Three regular articles on outcome prediction in oropharyngeal squamous cell carcinoma, concurrent detection of mitochondrial DNA copy number and mutation, and improving genetic testing in hereditary cancer by RNA analysis were selected for the November and December 2020 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 #3-6 are based on Zhou K, Mo Q, Guo S, Liu Y, Yin C, Ji X, Guo X, and Xing J; A Novel Next-Generation Sequencing–Based Approach for Concurrent Detection of Mitochondrial DNA Copy Number and Mutation. J Mol Diagn 2020, 22: 1408-1418


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

- Define cell-free DNA and describe its characteristics.
- Understand the use of cell-free (cf) human papillomavirus (HPV)-DNA in detection of HPV.
- Understand the use of cfHPV-DNA in plasma of patients with HPV-driven oropharyngeal squamous cell carcinoma as a tool to monitor the course of the disease and possibly detect recurrence before classic diagnostics.
- Describe features of human mitochondrial DNA (mtDNA).
- Define heteroplasmy.
- List the advantages of the streamlined capture-based next-generation sequencing approach for concurrent detection of mtDNA copy number and mutations.
- Discuss approaches for the detection of hereditary cancers.
- Discuss the clinical utility of RNA studies in resolving the significance of copy number gains.
- Understand the importance of RNA studies to improve variant classification.

1. Cell-free DNA (cfDNA) in the blood was first described by Mandel and Metais in 1948. Based on the referenced article, select the ONE best TRUE statement: [J Mol Diagn 2020, 22: 1333-1343]

   a. cfDNA is released into the bloodstream from nucleated blood cells and apoptotic and necrotic tissue cells.
   b. The size of circulating cfDNA varies from relatively small to large fragments of up to 10 kb. Most cfDNA fragments are approximately 10 to 70 bp in size, which corresponds to twice the length of DNA wrapped around a nucleosome and the histone H1.
   c. The total cfDNA concentration is lowered in patients of different cancer entities compared with healthy controls.
   d. Because of its size and foreign nature, cell-free (cf) human papillomavirus (HPV)-DNA is hard to detect tumor-specific DNA as distinguishing it from cellular DNA is challenging.
2. The outcome of HPV-driven oropharyngeal squamous cell carcinoma (OPSCC) is much more favorable than that of HPV-negative OPSCC following current therapy standards. This is the rationale for the investigation of treatment de-escalation to spare treatment-related toxicity in patients with HPV-driven OPSCC. However, the failure of two phase 3 de-escalation chemoradiotherapy trials demonstrates the need to monitor treatment response to identify early on during treatment those patients who do not benefit from de-escalation. Based on the referenced article, select the ONE best TRUE statement: [J Mol Diagn 2020, 22: 1333-1343]

a. Patients with HPV-driven OPSCC who develop recurrence following current standard treatment have yet to be reported.

b. Liquid biopsies might be particularly suitable for disease detection in patients with virus-driven malignancies.

c. This study investigated the usefulness of cfHPV-DNA in plasma of patients with HPV-driven OPSCC as a useful tool to confirm recurrence after classic diagnostics.

d. Like tumor-specific mutations, the detection of cfHPV-DNA requires knowledge of the presence of individual mutations that serve as markers.

3. Mitochondria contain their own genome, a maternally inherited double-strand circular DNA molecule. The human mitochondrial DNA (mtDNA) contains 16,569 bp, which encode 13 polypeptides of the respiratory chain, 22 transfer RNAs, and two ribosomal RNAs. Based on the referenced article, select the ONE best TRUE statement: [J Mol Diagn 2020, 22: 1408-1418]

a. Compared with two copies of nuclear DNA (nDNA), the copy number of mtDNA per mitochondrion ranges from 2 to 5, with 10 to 100 copies per cell depending on the different cell types.

b. mtDNA is sturdy and immune to replication error or damage by endogenous reactive oxygen species.

c. The mutation rate in mtDNA is approximately 10 times lower than in nDNA.

d. Numerous mutated and normal mtDNA co-exist in the same cell, this phenomenon of intracellular mtDNA mixture is defined as heteroplasmy.

4. Numerous studies have identified essential contributions of altered mtDNA copy number and mutations in many common disorders, including cancer. To date, capture-based next-generation sequencing (NGS) has been widely applied to detect mtDNA mutations, although it lacks the ability to assess mtDNA copy number. The discussed study developed a streamlined capture-based NGS approach for concurrent detection of mtDNA copy number and mutations, which enables comprehensive mtDNA profiling in different sample types, thereby reducing the time and cost of detection. Based on the referenced article, select the ONE best TRUE statement: [J Mol Diagn 2020, 22: 1408-1418]

a. This novel approach has one advantage and two disadvantages.

b. The advantage to this approach is that the information of both mtDNA copy number and mtDNA mutations can be obtained simultaneously by only one detection protocol, which combines the functions of both qPCR and sequencing.

c. One disadvantage of this novel approach is that it has limited utility.

d. The other disadvantage of this approach is that the effective detection of mtDNA can be performed in DNA samples with only high quality.

5. In this innovative capture-based NGS approach, nuclear DNA fragments were selected for probe preparation and further used as internal references for mtDNA copy number calculation based on capture sequencing data of mtDNA. The results show that, by applying mtDNA probes and nDNA probes within the same reaction system, it is practical to detect the mtDNA copy number and mutations concurrently in a reproducible and optimized manner. This approach was further optimized, and the accuracy and reproducibility in detecting mtDNA copy number and mutation was assessed. Based on the referenced article, select the ONE best TRUE statement: [J Mol Diagn 2020, 22: 1408-1418]

a. Measurement of the mtDNA copy number by whole-genome sequencing (WGS) (genome reference (GR)-based approach) has limited applicability in mtDNA copy number quantitation.
b. This limited utility of WGS is due to the somewhat biased nature of WGS sequencing.
c. For the capture-based approach (fragment reference (FR)–based approach), the target-enrichment procedure always results in unbiased estimation of mtDNA copy number.
d. The capture-based approach exhibits different capture efficiency of the probes targeting mtDNA and nDNA fragments, leading to the systematically increased ratio of mtDNA to reference nDNA.

6. The method described in the discussed study can be used for reporting a relative mtDNA copy number but not for the absolute quantification. Based on the referenced article, select the ONE best TRUE statement: [J Mol Diagn 2020, 22: 1408-1418]

a. Compared with the WGS-based approach, the described capture-based novel approach is slightly more expensive though it is equally sensitive.
b. Compared with the WGS-based approach, the described capture-based novel approach has poor accuracy.
c. Given the cost and diminished accuracy, WGS-based approach is a better choice over the described capture-based novel approach.
d. The described novel approach can also be used for copy number and mutation profiling of mtDNA in the frame of genetic screens and lineage tracing.

7. Hereditary cancers (HCs) account for approximately 3% of all diagnosed cancers, and most are explained by pathogenic germline mutations in high-penetrance predisposing genes. Based on the referenced article, select the ONE best TRUE statement: [J Mol Diagn 2020, 22: 1453-1468]

a. The introduction of NGS has changed HC genetic testing workflows, allowing the simultaneous analysis of multiple genes.
b. Though NGS is more cost-effective, it decreases the mutation detection rate.
c. NGS is the best approach to identify higher number of variants of uncertain significance (VUSs).
d. NGS-based approaches are gold standards in clinical use to adjust cancer prevention measures, risk reduction operations, or mutation-specific therapeutic strategies.

8. Several in silico tools have been developed with the aim of predicting whether a DNA change could affect the correct splicing of the expressed RNA. Based on the referenced article, select the ONE best TRUE statement: [J Mol Diagn 2020, 22: 1453-1468]

a. Spliceogenic effects related to VUSs have yet to be reported.
b. Discordances between different algorithms may arise, along with inconclusive interpretations; hence, experimental validation is always required.
c. mRNA analyses have emerged to assess the effect of protein changes on RNA processing.
d. mRNA analyses to assess the effect of DNA changes on RNA processing are based on the characterization of the similar transcripts derived from different variants.

9. Regarding copy number variations, MLPA has been established as the gold standard technique for the screening of large rearrangements within genes. Based on the referenced article, select the ONE best TRUE statement: [J Mol Diagn 2020, 22: 1453-1468]

a. Frameshift alterations that result in premature termination codons are uncommon genomic deletions.
b. Non-frameshift alterations that usually remove clinically important domains are uncommon genomic deletions.
c. The clinical significance of copy number gains is more difficult to ascertain, considering that the duplicated segment could have been inserted in a noncoding region and the expression of the gene involved would not be affected.
d. RNA studies have limited utility in resolving the clinical significance of copy number gains.
10. Reclassification of VUSs has a tremendous impact on the clinical management of probands and their relatives affected by hereditary cancers. Based on the referenced article, select the ONE best TRUE statement: [J Mol Diagn 2020, 22: 1453-1468]

a. Sixty percent of the variants analyzed in the discussed study changed their clinical classification to (likely) pathogenic, highlighting the importance of using RNA analysis after in silico prioritization in genetic testing algorithms.
b. RNA genetic testing has yet to be used to resolve VUSs in individuals previously tested by DNA analysis.
c. The clinical surveillance of VUSs is hard to adjust to a more precise risk.
d. The clinical surveillance of VUSs accesses similar therapeutic schemes and their genetic results are of low value in reproductive decisions.

11. All hereditary cancer-associated variants identified in the routine clinical setting were submitted to Alamut and SPICE splicing predictors. A total of 20 variants were prioritized for in vitro RNA analysis because of their putative spliceogenic effect according to at least three in silico tools. Based on the referenced article, select the ONE best TRUE statement: [J Mol Diagn 2020, 22: 1453-1468]

a. All variants including the ATM c.3577-13T>C reached this threshold.
b. The variant ATM c.3577-13T>C was prioritized because of the identification of another substitution in the same intronic position with a stronger prediction of splicing disruption (ATM c.3577-13T>G).
c. SPICE defines donor splice sites from positions +2 to +8 and acceptor splice sites from -12 to -2.
d. SPICE predictions for variants between the donor splice sites and the acceptor splice sites were discarded.

12. In all cases, total RNA was isolated from cultured lymphocytes of hereditary cancer patients harboring the variant under study and controls. The transcriptional profiles of patients were compared with 10 control individuals by agarose gel electrophoresis and Sanger sequencing. In addition, allele-specific expression (ASE) was assessed by single-nucleotide primer extension (SNuPE) in 4 variants. Based on the referenced article, select the ONE best TRUE statement: [J Mol Diagn 2020, 22: 1453-1468]

a. Among the 10 single-nucleotide variants (SNVs) analyzed, mRNA analysis identified an aberrant splicing pattern in all (100%).
b. The studied variants precluded any variants that disrupted the natural splice site leading to whole exon skipping.
c. De novo splice sites were generated for variants ATM c.7135C>G and MUTYH c.577-5A>G, whereas cryptic splice sites were activated for RAD51C c.404G>A and TP53 c.375G>A, giving rise in these cases to partial exon skipping or partial intron retention.
d. The 10 SNVs analyzed produce aberrant transcripts that carry gain of function proteins.