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ORIGINAL ARTICLE

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Phase I study of weekly cisplatin, vinorelbine, and concurrent thoracic radiation therapy in patients with locally advanced non-small-cell lung cancer

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Abstract
Background. The combination of chemotherapy and thoracic radiation therapy (TRT) is considered as a standard treatment for locally advanced non-small-cell lung cancer (NSCLC). Although the frequent interaction of anticancer agents and irradiation may produce stronger radio-sensitizing effects, the daily administration of these agents is complicated. We therefore used weekly administration of these agents, and conducted a phase I study of weekly cisplatin, vinorelbine, and concurrent TRT. The purpose of this study was to identify the maximum tolerated dose (MTD), the dose-limiting toxicity (DLT), and the recommended dose of this treatment.
Methods. Patients with locally advanced NSCLC were enrolled in this study. Both cisplatin and vinorelbine were given intravenously on a weekly schedule for 6 weeks, starting on the first day of TRT, i.e., on days 1, 8, 15, 22, 29, and 36. The total dose of TRT was 60 Gy. The dose of cisplatin was fixed at 20 mg/m^2 per week. The starting dose of vinorelbine was 15 mg/m^2 per week (dose level 1).
Results. Nine patients were enrolled in this study. All three patients at dose level 1 experienced DLTs. We decreased the dose of vinorelbine to 10 mg/m^2 per week (dose level 0). Two of the six patients at dose level 0 experienced DLTs. Therefore, dose level 1 was considered as the MTD, and dose level 0 as the recommended dose. The DLTs of this treatment were esophagitis, fatigue, infection, and hyponatremia.
Conclusion. The recommended dose of cisplatin is 20 mg/m^2 per week and that of vinorelbine is 10 mg/m^2 per week with standard TRT. A phase II study of this treatment is warranted.

Key words Cisplatin · Vinorelbine · Chemoradiotherapy · Non-small-cell lung cancer

Introduction

Lung cancer is a leading cause of cancer mortality in Western industrialized countries.[1] Approximately 80% of lung cancer is of the non-small-cell histologic type, such as squamous cell carcinoma, adenocarcinoma, and large cell carcinoma. Surgery, if possible, is the mainstay of treatment for patients with non-small-cell lung cancer (NSCLC); however, the majority of NSCLC is considered as unresectable due to the local or systemic spread of the cancer. Approximately 30% of NSCLC is locally advanced, unresectable stage IIIA or IIIB disease. The American Society of Clinical Oncology (ASCO) published their guideline (update 2003) for the treatment of unresectable NSCLC.[2] This guideline recommends the following treatment for locally advanced NSCLC: chemotherapy in association with definitive thoracic irradiation is appropriate for selected patients with unresectable, locally advanced NSCLC; radiation therapy should be included as part of the treatment for selected patients with unresectable locally advanced NSCLC; and chemotherapy given to

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NSCLC patients should be a platinum-based combination regimen.

The combination of cisplatin and vinorelbine is more effective than single-agent cisplatin, or cisplatin plus vindesine, for advanced NSCLC.[3,4] Furthermore, some randomized trials have shown that cisplatin plus vinorelbine is as effective as carboplatin plus paclitaxel, cisplatin plus gemcitabine, or cisplatin plus irinotecan.[5–7] Therefore, the cisplatin plus vinorelbine combination is considered as one of the standard platinum-based chemotherapy regimens.

There are two possible advantages of the combination of chemotherapy and radiation therapy. One is spatial cooperation (which means that radiation is effective against the loco-regional tumor, and chemotherapy eradicates micrometastases independently) and the other is the radiosensitizing effects.[8–10] Cisplatin is one of the anticancer agents whose radio-sensitizing effects have been studied extensively, and many preclinical studies have shown that cisplatin enhanced the cytotoxic effects of irradiation.[11] The European Organization for Research and Treatment of Cancer (EORTC) performed a randomized trial comparing the following three arms: thoracic radiation therapy (TRT) alone, TRT combined with weekly cisplatin, and TRT combined with daily cisplatin, for locally advanced NSCLC.[12] The survival rate was 54% at 1 year, 26% at 2 years, and 16% at 3 years for the TRT+daily-cisplatin group, as compared with 44%, 19%, and 13% for the TRT+weekly-cisplatin group, and 46%, 13%, and 2% for the TRT-alone group, respectively. The EORTC concluded that TRT+daily cisplatin had the greatest survival benefit of the three treatment arms and this benefit was due to the improvement of local control. On the other hand, some preclinical studies have shown that vinorelbine also had radio-sensitizing effects.[13–15] Vinorelbine is a potent inhibitor of mitotic microtubule polymerization, and this effect synchronizes cells at the G2/M phase of the cell cycle. This phase is considered as the most radio-sensitive phase; thus, vinorelbine can exhibit radio-sensitizing effects.

Although the frequent interaction of anticancer agents and irradiation may produce stronger radio-sensitizing effects, daily administration of these agents is complicated. Weekly administration is more convenient than daily administration. Therefore, we conducted a phase I study of weekly cisplatin, vinorelbine, and concurrent TRT for locally advanced NSCLC. The purpose of this study was to identify the maximum tolerated dose (MTD), the dose-limiting toxicity (DLT), and the recommended dose of this treatment.

Patients and methods

Eligibility criteria

Patients with histologically or cytologically confirmed locally advanced NSCLC were enrolled in this study. All patients were deemed suitable for definitive TRT by a radiation oncologist (T.T.). Other eligibility criteria included the following: age, 20 years or older; Eastern Cooperative Oncology Group (ECOG) performance status, 0 or 1; unresectable stage IIIA or IIIB; absence of malignant pleural or pericardial effusion; absence of involvement of contralateral hilar lymph nodes; no prior chemotherapy or TRT; adequate bone marrow function (leukocyte count ≥4000/μl, neutrophil count ≥2000/μl, hemoglobin level ≥10 g/dl, and platelet count ≥100000/μl), renal function (creatinine level ≤ upper limit of normal and creatinine clearance ≥50 ml/min), hepatic function (aspartate aminotransferase/alanine aminotransferase [AST/ALT] ≤ twice upper limit of normal and bilirubin level ≤ upper limit of normal), and pulmonary function (arterial partial pressure of oxygen [PaO_2] ≥70 mmHg); absence of interstitial pneumonitis or pulmonary fibrosis, or other serious illnesses; and no pregnancy or lactation. Written informed consent was obtained from all patients. This protocol was approved by the institutional review board of Osaka Prefectural Medical Center for Respiratory and Allergic Diseases. All patients received the protocol treatment at the same institution.

Chemotherapy

Both cisplatin and vinorelbine were given intravenously on a weekly schedule for 6 weeks, starting on the first day of TRT, i.e., on days 1, 8, 15, 22, 29, and 36 (Fig. 1). The doses of cisplatin and vinorelbine are described later. Cisplatin was administered as a 60-min infusion with adequate hydration (at least 1000 ml of fluid). Antiemetic drugs, such as 5-hydroxytryptamine type 3 ($5\text{-}HT_3$) receptor antagonists and dexamethasone 8 mg, were given intravenously before the administration of cisplatin. Vinorelbine was administered as a 5-min infusion. The minimum requirements for the administration of cisplatin and vinorelbine were as follows: leukocyte count 2000/μl or more, neutrophil count 1000/μl or more, platelet count 50000/μl or more, nonhematological toxicity grade 2 or less, and no suspension of TRT.

Subsequently, consolidation chemotherapy was given, starting 1 week after the completion of irradiation. If creatinine clearance was 60 ml/min or greater, cisplatin 80 mg/m^2 was given intravenously as a 60-min infusion on day 1 and vinorelbine 20 mg/m^2 was given intravenously as a 5-min infusion on days 1 and 8 of a 3-week cycle. Standard hydration and antiemetics were also given. If creatinine clearance was less than 60 ml/min, vinorelbine 25 mg/m^2 was given intravenously as a 5-min infusion on days 1, 8, and 15 of a 4-

Fig. 1. Treatment schedule of weekly cisplatin, vinorelbine, and concurrent thoracic radiation therapy

Discussion

Some randomized trials and meta-analyses have shown that the combination of chemotherapy and TRT has survival benefits compared with TRT alone for locally advanced NSCLC.[12,19–26] However, long-term survival was rare, with a median survival of 12 to 13.7 months and a 5-year survival rate of only 8% to 17%.

The West Japan Lung Cancer Group conducted a phase III study to compare concurrent chemoradiotherapy with sequential therapy.[27] The chemotherapy consisted of cisplatin, vindesine, and mitomycin, and TRT delivered a total of 56 Gy. The median survival in the concurrent arm was significantly longer than that in the sequential arm (16.5 versus 13.3 months). The Radiation Therapy Oncology Group (RTOG) and a Czech group conducted similar randomized trials and confirmed the superiority of the concurrent therapy over the sequential therapy.[28,29] Furthermore, Choy et al.[30] conducted a randomized phase II study of three regimens: sequential chemoradiotherapy versus induction chemotherapy followed by concurrent chemoradiotherapy versus concurrent chemoradiotherapy followed by consolidation chemotherapy; this was the so-called locally advanced multimodality protocol (LAMP) study. They used the combination of paclitaxel, carboplatin, and TRT. The median survival was 12.5 months for the sequential arm, 11 months for the induction/concurrent arm, and 16.1 months for the concurrent/consolidation arm. These results suggested that concurrent chemoradiotherapy, or possibly concurrent chemoradiotherapy followed by consolidation chemotherapy, was the most effective treatment in patients with locally advanced NSCLC. However, it is undetermined what regimen or what schedule is optimal for chemoradiotherapy.

Several schedules and doses of cisplatin, vinorelbine, and concurrent TRT have been reported. Masters et al.[31] recommended that cisplatin should be administered at 80 mg/m^2 on day 1 and vinorelbine at 15 mg/m^2 on days 1 and 8 every 3 weeks with standard TRT. After that, the Cancer and Leukemia Group B (CALGB) performed a randomized phase II study of cisplatin with gemcitabine or paclitaxel or vinorelbine as induction chemotherapy followed by concomitant chemoradiotherapy.[32] In the cisplatin-vinorelbine arm, the doses reported by Masters et al.[31] were used, and the CALGB concluded that induction chemotherapy followed by concomitant chemoradiotherapy was feasible, with the observed survival rates exceeding those of previous CALGB trials for all treatment arms. Sekine et al.[33] conducted a phase I study and reported that the recommended dose of cisplatin was 80 mg/m^2 on day 1 and that of vinorelbine was 20 mg/m^2 on days 1 and 8 every 4 weeks with TRT including a 4-day interval. The Czech group[29] used the following schedule and dose: cisplatin 80 mg/m^2 on day 1 and vinorelbine 12.5 mg/m^2 on days 1, 8, and 15 every 4 weeks with standard TRT starting on day 4.

Although the frequent interaction of anticancer agents and irradiation may produce stronger radio-sensitizing effects, there has been no report of a weekly schedule to date. Therefore we conducted a phase I study of weekly cisplatin and vinorelbine with standard TRT. The studies described above[29,31–33] reported that esophagitis and neutropenia were the major toxicities. The present study showed that the DLTs of our regimen were esophagitis, fatigue, infection, and hyponatremia. All patients at dose level 1 experienced grade 3 esophagitis, so this dose was considered an overdose. The strong radio-sensitizing effects may have resulted in the severe esophagitis. On the other hand, no severe neutropenia was observed. The recommended dose of cisplatin is 20 mg/m^2 per week and that of vinorelbine is 10 mg/m^2 per week in the present study.

The response rate and the median overall survival in this study were 56% and 11.9 months, respectively. Some concurrent chemoradiotherapy studies have reported better results, with response rates of 63% to 85% and median overall survivals of 11 to 18.3 months.[12,27–30,32] As our study had a very small sample size, of only nine patients, we cannot draw conclusions on the efficacy of this treatment from our present results.

In conclusion, our phase I study of weekly cisplatin, vinorelbine, and concurrent TRT showed that the DLTs of this treatment were esophagitis, fatigue, infection, and hyponatremia. The recommended dose of cisplatin is 20 mg/m^2 per week and that of vinorelbine is 10 mg/m^2 per week, i.e., on days 1, 8, 15, 22, 29, and 36, with standard TRT. We believe a phase II study of this treatment is warranted.

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Angled Forceps Used for Transbronchial Biopsy in Which Standard Forceps Are Difficult To Manipulate*

A Comparative Study

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Objectives: **To evaluate the usefulness of the Sasada transbronchial angled forceps (STAF) in patients with peripheral pulmonary lesions (PPLs), which are difficult to manipulate with standard forceps.**

Methods: **We have invented the STAF, a forceps with an angled tip. One hundred ten patients with PPLs that were difficult to reach with standard forceps were retrospectively evaluated. The patients first underwent bronchoscopy with a standard forceps and then with the STAF. The specimens obtained with standard forceps and those obtained with STAF were separately fixed and analyzed histologically. We compared the histologic diagnosis of the specimens obtained by STAF with that obtained by the specimens obtained with standard forceps. Statistical significance was calculated with the McNemar χ^2 statistic.**

Results: **The diagnostic yield of all lesions from the specimens obtained with STAF (86 of 110 lesions; 78.2%) was significantly higher than that of lesions from the specimens obtained with standard forceps (43 of 110 lesions; 39.1%; $p < 0.001$). Among malignant lesions, the yield obtained with STAF (60 of 72 lesions; 83.3%) was significantly higher than that obtained with standard forceps (32 of 72 lesions; 44.4%; $p < 0.001$). Among benign lesions, the yield obtained with STAF (26 of 38 lesions; 68.4%) was also significantly higher than that obtained with standard forceps (11 of 38 lesions; 28.9%; $p < 0.001$). Among the different lesion areas, the right upper lobe plus the left upper division gave the greatest difference in yield (STAF, 46 of 60 lesions; 76.7%; standard forceps, 22 of 60 lesions; 36.7%; $p < 0.001$). Among the different size ranges, the diagnostic yields obtained with STAF were significantly higher than that obtained with standard forceps except for the size range of ≤ 10 mm. There were two complications, pneumothorax and bronchial bleeding, both of which were controlled easily.**

Conclusions: **The STAF was shown to be useful for obtaining specimens that were sufficient for histologic diagnosis from PPLs that were difficult to manipulate with standard forceps.**

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Key words: angled forceps; peripheral pulmonary lesions; transbronchial biopsy

Abbreviations: CS = curve-shaped; CTGNB = CT scan-guided needle biopsy; NSCLC = non-small cell lung cancer; PPL = peripheral pulmonary lesion; STAF = Sasada transbronchial angled forceps; TBB = transbronchial biopsy; VATS = video-assisted thoracic surgery

Since the 1970s, transbronchial biopsy (TBB) of the lung performed through a flexible bronchoscope has gained wide acceptance and has become the most common method of performing lung tissue biopsy.[1–4] The numbers of patients with peripheral pulmonary lesions (PPLs) have increased along with the incidence of lung adenocarcinoma.[5,6] Patients in whom a diagnosis cannot be made by flexible fiberoptic bronchoscopy need to undergo CT scan-guided needle biopsy (CTGNB) or video-assisted thoracic surgery (VATS).[7,8] However, CTGNB is associated with critical complications, including air embolism

and pleural dissemination.[9,10] On the other hand, in patients with poor performance status or in elderly patients, VATS is not always performed. Therefore, TBB with a flexible bronchoscope is still the recognized first-choice procedure used to diagnose PPLs. Nevertheless, we have experienced diagnostic failure with PPLs, even though PPLs can be visualized by radiographic fluoroscopy. With such lesions, conventional straight forceps are difficult to reach, and we cannot obtain a sufficient amount of material for histologic diagnosis. To solve this problem, we invented the Sasada transbronchial angled forceps (STAF). Our experience with 110 patients is reported in the present study.

Materials and Methods

Patient Eligibility

We enrolled patients with PPLs that had been visualized by radiographic fluoroscopy and were difficult to manipulate by using standard forceps. Difficult-to-manipulate lesions presented in some situations as follows: the forceps could not really reach them, could hardly reach them, or could barely reach them. Such lesions were defined as *difficult PPLs*, and they mainly included solitary pulmonary lesions and mediastinum-involved tumors. Patients with diffuse pulmonary lesions or invasive shadows were excluded from the study. We judged the eligibility of the patient during bronchoscopy.

Study Design

The study was designed to retrospectively evaluate the usefulness of TBB with STAF in patients with difficult PPLs. Patients with difficult PPLs first underwent bronchoscopy with a standard forceps and then with STAF. The specimens obtained with standard forceps and those obtained with STAF were separately fixed and analyzed histologically. When either forceps absolutely could not reach a lesion that had been visualized by radiographic fluoroscopy, we did not perform a biopsy for safety reasons. Both specimens were diagnosed by two pathologists. We compared the histologic diagnosis obtained from the specimens by STAF with that from the specimens obtained by standard forceps. Cytologic and bacterial examinations were excluded in this study, because technical contamination was possible and could have caused misdiagnosis. Informed consent was obtained from all patients prior to undergoing the procedure.

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Equipment

We have invented a new forceps, called STAF (HBF-2010SH; Machida; Tokyo, Japan), that has an angled tip for obtaining adequate amounts of tissue from PPLs for histologic diagnosis. The structure of STAF is basically the same as that of standard forceps. STAF has a 12° angle 10 mm from the tip, so that the tip is perpendicular to the direction of the opening and shutting of the cup (Fig 1). STAF is a reusable product that can be fitted to any standard bronchoscope with channels having a diameter of $\geq$ 2.2 mm (*eg*, BF 1T-30, 40, or 1T-200, 240; Olympus; Tokyo, Japan). The cost is almost the same as that of standard forceps.

A New Biopsy Technique

To obtain adequate amounts of tissue for diagnosis, we invented a new biopsy technique called curve-shaped (CS) TBB. The CS-TBB method consists of five steps (Fig 2), which are clearly different from those of the conventional TBB method. First, we use standard forceps to identify the bronchus nearest to the lesion. Second, we switch to STAF and open the cup in front of the lesion. We search for a part of the lesion while letting the STAF turn and slide. Next, we make a CS motion by operating the bronchoscope, enabling a more perpendicular approach to the lesion. Finally, we push the forceps forward and perform the biopsy.

Statistical Analysis

The proportion of positive samples using STAF was compared with the proportion of positive samples using standard forceps. The patients who underwent bronchoscopy using STAF were exactly the same as those who underwent bronchoscopy using standard forceps. The statistical significance was calculated with the McNemar χ^2 statistic. A difference with a p value of < 0.05 was considered to be significant. The statistical analysis software was used for the analysis.

Representative Cases

Case 1

A 25-year-old woman had a 23-mm lesion in the right upper lobe (Fig 3, *top, A*). Bronchoscopy was performed to confirm the diagnosis of the lesion. TBB with standard forceps failed to obtain specimens through the right B1a because the forceps could not reach the mass (Fig 3, *bottom left, B*). However, TBB with STAF succeeded in obtaining a sufficient amount of tissue for the specimens (Fig 3, *bottom right, C*), and the diagnosis of tuberculosis was histologically confirmed. The culture from the specimen was negative for tuberculosis.

Case 2

A 44-year-old man had a 30-mm lesion in the left lower lobe (Fig 4, *left, A*). Bronchoscopy was performed, and TBB performed with standard forceps failed to obtain specimens through the left B10e. However, TBB with performed STAF succeeded in obtaining a sufficient amount of tissue from the specimens to confirm a diagnosis of hamartoma (Fig 4, *right, B*).

Case 3

A 54-year-old man had a mediastinum-involved tumor in the left lung (Fig 5, *left, A*). Only STAF was able to reach the lesion

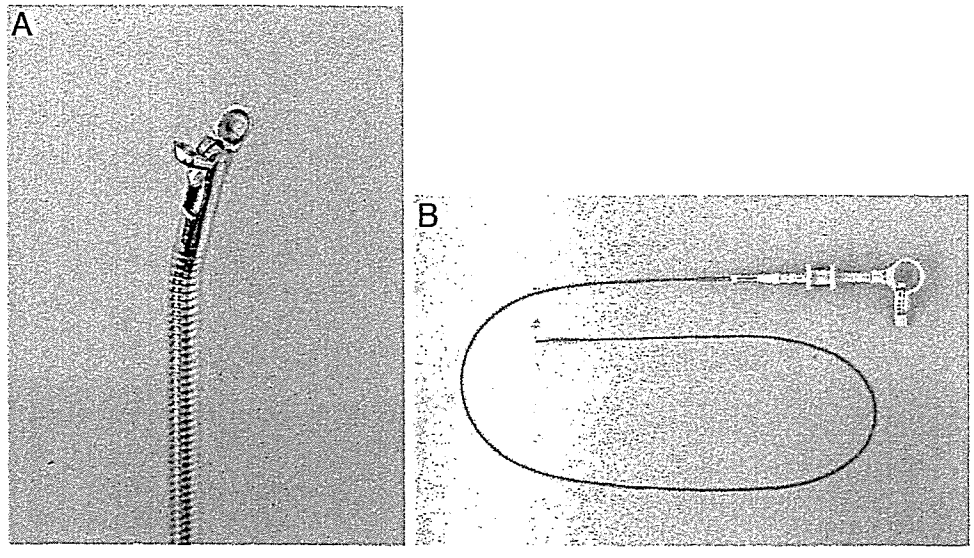

FIGURE 1. STAF. *Left, A*: Open position; the STAF has an angled tip. *Right, B*: the general shape of the STAF is almost the same as that of a standard forceps except for the angled tip.

(Fig 5, *right, B*), and a sufficient amount of tissue was obtained for the specimens to confirm a diagnosis of non-small cell lung cancer (NSCLC).

Case 4

A 74-year-old man had a 15-mm nodule in the right upper lobe (Fig 6, *top, A*). Specimens were obtained with standard forceps and STAF through the right B3a. The specimens obtained with standard forceps revealed only a normal bronchial wall (Fig 6, *bottom left, B*); in contrast, the specimens obtained with STAF revealed adenocarcinoma (Fig 6, *bottom right, C*).

Case 5

A 34-year-old man had a 14-mm nodule in the right lower lobe (Fig 7, *top, A*). Specimens were obtained with standard forceps and STAF through the right B6b. The specimens obtained with standard forceps were insufficient for pathological diagnosis, revealing only normal bronchial wall (Fig 7, *bottom left, B*). However, the specimens obtained with STAF revealed necrotizing epithelioid granuloma (Fig 7, *bottom right, C*), which suggested pulmonary tuberculosis. TBB with STAF succeeded in obtaining a specimen large enough for histologic examination.

RESULTS

One hundred ten consecutive patients with difficult PPLs who underwent bronchoscopy between August 2001 and July 2004 at the Osaka Prefectural Medical Center for Respiratory and Allergic Diseases were enrolled into the study. Of the 110

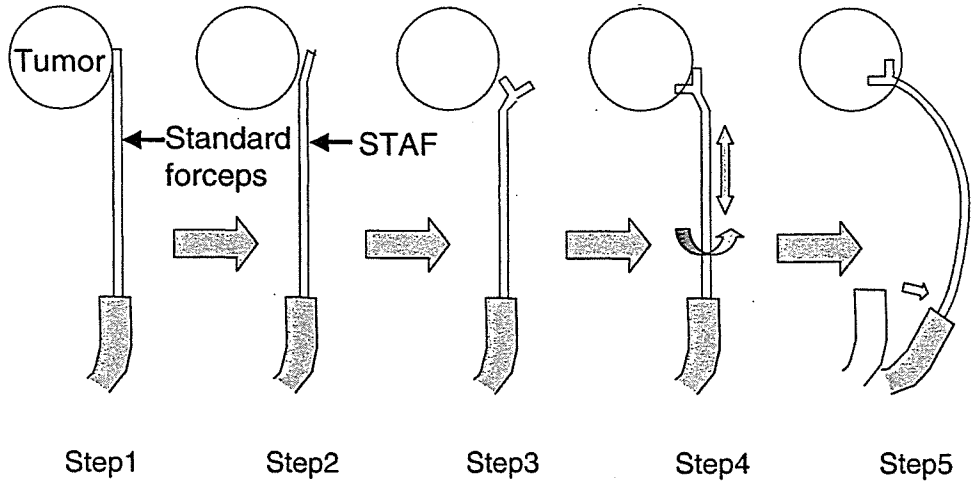

FIGURE 2. Method of performing CS-TBB. Step 1: search for the bronchus nearest to the lesion by using a standard forceps. Step 2: switch to STAF. Step 3: open the cup in front of the lesion. Step 4: turn and slide the STAF at the same time, and push forward. Step 5: make a CS motion by operating the bronchoscope, and obtain some tissue.

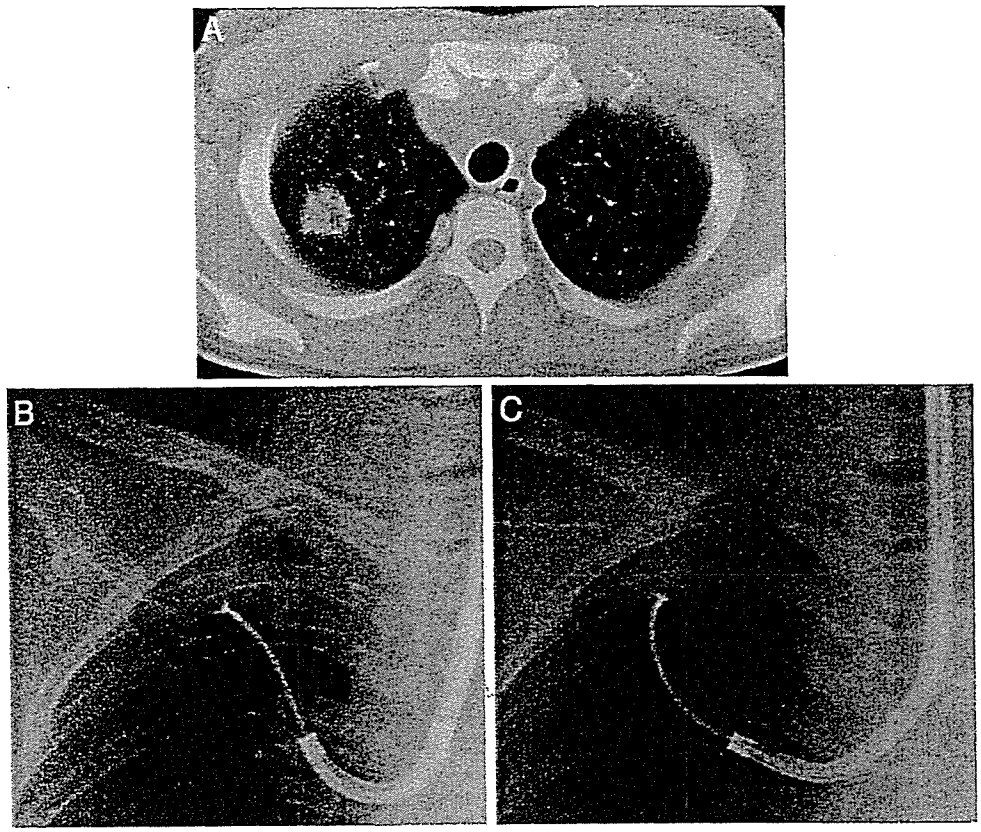

FIGURE 3. Case 1. A tuberculoma. *Top, A*: a chest CT scan reveals a coin lesion in the right upper lobe. *Bottom left, B*: a bronchoscopic image shows a standard forceps approaching a tumor through the right B1a. *Bottom right, C*: bronchoscopic image shows the STAF approaching the tumor. It forms a CS (*ie*, J-shape).

patients, 64 were men and 46 were women. The median age of the group was 67 (range, 25 to 86). The median size of the longest diameter of a lesion in the group was 20 mm (range, 6 to 60 mm). The longest diameters of all lesions revealed by chest CT scan were recorded.

Table 1 shows the diagnostic yields, and Table 2 shows the pathologic diagnoses in the 110 patients. The diagnostic yield of all lesions from the specimens obtained with STAF (86 of 110 lesions; 78.2%) was significantly higher than the that of lesions from the specimens obtained with standard forceps (43 of 110

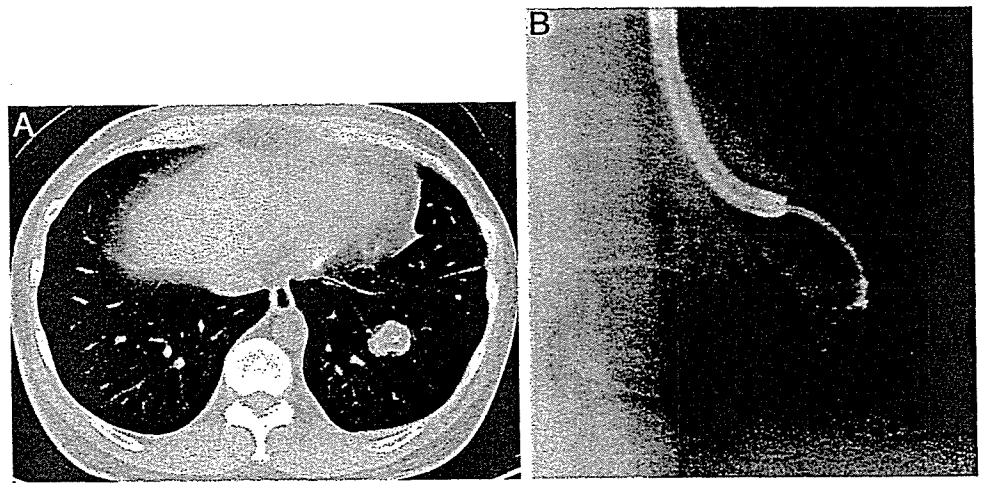

FIGURE 4. Case 2. A hamartoma. *Left, A*: a chest CT scan reveals a lesion in the left lower lobe. *Right, B*: a bronchoscopic image shows the STAF approaching a tumor through the left B10e. It forms a CS (*ie*, S-shape).

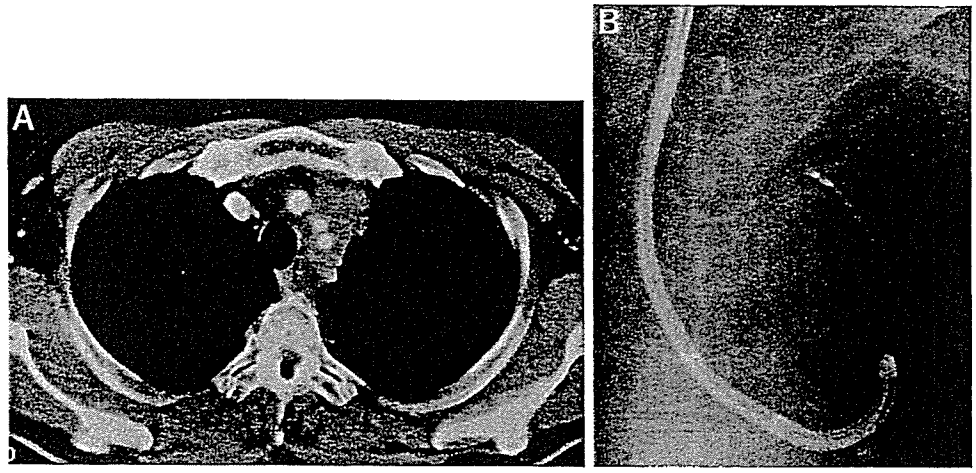

FIGURE 5. Case 3: NSCLC. *Left, A*: a chest CT scan reveals a mediastinum-involved tumor shadow on the left side. *Right, B*: a bronchoscopic image shows the STAF approaching the tumor through the left B3c.

lesions; 39.1%; $p < 0.001$). In malignant lesions, the yield obtained with STAF (60 of 72 lesions; 83.3%) was significantly higher than that obtained with standard forceps (32 of 72 lesions, 44.4%; $p < 0.001$). The pathologic diagnoses and the yields obtained with STAF included the following: adenocarcinoma, 86% (43 of 50 lesions); squamous cell carcinoma, 90% (9 of 10 lesions); small cell carcinoma, 100% (2 of 2 lesions); undifferentiated carcinoma, 80% (4 of 5 lesions); metastasis, 50% (2 of 4 lesions); and carcinoid tumor, 0% (0 of 1 lesion). In benign lesions, the yield obtained with STAF (26 of 38 lesions; 68.4%) was significantly higher than that obtained with standard forceps (11 of 38 lesions;

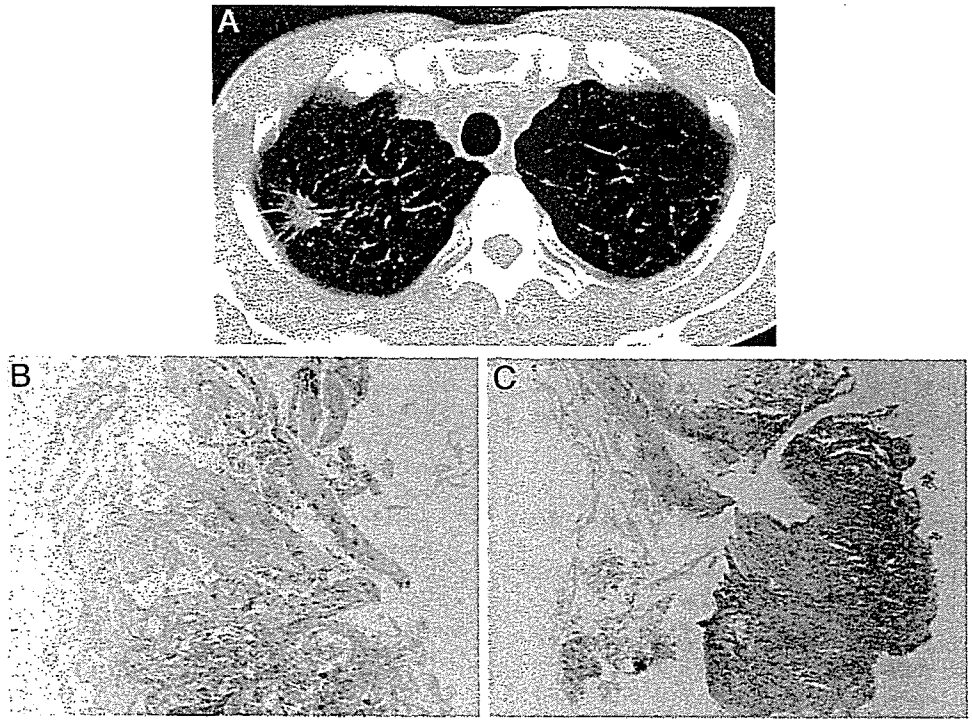

FIGURE 6. Case 4: a lung adenocarcinoma. *Top, A*: a chest CT scan reveals a coin lesion in the right upper lobe. *Bottom left, B*: the biopsy specimen obtained with standard forceps through the right B3a reveals only normal bronchial wall (hematoxylin-eosin, original ×40). *Bottom right, C*: STAF biopsy specimen reveals adenocarcinoma (hematoxylin-eosin, original ×40).

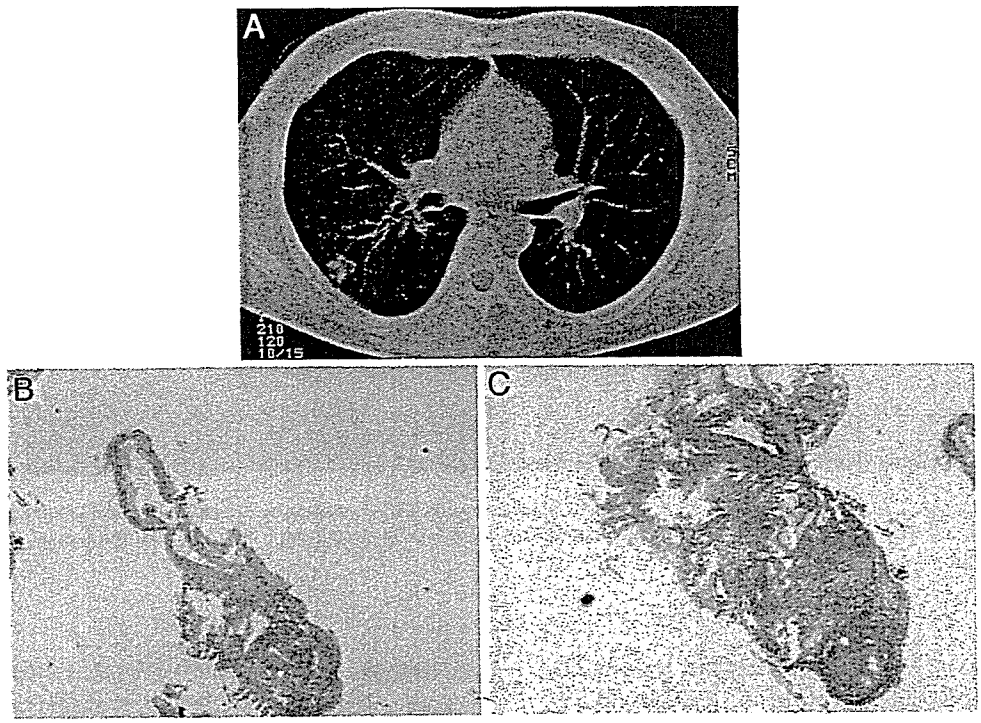

FIGURE 7. Case 5: a tuberculoma. *Top, A*: a chest CT scan reveals a coin lesion in the right lower lobe. *Bottom left, B*: the biopsy specimen obtained with standard forceps through the right B6b reveals a normal bronchial wall (hematoxylin-eosin, original ×40). *Bottom right, C*: STAF biopsy specimen reveals necrotizing epithelioid granuloma (hematoxylin-eosin, original ×40).

28.9%; $p < 0.001$). The diagnoses and yields obtained with STAF included the following: nonspecific inflammation, 57.1% (8 of 14 lesions); mycobacteriosis, 77.8% (7 of 9 lesions); hamartoma, 50% (4 of 8 lesions); organizing pneumonia, 100% (4 of 4 lesions); cryptococcosis, 100% (2 of 2 lesions); and lung abscess, 100% (1 of 1 lesion). Three patients underwent a second bronchoscopy when the first procedure failed to yield a specific diagnosis. Two of the specimens obtained in the second bronchoscopy resulted in a diagnosis.

Table 3 shows the diagnostic yield for each lesion area. Among the different lesion areas, the right upper lobe plus the left upper division gave the greatest difference in yield (STAF, 46 of 60 lesions [76.7%]; standard forceps, 22 of 60 lesions [36.7%]; $p < 0.001$). Table 4 shows the diagnostic yield for each size range. Among the different size ranges, the diagnostic yields obtained with STAF were significantly higher than that obtained with standard forceps except for those with a size of ≤ 10 mm.

Of 24 patients in whom a diagnosis could not be established, 11 were operated on. Diagnosis was

Table 1—*Diagnostic Yields From PPLs Biopsied With STAF and Standard Forceps*

	STAF		Standard Forceps		
Variables	No./Total No.	%	No./Total No.	%	p Value*
Positive sample	86/110	78.2	43/110	39.1	< 0.001
Malignant	60/72	83.3	32/72	44.4	< 0.001
Benign	26/38	68.4	11/38	28.9	< 0.001

*By McNemar test.

Table 2—*Clinical Diagnosis of PPLsin 110 Patients Who Underwent Bronchoscopy With STAF*

Lesions	No./Total No. (%)
Malignant	
Adenocarcinoma	43/50 (86)
Squamous cell carcinoma	9/10 (90)
Small cell carcinoma	2/2 (100)
Undifferentiated carcinoma	4/5 (80)
Metastasis	2/4 (50)
Carcinoid	0/1 (0)
Total	60/72 (83.3)
Benign	
Nonspecific inflammation	8/14 (57.1)
Mycobacteriosis	7/9 (77.8)
Hamartoma	4/8 (50)
Organizing pneumonia	4/4 (100)
Cryptococcosis	2/2 (100)
Lung abscess	1/1 (100)
Total	26/38 (68.4)

Table 3—*Effect of Lesion Area on Diagnostic Yield of PPLs Obtained With STAF and Standard Forceps**

Lesion Area	STAF No./Total No.	STAF %	Standard Forceps No./Total No.	Standard Forceps %	p Value†
RUL + LUD	46/60	76.7	22/60	36.7	< 0.001
RML + Lingula	13/17	76.5	5/17	29.4	0.004
Lower superior (B6)	10/11	90.9	4/11	36.4	0.014
Lower basal	17/22	77.3	12/22	54.5	0.025

*RUL = right upper lobe; LUD = left upper division; RML = right middle lobe.
†By McNemar test.

made by transbronchial needle aspiration in two further patients. In 1 patient diagnosis was made by transcutaneous needle biopsy, and in 10 further patients it was made by serial observations. There were two complications (pneumothorax and bronchial bleeding), both of which were controlled easily.

Discussion

Flexible fiberoptic bronchoscopy is routinely used for the diagnosis of PPLs. Flexible fiberoptic bronchoscopy in conjunction with TBB, bronchial brushing, or bronchial washing cytology has given physicians an additional procedure to aid in diagnosis. In patients with peripheral lung cancer, transbronchial needle aspiration has been developed.[11,12] However, benign pulmonary lesions are not usually diagnosed by cytologic examination, and thus lung resections are ultimately needed. Active tissue biopsy is required for the improvement of the diagnostic efficiency of PPLs, including benign lesions.

We had a patient in whom a critical air embolism developed during CTGNB, and we were thus confronted with the necessity of developing a new diagnostic procedure. We considered that the following three conditions are required in a new method. The first is that it must be safe for the patient and the staff. The second is that it should be inexpensive and easily introduced into many institutions. The final condition is that it is able to obtain specimens that can be used to histologically diagnose even benign lesions. Therefore, to satisfy these requirements, we should approach a lesion more perpendicularly by a standard bronchoscope to improve tissue collection.

Table 4—*Effect of Lesion Size on Diagnostic Yield of PPLs Obtained With STAF and Standard Forceps**

Lesion Size, mm	STAF No./Total No.	STAF %	Standard Forceps No./Total No.	Standard Forceps %	p Value†
≤ 10	2/7	28.6	2/7	28.6	NS
> 10 to ≤ 20	39/49	79.6	17/49	34.7	< 0.001
< gt>20 to ≤ 30	28/31	90.3	16/31	51.6	< 0.001
> 30	17/23	73.9	8/23	34.8	0.002

*NS = not significant.
†By McNemar test.

We invented STAF as a new device for diagnosis to be used with a standard bronchoscope (Fig 1). STAF has a 12° angle 10 mm from the tip, and this structure was the most controllable in our experience. If the tip angle is ≤ 10°, the forceps cannot grasp the edge of the lesion. On the other hand, if the tip angle is > 15°, the forceps flexes too much during the approach. We also invented a new biopsy technique to control STAF more effectively (Fig 2). The CS-TBB method enables a perpendicular approach. The slight angle of the tip, the flexural direction perpendicular to the opening, and the shutting of the cup are necessary conditions to make a CS motion successfully. The CS-TBB method has two typical approach patterns, called the *J-shape* (Figs 3, *bottom right*, *C*, and 5, *right*, *B*) and the *S-shape* (Fig 4, *right*, *B*) in bronchoscopic images. These approach patterns are thought to be very effective for obtaining specimens from PPLs, because we can apply force directly to the lesion.

In this study, the diagnostic yield from the malignant lesions obtained with STAF was 83.3%. This result is thought to be almost the same as or greater than that obtained in patients with PPLs, counting what a standard forceps can easily reach.[13,14] Previous studies[15,16] have reported that the diagnostic yields in peripheral pulmonary benign lesions were 50 to 65.8%. In the present study, the diagnostic yield obtained with STAF was 84.6%. This result is significantly higher than that obtained in previous studies. In the diagnostic analysis of lesions in each area of the lung (Table 3), the STAF was often used to obtain a specimen from a lesion in the right upper lobe and the left upper division. This reflects the fact that the upper lobe is difficult to reach anatomically. STAF was found to be effective for obtaining specimens form these lesions. In the diagnostic analysis of each size range (Table 4), efficacy was poor for lesions < 10 mm, which cannot be clearly visualized by radiographic fluoroscopy. STAF is thought to be effective for use with all lesions that can be visualized by radiographic fluoroscopy. Using the STAF, the lesion can be visualized by radiographic fluoroscopy; it seems that using STAF has a benefit even if it is any difficult area.

There are three principal reasons why the diagnostic yield from TBB specimens obtained with

STAF was superior to that from specimens obtained with standard forceps. First, STAF was able to approach the lesion after being advanced into the bronchus of choice by flexure of the tip. Second, STAF was able to grasp the lesion well by a more perpendicular application of the conventional biopsy method. Third, STAF was able to obtain enough tissue for histologic examination. STAF also has some original applications. In patients with a mediastinum-involved tumor that a standard forceps cannot really reach, STAF easily reaches the lesion and can obtain sufficient material for histological examination. STAF is also useful for benign tumors or metastatic lesions, which communicate poorly with the bronchi. In these lesions, we expect that STAF can break a surrounding bronchial wall and grasp a lesion. We think that these types of lesions are most effectively approached using the STAF. To obtain further effects, several variations of a product are essential, such as shaft flexibility, flexure angle of the tip, and shape of the cup.

The complications from TBB performed with STAF, such as bronchial bleeding and pneumothorax, were mostly mild. These were similar to those from conventional TBB. We think that this is because our new method is basically conventional bronchoscopy performed with a new device and a new technique. But a carelessly performed operation could cause critical complications; for example, the rupture of great vessels due to approaching adjacent mediastinal lesions. In addition, if the channel of the bronchoscope is ≤ 2 mm in diameter, a careless operation may cause fiber damage. The use of bronchoscopes with a channel diameter of > 2.2 mm is preferable.

CTGNB and VATS have been performed worldwide.[7,8] But CTGNB is considered to be more invasive than TBB because of the possibility of critical complications, including air embolism and pleural dissemination,[9,10] while VATS is not always performed in patients with poor performance status or in the elderly. Other transbronchial diagnostic procedures include bronchoscopy with an ultrathin bronchoscope coupled with virtual navigation,[17] and endobronchial ultrasonography-guided TBB.[18] But these procedures involve complicated methods and are not yet widely used. Widely used diagnostic procedures should be safe and easy to use. When we compare our new diagnostic procedure with other diagnostic procedures, we find that its safety characteristics are superior to those of CTGNB and VATS, while its ease of operation is superior to that of the ultrathin bronchoscope with virtual navigation and endobronchial ultrasonography-guided TBB. Furthermore, we do not require a new investment because the cost is almost the same as that of standard forceps. Consequently, from the point of view of safety, ease of operation, and cost-effectiveness, STAF can be used in any patients at any institution. However, physicians must learn and understand this biopsy technique well, and training is necessary.

In some studies,[19,20] a subgroup of patients with NSCLC have had specific mutations in the epidermal growth factor receptor gene that correlated with clinical responsiveness to the tyrosine kinase inhibitor gefitinib. But the accuracy of histologic diagnosis of peripheral lung cancer with TBB specimens is not always sufficient. We achieved a superior accuracy of histologic diagnosis in peripheral lung cancer (89.5%) between April 2002 and March 2003, and it was thought to be an additive effect of the innovation of STAF. Successful TBB with a low risk of severe complications is also important for predicting the efficacy of target therapies, because such information requires repeated biopsies. The adequate TBB specimens that were obtained with STAF are expected to contribute to the performance of gene analysis in patients with NSCLC in the future.

Conclusions

STAF was shown to be useful for obtaining sufficient specimens for histologic diagnosis from PPLs, which are difficult to manipulate with standard forceps, and the use of STAF resulted in a significant improvement in the diagnostic efficiency of TBB. CS-TBB performed with STAF can provide a high accuracy with safety and ease, so that this new device and technique may become widespread.

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EKB-569, a new irreversible epidermal growth factor receptor tyrosine kinase inhibitor, with clinical activity in patients with non-small cell lung cancer with acquired resistance to gefitinib

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KEYWORDS
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EGFR mutation;
Resistance to gefitinib;
Irreversible inhibitor of EGFR

Summary EKB-569 is a potent, low molecular weight, selective, and irreversible inhibitor of epidermal growth factor receptor (EGFR) that is being developed as an anticancer agent. A phase 1, dose-escalation study was conducted in Japanese patients. EKB-569 was administered orally, once daily, in 28-day cycles, to patients with advanced-stage malignancies known to overexpress EGFR. Two patients with advanced non-small cell lung cancer with EGFR mutations and acquired gefitinib resistance from the phase 1 study are described in detail. *Case #1* is a 63-year-old man with smoking history. He received treatment from 4 March 2004. Because he had no severe adverse events, a total of 10 courses of therapy were completed through December 16. Grade 2 skin rash and ALT elevation, and grade 1 diarrhea and nail changes developed. A chest CT scan on 4 August 2003 revealed multiple pulmonary metastases that had decreased in size. *Case #2* is a 49-year-old woman with no smoking history. She received therapy from 9 February 2004. She received a total of five courses of the therapy until 22 June 2004. Grade 3 nausea and vomiting

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and grade 1 diarrhea and dry skin developed. A chest CT scan on March 3 revealed multiple pulmonary metastases that had decreased in size. A brain MRI on March 4 showed that multiple brain metastases also had decreased in size. Based on RECIST criteria, they had stable disease but radiographic tumor regression was observed.

1. Introduction

1.1. Efficacy of gefitinib

The epidermal growth factor receptor (EGFR) autocrine pathway contributes to a number of processes important to cancer development and progression, including cell proliferation, apotosis, angiogenesis, and metastatic spread [1]. EGFR-tyrosine kinase has become a particularly promising drug targeting for treating non-small cell lung cancer. Gefitinib is an orally active, selective EGFR tyrosine kinase inhibitor that blocks signal transduction pathways implicated in proliferation and survival of cancer cells [2]. Responsiveness characteristics include distinct subgroups of women, patients who have never smoked, patients with adenocarcinoma, and Asians [3–5]. Molecular predictive markers have also been investigated. It is suggested that MAPK is a predictive marker for survival after treatment with gefitinib in chemo-naive patients with bronchioloalveolar carcinoma [6]. Patients with P-Akt-positive tumors who received gefitinib had a better response rate, disease control rate, and time to progression than patients with P-Akt-negative tumors, suggesting that gefitinib may be most effective in patients with basal Akt activation [7]. However, it was not possible to predict gefitinib sensitivity by the level of EGFR overexpression as determined by immunohistochemistry [8] or immunoblotting [9]. Recently it has been reported that somatic mutations in the tyrosine kinase domain of the *EGFR* gene occur in a subset of patients with lung cancer who showed a dramatic response to the EGFR tyrosine kinase inhibitors gefitinib and erlotinib [10–12]. All of these mutations were within exons 18 through 21 of the kinase domain of the *EGFR* gene.

1.2. Drug summary

EKB-569 (Wyeth Research, Collegeville, PA) is a potent, low molecular weight, selective, and irreversible inhibitor of EGFR that is being developed as an anticancer agent. EGFR is a receptor tyrosine kinase that is activated by a variety of growth factors. Upon binding ligands, including epidermal growth factor (EGF) or transforming growth factor alpha (TGF-α), EGFR dimerizes and its intracellular kinase domain is activated, leading to the recruitment and phosphorylation of a number of proteins that ultimately lead to cell growth [13,14]. Several features of EKB-569 may provide certain advantages over other EGFR inhibitors. First, EKB-569 is an orally available, small-molecule EGFR inhibitor, whereas antibody-targeted EGFR inhibitors require intravenous (IV) administration. Second, EKB-569 is an irreversible inhibitor of EGFR, while other small-molecule EGFR inhibitors bind EGFR reversibly [15].

1.3. Effects in humans (Japanese)

A phase 1, open-label, dose-escalation study to assess the safety, tolerability, and pharmacokinetics of EKB-569 was conducted in Japanese patients. EKB-569 was administered orally, once daily, in 28-day cycles, to patients (pts) with advanced-stage malignancies known to overexpress EGFR. Enrollment and treatment are completed; 15 pts (six men, nine women) were treated with 25 mg (3 pts), 35 mg (8 pts), or 50 mg (4 pts) of EKB-569. Their median age was 62 years (range 47–72); ECOG performance status varied: 0 = 4/15 (26.7%) or 1 = 11/15 (73.3%).

The most frequently occurring tumor types included non-small cell lung (10 pts) and breast (2 pts). The remaining tumors were renal, leiomyosarcoma, and malignant thymoma (1 pt each). The most frequently reported EKB-569-related adverse events were diarrhea (86.7%), rash (53.3%), anorexia (40.0%), and dry skin (40.0%). Dose-limiting toxicities were observed at the 50-mg dose level with grade 4 interstitial lung disease and grade 3 diarrhea, stomatitis, and increased blood calcium levels. Thus, the maximum tolerated dose was 35 mg EKB-569 per day.

1.4. Molecular analysis of lung cancer specimens

We obtained appropriate approval from the institution and written informed consent from the patients for the comprehensive use of tumor samples for molecular and pathologic analyses. Surgically resected tumor samples were obtained retrospectively before the patients received

any systemic treatment. All of these tumors were formalin fixed and paraffin embedded by the Department of Pathology. To minimize non-neoplastic tissue contamination, the tumor portion was first selected and marked on an H&E-stained tissue section slide by a pathologist. Only the tumor portion was dissected from the unstained tissue section and sent for DNA extraction.

DNA was extracted from the paraffin section containing a representative portion of each tumor, using the QIAamp DNA Mini kit (Qiagen, Hilden, Germany). For mutational analysis of the kinase domain of the *EGFR* coding sequence, exons 19, 20, and 21 were amplified with three pairs of primers (exon 19, F: 5′-TCACAATTGCCAGTTAACGTCT-3′-(this is the convention for writing a primer), R: 5#-cagcaaagcagaaactcacatc; exon 20, F: 5#-tgaaact-caagatcgcattcat, R: 5#-catggcaaactcttgctatcc; exon 21, F: 5#-gagcttcttcccatgatgatct, R: 5#-gaaaatgctggctgacctaaag). The PCR conditions were one cycle at 95 °C for 11 min, 46 cycles at 95 °C for 30 s, 60 °C for 30 s, 72 °C for 40 s, followed by one cycle at 72 °C for 7 min. PCR products were diluted and cycle-sequenced using the Big Dye Terminator v3.1/1.1 cycle sequencing kit (Applied Biosystems, Forster City, CA) according to the manufacturer's instructions. Sequencing products were electrophoresed on an ABI PRISM 3100 Genetic Analyzer (Applied Biosystems). All sequencing reactions were performed in both forward and reverse directions and chromatograms were reviewed manually and analyzed by BLAST (basic local alignment search tool). High-quality sequence variations found in both directions were scored as candidate mutations.

2. Clinical cases

Two patients from the Japanese phase 1 study are described in detail.

2.1. Case #1

A 63-year-old man with smoking history (BI: 720) who was treated for hyperlipidemia and hypertension showed an abnormal chest X-ray in February 1996. Further examinations including a chest computed tomography (CT) scan and bronchoscopy revealed an adenocarcinoma of the lung, c-T1N0M0, stage Ia, in the right upper lobe. He had undergone a right upper lobectomy with mediastinal lymph node dissection in July 1996 and was proven to have a well-differentiated adenocarcinoma, p-T1N0M0, stage Ia. After further follow-up, multiple pulmonary metastases in both lungs were found in January 2000. Then he was given first-line chemotherapy of cisplatin and docetaxel beginning in May 2000. After two courses of this regimen, multiple pulmonary metastases had not increased in size by CT scan; however skin metastases were found. He was started on oral gefitinib 250 mg/day on November 2000. After 4 weeks, a CT scan indicated a reduction of multiple pulmonary metastases. During this treatment, grade 2 rash and grade 1 nail changes, AST/ALT elevations, and diarrhea were observed. On June 2002, multiple pulmonary metastases had increased, and this treatment was discontinued. The patient entered a phase I study of a new EGFR tyrosine kinase inhibitor (TAK-165), starting treatment on October 2002. After 2 weeks of treatment, grade 3 anorexia was observed and the therapy was stopped. On February 2003, multiple pulmonary metastases had more increased, and on March 2003, he entered a phase I study of EKB-569, receiving treatment from 4 March 2004. EKB-569 (25 mg) was administered orally, once daily, in 28-day cycles. Because he had no severe adverse events, a total of 10 courses of therapy were completed through December 16. Grade 2 skin rash and ALT elevation, and grade 1 diarrhea and nail changes developed during this therapy. Based on RECIST criteria, the patient had stable disease (SD) but radiographic tumor regression was observed on 4 August 2003 (day 27 in the sixth course) (Fig. 1). The size of multiple pulmonary metastases increase by CT scan on 8 December 2003, and the treatment was stopped on 17 December 2003.

A lung cancer specimen was obtained at surgery and studied by immunohistochemistry. EGFR overexpression was detected. In addition, we found the heterozygous in-frame deletion E746-A750 in exon 19 of the *EGFR* gene by direct sequencing of the specimen.

2.2. Case #2

A 49-year-old woman with no smoking history, who was treated for Basedow's disease, insomnia, and bronchial asthma, had an abnormal chest X-ray in October 2000. Further examinations including a chest CT scan and bronchoscopy revealed lung cancer in the left upper lobe. She was diagnosed with adenocarcinoma, c-T1N0M0, stage Ia. She had a left-upper lobectomy with mediastinal lymph node dissection, which revealed a well-differentiated adenocarcinoma, p-T4N2M1, stage IV. She was then given first-line chemotherapy of carboplatin and paclitaxel beginning in January 2001. After two courses of therapy, she discontinued treatment because of adverse events. Right supraclavicular lymph node metastases were found on August

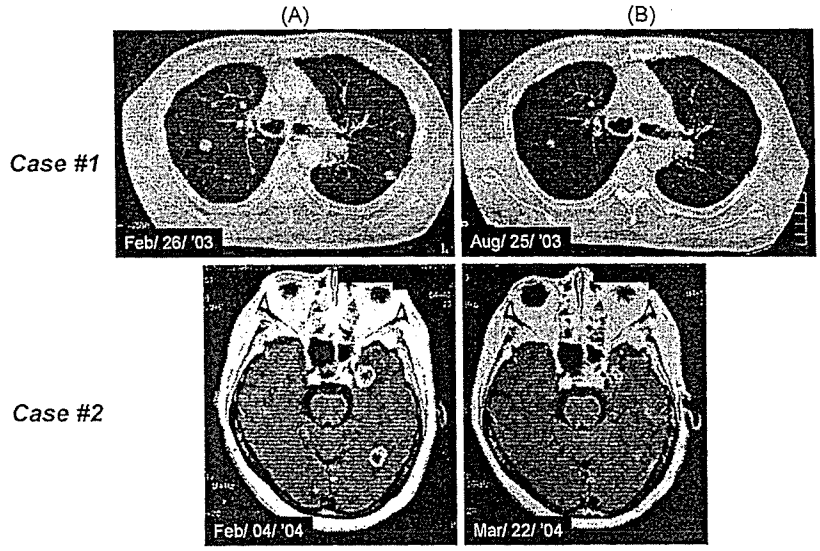

Fig. 1 *Clinical case* #1: a 63-year-old man with adenocarcinoma of lung. CT scan before treatment (A) and after initiation of EKB-569 (B). *Clinical case* #2: a 49-year-old woman with adenocarcinoma of brain metastasis. MRI scan before treatment (A) and after initiation of EKB-569 (B).

2001. Radiotherapy for the metastases (60 Gy/30 fractions) was done, and they decreased in size. On March 2002, right supraclavicular lymph node metastases increased and left clavicular lymph node metastases were found. On April 2002, the patient enrolled in a phase II trial of cisplatin, gemcitabine, and irinotecan for non-small-cell lung cancer. After two courses of therapy, bone metastases were found and pulmonary metastases had grown slowly so the treatment was stopped. She entered a phase I study of a new EGFR tyrosine kinase inhibitor (TAK-165) and started treatment on July 2002. The treatment was stopped after a week later due to grade 3 fatigue. In September 2002, the patient was started on oral gefitinib 250 mg/day. While she was taking 250 mg gefitinib daily for 15 months, the size of multiple pulmonary and bone metastases did not increase by CT scan and she had SD. On December 2003, the patient developed grade 3 oral mucositis and discontinued treatment. On January 2004, the size of multiple pulmonary and bone metastases increase by CT scan. She then entered a phase I study of EKB-569 and received therapy from 9 February 2004. EKB-569 (35 mg) was administered orally, once daily, in 28-day cycles. She received a total of five courses of the therapy until 22 June 2004. Grade 3 nausea and vomiting and grade 1 diarrhea and dry skin developed during the therapy. A chest CT scan on March 3 (day 24 in the first course) revealed multiple pulmonary metastases that had decreased in size. A brain MRI on March 4 (day 25 in the first course) showed that multiple brain metastases also had decreased in size (Fig. 1). The response was SD by RECIST criteria, although tumor regression was observed. The size of bone metastases increase by CT scan on 18 June 2004, and the treatment was stopped on 22 June 2004.

A lung cancer specimen was obtained by surgery and studied by immunohistochemistry. EGFR overexpression was detected. This lung cancer specimen had a heterozygous point mutation in exon 21 (L858R, CTG to CGG) of the *EGFR* gene.

3. Discussion

This is the first case report to describe the effects of EKB-569 on patients with adenocarcinoma of the lung. Case 1 is a 63-year-old man with a smoking history (BI: 720), and case 2 is a 49-year-old woman with no smoking history. Case 1 had an exon 19 deletion of E746-A750, and case 2 had an exon 21-point mutation. These patients underwent surgery and were treated with platinum-based chemotherapy and EGFR tyrosine kinase inhibitors. The treatment with EKB-569 was effective in these two patients after resistance to gefitinib and cytotoxic chemotherapy. These cases suggest that EKB-569 is effective in patients with *EGFR* mutations as has been reported for gefitinib and erlotinib. Despite initial responses to these EGFR inhibitors, patients eventually progress by unknown mechanisms of "acquired" resistance.

Recently, a second mutation in the *EGFR* kinase domain, which is associated with acquired resistance of non-small cell lung cancer to gefitinib or erlotinib, was reported [16,17]. Pao et al. showed that in two of five patients with acquired resistance

to gefitinib or erlotinib, progressing tumors contained, in addition to a primary drug-sensitive mutation in EGFR, a secondary mutation in exon 20. This mutation leads to a substitution of methionine for threonine at position 790 (T790M) in the kinase domain [16]. Kobayashi et al. reported the case of a patient with EGFR-mutant, gefitinib-responsive, advanced non-small cell lung cancer who relapsed after two years of complete remission during treatment with gefitinib. The DNA sequence of the EGFR gene in his tumor biopsy specimen at relapse also revealed the presence of the secondary point mutation, T790M [17]. Kurata et al. reported an interesting case in which acquired resistance to gefitinib could be overcome [18]. In this case, the patient received gefitinib, then a combination of nedaplatin and gemcitabine, and then gefitinib again. The cytotoxic agents may have altered the *EGFR* gene or associated genes to produce acquired sensitivity to gefitinib.

Kobayashi et al. also found that CL-387,785, a specific and irreversible, anilinoquinoline EGFR inhibitor [19], strongly inhibited the EGFR kinase in cells transfected with DNA containing the L747-S752 deletion in the *EGFR* gene or a double mutation with the L747-S753 deletion and the T790M point mutation. They speculated that CL-387,785 inhibited the EGFR kinase of the double mutant because of its altered binding to the kinase domain or its covalent binding to EGFR [17]. Kwak et al. used a bronchoalveolar cancer cell line with an L746-A750 deletion in the *EGFR* gene to isolate gefitinib-resistant clones. These clones had not acquired secondary EGFR mutations but were sensitive to the irreversible, anilinoquinoline EGFR inhibitor EKB-569 [20].

We have shown that EKB-569 had clinical activity in two patients with advanced non-small cell lung cancer with EGFR mutations and acquired gefitinib resistance. Thus, irreversible EGFR inhibitors may be an effective therapy for patients with EGFR-mutant advanced non-small cell lung cancer who have relapsed after treatment with gefitinib.

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