Cytopathology in focus: Updated NSCLC guideline moves molecular cytopathology forward

Sinchita Roy-Chowdhuri, MD, PhD

May 2019—The genomic landscape of non-small cell lung carcinoma is evolving constantly with the discovery of a growing number of molecular alterations and associated targeted therapies that have an impact on patient care. The CAP, International Association for the Study of Lung Cancer, and Association for Molecular Pathology issued a guideline in 2013 to provide a road map for molecular testing to select patients for treatment with targeted tyrosine kinase inhibitors.[1] The guideline was updated in 2018 and endorsed by the American Society of Clinical Oncology. It provides recommendations that affect cytopathology and the role of the cytopathologist in providing clinically relevant genomic information for the treatment of patients with NSCLC.[2,3]

The main updated recommendations pertaining to cytologic specimens are summarized in **Table 1**. One is the endorsement of "any cytology sample with adequate cellularity and preservation" for molecular testing. This is a significant update from the 2013 guideline in which cell blocks were recommended as a *preferred* substrate for testing. The updated guideline opens the door to better utilization of non-cell block (i.e. non-formalin-fixed, paraffin-embedded cytology) preparations such as direct smears, cytospin preparations, touch preparations, and liquid-based cytology. Several studies have indicated the limitations of restricting molecular testing to cell blocks alone, including a lack of on-site assessment for adequacy, variable cellularity, and suboptimal nucleic acid quality subject to formalin fixation and paraffin embedding.[4-6] To this effect, multiple institutions have validated non-FFPE cytology substrates for molecular testing and have shown in some instances that it may provide a superior substrate than their FFPE counterparts.[7-10] This may be due in part to the absence of cross-linking formalin artifacts in non-FFPE cytologic preparations resulting in the retrieval of better quality nucleic acids.

ASCO's endorsement of the 2018 guideline mentions the use of direct smears as the cytologic specimen of choice for lung cancer molecular testing.[3] One significant advantage of direct smears over other specimen preparations is the ability to perform rapid on-site evaluation for adequacy assessment, leading to adjustments in specimen collection and appropriate triage to ensure testing success. Dedicated smears and/or touch preparations (in cases with core biopsies) can be sequestered for molecular testing at the time of the procedure, thus reducing turnaround time and additional processing of slides.[11] However, any change in the current workflow in the majority of laboratories that are not equipped for the use of non-FFPE cytology preparations for molecular testing will require communication and coordination with significant infrastructure support. In addition, molecular laboratories must be willing to perform the necessary validation for non-FFPE material to allow for testing of cytology preparations.[4] Notwithstanding, the importance of this change in the updated 2018 guideline cannot be overstated because it will likely allow for testing in patients who otherwise may not have adequate tumor on FFPE material.

While the 2013 guideline acknowledged the potential of next-generation sequencing as a promising option for simultaneous detection of multiple targetable molecular alterations, the data at that time were insufficient to recommend NGS implementation in a clinical setting. Since then, however, numerous studies have outlined the feasibility and utility of high-sensitivity, high-throughput, and multi-gene assays for interrogating NSCLC for comprehensive genomic profiling. The ability to detect multiple molecular alterations from small amounts of nucleic acids in a single NGS assay led to the revised recommendation in 2018 that says "multiplexed genetic sequencing panels are preferred over multiple single-gene tests." Several studies using cytologic material including cell blocks as well as non-FFPE substrates have shown them to be equally effective in genomic profiling of NSCLC by NGS analysis.9,12-18 In fact, some studies have indicated better quality metrics when comparing NGS analysis in non-FFPE cytologic substrates versus FFPE material.[7,19]

The 2018 guideline includes *ROS1* testing in addition to *ALK. ALK* and *ROS1* testing can be performed by fluorescence in situ hybridization on both FFPE and non-FFPE cytology specimens such as direct smears and liquid-based cytology preparations.[20] The 2018 guideline also recommends ALK immunohistochemistry as a valid alternative to *ALK* FISH. The Food and Drug Administration has approved the Ventana ALK (D5F3) CDx Assay IHC kit (Ventana Medical Systems, Tucson, Ariz.) only for "routinely processed, paraffin-embedded specimens that have been fixed in neutral-buffered formalin." However, several studies have demonstrated the feasibility of ALK immunocytochemistry on direct smears and liquid-based cytology preparations (off-label use).[21,22] The updated guideline recommends using ROS1 IHC using D4D6 (Cell Signaling Technology, Danvers, Mass.) only as a screening test that requires confirmation by a molecular or cytogenetic method. A limited number of studies using *ROS1* FISH in cytologic specimens (FFPE as well as non-FFPE preparations) are available.[23,24] Studies showing the use of ROS1 immunocytochemistry in cytology preparations are currently limited in the literature.[25,26]

Table 1. Updated recommendations from the CAP/IASLC/AMP for molecular testing of cytologic specimens

2018 guideline statement	Strength of recommendation	New/changed statement
Any cytology sample with adequate cellularity and preservation may be tested.	Recommendation	Change in statement (2013 expert consensus opinion: Cytologic samples are also suitable for *EGFR* and *ALK* testing, with cell blocks being preferred over smear preparations.)
Multiplexed genetic sequencing panels are preferred over multiple single-gene tests to identify other treatment options beyond *EGFR, ALK,* and *ROS1.*	Expert consensus opinion	New statement
ROS1 testing must be performed on all lung adenocarcinoma patients, irrespective of clinical characteristics.	Strong recommendation	New statement
ROS1 IHC may be used as a screening test in lung adenocarcinoma patients; however, positive ROS1 IHC results should be confirmed by a molecular or cytogenetic method.	Expert consensus opinion	New statement
IHC is an equivalent alternative to FISH for ALK testing.	Recommendation	New statement

Table 2. Emerging biomarkers for molecular testing in lung cancer

■ *MEK1/MAP2K1*	■ *AKT1*
■ *FGFR 1-4*	■ *NRAS*
■ *NTRK 1-3*	■ *MTOR*
■ *NRG1*	■ *TSC 1-2*
■ *RIT1*	■ *KIT*
■ *NF1*	■ *PDGFRA*
■ *PIK3CA*	■ *DDR2*

While the updated guideline was unable to provide specific recommendations for standalone testing of molecular alterations such as *BRAF, MET, RET, ERBB2 (HER2)*, and *KRAS*, it recommends including those alterations in an expanded panel if adequate tissue is available.[2] The guideline also includes a list of emerging biomarkers for potential clinical use that may be of value in treating NSCLC patients (**Table 2)**; however, the current evidence is insufficient to provide guideline recommendations.

The guideline also does not provide recommendations for PD-L1 testing. Immunotherapy, together with the companion diagnostic biomarker IHC assay for PD-L1, has shown promising results in the treatment of advanced-stage NSCLC patients.[27,28] While cytology specimens were not included in the initial clinical validation studies for PD-L1, several groups have evaluated the feasibility of PD-L1 on cytology specimens and have demonstrated results that are comparable to those of paired histologic samples.[29,30] Therefore, it is conceivable that cytology specimens can be used for PD-L1 immunohistochemistry/immunocytochemistry to determine eligibility of NSCLC patients for immunotherapy. Notably, testing for *BRAF* and PD-L1 is currently included in the National Comprehensive Cancer Network guidelines for management of NSCLC patients.[31]

Molecular cytopathology plays a critical role in biomarker testing and management of lung cancer patients. The 2018 guideline for lung molecular testing is a leap in the right direction by encouraging better use of cytology materials for molecular testing. Molecular pathology laboratories and cytopathology groups need to come together

to implement a molecular cytopathology approach to lung cancer biomarker testing for better patient care.

1. 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(4):415–453.
2. Lindeman NI, Cagle PT, Aisner DL, et al. Updated molecular testing guideline for the selection of lung cancer patients for treatment with targeted tyrosine kinase inhibitors: guideline from the College of American Pathologists, the International Association for the Study of Lung Cancer, and the Association for Molecular Pathology. *Arch Pathol Lab Med.* 2018;142(3):321–346.
3. 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(9):911–919.
4. Roy-Chowdhuri S, Aisner DL, Allen TC, et al. Biomarker testing in lung carcinoma cytology specimens: a perspective from members of the Pulmonary Pathology Society. *Arch Pathol Lab Med.* 2016;140(11):1267–1272.
5. Bellevicine C, Vita GD, Malapelle U, Troncone G. Applications and limitations of oncogene mutation testing in clinical cytopathology. *Semin Diagn Pathol.* 2013;30(4):284–297.
6. Roh MH. The utilization of cytologic fine-needle aspirates of lung cancer for molecular diagnostic testing. *J Pathol Transl Med.* 2015; 49(4):300–309.
7. Roy-Chowdhuri S, Chen H, Singh RR, et al. Concurrent fine needle aspirations and core needle biopsies: a comparative study of substrates for next-generation sequencing in solid organ malignancies. *Mod Pathol.* 2017;30(4):499–508.
8. Bellevicine C, Malapelle U, de Luca C, Iaccarino A, Troncone G. EGFR analysis: current evidence and future directions. *Diagn Cytopathol.* 2014;42(11):984–992.

9. Karnes HE, Duncavage EJ, Bernadt CT. Targeted next-generation sequencing using fine-needle aspirates from adenocarcinomas of the lung. *Cancer Cytopathol.* 2014;122(2):104–113.
10. Gailey MP, Stence AA, Jensen CS, Ma D. Multiplatform comparison of molecular oncology tests performed on cytology specimens and formalin-fixed, paraffin-embedded tissue. *Cancer Cytopathol.* 2015;123(1):30–39.
11. Aisner DL. The revised College of American Pathologists/International Association for the Study of Lung Cancer/Association for Molecular Pathology guideline: a step forward for molecular cytopathology. *Arch Pathol Lab Med*. 2018;142(6):684–685.
12. Baum JE, Zhang P, Hoda RS, et al. Accuracy of next-generation sequencing for the identification of clinically relevant variants in cytology smears in lung adenocarcinoma. *Cancer Cytopathol*. 2017;125(6):398–406.
13. Buttitta F, Felicioni L, DelGrammastro M, et al. Effective assessment of egfr mutation status in bronchoalveolar lavage and pleural fluids by next-generation sequencing. *Clin Cancer Res*. 2013;19(3):691–698.
14. Doxtader EE, Cheng YW, Zhang Y. Molecular testing of non-small cell lung carcinoma diagnosed by endobronchial ultrasound-guided transbronchial fine-needle aspiration. *Arch Pathol Lab Med*. Epub ahead of print Jan. 26, 2018. doi:10.5858/arpa.2017-0184-RA.
15. Reynolds JP, Zhon Y, Jakubowski MA, et al. Next-generation sequencing of liquid-based cytology non-small cell lung cancer samples. *Cancer Cytopathol*. 2017;125(3):178–187.
16. Scarpa A, Sikora K, Fassan M, et al. Molecular typing of lung adenocarcinoma on cytological samples using a multigene next generation sequencing panel. *PLOS One*. 2013;8(11):e80478.
17. Treece AL, Montgomery ND, Patel NM, et al. FNA smears as a potential source of DNA for targeted next-generation sequencing of lung adenocarcinomas. *Cancer Cytopathol*. 2016;124(6):406–414.
18. Velizheva NP, Rechsteiner MP, Wong CE, et al. Cytology smears as excellent starting material for next-generation sequencing-based molecular testing of patients with adenocarcinoma of the lung. *Cancer Cytopathol*. 2017;125(1):30–40.
19. Hwang DH, Garcia EP, Ducar MD, Cibas ES, Sholl LM. Next-generation sequencing of cytologic preparations: an analysis of quality metrics. *Cancer Cytopathol*. 2017;125(10):786–794.

20. Pisapia P, Lozano MD, Vigliar E, et al. ALK and ROS1 testing on lung cancer cytologic samples: perspectives. *Cancer Cytopathol*. 2017; 125(11):817–830.
21. Savic S, Bode B, Diebold J, et al. Detection of ALK-positive non-small-cell lung cancers on cytological specimens: high accuracy of immunocytochemistry with the 5A4 clone. *J Thorac Oncol*. 2013;8(8):1004–1011.
22. Rosenblum F, Hutchinson LM, Garver J, Woda B, Cosar E, Kurian EM. Cytology specimens offer an effective alternative to formalin-fixed tissue as demonstrated by novel automated detection for ALK break-apart FISH testing and immunohistochemistry in lung adenocarcinoma. *Cancer Cytopathol.* 2014;122(11):810–821.
23. Bozzetti C, Nizzoli R, Tiseo M, et al. ALK and ROS1 rearrangements tested by fluorescence in situ hybridization in cytological smears from advanced non-small cell lung cancer patients. *Diagn Cytopathol.* 2015;43(11):941–946.
24. Fernandez-Bussy S, Labarca G, Pires Y, Caviedes I, Burotto M. Molecular testing of EGFR, EGFR resistance mutation, ALK and ROS1 achieved by EBUS-TBNA in Chile. *Arch Bronconeumol*. 2017;53(3):172–174.
25. Frankel D, Bourlard D, Garcia S, et al. Detection of *ALK* and *ROS1* rearrangements by immunocytochemistry on cytological samples [in French]. *Ann Pathol.* Epub ahead of print Jan. 30, 2019. doi:10.1016/j.annpat.2018.12.003.
26. Vlajnic T, Savic S, Baraseud A, et al. Detection of ROS1-positive non-small cell lung cancer on cytological specimens using immunocytochemistry. *Cancer Cytopathol.* 2018;126(6):421–429.
27. Dolled-Filhart M, Roach C, Toland G, et al. Development of a companion diagnostic for pembrolizumab in non-small cell lung cancer using immunohistochemistry for programmed death ligand-1. *Arch Pathol Lab Med*. 2016;140(11):1243–1249.
28. Garon EB, Rizvi NA, Hui R, et al. Pembrolizumab for the treatment of non-small-cell lung cancer. *N Engl J Med*. 2015;372(21):2018–2028.
29. 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(5):342–352.
30. Skov BG, Skov T. Paired comparison of PD-L1 expression on cytologic and histologic specimens from malignancies in the lung assessed with PD-L1

IHC 28-8pharmDx and PD-L1 IHC 22C3pharmDx. *Appl Immunohistochem Mol Morphol*. 2017;25(7):453–459.

31. Ettinger DS, Wood DE, Aisner DL, et al. NCCN Clinical Practice Guidelines in Oncology: Non-Small Cell Lung Cancer. Version 8.2018. https://www.nccn.org/professionals/physician_gls/pdf/nscl.pdf. Accessed December 2018.

Dr. Roy-Chowdhuri, a member of the CAP Cytopathology Committee, is associate professor, Department of Pathology and Laboratory Medicine, University of Texas MD Anderson Cancer Center, Houston.