A technical advance on detection of status of programed death ligand 1 and identification of multiple mutations using a single bronchoscopy specimen and two research articles on the use of next-generation sequencing in acute myeloid leukemia and breast cancer diagnostics were selected for the March 2019 JMD CME Program in Molecular Diagnostics. The authors of the referenced articles, the planning committee members, and staff have no relevant financial relationships with commercial interests to disclose.


Upon completion of this month’s journal-based CME activity, you will be able to:

- Define radial probe endobronchial ultrasound (EBUS).
- Discuss the uses of EBUS in the diagnosis and staging of lung cancer.
- Discuss the use of biomarkers for personalized immunotherapy.
- Discuss the roles of matrix metalloproteinase (MMP)-9 and its endogenous inhibitor (tissue inhibitor of metalloproteinase-3; TIMP3) in non–small-cell lung cancer (NSCLC) progression.
- Discuss the importance of MMP9:TIMP3 transcript ratio in NSCLC diagnostics.
- Discuss the clinical utility of a next-generation sequencing (NGS) panel for acute myeloid leukemia diagnostics.
- Discuss the clinical use of NGS in clinical management of breast cancer.

1. **Radial probe endobronchial ultrasound (EBUS)** is a minimally invasive procedure that improves localization of peripheral pulmonary lesions. Based on the referenced technical advance, select the ONE best TRUE statement: [J Mol Diagn 2019, 21:186-197.]
   a. Like cancer detection by histology, cytology samples of the suspect lesion site collected by radial EBUS bronchoscopy provide good diagnostic sensitivity – estimated at 45%.
   b. Combining rapid on-site examination (ROSE) of cytology samples with EBUS increases procedure times.
   c. Application of EBUS-guided procedures is limited in diagnosis of lung cancer due to the requirement of large sample size.
   d. EBUS-guided fine needle aspirates provide sufficient material for both immunohistochemical studies and mutation sequencing of EGFR and KRAS.

2. Demand is increasing on molecular and sequencing-based technologies to adapt to limited quantities of fixed tumor tissues for diagnostic purposes. Fixed tumor samples are becoming the most commonly used samples for diagnostic and molecular pathology applications. Based on the referenced technical advance, select the ONE best TRUE statement: [J Mol Diagn 2019, 21:186-197.]
   a. Fixed tumor tissue samples are optimal in all settings and for all downstream analyses.
   b. Unfixed frozen tumor tissue produces a high yield of DNA that is of high integrity, and useful for many molecular applications.
c. Though fixatives may fragment DNA, they do not affect mutation status in the cancer genome.

d. Most non–small-cell lung cancer (NSCLC) patients harbor oncogenic mutations (such as those affecting EGFR), impeding the use of checkpoint inhibitors for treatment.

   a. Biomarkers are being developed to identify patients who are most likely to respond to immunotherapy.
   b. Pembrolizumab is a humanized polyclonal antibody against programmed death 1 (PD-1).
   c. Pembrolizumab significantly increases progression-free and overall survival compared with platinum-based chemotherapy in patients with at least 25% programmed death ligand 1 (PD-L1) cancer cell expression.
   d. The combination of nivolumab (anti–PD-1) plus ipilimumab (anti–cytotoxic T lymphocyte–associated protein 4) is effective in increasing progression-free survival in patients with a low tumor mutational burden.

4. Matrix metalloproteinase (MMP)-9 and its endogenous inhibitor (tissue inhibitor of metalloproteinase-3; TIMP3) are implicated in cancer matrix remodeling, angiogenesis, cancer cell migration, and signaling. Based on the referenced technical advance, select the ONE best TRUE statement: [J Mol Diagn 2019, 21:186-197.]
   a. MMP-9 may contribute to the establishment of a metastatic niche.
   b. Immunohistochemical staining for MMP-9 in resected primary lung cancers has identified a weak association between MMP-9 expression and shortened patient survival.
   c. MMP9 immunoreactivity is seen in cancer cells, tumor-infiltrating neutrophils, as well as the adjacent tumor-free tissue.
   d. The loss of TIMP3 expression and/or activity can contribute to cancer progression by sequestering active MMPs in the tumor microenvironment.

5. The MMP9:TIMP3 transcript ratio is markedly increased in NSCLC tissue specimens. Based on the referenced technical advance, select the ONE best TRUE statement: [J Mol Diagn 2019, 21:186-197.]
   a. It is difficult to discriminate between benign lung lesions and NSCLC specimens using the MMP9:TIMP3 transcript ratio.
   b. The MMP9:TIMP3 ratio is less sensitive than cytology in detecting the presence of malignant cells in small bronchoscopy specimens.
   c. While considering the MMP9:TIMP3 transcript ratio, NSCLC tissue specimens need to be fixed for histologic assessment as well.
   d. The MMP9:TIMP3 ratio can confirm malignant cell content for subsequent downstream analysis.

6. Next-generation sequencing (NGS) has redefined the genetic landscape of acute myeloid leukemia (AML), providing new molecular markers for diagnostic and prognostic classifications. Based on the referenced article, select the ONE best TRUE statement: [J Mol Diagn 2019, 21:228-240.]
   a. The 2016 World Health Organization (WHO) classification has underlined the importance of molecular alterations by establishing RUNX1 and BCR-ABL as consolidated entities.
   b. The 2016 WHO classification has underlined the importance of molecular alterations by recognizing NPM1 and biallelic CEBPA as provisional categories.
   c. The 2017 European LeukemiaNet (ELN) recommendations have recognized mutations in ASXL1, TP53, and RUNX1 to influence patient outcome.
   d. The 2016 WHO diagnostic categories and 2017 ELN and Genomic classification often preclude some of the patients.

7. NGS is a valuable tool for AML diagnostics, with higher sensitivity and diagnostic yield than conventional molecular techniques. Based on the referenced article, select the ONE best TRUE statement: [J Mol Diagn 2019, 21:228-240.]
   a. Because of the increasing number of genes required for diagnostic and prognostic classification, conventional approaches may be insufficient to stratify AML patients into recently proposed disease classifications.
   b. Comprehensive routine molecular screening in hematooncology is easy but time intensive with gene panel–based NGS technologies.
   c. Establishment of universal standard quality criteria for NGS precludes the need for individual validation for its application to routine diagnostic laboratories.
   d. NGS technologies are limited in obtaining appropriate read depth.

8. NGS panels can detect mutations at a low variant allele frequency (VAF). Based on the referenced article, select the ONE best TRUE statement: [J Mol Diagn 2019, 21:228-240.]
   a. The reported panel detected all point mutations tested below 2% VAF.
   b. The cutoff threshold for variant reporting was set at 2% in the discussed study.
   c. For clinical reporting, a 5% VAF threshold is generally established.
   d. Actionable variants with VAF between 2% and 6% can be considered as true.
9. Genomic amplification at 9p24.1 holds significant promise as a prognostic biomarker and has implications for targeted therapy with JAK2 inhibitors, as well as with immunotherapy. Based on the referenced article, select the ONE best TRUE statement: [J Mol Diagn 2019, 21:307-317.]
   a. Genomic amplification at 9p24.1 has recently been described as a mechanism of resistance in post-chemotherapy, triple-negative breast cancer.
   b. Genomic amplifications at chromosome 9p24.1 were initially described in Epstein Barr virus–positive gastric adenocarcinoma.
   c. Genomic amplifications at chromosome 9p24.1 are infrequent in classical Hodgkin lymphoma and mediastinal large B-cell lymphoma.
   d. The association between genomic amplifications at chromosome 9p24.1 and small cell lung carcinoma is missing.

10. Genomic amplification at 9p24.1 has significant implications for therapy with both JAK2 kinase inhibitors and immune checkpoint inhibitors. Based on the referenced article, select the ONE best TRUE statement: [J Mol Diagn 2019, 21:307-317.]
   a. Amplification at 9p24.1 precludes the locus for Janus kinase 2 (JAK2).
   b. Amplification at 9p24.1 precludes the locus for programmed cell death 1 ligand 1 (PD-L1).
   c. Amplification at 9p24.1 involves programmed cell death 1 ligand 2 (PD-L2).
   d. Amplification at 9p24.1 involves the locus for Janus kinase 3 (JAK3).

11. **JAK2** is one of the four JAK-domain–containing tyrosine kinase genes. Based on the referenced article, select the ONE best TRUE statement: [J Mol Diagn 2019, 21:307-317.]
   a. The JAK-domain–containing tyrosine kinase genes include JAK1, JAK3, and JAK4.
   b. TYK2 is a JAK-domain–containing tyrosine kinase gene.
   c. JAK2 p.V617F mutation decreases JAK2 signaling.
   d. The downstream signaling of JAK2 is independent of STAT proteins.

12. TNBC are often managed with neoadjuvant chemotherapy. Based on the referenced article, select the ONE best TRUE statement: [J Mol Diagn 2019, 21:307-317.]
   a. Almost all TNBC patients respond to neoadjuvant chemotherapy.
   b. Genomic amplifications at the 9p24.1 locus contribute to resistance to chemotherapy in TNBC.
   c. The shortest region of overlap in the 9p24.1 locus spans approximately 250 kb, where immunohistochemistry for markers such as PD-L1 have shown great sensitivity.
   d. Fluorescence in situ hybridization (FISH)-based methodologies are the most detailed screening strategy for TNBCs.