Molecular Pathology of Lung Cancer Cytology Specimens

A Concise Review

Deepali Jain, MD, FIAC; Sinchita Roy-Chowdhuri, MD, PhD

• ***Context.***—**There has been a paradigm shift in the understanding of molecular pathogenesis of lung cancer. A number of oncogenic drivers have been identified in non–small cell lung carcinoma, such as the epidermal growth factor receptor (*EGFR*) mutation and anaplastic lymphoma kinase (*ALK*) gene rearrangement. Because of the clinical presentation at an advanced stage of disease in non-small cell lung carcinoma patients, the use of minimally invasive techniques is preferred to obtain a tumor sample for diagnosis. These techniques include image-guided biopsies and fine-needle aspirations, and frequently the cytology specimen may be the only tissue sample available for the diagnosis and molecular testing for these patients.**

Objective.—**To review the current literature and evaluate the role of cytology specimens in lung cancer mutation testing. We reviewed the types of specimens received in the laboratory, specimen processing, the effect of preanalytic factors on downstream molecular studies, and the commonly used molecular techniques for biomarker testing in lung cancer.**

Data Sources.—**PubMed and Google search engines were used to review the published literature on the topic.**

Conclusions.—**Mutation testing is feasible on a variety of cytologic specimen types and preparations. However, a thorough understanding of the cytology workflow for the processing of samples and appropriate background knowledge of the molecular tests are necessary for triaging, and optimum use of these specimens is necessary to guide patient management.**

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Molecular profiling of lung cancer has identified multiple driver mutations occurring in several oncogenes that have led to an increasing number of US Food and Drug Administration (FDA)–approved targeted therapies for non–small cell lung carcinoma (NSCLC) patients.[1,2] With a large fraction of NSCLC patients receiving a diagnosis of advanced-stage disease on cytology specimens alone, these cytologic samples play an important role in providing diagnostic, prognostic, and predictive information for appropriate management of NSCLC patients.[3,4] The clinical utility of cytology specimens for molecular testing in lung cancer has been validated and widely published in the literature.[5,6] In this review, we attempt to summarize the molecular cytopathology of NSCLC, including the various types of lung cytology specimens received in a cytopathology laboratory, the specimen preparations and various preanalytic factors affecting nucleic acid yield and downstream molecular testing, the variety of molecular techniques applied to cytology samples, and the implications of these molecular testing results in the management of NSCLC patients.

LUNG CYTOLOGY SPECIMEN TYPES

Lung cytology specimens are either fine-needle aspirations (FNAs) obtained by minimally invasive procedures, or exfoliative—including sputum, bronchial brushings and bronchial washings, bronchoalveolar lavages, and body cavity fluids/effusions.[7]

1. Fine-needle aspirations include transbronchial or endobronchial and/or tracheal FNAs, which are obtained through a flexible bronchoscope under ultrasound guidance. A needle is inserted through the bronchoscope into the lesion, and material is aspirated under image guidance. Transthoracic (percutaneous) FNAs, on the other hand, are performed under ultrasound guidance, if the lesion is subpleural, or under computed tomography guidance, if the lesion is deep parenchymal.[8] The FNA procedures may or may not have rapid on-site evaluation for the evaluation of adequacy, and the aspirated material is processed as direct smears with or without cell block (CB) preparation, or as liquid-based cytology (LBC) preparations.[9]
2. Bronchial brushings are obtained from peripheral lesions using a brush under direct visualization through a bronchoscope. Bronchial washings are collected using saline, typically following the brushing.[7]

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From the Department of Pathology, All India Institute of Medical Sciences, New Delhi (Dr Jain); and the Division of Pathology and Laboratory Medicine, Department of Pathology, The University of Texas MD Anderson Cancer Center, Houston (Dr Roy-Chowdhuri).

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Corresponding author: Sinchita Roy-Chowdhuri, MD, PhD, Department of Pathology, Division of Pathology and Laboratory Medicine, The University of Texas MD Anderson Cancer Center, 1515 Holcombe Blvd, Unit 85, Houston, TX 77030 (email: sroy2@mdanderson.org).

3. Bronchoalveolar lavage is performed by instilling 100 to 300 mL of saline into the alveolar spaces and aspirating at least 30% of the total volume in small aliquots, with the goal of sampling bronchioles as well as the alveolar spaces.[10]
4. Sputum can be examined either fresh or prefixed with alcohol and carbowax. Smears are made from centrifuged cell buttons.[7]
5. Body cavity fluids and effusions can be obtained via thoracocentesis as a minimally invasive procedure and have the advantage of serial collection, if needed, for diagnosis and/or prognostic and predictive biomarker studies.[11]

LUNG CYTOLOGY SPECIMEN COLLECTION

The choice of specimen collection technique in NSCLC diagnosis frequently depends on the location of the lesion (Table 1).[7] Proximal and centrally located tumors are best sampled by sputum examination and FNA procedures. Sputum cytology is the least invasive procedure, with limited sensitivity but high specificity in centrally located lesions. Endobronchial lesions that can be directly visualized through a bronchoscope are commonly sampled using bronchial washings and brushings, and transbronchial needle aspiration. Conventional transbronchial needle aspiration is an established method for cytologic evaluation of thoracic mass lesions, but it is limited by the fact that it is a blind procedure. With the advent of ultrasound-guided techniques, endobronchial ultrasound–guided transbronchial needle aspiration has been introduced to sample mediastinal lymph nodes and lesions under real-time visualization.[12,13] Transbronchial needle aspiration is usually preferred for central peribronchial lesions, whereas the diagnostic sensitivity of transthoracic needle aspiration is higher for the larger peripheral lung lesions.[14]

Table 1. Cytology Specimen Types and Collection Techniques Based on Location of the Lesion

Specimen Type and Collection Technique	Site of Lesion
Sputum, BW, BB	Proximal mucosal lesion
TBNA or transtracheal FNA with (EBUS) or without guidance	Proximal submucosal lesion Peribronchial, tracheal, carinal lesion Mediastinal lesion
TTNA, BB, BAL	Peripherally located parenchymal lesion

Abbreviations: BAL, bronchoalveolar lavage; BB, bronchial brushing; BW, bronchial washing; EBUS, endobronchial ultrasound; FNA, fine-needle aspiration; TBNA, transbronchial needle aspiration; TTNA, transthoracic needle aspiration.

LUNG CYTOLOGY SPECIMEN PROCESSING

Cytology specimens are processed using a variety of different methods depending on the individual laboratory preference, infrastructure, feasibility, and resources. The most common cytology specimen preparations used for molecular testing include direct smears; cytospin preparations; formalin-fixed, paraffin-embedded (FFPE) CB preparations; and LBC.

The direct smears prepared from bronchial brushing are immediately fixed for optimal cellular preservation. Alternatively, the brush can be rinsed in a cell-preserving solution (eg, saline, RPMI 1640 medium, CytoLyt [Hologic Inc, Marlborough, Massachusetts], etc) and sent to the laboratory for further processing. The FNA direct smears are prepared as both air-dried and alcohol-fixed methods and are most commonly stained using Diff-Quik and/or Papanicolaou stains. The LBC is processed as a monolayer slide as per manufacturer guidelines, and the rinse is used for ancillary studies or processed as a CB. Cell block preparations are the most commonly used preparations for molecular testing of cytology specimens.[15] This is primarily because most molecular laboratories are validated to perform tests on FFPE histologic specimens, which can be extended easily to cytologic FFPE samples. Although the current literature shows that CB is the most commonly employed cytologic specimen preparation used for molecular assays, multiple studies have demonstrated that other non-FFPE cytology specimen preparations also give optimum results, with the failure rate of CB being comparable to that of direct smears.[16] Despite mounting data showing the utility of non-FFPE cytology specimen preparations in a variety of molecular assays, including mutation analysis and fluorescence in situ hybridization studies, the 2013 lung molecular testing guidelines from the College of American Pathologists/International Association for the Study of Lung Cancer/Association for Molecular Pathology (CAP/IASLC/AMP) recommend the use of CB rather than other cytology specimen preparations.[17] However, a revised guideline scheduled to be published in 2018 will likely address this issue of specimen preparation and may modify the recommendation of using CB as opposed to other cytology preparations.

The advantages and limitations of the different specimen preparation methods are shown in Table 2.

LUNG CYTOLOGY DNA EXTRACTION

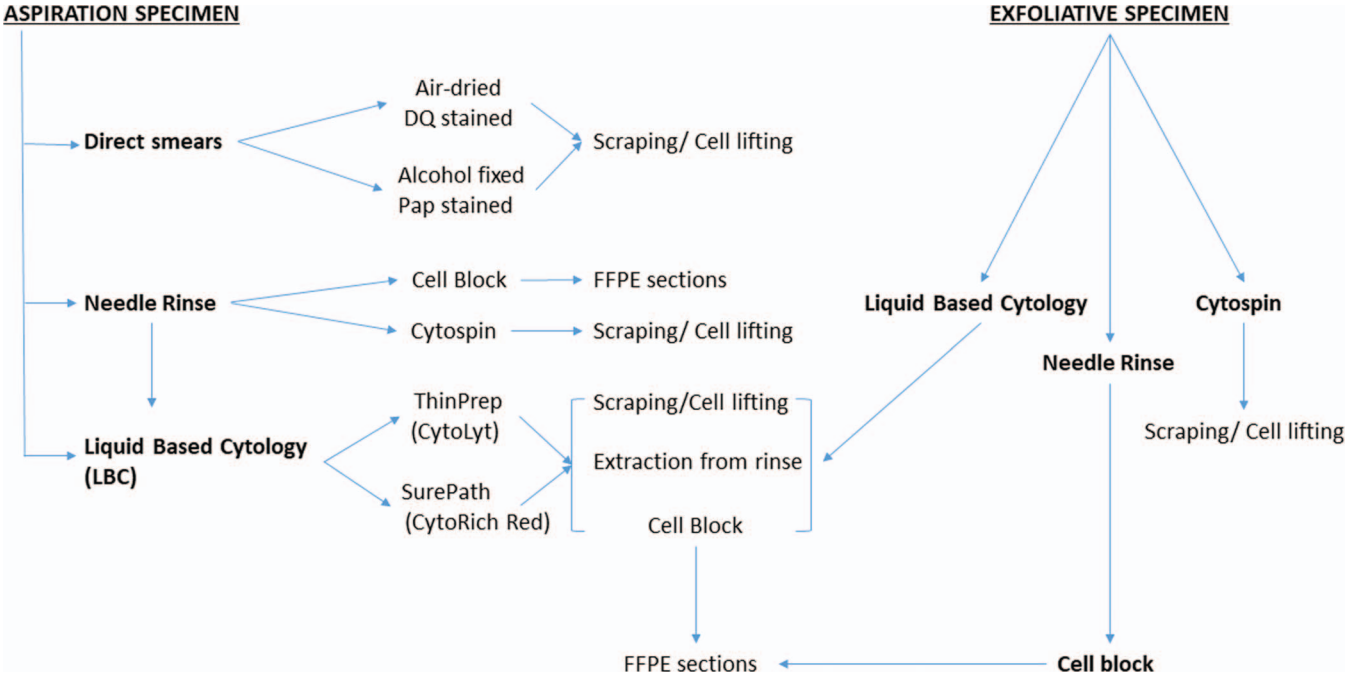
For molecular testing from smears, the tumor cells can be scraped (using a scalpel blade or needle) or cell-lifted into a buffer for DNA extraction.[18] The FNA needle rinse is typically collected in a variety of media, including saline, RPMI, formalin, and alcohol-based fixatives, such as CytoLyt or CytoRich Red (Fisher Scientific, Loughborough, United Kingdom).[19] The needle rinse is processed as a CB preparation after formalin fixation and paraffin embedding. Unstained sections from the CB can also be used for molecular testing by scraping off the designated areas based on tumor mapping of a corresponding hematoxylin-eosin–stained section. Alternatively, the needle rinse collected in CytoLyt solution or CytoRich Red fixatives can be used to prepare a cell monolayer LBC slide, and either the LBC slide can be scraped or the residual LBC sample can be effectively used for molecular testing. For specimens with cytospin preparations, similar to direct smears, the slides can be used for molecular testing by scraping or cell lifting, or cytospin pellets can be used by directly extracting from the pellet (Figure).[15,20]

As with any molecular assay, preanalytic variables play a critical role in the success of molecular testing in lung cytology specimens. The vast spectrum of interlaboratory variables in specimen collection, handling, fixation, and processing, underscores the need for standardization across laboratories to optimize preanalytic factors and validate results.[21–28] Some of these preanalytic factors are discussed in Table 3.

Table 2. Advantages and Limitations of Various Cytologic Preparations for Molecular Testing

Specimen Type and Preparation	Advantages	Disadvantages	Comments
Aspiration			
Stained direct-smear scraping	Immediate visualization and morphologic assessment for tumor adequacy Acquisition of whole cells Higher-quality nucleic acids due to no formalin fixation effects	Medicolegal: sacrificing of slides from the patient archival material Obtained tissue may be low volume to proceed with downstream processes	Slides can be digitally scanned or photographed to meet medicolegal requirements for preserving archival material
Rinse in LBC or LBC slide scraping	Optimal preservation of cells Recovery of good quality of DNA	Different preservatives (CytoLyt versus CytoRich Red) can have quantitative/qualitative differences in DNA yield[24] DNA degradation after long-term storage[19]	Can be optimized and validated in individual laboratories
Needle-core clot biopsy or cell block (FFPE)	Serial sections for downstream testing Does not require additional validation for molecular assays	Formalin artifacts in nucleic acids may affect quality of extracted DNA 4- to 5-micron sections are not representative of the entire nucleus Inability to perform on-site adequacy assessment, and therefore cannot predict cellularity Variability in CB preparations across various laboratories[15,20]	2013 CAP/IASLC/AMP clinical practice guidelines recommend use of CB rather than smears for all molecular testing, but this may be addressed in the revised guidelines
Exfoliative			
Stained cytospin/smear scraping	High-quality nucleic acids (non–formalin fixed) Direct assessment of adequacy and cellularity	Low tumor fraction due to contamination with lymphocytes, mesothelial cells	Tumor enrichment or microdissection is required to avoid nontumor cells
Cytospin pellet	High-quality nucleic acids (non–formalin fixed) Direct extraction, so no preanalytic factors associated with scraping/cell lifting	Inability to assess presence of tumor and tumor fraction	An aliquot of the sample can be used for cytomorphology and to quantify tumor fraction
LBC and CB	As described above	As described above	As described above

Abbreviations: CAP/IASLC/AMP, College of American Pathologists/International Association for the Study of Lung Cancer/Association for Molecular Pathology; CB, cell block; FFPE, formalin-fixed, paraffin-embedded; LBC, liquid-based cytology.



Schematic workflow of various cytology specimen preparations and processing methods. Abbreviations: DQ, Diff-Quik; FFPE, formalin-fixed, paraffin-embedded; LBC, liquid-based cytology.

Table 3. Influence of Preanalytic Factors on DNA Yield and Quality	
	Influence on DNA Yield and Quality
Preanalytic factors	
Fixatives: alcohol versus formalin	No quantitative difference between these 2 fixation methods on DNA yield, but formalin may cause degradation of nucleic acids (qualitative difference). However, most molecular assays are optimized and validated with formalin.[17,21,22]
Collection media: saline/RPMI/ CytoLyt/formalin	Initial placement in saline or RPMI with subsequent transfer in formalin or CytoLyt gives satisfactory results. Fixation for 6 to 12 hours is recommended. RPMI can be used for NGS.[23] Advantages of preservatives used in LBC are minimal contamination by blood, inflammation, and cellular debris; and cellular preservation for an extended period of time (3 weeks in CytoRich Red and 3 months in PreservCyt[a]). CytoLyt is better than CytoRich Red for DNA yield, possibly because of a small amount of formaldehyde in the latter. However, DNA preservation with CytoRich Red is still satisfactory.[24,25]
Staining: Diff-Quik versus Pap	Use of Pap staining along with water-soluble mounting medium yields a relatively better quantity of DNA, even with lesser cellularity. Archival (10–14 years old) Pap-stained FNA smears can also yield amplifiable DNA. However, Diff-Quik could be superior in terms of DNA preservation and integrity.[26] Diff-Quik smears can be visualized without coverslip and can be immediately triaged for molecular testing.[27] Recent studies point out that both Diff-Quik–stained and Pap-stained smears are equally feasible for molecular testing.[24] The DNA quality in terms of fragment length is marginally better with alcohol-based fixatives than with air-dried smears.[24]
Mounting media: non–xylene-based versus xylene-based	Non–xylene-based (water-soluble) mounting medium (eg, EcoMount[a]) yields significantly higher DNA quantity. EcoMount is a low-hazard, organic, polymer-based mounting medium that can be used as a substitute for xylene-based media.[24]
Tissue-retrieval method: scraping versus cell lifting	Scraping using a scalpel/razor blade/dissecting needle from the slides yields more DNA than cell lifting does.[18]
Type of slides	In comparison with nonfrosted, adhesive-coated, and positively charged slides, fully frosted slides yield less DNA because of the difficult dislodgement of cells from the rough surface of these slides.[3,18]
Sample preparation methods	
FNA, rinse versus scraping	FNA scraping provides high-quality nucleic acids. DNA in rinse may be affected by the use of different preservatives that yield different quantity/quality of DNA, but there is improved turnaround time because extraction is performed directly from the rinse.
LBC, rinse versus scraping	DNA extraction in LBC preparations may be influenced by the use of specific fixative and type of technique. Some studies reported scraping yields more DNA.[28]
CB, cytospin (BB/BW,BL/PE), sputum	Satisfactory DNA yield can be obtained with these cytology samples, and DNA quantity varies with the volume of the samples and specimen cellularity.

Abbreviations: BB, bronchial brush; BL, bronchial lavage; BW, bronchial wash; CB, cell block; FNA, fine-needle aspiration; LBC, liquid-based cytology; NGS, next-generation sequencing; Pap, Papanicolaou staining; PE, pleural effusion.

[a] EcoMount is from Biocare Medical LLC (Concord, California); PreservCyt is from Hologic Inc.

BIOMARKER TESTING IN LUNG CANCER

The 2013 CAP/IASLC/AMP guidelines for biomarker testing in lung cancer recommended testing for epidermal growth factor receptor (*EGFR*) and anaplastic lymphoma kinase (*ALK*). However, with rapid progress being made in our understanding of the genomic landscape in NSCLC and with clinical trials establishing the role of biomarkers beyond *EGFR* and *ALK* in these patients, the revised guidelines are likely to include recommendations for additional biomarkers. The current National Comprehensive Cancer Network (NCCN) guidelines for NSCLC patients recommend testing for *ROS-1*, *BRAF*, and PD-L1 (category 2A recommendation). Guidelines strongly advise for a broader molecular profiling to identify rare mutations for which drugs may be available or for which the patient can be registered under a clinical trial. The NCCN guidelines enlist driver events or genetic alterations in lung cancer against which emerging targeted agents are available. These include high-level *MET* amplification or *MET* exon 14 skipping mutation, *RET* rearrangements, and *ERBB2* (formerly *HER2/neu*) mutations (Table 4).[29]

All prognostic/predictive markers can be tested using cytology specimens with appropriate laboratory validation. Although PD-L1 testing has not been validated on cytology samples in a clinical trial, PD-L1 immunostaining and quantitation are feasible, if validated appropriately.[30]

The molecular assay employed for the detection of genomic alterations in lung cancer depends largely on the type of specimen, the overall cellularity versus the tumor cellularity, and the genomic alteration to be detected. Although the current CAP/IASLC/AMP guidelines recommend testing from samples with at least 50% tumor cellularity,[17] the revised guidelines may recommend using a testing methodology that is sensitive enough to detect mutations in specimens with as little as 20% tumor cells.

Polymerase chain reaction (PCR)–based methodologies are most commonly used for the detection of mutational changes, whereas chromosomal alterations are detected by conventional cytogenetics, fluorescence in situ hybridization, and/or next-generation sequencing–based assays. Assays for mutation analysis either employ a mutation-specific amplification method (ie, where the mutations are known), or methods that can detect mutations without prior knowledge of the exact mutation or the specific location in the sequence and can detect unknown novel mutations (Table 5).

Sanger sequencing, the most widely available PCR-based sequencing assay, uses fluorescently labeled nucleotides that

Table 4. List of Predictive Gene Biomarkers in Non–Small Cell Lung Carcinoma		
Gene	**Testing Method**	**Comments**
EGFR	PCR-based methods followed by sequencing Commercially available kits Digital PCR	PCR and sequencing are considered the standard reference method for the detection of *EGFR* mutations, along with *KRAS*, *ERBB2*, and *BRAF*, but may be limited by low analytic sensitivity when there is low tumor fraction in the sample
ALK	FISH IHC is an equivalent alternative to FISH	IHC validated for FFPE samples, so it may not be optimized to use on direct or spin smears
ROS1	FISH IHC can be used as a screening tool. IHC-positive tumors should be tested by molecular (RT-PCR) or cytogenetic (FISH) tests	IHC validated for FFPE samples, so it may not be optimized to use on direct or spin smears
HER2/neu, *KRAS*, *BRAF*, *RET*, *MET*	Used typically as part of a multiplexed sequencing panel (eg, next-generation sequencing)	
PD-L1	IHC	Not yet validated for cytology specimens, but few studies have shown comparability with histologic specimens with appropriate validation[30]

Abbreviations: ALK, anaplastic lymphoma kinase; EGFR, epidermal growth factor receptor; FFPE, formalin-fixed, paraffin-embedded; FISH, fluorescence in situ hybridization; IHC, immunohistochemistry; PD-L1, programmed death ligand-1; RT-PCR, reverse transcriptase–polymerase chain reaction.

are incorporated into the amplified DNA, and the sequence is visualized as an electropherogram. The advantage of Sanger sequencing is the ability to detect any mutation within the PCR amplification product without prior knowledge of the location or the type of mutation; however, Sanger sequencing is limited by a low analytic sensitivity of 15% to 20%, which limits its ability to analyze samples with low tumor cellularity.[31,32] A number of other sensitive techniques, such as coamplification at a lower denaturation temperature PCR (COLD-PCR), peptide nucleic acid–locked PCR (PNA-PCR), amplification refractory mutation system (ARMS), Competitive Amplification of Differentially Melting Amplicons (CADMA), and droplet digital PCR, have been employed by various laboratories in an attempt to increase the sensitivity of detection of mutations from low–tumor cellularity samples.

Variations of PCR-based sequencing assays have been successfully used by various laboratories for interrogating genes of interest, including real-time PCR, restriction fragment length analysis, and pyrosequencing. Although these methodologies offer much higher sensitivity than Sanger sequencing and are amenable to testing low–tumor content samples, they are restricted to analyzing only the mutations of interest and lack the ability to detect additional novel mutations. Thus, the clinical utility of screening multiple hot spot mutations of interest is limited in these targeted assays. On the other hand, high-throughput multiplexed assays, such as Sequenom MassARRAY (Agena Bioscience, San Diego, California), and next-generation sequencing, have gained popularity because of the simultaneous screening of multiple genes. Although Sequenom remains largely a targeted assay designed to detect known hot spot mutations, the multigene approach of next-generation sequencing coupled with a relatively high analytic sensitivity and minimal DNA requirements has made it a popular choice for mutation analysis in lung cancer samples.[3,4,6,16,18,33–41]

Table 5. Commonly Used Mutation Detection Assays in Lung Cancer Cytology Samples			
	Type of Assay	**Sensitivity, %**	**Advantage/Disadvantage**
Sanger sequencing	General	15–20	Ability to identify all possible mutations in the analyzed fragment Low-sensitivity assay requiring high tumor fraction in the sample to be analyzed
Real-time PCR	Targeted	0.5–5	High sensitivity Rapid and cost-effective MAF quantification
RFLP/CE	Targeted	1–5	High sensitivity Analyzes only the mutations that it was designed to detect
HRMA	General	5–10	Can detect the presence of mutations in the analyzed region but may not identify the specific mutations
Pyrosequencing	Targeted	5–10	High sensitivity Restricted to analyzing relatively short read lengths of DNA sequence
Sequenom	General/targeted	5–10	Multiplexed but targeted and will identify only mutations that the assay is designed to detect
NGS	General	5–10	High sensitivity Multiplexed with simultaneous screening of multiple genes and ability to detect all possible mutations Single platform to evaluate SNPs, insertion/deletions, CNVs, gene fusions, methylation, etc Higher cost, complex testing, and high analytic and bioinformatics needs

Abbreviations: CE, capillary electrophoresis; CNV, copy number variation; HRMA, high-resolution melting curve analysis; MAF, mutant allele frequency; NGS, next-generation sequencing; PCR, polymerase chain reaction; RFLP, restriction fragment length polymorphism; SNP, single-nucleotide polymorphism.

Table 6. Advantages and Disadvantages of Circulating Tumor Cells (CTCs) and Cell-Free DNA (cfDNA)	
CTCs	**cfDNA**
Complete DNA	Fragmented DNA
Low abundance, 1–10 CTCs per 10 mL of blood	Desirable because it appears in serum before the appearance of CTCs
Concentration strategies required for CTC extraction	Extraction easier than CTC extraction
Low sensitivity for mutation detection	High sensitivity for mutation detection
Large sample volume requirement	Lower volume gives optimum results
EPCAM cannot detect EMT-induced aggressive cancer cells	DNA from normal lysed cells may interfere with detection
Difficult to differentiate between benign CTCs and malignant CTCs	Unable to do morphologic analysis

Abbreviations: EMT, epithelial mesenchymal transformation; EPCAM, epithelial cell adhesion molecule.

Liquid biopsy assays refer to molecular assays involving circulating tumor cells (CTCs), circulating tumor DNA or circulating cell-free DNA (cfDNA), exosomes (50- to 150-nm microvesicular structures sequestering cfDNA), or cell-free and platelet-bound RNAs. It is considered either as complementary to or an alternative to high-risk invasive tumor sampling through needle aspiration or biopsy.[42,43] These CTCs or cell-free nucleic acids can be detected not only in blood serum or plasma but other nonhematologic body secretions or fluids, such as urine, saliva, and sputum, and respiratory tract secretions, including bronchial tree aspirations and effusions.[44–47] Some of the main differences between molecular assays involving CTCs and cfDNA are summarized in Table 6.[48] There are several methods and platforms available for detection of CTCs or their components in body fluids (Table 7).[49–54] The FDA has approved the CellSearch System, a CTC-selection platform, for metastatic breast, prostate, and colon cancer patients.[55–57] However, this method, based on recognition of CTCs using epithelial markers (epithelial cell adhesion molecule and cytokeratin), is frequently unable to detect lung cancer CTCs because of the epithelial mesenchymal transformation that occurs frequently in patients with advanced NSCLC. Therefore, currently, there is no recommendation about using CTCs in lung cancer diagnosis or for mutation detection. Liquid biopsy to detect driver mutations in lung cancer in clinical practice is mainly based on droplet digital PCR and/or next-generation sequencing technologies, with good concordance with tissue mutations.[58] Recently the FDA has approved the cobas EGFR Mutation Test v2 (Roche Molecular Diagnostics, Pleasanton, California), a liquid biopsy assay for testing plasma in lung cancer patients to detect *EGFR* mutations for diagnosis (in the absence of a tissue biopsy sample) and the monitoring of tumor burden.[59] This approach uses oncogenic mutations in plasma cfDNA as a surrogate for mutation profiling of original tumor.

There is currently insufficient evidence to support the use of liquid biopsy for establishing a primary diagnosis of lung adenocarcinoma. However, NCCN guidelines recommend the use of cfDNA for *EGFR* testing if biopsy has insufficient tissue. In addition, plasma cfDNA can be used to identify *EGFR* T790M mutations in lung adenocarcinoma patients with disease progression or *EGFR* TKI resistance; however, tissue biopsy is advised if the plasma cfDNA result is negative.[29]

Table 7. Molecular Platforms of Liquid Biopsy	
Methods	**Analytic Sensitivity**
Beads, Emulsions, Amplification, and Magnetics (BEAMing)	0.01%[49,50]
PCR-based techniques	
Real time	0.025%[51]
Droplet digital PCR	0.0045%[52]
NGS	0.2%[53]
Emerging method: EFIRM[54]	

Abbreviations: EFIRM, electric field–induced release and measurement; NGS, next-generation sequencing; PCR, polymerase chain reaction.

CONCLUSIONS

In summary, molecular testing is feasible on a variety of cytologic specimen types and preparations. This becomes of the utmost importance in a large fraction of NSCLC patients where the cytology specimen may be the only tissue sample available for diagnosis and ancillary studies. Therefore, a thorough understanding of the potential and the limitations of these substrates is necessary to appropriately triage and use them for molecular studies that can guide patient management.

Editor's Note.—After acceptance of this article, the updated CAP/IASLC/AMP guideline was published in the March 2018 issue of the *Archives* (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; doi: 10.5858/arpa.2017-0388-CP).

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