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Feasibility and Safety of Intrathoracic Biopsy and Repeat Biopsy for Evaluation of Programmed Cell Death Ligand–1 Expression for Immunotherapy in Non–Small Cell Lung Cancer[1]

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Purpose: To determine feasibility and safety of biopsy and repeat biopsy for assessment of programmed cell death ligand-1 (PD-L1) status.

Materials and Methods: This retrospective analysis reviewed 101 patients who underwent transthoracic core needle biopsy for the KEYNOTE-001 (MK-3475) clinical trial of pembrolizumab, an antiprogrammed cell death–1 therapy for non–small cell lung cancer, from May 2012 to September 2014. Sixty-one male patients (mean age, 66.1 years; range 36–83 years) and 40 female patients (mean age, 66.8 years; age range, 36–90 years) were included. Data collected included population characteristics, treatment history, target location, size, and depth from pleura. Adequacy of the tissue sample for diagnostic testing and rates of biopsy-related complications were assessed. Statistical analysis was performed by using univariate and multivariate generalized linear models to determine significant risk factors for biopsy complications.

Results: A total of 110 intrathoracic biopsies were performed, and 101 (91.8%) were performed as repeat biopsies subsequent to a previous percutaneous or bronchoscopic biopsy or previous surgical biopsy or resection. More than 84.5% (93 of 110) of biopsies were performed in patients who had undergone previous local or systemic therapy. Specimens were adequate for evaluation of PD-L1 expression in 96.4% of biopsies. Procedure-related complications occurred in 28 biopsies (25.4%); pneumothorax was most common (22.7%). Overall mean number of core needle biopsy samples obtained was 7.9 samples.

Conclusion: Image-guided transthoracic core needle biopsy is an effective method for obtaining tissue for PD-L1 expression analysis.

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n 2015, the U.S. Food and Drug Administration approved antiprogrammed cell death–1 (PD-1) inhibitors nivolumab and pembrolizumab for use in advanced non–small cell lung cancer after failure of platinum-based chemotherapy (1). Importantly, the degree of expression of programmed cell death ligand–1 (PD-L1) in tumor cells was shown to be an informative biomarker in predicting progression-free survival with pembrolizumab compared with standard of care platinum-based chemotherapy (2). U.S. Food and Drug Administration approval for pembrolizumab was therefore expanded to include first-line therapy in those patients with high PD-L1 expression ($\geq$50% tumor proportional score) with absence of epidermal growth factor receptor and anaplastic lymphoma kinase mutations, and second-line therapy in patients who progressed with standard therapy and in whom tumors expressed at least 1% PD-L1 (3).

Compared with molecular assays that determine the presence or absence of a mutation, as is the case for anaplastic lymphoma kinase and epidermal growth factor receptor, immunohistochemical assays for PD-L1 determine the protein expression across a range from 0% to 100% (4). Many challenges in the determination of PD-L1 expression have become apparent, including technical variability across available assays and heterogeneity of PD-L1 expression not only within tumors but also within metastases and the surrounding tumor microenvironment, particularly after treatment (5–7). Studies (8–10) that correlate PD-L1 expression in biopsy samples with surgically resected specimens have shown that small biopsy samples, with a mean of four biopsy samples, may yield false-negative PD-L1 expression in up to 2%–46% of cases.

To our knowledge, the assay used in our study is the first U.S. Food and Drug Administration–approved immunohistochemical test for treatment selection in patients with lung cancer. Previous studies (11–15) have shown percutaneous computed tomography (CT)–guided lung biopsy to be feasible and safe in patients with non–small cell lung cancer requiring repeat biopsies for mutational analysis who developed resistance to conventional chemotherapy or epidermal growth factor receptor–tyrosine kinase inhibitors. However, to the best of our knowledge, no publications exist that address the adequacy and safety of biopsy and repeat biopsy for immunohistochemical testing in non–small cell lung cancer. We hypothesize that percutaneous CT-guided core-needle lung biopsy is a safe and technically feasible method to obtain adequate tissue to determine PD-L1 status for immunotherapy in patients with non–small cell lung cancer. We also hypothesize that CT-guided lung biopsy is an effective method to determine PD-L1 status in patients undergoing repeat biopsy who have already undergone previous biopsies and previous local and systemic treatment.

Materials and Methods

A retrospective chart review was performed for all patients at our institution ($n = 101$) who underwent intrathoracic imaging-guided percutaneous core-needle biopsy for enrollment or during the KEYNOTE-001 trial (MK-3475; sponsored by Merck) from May 2012 to September 2014 following an institutional review board–approved protocol. The mean patient age at the time of biopsy was 66.4 years (age range, 36–90 years). The mean age at biopsy was not statistically different between male and female patients (unpaired t test, $P = .76$); the mean age of male patients ($n = 61$) was 66.1 years (age range, 36–83 years) and the mean age of female patients ($n = 40$) was 66.8 years (age range, 36–90 years). The population characteristics and biopsy characteristics are shown in Table 1. Seven patients underwent two CT-guided biopsies, and one patient underwent three CT-guided biopsies during the trial (Figure). Of the eight patients who

Advances in Knowledge

- Because of the U.S. Food and Drug Administration approval of antiprogrammed cell death-1 (PD-1) inhibitor therapy as second-line therapy, patients who require biopsy to determine eligibility for PD-1 inhibitor therapy may have had previous diagnostic biopsy (92%; 101 of 110) or resection and/or prior local or systemic treatment (<84.5% [93 of 110]).

- Regardless of whether patients are undergoing biopsy for initial determination of PD-1 status or subsequent repeat biopsy, complication rates in this patient population (25% in both groups) are similar to complication rates in the general population.

Implications for Patient Care

- High adequacy rates (96%) of percutaneous transthoracic biopsy and repeat biopsy ensure that patients who may benefit from PD-1 inhibitors are eligible for treatment.

- Obtaining a higher number of core needle biopsy samples is safe in the patient population presenting for evaluation of programmed cell death ligand–1 (PD-L1) expression and may be necessary because of the heterogeneous expression of PD-L1.

Table 1

Population and Biopsy Characteristics
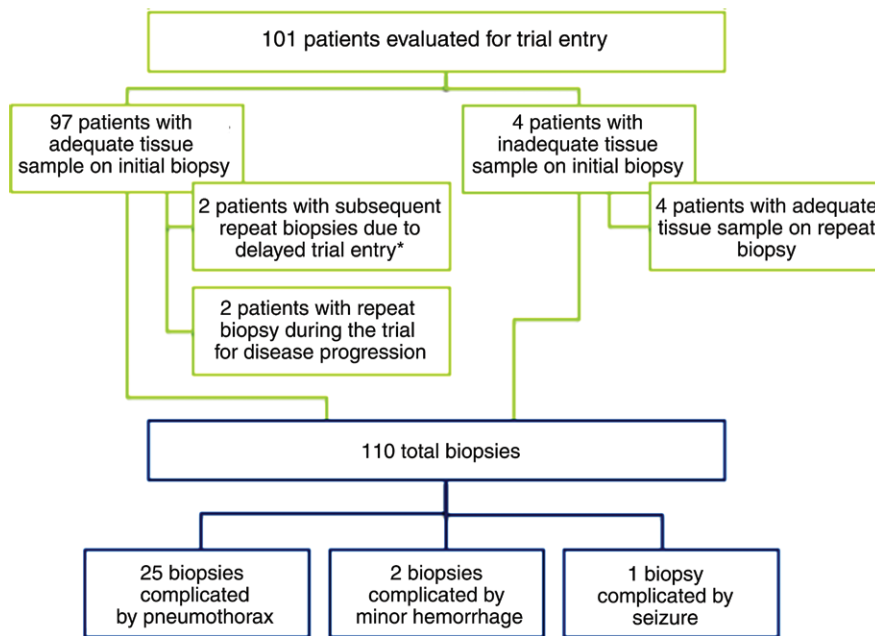
Parameter	Result
Patients who underwent biopsy as part of the KEYNOTE-001 trial	101
Biopsies performed	110
Repeat biopsies performed	101 (92)
Male patients	55 (54)
Mean age at biopsy (y)*	66.4 (36–90)
Male patient (y)*	66.1 (36–83)
Female patient (y)*	66.8 (36–90)
Biopsy treatment history	
Systemic therapy	89 (81)
Radiation	23 (21)
Surgery	20 (18)
Target biopsy location	
Lung biopsy	95 (86)
Not lung biopsy	15 (14)
Pleura or chest wall	9 (8)
Mediastinum	6 (5)
Mean longest lesion diameter (mm)*	42.1 (7–132)
Mean distance from pleura (mm)*	28.0 (0–85)
Solid lesions	98 (89)
Biopsies that crossed aerated lung	61 (55)
Lung target	57 (93)
Not lung target	4 (7)
Mean no. of cores obtained*	7.9 (1–18)
Use of closure device	51 (46)

Note.—Unless otherwise indicated, data are number with percentage in parentheses. Closure device indicates, for example, a blood patch or BioSentry sealant (Surgical Specialties, Wyomissing, Pa).

*Data in parentheses are range.

Flowchart of all patients and the biopsies performed during the trial, with breakdown of complications. *One patient underwent three biopsies before they entered the trial.

underwent repeat biopsies during the trial, the time between biopsies ranged from 38 to 412 days, and in three patients, a different lesion was targeted during the subsequent biopsy. Therefore, each biopsy was treated as an independent occurrence for a total of 110 biopsies performed in 101 patients.

Biopsies were performed primarily by the Thoracic Interventional Service (three interventionalists), with a small number of biopsies (*n* = 10) performed by the Vascular Interventional Service (five interventionalists). Informed consent was obtained from all patients. CT guidance was performed in all but one patient in whom ultrasonographic guidance was used for a chest wall mass. Coaxial technique was used with a 17-gauge outer needle with 18-gauge inner needle or 19-gauge outer needle with 20-gauge inner needle coaxial system (SuperCore; Argon Medical Devices; Plano, Tex). A cytopathologist was onsite to assess adequacy of the tissue sample by using touch preparation technique. A minimum required number of core biopsy samples was not specified by the KEYNOTE trial protocol. Samples were obtained according to routine practice at our institution with the aim of obtaining as many samples as safely possible to achieve the highest diagnostic yield. For this trial in particular, samples were sometimes required not only for immunohistochemical testing, but also for reconfirmation of previous histopathologic diagnosis. At the conclusion of the biopsy, manual compression, 2–10-mL blood patch, or synthetic sealant (BioSentry; Surgical Specialties Corporation) was used at the discretion of the interventionalists. Patients were observed in a procedure-treatment unit typically for 1–3 hours postprocedure and underwent either observation only or one or more chest radiographic examinations to exclude an immediate complication before discharge on the basis of the discretion of the interventionalists.

Documented patient characteristics included age, sex, and previous local or systemic therapy. Patients previously treated with targeted agents, such as erlotinib, were included in the group of patients who had undergone previous systemic antineoplastic therapy. Tumor characteristics, including histologic characteristics, anatomic location, size, and lesion consistency were also collected. A biopsy was categorized as an initial biopsy if no previous intrathoracic percutaneous or bronchoscopic biopsy and no previous intrathoracic surgical biopsy or resection had been performed either at our institution or documented as performed at an outside institution. A biopsy was categorized as a repeat biopsy if it occurred subsequent to any known previous intrathoracic biopsy or resection. Therefore, a broad range of patients were included in the repeat biopsy group, including patients who underwent initial testing

Table 2

Biopsy Outcomes: Adverse Events

Parameter	Result
Total adverse events (% of all biopsies)	28 (25)
Pneumothorax	25 (23)
Category 1: Clinical management only	20 (18)
Category 2: Intervention required (chest tube)	4 (4)
Category 3: Hospitalization indicated	1 (1)
Hemorrhage	2 (2)
Seizure	1 (1)

Note.—Data are number of complications; data in parentheses are range.

Table 3

Factors that Determined Intraprocedural or Postprocedural Complications and Univariate Regression Analysis

Parameter	Complication Status		P Value
	No Complication (n = 82)	Complication (n = 28)	
Initial biopsies*	6 (7)	3 (11)	.57
Repeat biopsies†	76 (93)	25 (89)	
Sex			
Men	46 (56)	15 (54)	.82
Mean age at biopsy (y)‡	65.4 (36–90)	69.2 (40–84)	.11
Treatment history			
Systemic therapy	66 (80)	23 (82)	.85
Radiation	15 (18)	8 (29)	.25
Surgery	14 (17)	6 (11)	.62
Target biopsy location			
Lung	70 (85)	25 (89)	.60
Pleura, chest wall, or mediastinum	12 (15)	3 (11)	
Mean longest lesion diameter (mm)‡	42.2 (7–132)	41.5 (12–87)	.95
Mean distance from pleura (mm)‡	25.9 (0–85)	34.0 (7–79)	.05§
Solid lesion	77 (94)	21 (75)	.01§
Biopsies that crossed aerated lung	38 (46)	23 (82)	.002§
Location of biopsies that crossed aerated the lung			
Lung	36 (95)	21 (91)	
Not lung	2 (5)	2 (9)	
Mean no. of cores obtained‡	8.2 (1–18)	7.2 (1–10)	.12
Use of closure device	31 (38)	20 (71)	.003§

Note.—Unless otherwise indicated, data are number of biopsies and data in parentheses are percentages. Closure device refers to blood patch or BioSentry sealant (Surgical Specialties).

* There were nine initial biopsies.

† There were 101 repeat biopsies.

‡ Data in parentheses are range.

§ $P \leq .05$.

for PD-1 status after prior diagnosis and/or treatment, as well as patients with a previously nondiagnostic biopsy for evaluation of PD-1 status.

Data elements regarding the biopsy technique were obtained from biopsy reports or directly from images stored in our picture archiving and communication system (Centricity; GE Healthcare, Barrington, Ill), including patient positioning, depth of target lesion (distance along the needle shaft from the pleural surface to the needle tip), number of cores obtained, and whether a closure device was used. Assessment of whether the needle crossed the aerated lung before entering the target lesion was on the basis of review of biopsy images.

Procedure-related complications included complications encountered during the procedure and up to 24 hours from time of biopsy, as noted on the biopsy report or subsequent imaging. Pneumothoraxes were graded by using the National Institutes of Health categorization of adverse events (Table 2) (16).

PD-L1 positivity was assessed by a prototype immunohistochemical assay developed by an outside laboratory by using the 22C3 antibody clone (Dako, Carpinteria, Calif). The percentage of cells with membranous PD-L1 staining was graded by pathologists by using a proportion score of any, moderate, or strong intensity of staining, as well as a numeric score to account for the proportion of cells staining for PD-L1. PD-L1 positivity

was defined as membranous staining in at least 1% of tumor cells (2).

Statistical Analysis

Statistical analysis was performed by using a generalized linear model function with statistical software (R version 3.1.2; R Foundation for Statistical Computing, Vienna, Austria). Univariate analysis was performed on all variables shown in Table 3. Backward feature selection with a threshold α value of .15 was used to determine which variables to set as control variables and which variables to include in the multivariate analysis. Our α threshold of .15 falls within recommended range of thresholds of .10 for backward selection and .25 for forward selection (17–19). In the final model, a P value of less than

.05 was considered to indicate statistical significance.

Results

Tissue Adequacy

Of the 110 biopsies performed, tissue was adequate for immunohistochemical analysis in 106 specimens (96%). Four specimens were inadequate for PD-L1 analysis, thus requiring repeat biopsy for the trial. Comparison of the characteristics of adequate versus inadequate biopsies is shown in Table 4. Of the patients who required repeat biopsy for inadequacy, three of the patients were men and the average age was 70.0 years (age range, 67–73 years). All four biopsies were performed in

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Table 4

Biopsy Outcomes: Adequacy Rates and Associated Factors

Description	Adequate Biopsy (n = 106)	Inadequate Biopsy (n = 4)
Initial biopsies*	8 (8)	1 (25)
Repeat biopsies†	98 (92)	3 (75)
No. of men	58 (55)	3 (75)
Mean age at biopsy (y)‡	66.3 (36–90)	70.0 (67–73)
Treatment type		
Chemotherapy	85 (80)	4 (100)
Radiation	21 (20)	2 (50)
Surgery	20 (19)	0 (0)
Target biopsy location		
Lung	92 (87)	3 (75)
Not lung	14 (13)	1 (25)
Lesion diameter		
Lesion diameter: longest dimension (mm)‡	42.2 (7–132)	39.0 (25–65)
Distance from pleura (mm)‡	27.2 (0–79)	48.8 (30–85)
Solid lesion	95 (90)	3 (75)
No. of biopsies that crossed aerated lung	57 (54)	4 (100)
Location of biopsies that crossed aerated lung		
Lung	54 (51)	3 (75)
Not lung	3 (3)	1 (25)
Service type		
Thoracic Interventional Service	96 (91)	4 (100)
Vascular Interventional Service	10 (9)	0 (0)
Mean no. of cores obtained‡	8.0 (1–18)	5.8 (2–10)
Use of closure device	48 (45)	3 (75)

Note.—Unless otherwise indicated, data are number of biopsies and data in parentheses are percentage. "Not lung" refers to pleura, chest wall, or mediastinum. Closure device refers to a blood patch or BioSentry Sealant (Surgical Specialties).

* n = 9

† n = 101

‡ Data in parentheses are range.

Table 5

Factors that Determined Intraprocedural or Postprocedural Complications

Parameter	P Value
Repeat biopsy	.97
Men	.12
Age at biopsy	.34
Target biopsy location, lung	.05*
Consistency of lesion, solid	.002*
Biopsies that crossed aerated lung	.02*
Closure device used	.05*

Note.—Parameters were analyzed by using multivariate regression analysis. Closure device refers to a blood patch or BioSentry Sealant (Surgical Specialties).

* $P \leq .05$.

patient, intraprocedural seizure related to intracranial metastasis.

Univariate and Multivariate Analysis

Statistical modeling to determine significant contributing factors was performed for biopsies without and with complications. The small number of inadequate biopsies precluded statistical analysis of factors that contributed to adequacy or inadequacy of biopsies.

Univariate P values for factors that determined intraprocedural and postprocedural biopsy complications are shown in Table 3. A higher percentage of patients who experienced complications were women and the average age was higher, but the differences between these groups were not statistically significant. The lesion was located farther from the pleura in biopsies with complications ($P = .05$). Complications occurred more often when biopsy needles crossed aerated lung ($P = .002$). In biopsies in which there was a complication, a higher percentage of target lesions were subsolid in consistency ($P = .01$). Additionally, a closure device was used more often in biopsies with procedure-related complication ($P = .03$).

Variables included in the multivariate analysis and corresponding P values are shown in Table 5. Crossing aerated lung with the biopsy needle was significantly correlated to occurrence of complications in the univariate analysis and remained

patients who had undergone previous local or systemic therapy. Three of the biopsy target lesions were located in the lung and one was in the mediastinum. The average number of core biopsy samples obtained in the group with adequate tissue samples was 8.0 (range, 1–18), compared with an average of 5.8 core biopsy samples (range, 2–10) in the group with inadequate tissue samples.

Complications

Rates of complications are shown in Table 2, and comparison of the characteristics of biopsies without and with complications is shown in Table 3. Complications occurred in 28 (25.4%) of 110 biopsies, and one patient underwent complications at two separate biopsy occasions. Pneumothorax was observed in 25 biopsies (22.7% of all biopsies

performed). Of the seven biopsies performed by using the 17-gauge outer and 18-gauge inner needle coaxial system (6.4% of all biopsies), one biopsy was complicated by pneumothorax (4.0% of all biopsies complicated by pneumothorax). Five of the biopsies complicated by pneumothorax required chest tube placement (5.0%), and one patient with chest tube placement also required admission to the hospital (0.9% of all biopsies). The patient who required chest tube placement and admission had previously undergone chemotherapy and radiation therapy. Delayed pneumothorax was detected in two patients at routine staging CT imaging performed the following day. Neither of these patients required intervention. Other less common complications included minor pulmonary hemorrhage and, in one

significant after adjusting for the constant parameters of sex and age. Targeting a lesion in the lung versus in the pleura, chest wall, or mediastinum and targeting a nonsolid lesion were significant factors associated with complications. The use of a closure device was also significantly associated with complications.

Repeat Biopsy Adequacy and Complications

Subsequent to a known previous intrathoracic percutaneous or bronchoscopic biopsy or previous intrathoracic surgical biopsy or resection procedure, 91.8% (n = 101) of the biopsies performed as part of the trial were performed as repeat biopsies. Adequacy rate in the 101 repeat biopsies was 97%, similar to the overall adequacy rate of 96%. Procedure-related complication rates were also similar in the repeat biopsy group compared with all biopsies (25% in both groups).

Among the eight patients who underwent more than one biopsy during the trial, five patients experienced a procedure-related complication, and one patient's biopsies were complicated by pneumothorax at both procedures. One patient's biopsy was halted because of an intraprocedural seizure and another patient was noted to have minor self-limiting hemoptysis during the biopsy. No complications were encountered during the second biopsy for any of the four patients who required repeat biopsy for inadequate tissue.

Discussion

Our study demonstrates that biopsy and repeat biopsy of tumors via image-guided core needle biopsy is an effective and safe method to obtain tissue samples needed for immunohistochemical analysis in this cohort of patients.

Our overall adequacy rate for obtaining tissue samples for PD-L1 immunohistochemical analysis was 96%, which falls within the higher range of published rates of adequacy for tissue obtained by CT-guided core needle biopsy. For the patients in this trial with insufficient tissue at initial biopsy, all repeat biopsies yielded diagnostic results. In some cases,

the intraprocedural complication during the initial biopsy appears to have led to earlier termination of the procedure necessitating a second biopsy for additional tissue. Studies have shown that obtaining more tissue volume improves diagnostic yield (20–22), so at our institution, we routinely obtain more core biopsy samples than the typical published two to four passes with a core biopsy needle (23), without an increase in complication rates. Particularly for evaluating PD-L1 status, which has shown to be heterogeneously expressed and can be undersampled by small biopsies, obtaining as many core needle samples as is feasible and safe is important to ensure that patients who may benefit from PD-1 inhibitors are eligible for treatment.

Our cohort of patients experienced complication rates similar to previously published rates of complication (20,24), and pneumothorax was the most common. The risk factors in this specific patient cohort were similar to those of the general population of patients who presented for image-guided percutaneous biopsy. The most significant risk factors related to complications in our cohort were the biopsy needle crossing aerated lung and increasing the distance of the target lesion from the pleura. In our single patient who experienced complication by pneumothorax requiring chest tube insertion and admission, both of these risk factors were present. Additional characteristics that were not statistically significant but showed expected trends associated with complications included higher average age and higher proportion of previous radiation therapy.

Biopsies of subsolid lesions were associated with a higher risk of procedure-related complications; however, these lesions represented an overall small population of our total cohort. In those biopsies, closure devices were more often used, which may reflect the relative complexity of the biopsy because the use of a closure device was associated with procedure-related complications.

Our results show that the rate of complications in patients who underwent multiple and repeat biopsies was similar to the rates of complications in both our overall biopsy population

and in the published literature (20,24). Drug-induced lung disease is reported to be as high as 3%–4% both with systemic agents and in trials of PD-1 pathway inhibitors (25–28), potentially adding to the challenge of repeated biopsies. Although we observed higher complication rates in patients with history of systemic therapy, radiation therapy, or surgical treatment, the difference was not significant and treatment history was not a predictive variable for complications.

A limitation of this study is that the data were reviewed retrospectively. Data were obtained primarily from radiology reports and by reviewing biopsy images on our picture archiving and communication system. Some data were incompletely documented. Reporting templates have changed over time, which resulted in slight variation in how data were reported. The biopsies were performed by eight different interventionalists in two different interventional radiology sections, which resulted in some inconsistency in technique as well, though the majority of the biopsies (90.9%) were performed by three thoracic interventionalists. Reporting of intraprocedural complications is variable because some interventionalists may not consider a self-limiting event to be a true complication and thus may not report these events. Although other studies focus only on postbiopsy complications or symptomatic complications, we took a more conservative approach and included any complication mentioned in the biopsy reports and any pneumothorax described in postbiopsy imaging. Additionally, we included all intrathoracic biopsies, which included chest wall lesions and lung lesions that contact the pleura, which have been reported to have slightly lower rates of biopsy complications (20). We opted not to exclude biopsies on the basis of specific target locations to capture the heterogeneity of this patient population.

To conclude, anti-PD-1 therapy is a promising therapy for patients with non–small cell lung cancer in whom tumors express PD-L1, and the need for tissue samples for treatment consideration will likely result in increased referrals for image-guided percutaneous

biopsy and repeat biopsy in this patient population. Percutaneous transthoracic core needle biopsy and repeat biopsy are feasible and adequate for immunohistochemical analysis in more than 95% of patients with complication rates comparable to the general population.

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