Three research articles on the use of tumor heterogeneity index for a direct comparison between the results of human growth factor receptor 2 immunohistochemistry and next-generation sequencing–based cancer panel tests in gastric cancer, direct detection of single-nucleotide polymorphism markers of antimalarial drug resistance from whole blood, and identification of a simple two-gene (RASSF1/LATS2) methylation signature to stratify different prognostic groups of patients with diffuse gliomas were selected for the July 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:

- Discuss the use of immunohistochemistry (IHC) and next-generation sequencing (NGS) in detecting intratumoral heterogeneity of human growth factor receptor 2 (HER2) in gastric cancer (GC).
- Discuss the use of tumor heterogeneity index for a direct comparison between the results of HER2 IHC and NGS-based cancer panel tests in GC.
- Understand the challenges associated with developing drugs to treat malaria.
- Discuss a novel approach for detection of single-nucleotide polymorphism markers of antimicrobial drug resistance directly from whole blood.
- Discuss the use of methylation-specific PCR or PCR coupled to combined bisulfite restriction analysis to assay promoter methylation of Ras association domain family (RASSF)/Hippo pathway genes.
- Discuss the use of a simple two-gene (RASSF1/LATS2) methylation signature to stratify different prognostic groups of patients with diffuse gliomas.

1. **Gastric cancer (GC) is one of the most common malignancies and remains the second leading cause of cancer-related death worldwide.** Given the tendency towards poor patient outcomes in GC, intensive efforts have been directed towards molecular characterization of these cancers. Based on the referenced article, select the ONE best TRUE statement: [J Mol Diagn 2019, 21: 612-622]
   a. Physicians as well as GC patients are limited by the lack of recent advancements in targeted therapeutics of GC.
   b. Though powerful in exploring potential cancer-driving genes, next-generation sequencing (NGS) technology has failed to identify oncogenic mutations in genes that drive GC.
   c. There is a lack of reports on the correlation between NGS results with human growth factor receptor 2 (HER2) immunohistochemistry (IHC) and/or fluorescent in situ hybridization (FISH) results in GC.
   d. Trastuzumab has been approved for treatment of HER2-negative GC based upon HER2 IHC/ISH and NGS results.
2. Intratumoral heterogeneity in overexpression of HER2 is common in GC and poses a challenge for identifying patients who would benefit from anti-HER2 therapy. Based on the referenced article, select the ONE best TRUE statement: [J Mol Diagn 2019, 21: 612-622]
   a. In GC, intratumoral heterogeneity of HER2 overexpression is found in 10% to 25% of cases.
   b. Results from biopsy and surgical resection specimens are always consistent in determining intratumoral heterogeneity.
   c. HER2 heterogeneity and low tumor purity can cause discrepancy between IHC and NGS results leading to challenges in patient selection for anti-HER2 targeted therapy.
   d. Detection rate of **ERBB2** copy number alteration (CNA) by the NGS test is independent of the tumor volume and H score of HER2 overexpression obtained by IHC.

3. Recently, CNA has gained considerable interest as a type of genomic/genetic variation that plays an important role in disease susceptibility. Based on the referenced article, select the ONE best TRUE statement: [J Mol Diagn 2019, 21: 612-622]
   a. Detection of CNA of specific genes is common for hereditary diseases.
   b. Detection of cancer-specific CNAs is widely used in cancers like breast cancers and GC with homogenous HER2 overexpression.
   c. Compared with whole genome sequencing, whole exome sequencing or targeted cancer panel sequencing introduces less biases and noise that may complicate CNA detection.
   d. Cancer-specific CNAs can be reliably detected from archival (formalin fixed) tissues.

4. Coexisting molecular alterations other than **ERBB2** amplification have not been fully explored in GC for clinical purposes. Based on the referenced article, select the ONE best TRUE statement: [J Mol Diagn 2019, 21: 612-622]
   a. In GC with HER2 overexpression, concomitant single-nucleotide variants (SNVs) were found in **CCNE1**, **MYC**, and **EGFR**.
   b. In HER2-negative GC, SNV was most frequent in **TP53**.
   c. In GC with HER2 overexpression, concomitant SNVs were absent in **TP53**, **PIK3CA**, **KRAS**, **ERBB2**, **FBXW7**, and **RB1**.
   d. In HER2-negative GC, SNVs were absent for **PIK3CA**, **KRAS**, and **PTEN**.

5. Because of the interference of blood on fluorescence detection and intensity, the choice of the fluorescent reporter dyes employed in molecular assays is critical. Based on the referenced article, select the ONE best TRUE statement: [J Mol Diagn 2019, 21: 623-631]
   a. Minor groove binder (MGB) probes with the reporter dyes FAM and VIC are ideal for genotyping for c.227A>C (p.Lys76Thr) from blood as they are orange-red dyes, which are found to be more compatible with the blood absorbance spectra than traditional MGB probes.
   b. MGB probes with the reporter dyes Cy5 and TEX615 have poor performance for c.227A>C (p.Lys76Thr) genotyping from blood because of the high overlap between blood absorption and the reporter dyes' excitation and emission wavelengths.
   c. Adaptive PCR is a real-time PCR platform that uses right-handed DNA (R-DNA) additives to monitor the reaction for more reliable point-of-care performance from blood.
   d. Single nucleotide polymorphism (SNP) genotyping directly from blood allows drug-resistance testing for malaria without an additional DNA extraction step, which significantly decreases the test time and complexity.

6. Monitoring of antimalarial resistance is important to prevent its further spread, but the available options for assessing resistance are less than ideal for field settings. Molecular detection is perhaps the most efficient method, but it is also the most complex because it requires DNA extraction and PCR instrumentation. Although the reagents outlined in this study may also be used as a molecular detection test, they are likely less sensitive than is required to detect low copy numbers. Based on the referenced article, select the ONE best TRUE statement: [J Mol Diagn 2019, 21: 623-631]
   a. PCR-based detection of malaria infection usually relies on amplification of targets that appear multiple times in the genome.
   b. Molecular markers of drug resistance have high starting copy numbers making their detection easy.
   c. The described test has very high sensitivity for the detection of malaria and may be used as gold standard going forward.
   d. The described test is best suited for assessing antimalarial resistance status from patients at early stages of malarial infections.
7. Ras association domain family (RASSF)/Hippo pathway alterations are poorly characterized in diffuse gliomas. Based on the referenced article, select the ONE best TRUE statement: [J Mol Diagn 2019, 21: 695-704]
   a. Diffuse gliomas account for 50% of primary brain tumors in adults.
   b. Diffuse gliomas are characterized by recurrent molecular alterations.
   c. Diffuse gliomas are characterized by mutations in the LATS1 and LATS2 genes and co-deletion of 1p/19q.
   d. IDH mutant gliomas lack a CpG island methylator phenotype (GCIMP).

8. Among epigenetically silenced genes present in gliomas, genes that encode Ras association domain family (RASSF)/Hippo pathway proteins, a pathway required for cell homeostasis, are common. Based on the referenced article, select the ONE best TRUE statement: [J Mol Diagn 2019, 21: 695-704]
   a. The RASSF superfamily consists of 15 genes.
   b. The RASSF superfamily encodes proteins with several protein-binding domains, enabling their interaction with a multitude of partners and their subsequent participation in several cellular processes.
   c. The SARAH domain allows RASSF7 to RASSF15 proteins to regulate the Hippo kinases.
   d. After LATS1/2 down-regulation in gliomas, Yes-associated protein (YAP) and transcriptional co-activator with PDZ-binding motif (TAZ) promote mesenchymal differentiation and growth, respectively.

9. RASSF or hippo kinase inactivation triggering the YAP/TAZ activation mechanism, followed by proliferation and cell migration in many different tumor types, has not been systematically investigated in the glioma setting. Based on the referenced article, select the ONE best TRUE statement: [J Mol Diagn 2019, 21: 695-704]
   a. RASSF1 and RASSF10 gene promoter are known to be frequently silenced in adult diffuse gliomas.
   b. RASSF2 and NORE1A/RASSF5 gene promoters are found hypermethylated in 4% and 10.6% of diffuse gliomas, respectively.
   c. RASSF4 and RASSF6 gene promoter methylation frequency is very high in gliomas.
   d. Common hypermethylation of LATS kinase genes is absent in astrocytomas.

10. RASSF1A/Hippo gene promoters are frequently hypermethylated in gliomas resulting in gene silencing. Based on the referenced article, select the ONE best TRUE statement: [J Mol Diagn 2019, 21: 695-704]
   a. Most gliomas studied (75.2%) exhibit at least four RASSF/Hippo promoter gene hypermethylations.
   b. LATST1 promoter hypermethylation is a hallmark of oligodendrogial tumors.
   c. Combination of RASSF1 or LATS2 promoter hypermethylation enables classification of patients into three prognostic groups with high, intermediate, or low risk of death.
   d. RASSF1/LATS1 silencing predicts longer survival.

11. Promoter hypermethylation of RASSF/Hippo pathway member genes is observed commonly in diffuse gliomas. Based on the referenced article, select the ONE best TRUE statement: [J Mol Diagn 2019, 21: 695-704]
   a. Only 2 of 50 gliomas lacked hypermethylation of the studied gene promoters.
   b. Among the RASSF family member genes, the RASSF1 promoter was the least frequently hypermethylated promoter.
   c. Among the Hippo pathway member genes, LATS2 promoter hypermethylation was the most common event.
   d. The RASSF10 promoter is the most frequently hypermethylated promoter in diffuse glioma.

12. Hypermethylation status of RASSF genes can be used for stratifying patients with diffuse glioma. Based on the referenced article, select the ONE best TRUE statement: [J Mol Diagn 2019, 21: 695-704]
   a. Hypermethylation of RASSF1 gene promoter is uncommon in diffuse gliomas when compared with other tumor tissues.
   b. Hypermethylation of RASSF10 gene promoter is uncommon in diffuse gliomas when compared with other tumor tissues.
   c. RASSF2 and RASSF5 gene promoters are also frequently hypermethylated in gliomas.
   d. RASSF1A/Hippo signaling pathway alterations are associated with a more favorable prognosis as opposed to that reported from other human cancers.