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CME May Questions # 1-12

An article and associated Commentary on detection of hepatitis B virus DNA, an article on targeted next-generation sequencing (tNGS) of myelodysplastic syndromes, and an article on elevated microsatellite alterations at selected tetra/pentanucleotide repeats in colorectal cancers were selected for the **May 2018 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.

Questions #1-5 are based on: Huang J-T, Yang Y, Hu Y-M, Liu X-H, Liao M-Y, Morgan R, Yuan E-F, Li X, Liu S-M: A highly sensitive and robust methods for hepatitis B virus covalently closed circular DNA detection in single cells and serum. *J Mol Diagn* 2018, 20:334-343; <http://dx.doi.org/10.1016/j.jmoldx.2018.01.010> and associated Commentary by Shen F, Sergi C, Sun H-L: Hepatitis B virus covalently closed circular DNA—selective droplet digital PCR: A sensitive and noninvasive method for hepatocellular carcinoma diagnosis? *J Mol Diagn* 2018, 20:277-278; <http://dx.doi.org/10.1016/j.jmoldx.2018.03.001>

Questions #6-8 are based on: Bräuninger A, Blau W, Kunze K, Desch A-K, Brobeil A, Tur MK, Etschmann B, Günther U, Körholz D, Schliesser G, Käbisch A, Kiehl M, Rummel M, Gattenlöhner S: Targeted next-generation sequencing is a sensitive tool for differential diagnosis of myelodysplastic syndromes in bone marrow trephines. *J Mol Diagn* 2018, 20:344-354; <http://dx.doi.org/10.1016/j.jmoldx.2018.01.006>.

Questions #9-12 are based on: Wang Y, Vnencak-Jones CL, Cates JM, Shi C: Deciphering elevated microsatellite alterations at selected tetra/pentanucleotide repeats, microsatellite instability and loss of heterozygosity in colorectal cancers. *J Mol Diagn* 2018, 20:366-372; <http://dx.doi.org/10.1016/j.jmoldx.2018.02.001>

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

- Understand the global health impact of chronic hepatitis B virus (CHB) infection.
- Describe the life cycle of hepatitis B virus (HBV).
- Define the hepatocellular damage caused by HBV and the development of hepatocellular carcinoma (HCC).
- Describe potential therapies for CHB.
- Discuss molecular testing methods for covalently closed circular DNA (cccDNA) of HBV.
- Define myelodysplastic syndrome (MDS).
- Discuss the role of MDS-associated mutations for diagnosis of clonal MDS.
- Describe microsatellite instability (MSI) and DNA mismatch repair (MMR) proteins.
- Understand the role of MSI in colorectal cancer (CRC).
- Define mono- and dinucleotide as well as short tandem repeats (STRs).
- Describe instability of tetra- and pentanucleotide repeats and their potential role in the pathogenesis of CRC.

1. Chronic hepatitis B virus (HBV) infection is a major health burden worldwide. Based on the referenced article and associated Commentary, select the ONE best TRUE statement: [See *J Mol Diagn* 2018, 20:334-343 and *J Mol Diagn* 2018, 20: 277-278.]

- a. Approximately 240 million patients have chronic hepatitis B virus (CHB) infection worldwide.
- b. There are 450,000 CHB-related deaths yearly.
- c. Approximately 1 billion individuals worldwide are infected with HBV.
- d. The global prevalence of CHB is distributed unevenly and is most concentrated in South America.

2. CHB can cause hepatocellular damage. Based on the referenced article and associated Commentary, select the ONE best TRUE statement: [See J Mol Diagn 2018, 20:334-343 and J Mol Diagn 2018, 20: 277-278.]

- a. CHB patients are at risk of developing hepatocellular carcinoma (HCC), which ranks eighth in terms of malignant cancer mortality.
- b. Currently, clinical therapies of CHB include six approved nucleos(t)ide analog (NUC) treatments.
- c. Currently, clinical therapies of CHB are limited to pegylated interferon- α or five approved NUC treatments.
- d. More than half of CHB patients achieve hepatitis B s antigen (HBsAg) loss by pegylated interferon- α and/or NUC after a follow-up of 5 years.

3. Long-term NUC administration for CHB patients is mainly attributable to the persistence of viral covalently closed circular DNA (cccDNA) and defective immune responses. Based on the referenced article and associated Commentary, select the ONE best TRUE statement: [See J Mol Diagn 2018, 20:334-343 and J Mol Diagn 2018, 20: 277-278.]

- a. The cccDNA serves as a template for the transcription of HBV RNAs.
- b. Several new antiviral strategies targeting cccDNA have been established to improve HBV clearance; two siRNA molecules are under clinical trial, including ARC-840 and TLN-HBV.
- c. Although high-dose interferon- α can degrade cccDNA, the treatment is hepatotoxic.
- d. In chimpanzees, NUC administration decreased cccDNA by $0.9 \pm 0.3 \log_{10}$ compared to baseline.

4. The low sensitivity of existing methods limits cccDNA detection in clinical samples. Based on the referenced article and associated Commentary, select the ONE best TRUE statement: [See J Mol Diagn 2018, 20:334-343 and J Mol Diagn 2018, 20: 277-278.]

- a. The cccDNA in CHB patients averages 10 copies per hepatocyte.
- b. The limit of detection (LOD) of Southern blotting is approximately 3 cccDNA copies per infected cell.
- c. Digital droplet PCR (ddPCR) has recently been approved by the Food and Drug Administration (FDA).
- d. HBV virions contain partially double-stranded DNA [alias relaxed circular DNA (rcDNA)].

5. The presence of cccDNA in the nucleus of infected cells is the major obstacle for the clearance of HBV during antiviral therapy of CHB. Based on the referenced article and associated Commentary, select the ONE best TRUE statement: [See J Mol Diagn 2018, 20:334-343 and J Mol Diagn 2018, 20: 277-278.]

- a. The authors of the referenced article claim that intrahepatic cccDNA is the best diagnostic marker for CHB.
- b. Most serum cccDNA originates from HBV-infected hepatocyte death, which releases the cccDNA into blood.
- c. cccDNA copy number is consistent in each hepatocyte.
- d. Serum cccDNA was negatively correlated with intrahepatic cccDNA levels.

6. The prognosis and treatment of myelodysplastic syndrome (MDS) varies from patient to patient on the basis of individual genetic risk factors and clinical circumstances. Based on the referenced article, select the ONE best TRUE statement: [See J Mol Diagn 2018, 20:344-354.]

- a. MDSs are a heterogeneous group of clonal hematological disorders characterized by morphologic dysplasia, ineffective hematopoiesis, cytopenia, and increased risk of development of secondary acute myeloid leukemia.
- b. Approximately 35 genes have been identified as recurrently mutated in 90% of MDS patients.
- c. The most frequently affected genes in MDS are tyrosine kinases.
- d. Immunohistologic examination of bone marrow trephines can easily distinguish between clonal neoplastic MDS and myelodysplasia attributable to reactive changes caused by toxic insults.

7. Detection of MDS-associated mutations can support the diagnosis of clonal MDS. Based on the referenced article, select the ONE best TRUE statement: [See J Mol Diagn 2018, 20:344-354.]

- a. Genes involved in DNA methylation such as *DNMT3A*, *SF3B1*, *IDH1*, and *IDH2* are recurrently mutated in the majority of MDS patients.
- b. Genes involved in RNA splicing such as *SRSF2* and *ZRSR2* are recurrently mutated in the majority of MDS patients.
- c. Genes involved in regulation of differentiation of myeloid cells such as *U2AF1* and *RUNX1* are recurrently mutated in the majority of MDS patients.
- d. The authors of the referenced article selected 16 recurrently mutated genes that were mutated in at least 10% of cases to develop a targeted NGS (tNGS) tool for differential diagnosis of MDS in bone marrow trephines.

8. Analysis of mutation patterns in MDS samples may provide insights into the pathophysiology of MDS. Based on the referenced article, select the ONE best TRUE statement: [See J Mol Diagn 2018, 20:344-354.]

- a. Considering the 60 samples with a final diagnosis of MDS carrying mutations, the average case had 3 mutations.
- b. *TP53* was the most frequently mutated gene.
- c. Most mutations detected in *TET2*, *STAG2*, *ASXL1*, *ZRSR2*, and *RUNX1* were stop and frame shift mutations and mutations destroying splice sites.
- d. The majority of cases with *TET2* mutations carried three *TET2* mutations.

9. Microsatellite instability (MSI) due to deficiency in DNA mismatch repair (MMR) proteins is a major pathway leading to colorectal cancers (CRCs). Based on the referenced article, select the ONE best TRUE statement: [See J Mol Diagn 2018, 20:366-372.]

- a. Sporadic MSI-high (MSI-H) CRCs result from *MLH1* germline mutations.
- b. MutL protein homologue 1 (MLH-1) repairs trinucleotide mismatch replication errors.
- c. Hypermethylation of the *MLH2* promoter region is responsible for Lynch-syndrome—associated cancers.
- d. Germline mutation in one of the four MMR genes, coupled with loss of function of the second allele, leads to most cases of inherited MSI-H cancers.

10. Stability of short tandem repeats (STRs) of >2 nucleotides is likely maintained by distinct mechanisms from that of mono- and dinucleotide repeats. Based on the referenced article, select the ONE best TRUE statement: [See J Mol Diagn 2018, 20: 366-372.]

- a. MSI at tetranucleotide repeats has been observed in gliomas.
- b. Elevated microsatellite alterations at selected tetranucleotide repeats (EMAST) has been reported in up to a quarter of CRCs.
- c. EMAST predicts poor prognosis in CRCs.
- d. EMAST is associated with well differentiated tumors.

11. Pentanucleotide repeat instability has been observed in some CRCs. Based on the referenced article, select the ONE best TRUE statement: [See J Mol Diagn 2018, 20: 366-372.]

- a. The authors of the referenced article used a 12-locus human STR panel to test for elevated microsatellite alterations at selected tetra- and pentanucleotide repeats (EMASTP).
- b. The 12 markers in the STR panel included eight tetranucleotide and four pentanucleotide markers.
- c. EMASTP status was determined as high (EMASTP-H), low (EMASTP-L), or stable (EMASTP-S), depending on the number of markers demonstrating instability, corresponding to $\geq 25\%$, $< 25\%$, or 0%, respectively.
- d. EMASTP instability was detected in all MSI-H CRCs that were tested, whereas among randomly selected microsatellite stable (MSS) CRCs, 28% of MSS CRCs were EMASTP-L and 72% were EMASTP-S.

12. Different STR markers were altered in CRCs. Based on the referenced article, select the ONE best TRUE statement: [See J Mol Diagn 2018, 20: 366-372.]

- a. D5S818 was the most frequently altered EMASTP locus in CRCs.
- b. VWA is located at 12p13 and has a complex TCTA repeat sequence.
- c. MSI-H CRCs were more likely to display instability at TH01 compared to MSS CRCs with EMASTP instability.
- d. VWA, FGA, and D5S818 were the three most commonly unstable markers in MSI-H CRCs.