival. All analyses were performed using StatView (Version 5, SAS Institute Inc., Cary, NC) software.

RESULTS

Mutations of *KRAS*, *PIK3CA*, and *EGFR* Gene

KRAS mutations at codon 12 (three G12C and one G12A, G12D, G12S, and G12V each) were found in seven patients. *PIK3CA* mutations occurring at codon 545 (E545K) in exon 9 were found in two patients (2.5%). These two patients were females who had never smoked and who had adenocarcinomas harboring an EGFR mutation (L858R). Of 78 patients, EGFR mutations were found in 44 (56%): two point mutations in exon 18 (G719A, G719C), 23 deletions in exon 19, two insertions in exon 19 (I744KIPVAI), one point mutation in exon 20 (S768I), and 16 point mutations in exon 21 (15 L858R, one L861Q). In three patients, there was a second mutation (L858R + A871G, L858R + E709G, G719C + E709H). Of the 44 patients with EGFR mutations, 23 were female, 23 were never smokers, and 43 had an adenocarcinoma. As reported previously, EGFR mutations and *KRAS* mutations were mutually exclusive.[12]

Expression Analysis of *EGFR*, *PIK3CA*, and *PTEN* Gene

Real-time RT-PCR analyses were performed for the *EGFR*, *PIK3CA*, and *PTEN* genes. Quantitation was performed in duplicate, and correlations between these two assays were high (R^2 were 0.89 for rRNA, 0.95 for *EGFR*, 0.92 for *PIK3CA*, and 0.89 for *PTEN*; Pearson's test). EGFR expression was significantly higher in tumors with *EGFR* mutations than in those without *EGFR* mutations; the relative value of the mutation group was 1.876 times that of the wild types ($p = 0.0075$; t test). However, the expressions of *PIK3CA* and *PTEN* were not associated with EGFR mutation; the expression ratio of *PIK3CA* between EGFR mutations and the wild types was 0.889 ($p = 0.5911$), whereas the expression ratio of *PTEN* between *EGFR* mutations and the wild types was 0.954 ($p = 0.8481$).

Relationship between Response and Mutations

Of 52 patients with measurable disease, 24 showed partial response (PR), 19 showed progressive disease (PD), and the remaining nine patients exhibited stable disease (SD). Of the 27 patients with EGFR mutation, 22 (81%) patients showed PR, three SD, and two PD. However, in the 25 patients without EGFR mutations, only two patients (8%) showed PR, six SD, and 17 PD (Table 1). In contrast, none of six tumors with *KRAS* mutations responded to gefitinib treatment (Table 1).

TABLE 1. Relationship between Gene Mutation/Expression and Gefitinib Effectiveness

Gene	Category	Imaging evaluation				
		Total	PR	SD	PD	p^a
EGFR	Wild type	25	2	6	17	<0.0001
	Mutation	27	22	3	2	
EGFR mutation	L858R	8	6	1	1	0.3261
	Deletion	17	15	2	0	
KRAS	Wild type	46	24	8	14	0.0274
	Mutation	6	0	1	5	
PIK3CA	Wild type	50	22	9	19	0.2972
	Mutation	2	2	0	0	
EGFR	High expression	26	18	2	6	0.0034
	Low expression	26	6	7	13	
PIK3CA	High expression	26	13	4	9	0.8477
	Low expression	26	11	5	10	
PTEN	High expression	26	11	5	10	0.8477
	Low expression	26	13	4	9	

[a] $p = \chi^2$ test.
PR, partial response; SD, stable disease PD, progressive disease.

There were only two patients with a *PIK3CA* mutation, and they also had an *EGFR* mutation (L858R). These two patients showed PR. Due to a limited number of *PIK3CA* mutations, it was difficult to evaluate the isolated effect of a *PIK3CA* mutation on tumor response.

Relationship between Response and Expressions

When the patients were divided into two groups according to the median value of gene expression, tumors with high EGFR expression were significantly more responsive to gefitinib than those with low EGFR expression (18 cases of 26 high expressions showed PR, and 13 cases of 26 low expressions showed PD, $p = 0.0034$; χ^2 test) (Table 1). When we compared to the groups as mean expression value of EGFR, expression in PR group was significantly higher than that in SD or PD groups (Table 2). On the other hand, expression of *PIK3CA* or *PTEN* did not affect the response rate.

TABLE 2. Relationship between Average of Gene Expressions and Gefitinib Effectiveness

Gene	Imaging evaluation			
	PR (n = 24)	SD (n = 9)	PD (n = 19)	p^a
EGFR	1.917 ± 1.467	1.027 ± 1.553	0.977 ± 0.855	0.0053
PIK3CA	0.415 ± 0.322	0.465 ± 0.409	0.536 ± 0.640	0.9332
PTEN	0.248 ± 0.296	0.278 ± 0.281	0.252 ± 0.240	0.7415

[a] p = Kruskal-Wallis test.
PR, partial response; SD, stable disease PD, progressive disease.

Effect of Molecular Alterations on Patient Survival after Gefitinib Treatment

Patients with *KRAS* mutations survived for a significantly shorter time from the day of starting gefitinib than those without *KRAS* mutations ($p = 0.0323$, Fig. 1B). We also confirmed the favorable effect of *EGFR* mutations on patient survival as reported previously ($p = 0.0040$; log-rank test, Fig. 1A).

On the other hand, while *EGFR* expression did not appear to affect survival time (Fig. 2A), high expressions of *PIK3CA* and *PTEN* were associated with prolonged survival (Fig. 2B and C). Of interest, when effects of *EGFR* mutations with or without high expression of *PIK3CA* or *PTEN* were simultaneously analyzed, only patients in the group with *EGFR* mutations and high expression of *PIK3CA* or *PTEN* showed longer survival (*PIK3CA*: $p = 0.0071$, *PTEN*: $p = 0.0614$; log-rank test; Fig. 3A and B).

Because a cutoff line of gene expression is hard to determine, univariate analyses of sequential expression data were also performed to assess the correlations among expressions and clinical outcome. High expression of each gene was associated with prolonged survival; hazard ratio (HR) of *EGFR* was 0.797 ($p = 0.1115$), HR of *PIK3CA* was 0.367 ($p = 0.0946$), and HR of *PTEN* was 0.108 ($p = 0.0685$) (Table 3). However, Cox's proportional hazards model failed to show an independent contribution of these variables to overall survival (data not shown).

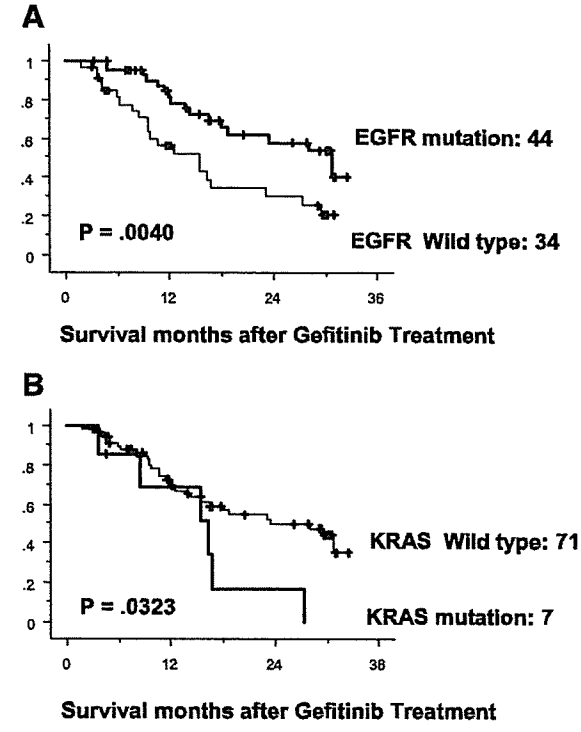

FIGURE 1. Effect of *EGFR* mutations (*A*) and *KRAS* mutations (*B*) on survival of patients who received gefitinib treatment.

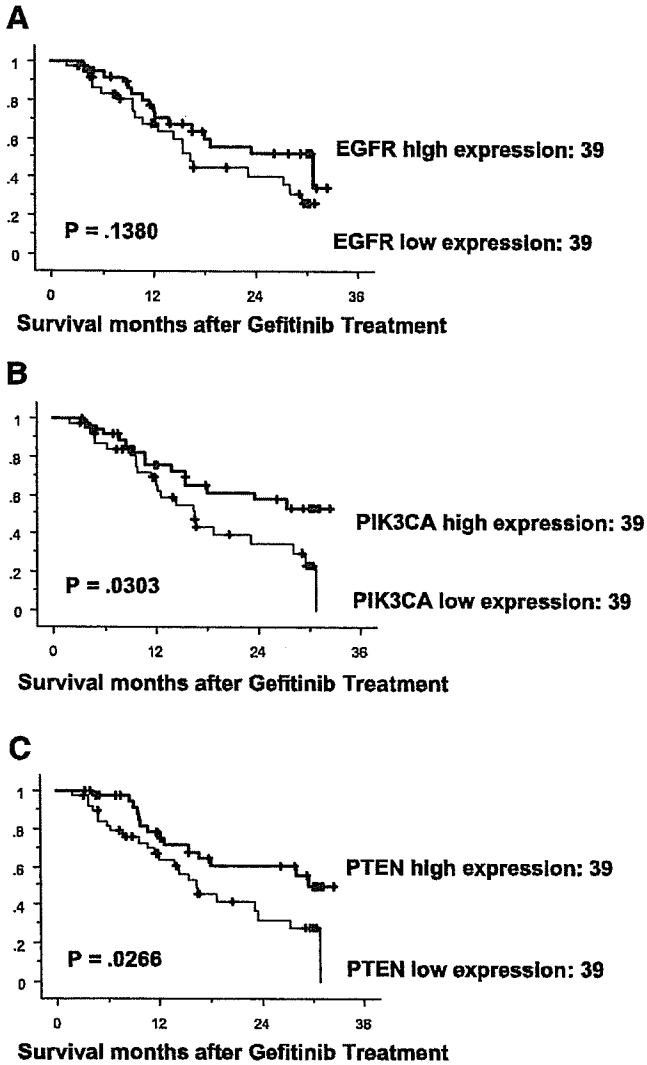

FIGURE 2. Effect of expression of *EGFR* (*A*), *PIK3CA* (*B*), and PTEN (C) gene expression status on survival of patients who received gefitinib treatment. Cutoff values of expression were median (i.e., 0.915 for *EGFR*, 0.324 for *PIK3CA*, and 0.152 for *PTEN*).

DISCUSSION

We showed that, in addition to EGFR mutations, mutations of the KRAS gene, as well as high expression of *PIK3CA* and *PTEN* genes, had a significant impact on the survival of lung cancer patients after gefitinib treatment. We were able to confirm the recent report by Pao et al.[14] that lung adenocarcinomas with *KRAS* mutation are resistant to gefitinib treatment and to further extend their observation by showing that patients with a *KRAS* mutation had a significantly poorer survival, as well as a finding in a phase III trial of erlotinib, another EGFR-TKI (TRIBUTE).[15]

The role of EGFR expression in predicting gefitinib treatment has been denied in earlier studies.[16,17] However, several investigators have recently reported on the importance of EGFR amplification or overexpression. Cappuzzo et al.[18] recently reported that *EGFR* gene amplification and expression as determined by immunohistochemistry were significantly associated with prolonged survival. Tsao et al.[19] also reported that the expression and amplification of EGFR was associated with an objective response or survival in a phase III trial of erlotinib versus placebo. However, we did not observe a significant contribution of EGFR expression to prolonged survival, although we used a different RT-PCR than that used by previous investigators.

Sordella et al.[20] reported that the PIK3CA-AKT or the STAT pathway is activated by EGFR mutations, whereas the RAS-MAPK pathway is not. In line with this observation, phosphorylated Akt but not MAPK was reported to be significantly associated with gefitinib activity.[11] Han et al.[21] also reported that intense nuclear staining of p-Akt (Ser473) was associated with prolonged time to progression and overall survival. Because *PIK3CA* and *PTEN* regulate phosphorylation of Akt, we hypothesized that alteration of these genes might have some relevance to the response seen with gefitinib treatment.

The *PIK3CA* gene is somatically mutated in several types of human cancer. Although mutations were relatively common in colorectal cancer (32%),[10] breast cancer (26%–40%),[13,22] hepatocellular carcinoma (35%),[13] and some ovarian cancers (20%),[22] it is rare in NSCLC (1%–4%).[10,13] Although we detected *PIK3CA* mutation at a similar frequency in our cohort, *PIK3CA* did not appear to affect sensitivity to gefitinib. Interestingly, there was no mutually exclusive relationship with *EGFR* mutations, as was seen in the *KRAS* gene. However, we found that high expression of *PIK3CA* significantly correlated with overall survival, especially in patients with an *EGFR* mutation. Tumors constitutively activated by an *EGFR* mutation and additional PI3K overexpression might be more sensitive to gefitinib. The mechanism of high *PIK3CA* expression might be attributable to the amplification of 3q23-29 where the *PIK3CA* gene is located because this region is amplified in 12 of 19 NSCLC cell lines (63%) by comparative genomic hybridization (CGH).[23]

In NSCLC, although mutation or methylation of *PTEN* was reported to be rare, high expression of *PTEN* has been shown to be a significantly favorable prognostic marker.[24] In vitro assay has proven that gefitinib-resistant cell lines have reduced *PTEN* expression compared to parental PC9 cell line that had an *EGFR* mutation (in-frame deletion).[25] Breast cancer cell lines lacking PTEN function have elevated Akt activity and are resistant to ZD1839 (gefitinib). Reconstitution of PTEN function decreases Akt activity and can reestablish sensitivity to ZD1839.[26] In glioblastoma, PTEN was also shown to be a key molecule in the PI3K pathway.[27] We found that PTEN expression did not correlate with the response to gefitinib. The response was merely a surrogate for the overall effect on survival. Indeed, we found that longer survival was only seen in patients with high expression of PTEN, even though they had *EGFR* mutations.

In conclusion, *EGFR* mutations are generally associated with a positive response to gefitinib treatment, as we and others have previously reported. In patients without *EGFR*

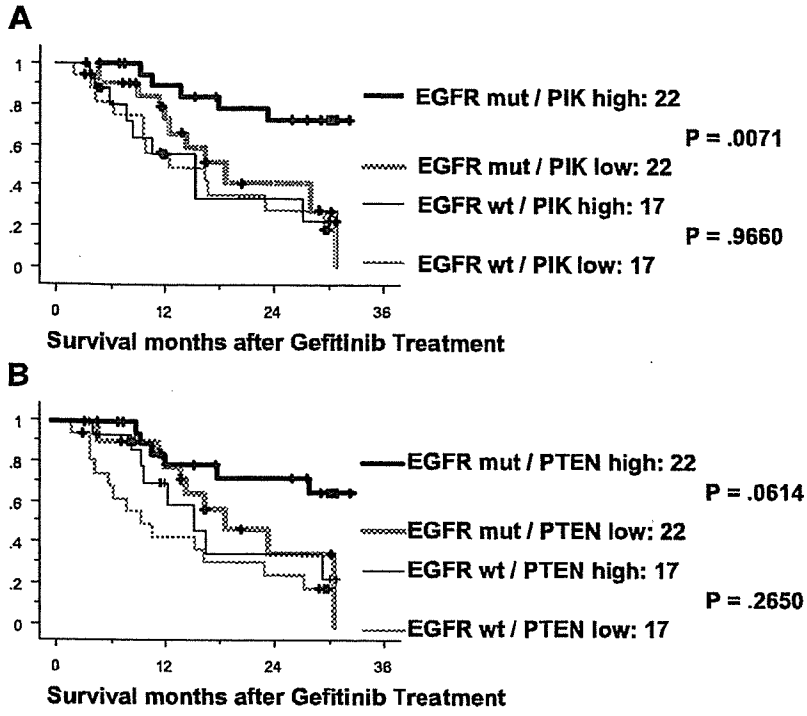

FIGURE 3. Combined effect of *EGFR* mutation and *PIK3CA* expression (*A*) or *EGFR* mutation and *PTEN* expression (*B*) on survival of patients who received gefitinib treatment.

TABLE 3. Univariate Analysis of Overall Survival Using the Cox Proportional Hazards Model

Variable	HR	95% CI	*p*
EGFR wild/mutant[a]	2.526	1.314–4.858	0.0055
KRAS mutant/wild[a]	2.542	1.048–6.168	0.0390
EGFR expression[b]	0.797	0.603–1.054	0.1115
PIK3CA expression[b]	0.367	0.113–1.189	0.0946
PTEN expression[b]	0.108	0.010–1.184	0.0685

[a] Split data (nominal variable).
[b] Sequential data (continuous variable).

mutations, tumors with *KRAS* mutations clearly identify patients who do not benefit from gefitinib treatment. Among patients with *EGFR* mutations, those with high expression of *PIK3CA* and *PTEN* survive for a longer period. Thus, evaluating *KRAS* mutations, *PIK3CA* and *PTEN* expressions, in addition to *EGFR* mutations, might help identify lung cancer patients who are most suitable for gefitinib treatment.

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Pleural Lavage Cytology Before and After Lung Resection in Non-Small Cell Lung Cancer Patients

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Background. The aim of this study was to analyze on a multivariate basis the prognostic significance of pre-resection and post-resection pleural lavage cytologies in surgically resected primary non-small cell lung cancer (NSCLC) patients, in relation to pathologic TNM factors in a large cohort of almost 1,200 patients.

Methods. From August 1992 through March 2001, pleural lavage cytology (PLC) was performed in 1,214 NSCLC patients without pleural effusion or dissemination undergoing pulmonary resection. The cytologic evaluation was classified into three categories: negative, suggestive, and positive. To investigate the impact on patient survival, PLC results were analyzed with conventional clinicopathologic factors.

Results. Definitive pre-resection PLC result was obtained in 1,194 patients and 38 had a positive result. The 5-year survival rates were 27% if pre-resection PLC was positive and 71% if negative. Of 1,198 patients 54 had a positive post-resection PLC result. The 5-year survival rates were 10% if post-resection PLC was positive and 73% if negative. On multivariate analysis, post-resection PLC was an independent prognostic factor as significant as established clinicopathologic factors.

Conclusions. Pre-resection and post-resection PLC should be recognized as an essential prognostic factor and should be performed in NSCLC patients without pleural effusion and dissemination. Post-PLC, compared with pre-PLC, had a greater and independent impact on survival and needs to be incorporated in the pathologic staging of NSCLC in the future.

(Ann Thorac Surg 2006;81:298–304)

Pleural lavage cytology (PLC) has been reported to be a possible prognostic factor in patients with resected non-small cell lung cancer (NSCLC). However, many of the reports are that only PLC immediately after thoracotomy, before lung resection, (pre-PLC) has been studied in detail. The pre-PLC impact on patient outcome has been studied, chiefly, on a univariate basis and has not been studied in relation to the conventional pathologic TNM by multivariate analysis. Although pre-PLC has been reported to be a poor prognosis predictor, a positive result is currently not recognized as equivalent to T4 or a factor indicating incomplete resection. Although PLC after radical NSCLC resection, before chest closure, (post-PLC) has also been studied, significance of post-PLC remains controversial. Higashiyama and associates [1] performed pre-PLC and post-PLC in 325 lung cancer patients, but neither pre-PLC nor post-PLC results were an independent prognostic factor. Dresler and associates [2], who reported the pre-PLC and post-PLC analysis in 137 patients, stated that the 3-year survival rate was significantly better in negative post-PLC patients than in positive patients. We thought further analyses on post-PLC were needed. In the present study, we analyzed both pre-PLC and post-PLC on a multivariate basis, in relation to pathologic TNM factors in a large cohort of almost 1,200 patients.

Material and Methods

From August 1992 through March 2001, a total of 1,387 patients underwent surgical resection for primary NSCLC at the National Cancer Center Hospital East. Intraoperative PLC, which was approved for this observational study by the institutional review board, was prospectively performed in all patients without pleural effusion and dissemination, totaling 1,214 patients, and all were enrolled in this study. As the largest sample size for PLC study was 1,000 before this study, we aimed at accruing well more than 1,000 patients before analysis. Preoperative evaluation included a detailed history, physical examination, bronchoscopy, contrast-enhanced computed tomography (CT) of the chest, and distant metastasis screening (bone, brain, liver, and adrenals). Histologic typing was determined according to the World Health Organization classification [3]. Disease stages were determined based on the TNM classification of the International Union Against Cancer [4]. Immediately after

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thoracotomy, the pleural cavity was carefully washed with 500 mL physiologic saline before any pulmonary parenchyma manipulation. A sample of 50 mL was retrieved for cytologic evaluation (pre-PLC). We performed lung resection (segmentectomy or greater) and complete mediastinal lymph node dissection in 1,199 patients, and lung resection and mediastinal lymph node sampling in 15 patients. Before chest closure, a pleural cavity lavage sample was also retrieved (post-PLC) in the same fashion as pre-PLC. Samples were centrifuged at 1,500 rpm for 5 minutes. The sediment was stained using Papanicolaou's methods. A single cytologist blinded to the clinical-pathologic information evaluated the specimen and classified it into three categories: Papanicolaou classes I and II as negative, class III as suggestive, and classes IV and V as positive. In the survival analyses, we studied only cases with definitive cytologic diagnoses, excluding Papanicolaou class III. To investigate the impact on patient survival, the following conventional clinicopathologic factors were reviewed and analyzed: age, gender, smoking index (< 400 vs ≥ 400), serum carcinoembryonic antigen (CEA) level (< 5.0 mg/mL vs ≥ 5.0 mg/mL), clinical T factor (cT: cT2-4 vs cT1), clinical lymph node status (cN: mediastinal node involvement as cN2 vs less extensive as cN0-1), histologic type of tumor (adenocarcinoma versus others), pleural involvement of surgical (sP0-1 vs sP2-3) and pathologic finding (p0 vs p1-3), lymphatic invasion (positive versus negative), vascular invasion (positive versus negative), pathologic N status (pN: pN2-3 vs pN0-1), degree of fibrotic scarring (scar grade 1-2 vs grade 3-4), nuclear atypia (grade 1 or 2 vs grade 3), mitotic activity (mitotic index 1 or 2 vs 3), and surgical resection completeness (incomplete versus complete). Complete resection was defined as negative surgical margin and no highest mediastinal lymph node involvement. Incomplete resection was defined as positive surgical margin or highest mediastinal lymph node involvement. The smoking index was defined as the product of the number of cigarettes smoked per day and the number of years of smoking. We defined cN2 as mediastinal lymph node(s) greater than 1.0 cm in the shortest dimension on preoperative conventional CT. Pleural involvement was classified according to the Japan Lung Cancer Society criteria: p0; tumor did not extend beyond the elastic pleural layer, p1; tumor invaded the visceral pleura elastic layer but was not exposed on the pleural surface, p2; tumor was exposed on the pleural surface and p3; tumor invaded the parietal pleura or chest wall. Surgeons determined pleural involvement (sP factor) macroscopically before resection. Pathologic pleural involvement (p factor) were diagnosed on the resected specimens by a single pathologist blinded to the surgeons' findings [5]. Lymphatic invasion and vascular invasion indicated tumor cells identifiable in the lymphatic and vascular vessel lumen, respectively. Scar grade was classified into 4 grades: grade 1; tumor had foci of alveolar collapse with resulting condensation of elastic fibers but no or minimal fibroblastic tissue with collagen, grade 2; tumor had fibroblastic tissue with a small amount of collagen fibers, grade 3; tumor had fibroblastic tissue with moderate or abundant amount of collagen fibers, and grade 4; tumor showed hyalinization [6]. Nuclear atypia categorization was based on the most atypical nuclei on sections and divided into 3 grades as follows: grade1; nuclei that were uniform in size and equal to or only slightly larger than those of reactive type II alveolar epithelial

Table 1. Patient Characteristics ($n = 1,214$)

Characteristics	Results	
Gender		
Male	781	(64)
Female	433	(36)
Histology		
Adenocarcinoma	792	(65)
Squamous cell carcinoma	284	(23)
Others	138	(12)
Clinical T factor		
T1	593	(49)
T2	490	(40)
T3	111	(9)
T4	20	(2)
Clinical N factor		
N0	1,005	(83)
N1	116	(10)
N2	92	(8)
N3	1	(<1)
Clinical stage		
IA	550	(45)
IB	376	(31)
IIA	17	(1)
IIB	129	(11)
IIIA	113	(9)
IIIB	24	(2)
IV	5	(<1)
Pathologic T factor		
T1	543	(45)
T2	434	(36)
T3	126	(10)
T4	111	(9)
Pathologic N factor		
N0	801	(66)
N1	204	(17)
N2	202	(17)
N3	7	(1)
Pathologic stage		
IA	438	(36)
IB	256	(21)
IIA	51	(4)
IIB	147	(12)
IIIA	196	(16)
IIIB	113	(9)
IV	13	(1)

(Numbers in parentheses are percentages)

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Table 2. Pre-PLC Result and Clinicopathologic Characteristics

Factors	Pre-PLC (n = 1,194)		P Value
	Positive (n = 38)	Negative (n = 1,156)	
Age	63	63	0.740
Gender			
Male	25	746	
Female	13	410	0.873
Treatment modality (resection type)			
Lobectomy	34	1,049	
Pneumonectomy	1	64	0.177
Limited resection	3	43	(limited resection vs others)
Pathologic stage			
I	16	667	
II	3	193	0.056
III	19	283	(stage I vs others)
IV	0	13	
Histology			
Adenocarcinoma	26	751	
Squamous cell carcinoma	6	274	0.660
Large cell carcinoma	3	47	(adenocarcinoma vs others)
Other	3	84	
Pathologic pleural involvement			
p0	11	754	
p1–3	27	402	<0.001
Pathologic N status			
N0	17	774	
N1–3	21	382	0.041
Lymphatic invasion			
Positive	27	481	
Negative	11	675	<0.001
Vascular invasion			
Positive	30	633	
Negative	8	523	0.003
Resection completeness			
Complete	28	1,067	
Incomplete	10	89	<0.001
Scar grade			
1–2	0	191	
3–4	35	844	0.001
NA	3	121	
Nuclear atypia			
1–2	15	432	
3	20	607	0.863
NA	3	117	
Mitotic index			
1–2	26	813	
3	9	226	0.539
NA	3	117	

NA = data not available.

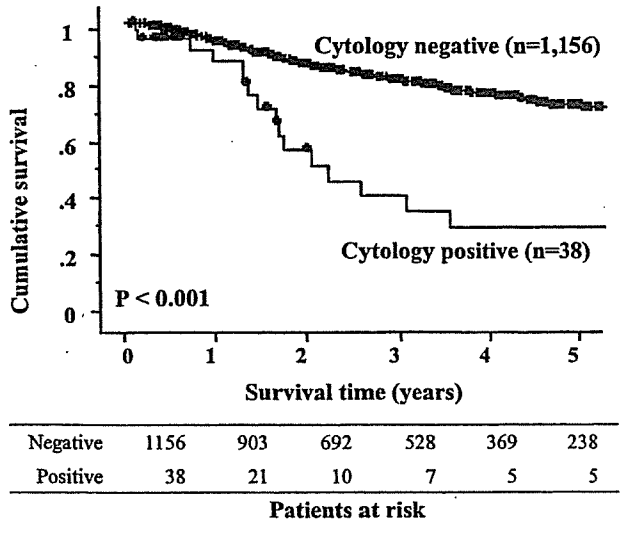
cells, grade 2; nuclei that were uniform in size and up to twice the size of those of reactive type II alveolar epithelial cells, and grade 3; presence of giant tumor cells. Mitotic index was classified into three grades based on the findings of several sections: index 1; up to 5 mitotic cells per 10 high-power fields (HPF), index 2; 6–15 mitotic cells per 10 HPF, and index 3; greater than 15 mitotic cells per 10 HPF [7]. The length of survival was defined as the interval in months between the day of surgical intervention and the date of death due to

Table 3. Post-PLC Results and Clinicopathologic Characteristics

Factors	Post-PLC (n = 1,182)		p Value
	Positive (n = 54)	Negative (n = 1,128)	
Age	61	63	0.363
Gender			
Male	37	725	
Female	17	403	0.524
Treatment modality (resection type)			
Lobectomy	48	1,026	
Pneumonectomy	2	63	0.129
Limited resection	4	39	(limited resection vs others)
Pathologic stage			
I	7	673	
II	3	191	<0.001
III	42	253	(stage I vs others)
IV	2	11	
Histology			
Adenocarcinoma	41	731	
Squamous cell carcinoma	4	270	0.094
Large cell carcinoma	4	46	(adenocarcinoma vs others)
Other	5	81	
Pathologic pleural involvement			
p0	26	732	
p1–3	28	396	0.019
Pathologic N status			
N0	10	776	
N1–3	44	352	<0.001
Lymphatic invasion			
Positive	43	463	
Negative	11	665	<0.001
Vascular invasion			
Positive	41	614	
Negative	13	514	0.002
Resection completeness			
Complete	25	1,001	
Incomplete	29	127	<0.001
Scar grade			
1–2	3	186	
3–4	47	821	0.022
NA	4	121	
Nuclear atypia			
1–2	18	423	
3	32	588	0.465
NA	4	117	
Mitotic index			
1–2	42	790	
3	8	221	0.382
NA	4	117	

NA = data not available.

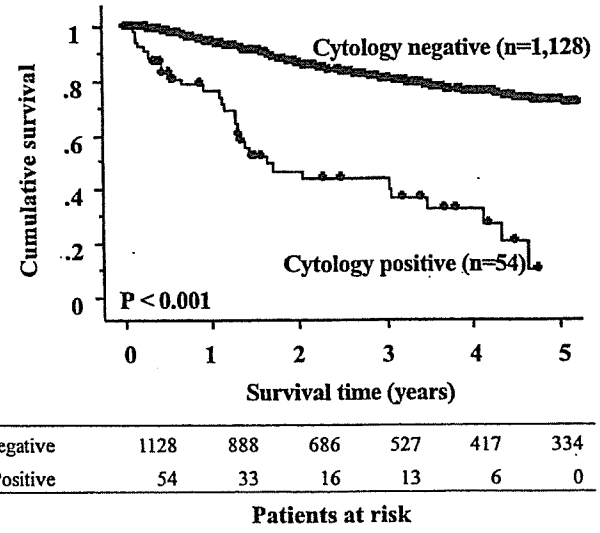
any cause or the last follow-up. An observation was censored at the last follow-up when the patient was alive or lost to follow-up. The survival rates were calculated by the Kaplan-Meier method [8] and univariate analyses were performed by means of the log-rank test. Multivariate analyses were performed using the Cox proportional hazards model [9]. Forward and backward stepwise procedures were used to determine the combination of prognostic factors (StatView: version 5.0; SAS Institute, Inc, Cary, NC). A *p*



Negative	1156	903	692	528	369	238
Positive	38	21	10	7	5	5

Patients at risk

Fig 1. Survival curves of patients according to pre-PLC results. The 5-year survival rate was 27% for positive pre-PLC patients and was significantly worse (71%) for negative pre-PLC patients. The crosses indicate censored cases at the respective points. (PLC = pleural lavage cytology.)



Negative	1128	888	686	527	417	334
Positive	54	33	16	13	6	0

Patients at risk

Fig 2. Survival curves of patients according to post-PLC results. The 5-year survival rate was 10% for positive post-PLC patients and was significantly worse (73%) for negative post-PLC patients. The crosses indicate censored cases at the respective points. (PLC = pleural lavage cytology.)

value less than 0.05 was taken to indicate a statistical significance.

Results

Patient clinicopathologic characteristics are shown in Table 1. There were 781 men and 433 women. Their ages ranged from 22 to 89, with a median of 65 years. Clinicopathologic characteristics for pre-PLC and post-PLC are shown in Tables 2 and 3, respectively. For pre-PLC, definitive cytologic results were obtained in 1,194 patients, with a positive result in 38 (3.2%). Univariate analyses revealed significant differences between pre-PLC positive and negative patients in pathologic pleural involvement, pathologic N status, lymphatic permeation, vascular invasion, resection completeness, and scar grade. For post-PLC, definitive cytologic result was obtained in 1,182 patients, 54 (4.6%) of which showed a positive result. Significant differences were observed in pathologic stage, pathologic pleural involvement, pathologic N status, lymphatic permeation, vascular invasion, resection completeness, and scar grade between post-PLC positive and negative patients. The 5-year survival rate was 27% for positive pre-PLC patients, which was significantly worse than 71% for negative pre-PLC patients (Fig 1). The 10% 5-year survival rate for positive post-PLC patients was significantly worse 73% for negative post-PLC patients (Fig. 2).

Five-year survival rates for patients with negative pre-PLC and post-PLC (n = 1,094), positive pre-PLC and negative post-PLC (n = 21), negative pre-PLC and positive post-PLC (n = 37), and positive pre-PLC and positive post-PLC (n = 13) were 81, 50, 12, and 0%, respectively. Multivariate analyses revealed 6 independent prognostic factors when only factors available before lung resection were analyzed (Table 4): age, CEA level, cT factor, cN factor, sP factor, and pre-PLC result. When factors available after postoperative pathologic evaluation were included in multivariate analyses, however, 10 independent prognostic factors were recognized, but pre-PLC result was not (Table 5): Age, CEA level, cT factor, pT factor, pN factor, p factor, lymphatic invasion, vascular invasion, resection completeness, and post-PLC result.

Comment

The first report on PLC was in 1958 by Spjut and associates [10]. They reported the results of post-PLC in 49 patients with lung cancer undergoing surgical resection. The cytologic results were positive for malignant cells in 16 (33%) of them, but outcomes were not analyzed. In 1984, Eagan and colleagues [11] reported positive post-PLC in 12 (8.9%) of 135 patients. Lung cancer recurred in nine of the 12 patients, with only two in the

Table 4. Multivariate Analysis Results for Prognostic Factors Available Before Lung Resection

Variable	Hazard Ratio (95% CI)	*p* Value
Age	1.020 (1.006–1.035)	0.005
Gender	0.958 (0.638–1.436)	0.833
Smoking (S.I > 400)	0.963 (0.648–1.433)	0.853
CEA	1.732 (1.320–2.272)	<0.001
cT factor (2–4 vs 1)	0.624 (0.475–0.814)	0.002
cN factor (1–3 vs 0)	0.512 (0.379–0.691)	<0.001
sP factor (2–3 vs 1–2)	0.621 (0.475–0.814)	<0.001
Pre-PLC	2.980 (1.683–5.277)	<0.001

CEA = serum carcinoembryonic antigen; CI = confidence interval; PLC = pleural lavage cytology; S.I = smoking index.

Table 5. Multivariate Analysis Results Including Factors Available After Lung Resection

Variable	Hazard Ratio (95% CI)	*p* Value
Age	1.021 (1.006–1.037)	0.006
CEA	1.301 (0.970–1.744)	0.079
cT factor (2–4 vs 1)	0.971 (1.411–2.051)	0.071
cN factor (1–3 vs 0)	0.951 (0.652–1.388)	0.796
sP factor (1–3 vs 0)	1.244 (0.834–1.856)	0.284
pT factor (2–4 vs 1)	1.285 (1.181–1.399)	<0.001
pN factor (1–3 vs 0)	0.446 (0.316–0.629)	<0.001
p factor (1–3 vs 0)	0.726 (0.527–1.001)	0.050
Histology (Ad.[a] vs others)	1.100 (0.769–1.573)	0.602
Lymphatic invasion	1.495 (1.058–2.114)	0.023
Vascular invasion	2.161 (1.410–3.311)	<0.001
Scar grade (3–4 vs 1–2)	0.792 (0.453–1.383)	0.412
Nuclear atypia (3 vs 1–2)	0.634 (0.447–0.898)	0.010
Mitotic index (3 vs 1–2)	0.875 (0.617–1.239)	0.452
Resection completeness	0.676 (0.472–0.968)	0.033
Pre-PLC	1.833 (0.949–3.541)	0.071
Post-PLC	1.803 (1.077–3.018)	0.024

Ad.[a] = adenocarcinoma; CEA = serum carcinoembryonic antigen; PLC = pleural lavage cytology.

ipsilateral pleural space. Eight patients died of lung cancer, one recurring locally and seven having distant metastases. They concluded the prognostic role of PLC needed further study. The first report on pre-PLC was by Kondo and associates in 1989 [12], followed by their expanded result analyses in 1993 [13]. They reported that 42 (9.0%) of 467 lung cancer patients undergoing surgery with little or no pleural effusion had a positive pre-PLC result. The 3-year survival rates of the patients with negative and positive cytology results were 68.7% and 22.9%, respectively. The prognosis of the positive cytology group was as poor as that of stage IIIB or IV patients. They concluded that pre-PLC was an important prognostic factor, indicating microscopic cancer cell exfoliation into the pleural cavity and subclinical malignant pleural effusion. Okada and associates [14] reported, based on 1,000 patients in 2003, that 45 (4.5%) patients had positive pre-PLC findings. Positive cytologic findings were observed more frequently in patients with adenocarcinoma, advanced stage, extended lymph node involvement, pleural involvement, lymphatic invasion, vascular invasion, high serum CEA level, and male gender. The survival rate at 5 years was 28% in patients with a positive result and 67% in negative patients ($p < 0.001$). Multivariate analysis demonstrated that pre-PLC was an independent prognostic determinant ($p = 0.0290$). Higashiyama and associates [1] performed pre-PLC and post-PLC in 325 lung cancer patients without malignant pleurisy. Positive post-PLC patients especially with adenocarcinoma resulted in a poor outcome. The survival rate at 5 years was 71% in 250 patients with negative pre-PLC and post-PLC results, while it was 33% in 19 patients with positive results. However, in multivariate analyses, neither pre-PLC nor post-PLC result was an independent prognostic factor in their study. Dresler and associates [3] reported the pre-PLC and post-PLC analysis in 137 patients in 1999. The 3-year survival rates of the patients with negative and positive pre-PLC results were 55% and 0%, respectively ($p = 0.088$). The 3-year survival rates of the patients with negative and positive post-PLC results were 50% and 0%, respectively ($p < 0.04$). In the present study, we analyzed both pre-PLC and post-PLC in almost 1,200 patients, the largest cohort ever studied with regard to PLC. Both pre-PLC and post PLC were analyzed in a multivariable setting, together with conventional significant clinicopathologic prognostic factors we reported previously [15]. Although our study yielded results similar to previous studies and post-PLC proved to be an important prognostic predictor, we found no difference in PLC results in relation to histologic characteristics. There have been a considerable number of reports concluding positive pre-PLC to be a poor prognosis predictor since pre-PLC was first reported by Kondo and associates in 1989 [12]. However, positive pre-PLC is currently not recognized as equivalent to T4 or a factor indicating incomplete resection [16–18]. In our study, pre-PLC was an independent prognostic factor when analyzed with prognostic factors available before lung resection, but not when postoperative pathologic factors and post-PLC results were combined in analyses. Positive pre-PLC patient outcome, when post-PLC was negative, was not very poor, with the 5-year survival rate reaching almost 60%. Therefore, positive pre-PLC result alone does not contraindicate surgical resection. In contrast, post-PLC proved to be an independent prognostic factor as significant as other established prognostic factors, including pathologic TNM status. No positive post-PLC patients survived beyond 4 years. As the patient outcome was extremely poor when pre-PLC was also positive, adjuvant therapy may be needed in these patients. We conclude PLC should be recognized as an essential prognostic factor and should be performed in NSCLC patients without pleural effusion and dissemination. And post-PLC, compared with pre-PLC, had a greater and independent impact on survival and needs to be incorporated in the pathologic staging of NSCLC in the future. As Vicidomini and associates referred to in their recent article on PLC [19], the results of the American College of Surgeons Oncology Group's Z0040 trial, which has completed a 1,200 patient accrual, will further define the potential implications of PLC in the management of lung cancer.

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Is surgical resection indicated for a solitary non–small cell lung cancer recurrence?

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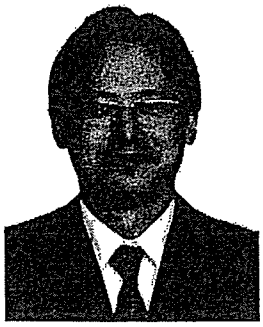

Dr T. Hishida

Objectives: Some investigators have reported long-term survival after surgical resection of a solitary non–small cell lung cancer recurrence in various sites. However, the role and indications of the second operation remain unclear.

Methods: We reviewed 28 patients with a solitary recurrence after successful initial resection of primary non–small cell lung cancer who underwent resection of the recurrent lesion. The clinicopathologic factors associated with outcome were analyzed.

Results: There were 17 men and 11 women. Recurrence resection was performed for the following sites: 16 in the lung, 5 in the brain, 2 in the adrenal gland, and 1 each in the chest wall, stomach, skin, pelvic lymph node, and malar bone. The median survival time was 25 months, and the 1-, 2-, and 5-year survival rates after recurrence were 89%, 59%, and 32%, respectively. Advanced p-stage (p-stage II and III, n = 14) of the primary tumor was the significant negative prognostic factor. Patients with p-stage II or III had survival equivalent to that of those who had multiple recurrences or were unfit for further surgical intervention.

Conclusions: Resection of a solitary non–small cell lung cancer recurrence might provide long-term survival in highly selected patients. However, surgical resection might be contraindicated if the primary tumor is stage II or III.

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Five-year survival rates of patients with non–small cell lung cancer (NSCLC) have been disappointing, even after successful complete resection, with about 50% of patients eventually experiencing recurrence and death from the disease.[1] Recurrent lesions are generally multiple and disseminated, and additional surgical intervention is usually not indicated. Some investigators have reported long-term survivals after solitary recurrence resection of the brain, adrenal gland, spleen, liver, and bone.[2-10] However, the role and indication of surgical intervention remain unclear. The aim of this study is to investigate clinicopathologic characteristics of patients with NSCLC who underwent resection of a solitary recurrent lesion and to identify prognostic factors.

Patients and Methods

Patients

We retrospectively reviewed the clinical and pathologic files of 1698 consecutive patients with NSCLC who had undergone complete surgical resection at the National Cancer Center Hospital East from 1989 through 2002. Data collection and analyses were approved, and the need for obtaining informed consent from each patient was waived by the institutional review board in January 2004. Patients with synchronous metastasis (M1) were excluded. Among them, we identified 592 (35%) patients with locoregional or distant recurrence in 2003 or earlier. We excluded patients with second pulmonary lesions that were not clearly distinguished from metachronous second primary NSCLC on the basis of the criteria of Martini and Melamed.[11] Our follow-up procedures included physical examination, chest roentgenogra-

Abbreviations and Acronyms
FDG = ^{18}F fluorodeoxyglucose
NSCLC = non–small cell lung cancer
PET = positron emission tomography
RFI = recurrence-free interval

phy, and blood testing, including tumor markers, 1 month after the initial operation, every 3 to 6 months during the first 3 years, and every half year to 1 year thereafter. If any abnormality was found, we performed computed tomographic scans. We did not routinely perform bone scanning and brain examinations for asymptomatic patients. When a lesion suggesting locoregional or distant recurrence was detected, we scrutinized the whole body radiologically.

Thirty of the 592 patients underwent resection of a solitary recurrent lesion. Among them, 2 patients who had recurrence-free intervals (RFIs) of 1 and 4 months, respectively, were eliminated from this study because they possibly had undetectable "missed" M1 disease at the initial operation. Nine of the 28 patients were symptomatic at the time of recurrence detection. All but 1 patient, who had intrapulmonary recurrence, were asymptomatic. The median period from recurrence detection to the second operation was 2.6 months (range, 0.2-24 months). The follow-up protocols were the same before and after recurrence resection. The median follow-up period after recurrence resection was 33 months, ranging from 11 to 128 months.

For the remaining 562 patients, surgical intervention was not indicated because recurrences were multiple, patients were unfit for further surgical intervention, or both. They underwent palliative chemotherapy, radiotherapy, or best supportive care. Patients with multiple recurrences who underwent palliative operations for symptomatic sites were included in this group.

Prognostic Evaluation

We attempted to identify prognostic factors associated with subsequent survival after resection of a solitary recurrent lesion. We evaluated the following factors: clinical characteristics at recurrence (sex, age, carcinoembryonic antigen level, time from initial resection to recurrence detection [RFI], symptoms at the time of recurrence, site of recurrence, and mode of recurrence [locoregional or distant]) and pathologic findings of the primary lung cancer (histology, tumor size, lymph node status, p-stage, and lymphatic and vascular permeations). We defined locoregional recurrence as recurrence within the ipsilateral thorax and distant recurrence as all other recurrences. Each pathologic specimen was reviewed by a board-certified pathologist who was blinded to the clinical outcome. Histology was specified on the basis of the World Health Organization classification for cell types.[12] Pathologic stages were determined on the basis of the TNM classification of the International Union Against Cancer.[13]

Survival Analysis

Survivals were calculated by using the Kaplan-Meier method and were compared with the log-rank test. Zero time was the date of recurrence identification, and the terminal event was defined as death from any cause. An observation was censored at the last follow-up when the patient was alive or lost to follow-up. Factors with a P value of less than .15 were entered into the multivariate analysis by using the Cox proportional hazards stepwise model. All statistical analyses were performed with a software package (JMP, release 5.0; SAS Institute Inc, Cary, NC).

TABLE 1. Clinicopathologic characteristics of 28 patients with NSCLC who underwent resection of a solitary recurrent lesion

Characteristics	Value	No.
Clinical characteristics at recurrence		
Age at recurrence resection (y)	Median	65
	Range	39-73
Sex	Male	17
	Female	11
RFI (mo)	Median	23
	Range	6-82
CEA level (ng/mL)	Median	3.5
	Range	0.5-3286
Recurrent site	Ipsilateral lung	8
	Contralateral lung	8
	Brain	5
	Adrenal grand	2
	Chest wall	1
	Stomach	1
	Skin	1
	Abdominal lymph node	1
	Bone (malar bone)	1
Pathologic characteristics of primary tumor		
Histology	Adenocarcinoma	21
	Squamous cell carcinoma	5
	Adenosquamous carcinoma	1
	Pleomorphic carcinoma	1
Size of primary tumor (cm)	Mean ± SD	4.1 ± 1.7
p-Stage of primary tumor	IA/IB	4/10
	IIA/IIB	2/6
	IIIA/IIIB	4/2
Nodal status of primary tumor	N0/N1/N2	18/7/3

RFI, Recurrence-free interval; *CEA*, carcinoembryonic antigen; *SD*, standard deviation.

Results

Patient Characteristics

Clinicopathologic characteristics of 28 patients who underwent resection of a solitary recurrent lesion are shown in Table 1. There were 17 men and 11 women, with a median age of 65 years (range, 39-73 years) at the time of resection of the recurrent lesion. At the initial operation, 26 of 28 patients underwent lobectomy and systemic mediastinal lymph node dissection. Two patients underwent limited

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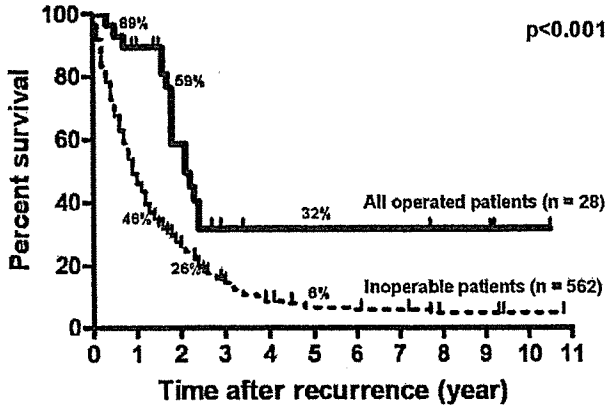

Patients at risk

All operated patients	28	24	13	4	1
Inoperable patients	365	154	63	8	1

Figure 1. Comparative survival curves among 28 resected patients and 562 patients without resection. The difference in survival probability after recurrence is significant (1-, 2-, and 5-year survivals after recurrence: 89%, 59%, and 32% vs 46%, 26%, and 6%; $P < .001$).

GTS

lung resection because of insufficient pulmonary reserve. Neoadjuvant platinum-based chemotherapy was administered to 1 patient because of clinical N2 status. All patients achieved macroscopically complete surgical removal of their primary NSCLC tumor, but the resection margin was pathologically positive in 1 patient. The patient had recurrence in the adrenal gland. RFI was almost 2 years (median, 23 months; range, 6-82 months). The lung (n = 16) was the most frequent site of recurrence. The mode of resection for intrapulmonary recurrences included 3 completion pneumonectomies, 1 lobectomy, and 12 limited resections. Distal gastrectomy was performed for the patient who had gastric recurrence with severe progressive anemia, and open lymph node resection was performed for the patient with pelvic lymph node recurrence. Complete removal of the recurrence was accomplished in all patients. There was no complication after resection of the recurrent lesion. One of 5 patients with brain recurrence received whole-brain irradiation postoperatively. No patients underwent systemic chemotherapy after resection of the recurrent lesion.

Survival and Prognostic Factors After Resection of the Solitary Recurrent Lesion

Figure 1 shows comparative survival curves after recurrence among 28 patients who underwent resection of the solitary recurrent lesion and 562 patients in whom an additional operation was not indicated. Overall 1-, 2-, and 5-year survivals after recurrence were significantly better in patients who underwent resection of a solitary recurrent lesion than in those who did not undergo resection (89%, 59%, and 32% vs 46%, 26%, and 6%; $P < .001$). The median survival times after recurrence were 25 and 11 months, respectively.

Table 2 shows the relationship between survival after resection of the recurrent lesion and the clinicopathologic characteristics of the 28 patients. Multivariate analysis demonstrated that advanced p-stage (stage II-III) of the primary lung cancer was the significant negative prognostic factor associated with survival after recurrence detection (hazard ratio, 6.15; 95% confidential interval, 1.09-30.8; $P = .04$). As shown in Figure 2, the patients with p-stage II or III disease demonstrated survival statistically equivalent to that of patients not undergoing resection after recurrence detection ($P = .11$). In 14 patients with p-stage I disease, 10 and 3 patients survived for more than 2 and 5 years, respectively, after recurrence detection. One with recurrence in the malar bone is surviving for 7 years without a distant failure. In contrast, 3 and 1 of 14 patients with p-stage II or III disease survived for more than 2 and 5 years, respectively, but with a distant failure.

Discussion

Most recurrences after primary NSCLC resection are multiple and disseminated and are usually treated with systemic chemotherapy when patients can tolerate it. Although many studies have shown that systemic chemotherapy prolongs survival in unresectable stage IV NSCLC, there have been no large-scale, randomized prospective trials addressing whether chemotherapy improves survival of patients with recurrence.[14] In an effort to improve long-term tumor control and subsequent survival, attempts have been made to incorporate surgical intervention in selected cases of solitary NSCLC recurrence. Evidence that a solitary recurrent lesion can be effectively treated with surgical intervention exists for malignancies other than lung cancer. For colorectal cancer, melanoma, and thyroid cancer, resection of recurrent lesions can offer prolonged survival.[15-17] For lung cancer, some investigators have reported acceptable survival after resection of the recurrent lesion, but others have contradicted these conclusions. Abrahams and coworkers[4] demonstrated a satisfactory outcome in brain recurrence, with a median survival time of 18 months and a 5-year survival rate of 28.9%. In contrast, Saitoh and associates[2] conducted 24 brain resections, with a 5-year survival rate of only 8.3%. Prognostic factors for survival after resection of the recurrent lesion have not been clarified.

Although our patient population was heterogeneous, with a variety of recurrence sites, the overall survival after resection of the recurrent lesion was acceptable by current standards, with a median survival time of 25 months and a 5-year survival rate of 32%. The patients with a solitary NSCLC recurrence arising from an advanced primary tumor