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CME May Questions # 1-12

A Review on human papillomavirus carcinogenesis and genomics, and research articles on *NTRK3* fusions in childhood melanocytic neoplasms and minimal residual disease analysis in acute promyelocytic leukemia were selected for the **May 2017 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 #1-8 are based on: Leal SM, Gulley ML: Current and emerging molecular tests for human papillomavirus-related neoplasia in the genomic era. *J Mol Diagn* 2017, 19:366-377; <http://dx.doi.org/10.1016/j.jmoldx.2017.01.006>.

Questions #9-10 are based on: Wang L, Busam KJ, Benayed R, Cimera R, Wang J, Denley R, Rao M, Aryeequaye R, Mullaney K, Cao L, Ladanyi M, Hameed M: Identification of *NTRK3* fusions in childhood melanocytic neoplasms. *J Mol Diagn* 2017, 19:387-396; <http://dx.doi.org/10.1016/j.jmoldx.2017.11.005>.

Questions #11-12 are based on: Brunetti C, Anelli L, Zagaria A, Minervini A, Minervini CF, Casieri P, Coccaro N, Cumbo C, Tota G, Impera L, Orsini P, Specchia G, Albano F: Droplet digital PCR is a reliable tool for monitoring minimal residual disease in acute promyelocytic leukemia. *J Mol Diagn* 2017, 19:437-444; <http://dx.doi.org/10.1016/j.jmoldx.2017.01.004>.

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

- Define viruses that have been identified as etiologic agents of human malignancies.
- Describe human papillomavirus (HPV).
- Understand where HPV is most prevalent worldwide.
- Describe the steps of HPV infection.
- Explain the role of HPV in immune responses.
- Describe the integration of the HPV genome into host chromosomal DNA.
- Understand that HPV infection induces epigenetic changes in viral and host DNA.
- Explain the screening ages of women for possible HPV infection.
- Describe spitzoid melanocytic neoplasms.
- Explain spitzoid neoplasm gene mutations/fusions.
- Define acute promyelocytic leukemia (APL).
- Understand APL treatment.

1. In 1911 the pathologist Peyton Rous showed that cell-free tumor extracts containing the Rous sarcoma virus were able to transmit tumors between chickens. Based on the referenced Review, select the ONE best TRUE statement: [See *J Mol Diagn* 2017, 19:366-377.]

- a. Rous also identified papillomavirus as the cause of benign warts in rabbits. Despite his extraordinary work, microbe-mediated oncogenesis was considered a scientific curiosity until Epstein and Barr's 1967 identification of viral particles in human Burkitt lymphoma.
- b. Since the discovery of the Epstein-Barr virus (EBV), four more viruses have been identified as the etiologic agents of human malignancies.
- c. Current estimates link a viral etiology to 20% to 25% of all human malignancies.
- d. The bacterium *Helicobacter pylori* and the parasite *Clonorchis haematobium* are associated with human malignancies.

2. Human papillomavirus (HPV), a nonenveloped circular double-stranded (ds) DNA virus in the papillomaviridae family, infects basal epithelial cells in the epidermis and mucosae. Based on the referenced Review, select the ONE best TRUE statement: [See J Mol Diagn 2017, 19:366-377.]

- a. There are now three genera, 19 species, and 300 HPV types recognized by the International Human Papillomavirus Reference Center (IHPRC).
- b. Genera exhibit <30% sequence homology in the L1 gene encoding the major capsid protein, and are designated α , β , γ , μ , and ν .
- c. Species exhibit 80% to 90% homology.
- d. High-risk HPV types typically refer to those having a causal association with cervical cancer.

3. A meta-analysis of 423 published studies reveals the percentage of each high-risk type in cervical carcinoma and premalignant lesions. Based on the referenced Review, select the ONE best TRUE statement: [See J Mol Diagn 2017, 19:366-377.]

- a. In North America, HPV is identified in 40%, 8%, and 25% of carcinomas arising in the oropharynx, oral cavity, and larynx, respectively.
- b. HPV16 is the most common type at all geographic locations.
- c. Notable geographic variations include identification of HPV18 in 30% of HPV⁺ carcinomas in the oral cavity worldwide versus 17% in North America and up to 60% in Asia.
- d. HPV types associated with oropharynx cancer in Central and South America differ from other geographic locations with higher rates of HPV58, 45, 51, 59, and 66 and minimal HPV18.

4. HPV infection requires abrasion of the surface epithelium with exposure of the basement membrane, enabling viral binding to exposed heparan sulfate proteoglycans. Based on the referenced Review, select the ONE best TRUE statement: [See J Mol Diagn 2017, 19:366-377.]

- a. Furin bound to the basement membrane cleaves L2 on the viral capsid, exposing the major viral capsid protein L1, which is the target of prophylactic vaccines.
- b. L1 binds to an unknown receptor on adjacent basal epithelial cells resulting in viral entry via endocytosis in the first six to eight hours, uncoating in the late endosome, and release of viral dsDNA complexed with L2 into the cytosol.
- c. Viral DNA enters the nucleus within 12 hours, with transcription of six early genes: E1- 6.
- d. E1 and E2 are required for viral episome replication to yield approximately 65 viral genomes per cell.

5. HPV infection poorly elicits innate and adaptive immune responses. Based on the referenced Review, select the ONE best TRUE statement: [See J Mol Diagn 2017, 19:366-377.]

- a. Traumatic injury to leukocytes during initial infection releases danger-associated molecular patterns (DAMPs), which activate pattern recognition receptors on leukocytes leading to inflammation.
- b. After healing, the virus spreads with maximal DAMP production and inflammation.
- c. Although the cytoplasm contains innate dsDNA sensors, and endosomes harbor toll-like receptors, HPV dsDNA seems unavailable for recognition.
- d. HPV dsDNA may be either restricted to the cytoplasm or complexed to L1 proteins, making it unavailable for innate immune recognition.

6. Integration of the viral genome into host chromosomal DNA can disrupt viral gene expression patterns leading to lack of immunogenic viral proteins and escape from adaptive immune control. Based on the referenced Review, select the ONE best TRUE statement: [See J Mol Diagn 2017, 19:366-377.]

- a. Integration events occur at a rate of about 16 per cervical carcinoma clone, or 1 to 26 per head and neck carcinoma clone.
- b. HPV DNA *in situ* hybridization staining patterns can distinguish episomal versus integrated viral genomes.
- c. The breakpoint in the circular HPV16 genome occurs most frequently in E2>L2>L1>E1.
- d. E2 disruption favors oncogenesis since E2 negatively regulates E3 and E4 transcription.

7. HPV infection induces epigenetic changes in viral DNA as well as in the host genome. Based on the referenced Review, select the ONE best TRUE statement: [See J Mol Diagn 2017, 19:366-377.]

- a. Viral E6 and E7 both up-regulate the enzyme DNA methyltransferase 3B, resulting in methylation of multiple genomic sites.
- b. HPV infection up-regulates DNA methyltransferase 1 and histone lysine methylases, which function in concert to alter gene transcription and promote carcinogenesis.
- c. Methylation of E2 binding sites on the HPV genome interferes with E2-mediated inhibition of E1 and E4 expression.
- d. In head and neck carcinoma, an HPV-related methylation signature is prognostic.

8. Prevention of cervical carcinoma is the primary goal of screening programs, which continue to be refined as new evidence emerges. Based on the referenced Review, select the ONE best TRUE statement: [See J Mol Diagn 2017, 19:366-377.]

- a. Cytology alone every five years is recommended for women from age 21 to 29 years.
- b. Women aged 30 to 45 years have the option for co-testing every three years.
- c. Recent Federal Drug Administration (FDA) approval of a standalone high-risk HPV molecular assay every three years is an option for women > 25 years.
- d. Women with pathology of moderate-to-high grade cervical intraepithelial neoplasia should receive continued screening for at least 10 years using co-testing, even after age 45 years.

9. Spitzoid melanocytic neoplasms are a distinctive group of melanocytic tumors. Based on the referenced article, select the ONE best TRUE statement: [See J Mol Diagn 2017, 19:387-396.]

- a. In 1850, Sophie Spitz coined the term "melanoma of childhood" for a group of melanocytic skin tumors composed of spindled or epithelioid melanocytes that developed exclusively in children and adolescents.
- b. Approximately 50% of spitzoid melanocytic neoplasms behaved in an indolent manner and they were subsequently termed 'Spitz nevus' to indicate their benign nature.
- c. Malignant tumors with spitzoid histological features were termed 'spitzoid malignant tumors', and these tumors often showed aggressive clinical behavior atypical of conventional melanomas.
- d. Tumors with ambiguous histological features, overlapping between those of Spitz nevus and melanoma, are termed 'atypical Spitz tumors'.

10. The majority of spitzoid neoplasms lack mutations in common melanoma-associated oncogenes. Based on the referenced article, select the ONE best TRUE statement: [See J Mol Diagn 2017, 19:387-396.]

- a. Spitzoid tumors lack mutations in melanoma-associated genes, such as *ROS1* or *MET*.
- b. Recent studies have uncovered *NRAS*, *KIT*, *GNAQ*, and *GNA11* fusions in spitzoid tumors.
- c. A genome-wide high-resolution single nucleotide polymorphism (SNP)-array analysis was performed as an adjunct to the histopathological diagnosis for diagnostically challenging melanocytic tumors.
- d. Over 300 samples have been tested, a majority of which were diagnosed histologically as atypical Spitz tumors.

11. Acute promyelocytic leukemia (APL) is a rare hematologic malignancy commonly associated with the chromosomal translocation t(15;17)(q24;q21). Based on the referenced article, select the ONE best TRUE statement: [See J Mol Diagn 2017, 19:437-444.]

- a. APL involves the promyelocytic leukemia (*PML*) and the retinoic acid receptor- α (*RARA*) genes, resulting in the oncogenic fusion transcript *PML-RARA*.
- b. The oncogenic fusion breakpoints on chromosome 17 are localized within a 10 kb fragment of the *RARA* intron 2.
- c. Up to three regions of the *PML* locus may be involved in the translocation: intron 6, exon 7 and intron 3, accounting for 5%, 40%, and 55% of cases, respectively.
- d. The different breakpoints lead to two possible *PML-RARA* isoforms, referred to as long (L or bcr1) and short (S or bcr2).

12. Current APL treatment is highly successful. Based on the referenced article, select the ONE best TRUE statement: [See J Mol Diagn 2017, 19:437-444.]

- a. APL treatment leads to long-term remission and possibly a cure for about 50% of newly diagnosed patients.
- b. Approximately 50% of patients are at particular risk for relapse.
- c. The detection of the *PML-RARA* transcript, performed at the post-consolidation phase, provides an independent prognostic factor in APL.
- d. *PML-RARA* amplification by nested PCR is the method most commonly employed to confirm the morphological diagnosis of APL.