Published in final edited form as:

AJR Am J Roentgenol. 2010 January ; 194(1): 266–269. doi:10.2214/AJR.09.2858.

Core Needle Lung Biopsy Specimens: Adequacy for *EGFR* and *KRAS* Mutational Analysis

Stephen B. Solomon¹, Maureen F. Zakowski², William Pao³, Raymond H. Thornton¹, Marc Ladanyi², Mark G. Kris³, Valerie W. Rusch⁴, and Naiyer A. Rizvi²

¹Department of Radiology, Memorial Sloan-Kettering Cancer Center, 1275 York Ave., Howard 118, New York, NY 10021

²Department of Pathology, Memorial Sloan-Kettering Cancer Center, New York, NY

³Department of Medicine, Memorial Sloan-Kettering Cancer Center, New York, NY

⁴Department of Surgery, Memorial Sloan-Kettering Cancer Center, New York, NY

Abstract

OBJECTIVE—The purpose of this study was to prospectively compare the adequacy of core needle biopsy specimens with the adequacy of specimens from resected tissue, the histologic reference standard, for mutational analysis of malignant tumors of the lung.

SUBJECTS AND METHODS—The first 18 patients enrolled in a phase 2 study of gefitinib for lung cancer in July 2004 through August 2005 underwent CT- or fluoroscopy-guided lung biopsy before the start of gefitinib therapy. Three weeks after gefitinib therapy, the patients underwent lung tumor resection. The results of *EGFR* and *KRAS* mutational analysis of the core needle biopsy specimens were compared with those of *EGFR* and *KRAS* mutational analysis of the surgical specimens.

RESULTS—Two specimens were unsatisfactory for mutational analysis. The results of mutational assay results of the other 16 specimens were the same as those of analysis of the surgical specimens obtained an average of 31 days after biopsy.

CONCLUSION—Biopsy with small (18- to 20-gauge) core needles can yield sufficient and reliable samples for mutational analysis. This technique is likely to become an important tool with the increasing use of pharmacotherapy based on the genetics of specific tumors in individual patients.

Keywords

biopsy; lung cancer; molecular typing; personalized medicine; targeted therapy

With the advent of targeted cancer therapy, mutational analysis is becoming an increasingly important component of clinical care. For example, patients with breast cancer with tumors overexpressing the ERBB2 (formerly HER2 or HER2/neu) cell surface growth factor receptor have improved responses when treated with the targeted therapy trastuzumab [1]. In other instances results of tumor profiling in the midst of treatment can be an early indication of drug resistance [2]. All of these molecular profiling analyses require adequate and representative tissue from the tumor.

Clinical diagnosis increasingly relies on imaging-guided needle biopsy. Morphologic and histochemical analyses of core samples facilitate diagnosis and appropriate staging of disease. However, for future incorporation of molecular profiling into clinical care, it is important that profiling be feasible with samples obtained at needle biopsy. Few studies have been conducted to analyze the sufficiency of core needle biopsy for molecular profiling, and most of these studies have been limited to breast tissue and large-gauge needles. Unlike surgical specimens, needle biopsy specimens can be inadequate because of insufficient material, targeting error, and tumor heterogeneity.

Gefitinib is an inhibitor of the epidermal growth factor receptor (EGFR) tyrosine kinase. Somatic mutations in the DNA-encoding portions of the kinase domain of the *EGFR* gene have been found in lung adenocarcinomas and are associated with increased sensitivity to the drug [3]. Similarly, *KRAS* mutations have been associated with lack of response to tyrosine kinase inhibitor therapy [4]. Therefore, to optimize treatment of patients with non-small cell carcinoma of the lung, it is important to conduct molecular profiling before therapy is started. Ideally, this profiling would be accurately and reliably performed with core biopsy specimens obtained with the small-gauge needles typically used for lung biopsy. Using archived specimens, Boldrini et al. [5] found retrospectively that mutational analysis is feasible. Chen et al. [6] similarly found that CT-guided biopsy can be used to analyze core needle biopsy specimens for *EGFR* mutation. These studies, however, were performed without resected specimens for validation.

The purpose of this study was to prospectively compare the adequacy of core needle biopsy specimens with the adequacy of resected specimens, the histologic reference standard, for mutational analysis of malignant tumors of the lung.

Subjects and Methods

Study Overview

This study was correlative and part of a broader prospective institutional review board–approved, phase 2 study of the correlation between gefitinib response and the presence of mutations in the *EGFR* gene. Eligible patients had resectable stage I or II adenocarcinoma of the lung with less than a 15-pack-year smoking history or had resectable bronchioloalveolar carcinoma. For eligibility in the study, all patients underwent biopsy at the start of the study. Mutational analysis was performed on the biopsy specimen. According to the broader protocol, patients then underwent 3 weeks of gefitinib therapy, after which they underwent surgical resection. Mutational analysis was performed on the resected specimens. Results of the mutational analyses were compared.

Core Needle Biopsy

The first 18 patients enrolled in the phase 2 study (July 2004–August 2005) underwent CT- or fluoroscopy-guided lung biopsy before the start of gefitinib therapy. The core needle biopsy device and imaging approach were selected by one of the seven interventional radiologists performing the procedure. The number of passes and determination of specimen adequacy were subject to the individual operator's discretion and were guided by the findings at on-site cytologic examination. Targeted tumor size, number of passes, and core needle device used were recorded. Complications of needle biopsy, such as pneumothorax requiring a chest tube, were noted. Cytopathologic technologists were present for all biopsies.

Mutational Analysis

EGFR mutational analysis of DNA extracted from tumor samples was performed by either direct sequencing of exons 18–24 [3] or with more sensitive polymerase chain reaction–based assays [7]. *KRAS* mutations were assessed with a direct sequencing described previously [6].

Results

Study Overview and Patients

Fifty patients were enrolled in the phase 2 study of gefitinib. The first 18 patients were the subjects of this analysis because both a CT-guided core needle biopsy specimen and a surgically resected specimen were available for genetic mutational analysis. The average longest cross-sectional diameter of the tumor was 3.2 cm (range, 1.5–6.4 cm), and the average depth of the lesion from the skin was 61 mm. The clinical characteristics of the patients are shown in Table 1.

Procedure

One of seven interventional radiologists, who had a minimum of 5 years of experience, performed the lung biopsies. Fourteen biopsies were guided by fluoroscopy and four by CT (Fig. 1). Three biopsies were performed with a semiautomatic coaxial system, 2-cm-long blade, and 20-gauge core biopsy needle. Fifteen procedures were performed with a semiautomatic coaxial system, 2-cm-long blade, and 18-gauge core biopsy needle (Temno Evolution, Cardinal Health). An average of 1.8 needle passes (range, 1–4; median, 2) were made. Three patients had a small pneumothorax, but none needed chest tube placement or hospitalization (Table 2). A cytotechnologist was on site, and touch preparation technique was used for immediate inspection of the sample.

Mutational Analysis Comparison

Two biopsy specimens were unsatisfactory for mutational analysis. Both of these were obtained at fluoroscopically guided procedures. The other 16 biopsy specimens were analyzed successfully and harbored the same genotypes as the matching surgical specimens, which were acquired an average of 31 days after biopsy. The tumors from three patients had an *EGFR* exon 19 deletion. The specimen from one patient had an exon 21 L858R point mutation, and that from another patient had an exon 18 deletion. All patients had the wild-type *KRAS* gene. Because the mutation results were highly concordant, the other 32 patients in the larger gefitinib study did not undergo pretreatment biopsy before starting gefitinib therapy.

Discussion

Mutational analysis of tumors is becoming an important factor in the clinical care of cancer patients. Drug selection is often determined by the presence or absence of a particular genetic mutation. For example, breast cancer patients with tumors overexpressing the *ERBB2* cell surface growth factor receptor have improved responses to targeted therapy with trastuzumab [1].

Although imaging-guided core needle biopsy has become the accepted minimally invasive technique for histopathologic diagnosis, it is uncertain whether core needle biopsy yields sufficient and reliable material for mutational analysis. Ellis et al. [8] reported that in breast tissue, single-pass core biopsy with a 14-gauge needle yielded a median of 1.34 μg of total RNA, sufficiently greater than the 1 μg of RNA required for microarray analysis. In that study, core needle biopsy yielded suitable material for RNA analysis 93% of the time. In a

study of core biopsy of the breast [9], sufficient material was obtained in only 75% of cases. All of the biopsies in those two studies were performed with relatively larger-gauge needles than are used in biopsy of tissue other than breast. Chen et al. [6], however, found it possible to perform *EGFR* mutational analysis of lung cancer with three cores obtained with an 18-gauge core biopsy needle. However, their results were not validated with the reference standard surgical specimen.

Another challenge at imaging-guided core needle biopsy is the risk of sampling error, which occurs when a tumor is composed of distinct cell populations. Needle biopsy sampling error in standard pathologic analysis has been reported [10], showing that different parts of a tumor can have different genetic expressions.

In this study, we prospectively compared the results of mutational analysis of specimens obtained from lung cancer patients at imaging-guided 18- and 20-gauge core needle biopsy with the results of analysis of specimens obtained at surgical resection. There was 100% agreement (16 of 16 cases) in mutational analysis results for the two types of specimens when satisfactory material was present. Although the study had a relatively small sample size, the data suggest that imaging-guided core biopsy can be used reliably to obtain molecular information for guiding therapy, even when thin needles (18- or 20-gauge) are used. The needle samples in our study contained approximately 2 μ g of material, which is satisfactory for analysis. Although other investigators [8] found high correlation using 14-gauge breast biopsy needles, the results of our study suggest broader applicability of core needle biopsy to tissue other than breast.

That there was complete agreement in our specimens suggests that the *EGFR* mutation, believed to be an early event in the types of lung cancer studied, is not subject to substantial intratumor heterogeneity. This consistency is similar to breast tumor *ERBB2* status [11] and is in contrast to the heterogeneity of estrogen and progesterone receptors [12].

Two of 18 specimens (11.1%) in this study were unsatisfactory for analysis. A number of explanations for unsatisfactory biopsy are possible. The two unsatisfactory specimens in our study were obtained with fluoroscopic guidance, and the lower tissue resolution of fluoroscopy than of CT may lead to uncertainty in needle tip localization. CT has greater tissue contrast resolution, and the 3D capability of the technique can increase confidence in needle placement [13]. On-site cytologic inspection is another factor in higher success rates of diagnostic biopsy [14] and may aid in additional yield of mutational analysis specimens.

This study was limited by its small sample size and focus on limited mutational analysis. Nevertheless, the 100% agreement with surgical specimens when adequate material was available suggests that core needle biopsy can yield sufficient and reliable samples for mutational analysis. In addition, the study sample consisted mainly of patients with adenocarcinoma because this tumor occurs more frequently in patients with a limited smoking history, which was one of the inclusion criteria for the study from which the patient sample was drawn. It is unlikely that this factor affected the results.

The ability to gain accurate mutational information from needle biopsy specimens is an important realization. With this understanding, the importance of needle biopsy is likely to increase with development of pharmacotherapy based on the genetic makeup of individual tumors rather than on morphologic histologic features alone.

References

1. Piccart-Gebhart MJ, Procter M, Leyland-Jones B, et al. Trastuzumab after adjuvant chemotherapy in *HER2*-positive breast cancer. *N Engl J Med*. 2005; 353:1659–1672. [PubMed: 16236737]

2. Heinrich MC, Corless CL, Blanke CD, et al. Molecular correlates of imatinib resistance in gastrointestinal stromal tumors. *J Clin Oncol*. 2006; 24:4764–4674. [PubMed: 16954519]
3. Pao W, Miller V, Zakowski M, et al. EGF receptor gene mutations are common in lung cancer from “never smokers” and are associated with sensitivity of tumors to gefitinib and erlotinib. *Proc Natl Acad Sci USA*. 2004; 101:13,306–13,311.
4. Pao W, Miller VA, Politi KA, et al. Acquired resistance of lung adenocarcinomas to gefitinib or erlotinib is associated with a second mutation in the EGFR kinase domain. *PLoS Med*. 2005; 2:e73. [PubMed: 15737014]
5. Boldrini L, Gisfredi S, Ursino S, et al. Mutational analysis in cytological specimens of advanced lung adenocarcinoma: a sensitive method for molecular analysis. *J Thorac Oncol*. 2007; 2:1086–1090. [PubMed: 18090579]
6. Chen CM, Cheng JW, Cheung YC, et al. Computed tomography-guided core-needle biopsy specimens demonstrate epidermal growth factor receptor mutations in patients with non-small-cell lung cancer. *Acta Radiol*. 2008; 49:991–994. [PubMed: 18651255]
7. Pan Q, Pao W, Ladanyi M. Rapid polymerase chain reaction-based detection of epidermal growth factor receptor gene mutations in lung adenocarcinomas. *J Mol Diagn*. 2005; 7:396–403. [PubMed: 16049312]
8. Ellis M, Davis N, Coop A, et al. Development and validation of a method for using breast core needle biopsies for gene expression microarray analyses. *Clin Cancer Res*. 2002; 8:1155–1166. [PubMed: 12006532]
9. Symmans WF, Ayers M, Clark EA, et al. Total RNA yield and microarray gene expression profiles from fine-needle aspiration biopsy and core-needle biopsy samples of breast carcinoma. *Cancer*. 2003; 97:2960–2971. [PubMed: 12784330]
10. Rinas AC, Ward WG, Kilpatrick SE. Potential sampling error in fine needle aspiration biopsy of dedifferentiated chondrosarcoma: a report of 4 cases. *Acta Cytol*. 2005; 49:554–559. [PubMed: 16334036]
11. Mueller-Holzner E, Fink V, Frede T, Marth C. Immunohistochemical determination of *HER2* expression in breast cancer from core biopsy specimens: a reliable predictor of *HER2* status of whole tumor. *Breast Cancer Res Treat*. 2001; 69:13–19. [PubMed: 11759824]
12. Howell A, Harland RN, Barnes DM, et al. Endocrine therapy for advanced carcinoma of the breast: effect of tumor heterogeneity and site of biopsy on the predictive value of progesterone receptor. *Cancer Res*. 1987; 47:296–299. [PubMed: 3791214]
13. Yankelevitz DF, Henschke CI, Koizumi JH, Altorki NK, Libby D. CT-guided transthoracic needle biopsy of small solitary pulmonary nodules. *Clin Imaging*. 1997; 21:107–110. [PubMed: 9095385]
14. Santambrogio L, Nosotti M, Bellaviti N, Pavoni G, Radice F, Caputo V. CT-guided fine-needle aspiration cytology of solitary pulmonary nodules: a prospective, randomized study of immediate cytologic evaluation. *Chest*. 1997; 112:423–425. [PubMed: 9266878]

Fig. 1. 63-year-old man with adenocarcinoma of lung. CT scan obtained during 20-gauge core needle biopsy shows 1.5-cm lung nodule. Patient is prone, and targeted nodule is at right lung base.

TABLE 1

Patient and Tumor Characteristics

Patient No.	Sex	Age (y)	Tumor Stage	Histologic Finding	Lesion Size (cm)	Lesion Depth (mm)
1	M	53	I	Adenocarcinoma with bronchioloalveolar carcinoma	2.7 × 3.7 (solid, 1.5 × 1.1)	88
2	F	75	IA	Adenocarcinoma	2.0 × 1.1	36
3	M	64	IA	Adenocarcinoma	1.7 × 0.8	66
4	F	72	IA	Adenocarcinoma	1.3 × 1.8	60
5	F	59	IA	Adenocarcinoma with bronchioloalveolar carcinoma	3.1 × 2.1	85
6	F	38	IA	Adenocarcinoma	2.0 × 1.8	37
7	M	67	IIIA	Adenocarcinoma	3.5 × 2.5 (solid, 3.5 × 1.5)	92
8	F	74	IB	Adenocarcinoma	3.4 × 2.3	32
9	F	78	I	Adenocarcinoma with bronchioloalveolar carcinoma	3.0 × 2.6	60
10	F	56	IB	Bronchioloalveolar carcinoma	6.4 × 2.7	40
11	F	78	IA	Bronchioloalveolar carcinoma	1.5 × 1.1	70
12	F	51	IA	Adenocarcinoma with bronchioloalveolar carcinoma	1.5 × 0.9	62
13	M	66	IB	Adenocarcinoma	4.6 × 3.6	70
14	M	70	IB	Squamous cell carcinoma	4.9 × 4.1	67
15	F	84	IA	Adenocarcinoma	4.6 × 2.2	30
16	F	51	I	Adenocarcinoma with bronchioloalveolar carcinoma	2.2 × 1.3	70
17	M	67	IA	Adenocarcinoma with bronchioloalveolar carcinoma	2.1 × 1.7	64
18	F	82	IA	Adenocarcinoma	3.5 × 2.5	65

TABLE 2

Procedure Characteristics

Patient No.	Imaging	Needle Gauge	No. of Passes	Complications	Sample Quality
1	Fluoroscopy	20	3	No pneumothorax	Satisfactory
2	Fluoroscopy	18	2	No pneumothorax	Satisfactory
3	Fluoroscopy	18	2	No pneumothorax	Satisfactory
4	Fluoroscopy	18	1	Trace pneumothorax, no chest tube	Satisfactory
5	Fluoroscopy	18	1	No pneumothorax	Satisfactory
6	Fluoroscopy	18	1	Trace pneumothorax, no chest tube	Satisfactory
7	Fluoroscopy	18	2	No pneumothorax	Satisfactory
8	Fluoroscopy	18	1	No pneumothorax	Satisfactory
9	Fluoroscopy	18	1	No pneumothorax	Satisfactory
10	CT	18	2	No pneumothorax	Satisfactory
11	CT	20	3	No pneumothorax	Satisfactory
12	CT	20	2	No pneumothorax	Satisfactory
13	Fluoroscopy	18	1	No pneumothorax	Satisfactory
14	Fluoroscopy	18	4	No pneumothorax	Unsatisfactory
15	CT	18	2	No pneumothorax	Satisfactory
16	Fluoroscopy	18	3	No pneumothorax	Unsatisfactory
17	Fluoroscopy	18	1	No pneumothorax	Satisfactory
18	Fluoroscopy	18	1	Trace pneumothorax, no chest tube	Satisfactory