A Review on annotating somatic variants in cancer and an article on the evaluation of mutational testing of pre-neoplastic Barrett's mucosa were selected for the July 2015 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 #1-6 are based on: Lee LA, Arvai KJ, Jones D: Annotation of sequence variants in cancer samples: Processes and pitfalls for routine assays in the clinical laboratory. J Mol Diagn 2015, 17:339-351; http://dx.doi.org/10.1016/j.jmoldx.2015.03.003. Please note that at the time the Review was composed, all the authors were employees of Quest Diagnostics, which offers sequencing assays commercially.


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

- Describe the genetic changes that are associated with clinical syndromes such as cancer.
- Define minor allele frequencies (MAFs).
- Describe driver and passenger mutations and their role in tumor development.
- Explain next-generation sequencing (NGS) assays and their use.
- Define TET2 and the mutations that occur in this gene.
- Describe pathway analysis and co-occurring mutations.
- Describe the characteristics of Barrett's esophagus.
- Understand the risk factors, prevention, and detection of esophageal adenocarcinoma (EAC).

1. During tumor development, there is a complex interplay between somatic or acquired mutations in oncogenes, tumor suppressors, and epigenetic regulators and germline, or inherited, genetic variation. Based on the referenced Review, select the ONE statement that is NOT true: [See J Mol Diagn 2015, 17:339-351.]

   a. Initial work on localizing genetic variants associated with cancer susceptibility focused on well-defined clinical syndromes.
   b. Oncogenes were initially localized using targeted DNA sequencing.
   c. Genetic changes observed in affected individuals include frameshift and nonsense mutations.
   d. Additional genetic changes involve inactivating mutations with loss of function linked to tumor initiation.
2. Translation of germline variant calls into clinical decisions relies on proper annotation. Based on the referenced Review, select the ONE statement that is NOT true: [See J Mol Diagn 2015, 17:339-351.]

a. Presumed benign variants are typically regarded as those with minor allele frequencies (MAFs) of 10% to 15%.
b. Most single nucleotide polymorphisms (SNPs) occur at MAFs under 0.5% (<1% of the population), highlighting the difficulty of variant annotation.
c. At this time, curated gene-specific databases cover only a few cancer-associated genes.
d. The best-studied cancer susceptibility genes, particularly BRCA1, BRCA2, and the Lynch syndrome-associated mismatch repair genes, have publicly available and curated databases.

3. Genetic changes that arise during the development of a tumor are termed somatic mutations and possess commonalities and differences with germline changes. Based on the referenced Review, select the ONE statement that is NOT true: [See J Mol Diagn 2015, 17:339-351.]

a. Acquired somatic mutations in cancer cells are propagated through clonal expansion from founder cancer stem cells or tumor subpopulations.
b. If a given genetic change promotes tumor development, it is regarded as a driver mutation and is typically retained during the disease course.
c. Driver mutations are typically classified as loss-of-function changes in tumor-promoting oncogenes or gain-of-function changes in tumor suppressor genes.
d. Once a tumor becomes established, additional mutations that were present in the selected abnormal cell population but that are not integral to tumorigenesis can arise as passengers.

4. Approaches to somatic variant annotation in cancers differ based on type of assay (full exome versus hotspot or targeted panels) and assay goal(s). Based on the referenced Review, select the ONE statement that is NOT true: [See J Mol Diagn 2015, 17:339-351.]

a. Next-generation sequencing (NGS) clonality assays include T-cell receptor and B-cell antigen receptor profiling in lymphoproliferative disorders, identifying hematologic neoplasms in patients with blood cell abnormalities and distinguishing atypical hyperplasia from early-stage precancers lesions.
b. If a sequencing assay is used for risk stratification, annotation must be tied to an underlying data set with strong statistical power and a similar clinical management strategy as the target population.
c. For theranostic indications, annotation focuses on identifying important driver or resistance mutations that can guide therapy decisions.
d. NGS theranostic panels comprising thousands of genes are now routinely used, particularly for relapsed or refractory solid tumors for which off-label or compassionate use of targeted agents is more common.

5. Mutations in TET2 are now commonly used to help in the diagnosis and classification of myelodysplastic syndrome and myeloproliferative neoplasm. Based on the referenced Review, select the ONE statement that is NOT true: [See J Mol Diagn 2015, 17:339-351.]

a. TET2 belongs to the TET family of epigenetic regulatory enzymes that convert 5-methyl-cytosine to 5-hydroxymethylcytosine.
b. Somatic or acquired TET2 mutations occur at high frequency across a spectrum of myeloid and lymphoid malignant tumors.
c. Most pathogenic mutations in TET2 result in complete or partial loss of function.
d. TET2 mutations are commonly missense mutations occurring anywhere within the coding region of the gene.

6. Pathway analysis is a tool in which genes that are complementary are frequently mutated together, whereas those that act along the same pathway or in the same complex are not. Based on the referenced Review, select the ONE statement that is NOT true: [See J Mol Diagn 2015, 17:339-351.]

a. In hematopoietic malignant tumors, TET2 mutations typically co-occur with mutations in the epigenetic regulators DNMT3B and DNMT1.
b. TET2 mutations are mutually exclusive with IDH1 and IDH2 mutations in hematopoietic malignant tumors.
c. The frequency of complementing mutations increases with tumors progression.
d. As knowledge of mutational patterns characteristic for specific cancer types increases, the pattern of co-occurring alterations in other genes can help resolve the nature of indeterminate calls.
7. Esophageal adenocarcinoma (EAC) most frequently develops in patients with Barrett’s esophagus (BE), estimated to affect 3.3 million adults in the United States. Based on the referenced article, select the ONE statement that is NOT true: [See J Mol Diagn 2015, 17:412-419.]

   a. BE results from injury of the esophageal mucosa associated with gastroesophageal reflux, which leads to esophagitis and eventually BE.
   b. The incidence of EAC has doubled over the past four decades in the United States, paralleling the increase in detection of esophageal reflux and diagnosis of BE.
   c. Barrett’s intestinal metaplasia (BIM) is characterized by the replacement of normal squamous esophageal mucosa by columnar epithelium with intestinal metaplasia.
   d. BIM often occurs in the background of patches of cardiac, oxyntic, or cardio-oxyntic type mucosa along the length of BE.

8. BE may harbor genomic mutations before histologic appearance of dysplasia and cancer and requires frequent surveillance. Based on the referenced article, select the ONE statement that is NOT true: [See J Mol Diagn 2015, 17:412-419.]

   a. Patients with BE without dysplasia have the same EAC risk as those with high-grade dysplasia.
   b. Current guidelines for prevention of EAC require repeat surveillance endoscopies with biopsies of the Barrett’s mucosa, followed by pathological examination to detect Barrett’s intestinal metaplasia and dysplasia.
   c. The detection of dysplasia is hampered by sampling errors and high interobserver diagnostic variability.
   d. Known risk factors associated with EAC include male sex, older age, white race, hiatal hernia size, length of Barrett’s epithelium, smoking, and high body mass index.