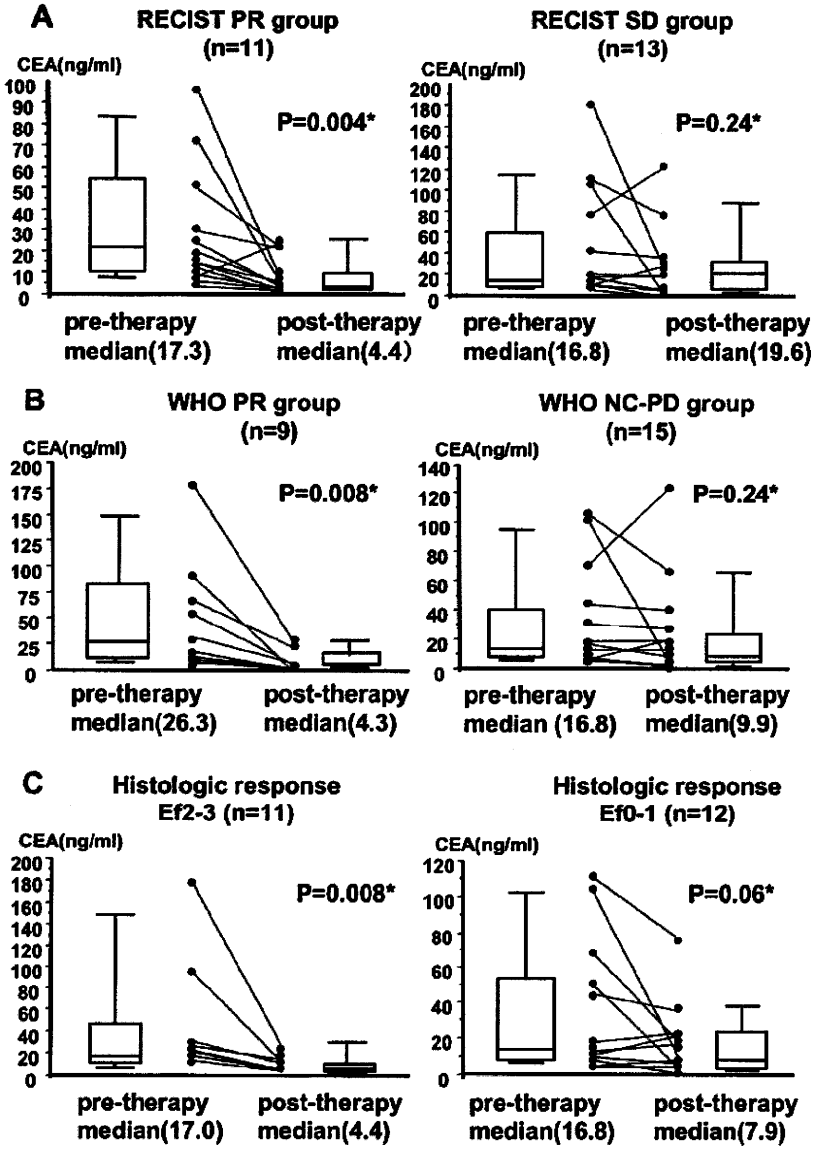

Fig 1. Correlation between serum CEA levels before and after chemotherapy, and responsiveness to treatment as defined by RECIST (A), WHO criteria (B), and histological response (C). The box shows lower quartile, median, and upper quartile. Each bar shows the largest and smallest nonoutlier observation. *The *P* value was calculated using the Wilcoxon signed rank test.

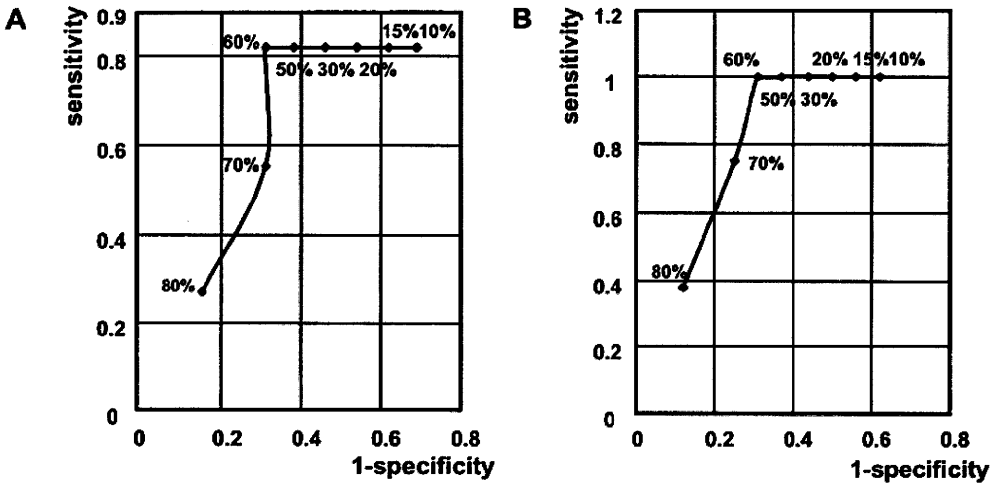

Fig. 2. Receiver operating characteristic (ROC) curve analysis to determine the cutoff value of CEA concentration. Each curve was evaluated using RECIST (A) and WHO criteria (B).

pleuritis carcinomatosa, pleural effusion, or small lesions. Because it is also difficult to evaluate antitumor effects by RECIST when the patient has minute multiple pulmonary metastases or irregularly shaped tumors, we evaluated changes in serum CEA levels as a surrogate marker for tumor response to chemotherapy. A previous report compared the evaluation of responsiveness by chest CT with that performed by histological examination.[13] Only one report analyzed changes in CEA value in relation to tumor response.[14] In this study, we showed that changes in CEA levels in the serum reflected tumor response evaluated by RECIST, WHO criteria, or histological examination.

We also used an ROC curve to show that a 60% reduction in CEA levels was appropriate for predicting a good response to chemotherapy. In RECIST and WHO criteria, a similar volume reduction ratio is thought to be adopted. Partial response evaluated by RECIST (i.e., a 30% decrease of the "longest diameter" of target lesions) is equivalent to a 50% reduction of the tumor area (i.e., $1 - 0.7^2$), which is a definition of PR by WHO criteria. This degree of tumor shrinkage is considered to be equal to a 65% reduction in tumor volume (i.e., $1 - 0.7^3 = 0.657$). If the CEA reduction ratio is assumed to reflect shrinkage of the tridimensional volume of the tumor, the cutoff value of 60% obtained here appears reasonable.

Histological response should reflect tumor response precisely and is expected to be the best surrogate marker for the survival of patients; however, the correlation obtained in this study between histological response and other criteria was not significant. One possible explanation for this discrepancy is the possibility that the interstitial tissue remained as scar or necrotic tissue after treatment, which would mask tumor shrinkage even if tumor cells had disappeared. Another explanation could be that the tumor was resected before the maximum reduction effect was achieved because the interval between the end of chemotherapy and surgery was relatively short. Because the tumors in the 11 patients in our cohort had invaded thoracic walls, they could not shrink uniformly, and the effect of tumor reduction was not reflected appropriately in chest CT imaging. Another report suggested that CEA levels increase transiently for the first 20–60 days after the onset of chemotherapy or radiotherapy.[15]

In this study, we reviewed only patients with high CEA levels (>5 ng/ml). Because the proportion of NSCLC patients with elevated CEA was suggested to be less than 50%, the possibility arises that patients with normal preoperative serum CEA levels should also be evaluated. Moreover, the targets of this study were surgically treated

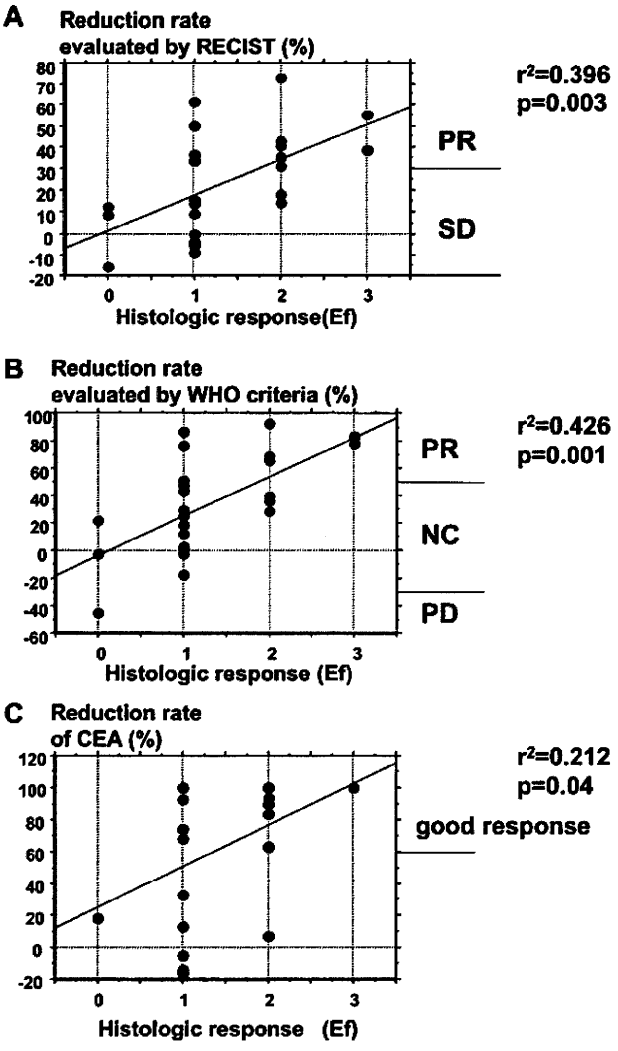

Fig. 3. (A) Correlation between histological response and tumor reduction rate evaluated by RECIST. (B) Correlation between histological response and tumor reduction rate evaluated by WHO criteria. (C) Correlation between histological response and reduction rate of serum CEA levels.

patients: it remains unclear whether these patients and highly advanced NSCLC patients were equally treated, though the chemotherapy regimen was similar in both scenarios. Larger-scale studies targeting postoperative recurrence cases and unresectable NSCLC cases are expected in the near future.

RECIST is a "ruler" standardized as the criteria of evaluation of tumor response. The reproducibility of RECIST is clear, and the evaluation of tumor response using RECIST can be easily carried out by surgeons, pulmonologists, or radiation oncologists, but not by radiologists.[16] On the other hand, we frequently measure

serum CEA levels during the treatment of NSCLC patients because this parameter can be assessed easily; therefore serum CEA concentration may also be a useful surrogate marker for patients with lesions that are not measurable.

Conclusion

Serum CEA levels appeared to be a useful surrogate marker for the evaluation of tumor response to chemotherapy, and it seemed to be comparable with RECIST in patients with NSCLC who had elevated CEA levels prior to treatment.

Note: The main results of this paper were previously published in Japanese in the *Japanese Journal of Lung Cancer*.[17]

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Salvage surgery for advanced non–small cell lung cancer after response to gefitinib

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Epidermal growth factor receptor (EGFR) tyrosine kinase inhibitor (EGFR-TKI) gefitinib has dramatic efficacy in more than 70% of advanced non–small cell lung cancers with EGFR gene mutations.[1] Some patients with inoperable systemic non–small cell lung cancers demonstrate a downstaging of their cancer to operable disease status after gefitinib treatment. Despite high response rates for EGFR mutant tumors, the median time to progression is about 1 year.[1] The EGFR T790M mutation and *MET* amplification are thought to be the underlying mechanisms of the acquired resistance to EGFR-TKIs. When complete resection of residual disease is possible, the patients can then be considered disease free. We have aggressively performed salvage lung resections for patients with gefitinib responses and demonstrated downstaging to N0M0. The purpose of this study was to assess

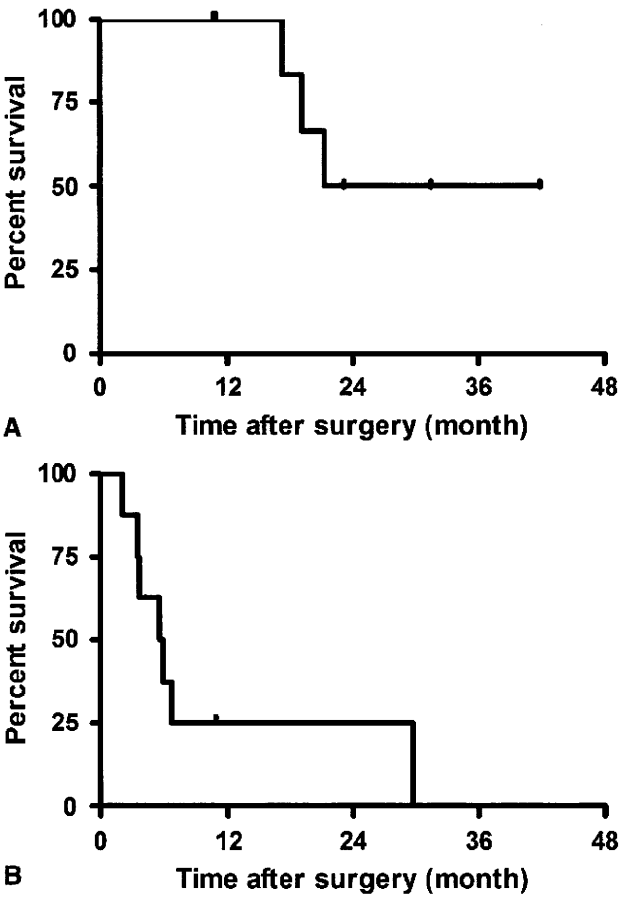

FIGURE 1. A, Overall survival curve of patients who underwent surgical resection after response to gefitinib administration. Median overall survival after surgery was 32 months. B, Recurrence-free survival curve of patients who underwent surgical resection after response to gefitinib administration. Median recurrence-free survival after surgery was 6 months.

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TABLE 1. Patient characteristics

Case	Age (y), sex	Initial CS	Treatment before gefitinib (response)	Gefitinib duration	CS before surgery
1	73, F	cT4(PM)N2M0, IIIB	None	3 mo	cT2N0M0, IB
2	51, F	cT2N3M1(brain), IV	None	3 y	cT1N0M0, IA (local regrowth)
3	58, F	cT2N2M0, IIIA	CDDP+VNR (SD), CBDCA+PTX (SD)	5 mo	cT1N0M0, IA
4	58, F	cT4(D+)N0M0, IIIB	CDDP+GEM (SD)	2 y, 10 mo	cT1N0M0, IA (local regrowth)
5	63, F	cT2N3M1(abd LN), IV	CDDP+TS-1 (PR)	1 y, 4 mo	cT1N0M0, IA (local regrowth)
6	33, M	cT4 (PM, E+)N0M0, IIIB	CBDCA+PTX (SD)	2 mo	cT1N0M0, IA
7	54, M	cT4N3M1(PM), IV	CDDP+DTX (SD)	1 y, 10 mo	cT1N0M0, IA
8	71, F	cT2N3M0, IIIB	None	1 y, 6 mo	cT2N0M0, IB
9	57, F	cT4N0M1(PM), IV	CBDCA+DTX (SD)	Unknown	cT1N0M0, IA

CS, Clinical stage; *EGFR*, epidermal growth factor receptor; *PM*, pulmonary metastasis; *DWD*, died with disease; *AWD*, alive with disease; *CDDP*, cisplatin; *VNR*, vinorelbine tartrate; *SD*, stable disease; *CBDCA*, carboplatin; *PTX*, paclitaxel; *CR*, complete response; *D*, pleural dissemination; *GEM*, gemcitabine; *AWOD*, alive without disease; *abd LN*, abdominal lymph node; *TS-1*, tegafur/gimeracil/oteracil potassium; *PR*, partial response; *E*, malignant pleural effusion; *DTX*, docetaxel. *. Endothelial growth factor receptor mutational analysis was performed on pretreatment biopsy specimens obtained by bronchoscopy.

the perioperative safety and survival benefit of these salvage lung resections.

CLINICAL SUMMARY

After institutional review board approval at each institution, the clinicopathologic profiles of a total of 9 patients were collected by a questionnaire survey in 2009 from 7 institutions belonging to the Lung Cancer Surgical Study Group of the Japan Clinical Oncology Group. The questionnaire included the following items: sex, age, smoking history, clinical (pretreatment) stage, response to therapy before gefitinib monotherapy, response to and adverse effects of gefitinib monotherapy, duration of gefitinib administration, withdrawal period of gefitinib before surgery, preoperative clinical stage, surgical procedure, morbidity and mortality of surgery, primary site by lobe, histology, pathologic stage, EGFR mutation status, postoperative therapy, survival time, recurrence, and cause of death.

The patient characteristics are shown in Table 1. All cases were adenocarcinoma, and all had been initially diagnosed as inoperable. Surgery was performed to eradicate residual tumors or local recurrence and regrowth, with a median administration period of 17 months (range, 2–36 months). Gefitinib was terminated before surgery in all cases, with a median withdrawal period of 7 days (range, 1–21 days). Resection was accomplished in all cases, with a median hospital stay of 9 days (range, 6–34 days). There was 1 case of mild liver dysfunction, and there were no deaths. An EGFR mutational analysis of resected specimens or of the pretreatment biopsy specimen (patient 3) was performed in 7 cases. Six of 7 patients harbored EGFR mutations, exon 19 deletions or exon 21 L858R. Two patients also had EGFR-TKI–resistant exon 20 T790M mutations. Four patients who underwent surgery in late study period received gefitinib postoperatively for various durations. Despite the remarkable downstaging of the patients' disease after gefitinib treatment, 7 of 9 patients showed a more advanced pathologic stage than their preoperative clinical stage. Six patients with initial N2-3 disease all had radiologic downstaging to N0 status before attempted resection. Pathologically, 2 patients had persistent N2 disease and 1 had N1 disease. The recurrence-free and overall survivals are shown in Figure 1 (*A* and *B*). The most common site of recurrence was the brain. One patient has been alive without disease for 11 months with the use of adjuvant gefitinib.

DISCUSSION

Our patient population had no serious immediate postoperative morbidity or mortality. Among a total of 41 patients in the literature who underwent lung resection after EGFR-TKI treatment, none died perioperatively.[2-5] Although there has been some concern that preoperative EGFR-TKIs may be associated with impaired wound healing, major lung resection after EGFR-TKI therapy may be feasible.

On the other hand, postoperative survival in the this series was not satisfactory, with a median recurrence-free survival of 6 months. Despite dramatic radiographic downstaging after gefitinib treatment, 7 of 9 patients had further advanced pathologic stages than their preoperative clinical stages. Dramatic radiologic response does not necessarily correlate with cell death. Our results suggest that initially expressed systemic disease was essentially unchanged even after dramatic radiologic response to gefitinib. Surgery after gefitinib treatment should be limited to patients without initial evidence of disseminated and distant metastases. EGFR-TKIs have both higher and more rapid responses, and better toxicity profiles than standard chemotherapy for non–small cell lung cancers harboring EGFR mutation. Preoperative EGFR-TKI treatment strategy should be reevaluated in the neoadjuvant setting for early to locally advanced but operable disease. The optimal duration of EGFR-TKI treatment,

TABLE 1. Continued

Mode of resection	Pathologic stage	EGFR gene status	Adjuvant therapy	Outcome
Lobectomy	pT2N1M0, IIB	Wild type	None	Bone metastasis (6 mo), DWD (1 y, 5 mo)
Lobectomy	pT1N0M0, IA	Exon 19 (del)	None	Brain metastasis (2 mo), AWD (3 y, 6 mo)
Left pneumonectomy	Pathologic CR	Exon 19 (del)*	Gefitinib (2 y)	Brain metastasis (2 y, 4 mo), AWD (2 y, 7 mo)
Bilobectomy	pT1N1M0, IIA	Exon 19 (del)	Gefitinib (11 mo)	AWOD (11 mo)
Lobectomy	pT1N2M0, IIIA	Unknown	None	Brain metastasis (5 mo), AWD (2 y)
Left extrapleural pneumonectomy	pT4N2M0, IIIB	Exon 19 (del)	Gefitinib (3 mo)	Brain metastasis (3 mo), DWD (1 y, 7 mo)
Lobectomy	pT4N0M0, IIIB	Exon 19 (del) Exon 20 (T790M)	None	Metastasis in thorax (6 mo), AWD (10 mo)
Lobectomy	pT2N2M0, IIIA	Exon 21 (L858R) Exon 20 (T790M)	None	Metastasis in thorax (4 mo), DWD (1 y, 9 mo)
Lobectomy	pT2N0M0, IB	Unknown	Gefitinib	Unknown

the timing of surgery, and the role of adjuvant EGFR-TKI treatment should be also investigated in the future.

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Aortic dissection and rupture in adolescents after tetralogy of Fallot repair

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Aortic dissection in children and adolescents is rare, yet it is associated with high mortality. A recent article[1] describing 13 patients with aortic dissections operated between 1970 and 2000 reported an operative mortality of 38%. Progressive aortic root dilatation is a recognized feature of tetralogy of Fallot (TOF)[2,3] and generally managed conservatively. However, 2 recent reports of aortic dissection in patients with aortic aneurysm after TOF repair[4,5] together with the case presented reemphasize the fact that aortic root dilatation must be monitored closely in patients with TOF.

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SURGERY TODAY

Review Articles

Small Peripheral Lung Adenocarcinoma: Clinicopathological Features and Surgical Treatment

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Abstract
The clinical use of high-resolution computed tomography (CT) has greatly advanced diagnosis of small peripheral lesions of the lung. In CT images, these lesions often exhibit ground-glass opacity (GGO). Ground-glass opacity is typical of noninvasive bronchioloalveolar carcinoma (BAC), which is characterized by a lepidic pattern of cells that line the alveoli but do not invade neighboring structures. Bronchioloalveolar carcinoma is classified as a subset of lung adenocarcinoma, but has distinct clinical and pathological features and a favorable prognosis. Most small peripheral lung lesions, including BAC, probably originate in the epithelium of the peripheral airway. As with other subsets of non-small cell lung cancer, surgical resection is a potentially curative treatment. However, it is questionable whether a lobectomy is necessary for small lesions that exhibit GGO, particularly when they are <1 cm in diameter. Although several Japanese investigators have suggested that a limited resection, including a wedge resection and a segmentectomy without nodal dissection, is an appropriate treatment for small lung adenocarcinomas, this approach should be validated by clinical trials.

Key words Bronchioloalveolar carcinoma · Ground-glass opacity · Limited resection

Introduction

The development of radiological mass screening methods for lung cancer, particularly low-dose helical computed tomography (CT), has increased the incidence of detecting small peripheral lung lesions.[1,2] Ground-glass opacity (GGO) is usually a nonspecific finding in CT images of the lung, because it is associated with a variety of pulmonary diseases such as inflammation, bleeding, pulmonary lymphoproliferative disorder, atypical adenomatous hyperplasia (AAH), bronchioloalveolar carcinoma (BAC), and well-differentiated adenocarcinoma.[3,4] An early adenocarcinoma or BAC is highly suspected if focal GGO persists for several months.[5,6] Bronchioloalveolar carcinoma is classified as a noninvasive carcinoma with no evidence of stromal, vascular, or pleural invasion in the revised histological classification of the World Health Organization (WHO).

In a large collaborative study, the International Early Lung Cancer Action Program, low-dose helical CT was used to screen more than 30000 asymptomatic persons per year who were considered at risk of developing lung cancer.[7] This screening detected 412 cases of clinical stage I lung cancer. The estimated 10-year survival rate was 92% regardless of the type of surgical procedure. Although a lobectomy with systematic mediastinal lymph node dissection remains the standard of care for resection of T1 N0 M0 non-small cell lung cancer, it has not been established whether a lobectomy or a pulmonary resection are necessary for the GGO type of small lesion.

A clear understanding of the features of these tumors would facilitate the successful treatment of this increasingly prevalent small lung adenocarcinoma. This article reviews the current information on the histopathology, natural history, radiological findings, and surgical treatment of early-stage peripheral lung adenocarcinomas. The subject of this article is small lung adenocarcinomas including BAC, adenocarcinoma of mixed subtypes, and invasive adenocarcinoma.

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Histopathology

Most small lesions of the peripheral lung that are undetectable with plain chest X-ray films are diagnosed pathologically as focal BAC or AAH, a precancerous lesion.[5,8] In contrast, small lung lesions that can be detected using X-ray films include squamous cell carcinomas and poorly differentiated adenocarcinomas, which usually form solid nodules.

In a study of stage I adenocarcinomas <2 cm in diameter, Noguchi et al. found that patients with pure BAC (type A) and BAC with foci involving structural collapse of the alveoli (type B) have a greater 5-year survival rate (100%) than patients with foci involving active fibroblastic proliferation (type C, 75% survival rate) or patients with pure adenocarcinomas (types D–F, 52% survival rate).[9] These findings greatly influenced the panel of the 1999 WHO/International Association for the Study of Lung Cancer Classification, which proposed a new, more stringent definition of BAC by including the presence of pure lepidic growth without invasion of the stroma, blood vessels, or pleura.[8]

In 1960, Liebow published the first report on BAC, describing it as a well-differentiated adenocarcinoma with neoplastic cells spread along the alveoli, but with little stromal reaction, no invasion, and preservation of alveolar structure.[10] According to the WHO criteria, BAC is a carcinoma in situ, and a tumor should not be classified as a BAC if it is associated with lymphatic or systemic metastases. Bronchioloalveolar carcinoma is classified further into nonmucinous (60%–80%) and mucinous (20%–40%) types. Nonmucinous BAC is associated with proliferation of Clara cells, nonciliated bronchial epithelium, and type II pneumocytes. The histological findings in mucinous BAC include goblet cells or mucin-producing columnar tumor cells. Tumors showing both BAC features and areas of invasion are classified as adenocarcinomas of mixed subtypes, and there is evidence that the presence of any BAC features is predictive of better survival than that of pure invasive adenocarcinoma.[8,9,11–14] However, it is still unclear whether a high proportion of BAC features is a better predictor of survival than a low proportion of BAC features.

A diagnosis of BAC requires a histological examination to determine the presence or absence of invasion, thus cytology or frozen-section diagnosis of BAC, which involves examination of only a small portion of the tumor, is probably unreliable. Therefore, diagnosis and treatment options, including surgery, are usually based on radiographic findings, which correlate closely with a diagnosis based on pathological examination.

Another major change made in 1999 to the WHO classification involved the addition of AAH as a precursor lesion for lung adenocarcinoma. This was preserved in the 2004 WHO classification. AAH is a solitary alveolar lesion, usually <5 mm in diameter, and is accompanied by proliferation of type II pneumocytes or Clara-cell-like cells with various cellular atypia.[8] A diagnosis of BAC may be warranted for some lesions that exhibit foci of elevated atypia. Moreover, AAH lesions are occasionally seen in the periphery of indisputable adenocarcinoma. Miller et al.[15] suggested that AAH might be analogous to an adenomatous lesion of the colon, and subsequent studies have supported this hypothesis.[16,17]

Natural History of Small Lesions Showing Ground-Glass Opacity

The timeframe for progression of small GGO lesions of the lung to solid tumors is unclear. Although it has been suggested that the adenoma–carcinoma sequence also applies to tumorigenesis in adenocarcinoma of the lung, no persuasive data have been presented on the percentage of indolent nonsolid GGO lesions over a long period, or the percentage of GGO lesions that progress to become solid lesions. Information on the dormancy of lesions that exhibit pure GGO is important for effective management of small lung tumors. Occasionally, tumors do not change in size over long periods. Patients diagnosed with the Noguchi type A, which has a 100% 5-year survival rate, and who do not need resection, may be included in the dormant subgroup. It is important to elucidate the natural history of the small lung nodules with GGO features to avoid over diagnosis.

Takashima et al. conducted a serial CT study and described the progression of lung adenocarcinomas with GGO components. Lung adenocarcinomas that initially present with GGO subsequently increase in size in 75% of patients, and 17% of patients develop solid components within the nodule. The solid portions increase in 23% of patients, and spiculation appears in 6% of patients.[18] Another investigator reported three types of progression for BAC: (1) an increase in the size of the BAC, (2) a decrease in size and the appearance of a solid component in one BAC and one adenocarcinoma of mixed subtype, and (3) a BAC with stable size and increasing density. All but one of the follow-up cases of lung cancers were noninvasive, whereas the remaining GGO tumor had a solid component and was minimally invasive.[19]

In a study of 19 patients, the size of pure GGO tumors did not change in eight patients, increased slightly (up to 5 mm) in six patients, and increased by >5 mm in five patients during a follow-up of 2 years or more.[4] Among the 10 patients who underwent a limited resection, five had adenocarcinoma, three had pulmonary lymphoproliferative disorder, one had AAH, and one had focal

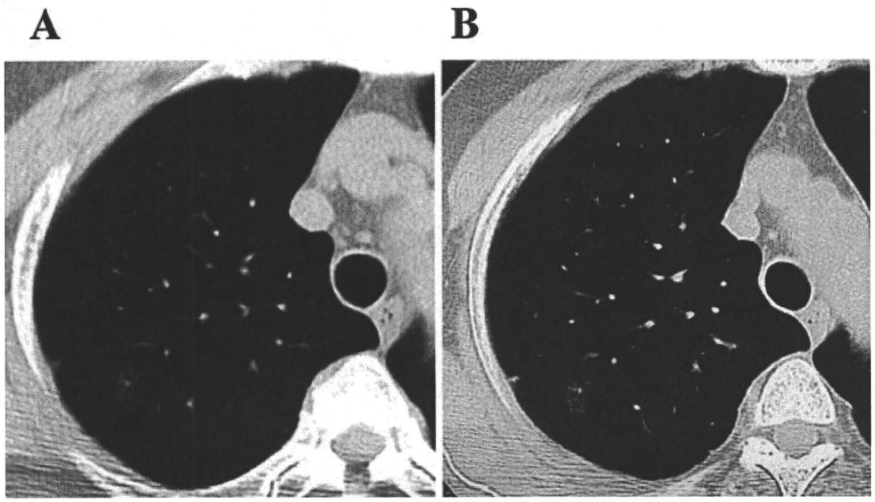

Fig. 1A,B. Computed tomography (CT) image of a representative case of a small lung lesion. The 68-year-old woman was available for long-term follow-up. **A** Thin-section CT scan of a 10-mm diameter tumor in the right upper lung lobe with pure ground-glass opacity (GGO), an indistinct boundary, and hazy attenuation. **B** The size of GGO was unchanged at the 27-month follow-up. The lesion size either did not change or increased only slightly (up to 5 mm) in 9 of 13 GGO patients who we observed for more than 2 years

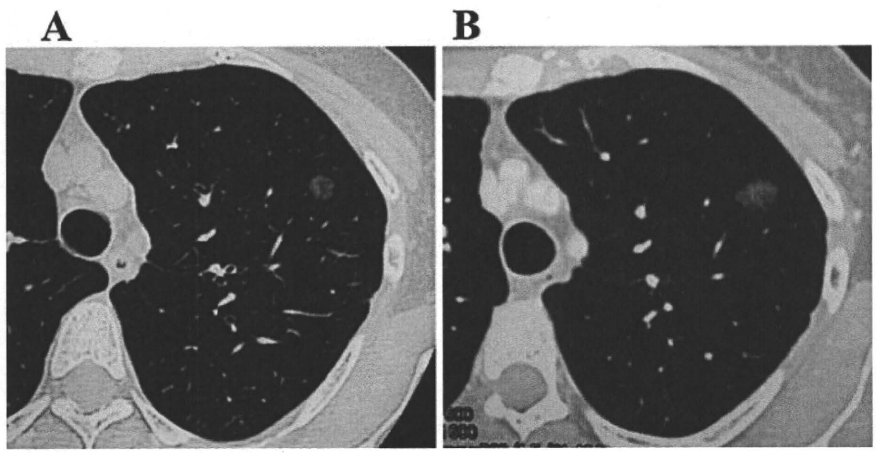

Fig. 2A,B. Computed tomography images from a 41-year-old woman. **A** Thin-section CT scan showing a pure GGO 10 mm in diameter in the left upper lobe of the lung. **B** At the 51-month follow-up, the diameter of the lesion had increased to 15 mm

fibrosis. The authors suggested that some pure GGOs never progress to clinical disease.

Thirteen patients with GGO who had not experienced an intervention for more than 2 years were reviewed (median follow-up, 48 months; range 24–96 months). At the time of their discovery, the lung lesions were 8–21 mm in diameter. At the follow-up, the size of the lesions had not changed or had increased slightly (up to 5 mm) in all but four of the 13 patients (a representative case is shown in Fig. 1). In two of the 13 patients, the lesion increased in diameter; one increased from 15 mm to 23 mm over 33 months and the other increased from 10 mm to 15 mm over 51 months (one is shown in Fig. 2). In the other two patients, the lesion became slightly larger and multiple new GGOs appeared over 45 and 32 months (one is shown in Fig. 3). Figure 4 shows the natural history of a small, pure GGO based on previous reports and our study. The management of such GGO lesions should differ from that of noncalcified solitary nodules with soft-tissue density, while wide wedge resection or segmentectomy is a safe and minimally invasive procedure for treating small GGO lesions.

Radiographic Evaluation

Many radiologic studies on solitary, peripheral, and small lung adenocarcinomas have shown a strong correlation between the CT findings and pathologic features.[20–22] Asamura et al. studied 48 lung carcinomas ≤1 cm in diameter and observed three types of high-resolution CT patterns: a nonsolid GGO type ($n = 19$), a partly solid GGO type ($n = 9$), and a solid type ($n = 20$). They reported that there were no recurrences or BAC histological types among all 28 GGO (nonsolid and partly solid) lesions. However, not all pure GGO lesions are histologically pure BACs, and some have an invasive adenocarcinoma component.[23] In a comprehensive analysis of hilar and mediastinal lymph nodes that were systematically dissected, nodal micrometastasis was found in 20% of patients with adenocarcinomas

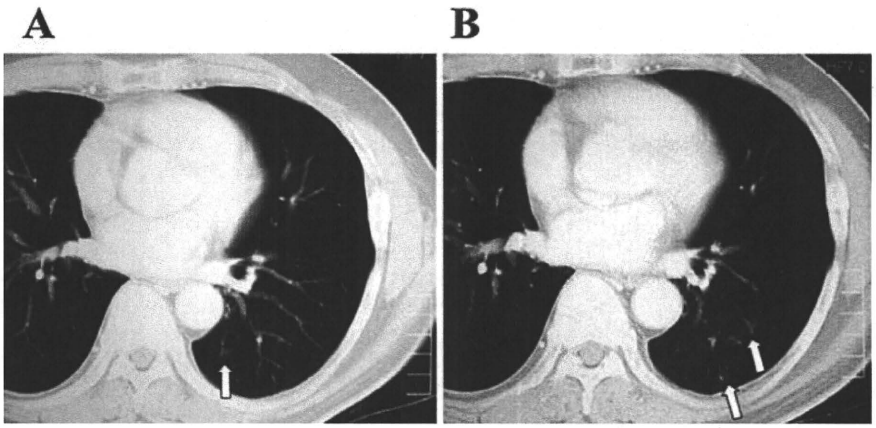

Fig. 3A,B. Computed tomography images from a 60-year-old man. **A** CT scan showing a small, pure GGO in the left lower lobe of the lung (*arrow*). **B** At the 45-month follow-up, the lesion had increased slightly in size and multiple new GGOs had appeared (*arrows*)

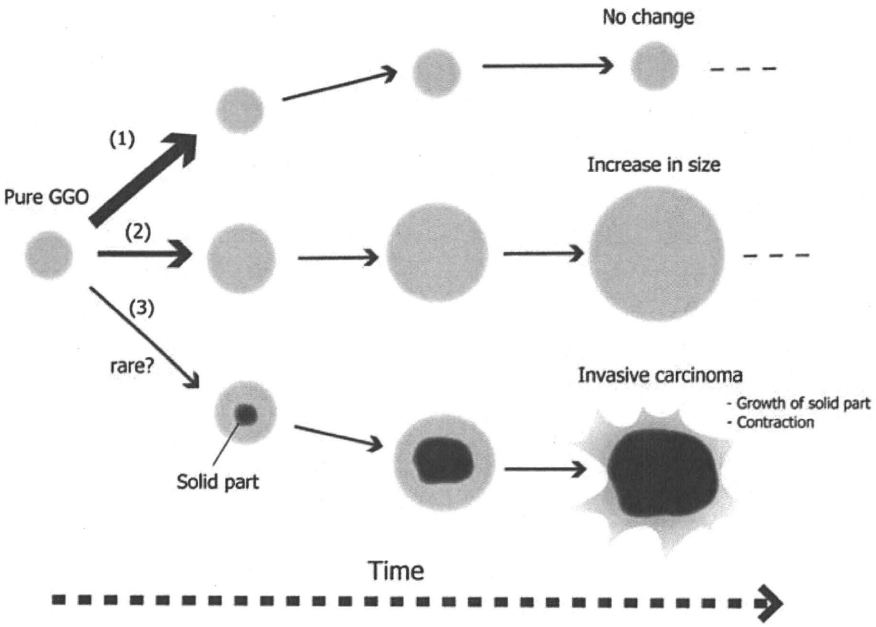

Fig. 4. Natural history of a small, pure GGO compiled from previous reports and our study. Three types of progression are suggested: (*1*) the size of the tumor is stable for a long period, (*2*) the size of the tumor increases but there is no solid component, and (*3*) the size of the tumor increases and a solid component appears, which is indicative of an invasive adenocarcinoma

Table 1. Frequency of lymph node metastasis in patients with peripheral lung cancer 1 cm or less in diameter

First author (year)[Ref.]	No. of patients	pN1	pN2	Total (%)
Ohta (2001)[24]	11	3	1	4 (36%)
Miller (2002)[25]	100	5	2	7 (7%)
Asamura (2003)[23]	48	2	1	3 (6%)
Ikeda (2004)[26]	47	0	1	1 (2%)
Okada (2005)[27]	50	0	1	1 (2%)

1.1–2.0 cm in diameter and in 4 of 11 patients with adenocarcinomas ≤1.0 cm in diameter.[24] The frequency of lymph node metastasis in small lung cancers ≤1 cm in diameter is shown in Table 1.[23–27] Therefore, the tumor size alone is not a reliable indicator of the noninvasive nature of small lung adenocarcinomas.

It has been suggested that increased lymph node metastasis and pleural and vascular invasion are associated with greater scarring and fibrotic foci in tumors. A summary of correlations between CT findings and pathological features is shown in Table 2.[21,28–30] Takashima et al. reported that a lesion size of 15 mm, a GGO area >57%, and a BAC histology are correlated with favorable prognosis according to a univariate analysis, but a multivariate analysis showed that the percentage of the area showing GGO was the only independent prognos-

Table 2. Correlation between the ratio of ground-glass opacity and pathological diagnosis

First author (year)[Ref.]	GGO	Pathological findings		
		Pure BAC	Mixed subtype	Adenocarcinoma
Aoki (2001)[28]	<10%	1	42	30
	10%–50%	8	21	1
	>50%	18	6	0
		BAC <50%	BAC >50%	
Kodama (2001)[21]	<50%	36	2	
	>50%	16	50	
		Pure BAC	Mixed subtype	Adenocarcinoma
Matsuguma (2002)[30]	0%	1	1	37
	1%–25%	1	2	19
	26%–50%	1	2	6
	51%–75%	6	3	2
	76%–100%	12	2	1
		Noguchi A, B	Noguchi C	
Takashima (2002)[29]	<10%	0	7	
	10%–50%	4	14	
	>50%	31	6	

BAC, bronchioloalveolar carcinoma; GGO, ground-glass opacity

tic factor.[29] These authors also used a multivariate analysis of 52 patients with mixed subtype lung adenocarcinomas with a BAC component to demonstrate that air bronchograms and histological grading are of prognostic importance. In addition, Okada et al. reported a correlation between the tumor shadow disappearance rate, the ratio of the tumor area of the mediastinal window to that of the lung window on high-resolution CT, and the percentage of BAC histology in resected specimens of tumors <3 cm in diameter.[31] Therefore, the classification of tumors according to the percentage of the area displaying GGO seems to be suitable for selecting patients for limited surgery, as described in a subsequent section.

On the other hand, Nomori et al. used CT and positron emission tomography (PET) to quantify and describe small lesions. They used histograms of CT pixel numbers for AAH, nonmucinous BAC, and adenocarcinoma to quantify peaks and mean numbers of CT pixels. They found that the peak CT pixel number is useful for differentiating between AAH and BAC. In addition, clinical T1 N0 M0 adenocarcinomas with a low peak CT pixel number seldom have lymph node metastasis or involvement of vessels or pleura.[32,33] Positron emission tomography is now widely used as a sensitive and qualitative imaging modality to differentiate benign from malignant lung lesions. It was recently reported that PET with ^{11}C-acetate (AC) is useful for detecting slow-growing tumors such as well-differentiated hepatocellular carcinomas and prostate cancers. A study of 54 pulmonary nodules 1–3 cm in size that appeared as GGO on CT using AC-PET showed that about one-third of well-differentiated adenocarcinomas of the lung were not detected by ^{18}F-fluorodeoxyglucose-PET.[34]

Surgical Treatment

According to the only prospective, randomized trial conducted by the Lung Cancer Study Group, the appropriate surgical procedure for T1 N0 M0 non-small cell lung cancer is a lobectomy with mediastinal lymph node dissection.[35] This study demonstrated that lesser surgery such as a wedge resection or segmentectomy resulted in three times as many local recurrences as a lobectomy. However, it may not be appropriate to apply these results to the treatment of small-sized lung adenocarcinomas that exhibit GGO on CT because such tumors were not well recognized in 1990, when the study was conducted. Is lobectomy necessary for small lesions of the GGO type, particularly those <1 cm in size? Essentially, a lobectomy and hilar–mediastinal lymph node dissection should be indicated only for tumors with a present or possible risk of nodal invasion.

For small lung cancers, limited resections, including wedge resections without lymph node dissection or segmentectomy, are minimally invasive. In several studies conducted by Japanese investigators, all patients were alive and none experienced a recurrence at a median follow-up interval of 29–50 months.[36–41] A comparison between the results from limited resection and conven-

Table 3. Comparisons of limited resection with conventional lobectomy

First author (year)[Ref.]	Case	Procedure	5-year survival rate				
			Limited		Lobectomy		
			%	*n*	%	*n*	*P*
Kodama (1997)[42]	Intentional	Segmentectomy	93	46	88	77	0.86
	Compromised	Segmentectomy	48	17			0.001
Landreneau (1997)[43]		Wedge (open)	58	42	70	117	0.005
		Wedge (VATS)	65	60			0.89
Miller (2002)[25]	Size < 10 mm	Wedge	27	13	71	71	0.04
		Segmentectomy	57	12			—
Koike (2003)[44]	Size < 20 mm	Wedge	89	14	90	159	0.91
		segmentectomy		60			
Okada (2005)[27]	Size < 20 mm	Wedge	86	37	92	154	0.92
		Segmentectomy	97	129			0.91
	Size 21–30 mm	Wedge	39	19	87	268	0.0001
		Segmentectomy	85	76			0.91
Schuchert (2007)[45]		Segmentectomy	Not given	182	Not given	246	0.88

VATS, video-assisted thoracic surgery

tional lobectomy studies is shown in Table 3.[25,27,42–45] Some of these studies had satisfactory 5-year survival rates. Information on the types of lung cancer that can be resected using such procedures without increasing the local recurrence rate would help to define standard treatments for small lung cancers.

Even if a limited resection is possible, it is difficult to draw conclusions from the aforementioned studies because most of them involved a relatively short follow-up interval in a clinical situation, whereas patients are at risk of recurrence for up to 10 years. In addition, the sample sizes were relatively small, the patients were not randomized, and tumor invasions were sometimes not detected until permanent sections were viewed. There are differences of opinions about the usefulness of limited resection.[25,46] Barlesi et al. indicated that completely resected BAC is associated with a 5-year survival rate of 48–69%. Of patients who experience recurrence, 76%–95% initially recur locally, a rate higher than that for other subtypes of NSCLC.[46] It is also unclear whether a high proportion of BAC histology predicts improved survival.[9,13,14,47]

Accordingly, there is still controversy concerning the limited resection of peripheral small lung cancers, and clinical evidence is needed to demonstrate the effectiveness of limited resections for such tumors. In a recent report from the Japan Lung Cancer Surgical Study Group, a radiological noninvasive cancer (NIC) was defined as a tumor having a consolidation of less than half of the maximum tumor dimension. A pathological NIC that is a future candidate for limited surgery was defined as a tumor with no lymph node metastasis and no lymphatic or vascular invasion. The study group reviewed 545 tumors that had been resected by a lobectomy. The specificity of the diagnosis of pathological NIC was 96.4%, i.e., 3.6% of pathologic invasive cancer appeared to be noninvasive cancer on radiology.[48] Although CT findings cannot rule out the presence of invasive cancer, limited resections may be justified by the extremely low chance of invasive features in the pure GGO type of small lung cancer. Based on the results of this exploratory analysis, a prospective phase II study comparing limited resection with conventional lobectomy for peripheral lung adenocarcinomas ≤2.0 cm in size is now planned by the Japan Clinical Oncology Group.

Conclusion

With the increased use of high-resolution CT, many more small lung lesions, especially adenocarcinomas and BACs, have been detected than in the past. These types of lung lesion are distinct in terms of their pathologies, radiological findings, natural histories, and treatments.

A great deal remains to be learned about tumor biology and surgical treatment before a minimally invasive category can be defined. Comprehensive radiology–pathology correlation studies should be conducted in many countries to validate existing criteria. The features of small noninvasive lung adenocarcinomas should be clarified in clinical multicenter studies to define the optimal CT findings for a limited resection without lymph node dissection.

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BRIEF REPORT

EML4-ALK Mutations in Lung Cancer That Confer Resistance to ALK Inhibitors

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SUMMARY

The EML4 (echinoderm microtubule-associated protein-like 4)–ALK (anaplastic lymphoma kinase) fusion-type tyrosine kinase is an oncoprotein found in 4 to 5% of non–small-cell lung cancers, and clinical trials of specific inhibitors of ALK for the treatment of such tumors are currently under way. Here, we report the discovery of two secondary mutations within the kinase domain of EML4-ALK in tumor cells isolated from a patient during the relapse phase of treatment with an ALK inhibitor. Each mutation developed independently in subclones of the tumor and conferred marked resistance to two different ALK inhibitors.

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EML4-ALK IS A FUSION-TYPE PROTEIN TYROSINE KINASE THAT IS PRESENT in 4 to 5% of cases of non–small-cell lung cancer and is generated as a result of a small inversion within the short arm of human chromosome 2.[1-3] EML4-ALK undergoes constitutive dimerization through interaction between the coiled-coil domain within the EML4 region of each monomer, thereby activating ALK and generating oncogenic activity. In transgenic mice that express EML4-ALK specifically in lung epithelial cells, hundreds of adenocarcinoma nodules develop in both lungs soon after birth, and oral administration of a specific inhibitor of ALK tyrosine kinase activity rapidly eradicates such nodules from the lungs.[4] These observations reveal the essential role of EML4-ALK in the carcinogenesis of non–small-cell lung cancer harboring this fusion kinase. Furthermore, clinical trials are investigating crizotinib (PF-02341066), an inhibitor of the tyrosine kinase activity of both ALK and the met proto-oncogene (MET), for the treatment of EML4-ALK–positive non–small-cell lung cancer.

In addition to crizotinib, other tyrosine kinase inhibitors have been shown to have pronounced therapeutic activity in patients with cancer. For instance, imatinib mesylate and gefitinib, tyrosine kinase inhibitors for the c-abl oncogene 1 nonreceptor tyrosine kinase (ABL) and epidermal growth factor receptor (EGFR), improve the outcome for patients who have chronic myeloid leukemia that is positive for the BCR (breakpoint cluster region protein)–ABL fusion kinase[5] and patients who have non–small-cell lung cancer that is associated with EGFR activation,[6]

respectively. Unfortunately, however, a fraction of the target tumors are either refractory to corresponding tyrosine kinase inhibitors from the start of treatment or become resistant after an initial response.

In a case of EML4-ALK–positive non–small-cell lung cancer that became resistant to crizotinib after successful treatment for 5 months, we have discovered two de novo mutations in EML4-ALK, each of which confers resistance to the drug.

CASE REPORT

The patient was a 28-year-old man without a history of smoking who had received a diagnosis of lung adenocarcinoma, at a tumor–node–metastasis (TNM) clinical stage of T4N3M1, in April 2008. Given that the tumor did not harbor any EGFR mutations, the patient was treated with conventional chemotherapy. However, his tumor progressed after six cycles of three two-drug combinations. In November 2008, the presence of *EML4-ALK* variant 1 messenger RNA (mRNA)[1] in the tumor was confirmed by means of reverse transcription–polymerase-chain-reaction (PCR) analysis of a sputum sample. At this stage, the patient had large tumor nodules in the hilum of the right lung, multiple enlarged lymph nodes in the mediastinum, atelectasis in the right lung, and a massive effusion in the right pleural cavity (Fig. 1 in the Supplementary Appendix, available with the full text of this article at NEJM.org).

The patient was enrolled in the A8081001 study of crizotinib (ClinicalTrials.gov number, NCT00585195) on November 28, 2008, with oral administration of the drug at a dose of 250 mg twice per day. Within 1 week after the start of crizotinib treatment, his symptoms improved markedly. Although he had a partial response to the treatment, his pleural effusion was not completely eradicated (Fig. 1 in the Supplementary Appendix). After 5 months of treatment, however, the tumor abruptly started to grow again, resulting in a rapid expansion of the pleural effusion and in the development of tumors in both lungs (Fig. 1 in the Supplementary Appendix). The patient was withdrawn from the trial on May 25, 2009, and a sample of the pleural effusion in the right lung was then obtained for molecular analysis.

METHODS

DNA sequencing and characterization of the EML4-ALK mutants are described in detail in the Supplementary Appendix.

RESULTS

Because our patient's tumor resumed growth despite sustained administration of the ALK inhibitor crizotinib, we speculated that it might have acquired secondary genetic changes that confer resistance to the drug. Furthermore, given that resistance to tyrosine kinase inhibitors often results from acquired mutations within the target kinases,[7-9] we first examined the possibility that EML4-ALK itself had undergone amino acid changes.

Sputum specimens were obtained before crizotinib treatment, and pleural-effusion specimens were obtained after relapse, when treatment was stopped, for molecular analysis. Given that the proportion of tumor cells in the two types of specimens may have differed, we performed deep (high-coverage) sequencing of *EML4-ALK* complementary DNA (cDNA) derived from the specimens, using a high-throughput sequencer (Genome Analyzer II, Illumina) (Fig. 2 in the Supplementary Appendix). The sensitivity of our sequencing system, examined with the use of cDNA corresponding to the Janus kinase 3 (JAK3) amino acid mutation V674A[10] as a control, revealed that the maximum detection sensitivity was no more than one mismatched read per 6.50×10^5 total reads (Table 1 in the Supplementary Appendix).

Using deep sequencing, we detected a known single-nucleotide polymorphism, rs3795850, in the cDNA from the four specimens that were positive for *EML4-ALK* (Table 2 and Fig. 3 in the Supplementary Appendix). In addition, a T→C change at a position corresponding to nucleotide 4230 of human wild-type *ALK* cDNA (GenBank accession number, NM_004304) was detected at a low frequency (8.9%) in the sputum cDNA from our patient. Furthermore, two new alterations, G→A and C→A changes at positions corresponding to nucleotides 4374 and 4493 of wild-type *ALK* cDNA, were detected at frequencies of 41.8% and 14.0%, respectively, in the patient's pleural-effusion cDNA. There were no other recurrent alterations (present in 5% of reads) in the kinase-domain cDNA derived from any of the specimens.

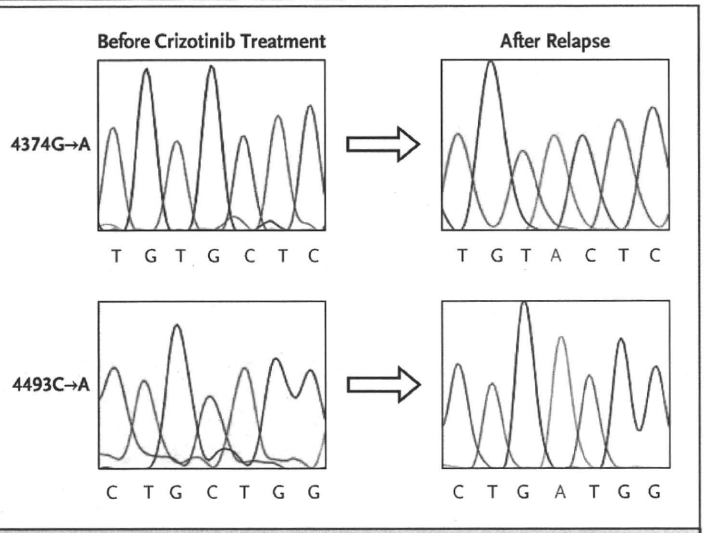

Figure 1. **Secondary Mutations within *EML4-ALK*.**
Electrophoretograms are shown for *EML4-ALK* cDNA clones prepared from sputum specimens obtained from our patient before crizotinib treatment and from pleural-effusion specimens obtained after relapse. The 4374G→A and 4493C→A mutations are present in the specimens obtained after relapse.

We next attempted to confirm these nucleotide changes by using Sanger sequencing. To rule out the possibility that the mutations had occurred in endogenous wild-type *ALK* rather than in *EML4-ALK*, we performed PCR with a forward primer targeted to *EML4* cDNA so that only the fusion cDNA would be amplified (Fig. 2 in the Supplementary Appendix). We did not detect the 4230T→C change among the 256 fusion cDNA clones derived from the patient's sputum specimens (data not shown), indicating that it was an artifact of the initial PCR or the deep-sequencing step. We did, however, readily confirm both 4374G→A and 4493C→A changes. Among 73 *EML4-ALK* cDNA clones from the patient's pleural-effusion specimens, 34 (46.6%) were positive for 4374G→A and 11 (15.1%) were positive for 4493C→A (Fig. 1). (The remaining 28 (38.4%) were negative for both point mutations.) These rates of detection are similar to those from the deep sequencing of *ALK*, indicating that wild-type *ALK* mRNA was present at a low level in lung tissue, as reported previously.[1]

The PCR analyses covered both nucleotide positions, yet none of the patient's specimens contained both mutations, indicating that each mutation occurred independently. Genomic fragments encompassing the 4374G and 4493C positions were also amplified by means of a PCR assay and were then subjected to nucleotide sequencing, which confirmed the presence of each of the two mutations in the tumor genome (Fig. 4 in the Supplementary Appendix).

The 4374G→A and 4493C→A substitutions result in cysteine→tyrosine (C→Y) and leucine→methionine (L→M) changes at the positions corresponding to amino acids 1156 and 1196, respectively, of wild-type human ALK (Fig. 2 in the Supplementary Appendix). We examined whether such amino acid changes affect the sensitivity of EML4-ALK to ALK inhibitors.

Cells of the mouse interleukin-3–dependent cell line BA/F3 that were made to individually express primary EML4-ALK and secondary mutant EML4-ALK (with the C1156Y or L1196M mutation) were exposed to ALK inhibitors. Crizotinib inhibited the growth of BA/F3 cells expressing primary EML4-ALK, in a concentration-dependent manner (Fig. 2A). In contrast, cells expressing either the C1156Y or L1196M mutant form manifested a markedly reduced sensitivity to the drug. Cells expressing the L1196M mutant form of EML4-ALK were more resistant to crizotinib than were those expressing the C1156Y mutant form (Fig. 2A, and Fig. 5 in the Supplementary Appendix).

We also examined whether cells expressing these EML4-ALK mutants are also refractory to other ALK inhibitors. A 2,4-pyrimidinediamine derivative (PDD) has a median inhibitory concentration for ALK of less than 10 nM,[11] and oral administration of PDD has been shown to eradicate lung-cancer nodules in transgenic mice with EML4-ALK expression.[4] BA/F3 cells expressing EML4-ALK with either the C1156Y or L1196M mutation were markedly less sensitive to PDD than were those expressing the primary EML4-ALK (Fig. 2A). Thus, although these mutations appear to develop during clinical treatment with crizotinib, their generation probably renders EML4-ALK resistant not only to crizotinib but also to other ALK inhibitors. In contrast to the resistance profile for crizotinib, BA/F3 cells expressing the EML4-ALK C1156Y mutant form were slightly more resistant to PDD than were those expressing the L1196M mutant form (Fig. 2A, and Fig. 6 in the Supplementary Appendix), indicating that the resistance profiles for the two mutations may be, in part, inhibitor-dependent, as was previously shown for BCR-ABL mutants.[12]

We examined tyrosine phosphorylation of

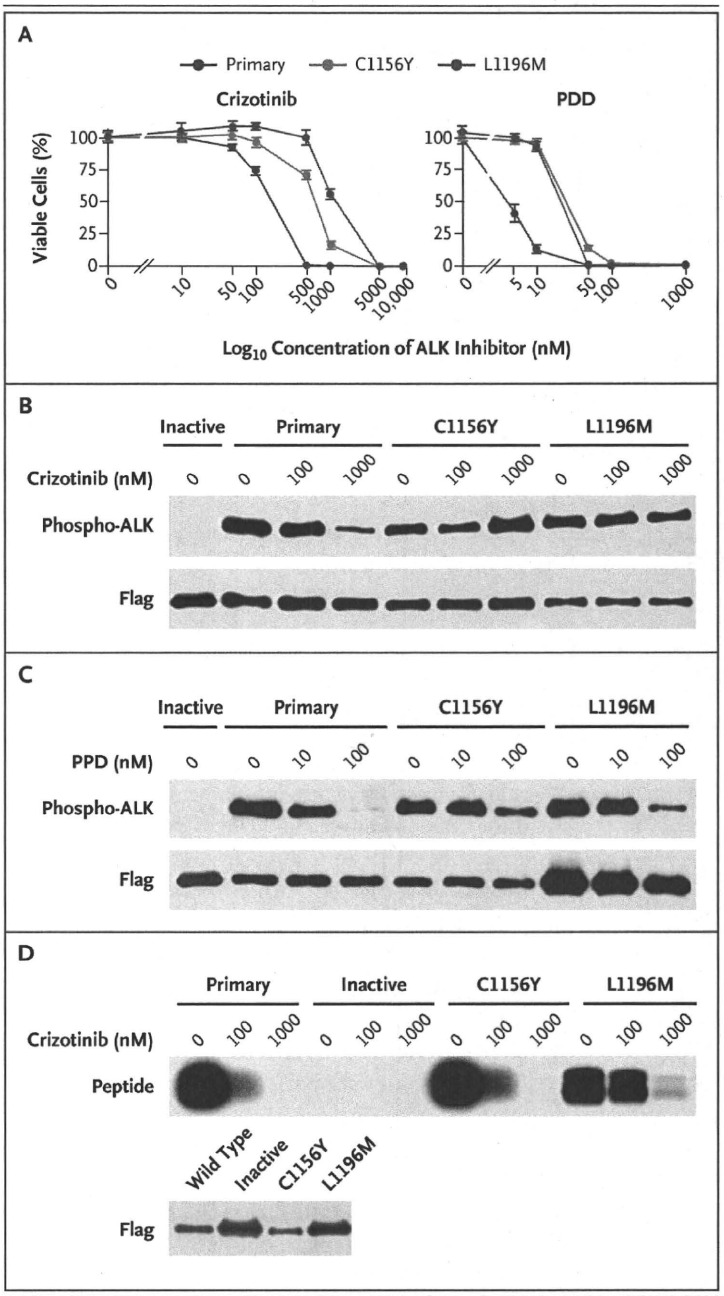

Figure 2. Properties of EML4-ALK with Secondary Mutations.

Panel A shows the percentage of viable BA/F3 cells expressing primary EML4-ALK, EML4-ALK with the C1156Y mutation, or EML4-ALK with the L1196M mutation, after 5×10^5 cells were incubated for 48 hours with the indicated concentration of crizotinib (left) or 2,4-pyrimidinediamine derivative (PDD) (right). Data are expressed as the mean value, from three separate experiments, for the percentage of cells expressing primary EML4-ALK after incubation in the vehicle (dimethyl sulfoxide) only. The I bars indicate standard deviations. Because primary EML4-ALK, EML4-ALK with the C1156Y mutation, and EML4-ALK with the L1196M mutation each abrogate the interleukin-3 dependence of BA/F3 cells, the assays were performed in the absence of the interleukin. Panels B and C show the effect of ALK inhibitors on EML4-ALK and its secondary mutant forms, tagged with the Flag epitope, in BA/F3 cells. Panel B shows the results of exposure to various concentrations of crizotinib for 15 hours, after which EML4-ALK was immunoprecipitated from cell lysates with antibodies against the Flag epitope and the immunoprecipitate was subjected to immunoblot analysis with the use of antibodies specific for ALK phosphorylated at the tyrosine at position 1604 (Phospho-ALK) or for the Flag epitope. Cells expressing an inactive mutant form of EML4-ALK were examined as a negative control. Panel C shows the results of a similar experiment, involving PDD instead of crizotinib. Panel D shows the results of an in vitro kinase assay for Flag-tagged EML4-ALK or its secondary mutants immunoprecipitated from BA/F3 cells with antibodies against the Flag epitope. The immunoprecipitates were incubated with [γ-^{32}P]ATP, a synthetic peptide, and various concentrations of crizotinib (top). Separate immunoprecipitate samples were subjected to immunoblot analysis with antibodies against the Flag epitope (bottom).

EML4-ALK by means of immunoblot analysis, using antibodies specific for ALK phosphorylated at the tyrosine at position 1604. The exposure of BA/F3 cells to crizotinib markedly inhibited the tyrosine phosphorylation of EML4-ALK but did not substantially affect that of the C1156Y and L1196M mutants (Fig. 2B). Exposure to PDD also inhibited the tyrosine phosphorylation of EML4-ALK, in a concentration-dependent manner, with a lesser effect on the mutants (Fig. 2C). The results of an in vitro kinase assay were consistent with these findings, showing pronounced inhibition of the enzymatic activity of primary EML4-ALK with crizotinib, whereas the effect on the C1156Y mutant was less pronounced and the effect on the L1196M mutant was much less pronounced (Fig. 2D).

Figure 3 shows the cysteine at position 1156 (C1156) and the leucine at position 1196 (L1196) of the kinase domain of ALK.[13] C1156 is positioned adjacent to the N-terminal of the predicted helix αC as well as close to the upper edge of the ATP-binding pocket. No activating mutations have been reported at this position in other tyrosine kinases in cancer specimens. L1196 of ALK corresponds to the threonine at position 315 in ABL and at position 790 in EGFR, each of which is the site of the most fre-

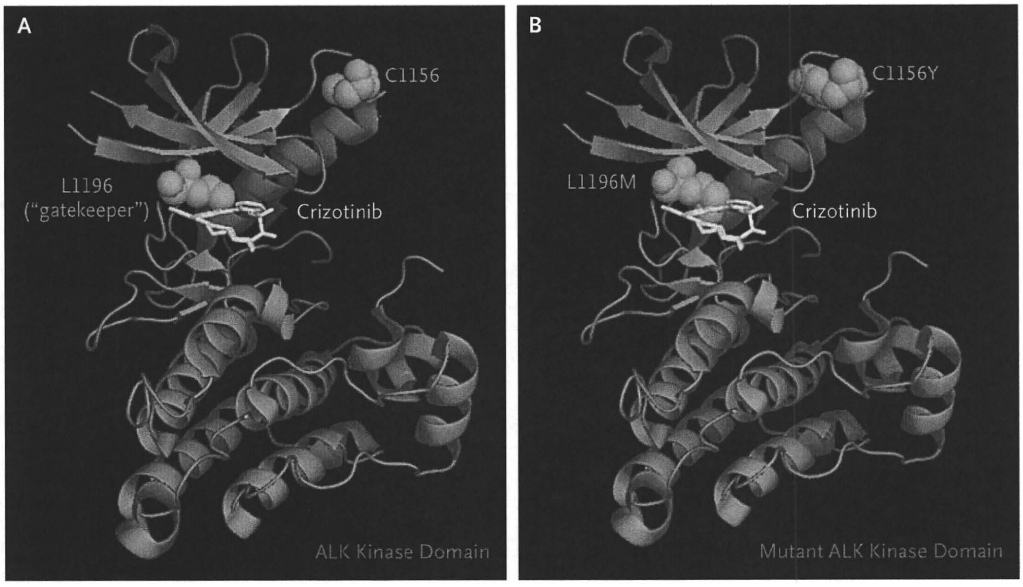

Figure 3. Predicted Crystal Structure of the Kinase Domain of ALK.
Panel A shows the nonmutant kinase domain and the site of binding of crizotinib. Panel B shows the EML4-ALK secondary mutations at positions 1156 and 1196. Adapted from Bossi and colleagues.[13]

quently acquired mutations that confer resistance to tyrosine kinase inhibitors in these kinases (Fig. 7 in the Supplementary Appendix).[14,15] This site is located at the bottom of the ATP-binding pocket (Fig. 3), and the presence of an amino acid with a bulky side chain at this "gatekeeper" position may interfere with the binding of many tyrosine kinase inhibitors.[7,16]

DISCUSSION

We identified two de novo mutations within the kinase domain of EML4-ALK from the tumor of a single patient that confer resistance to multiple ALK inhibitors. Given that we did not detect any *EML4-ALK* cDNA harboring both mutations, we propose that each mutation developed independently in distinct subclones of the tumor. Because we were not able to examine pleural-effusion specimens from the patient before he received crizotinib treatment, we do not know whether the resistant clones were present initially or developed secondarily, during the treatment.

Amino acid substitutions at the gatekeeper position of several tyrosine kinases have been detected in tumors treated with tyrosine kinase inhibitors (Fig. 7 in the Supplementary Appendix).[7-9,17,18] Whereas no mutations at this site have previously been reported for EML4-ALK or ALK, the effects of various artificial amino acid substitutions at the gatekeeper position of nucleophosmin (NPM)–ALK, another fusion-type "oncokinase" form of ALK, were recently examined.[19] The findings were consistent with the results of our analysis of tumor cells in vivo: the introduction of methionine at this position rendered NPM-ALK resistant to ALK inhibitors. It is therefore likely that gatekeeper alterations constitute a universal mechanism for the acquisition of tyrosine kinase–inhibitor resistance in oncogenic tyrosine kinases.

In contrast to gatekeeper substitutions, activating mutations at the position adjacent, on the N-terminal side, to the αC helix (e.g., C1156 in ALK) have not been confirmed for other tyrosine kinases in cancer specimens. Though a T→I change at the corresponding position of EGFR was described in one case of non–small-cell lung cancer, its relevance to drug sensitivity was not examined.[16] The importance of helix αC for allosteric regulation of enzymatic activity has been shown, however, for serine–threonine kinases.[20] A change at C1156 of ALK might therefore interfere allosterically with the binding of tyrosine

kinase inhibitors. Determination of the crystal structure of the ALK kinase domain with the C1156Y or L1196M mutation should shed light on these matters, as well as provide a basis for the development of next-generation ALK inhibitors that may effectively eradicate tumors harboring EML4-ALK with the acquired mutations.

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Disclosure forms provided by the authors are available with the full text of this article at NEJM.org.

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