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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Manuscript received March 31, 2005; revision accepted September 23, 2005.

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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

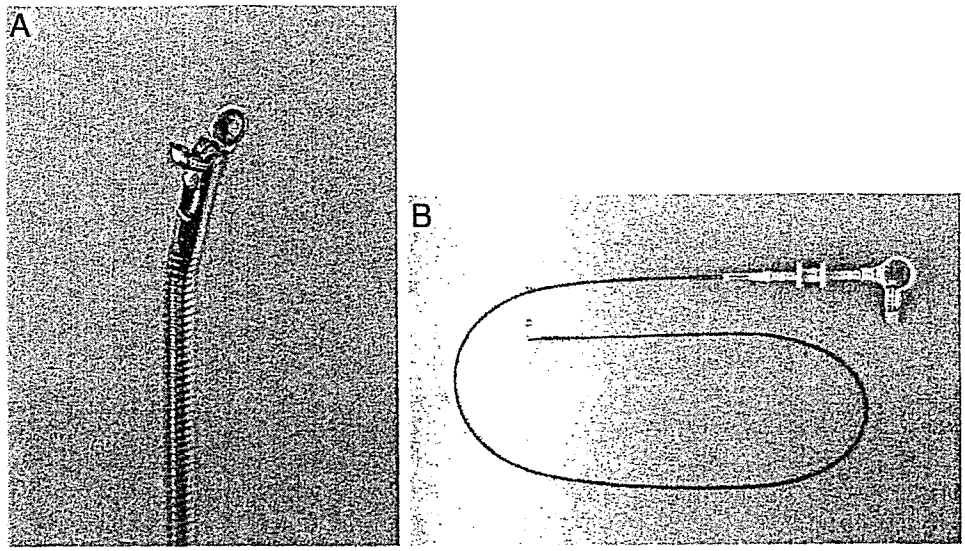

FIGURE 1. STAF. *Left, A*: Open position; the STAF has an angled tip. *Right, B*: the general shape of the STAF is almost the same as that of a standard forceps except for the angled tip.

(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

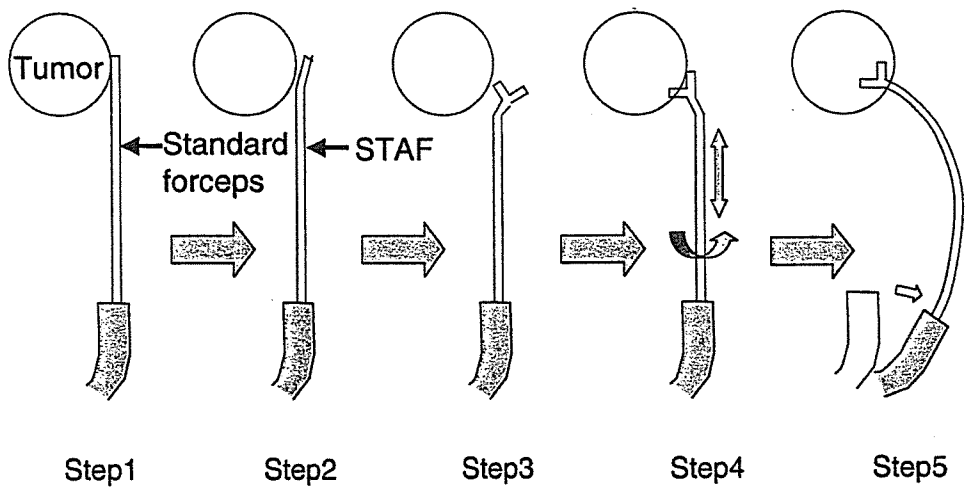

FIGURE 2. Method of performing CS-TBB. Step 1: search for the bronchus nearest to the lesion by using a standard forceps. Step 2: switch to STAF. Step 3: open the cup in front of the lesion. Step 4: turn and slide the STAF at the same time, and push forward. Step 5: make a CS motion by operating the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

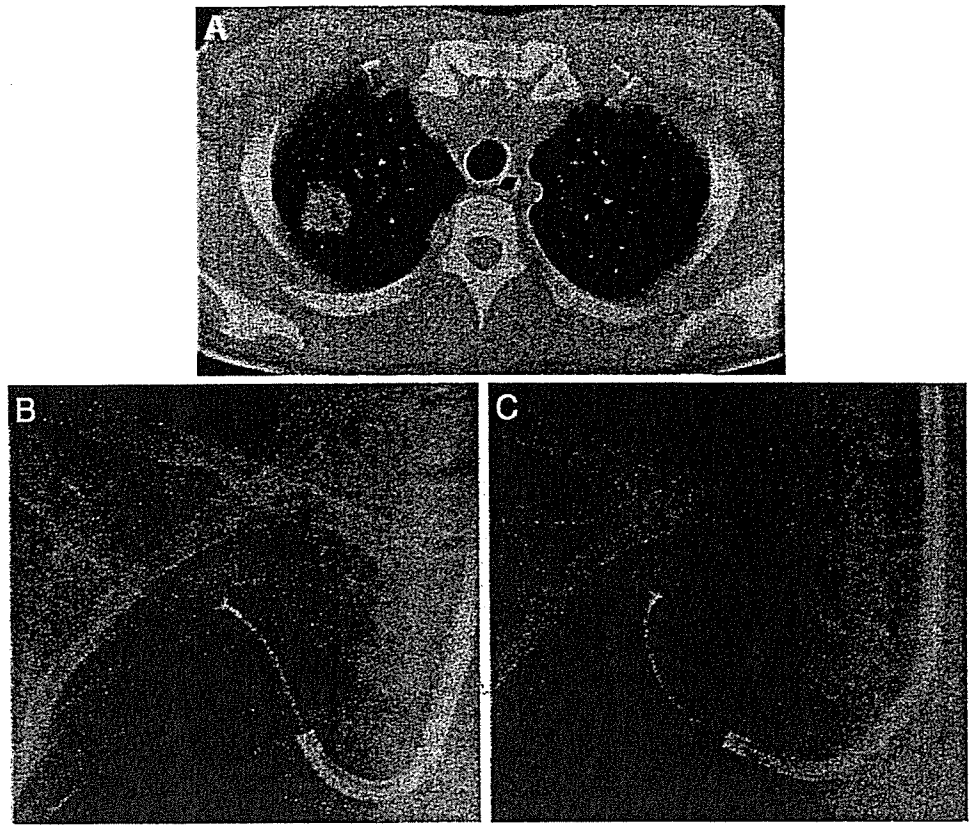

FIGURE 3. Case 1. A tuberculoma. *Top, A*: a chest CT scan reveals a coin lesion in the right upper lobe. *Bottom left, B*: a bronchoscopic image shows a standard forceps approaching a tumor through the right B1a. *Bottom right, C*: bronchoscopic image shows the STAF approaching the tumor. It forms a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

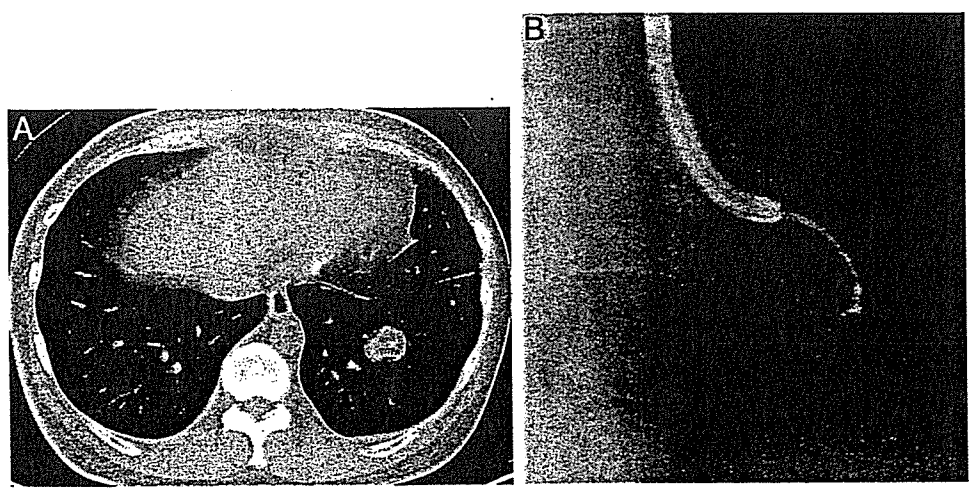

FIGURE 4. Case 2. A hamartoma. *Left, A*: a chest CT scan reveals a lesion in the left lower lobe. *Right, B*: a bronchoscopic image shows the STAF approaching a tumor through the left B10e. It forms a CS (*ie*, S-shape).

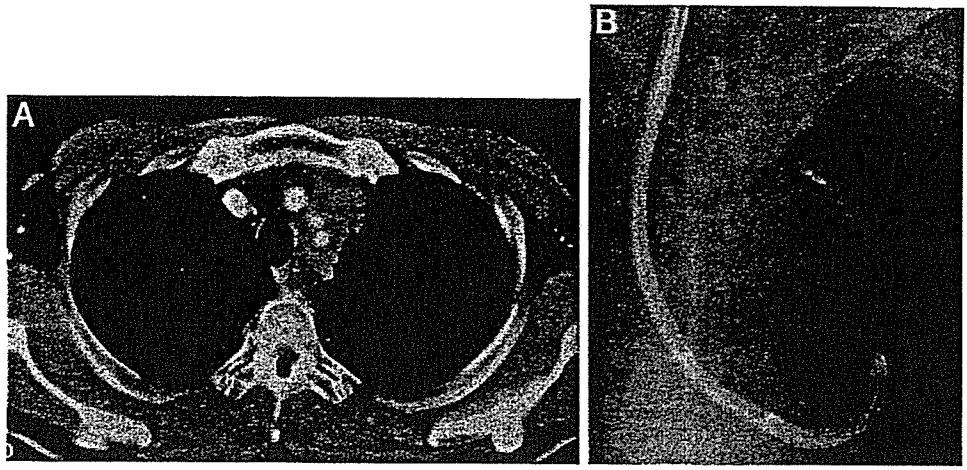

FIGURE 5. Case 3: NSCLC. *Left, A*: a chest CT scan reveals a mediastinum-involved tumor shadow on the left side. *Right, B*: a bronchoscopic image shows the STAF approaching the tumor through the left B3c.

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;

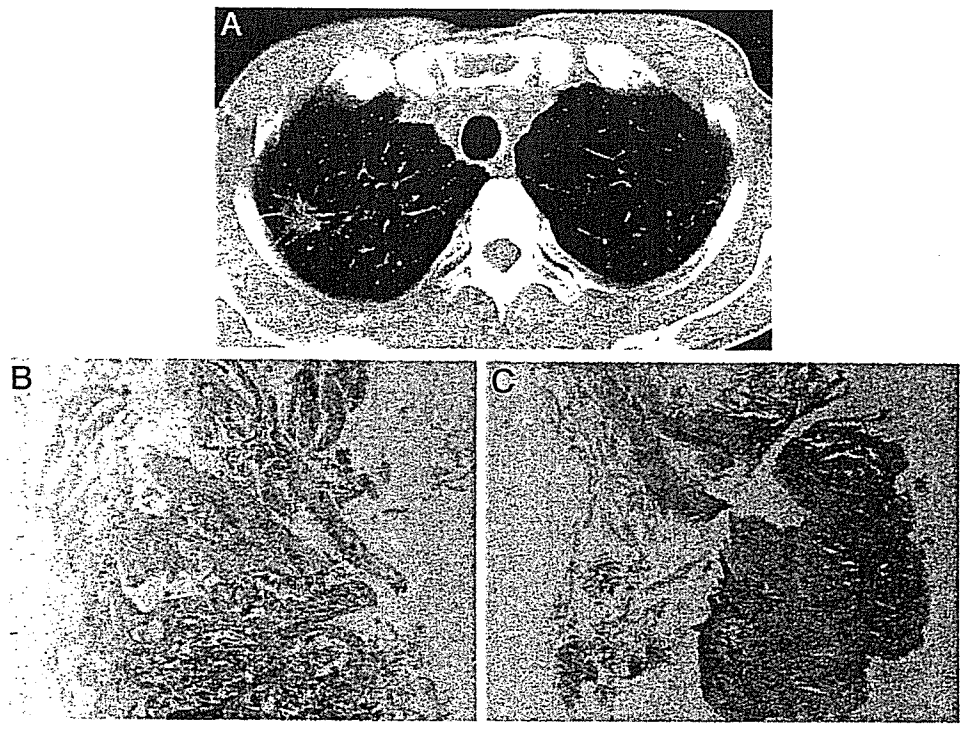

FIGURE 6. Case 4: a lung adenocarcinoma. *Top, A*: a chest CT scan reveals a coin lesion in the right upper lobe. *Bottom left, B*: the biopsy specimen obtained with standard forceps through the right B3a reveals only normal bronchial wall (hematoxylin-eosin, original ×40). *Bottom right, C*: STAF biopsy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

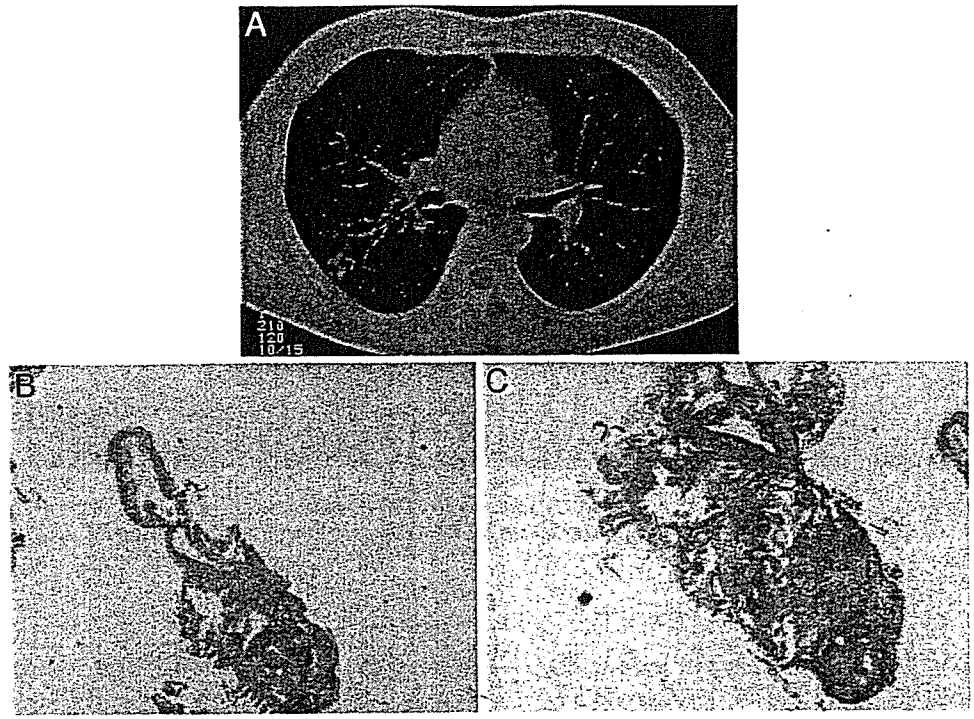

FIGURE 7. Case 5: a tuberculoma. *Top, A*: a chest CT scan reveals a coin lesion in the right lower lobe. *Bottom left, B*: the biopsy specimen obtained with standard forceps through the right B6b reveals a normal bronchial wall (hematoxylin-eosin, original ×40). *Bottom right, C*: STAF biopsy specimen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

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

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.

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.

ACKNOWLEDGMENT: We thank the Machida Corporation (K. Miyagi) for technical support.

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Lung Cancer (2006) 51, 363–368

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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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Received 2 August 2005; received in revised form 12 October 2005; accepted 18 October 2005

KEYWORDS
EKB-569;
Non-small cell lung cancer;
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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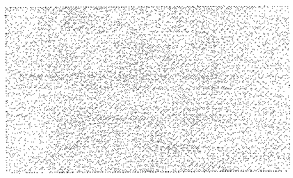
0169-5002/$ – see front matter
doi:10.1016/j.lungcan.2005.10.006



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

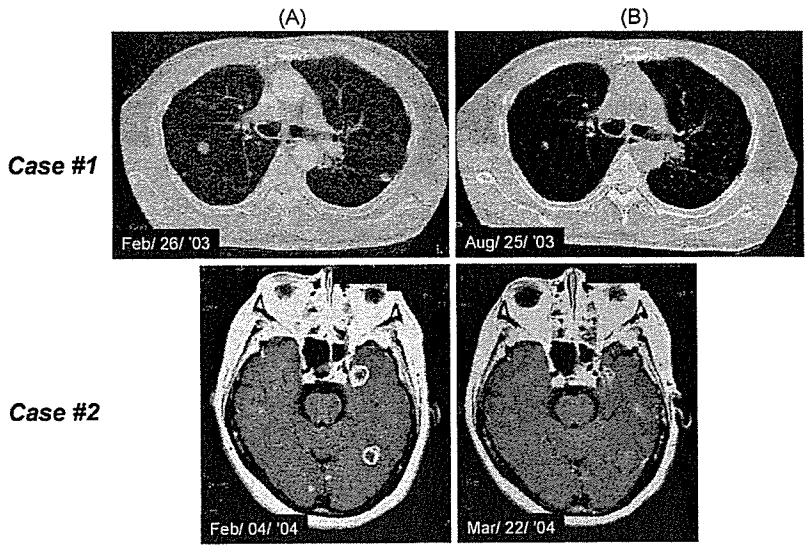


Fig. 1 *Clinical case* #1: a 63-year-old man with adenocarcinoma of lung. CT scan before treatment (A) and after initiation of EKB-569 (B). *Clinical case* #2: a 49-year-old woman with adenocarcinoma of brain metastasis. MRI scan before treatment (A) and after initiation of EKB-569 (B).

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.

Acknowledgments

We thank Tetsuya Mitsudomi and Yasushi Yatabe (Aichi Cancer Center Hospital) for technical assistance in molecular analysis of tumors.

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Jpn J Clin Oncol 2005;35(4)181–187
doi:10.1093/jjco/hyi057

A Phase I/II Study Comparing Regimen Schedules of Gemcitabine and Docetaxel in Japanese Patients with Stage IIIB/IV Non-small Cell Lung Cancer

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Received October 7, 2004; accepted January 31, 2005

Objective: Gemcitabine and docetaxel are non-platinum agents with activity in non-small cell lung cancer (NSCLC). This study was conducted to determine and evaluate the recommended regimen of gemcitabine–docetaxel and evaluated its efficacy and safety in chemonaive Japanese NSCLC patients.

Methods: In phase I, patients with stage IIIB/IV NSCLC were randomized and received either gemcitabine on days 1 and 8 plus docetaxel on day 1 or gemcitabine on days 1 and 8 plus docetaxel on day 8. The recommended regimen was the dose level preceding the maximum tolerated dose; once determined, patients were enrolled in phase II. Efficacy and toxicity were evaluated in all patients.

Results: Twenty-five patients were enrolled in phase I and six patients were given the recommended regimen; gemcitabine 1000 mg/m^2 on days 1 and 8 plus docetaxel 50 mg/m^2 on day 8. An additional 34 patients were enrolled into phase II and administered with the recommended regimen. The response rate was 32.2% [95% confidence interval (CI) 20.6–45.6%] overall and 30.0% (95% CI 16.6–46.5%) in patients with the recommended regimen (40 patients). Although grade 3 interstitial pneumonia was observed in two patients (5.0%) who received the recommended regimen, both recovered shortly after steroid treatment. No unexpected events were observed throughout this study.

Conclusions: Gemcitabine 1000 mg/m^2 on days 1 and 8 plus docetaxel 50 mg/m^2 on day 8 has comparable efficacy and more tolerable toxicities than previously reported platinum-based regimens. These results should be verified by a phase III study.

Key words: docetaxel – gemcitabine – non-small cell lung cancer

INTRODUCTION

Non-small cell lung cancer (NSCLC) is one of the most common malignant tumors, progresses in a short time period, has a bleak prognosis, and represents the leading cause of cancer death in the world. The number of patients with NSCLC is increasing, and most tumors are inoperable. Despite improvements in the detection and treatment of NSCLC, long-term survival is rare. Therefore, the development of new chemotherapy treatments is essential.

The use of single-agent and combination chemotherapy against NSCLC has been studied. Platinum-based regimens have shown high efficacy but at the cost of severe toxicities (1,2). Therefore, non-platinum agents such as gemcitabine, docetaxel, paclitaxel, irinotecan and vinorelbine have been developed and have proven their efficacies. Among the new agents, the combination of gemcitabine and docetaxel has emerged as one of the most promising, showing equivalent efficacy with, and less toxicity than, cisplatin-based chemotherapies (3).

Gemcitabine (2′-deoxy-2′,2′-difluorocytidine monohydrochloride) is a nucleoside antimetabolite against deoxycytidine. It is intracellularly metabolized to gemcitabine triphosphate,

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which inhibits DNA synthesis, and has shown potent cytocidal activity against solid tumors (4–8).

Docetaxel, an antineoplastic agent that acts on microtubules to promote formation of abnormal microtubule bundles, has also shown cytotoxicity (9–11). Gemcitabine and docetaxel have different mechanisms of action, but by combining them, there is the potential of synergistic antitumor activity (12).

Several studies have been conducted to evaluate the therapeutic benefits of gemcitabine and docetaxel (13–15). The efficacy of gemcitabine–docetaxel is similar to platinum-based regimens, but due to each drug's non-overlapping toxicities, their combination produces toxicities more tolerable than platinum-based regimens. Georgoulias et al. (16) compared gemcitabine 1100 mg/m^2 on days 1 and 8 plus docetaxel 100 mg/m^2 on day 8 with cisplatin 80 mg/m^2 on day 2 plus docetaxel 100 mg/m^2 on day 1 in 441 patients with NSCLC. They reported that the two regimens were equivalent in efficacy, but toxicities were more severe for the combination of docetaxel and cisplatin.

There has been no published report considering both administering dose and schedule for the combination of gemcitabine and docetaxel. Therefore, we conducted a phase I/II study to compare two schedules of gemcitabine–docetaxel in patients with NSCLC and determine the recommended regimen in phase II. We assessed the efficacy and safety in all 59 patients: the efficacy and detailed safety profile were also evaluated in 40 patients who were given the recommended regimen.

SUBJECTS AND METHODS

Eligibility Criteria

Japanese patients with histologically or cytologically confirmed unresectable TNM stage IIIB or IV NSCLC who met the following criteria were eligible for the study: suitable for first-line chemotherapy with no prior chemotherapy; measurable lesions that can be accurately measured in at least one dimension; aged 20–74 years; Eastern Cooperative Oncology Group (ECOG) performance status of 0–1; a life expectancy of at least 3 months; and adequate organ functions as indicated by white blood cell count $\geq 4.0 \times 10^9$/l, absolute neutrophil count $\geq 2.0 \times 10^9$/l, platelets $\geq 100 \times 10^9$/l, hemoglobin ≥ 9.5 g/dl, aspartate aminotransferase/alanine aminotransferase ≤ 2.5 times the upper limit of normal, total bilirubin ≤ 1.5 times the upper limit of normal, serum creatinine $\leq$ the upper limit of normal, PaO_2 in arterial blood ≥ 60 torr. If a patient had received radiotherapy during the 3 weeks before enrollment, the measurable disease had to be outside of the radiation port.

Patients were excluded from the study if they had radiologically and clinically apparent interstitial pneumonia or pulmonary fibrosis, intracavitary fluid retention requiring treatment, or grade 2–4 peripheral neuropathy or edema. Additional exclusion criteria included: superior vena cava syndrome; symptomatic brain metastasis; pregnancy or breast-feeding; active concurrent malignancy; any serious concurrent illness (e.g. uncontrolled diabetes mellitus, hepatopathy, angina pectoris, myocardial infarction within 3 months after onset, severe infection, or fever suggestive of severe infection); history of serious drug allergy; or any condition that, in the opinion of the investigator, disqualified the patient based on safety.

This study was conducted in accordance with the Declaration of Helsinki, Japanese Guidelines for Clinical Evaluation of Antineoplastic Agents (promulgated in February 1991) and good clinical practice. All patients who entered into this study were required to give written informed consent.

Study Design And Treatment

This was a multicenter, open-label, phase I/II study of gemcitabine and docetaxel in Japanese patients with advanced NSCLC.

In the phase I portion of this study, patients were randomized into two arms, each with a different treatment schedule. In both arms (Arm 1 and Arm 2), gemcitabine was administered in a 30-min infusion on days 1 and 8, every 21 days. In Arm 1, docetaxel was administered intravenously over at least 1 h on day 1; in Arm 2, docetaxel was given on day 8. The administration of docetaxel followed an intravenous infusion of dexamethasone 4 mg, and gemcitabine was given immediately after the docetaxel infusion.

Patients were discontinued from the study due to progressive disease; inability to initiate a treatment cycle even at 6 weeks after the start of the previous cycle; recurrence of a dose-limiting toxicity (DLT) after resumption of the study treatment at a reduced dose; occurrence of a serious adverse event or aggravation of a concomitant illness (e.g. interstitial pneumonia, pulmonary fibrosis, or severe infection) which caused rapid aggravation of disease and precluded continuation of the study treatment; patient's request to withdraw from the study; or any event that required discontinuation in the opinion of the investigator.

During study enrollment, the current approved maximum dosage of gemcitabine and docetaxel as single agents in Japan was 1000 mg/m^2 and 60 mg/m^2, respectively. In phase I, the sample size was determined to be six per cohort based on the conventional design of phase I clinical studies of antineoplastic agents. In this study, both arms were randomized according to a predetermined schedule, enrolled patients in cohorts of six, and were initially treated at dose level 1 (gemcitabine 1000 mg/m^2 and docetaxel 50 mg/m^2). For the first cycle of treatment, patients were treated on an inpatient basis; if their condition permitted, patients were treated on an outpatient basis thereafter. If fewer than 50% of the patients in dose level 1 experienced DLTs, patients were enrolled at dose level 2 (gemcitabine 1000 mg/m^2 and docetaxel 60 mg/m^2). If 50% or more of the patients in dose level 1 experienced DLTs, patients were enrolled at dose level 0 (gemcitabine 800 mg/m^2 and docetaxel 50 mg/m^2) (Fig. 1). The maximum tolerated dose (MTD) was defined as the dose level that produced any of the following DLTs (per the National Cancer Institute–Common

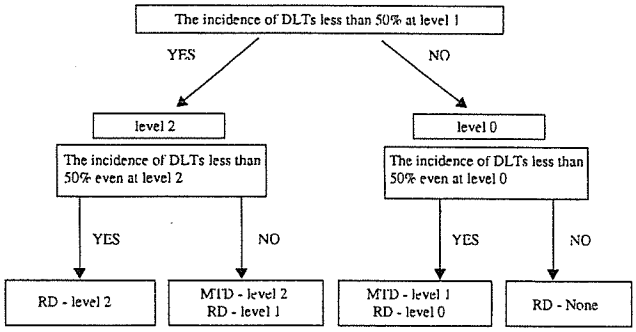

Figure 1. Recommended dosages in each arm. DLT, dose-limiting toxicity; RD, recommended dosage; MTD, maximum tolerated dose.

Toxicity Criteria scale) in 50% or more of patients during the first treatment cycle: grade 4 leukopenia or neutropenia persisting for at least 4 days; grade 3/4 neutropenia associated with a fever ≥38.0°C or infection; thrombocytopenia ($<20 \times 10^9$/l) or need of a platelet transfusion; or grade 3/4 non-hematological toxicities (excluding nausea/vomiting, anorexia, fatigue and hypersensitivity). G-CSFs were administrated for the treatment of grade 4 neutropenia or grade 3 neutropenic fever. A DLT was also reported if any day-8 doses were omitted and dosing requirements were not satisfied until after day 15, or if the second cycle was delayed until after day 29 because the dosing requirements were not satisfied.

The recommended dose for phase II had to be determined from the arm that reached the highest dose level. If at dose level 2 the incidence of DLTs was less than 50%, the recommended dose was defined as dose level 2. The arm that reached the higher dose level reflected the recommended regimen for phase II. If the recommended dose level for the two arms was identical, the recommended regimen would be decided according to the following steps: (i) if frequency of DLTs was 0% in one arm and 33.3% or more in the other arm, the former was selected. If this did not occur, then (ii) if the dose intensity for evaluable patients in one arm was higher by 10% or more than the other arm, the arm with the higher dose intensity was selected. If this did not occur, then (iii) the arm with the fewer day-8 dose omissions in first and second cycles was selected. If the recommended dosage regimen still could not be decided, the sponsor (Aventis Pharma Japan and Eli Lilly Japan K.K.) and the coordinating investigator determined the recommended phase II regimen. If the MTD was dose level 0 in both arms, the study was terminated (Fig. 1).

The sample size for the recommended regimen was determined as follows. The response rate of this regimen and gemcitabine single agent was assumed to be 35 and 20%, respectively, in view of the response rates previously achieved (9,10,17,18). If the sample size of the recommended regimen was set as 40 patients, the probability for the one-sided 90% lower limit of response rate to exceed 20% was 82%. Thus, the target sample size in the recommended regimen including six patients in phase I was set at 40 patients.

The phase II study was conducted with 34 patients. Forty patients who were given the recommended regimen were evaluated for the efficacy and detailed safety profile: these patients consisted of six and 34 patients who entered into the study at phase I and II, respectively.

In this phase I/II study, patients received a minimum of two cycles of gemcitabine–docetaxel and up to four additional cycles.

Dose Modifications

During a cycle, dose modifications were not allowed. If not all of the following requirements were satisfied on either the day of treatment or the previous day, administrations of gemcitabine and docetaxel were delayed until the patient completely recovered. For gemcitabine and docetaxel doses administered on day 1 of Arm 1 or gemcitabine on day 1 of Arm 2, delays occurred for patients with an absolute neutrophil count $<1.5 \times 10^9$/l, a platelet count $<70 \times 10^9$/l, any grade 3/4 non-hematologic toxicities (except PaO_2), or PaO_2 <60 torr. When gemcitabine was given on day 8 of Arm 1, exceptions included leukopenia $<2.0 \times 10^9$/l and an absolute neutrophil count $<1.0 \times 10^9$/l, a platelet count $<70 \times 10^9$/l, any grade 3/4 non-hematological toxicities. When gemcitabine was given on day 8 of Arm 2, exceptions included an absolute neutrophil count $<1.5 \times 10^9$/l, a platelet count $<70 \times 10^9$/l, any grade 3/4 non-hematological toxicities. If a patient developed a DLT, the subsequent doses were cancelled, and in the next cycle the patient could resume the study treatment at the next lower dose level. If a patient developed a DLT at dose level 0, gemcitabine 800 mg/m^2 and docetaxel 40 mg/m^2 were administered in the next cycle.

Baseline and Treatment Assessment

Assessments at baseline included tumor measurements by X-ray and computed tomography (CT) scan within 4 weeks before the day of starting the study treatment. Equally, grading performance status and physical examination were performed within a week; hematology, blood chemistries, urinalysis, arterial blood gas analysis and electrocardiogram were observed within 2 weeks.

After the start of treatment, tumor measurements were obtained every 2 weeks via X-ray and 4 weeks via CT scan. Tumor response was assessed with the World Health Organization (WHO) criteria. Safety assessments, including performance status, hematology, blood chemistries and urinalysis, were obtained weekly. Physical examination, arterial blood gas analysis and electrocardiogram were performed at any time. Adverse events were estimated according to National Cancer Institute–Common Toxicity Criteria version 2.0. All patients were assessed for efficacy and safety. An additional response rate was recorded for patients who received the recommended regimen in phase I and all phase II patients.

RESULTS

PATIENT CHARACTERISTICS

Between July 2000 and July 2002, 59 chemonaive patients (43 male, 16 female) with NSCLC were enrolled in phase I and II portions from the five hospitals after approval by the IRB. Twenty-five patients were enrolled in the phase I portion of the study, and 34 patients were enrolled in phase II. Baseline patient characteristics for all patients and patients who received the recommended regimen are summarized in Table 1.

PHASE I

Twenty-five patients were enrolled into the phase I portion of the study. The number of patients treated and the DLTs observed in the first cycle at each dose level of gemcitabine and docetaxel are shown in Table 2.

In Arm 1, 50% of patients had DLTs at dose level 1 and dose level 0, therefore Arm 1 could not be the recommended regimen: there were 2/6 and 3/6 patients who achieved partial response (PR) at dose level 1 and 0 in Arm 1, respectively.

Table 1. Baseline characteristics

Patient characteristics	All patients ($n = 59$), n (%)	Patients who received the recommended regimen ($n = 40$), n (%)
Gender		
Male	43 (72.9%)	26 (65.0%)
Female	16 (27.1%)	14 (35.0%)
Age		
Median	62	64
Range	38–74	38–74
ECOG performance status		
0	5 (8.5%)	2 (5.0%)
1	54 (91.5%)	38 (95.0%)
Stage		
IIIB	14 (23.7%)	8 (20.0%)
IV	33 (55.9%)	23 (57.5%)
Postsurgical recurrence	12 (20.3%)	9 (22.5%)
Histological type		
Adenocarcinoma	34 (57.6%)	25 (62.5%)
Squamous cell carcinoma	19 (32.2%)	14 (35.0%)
Large cell carcinoma	5 (8.5%)	1 (2.5%)
Other	1 (1.7%)	0 (0%)
Prior therapy		
None	45 (76.3%)	29 (72.5%)
Surgery	13 (22.0%)	11 (27.5%)
Radiotherapy	0 (0%)	0 (0%)
Radiotherapy and surgery	1 (1.7%)	0 (0%)

ECOG, Eastern Cooperative Oncology Group.

In Arm 2, no DLT was observed at dose level 1: 3/6 patients achieved PR. At dose level 2, one patient discontinued due to progressive disease; therefore, one patient was added. However, another patient discontinued due to grade 3 hypersensitivity (not a DLT). In this regimen, two DLTs had already been observed in five other patients, but the sponsors (Aventis Pharma Japan and Eli Lilly Japan K.K.) and investigators decided not to add one more patient to dose level 2 in Arm 2 in consideration of patients' safety. PRs were observed in 2/7 patients at dose level 2 of Arm 2.

Therefore, the recommended regimen was determined as gemcitabine 1000 mg/m^2 on days 1 and 8 plus docetaxel 50 mg/m^2 on day 8 due to the incidence of DLT.

DOSE ADMINISTRATION

In Arm 1, a total of 49 cycles were accomplished. One case delayed the date of administration on day 1 (defined as more than 8 days) as a matter of convenience; seven and four cases delayed their dates of administration on day 8 (defined as more than 1 day) because of adverse events and non-medical reasons, respectively; and four cases could not be treated on day 8 because of adverse events. In Arm 2, including phase I and II portions, a total of 145 cycles were accomplished. Four and five cases delayed their dates of administration on day 1 because of adverse events and non-medical reasons, respectively; 21 and nine cases delayed their dates of administration on day 8 because of adverse events and non-medical reasons, respectively; and two cases could not be treated on day 8 because of

Table 2. Phase I dose-limiting toxicities

Dose level	GEM/DOC (mg/m^2)	Arm 1	Arm 2
0	800/50	3/6 patients: • G3 ALT increased • G1 fever, G3 neutropenia • G2 infection, G3 neutropenia	N/A
1	1000/50	3/6 patients: • G3 infection, G3 neutropenia • G4 neutropenia, G1 fever, G3 infection • G3 neutropenia, G2 infection, G3 arrhythmia, G3 diarrhea	0/6 patients
2	1000/60	N/A	2/5 patients: • G3 ALT increased • G2 fever, G3 neutropenia

GEM, gemcitabine; DOC, docetaxel; G, grade; ALT, alanine aminotransferase; N/A, not applicable.

adverse events. The most common adverse event for a dose delay was neutropenia.

EFFICACY

All 59 patients were involved in the analysis for efficacy, and 19 of 59 patients achieved PR for an overall response rate of 32.2% [95% confidence interval (CI) 20.6–45.6%]. Of the 40 patients who received the recommended regimen in either phase I or phase II, 12 patients achieved PRs for a response rate of 30.0% (95% CI 16.6–46.5%).

The median time to progressive disease in all 59 patients was 111 days (95% CI 71–154 days). Median survival time was 11.9 months (95% CI 7.0–15.0 months), with 1-year survival rate at 47.1% (95% CI 34.0–60.2%).

SAFETY

All 59 patients were evaluable for safety. Grade 3 and 4 drug-related toxicities observed in all 59 patients are shown in Table 3. Grade 3 and 4 drug-related toxicities observed in 40 patients who received the recommended regimen are also shown in Table 4.

In all 59 patients, grade 3 and 4 neutropenia were observed in 19 (32.2%) and 20 (33.9%) patients, respectively. Grade 3 and 4 leukopenia were observed in 24 (40.7%) and four (6.8%) patients, respectively. Grade 3 non-hematological toxicities included infection in four patients (6.8%), anorexia in four patients (6.8%), and nausea, diarrhea, rash and constipation in three patients (5.1%) each. After starting docetaxel administration, grade 3 interstitial pneumonia was reported in three patients (5.1%), all of whom recovered shortly after steroid treatment; grade 4 anaphylaxis was reported in two patients (3.4%). There were no toxic deaths.

DISCUSSION

In this phase I/II study, we examined the activity and tolerability of gemcitabine and docetaxel. In phase I, the recommended regimen was determined as gemcitabine 1000 mg/m^2 on days 1 and 8 plus docetaxel 50 mg/m^2 on day 8. The response rate of all 59 patients was 32.2% (95% CI 20.6–45.6%). When re-evaluated in the 40 patients who received the recommended regimen, the response rate was 30.0% (95% CI 16.6–46.5%). Although the number of patients was limited, Arm 1 (docetaxel on day 1) had a numerically better response: for the 12 patients in Arm 1, five PRs were recorded for a response rate of 42%. However, Arm 1 had more toxicities than the docetaxel on day-8 schedule.

Overall, the toxicity associated with the gemcitabine–docetaxel regimen was manageable. In Arm 1, five patients (42%) had grade 3/4 neutropenia supervened with infection or fever, while only one patient (9%) had grade 3 neutropenia with infection or fever in Arm 2. This indicated that docetaxel was better tolerated on day 8 than on day 1 in a 21-day cycle. It is speculated that the influence of time to nadir of neutropenia is different in each agent: 14–20 days with gemcitabine and 9 days with docetaxel. The time to recover from nadir is 7–8 days with gemcitabine and 8 days with docetaxel. This could explain why docetaxel on day 8 was better tolerated.

Meta-analysis studies have reported that cisplatin-based regimens produce a significant survival benefit in NSCLC (20–23), improve median survival time by 6–8 weeks and 1-year survival rate from 15% to 25% when compared with the best supportive care (24). But studies with platinum-based combinations have also reported severe toxicities, so the deterioration of patients' quality of life is a major problem to be solved (3).

New effective non-platinum-based therapies have been used in various combinations in recent years, and the combination of gemcitabine and docetaxel has been established as one of the

Table 3. NCI–CTC grade 3/4 toxicities (n = 59)

Toxicities	Grade 3		Grade 4	
	n	%	*n*	%
Hematological toxicities				
Leukopenia	24	40.7	4	6.8
Neutropenia	19	32.2	20	33.9
Lymphopenia	10	16.9	0	0.0
Hemoglobin decreased	4	6.8	0	0.0
Thrombocytopenia	1	1.7	0	0.0
Thrombocytosis	1	1.7	0	0.0
Non-hematological toxicities				
ALT increased	5	8.5	0	0.0
Infection	4	6.8	0	0.0
Anorexia	4	6.8	0	0.0
Nausea	4	6.8	0	0.0
Diarrhea	3	5.1	0	0.0
Interstitial pneumonia	3	5.1	0	0.0
Rash	3	5.1	0	0.0
Constipation	3	5.1	0	0.0
AST increased	2	3.4	0	0.0
Fatigue	2	3.4	0	0.0
Vomiting	2	3.4	0	0.0
Hyperglycemia	1	1.7	0	0.0
Hyponatremia	1	1.7	0	0.0
Allergic reaction	1	1.7	0	0.0
Vasovagal reaction	1	1.7	0	0.0
Body temperature decrease	1	1.7	0	0.0
Weight increase	1	1.7	0	0.0
Hypotension	1	1.7	0	0.0
Pneumonia	1	1.7	0	0.0
Arrhythmia	1	1.7	0	0.0
Edema	1	1.7	0	0.0
Neuropathy peripheral	1	1.7	0	0.0
Anaphylaxis	0	0.0	2	3.4

NCI–CTC, National Cancer Institute–Common Toxicity Criteria version 2.0; ALT, alanine aminotransferase; AST, aspartate aminotransferase.

Table 4. NCI–CTC grade 3/4 toxicities (n = 40, recommended regimen)

Toxicities	Grade 3		Grade 4	
	n	%	*n*	%
Hematological toxicities				
Leukopenia	13	32.5	2	5.0
Neutropenia	12	30.0	11	27.5
Lymphopenia	5	12.5	0	0.0
Hemoglobin decreased	2	5.0	0	0.0
Thrombocytopenia	1	2.5	0	0.0
Thrombocytosis	1	2.5	0	0.0
Non-hematological toxicities				
ALT increased	2	5.0	0	0.0
Diarrhea	2	5.0	0	0.0
Infection	2	5.0	0	0.0
Interstitial pneumonia	2	5.0	0	0.0
Rash	2	5.0	0	0.0
Fatigue	2	5.0	0	0.0
Nausea	2	5.0	0	0.0
Vomiting	2	5.0	0	0.0
Hyperglycemia	1	2.5	0	0.0
Hyponatremia	1	2.5	0	0.0
AST increased	1	2.5	0	0.0
Allergic reaction	1	2.5	0	0.0
Vasovagal reaction	1	2.5	0	0.0
Anorexia	1	2.5	0	0.0
Body temperature decrease	1	2.5	0	0.0
Weight increase	1	2.5	0	0.0
Hypotension	1	2.5	0	0.0
Pneumonia	1	2.5	0	0.0
Edema	1	2.5	0	0.0
Constipation	1	2.5	0	0.0
Peripheral neuropathy	1	2.5	0	0.0
Anaphylaxis	0	0.0	2	5.0

NCI–CTC, National Cancer Institute–Common Toxicity Criteria version 2.0; ALT, alanine aminotransferase; AST, aspartate aminotransferase.

well-examined regimens. In recent studies using gemcitabine–docetaxel in NSCLC, response rates of 25–50% (19,25–29) and time-to-progression of disease of 106–132 days (31,32) have been reported. Georgoulias et al. (16) reported that the gemcitabine–docetaxel and docetaxel–cisplatin regimens they compared were equivalent in efficacy, but toxicity was severe in the latter. While docetaxel–cisplatin regimens showed severe toxicities of grade 3 anemia (5%), grade 3/4 neutropenia (13%/21%), grade 3 nausea/vomiting (10%) and grade 3 diarrhea (8%), gemcitabine–docetaxel regimens had grade 3/4 anemia (1%/1%), grade 3/4 neutropenia (11%/11%), grade 3 nausea/vomiting (2%) and grade 3/4 diarrhea (2%/1%) in 441 patients. However, the difference of efficacy and safety by the administration schedule and dosage of gemcitabine and docetaxel has not been well documented.

There are some studies that have examined the efficacy and safety of the same schedule as the recommended regimen in our study, namely gemcitabine on days 1 and 8 plus docetaxel on day 1. In these studies dosages were various: gemcitabine was 800–1100 mg/m^2 and docetaxel was 60–100 mg/m^2 (18,19,27–30). Response rates in these studies also varied from 16 to 38%, which indicates that the response rate of the recommended regimen in our study (30.0%) was clinically meaningful because the dosage of docetaxel (50 mg/m^2) in our study is less than that in any other studies. This might have contributed to the relatively mild toxicities of our recommended regimen.

In another study (26), a high response rate (50.0%) was achieved in patients with another administering schedule: gemcitabine 1000 mg/m^2 on days 1 and 10 plus docetaxel 80 mg/m^2 on day 1, administered every 21 days. The most common treatment-related toxicity was myelosuppression. Grade 3/4 leukopoenia and neutropenia occurred in only six (18%) and eight (24%) patients, respectively.

The median survival was 11.9 months in our study, being slightly better than the result from the median survival of the phase III study with gemcitabine and cisplatin, which was 8.7–9.1 months (33,34). This result suggests that the regimen we selected in the phase II portion of this study is comparable in survival with the cisplatin-based regimen.

In conclusion, the combination of gemcitabine 1000 mg/m^2 on days 1 and 8 plus docetaxel 50 mg/m^2 on day 8 is suggested to be better tolerated and has equivalent efficacy to cisplatin-based therapy. These results should be verified by a phase III study in Japanese patients.

CONCLUSION

In this phase I/II study, we studied the activity and tolerability of gemcitabine and docetaxel in Japanese patients. The combination of gemcitabine 1000 mg/m^2 on days 1 and 8 plus docetaxel 50 mg/m^2 on day 8 is suggested to be well tolerated and has equivalent efficacy to cisplatin-based therapy.

Acknowledgments

We thank Dr N. Masuda for his helpful comments with the preparation of the paper; and Drs T. Taguchi, Y. Ariyoshi, N. Hara and M. Kawahara for overseeing the management of the study. This work was supported by Eli Lilly Japan K.K.

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