Table 1. Patient characteristics (n = 502)

	Total	Developed ILD	No ILD Development	*P*-value
Gender				
Male	371	24	347	0.0361
Female	131	2	129	
Age				
Median (range)	65 (33–83)	66 (53–77)	65 (33–83)	0.5253
ECOG PS				
0–1	443	26	417	0.0590
2–4	59	0	59	
Pathological type				
Adenocarcinoma	279	14	265	0.8775
Squamous cell carcinoma	84	6	78	
Poorly differentiated carcinoma	56	3	53	
Small cell carcinoma	79	3	76	
Others	4	0	4	
Smoking status				
Current smoker	272	14	258	0.1085
Former smoker	124	10	114	
Never smoked	106	2	104	
Clinical stage				
IB	10	0	10	0.6633
IIB	7	0	7	
IIIA	21	0	21	
IIIB	128	8	120	
IV or recurrence after operation	336	18	318	
Treatment history				
Platinum-based	384	18	366	0.3505
Vinorelbine-containing	295	13	282	0.4145
Gemcitabine-containing	110	7	103	0.4758
Taxane-containing	236	14	222	0.5470
Irinotecan-containing	72	2	70	0.5624
Etoposide-containing	67	2	65	0.5573
TKI	188	14	174	0.0954
Number of chemotherapy regimens				
1	212	9	203	0.7733
2	155	9	146	
3	106	7	99	
4 or 5	29	1	28	

ILD, interstitial lung disease; TKI, tyrosine kinase inhibitor.

Table 2. Radiological findings of plain X-ray and computerized tomography films of the chest

Interstitial shadow on plain X-ray films	65 (13%)
Interstitial shadow on CT films	102 (20%)
Mild	37 (7%)
Moderate	42 (8%)
Severe	23 (5%)
Pulmonary emphysema on CT films	189 (38%)
Mild	92 (18%)
Moderate	49 (10%)
Severe	48 (10%)
Pulmonary bullae	101 (20%)

Table 3. Radiological findings and interstitial lung disease

Radiological findings	Developed ILD	No ILD Development	*P*-value
Interstitial shadow on plain X-ray films of the chest			
No	23	414	1.000
Yes	3	62	
Interstitial shadow on CT film of the chest			
No	15	385	0.0096
Yes	11	91	
Severity of the interstitial shadow			
No	15	385	<0.0001
Mild	8	29	
Moderate	1	41	
Severe	2	21	
Pulmonary emphysema			
No	14	299	0.4075
Yes	12	177	
Severity of the emphysema			
No	14	299	0.6468
Mild	7	85	
Moderate	2	47	
Severe	3	45	
Pulmonary bullae			
No	18	383	0.2052
Yes	8	93	

ILD, interstitial lung disease.

information for prescription, patients with obvious interstitial shadow on chest X-ray should avoid gemcitabine or irinotecan. Although patients with interstitial shadow on chest X-ray were excluded in previous clinical trials in Japan, unexpectedly frequent ILD has been reported, as in the case of combination chemotherapy with docetaxel and gemcitabine (7). Is interstitial shadow on chest X-ray an appropriate criterion to detect interstitial pneumonia or pulmonary fibrosis and avoid ILD? Generally, chest CT can detect interstitial shadow more clearly than chest X-ray. Specifically, high-resolution CT of the chest is essential in diagnosing interstitial pneumonia. However, it has not been determined exactly how much more interstitial shadow detected by CT reveals the onset of ILD. We analyzed CT films of consecutive lung cancer patients who underwent

Table 4. Multivariate analysis of risk factors associated with the onset of interstitial lung disease

Variable	Odds ratio	95% CI	*P*-value
Interstitial shadow on CT films of the chest	3.20	1.34–7.59	0.0086
Treatment history with TKI	3.17	1.36–7.36	0.0073
Male gender	4.33	0.970–19.38	0.0551

CI, confidence interval; TKI, tyrosine kinase inhibitor.

chemotherapy without thoracic radiation therapy. Retrospective review of medical records identified that 26 out of 502 patients developed ILD. We found that interstitial shadow on CT films was associated with onset of ILD, but that interstitial shadow on X-ray was not. We divided interstitial shadow into three classes: mild, moderate and severe. Interstitial shadow on X-ray means moderate to severe interstitial pneumonia. Eight out of 37 patients (22%) with mild interstitial shadow not detected on chest X-ray developed ILD. The reason for the high rate of ILD in patients with mild interstitial shadow is unknown. The criteria of no interstitial shadow on chest X-ray did not sufficiently reduce the risk of ILD. Treatment history with TKI, either gefitinib or erlotinib, was also associated with onset of ILD in multivariate analysis. Conversely, treatment with gemcitabine or irinotecan was not associated with onset of ILD.

Our retrospective analyses have several limitations. We avoided treatment with gemcitabine, irinotecan or TKI in the case of patients with moderate to severe interstitial shadow detectable on chest X-ray films. Some patients who were transferred to another hospital just after chemotherapy may have developed ILD, but detailed clinical courses after transfer were not available. Early death after chemotherapy due to disease progression might conceal the onset of ILD. Although these biases may exist, our analyses were made with an extensive cohort of patients, and therefore the results obtained are of significance.

The frequency of ILD in Japanese patients was reported to range between 3 and 15% in previous clinical trials (6–8). This rate appears to be higher than that observed in the rest of the world. Explanations include the possibility that ILD may be more prevalent among the Japanese or, alternatively, that a greater awareness of the disease could lead to more frequent diagnosis. Furthermore, there may be an increased genetic susceptibility to ILD specifically among the Japanese population (5).

Patients with interstitial shadow on chest X-ray have been excluded in previous clinical trials to avoid ILD caused by chemotherapeutic agents. However, this criterion alone is considered insufficient. It is recommended that patients with interstitial shadow on chest CT are excluded from future clinical trials until this issue is clarified, as it is anticipated that use of chemotherapeutic agents frequently mediate onset of ILD in this context. Therefore, physicians need to understand the associated risk of ILD in patients with interstitial shadow on chest CT and obtain informed consent from patients before administering chemotherapy in clinical practice.

Acknowledgments

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Improved Diagnostic Efficacy by Rapid Cytology Test in Fluoroscopy-Guided Bronchoscopy

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Background: Fluoroscopy-guided bronchoscopy is a safe and routine method used to obtain a histologic or cytologic specimen of peripheral lung nodules, but it has low sensitivity in diagnosing malignant tumors. Although feedback from rapid cytology tests are expected to improve diagnostic rates, the value of the routine use of rapid cytology tests has not been established.

Materials and Methods: We prospectively studied 657 patients with suspected peripheral malignant lung lesions on chest computed tomography who underwent fluoroscopy-guided bronchoscopy between January 2002 and December 2004. Rapid on-site cytopathologic examinations (ROSE) were performed during bronchoscopic examinations. The additional approach to the lesions was performed immediately after conventional bronchoscopic examinations when ROSE was not considered diagnostic.

Results: There were 528 patients diagnosed as having malignant lesions. In 477 of these patients (90.3%), final malignant diagnosis was established by the initial bronchoscopy. Among these, 84 patients (15.9%) were diagnosed only with the additional feedback from ROSE. Of 240 peripheral lesions ≤2 cm, 174 were found to be malignant. Without ROSE, 110 (63.2%) of peripheral malignant lesions were diagnosed by bronchoscopy. The integration of ROSE enabled us to diagnose an additional 40 patients (23.0%) by bronchoscopy. ROSE improved diagnostic yield independent of the site and histology of the lesions and experience of the operators.

Conclusion: ROSE increased the diagnostic yield of bronchoscopy from 74.4% to 90.3% and therefore is an effective reinforcement in bronchoscopic diagnosis of peripheral pulmonary malignancies. The use of ROSE in routine bronchoscopy should be encouraged.

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Examinations used to diagnose pulmonary malignant lesions should be safe, accurate, and optimal for obtaining adequate information. A flexible fiberoptic bronchoscope has become prevalent in obtaining specimen from lung lesions. Although central visible tumors can be diagnosed at high sensitivity, it is reported that the diagnostic rate for peripheral lung lesions is low, from 62% to 86%, even in combination with various techniques.[1–4] Brush, curette, forceps, and aspiration needles have been investigated as tools to obtain diagnostic specimens. Other reports recommend rapid on-site cytopathologic examinations (ROSE) in transbronchial needle aspiration of lymph nodes.[5–7] However, ROSE has not been introduced for diagnosing peripheral lung lesions. Recently, the combination of ultra-fast Papanicolaou staining and multiplanar reconstruction images has been recommended to improve diagnostic accuracy and safety in fluoroscopy-guided transbronchial biopsy.[8] In this prospective study, we integrated ROSE into routine bronchoscopy and evaluated the benefit of bronchoscopy combined with ROSE.

BRONCHOSCOPY

In our hospital, we foremost recommend bronchoscopy with a flexible bronchoscope in the diagnosis of pulmonary nodules because of its safety. If the lesions are not bronchoscopically invisible, procedures to obtain diagnostic materials are performed under fluoroscopic guidance. Transcutaneous fine-needle biopsy (TCNB) is recommended for patients with a negative result of preceding bronchoscopy or with negligible risk of pneumothorax by percutaneous puncture, such as those with lesions invading the thoracic wall. Video-assisted thoracic surgery (VATS) is usually recommended for patients with negative results of bronchoscopy and/or TCNB or lesions unrecognizable under fluoroscopy. For pure GGO, we recommend computed tomographic (CT) follow-up, otherwise VATS.

In bronchoscopy, the specimen for cytology was obtained by curetting or brushing. The material was smeared on two glass slides: one was subjected to ROSE (ROSE sample) and the other to conventional Papanicolaou staining. During ROSE, forceps biopsy was performed to obtain the specimen for histology and cytology. When ROSE was not diagnostic, additional bronchoscopic examinations, such as transbronchial needle aspiration (TBNA), bronchial washing, or ultrathin bronchoscopy, were performed to obtain additional samples just after conventional bronchoscopy. For the analysis, we defined both the material subjected to Papanicolaou staining and the material obtained by biopsy as conventional

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samples. The material obtained by additional bronchoscopic examinations after ROSE was defined as additional samples.

CYTOLOGY AND HISTOLOGY EXAMINATION

We used rapid Shorr stain as a rapid cytology test, which we have recently developed by modifying the Shorr stain.[9] Rapid Shorr stain completes staining very fast (approximately 1 minute) and presents similar coloring to Papanicolaou staining; therefore, it is familiar to the cytoscreeners in our institute. The cytopathologist was able to provide a preliminary diagnosis within a few minutes. Papanicolaou staining was performed after bronchoscopic examination. Tissue specimens obtained by forceps biopsy were fixed in formalin, embedded in paraffin, and stained with hematoxylin-eosin. Additional specific staining was performed when necessary.

PATIENTS

We performed 1900 flexible bronchoscopic examinations between January 2001 and December 2004. Based on the results of chest radiograph and CT, 795 patients were thought to have central lesions and underwent bronchoscopy without fluoroscopy; 1105 patients underwent fluoroscopy-guided bronchoscopy. ROSE was not performed in the examinations to obtain samples for bacterial testing, for visible lesions, or to evaluate lesions diagnosed before, etc. ROSE was not used for the patients entered into another study performed during the same period in which ROSE was not integrated. Other patients' samples were not subjected to ROSE because only a single trial to obtain bronchoscopic material was possible because of patients' stress during bronchoscopy. Excluding these from the 1105 patients who underwent fluoroscopy-guided bronchoscopy, 657 patients received fluoroscopy-guided bronchoscopy with ROSE. ROSE was repeated when we thought it possible and necessary. Despite negative ROSE results, the lesions of very likely malignant or difficulty except for bronchoscopy, we tend to repeat ROSE. If a diagnosis could not be made via bronchoscopy, further work-up for the lesions included surgical procedures, TCNB, follow-up by bronchoscopy, chest radiograph and CT, and sputum investigations.

RESULTS

Bronchoscopic examinations with ROSE were performed under fluoroscopic guidance for 657 peripheral lung lesions. Patient characteristics are listed in Table 1. The final diagnosis of malignant and benign disease was determined in 528 and 117 lesions, respectively. The remaining 12 lesions were not diagnosed and subjected to careful follow-up. Malignant lesions consisted of adenocarcinoma (n = 328), squamous cell carcinoma (n = 87), small cell carcinoma (n = 32), carcinoid (n = 20), large call carcinoma (n = 7), lymphoma (n = 3), metastatic carcinoma (n = 22), and other malignancies (n = 29).

As shown in Table 2, 393 lesions were diagnosed as malignant by using conventional samples alone. ROSE definitively detected malignant cells in 357 malignant lesions but failed to detect atypical cells in 36 malignant lesions. The false-negative rate of ROSE was 9.2% compared with diagnosis based on conventional samples. In ROSE, a limited time period is permitted for screening and diagnosis. However, cancer cells were detected in only one sample with a negative ROSE result by subsequent re-diagnosis with sufficient time. There was no false-positive result in ROSE. However, final diagnosis was obtained with the additional samples in 84 of 135 malignant lesions that were not diagnosed with conventional samples alone. Therefore, the integration of ROSE into bronchoscopic examination improved the diagnostic sensitivity from 74.4% to 90.3% (Figure 1*A*). The improvement of sensitivity was statistically significant ($p < 0.05$) and enabled effective diagnosis for peripheral lung lesions.

TABLE 1. Patient characteristics

Sex	All patients	Patients with malignancy
Male	411	344
Female	246	184
Age (year)		
Range	25–89	27–87
Average	65.7	66.5
Chance of discovery		
Annual screening	250	183
Tests for other diseases	223	176
Subjective symptoms	163	151
Others	21	18
Smoking status		
Smoker	223	190
Ex-smoker	161	136
Non-smoker	210	156
Unknown	63	46

Additional samples for diagnosis were collected by brushing, curetting, forceps biopsy, TBNA, ultra-thin-bronchoscopy, and washing from the same or other bronchi. Sometimes, several methods were combined for obtaining a specimen. The methods to obtain additional specimens were determined based on the bronchoscopic access to the lesions and the condition of patients. We analyzed additional approaches contribute to the improvement of diagnostic accuracy (Table 3). Whereas brushing showed low diagnostic yield, curetting or forceps biopsy from the other branch, TBNA, and forceps biopsy with ultra-thin bronchoscope yielded more than a 65% positive rate in additional approaches. Washing was also useful for diagnosis in additional approaches, but malignant cells were usually detected by the other methods conducted at the same time.

Surprisingly, ROSE provided more benefit for the diagnosis of small-sized lesions (≤2 cm) (Figure 1*B*). With conventional samples, 110 of 174 small-sized malignant lesions (63.2%) were diagnosed by bronchoscopy. With the help of ROSE, 40 lesions (23.0%) were diagnosed only with an additional sample. Improvement of diagnostic rate for small lesions was significantly greater than that for larger lesions (23.0% versus 12.4%; $p < 0.05$). No significant improvement was observed among the other factors in exam-

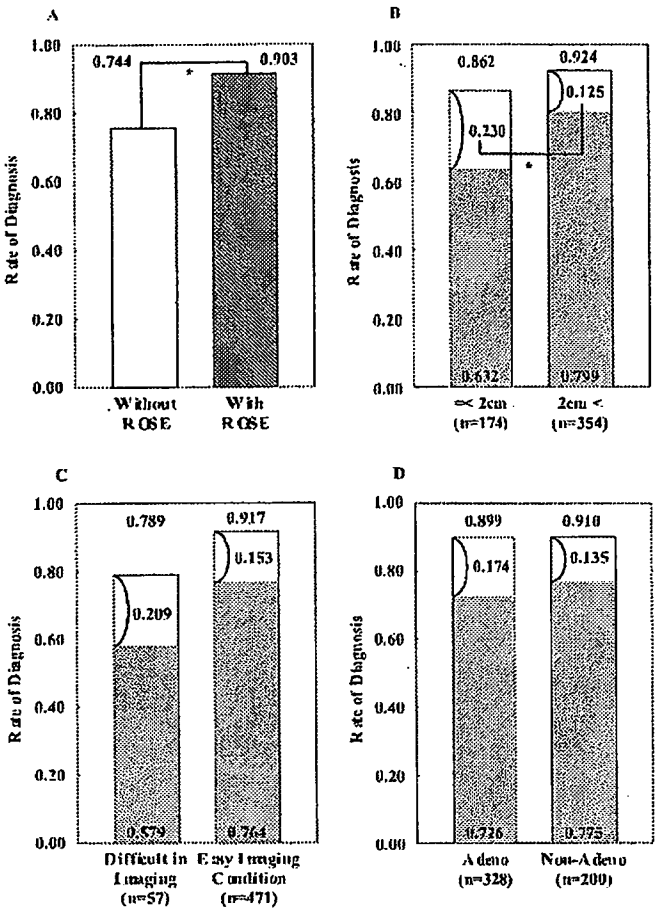

FIGURE 1. ROSE improved diagnostic yield and lesion features. *A*, ROSE improved diagnostic sensitivity. The *gray bar* shows the diagnostic sensitivity of fluoroscopy-guided bronchoscopy with ROSE; the *white bar* shows the diagnostic sensitivity of bronchoscopy without ROSE. The sensitivity is significantly different ($p < 0.05$). *B*, Tumor size and improvement of diagnostic sensitivity by ROSE. The *shaded area* indicates diagnostic sensitivity without ROSE. The improvement in small lesions was better than that in large lesions ($p < 0.05$). *C*, Imaging conditions of the lesions under fluoroscopy revealed diagnostic yield but little difference in improvement by ROSE. The *shaded area* indicates diagnostic sensitivity without ROSE. *D*, Histology type made little difference in diagnostic sensitivity and improvement by ROSE. The *shaded area* indicates diagnostic sensitivity without ROSE.

inations (Figure 1, *C* and *D*). Examination of poorly visible lesions in fluoroscopy had low sensitivity (n = 57, 78.9%) compared with that of clearly visible lesions (n = 471, 91.7%). The improvement by ROSE was slightly higher in examinations for poorly visible lesions (21.1% versus 15.3%), although the difference was not statistically significant. Little improvement by ROSE was shown between histology types of the lesions: adenocarcinoma 52.3%, squamous cell carcinoma 56.3%, small cell carcinoma 50%, and metastatic carcinoma 40.0% of ROSE-negative lesions. Our results also showed the difficulty in diagnosing lesions in the upper lobe and S6, especially in right lung with conventional samples. However, a comparable improvement of diagnostic yield was achieved with ROSE in most areas (from 40% to 60% of ROSE-negative lesions). We calculated the diagnostic yields with conventional samples and additional samples for each examiner to determine the effect of skill level of examiners on usefulness of ROSE. Although the skill level of the examiner tends to correlate to diagnostic yield with conventional samples, improved diagnosis by ROSE was observed similarly in almost all of the examiners (approximately 40% to 52% of ROSE-negative cases).

ROSE was repeated to make a decision for further examinations when access to the lesion was not satisfactory and an additional approach was considered to be possible. We calculated the effect of repeated ROSE on the diagnostic yield of peripheral lung cancer by fluoroscopy-guided bronchoscopy and found that a diagnostic improvement of 89.4% was attained by the first ROSE and 3.2% by the second ROSE (Table 4). Repeated ROSE improved diagnosis in only five of 107 examinations.

DISCUSSION

Bronchoscopic examination with fluoroscopic guidance is often used to obtain a diagnostic specimen of lung nodules. However, most reports have shown relatively low accuracy of diagnosing peripheral lesions by bronchoscopy.[10-12] Bandoh et al.[8] reported refined accuracy up to 91% by combining multiplanar reconstruction images and ultra-fast Papanicolaou staining. They used a historical control for comparison and multiplanar images for another tool. Our study was designed to improve the bronchoscopic diagnosis of peripheral malignant lesions by introducing only ROSE and was performed prospectively in routine bronchoscopic examinations. Therefore, more precise analysis could be performed to estimate ROSE's effectiveness. Our result shows that diagnostic sensitivity of peripheral malignant lesions was improved from 74.4% to 90.3% with ROSE only.

To obtain rapid diagnosis during bronchoscopy, the staining method should be convenient and fast and should present suitable coloring for diagnosis. Several staining methods are applied in ROSE.[8,14,15] We selected rapid Shorr staining for ROSE that we established recently[9] because it is simple, rapid, and similar in coloring to Papanicolaou staining, which is familiar to cytoscreeners and cytopathologists. Additionally, rapid Shorr staining requires only a small area for staining. Rapid Shorr staining is reliable, with low false-positive and false-negative rates.

To improve sensitivity, a method for obtaining additional samples should be carefully determined. When another visible bronchus could be a suitable path to the lesion, we selected this path. When the visible route to the lesion could not be improved, we changed the method for approaching to lesions to TBNA, ultra-thin bronchoscopy, or washing. Comparison among the methods indicates that TBNA and ultra-thin bronchoscopy were most effective in the approach through the same bronchus. In the approach through different bronchi, curetting and biopsy were effective for diagnosis, whereas TBNA was a good alternative (Table 3). Therefore,

TABLE 2. Results of bronchoscopic examinations with ROSE

ROSE	Final diagnosis	Diagnosis by conventional samples	Diagnosis by additional samples	Diagnosis by different examinations
Negative	279			
Malignant	154	26	80	48
Benign	113	13	2	98
Unknown	12	0	0	12
Positive suspected	21			
Malignant	17	10	4	3
Benign	4	1	0	3
Unknown	0	0	0	0
Positive	357			
Malignant	357	357	0	0
Benign	0	0	0	0
Unknown	0	0	0	0

ROSE, rapid on-site cytopathologic examinations.

TABLE 3. Methods of additional sampling for diagnosing malignant lesions

	Tested lesions	Sole positive	Positive
Brushing	16	0 (0.0%)	4 (26.7%)
(from other branch)	4	0 (0.0%)	1 (25.0%)
Curetting and forceps	101	33 (32.7%)	51 (50.5%)
(from other branch)	14	12 (85.7%)	13 (92.9%)
TBNA	35	16 (45.7%)	25 (71.4%)
(from other branch)	7	4 (57.1%)	6 (85.7%)
Washing	29	3 (10.3%)	12 (41.4%)
(from other branch)	4	1 (25.0%)	2 (50.0%)
Forceps with ultra-thin bronchoscope	20	14 (70.0%)	20 (100%)
Washing with ultra-thin bronchoscope	16	0 (0.0%)	11 (68.8%)

TABLE 4. Diagnostic yield of malignant lesions by repeated ROSE

ROSE	Bronchoscopic examinations	Additional examination	Diagnostic yield	Accumulated sensitivity
0	657		393	74.4%
1	657	214	79	89.4%
2	126	94	3	90.0%
3	20	12	2	90.3%
4	1	1	0	90.3%

ROSE, rapid on-site cytopathologic examinations.

alternative routes or methods such as TNBA or ultra-thin bronchoscopy should be considered when ROSE is not diagnostic. We do not recommend brushing and washing.

It has been reported that the size of the lesion has negative correlation to the sensitivity of bronchoscopy. Our results also showed low sensitivity for small lesions (≤2 cm). Surprisingly, however, improvement of diagnostic yield by ROSE was more prominent in diagnosing small lesions (Figure 1*B*). We analyzed the relationship between the size of lesions and the methods by which diagnosis could be made with additional samples. There was no distinct difference in frequency of usage of each method and its ability to yield additional diagnoses between the small and large lesions. Therefore, the reason why diagnostic yield improved more in smaller lesions is not known. One possible explanation is poor fluoroscopic targeting for smaller lesions in bronchoscopy. We used biplane fluoroscopy, but not CT, to determine whether the tip of sampling tools reached the lesions. It is reasonable that the error in targeting by this method is greater for small lesions than for large lesions. ROSE may have improved diagnostic yield partly by correcting the error in targeting.

There are several factors other than the size of tumors related to diagnostic yields. The experience of the examiners relates to the diagnostic sensitivity of bronchoscopic examinations.[16] The location of the lesion, histology type, and visibility under fluoroscopy can influence the yield. We analyzed the relationship between these factors and diagnostic yield. Experience of examiners, location of the lesion, and fluoroscopic visibility of lesions showed some relation to the diagnostic yield. However, improvement of diagnosis by ROSE was similarly observed for all examiners. Diagnostic yield of the lesions in the upper lobe and S6 was relatively low. However, we did not observe a clear difference of improvement by ROSE by location. Examinations for poorly

visible lesions under fluoroscopy showed low sensitivity compared with clearly visible lesions. The improvement by ROSE was slightly higher in the examinations for poorly visible lesions, although not statistically significant. Comparison among histology types of the lesions showed little difference in sensitivity and improvement by ROSE. We encourage the use of ROSE for diagnosing peripheral lesions, especially those of small size, regardless of their location, fluoroscopic visibility, or experience of the examiners.

We usually performed curetting and forceps biopsy only once before ROSE. Although repeated curetting and biopsy were thought to improve sensitivity, we repeated the collection of specimens only in negative ROSE cases, including false negatives. We performed additional examinations for only 214 cases with ROSE and showed an increased sensitivity by 14.9% instead of performing repeated curetting and biopsy in most of the 657 cases without ROSE. ROSE enabled us to avoid unnecessary examinations, even including false-negative cases. Considering the low effectiveness of repeated ROSE, single ROSE is recommended. Recently, CT screening and positron emission tomography have been experimentally introduced for the early detection of lung cancer.[16–18] We expect to diagnose peripheral lung nodules more safely and accurately in the future. The combination of ROSE with fluoroscopy-guided bronchoscopy is encouraged as a conventional method to enhance its safety and sensitivity.

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Is the Importance of Achieving Stable Disease Different between Epidermal Growth Factor Receptor Tyrosine Kinase Inhibitors and Cytotoxic Agents in the Second-Line Setting for Advanced Non-small Cell Lung Cancer?

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Background: It is controversial whether achieving stable disease leads to a survival benefit and whether the importance of achieving stable disease differs between cytotoxic agents and molecular targeted agents. To examine these questions, the authors retrospectively reviewed phase II and III studies in the second-line setting for advanced non-small cell lung cancer using epidermal growth factor receptor (EGFR) tyrosine kinase inhibitors (TKIs) and cytotoxic agents separately.

Methods: The authors chose 45 trials for the chemotherapy group and nine for the EGFR TKI group by searching the PubMed database. All nine trials in the EGFR TKI group concern gefitinib and erlotinib.

Results: The median survival time increased 0.0375 month with each 1% increase in stable disease rate ($p = 0.039$), and each 1% increase in response rate resulted in 0.0744 ($p < 0.001$) month of median survival time in the analysis combined with both cytotoxic agents and EGFR TKIs. Main and interaction terms for EGFR TKI treatment were not statistically significant. With respect to time to progression, only response rate showed a statistically significant relationship with survival.

Conclusions: To obtain response seems to be more important than to achieve stable disease for both cytotoxic agents and EGFR TKIs, although achieving stable disease is still valuable. The relationship between survival and response or stable disease appears similar for cytotoxic agents and EGFR TKIs.

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In 1995, a meta-analysis demonstrated a modest survival benefit for cisplatin-based chemotherapy compared with best supportive care as first-line therapy in patients with locally advanced or metastatic non-small cell lung cancer (NSCLC).[1] Equal survival improvement is provided by introducing several new agents with novel mechanisms and significant activity against NSCLC such as taxanes, gemcitabine, and vinorelbine, when used in combination with a platinum agent.[2–4] However, most patients relapse following platinum-based chemotherapy, leading to poor survival. Until recently, the role of second-line chemotherapy was not well defined because most patients had a poor performance status by the time of relapse. However, as newer agents in combination with platinum agents have increased, the number of patients with durable antitumor effects and the number of patients for second-line chemotherapy have increased. Therefore, second-line chemotherapy for advanced NSCLC is becoming increasingly important. Several chemotherapy agents have been evaluated in the second-line setting. Among them, docetaxel was the first agent to show a survival benefit and an improvement in quality of life in two large phase III studies[5,6] and has been approved as a second-line agent. A recent randomized phase III study reported that pemetrexed (a multitargeted antifolate, Alimta; Eli Lilly & Co., Indianapolis, IN) had comparable activity and better symptom relief than docetaxel.[7] Both of these cytotoxic agents demonstrated response rates of less than 10%, but both agents have demonstrated survival benefits and an improvement in quality of life. This indicates that it is important to achieve stable disease and objective response for second-line cytotoxic agents.

The molecular targeted agents are attractive because they promise to produce specific cytostatic action with a resultant mild toxicity profile. In many tumors, overexpression of the epidermal growth factor receptor (EGFR) is associated with a poor prognosis and chemoresistance,[8,9] and it is common in NSCLC.[10–12] The low-molecular-weight EGFR tyrosine kinase inhibitors (TKIs) gefitinib and erlotinib are the most advanced agents in clinical trials. The results of a recent phase III study in the second-line setting showed that erlotinib significantly improved survival compared with best supportive care,[13] although the overall response rate was only 9% on the erlotinib arm.

Because of their mechanism of action, it might be more important to achieve stable disease for most molecular targeted agents than for their cytotoxic counterparts. However, evaluating stable disease in clinical trials is very difficult, as patients with stable disease are not a homogeneous population.

Based on this background, we hypothesized that not only objective response but also stable disease could lead to survival benefit, in particular, with molecular targeted agents. Therefore, we retrospectively reviewed phase II and randomized phase III studies in the second-line setting using EGFR TKIs and cytotoxic agents separately to evaluate our hypothesis and ascertain whether the importance of achieving stable disease was different between EGFR TKIs and cytotoxic agents.

METHODS

Search and Selection for Trials

Data concerning response rates, rates of stable disease, time to progression, and survival from all published studies including phase II and randomized phase III studies assessing the activity of EGFR TKIs and cytotoxic agents in the second-line setting were identified electronically. We performed the search for trials through a computer-based search of the PubMed database using the following terms: "NSCLC," "chemotherapy (second or pretreated)," "advanced," "not radiation," "not adjuvant," "randomized controlled trial," "human," and "English," in the chemotherapy group. In the EGFR TKI group, we used the following terms: "NSCLC," "clinical trial," "human," "English," and the name of the EGFR TKI (e.g., gefitinib, referred from the review of Wendy et al.[14]). All trials that had been reported by September 30, 2004, were targeted. However, because there was no phase III study in the EGFR TKI group, only one abstract from the *Proceedings of the American Society of Clinical Oncology*, by Shepherd et al., was added. Among the retrieved studies, we excluded the trials that had missing outcomes data. We also excluded phase I/II studies. When we examined randomized phase III and randomized phase II studies, if both arms (experimental and reference arms) included cytotoxic agents or EGFR TKIs, both were included in our analysis.

Statistical Analysis

All the analyses were performed with Stata version 8 (Stata Corp., College Station, TX). Multiple linear regression analysis was applied to examine impacts on the proportion of subjects who responded and achieved stable disease on survival (median survival time [MST] and time to progression [TTP]). Scales in the models were percentages and months for proportion of subjects and survival, respectively. Two models were examined: model 1, including response rate and stable disease rate or disease control rate (response rate plus stable disease rate) as explanatory variables; and model 2, including EGFR TKI usage (yes/no) and interaction terms between EGFR TKI usage and response/stable disease rate or disease control rate in addition to model 1. In the models, each study was weighted by the number of subjects in an intent-to-treat analysis setting in each study. Thereafter, we chose model 1 based on the significance of interaction terms. To further evaluate the impact of stable disease rate considering response rate, we chose a linear regression model for residual (the observed median survival minus fitted median survival in the response rate only model) as a dependent variable with stable disease rate as a responsible variable. This approach was applied to MST and TTP separately (Figures 1 and 2). The statistical significance was defined as a value of $p < 0.05$, and adjustment for multiple comparison was not considered because of the exploratory setting of this study.

RESULTS

Study Characteristics

As a result of our search, we identified 219 references and chose 45 trials for the chemotherapy group and nine trials for the EGFR TKI group. The baseline characteristics of the 45 trials and nine trials are shown in Tables 1 and 2, respectively. There are four randomized phase II and three phase III studies for cytotoxic agents, and two randomized phase II studies and one phase III study for EGFR TKIs. In the analysis of cytotoxic agents, docetaxel, pemetrexed, other agents, and many types of combination regimens are included. In the analysis of EGFR TKIs, only monotherapies of gefitinib and erlotinib were detected. The median number of enrolled patients per study was 40 (range, 17–288) for the cytotoxic agents and 103 (range, 31–488) for the analysis of EGFR TKIs.

Median Survival Time

As shown in Table 3, both rate of stable disease and response rate were statistically significanty associated with MST in model 1 in the analysis that combined both cytotoxic agents and EGFR TKIs. The coefficient 0.0375 ($p = 0.039$) for stable disease in model 1 indicates that MST increases by 0.0375 month for each 1% increase in stable disease rate. Similarly, each 1% increase in response rate is associated with an increase of 0.0744 month in MST ($p < 0.001$). This trend was similarly observed in model 2, which considered the interaction between EGFR TKI treatment and two response parameters. As interaction terms for EGFR TKI treatment were not statistically significant, one may interpret that the relationship between survival and response rate or stable disease rate is not different between EGFR TKI and cytotoxic chemotherapy. We therefore took model 1 as the model

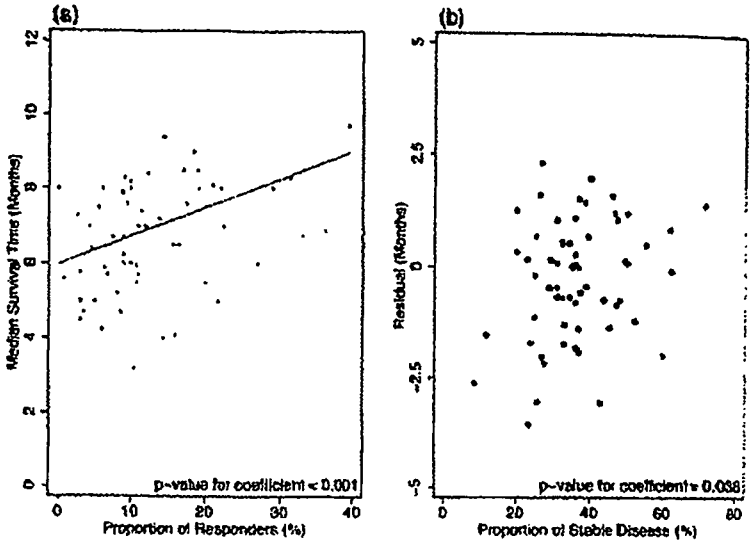

FIGURE 1. Scatterplot for MST and response/stable disease rates. (*A*) The observed MST corresponding to the percentage of responders. (*B*) The residuals (observed MST minus fitted MST in the model for *A*). The figure indicates that both response rate and stable disease rate significantly influence the prolongation of MST.

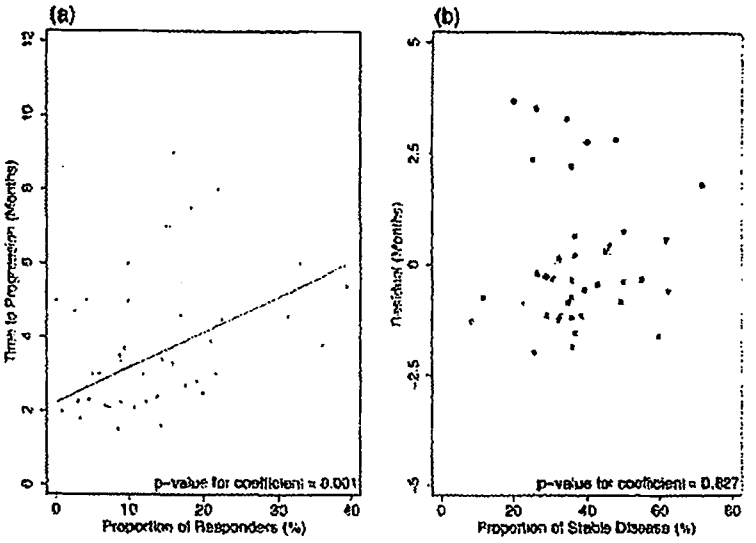

FIGURE 2. Scatterplot for TTP and response/stable disease rates. (*A*) The observed median TTP corresponding to the percentage of responders. (*B*) The residuals (observed TTP minus fitted TTP in the model for *A*). The figure indicates that the response rate but not the stable disease rate significantly influences the prolongation of TTPs.

explaining associations between MST and response variables. Figure 1A is a graphic presentation of observed MSTs corresponding to response rates with the fitted line. Figure 1B presents how well the stable disease rate explains the residual by the response rate only model. Both figures indicate that the response rate and the stable disease rate significantly contribute to MST prolongation. The coefficient for the disease control rate in model 1 was 0.05, indicating that a 1% increase in the disease control rate prolongs MST by 0.05 month ($p < 0.001$). Similar results regarding EGFR TKI terms are listed in Table 3.

Time to Progression

Table 4 shows similar analyses as MST for TTP considering stable disease rate and response rate. Contrary to MST analyses, only response rate showed a statistically significant association with TTP. The coefficient 0.0954 ($p = 0.001$) for response rate in model 1 indicates that TTP increases 0.0954 month with each 1% increase in response rates. Nonsignificant coefficient for stable disease rates indicates lack of impact of this factor on TTP after response rate has been accounted for. As interaction terms for EGFR TKI treatment were not statistically significant, we took model 1 as the model explaining associations between TTP and response variables. Figure 2 is a similar graphic presentation of observed TTPs. Although Figure 2A shows that response rate significantly influences the TTPs, there is no apparent association between TTPs and stable disease rate (Figure 2B). As shown in Table 4, disease control rate was not significantly associated with prolongation of TTP in model 1 and model 2. EGFR TKI interaction terms were not statistically significant.

DISCUSSION

Since the introduction of molecular targeted agents (especially epidermal growth factor receptor inhibitors) in clinical trials in recent years, the importance of achieving stable disease has become an important issue. For these

TABLE 1. Characteristics of the Trials with Cytotoxic Agents in the Second-Line Setting for NSCLC

Author	Phase	Regimen	No. (ITT)	RR (%)	SD (%)	DCR (%)	TTP (mo)	MST (mo)
Stewart et al., 1996[15]	II	Paclitaxel + hydroxyurea	30	3	52	55	—	5
Georgoulias et al., 1997[16]	II	Paclitaxel + gemcitabine	26	29	25	54	—	8
Gridelli et al., 1999[17]	II	Gemcitabine	30	20	60	80	2.5	5.5
Crino et al., 1999[18]	II	Gemcitabine	83	19	31	50	—	8.5
Stathopoulos et al., 1999[19]	II	Paclitaxel + cisplatin	36	38.9	58.3	97.2	—	—
Perng et al., 2000[20]	II	Docetaxel	14	28.6	—	—	4.75	11.7
Mattson et al., 2000[21]	II	Docetaxel	72	13.8	29.3	43.1	2.4	7.2
Rosati et al., 2000[22]	II	Paclitaxel + cisplatin + gemcitabine	26	27	27	54	—	6
Sculier et al., 2000[23]	II	Gemcitabine	77	6	27.7	33.7	—	4.25
Gridelli et al., 2000[24]	II	Docetaxel	23	21.7	8.7	30.4	3	5
Hainsworth et al., 2000[25]	II	Gemcitabine + vinorelbine	55	16.4	43.6	60	—	6.5
Shepherd et al., 2000[5]	III	Docetaxel	55	5.5	47.3	52.8	—	7.5
		Docetaxel	49	6.3	37.5	43.8	—	5.9
Fossella et al., 2000[6]	III	Docetaxel	125	10.8	33	43.8	2.1	5.5
		Docetaxel	125	6.7	36	42.7	2.13	5.7
		Vinorelbine/ifosfamide	123	0.8	31	31.8	1.98	5.6
Kosmas et al., 2001[26]	II	Gemcitabine + vinorelbine	43	33	37	70	6	8.5
Hainsworth et al., 2001[27]	II	Docetaxel + gemcitabine	40	10	48	58	6	6
		Docetaxel + vinorelbine	23	0	40	40	5	8
Agelaki et al., 2001[28]	II	Vinorelbine + carboplatin	37	16	30	46	9	—
Kakolyris et al., 2001[29]	II	Cisplatin + irinotecan	44	22	20	42	8	8
Huisman et al., 2001[30]	II	Cisplatin + epirubicin	27	33	33	66	—	6.75
Pectasides et al., 2001[31]	II	Gemcitabine + vinorelbine	39	2.6	35.9	38.5	4.7	7.3
Lilenbaum et al., 2001[32]	II	Docetaxel	30	10	20	30	—	8
Kosmas et al., 2001[33]	II	Gemcitabine + docetaxel	40	22.5	32.5	55	4.5	7
Kakolyris et al., 2001[34]	II	Docetaxel + gemcitabine	32	15.6	34.4	50	7	6.5
Spiridonidis et al., 2001[35]	II	Docetaxel + gemcitabine	40	32.5	—	—	—	8.1
Juan et al., 2001[36]	II	Paclitaxel	40	39.47	39.47	78.94	5.4	9.7
Chen et al., 2002[37]	II	Docetaxel + gemcitabine	36	36.1	36.11	72.21	3.8	6.9
Gonzalez et al., 2002[38]	II	Irinotecan + vinorelbine	35	9	39	48	—	6.25
Rinaldi et al., 2002[39]	II	Topotecan + gemcitabine	35	11	23	34	—	7
Socinski et al., 2002[40]	II	Paclitaxel	62	8.1	37	45.1	—	5.2
Herbst et al., 2002[41]	II	Gemcitabine + vinorelbine	36	17	50	67	4.6	8.5
Sculier et al., 2002[42]	II	Paclitaxel	67	3	24	27	—	4.5
Thongprasert et al., 2002[43]	II	Docetaxel	34	10.7	47	57.2	—	5.95
Han et al., 2003[44]	II	Irinotecan + capecitabine	37	11.4	34.3	45.7	—	7.4
Chen et al., 2003[45]	II	Docetaxel + ifosfamide	17	31.3	62.5	93.8	4.6	8.3
Font et al., 2003[46]	II	Irinotecan + docetaxel	51	6	37	43	3	8
Chen et al., 2003[47]	II	Vinorelbine + cisplatin	22	9.5	61.9	71.4	3.7	7.6
Smit et al., 2003[48]	II	Pemetrexed	45	4.5	36	40.5	2.3	6.4
		Pemetrexed	36	14.3	26	40.3	1.6	4
Chen et al., 2003[49]	II	Gemcitabine + vinorelbine	50	10	72	82	5	8.2
Dongiovanni et al., 2004[50]	II	Paclitaxel + gemcitabine	34	12	50	62	3	7
Georgoulias et al., 2003[51]	II	Irinotecan + gemcitabine	76	18.4	26.3	44.7	7.5	9
		Irinotecan	71	4.2	25.3	29.5	5	7
Park et al., 2003[52]	II	Gemcitabine + vinorelbine	38	21	55	76	3.9	8.1
Serke et al., 2003[53]	II	Docetaxel	36	11	25	36	—	5.7
Hanna et al., 2003[7]	III	Pemetrexed	283	9.1	45.8	54.9	3.4	8.3
		Docetaxel	288	8.8	46.4	55.2	3.5	7.9
Ceresoli et al., 2003[54]	II	Paclitaxel	53	15	21	36	7	—
Ardizzoia et al., 2003[55]	II	Docetaxel	42	10.5	23.5	34	—	3.2
Quoix et al., 2003[56]	II	Docetaxel	93	8.6	37.1	45.7	1.5	4.7
		Docetaxel	89	7.4	49.4	56.8	2.1	6.7

ITT, intention to treat; RR, response rate; SD, stable disease; DCR, disease control rate; TTP, time to progression; MST, median survival time.

TABLE 2. Characteristics of the Trials with EGFR TKIs in the Second-Line Setting for NSCLC

Author	Phase	Regimen	No. (ITT)	RR (%)	SD (%)	DCR (%)	MST (mo)
Gridelli et al., 2000[57]	II	Gefitinib	59	3.4	11.8	15.2	4.7
Cappuzzo et al., 2003[58]	II	Gefitinib	63	15.9	42.8	58.7	4.1
Pallis et al., 2003[59]	II	Gefitinib	31	3	29	32	5.75
Fukuoka et al., 2003[60]	II	Gefitinib	103	17.5	35.9	53.4	7.6
		Gefitinib	109	19.1	32.4	51.5	8
Kris et al., 2003[61]	II	Gefitinib	106	12	31	43	7
		Gefitinib	115	9	31	40	6
Shepherd et al., 2004[62]	III	Erlotinib	488	9	35	44	6.7
Pérez-Soler et al., 2004[63]	II	Erlotinib	57	12.3	38.6	50.9	8.4
Cappuzzo et al., 2004[64]	II	Gefitinib	106	14.4	26.8	41.2	9.4
Cappuzzo et al., 2000[65]	II	Gefitinib	40	5	45	50	5

ITT, intention to treat; RR, response rate; SD, stable disease; DCR, disease control rate; TTP, time to progression; MST, median survival time.

TABLE 3. Multiple Regression Models for Predicting MST by Study Parameters

	Model 1			Model 2		
	Coefficient	SE	*p* Value	Coefficient	SE	*p* Value
Models evaluating SD/RR and interactions with EGFR TKIs use No. 1*						
SD (%)	0.0375	0.0178	0.039	0.0500	0.0188	0.01
RR (%)	0.0744	0.0181	<0.001	0.0669	0.0190	0.001
SD_EGFR interaction	—	—	—	−0.0967	0.0703	0.175
RR_EGFR_interaction	—	—	—	0.1082	0.0591	0.073
EGFR TKI	—	—	—	2.2773	2.5364	0.373
_cons	4.6156	0.6532	<0.001	4.1579	0.7617	<0.001
			$R^2 = 0.214$			$R^2 = 0.284$
Models evaluating DCR and an interaction with EGFR TKIs use No. 2†						
DCR (%)	0.0501	0.0119	<0.001	0.0559	0.0132	<0.001
DCR_EGFR_interaction	—	—	—	−0.0226	0.0466	0.629
EGFR TKI	—	—	—	1.3146	2.0593	0.526
_cons	4.4323	0.6003	<0.001	4.0573	0.7019	<0.001
			$R^2 = 0.19$			$R^2 = 0.204$

*Coefficients for SD and RR denote increase of MST in months for 1% increase in SD/RR (model 1).
†Coefficients for DCR denote increase of MST in months for 1% increase in DCR (model 1).
SD, stable disease; RR, response rate; DCR, disease control rate.

agents, stabilization of disease without tumor shrinkage may represent a meaningful benefit. This phenomenon has been derived from two randomized phase II studies (Iressa Dose Evaluation in Advanced Lung Cancer [IDEAL]-1 and IDEAL-2).[60,61] In IDEAL-2, the median survival time of patients achieving stable disease was 9.4 months versus 5.2 months for those with progressive disease.[61] Moreover, when survival and symptom improvement were analyzed together, the median survival time for patients achieving stable disease with symptom improvement was 12.8 months versus 4.8 months for those without symptom improvement.

In contrast, the importance of achieving stable disease has been evaluated for cytotoxic agents. Docetaxel significantly improved overall survival compared with best supportive care as second-line therapy despite the overall response rate of only 6%.[5] In this study, 42.7% of patients achieved stable disease, which suggests that docetaxel also confers clinical benefit by producing stable disease.

In this retrospective review, we investigated the relationship between response rates and survival benefit and between the rates of stable disease and survival benefit in second-line treatment of NSCLC using both cytotoxic agents and EGFR TKIs. The more the rates of response and stable disease increase, the more the improvement of overall survival is obtained in the analysis that combined both cytotoxic agents and EGFR TKIs. However, as shown in Table 3, for both cytotoxic agents and EGFR TKIs, the survival improvement for a 1% increase in response rate is higher than for a 1% increase in stable disease rate. Moreover, for time to progression, only response rate showed a statistically significant association with TTP. These results indicate that it is more important to increase response rates than to achieve

TABLE 4. Multiple Regression Models for Predicting TTP by Study Parameters

	Model 1			Model 2		
	Coefficient	SE	*p* Value	Coefficient	SE	*p* Value
Models evaluating SD/RR and interactions with EGFR TKIs use No. 1*						
SD (%)	−0.0050	0.0229	0.828	−0.0248	0.0292	0.402
RR (%)	0.0954	0.0265	0.001	0.0963	0.0291	0.002
SD_EGRF_interaction	—	—	—	0.0297	0.0353	0.406
RR_EGFR_interaction	—	—	—	−0.0344	0.0391	0.385
EGFR TKIs	—	—	—	−1.9322	1.3858	0.172
_cons	2.4205	0.9348	0.014	3.5861	1.2925	0.009
			$R^2 = 0.183$			$R^2 = 0.325$
Models evaluating DCR and an interaction with EGFR TKIs use No. 2†						
DCR (%)	0.0281	0.1430	0.057	0.0166	0.0197	0.405
DCR_EGFR_interaction	—	—	—	0.0088	0.0210	0.677
EGFR TKIs	—	—	—	−1.5120	1.3021	0.253
_cons	1.9636	0.8734	0.03	2.8927	1.2334	0.024
			$R^2 = 0.047$			$R^2 = 0.148$

*Coefficients for SD and RR denote increase of TTP in months for 1% increase in SD/RR (model 1).
†Coefficients for DCR denote increase of TTP in months for 1% increase in DCR (model 1).
SD, stable disease; RR, response rate; DCR, disease control rate.

stable disease to improve overall survival for both cytotoxic agents and EGFR TKIs in the second-line setting, although increasing stable disease rates is still valuable.

In our analysis, we could not find a significant difference between cytotoxic agents and EGFR TKIs in terms of the relationship between survival and response and stable disease rate, as interaction terms for EGFR TKI treatment were not statistically significant. As a result, one may infer that the effect on survival of increasing response rates and stable disease rates is similar for cytotoxic agents and EGFR TKIs. However, this interpretation requires cautions on two points. First, our review contains many heterogeneous phase II studies with greatly different registered numbers of cases, and many heterogeneous patient characteristics with a greatly different administered number of regimens before these studies. The method of evaluating response is also different. These may possibly lead to a false conclusion. Moreover, the main effect of EGFR TKI was large but not statistically significant, indicating no evidence of a difference between EGFR TKIs and cytotoxic agents in terms of survival. However, there are very few EGFR TKI studies included in this review, and therefore the ability to detect such an effect may be low. Second, evaluating stable disease in clinical trials is very difficult, as patients with stable disease are not a homogeneous population. The Response Evaluation Criteria in Solid Tumors study defined stable disease as the longest diameter of tumor size from a less than 30% decrease to a less than 20% increase.[65] True disease stabilization inhibits tumor growth and metastasis and may be associated with improvement of survival, symptoms, and quality of life. However, it is difficult to distinguish true stable disease from nonstable disease. Therefore, it is crucial to classify a category of stable disease in the future.

CONCLUSIONS

In conclusion, our review indicated that although it is appropriate to adapt disease control rates to assess the effect of agents in the second-line setting, which is a new concept often used by clinical trials for molecular targeted agents, to obtain response seems to be more important than to achieve stable disease when new agents are developed, although achieving stable disease is still valuable. The relationship between survival and response and stable disease appears similar for cytotoxic agents and EGFR TKIs.

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Molecular Biology, Genomics, and Proteomics in Bronchioloalveolar Carcinoma

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Abstract: The charge of the Molecular Biology, Genomics, and Proteomics in Bronchioloalveolar Carcinoma Committee was to evaluate the molecular biology, genomic changes, and proteomic findings in patients with bronchioloalveolar carcinoma compared with other types of lung cancer. The literature was reviewed and unpublished information was presented by the committee members at the session. The molecular biology studies have included findings on epidermal growth factor receptor (*EGFR*) mutations, p53 mutations, K-*ras* mutations, and loss of heterozygosity. The genomic changes have mostly focused on the mRNA expression arrays as well as protein studies. The current state of knowledge was reviewed, the missing information was acknowledged, and proposals for future research were identified.

Key Words: Lung neoplasm, Adenocarcinoma, Bronchioloalveolar, Adenocarcinoma, Carcinoma, Non-small cell lung cancer.

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Little information is available about p53 mutations and p53 protein overexpression detected by immunohistochemistry, microsatellite loss of heterozygosity (LOH), and K-*ras* mutations in adenocarcinoma of the bronchioloalveolar subtype, according to the last World Health Organization (WHO) pathological classification proposed in 1999. However, the frequency of these molecular abnormalities seems to increase during the multistep process of carcinogenesis of peripheral adenocarcinoma going from atypical alveolar hyperplasia adenocarcinoma to bronchioloalveolar carcinoma (BAC) and to invasive adenocarcinoma.

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ATYPICAL ADENOMATOUS HYPERPLASIA

There is an increasing body of evidence to support the concept of atypical adenomatous hyperplasia (AAH) as the precursor of at least a subset of adenocarcinomas.[1] AAH is most frequently detected in lungs from patients bearing lung cancers (9–20%), especially adenocarcinomas (up to 40%) compared with squamous cell carcinomas (11%).[2] Several molecular changes frequently present in lung adenocarcinomas are also present in AAH lesions, and there is further evidence that AAH may represent true preneoplastic lesions.[1] The most important findings are the presence in AAHs of K-*ras* (codon 12) mutations (40%),[3] loss of *LKB1* function (20%),[4] allelic losses in chromosomes 3p (20%), 9p ($p16^{INK4a}$, 10%), 9q (50%), 17q, and 17p (*TP53*, 5%),[5,6] and overexpression of cyclin D1 (70%), p53 (ranging from 10 to 60%),[7] and survivin (50%).[8] Despite the evidence that AAH is a precursor lesion for a subset of lung adenocarcinomas, there is general consensus that the pathogenesis of most adenocarcinomas is still unknown. The findings of relatively infrequent tyrosine kinase domain epidermal growth factor receptor (*EGFR*) mutations in AAH lesions (three out of 40 examined)[9,10] and no *EGFR* mutation[11,12] or relatively low frequency in true BACs of the lung[9] support the concept that genetic abnormalities of *EGFR* are not relevant in the pathogenesis of alveolar types of lung neoplasia. In addition, Tang et al.[13] recently reported that *EGFR* mutation is an early event in the pathogenesis of lung cancer, being identified in histologically normal epithelium of small bronchi and bronchioles adjacent to *EGFR* mutant lung adenocarcinomas in nine out of 21 (43%) patients examined, but in none of the patients without mutation in the tumor. These data further support the notion than AAH lesions are not involved in the pathogenesis of *EGFR* mutant lung adenocarcinomas.

BAC, ADENOCARCINOMA WITH BRONCHIOLOALVEOLAR FEATURES, AND ADENOCARCINOMA OF THE LUNG

The frequency of *EGFR* mutations has also been studied in patients with BAC, adenocarcinoma with BAC features, and adenocarcinomas of the lung. Although responses to EGFR tyrosine kinase inhibitors have been reported to be higher[14] and EGFR mutations were preferentially observed in tumors having BAC features,[12,15] we did not find association with the BAC subtype of adenocarcinoma in 97 cases from

the United States[11] using the criteria stated by the 1999 WHO classification of lung tumors.[16,17]

In addition to the WHO system, Noguchi et al.[18,19] have classified adenocarcinomas into different categories that have different frequencies of genetic changes. Koga et al.[20] reported that p53 mutations were present in approximately 0% of 17 pure BAC, 11% of 27 mixed adenocarcinoma with BAC features, and 48% of 101 invasive adenocarcinomas. Similar to the frequency of mutations, the frequency of p53 protein overexpression detected by immunohistochemistry increased from 6% (2/32 tumors) in pure BAC to 28% (27/133) in BAC with foci of active fibroblastic proliferation (Noguchi type C) and to 40% (14/35) in adenocarcinoma.[21] p53 mutation and protein overexpression were also correlated with the size and invasive component of small peripheral adenocarcinomas (≥5 mm: 41%; <5 mm: 20%).[22,23]

The frequency of allelic losses also increased significantly during malignant progression. According to Noguchi's classification,[18,19] frequencies of allelic losses at chromosomal loci 3p, 17p, 18q, and 22q were significantly lower in BAC with or without alveolar collapse (Noguchi types A and B, respectively) than in BAC with active fibroblastic proliferation (Noguchi type C) in a series of 66 small peripheral adenocarcinomas.[24]

The frequency and type of K-*ras* mutation in BAC are related to the cytological features (mucinous versus nonmucinous). This raises the question of whether the mucinous form might represent a biological entity separate from the nonmucinous form. Small series of tumors (all <50) from patients with adenocarcinoma of the lung show that the K-*ras* mutation is present in 73 to 100% of the mucinous types and that the type of the mutation was usually G to A (codon 12), whereas it was seen in 10 to 43% in the nonmucinous types, usually in G to T transversions.[25–27] Mutations at codon 12 of the K-*ras* oncogene were found in 39% of 41 AAH, 42% of 18 adenocarcinomas, and none of five lung neoplasms that were not adenocarcinomas. Of the patients with both an AAH and a synchronous adenocarcinoma, more than half did not have the mutation in both the AAH and the synchronous lung adenocarcinoma, suggesting that peripheral adenocarcinomas arise not always from AAH but sometimes directly from a background of field cancerization.[27]

Adenocarcinomas with BAC features are also characterized by an intense inflammatory reaction especially containing alveolar neutrophils and macrophages. Increased numbers of tumor-infiltrating neutrophils are linked to poorer outcomes in these patients.[28] Tumor environment drives local neutrophil recruitment and activation via C-X-C chemokine release such as interleukin-8 and epithelial cell–derived neutrophil activating protein 78 but also prolongs alveolar neutrophil survival through the production of soluble antiapoptotic factors (granulocyte–macrophage colony–stimulating factor and granulocyte colony–stimulating factor).[29,30] The mechanisms by which neutrophils influence the prognosis of adenocarcinoma with BAC features could be multiple. It has been postulated that the persistence of neutrophil alveolitis would result in persistent release of proinflammatory mediators such as cytokines, proteases, and reactive oxygen and nitrogen species that can damage DNA and activate oncogenes.[31,32] Among these factors released by neutrophils, hepatocyte growth factor seems to be particularly involved in the progression of these types of tumors, especially through its mitogenic and scattering properties, favoring c-Met expressing tumor-cell migration along the alveolar basal membrane.[33] Lastly, neutrophils might be involved in luminal tumor spread by promoting tumor-cell shedding (M. Wislez, AACR 2004), described pathologically as the presence of micropapillary clusters that are also involved in the mechanism of aerogenous progression.[34]

GENOMIC AND PROTEOMIC STUDIES OF BAC

As mentioned before, BAC is thought to arise from AAH and is potentially an intermediate to invasive adenocarcinoma. Extensive analyses of BAC using gene-expression profiling and proteomic-based studies have not yet been performed and are only available for limited numbers of these cancers. These types of studies may have the potential to define similarity or differences in the observed types of adenocarcinoma of the lung. Of particular interest is the potential regulatory pathway involved in the lepidic growth patterns of BAC, which is different from most other adenocarcinomas of the lung. The observation that some adenocarcinomas can exhibit regions of BAC provides complexity and has resulted in multiple pathological-based classifications.[14,16–19] Genomic studies have the potential to define the similarities as well as key differences between BAC, adenocarcinomas with BAC features, and adenocarcinomas of the lung.

Recent studies examining individual genes have hinted at differences between BAC and adenocarcinomas. The tumor suppressor in the lung cancer-1 gene encodes an adhesion molecule and is frequently associated with LOH at that locus in non–small-cell lung cancer. Both normal lung cells and BAC retain expression of tumor suppressor in lung cancer-1, whereas 63% of adenocarcinomas demonstrated decreased expression detected by immunohistochemistry.[35] BACs have very low p53 DNA mutation frequencies compared with adenocarcinomas of the lung.[20] LOH at the 3p FHIT loci was observed in 43% of BAC, and 12th codon K-*ras* mutations are detected in the mucinous form of BAC.[36] A comparative LOH study between 14 BAC and 20 stage I lung adenocarcinomas using nine chromosomal regions revealed that the most frequently affected chromosomal regions in BAC were 8q and 17p.[37] In adenocarcinomas of the lung, LOH at 1p, 3p, 7q, and 18q was more frequent than in BAC, and fractional allele loss was greater in adenocarcinomas of the lung than BAC.

Using immunocytochemistry to examine protein expression, detection of the thyroid transcription factor-1 (TTF-1), cytokeratin 7, and cytokeratin 20 were measured in both mucinous and nonmucinous BAC.[38] TTF-1 was detected in 17% of mucinous and 94% of nonmucinous BAC, cytokeratin 7 was detected in 100% of mucinous and 23% of nonmucinous BAC, and cytokeratin 20 was detected in 60% of mucinous and 0% of nonmucinous BAC.[38] In a study that examined MUC protein expression in AAH, BAC, and adenocarcinomas with BAC features, MUC1 decreased from

AAH to BAC and from BAC to adenocarcinoma, whereas MUC2, MUC5AC, MUC6, and depolarized MUC6 increased.[39] Alterations in p53 and the increased expression of MUC1, MUC5AC, and MUC6 were noted.

ADDITIONAL GENOMIC AND PROTEOMIC STUDIES

A comparison of normal lung tissue and BAC using oligonucleotide arrays was reported by Goodwin et al.[40] and identified 12 up-regulated and six down-regulated genes in the BAC tumors. Although this analysis provides some information, a comparison of BAC and adenocarcinomas was not included, which may be most relevant in defining critical genes involved in the development of these cancers. We used oligonucleotide arrays to examine gene expression in 14 BAC and 73 adenocarcinomas.[41] The most highly expressed genes that were significantly different between the BAC tumors and adenocarcinomas and higher in BAC included the surfactant pulmonary-associated proteins A1, A2, C and D, MUC1, TTF-1 and TTF-3, villin 2, and prostaglandin D2 synthetase. Interestingly, higher mRNA expression for both fos and jun B were detected in BAC, which may reflect an elevated AP-1 activity and upstream signaling events in these tumors. The higher level of expression of surfactant genes is consistent with the well-differentiated phenotypic characteristics of BAC. TTF-1 was the most differentially expressed gene between BAC and adenocarcinomas, consistent with the high TTF-1 protein expression reported in BAC.[38] Because of the small numbers of tumors for our analyses, it was not possible to divide the BAC tumors into separate categories such as mucinous, nonmucinous, and mixed histology. Although we found MUC1 mRNA present in both BAC and adenocarcinomas of the lung, the significantly increased expression in BAC is consistent with the higher MUC1 protein levels that have been reported in these tumors.[39]

Analysis of survival-related genes revealed prostaglandin D2 synthetase and neutrophil elastase 2 to be more highly expressed in BAC than the other adenocarcinomas. In contrast, much lower levels of vascular endothelial growth factor were detected in the BAC, possibly reflecting a lesser level of angiogenesis and hypoxia in these tumors relative to the adenocarcinomas. Adenocarcinomas also expressed increased levels of metallothionein 2A and thioredoxin reductase mRNA. We speculate that these genes may correspond to smoking-related alterations because these genes may change in response to reactive oxygen species originating from tobacco smoking or in response to inflammatory cells. Alternately, the expression of thioredoxin reductase and metallothionein 2 may reflect the higher rates of cell proliferation in the lung adenocarcinomas relative to BAC.

Few, if any, large-scale proteomic analyses of BAC have been reported. We examined the same BAC and lung adenocarcinomas for mRNA using oligonucleotide arrays and also at the protein level with two-dimensional gel electrophoresis and mass spectrometry.[42] A total of 682 protein spots were quantified, and 75 proteins were found to differ significantly ($p < 0.05$) between BAC and lung adenocarcinomas. Thirty-eight protein spots were successfully identified using mass spectometry. Of interest were the relatively higher expression of the ras-related protein RAB-14, glutathione-s-transferase-pi, cytokeratin 7, and three isoforms of the selenium-binding protein 1 in BAC compared with adenocarcinomas of the lung. Adenocarcinomas expressed higher levels of phosphoglycerate kinase 1, pyruvate kinase M1/M2, and stathmin (OP-18) compared with BACs. Increased phosphoglycerate kinase 1 is consistent with higher hypoxia-induced glycolysis in the adenocarcinomas of the lung relative to BAC.[42]

Future studies that include sufficient numbers of the various histological subtypes of BAC are needed to provide insight into the similarities and differences among these tumors and as compared with lung adenocarcinomas. The NCI Director's Challenge: Validation Study of Lung Adenocarcinomas will examine gene expression using Affymetrix 133A oligonucleotide arrays among approximately 500 tumors. Thus, a relatively large number of BACs will be included in this study, allowing potential gene pathways to be defined that may be relevant to our understanding of the growth- and cell-signaling systems in BAC. These analyses will also incorporate detailed pathologic assessment of each tumor so that the subtypes of each BAC can be compared. It is expected that these data; made available to the research community, will then stimulate further research into potential new markers for early diagnosis and possible therapeutic intervention strategies that may be effective for BAC.

FUTURE DIRECTIONS

The Committee responsible for Molecular Biology, Genomics, and Proteomics in Bronchioloalveolar Carcinoma outlined studies that will provide further insights into BAC. The most important part of the meeting was partial agreement and understanding about the interpretation of the pathological classification. The participants in the meeting agreed on a common set of descriptors for the pathological interpretation of BAC that will be used more consistently in the future.

TABLE 1. Different Biological Properties in Atypical Adenomatous Hyperplasia, Pure Bronchioloalveolar Cancer, Adenocarcinoma with Bronchioloalveolar Cancer Features, and Adenocarcinoma

	Atypical Adenomatous Hyperplasia	Bronchioloalveolar Carcinoma	Adenocarcinoma with Bronchioloalveolar Carcinoma Features	Adenocarcinoma of the Lung
EGFR mutation	↓ <5%	10%		↑ 40%
TP53 mutations	Not reported	↓ 0%	↓ 10%	↑ 50%
p53 by immunohistochemistry	Not reported	↓ 5%	↑ 30%	↑ 50%

Upcoming technological improvements will provide additional insights into the biology of BAC. These will include the increasing ability to detect genetic changes in BAC and adenocarcinomas including, but not be limited to, *EGFR*, HER-2/neu, B-raf, K-*ras*, and *TP53*. In addition, there is the ability to detect genetic loss in the whole genome using studies with single-polynucleotide polymorphisms or array chromosomal genomic hybridization. There is increasing ability to use small and smaller amounts of DNA and DNA from paraffin-embedded tissues. Future studies will provide information on the degree of genetic changes seen in early lesions (<1cm) that are being detected more often as computerized tomographic scanning of the chest is becoming more widely used. These findings can be compared with the more advanced lesions. The genetic changes can also provide insights into the clonality of the BACs to determine whether the multiple lesions in the lungs arise from single or multiple clones. Table 1

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