Bronchoscopic tissue yield for advanced molecular testing: are we getting enough?

Pattraporn Tajarernmuang[1,2], Linda Ofiara[3], Stéphane Beaudoin[3], Anne V. Gonzalez[1,3]

[1]Respiratory Epidemiology and Clinical Research Unit, McGill University Health Centre Research Institute, Montreal, Quebec, Canada; [2]Respiratory, Critical Care and Allergy Division, Faculty of Medicine, Chiang Mai University, Chiang Mai, Thailand; [3]Respiratory Division, Montreal Chest Institute, McGill University Health Centre, Montreal, Quebec, Canada

Contributions: (I) Conception and design: P Tajarernmuang, AV Gonzalez; (II) Administrative support: AV Gonzalez; (III) Provision of study materials or patients: P Tajarernmuang, AV Gonzalez; (IV) Collection and assembly of data: P Tajarernmuang, AV Gonzalez; (V) Data analysis and interpretation: All authors; (VI) Manuscript writing: All authors; (VII) Final approval of manuscript: All authors.

Correspondence to: Dr. Anne V. Gonzalez, MD, MSc. Centre for Outcomes Research and Evaluation, McGill University Health Centre Research Institute, 5252 Boul. de Maisonneuve West, 3D.63, Montreal, Quebec H4A 3S5, Canada. Email: anne.gonzalez@mcgill.ca.

Abstract: The treatment of advanced lung cancer has become increasingly personalized over the past decade as a result of the improved understanding of tumor molecular biology and anti-tumor immunity. An adequate tumor sample is central to targetable mutation analysis, and immunologic profiling. The majority of lung cancer patients currently present at an advanced disease stage, so that diagnosis and staging are largely based on small biopsy and cytology specimens. Flexible bronchoscopy techniques play a prominent role in the acquisition of these diagnostic specimens. This narrative review summarizes the available evidence with regards to the role of various conventional and advanced flexible bronchoscopy techniques in acquiring sufficient tissue for mutation analysis and programmed death-ligand 1 (PD-L1) testing.

Keywords: Bronchoscopy; lung cancer; immunohistochemistry (IHC); molecular testing; programmed death-ligand 1 (PD-L1)

Introduction

The treatment of lung cancer has evolved beyond the simple histologic distinction of small cell and non-small cell lung cancer (NSCLC). Novel treatments for NSCLC target specific gene alterations. Trials have demonstrated the significant benefit of such targeted therapies over standard cytotoxic chemotherapy. Patients with epidermal growth factor receptor (EGFR) mutations, typically on exons 19 and 21, exhibit dramatic responses to specific tyrosine kinase inhibitors (TKI) such as gefitinib, erlotinib, afatinib and osimertinib (10–15% of NSCLC) (1,2). Rearrangement of the anaplastic lymphoma kinase (ALK) gene (5% of NSCLC) predicts response to treatment with ALK inhibitors such as crizotinib, ceritinib, alectinib, and brigatinib (3). ROS-1 rearrangement (1–2% of NSCLC)

also predicts response to crizotinib (4). KRAS mutations, detected predominately in smokers with adenocarcinoma, are associated with brain metastasis and a worse prognosis (5). KRAS inhibitors are currently undergoing early phase clinical trials.

Over the past decade, the understanding of anti-tumor immunity has remarkably improved. Interaction of the programmed death-ligand 1 (PD-L1) with its receptor (PD-1), has been recognized as an essential immune escape mechanism for tumor cells, and is now targeted in lung cancer treatment. The level of PD-L1 expression on tumor cells, as evaluated by immunohistochemistry (IHC), currently guides immunotherapy treatment decisions. Several immune checkpoint inhibitors are used in the treatment of advanced NSCLC (6). Patients with advanced NSCLC and PD-L1 expression on at least 50%

of tumor cells (a.k.a. tumor proportion score or TPS 50%) benefit from first-line treatment with the anti-PD-1 agent pembrolizumab, with improved survival and reduced adverse effects compared to standard chemotherapy (7). Pembrolizumab and platinum based chemotherapy is indicated as first-line treatment in patients with PD-L1 1–49% (8,9). Pembrolizumab can also be administered as second-line treatment in the presence of PD-L1 expression on at least 1% of tumor cells (10).

Advanced molecular testing, to identify targetable mutations and quantify PD-L1 expression, thus guides the personalized treatment of lung cancer. The majority of patients with advanced lung cancer are diagnosed and staged with minimally invasive techniques, and the resultant small biopsy and cytological samples must be judiciously processed for molecular testing. Small biopsy and cytopathology specimens also play a central role in the context of disease progression after treatment (11). The selection of a biopsy site is based on review of available CT and/or PET imaging, keeping in mind that invasive sampling should target the site associated with the highest suspected disease stage, whenever feasible (12,13). Bronchoscopic procedures must successfully sample target lesions (node, nodule or mass) and provide sufficient tissue for both histological characterization, and molecular analysis. This narrative review summarizes the available evidence with regards to the role and limitations of various conventional and advanced flexible bronchoscopy techniques in acquiring sufficient tissue for mutation analysis and PD-L1 testing.

Molecular testing of small biopsy and cytology specimens

The increasing role of small biopsy and cytology specimens was recognized in the 2015 World Health Organization Classification of Lung Tumors (14). The most recent guidelines from the College of American Pathologists (CAP), the International Association for the Study of Lung Cancer (IASLC), and the Association for Molecular Pathology (AMP) recommend molecular testing at the time of initial diagnosis in patients with advanced lung adenocarcinoma regardless of clinical characteristics (15). The NCCN has expanded the indication for EGFR and ALK testing to non-squamous NSCLC and NSCLC not otherwise specified (NOS), as well as patients with metastatic squamous cell lung carcinoma who are non-smokers (6). A basic panel, including EGFR mutation, ALK

rearrangement, mutations in V-raf murine sarcoma viral oncogene homolog B (BRAF), and ROS-1 mutation, is recommended for routine testing in lung adenocarcinomas. An expanded panel with RET, ERBB2 (HER2), KRAS, and MET can be tested in the context of clinical trials (15).

The accuracy of molecular profiling depends on the quality of tissue samples, both in terms of the quantity and quality of tumor cells acquired. Adequate technique for both specimen collection and processing are essential, and collaboration between proceduralists and pathologists is required (16). The required number of tumor cells for molecular analysis has not been clearly established, although studies have suggested a range of 100–400 tumor cells (17,18). Sun *et al.* reported that cytological specimens are comparable to histological samples, as long as they provide DNA concentrations >25 ng·mL^{-1}, >30 tumor cells, or >30% tumor cellularity (19). Cytology cell blocks are preferred to simple smears for the purpose of molecular analysis (20). Small biopsy and cytological samples generally provide lower amounts of DNA, so that sensitive methods are required to detect targetable mutations.

The CAP/IASLC/AMP guideline recommends using sensitive analytical assays that can detect mutations with tumor cell proportions as low as 20% of the test sample (15). The NCCN guideline lists a range of methods for tissue genotyping including next-generation sequencing (NGS), real-time polymerase chain reaction (PCR) for specific targetable mutations, Sanger sequencing, other multiplex approaches (e.g., SNaPshot, MassARRAY), fluorescence *in situ* hybridization (FISH) for detecting gene rearrangements, and immunohistochemistry (IHC) for certain analyses or (mutation) screening. Sanger sequencing, the first generation of DNA sequencing, examines one or a few genes at a time, and requires more than 25–30% of tumor cells (6). In contrast, NGS allows the detection of numerous mutations at the same time, making it particularly advantageous when limited tissue samples are available. The interested reader is referred to the website of the American Association for Clinical Chemistry for a user-friendly description of these techniques (https://labtestsonline.org/genetic-testing-techniques).

There is limited data on the molecular testing of samples acquired with conventional diagnostic bronchoscopy. Many studies have combined bronchoscopy samples with other small biopsy or cytology specimens, making specific evaluation more difficult. The percentage of malignant cells from bronchoscopic biopsies [endobronchial biopsy or radial-endobronchial ultrasound (R-EBUS) guided

transbronchial lung biopsy (TBB)], endobronchial ultrasound with transbronchial needle aspiration (EBUS-TBNA), and surgical biopsies was examined by Dooms et al: 57% of diagnostic endoscopic biopsies, 44% of EBUS-TBNA samples, and 90% of surgical biopsies provided >25% tumor cells, which is considered adequate for Sanger sequencing. Surgical biopsies provided more DNA than bronchoscopic biopsies and EBUS-TBNA, with medians of 2,500 *vs.* 1,610 and 1,440 ng, respectively (21). Hagmeyer and colleagues examined the role of brush specimens acquired from central lung cancers for molecular testing using NGS. The combined brush smear and brush tip washing provided diagnostic sensitivity of 69%, and NGS was feasible in 18 of 29 samples (62%) (22). Perhaps not surprisingly, NGS has been shown to be more sensitive than Sanger sequencing for detection of EGFR mutations in both BAL and pleural fluid samples (23).

EBUS-TBNA samples and molecular testing

Labarca and colleagues recently reviewed the adequacy of EBUS-TBNA samples for molecular analysis in patients with NSCLC. A total of 33 studies, encompassing 2,698 participants, were included in their meta-analysis. The pooled probability of obtaining an adequate sample for EGFR and ALK testing was 94.5% and 94.9%, respectively, and the prevalence of EGFR mutation and ALK rearrangement were 15.8% and 2.8%, respectively (24). A small study from Chile reported 10 of 12 (83.3%) EBUS-TBNA specimens were adequate for ROS1 testing (25). A retrospective review by Cicek *et al.* showed 90 of 98 (91.8%) EBUS-TBNA samples were adequate for the ROS1 FISH test (26). Xie *et al.* showed 100% concordance for EGFR, ALK, and ROS1 results between NGS and conventional analytical assays in EBUS-TBNA samples, however NGS provided information on 12 additional mutations (27).

The recent CHEST Guideline on technical aspects of EBUS-TBNA recommends that additional samples, beyond the minimum of 3 separate needle passes acquired to establish the diagnosis of lung cancer, be obtained for molecular analysis (28). Labarca *et al.* suggested that the minimum number of needle passes per lymph node in order to obtain adequate tissue for molecular analysis was 3 with rapid on-site evaluation (ROSE) or 4 without ROSE (24). Trisolini *et al.* conducted a randomized trial to evaluate whether ROSE increased the yield of EBUS-TBNA for molecular analysis. The result showed 90.8% of EBUS-TBNA specimens from the ROSE arm and 80% from non-ROSE arm were suitable for genotypic analysis (P=ns). Patients in the ROSE arm were more likely to have the bronchoscopy terminated after a single biopsy site (29). The available evidence does not suggest a difference in diagnostic yield and/or feasibility of molecular analysis according to needle size used (21 or 22G), and data is still limited on the newer 19G needles (30,31). Larger size needles, however, have been associated with more blood in specimens (30).

Guided bronchoscopy samples and molecular testing

Molecular testing guides the treatment of patients with advanced lung cancer, in whom isolated peripheral pulmonary nodules are rarely targeted for sampling. Accordingly, there is limited data on molecular analysis of specimens acquired with guided bronchoscopy techniques. Moon *et al.* demonstrated the feasibility of molecular testing using R-EBUS-guided TBB peripheral lung lesions: 63 of 64 specimens (98%) were found to be adequate for EGFR testing, 60 of 60 (100%) adequate for ALK IHC, and 16 of 17 (94%) adequate for PD-L1 IHC (32). Kim *et al.* compared EGFR mutations and ALK rearrangements of paired R-EBUS-guided TBB and subsequent surgical resection specimens. The result demonstrated 97% agreement in EGFR mutations and 100% in ALK translocation (33). Guisier *et al.* retrospectively reviewed the adequacy of R-EBUS-guided TBB and brushings for molecular testing. Molecular analysis was feasible in 88/113 non-squamous NSCLC samples, with 73 of 86 diagnostic biopsy specimens (85%) being suitable for testing. Factors associated with successful molecular testing included upper or middle lobe location and at least three biopsies taken (34). However, when diagnosis was based on brushings (smears) alone, only 15/27 smears were suitable for molecular analysis. In contrast, two studies from Taiwan and Spain demonstrated a 95% success rate of molecular analysis from R-EBUS-guided "waste" brushing samples; the brushes were immediately snipped off and immersed into fixative solution (35,36). The NAVIGATE study examined the diagnostic yield of ENB-guided sampling for peripheral pulmonary lesions, and reported 86.2% of samples (75/87 specimens) were adequate for molecular/genetic testing (37). If tissue sampling is successful, then advanced molecular testing is highly feasible in samples from peripheral pulmonary lesions acquired using guided bronchoscopy techniques.

PD-L1 testing of small biopsy and cytology specimens

The current NCCN guidelines recommend quantitative evaluation of tumor cell PD-L1 expression at initial diagnosis of advanced NSCLC (6). PD-L1 expression is quantified by the percentage of membrane stained viable tumor cells relative to all viable tumor cells in the sample, known as tumor proportion score (TPS). A minimum of 50–100 viable tumor cells is required, depending on the assay used. Several antibodies and platforms for PD-L1 IHC testing have been developed and marketed, and lack of standardization remains a significant limitation. Clinical trials have used different PD-L1 assays and varying cut-off levels (38). Most clinical trials supporting the use of immune checkpoint inhibitors performed PD-L1 testing on formalin-fixed paraffin-embedded (FFPE) core biopsy or surgical resection specimens, however this practice does not reflect the clinical care of most patients with advanced lung cancer (39).

Concerns have been raised with regards to the use of cytology specimens to evaluate with immune landscape in NSCLC (38). Small biopsies and cytology samples could increase the risk of false-negative PD-L1 results, based on the known temporal and spatial heterogeneity of its tumoral expression (40,41). Bigras et al. evaluated the impact of small biopsy size on PD-L1 results in vitro. More biopsies <2 mm^2 were associated with false-negative results (PD-L1 TPS <1%), while larger specimens more frequently demonstrated PD-L1 TPS 1–49%. However, the number of samples which detected PD-L1 TPS ≥50% was not different between small and large biopsies (42).

The concordance of PD-L1 expression as assessed by bronchoscopic small biopsies and surgical resection specimens has been examined. Kitazono et al. reviewed the PD-L1 expression of 79 patients in whom both small biopsy specimens and surgical resection samples were available. The bronchoscopic samples included 59 TBB, 12 TBNA, and 8 CT-guided needle biopsies. The concordance of PD-L1 expression was 92.4% concordance between small biopsies and surgical resections, using a hybrid IHC score assessment (43). The study of Ilie and colleagues provides contrasting results: 160 patients with both surgical specimens and small biopsies were considered, in whom 110 (69%) had TBB specimens. PD-L1 expression was underestimated by small biopsy specimens, with 48% overall discordance rate. However, overall concordance

was evaluated based on PD-L1 expression in both tumor cells and tumor-infiltrating immune cells using the PD-L1 SP142 antibody clone (44).

Several recent studies have examined the feasibility of PD-L1 testing using small biopsy and cytological samples (43-46), in particular the use of EBUS-TBNA samples for PD-L1 IHC analysis (Table 1). Sakakibara et al. demonstrated that a significantly larger number of tumor cells were obtained with EBUS-TBNA compared with TBB, with median numbers of 1,149 vs. 435 (P<0.001); crush rate was significantly lower with EBUS-TBNA vs. TBB (47). Sakata et al. retrospectively compared the results of PD-L1 testing in EBUS-TBNA samples vs. surgical resection specimens in 61 patients and reported that sensitivity, specificity, PPV, and NPV for PD-L1 ≥1% were 72%, 100%, 100%, and 80%, respectively. The concordance rate between EBUS-TBNA vs. surgical specimens was 87% for PD-L1 ≥1% and 82% for PD-L1 ≥50%. However, the sensitivity of EBUS-TBNA samples declined from 72% to only 47% at a PD-L1 expression cut-off of ≥50% (vs. ≥1%), raising concerns of false-negative PD-L1 results in EBUS-TBNA specimens (48). In contrast, we have reported a recent series of PD-L1 testing in 120 consecutive EBUS-TBNA samples. Comparison of PD-L1 results in the subgroup of 18 patients with both EBUS-TBNA and histological samples revealed moderate concordance, with no false-negative results (50).

PD-L1 IHC testing has been approved in formalin-fixed paraffin embedded (FFPE) tissue samples (54). Concerns have been raised with regards to the use of other agents such as methanol-based fixatives for subsequent PD-L1 assessment. Recent studies have reported on the feasibility of PD-L1 testing in Cytolyte-fixed specimens (50,51), with two recent studies reporting concordance rates of 78% and 91%, respectively, for PD-L1 expression in EBUS-TBNA specimens initially processed with methanol-based fixatives vs. FFPE histological specimens (49,50). Similar to molecular testing, limited data is currently available on the role of ROSE (55) and needle gauge in ensuring adequate EBUS-TBNA samples are acquired for assessment of PD-L1 expression.

The available evidence thus suggests that PD-L1 testing of EBUS-TBNA samples is feasible, and points towards at least moderate concordance with PD-L1 expression assessed in histological samples. Future work will need to determine the role of PD-L1 expression as assessed in cytological samples for predicting response to immunotherapy.

Table 1 PD-L1 testing on EBUS-TBNA specimens: feasibility and concordance with histology samples

Author, year	PD-L1 assay	TPS cut-offs	Number of EBUS-TBNA samples	Feasibility of PD-L1 testing	Number and type of paired samples	Agreement analysis	Comments
Sakakibara 2017 (47)	EPR1161	N/A	EBUS-TBNA 97	99%	Resected primary tumor 6, resected LN 5, TBB 16	Pearson correlation for lung resection r=0.75, for LN metastasis r=0.93, for TBB r=0.75	1 of 97 EBUS-TBNA sample had <100 tumor cells
Sakata 2018 (48)	22C3	≥1%, ≥50%	EBUS-TBNA 61	84%	Surgical resection 61	Concordance 87% for cut-off ≥1% and 82% for cut-off ≥50%	–
Wang 2019 (49)	22C3	≥50%	EBUS-TBNA	86.8%	Paired surgical biopsy 34	Concordance 91.3%	–
Smith 2019 (50)	22C3	≥50%	EBUS-TBNA	92%	Surgical resection 11, TBB 1, core biopsies 4, pleural biopsy 1, autopsy 1	Concordance 78%	–
Stoy 2018 (51)	28-8	<1%, 1–49%, ≥50%	EBUS-TBNA 16, endobronchial FNA 4, peripheral TBNA nodule 2	88% for 16 EBUS-TBNA samples	FOB biopsy 2 (same location), 1 (different location)	Concordance in 2 of 3 cases (for which cytology and histology were from same location)	–
Biswas 2018 (52)	22C3	<1%, 1–49%, ≥50%	EBUS-TBNA 50	86%	N/A	N/A	–
Fernandez-Bussy 2018 (53)	E1L3N	<1%, 1–50%, >50%	EBUS-TBNA 23	100%	N/A	N/A	–

Table adapted with permission from Smith et al. (50). PD-L1, programmed death-ligand 1; CT, computed tomography; EBUS-TBNA, endobronchial ultrasound with transbronchial needle aspiration; FNA, fine needle aspiration; FOB, flexible bronchoscopy; LN, lymph node; N/A, not available; TBB, transbronchial biopsy.

Summary

The treatment of advanced lung cancer has become increasingly personalized over the past decade, as a result of improved understanding of tumor molecular biology and anti-tumor immunity. Advanced diagnostic bronchoscopy techniques play a central role in the evaluation of patients with suspected lung cancer. An adequate tumor sample is central to targetable mutation analysis and immunologic profiling. To the question "Are we getting enough?" the answer is "it depends"—mainly on the procedure being performed, but also partly on the analysis being requested. Communication between bronchoscopists and pathologists is key: clinicians should know which technique is being used locally for molecular profiling, minimum number of tumor cells required, as well as preferred samples types and preparation. Minimally invasive needle techniques such as EBUS-TBNA have become the first-line procedures in patients with adenopathy, and/or centrally located tumors. The available data suggests that EBUS-TBNA samples are largely adequate for molecular testing, and seem to provide a reliable assessment of PD-L1 expression. Advanced diagnostic bronchoscopy techniques for sampling of peripheral pulmonary lesions still suffer from a limited and variable diagnostic yield, which technological advances actively seek to remedy. The role of conventional diagnostic bronchoscopy techniques is similarly limited to centrally located lesion that can be sampled under direct vision. Technological advances in both bronchoscopy procedures and analytical processes are expected to further consolidate the role of small biopsy and cytological specimens for molecular testing in patients with advanced lung cancer.

Acknowledgments

Footnote

Provenance and Peer Review: This article was commissioned by the Guest Editors (Fabien Maldonado and Robert Lentz) for the series "Novel Diagnostic Techniques for Lung Cancer" published in *Journal of Thoracic Disease*. The article was sent for external peer review organized by the Guest Editors and the editorial office.

Conflicts of Interest: All authors have completed the ICMJE uniform disclosure forms (available at http://dx.doi. org/10.21037/jtd-19-4119). The series "Novel Diagnostic Techniques for Lung Cancer" was commissioned by the editorial office without any funding or sponsorship. SB reports non-financial support from Olympus America Inc., outside the submitted work. Dr. AVG reports grant support from the Rossy Cancer Network. The other authors have no other conflicts of interest to declare.

Ethical Statement: The authors are accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved.

Open Access Statement: This is an Open Access article distributed in accordance with the Creative Commons Attribution-NonCommercial-NoDerivs 4.0 International License (CC BY-NC-ND 4.0), which permits the non-commercial replication and distribution of the article with the strict proviso that no changes or edits are made and the original work is properly cited (including links to both the formal publication through the relevant DOI and the license). See: https://creativecommons.org/licenses/by-nc-nd/4.0/.

References

1. Greenhalgh J, Dwan K, Boland A, et al. First-line treatment of advanced epidermal growth factor receptor (EGFR) mutation positive non-squamous non-small cell lung cancer. Cochrane Database Syst Rev 2016;(5):CD010383.

2. Holleman MS, van Tinteren H, Groen HJ, et al. First-line tyrosine kinase inhibitors in EGFR mutation-positive non-small-cell lung cancer: a network meta-analysis. Onco Targets Ther 2019;12:1413-21.

3. Fan J, Fong T, Xia Z, et al. The efficacy and safety of ALK inhibitors in the treatment of ALK-positive non-small cell lung cancer: A network meta-analysis. Cancer Med 2018;7:4993-5005.

4. Shaw AT, Riely GJ, Bang YJ, et al. Crizotinib in ROS1-rearranged advanced non-small-cell lung cancer (NSCLC): updated results, including overall survival, from PROFILE 1001. Ann Oncol 2019;30:1121-6.

5. Meng D, Yuan M, Li X, et al. Prognostic value of K-RAS mutations in patients with non-small cell lung cancer: a systematic review with meta-analysis. Lung Cancer 2013;81:1-10.

6. NCCN Clinical Practice Guidelines in Oncology (NCCN Guidelines®) Non-Small Cell Lung Cancer Version

6.2019 [Internet]. National Comprehensive Cancer Network. 2019. Available online: www.NCCN.org

7. Kim R, Keam B, Hahn S, et al. First-line Pembrolizumab Versus Pembrolizumab Plus Chemotherapy Versus Chemotherapy Alone in Non-small-cell Lung Cancer: A Systematic Review and Network Meta-analysis. Clin Lung Cancer 2019;20:331-8.e4.

8. Langer CJ, Gadgeel SM, Borghaei H, et al. Carboplatin and pemetrexed with or without pembrolizumab for advanced, non-squamous non-small-cell lung cancer: a randomised, phase 2 cohort of the open-label KEYNOTE-021 study. Lancet Oncol 2016;17:1497-508.

9. Gandhi L, Rodriguez-Abreu D, Gadgeel S, et al. Pembrolizumab plus Chemotherapy in Metastatic Non-Small-Cell Lung Cancer. N Engl J Med 2018;378:2078-92.

10. Herbst RS, Baas P, Kim DW, et al. Pembrolizumab versus docetaxel for previously treated, PD-L1-positive, advanced non-small-cell lung cancer (KEYNOTE-010): a randomised controlled trial. Lancet 2016;387:1540-50.

11. Kirita K, Izumo T, Matsumoto Y, et al. Bronchoscopic Re-biopsy for Mutational Analysis of Non-small Cell Lung Cancer. Lung 2016;194:371-8.

12. Ost DE, Jim Yeung SC, Tanoue LT, et al. Clinical and organizational factors in the initial evaluation of patients with lung cancer: Diagnosis and management of lung cancer, 3rd ed: American College of Chest Physicians evidence-based clinical practice guidelines. Chest 2013;143:e121S-41S.

13. Silvestri GA, Gonzalez AV, Jantz MA, et al. Methods for staging non-small cell lung cancer: Diagnosis and management of lung cancer, 3rd ed: American College of Chest Physicians evidence-based clinical practice guidelines. Chest 2013;143:e211S-50S.

14. Travis WD, Brambilla E, Nicholson AG, et al. The 2015 World Health Organization Classification of Lung Tumors: Impact of Genetic, Clinical and Radiologic Advances Since the 2004 Classification. J Thorac Oncol 2015;10:1243-60.

15. Kalemkerian GP, Narula N, Kennedy EB, et al. Molecular Testing Guideline for the Selection of Patients With Lung Cancer for Treatment With Targeted Tyrosine Kinase Inhibitors: American Society of Clinical Oncology Endorsement of the College of American Pathologists/International Association for the Study of Lung Cancer/Association for Molecular Pathology Clinical Practice Guideline Update. J Clin Oncol 2018;36:911-9.

16. Planchard D, Popat S, Kerr K, et al. Metastatic non-small cell lung cancer: ESMO Clinical Practice Guidelines for diagnosis, treatment and follow-up. Ann Oncol 2018;29:iv192-iv237.

17. Pirker R, Herth FJ, Kerr KM, et al. Consensus for EGFR mutation testing in non-small cell lung cancer: results from a European workshop. J Thorac Oncol 2010;5:1706-13.

18. Savic S, Tapia C, Grilli B, et al. Comprehensive epidermal growth factor receptor gene analysis from cytological specimens of non-small-cell lung cancers. Br J Cancer 2008;98:154-60.

19. Sun PL, Jin Y, Kim H, et al. High concordance of EGFR mutation status between histologic and corresponding cytologic specimens of lung adenocarcinomas. Cancer Cytopathol 2013;121:311-9.

20. Lindeman NI, Cagle PT, Beasley MB, et al. Molecular testing guideline for selection of lung cancer patients for EGFR and ALK tyrosine kinase inhibitors: guideline from the College of American Pathologists, International Association for the Study of Lung Cancer, and Association for Molecular Pathology. J Mol Diagn 2013;15:415-53.

21. Dooms C, Vliegen L, Vander Borght S, et al. Suitability of small bronchoscopic tumour specimens for lung cancer genotyping. Respiration 2014;88:371-7.

22. Hagmeyer L, Fassunke J, Engels M, et al. Bronchoscopic Brushing from Central Lung Cancer-Next Generation Sequencing Results are Reliable. Lung 2019;197:333-7.

23. Buttitta F, Felicioni L, Del Grammastro M, et al. Effective assessment of egfr mutation status in bronchoalveolar lavage and pleural fluids by next-generation sequencing. Clin Cancer Res 2013;19:691-8.

24. Labarca G, Folch E, Jantz M, et al. Adequacy of Samples Obtained by Endobronchial Ultrasound with Transbronchial Needle Aspiration for Molecular Analysis in Patients with Non-Small Cell Lung Cancer. Systematic Review and Meta-Analysis. Ann Am Thorac Soc 2018;15:1205-16.

25. Fernandez-Bussy S, Labarca G, Pires Y, et al. Molecular Testing of EGFR, EGFR Resistance Mutation, ALK and ROS1 Achieved by EBUS-TBNA in Chile. Arch Bronconeumol 2017;53:172-4.

26. Cicek T, Ozturk A, Yilmaz A, et al. Adequacy of EBUS-TBNA specimen for mutation analysis of lung cancer. Clin Respir J 2019;13:92-7.

27. Xie F, Zheng X, Mao X, et al. Next-Generation Sequencing for Genotyping of Endobronchial Ultrasound-Guided Transbronchial Needle Aspiration Samples in Lung Cancer. Ann Thorac Surg 2019;108:219-26.

28. Wahidi MM, Herth F, Yasufuku K, et al. Technical Aspects of Endobronchial Ultrasound-Guided Transbronchial Needle Aspiration: CHEST Guideline and Expert Panel Report. Chest 2016;149:816-35.

29. Trisolini R, Cancellieri A, Tinelli C, et al. Randomized Trial of Endobronchial Ultrasound-Guided Transbronchial Needle Aspiration With and Without Rapid On-site Evaluation for Lung Cancer Genotyping. Chest 2015;148:1430-7.

30. Dooms C, Vander Borght S, Yserbyt J, et al. A Randomized Clinical Trial of Flex 19G Needles versus 22G Needles for Endobronchial Ultrasonography in Suspected Lung Cancer. Respiration 2018;96:275-82.

31. Garrison G, Leclair T, Balla A, et al. Use of an Additional 19-G EBUS-TBNA Needle Increases the Diagnostic Yield of EBUS-TBNA. J Bronchology Interv Pulmonol 2018;25:269-73.

32. Moon SM, Choe J, Jeong BH, et al. Diagnostic Performance of Radial Probe Endobronchial Ultrasound without a Guide-Sheath and the Feasibility of Molecular Analysis. Tuberc Respir Dis (Seoul) 2019;82:319-27.

33. Kim I, Eom JS, Kim AR, et al. Molecular analysis of small tissue samples obtained via transbronchial lung biopsy using radial probe endobronchial ultrasound. PLoS One 2019;14:e0212672.

34. Guisier F, Salaun M, Lachkar S, et al. Molecular analysis of peripheral non-squamous non-small cell lung cancer sampled by radial EBUS. Respirology 2016;21:718-26.

35. Tsai TH, Yang CY, Ho CC, et al. Multi-gene analyses from waste brushing specimens for patients with peripheral lung cancer receiving EBUS-assisted bronchoscopy. Lung Cancer 2013;82:420-5.

36. Sánchez-Font A, Chalela R, Martin-Ontiyuelo C, et al. Molecular analysis of peripheral lung adenocarcinoma in brush cytology obtained by EBUS plus fluoroscopy-guided bronchoscopy. Cancer Cytopathol 2018;126:860-71.

37. Folch EE, Pritchett MA, Nead MA, et al. Electromagnetic Navigation Bronchoscopy for Peripheral Pulmonary Lesions: One-Year Results of the Prospective, Multicenter NAVIGATE Study. J Thorac Oncol 2019;14:445-58.

38. IASLC Atlas of PD-L1 Immunohistochemistry Testing in Lung Cancer: Editorial Rx Press; 2017. Available online: https://www.iaslc.org/Portals/0/iaslc_pd-l1_atlas_mar2018_lo-res.pdf?ver=2019-06-06-153849-143

39. Beattie J, Yarmus L, Wahidi M, et al. The Immune Landscape of Non-Small-Cell Lung Cancer. Utility of Cytologic and Histologic Samples Obtained through Minimally Invasive Pulmonary Procedures. Am J Respir Crit Care Med 2018;198:24-38.

40. Nakamura S, Hayashi K, Imaoka Y, et al. Intratumoral heterogeneity of programmed cell death ligand-1 expression is common in lung cancer. PLoS One 2017;12:e0186192.

41. Casadevall D, Clave S, Taus A, et al. Heterogeneity of Tumor and Immune Cell PD-L1 Expression and Lymphocyte Counts in Surgical NSCLC Samples. Clin Lung Cancer 2017;18:682-691.e5.

42. Bigras G, Mairs S, Swanson PE, et al. Small Biopsies Misclassify up to 35% of PD-L1 Assessments in Advanced Lung Non-Small Cell Lung Carcinomas. Appl Immunohistochem Mol Morphol 2018;26:701-8.

43. Kitazono S, Fujiwara Y, Tsuta K, et al. Reliability of Small Biopsy Samples Compared With Resected Specimens for the Determination of Programmed Death-Ligand 1 Expression in Non-Small-Cell Lung Cancer. Clin Lung Cancer 2015;16:385-90.

44. Ilie M, Long-Mira E, Bence C, et al. Comparative study of the PD-L1 status between surgically resected specimens and matched biopsies of NSCLC patients reveal major discordances: a potential issue for anti-PD-L1 therapeutic strategies. Ann Oncol 2016;27:147-53.

45. Noll B, Wang WL, Gong Y, et al. Programmed death ligand 1 testing in non-small cell lung carcinoma cytology cell block and aspirate smear preparations. Cancer Cytopathol 2018;126:342-52.

46. Wang H, Agulnik J, Kasymjanova G, et al. Cytology cell blocks are suitable for immunohistochemical testing for PD-L1 in lung cancer. Ann Oncol 2018;29:1417-22.

47. Sakakibara R, Inamura K, Tambo Y, et al. EBUS-TBNA as a Promising Method for the Evaluation of Tumor PD-L1 Expression in Lung Cancer. Clin Lung Cancer 2017;18:527-534.e1.

48. Sakata KK, Midthun DE, Mullon JJ, et al. Comparison of Programmed Death Ligand-1 Immunohistochemical Staining Between Endobronchial Ultrasound Transbronchial Needle Aspiration and Resected Lung Cancer Specimens. Chest 2018;154:827-37.

49. Wang G, Ionescu DN, Lee CH, et al. PD-L1 testing on the EBUS-FNA cytology specimens of non-small cell lung cancer. Lung Cancer 2019;136:1-5.

50. Smith A, Wang H, Zerbo A, et al. Programmed Death Ligand 1 Testing of Endobronchial Ultrasound-guided Transbronchial Needle Aspiration Samples Acquired For the Diagnosis and Staging of Non-Small Cell Lung Cancer. J Bronchology Interv Pulmonol 2020;27:50-7.

51. Stoy SP, Rosen L, Mueller J, et al. Programmed death-ligand 1 testing of lung cancer cytology specimens obtained with bronchoscopy. Cancer Cytopathol 2018;126:122-8.

52. Biswas A, Leon ME, Drew P, et al. Clinical performance of endobronchial ultrasound-guided transbronchial needle aspiration for assessing programmed death ligand-1 expression in nonsmall cell lung cancer. Diagn Cytopathol 2018;46:378-83.

53. Fernandez-Bussy S, Pires Y, Labarca G, et al. PD-L1 Expression in a Non-Small Cell Lung Cancer Specimen Obtained by EBUS-TBNA. Arch Bronconeumol 2018;54:290-2.

54. . Available online: https://www.accessdata.fda.gov/cdrh_docs/pdf15/P150013c.pdf

55. Doxtader E, Yachimiak T, Mukhopadhyay S, et al. Rapid On-site Evaluation (ROSE) of Endobronchial Ultrasound-guided Fine-needle Aspiration (EBUS-TBNA) Optimizes Tissue for Evaluation of PD-L1 Expression on Formalin-fixed Non-small Cell Lung Carcinoma. J Am Soc Cytopathol 2017;6:S45.