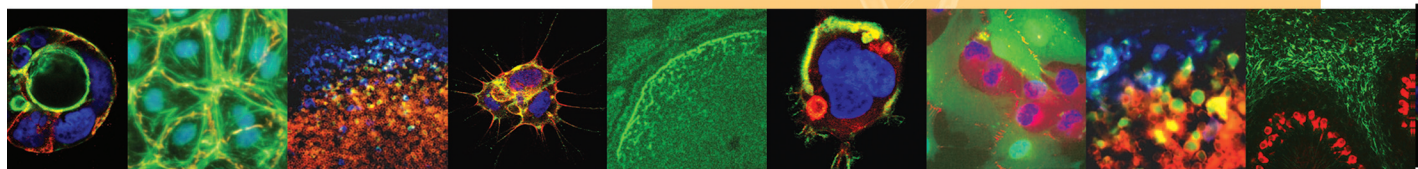




ASIP Journal CME Program

JMD CME Program in Molecular Diagnostics

Questions & Answers 2011



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Program Director: Mark E. Sobel, MD, PhD

NOTES

ASIP 2011 Journal CME Program

JMD 2011 CME Program in Molecular Diagnostics
<http://www.asip.org/CME/index.cfm>

American Society for Investigative Pathology *and the*
Association for Molecular Pathology

The Journal of Molecular Diagnostics (JMD) Volume 13

Mark E. Sobel, MD, PhD, Director of Journal CME Programs

Continuing Medical Education (CME) Accreditation Statement: This journal-based CME activity ("ASIP 2011 JMD CME Program in Molecular Diagnostics") has been planned and implemented in accordance with the Essential Areas and policies of the Accreditation Council for Continuing Medical Education (ACCME) through the joint sponsorship of the Federation of American Societies for Experimental Biology (FASEB) and the American Society for Investigative Pathology (ASIP). FASEB is accredited by the ACCME to provide continuing medical education for physicians.

FASEB designates this journal-based CME activity ("ASIP 2011 JMD CME Program in Molecular Diagnostics") for a maximum of 50 *AMA PRA Category 1 Credit(s)*[™]. Physicians should only claim credit commensurate with the extent of their participation in the activity.

The ASIP 2011 JMD CME Program in Molecular Diagnostics is an annual journal-based CME program consisting of a series of 50 questions based on selected articles in the 2011 issues (Volume 13) of *The Journal of Molecular Diagnostics* (JMD). Bimonthly exams, consisting of up to 10 questions that are based on selected articles appearing in each issue of the Journal, will be available online on the Journal website for registered participants. To receive CME credit for this journal-based CME activity, participants must achieve a score of at least 75% on each bimonthly exam and complete a Post-Test Evaluation. All exams must be completed by December 31, 2011 to receive CME credit. Participants will earn 10 *AMA PRA Category 1 Credit(s)*[™] for successful completion of the January 2011 exam (a minimum of 8 questions answered correctly) and will earn 8 *AMA PRA Category 1 Credit(s)*[™] for successful completion of each of the March – November 2011 exams (a minimum of 6 questions answered correctly for each bimonthly exam).

SAM Credit: The ASIP 2011 JMD CME Program in Molecular Diagnostics is approved by the American Board of Pathology for up to 50 SAM credits. Physicians should only claim credit commensurate with the extent of their participation in the activity. After successfully completing the CME exams as described above, participants may separately apply for SAM credit by completing SAM applications online on the ASIP website (www.asip.org). All SAM applications must be completed by December 31, 2011 for participants to receive SAM credit.

Objective/Target Audience: The objective of the ASIP 2011 JMD CME Program in Molecular Diagnostics is to increase basic and applied pathology knowledge, focusing on the molecular pathogenesis, diagnosis, prognosis, and the treatment of disease. The ASIP 2011 JMD CME Program in Molecular Diagnostics is designed to meet the participants' education needs in the physician competency area of Medical Knowledge, as defined by the Accreditation Council for Graduate Medical Education (ACGME) and the American Board of Medical Specialties (ABMS), and to support participants' lifelong learning towards a goal of promoting patient safety and improving patient care. This journal-based CME program is specifically targeted to trainees, clinicians and researchers investigating mechanisms of disease who wish to advance their knowledge of the cellular and molecular biology of disease.

Educational Objectives: At the completion of the ASIP 2011 JMD CME Program in Molecular Diagnostics, participants should be able to:

1. discuss the research underway and/or current molecular approaches to the diagnosis and prognosis of inherited diseases and syndromes;
2. discuss the research underway and/or current molecular approaches to pharmacogenetics, cytogenetics, DNA identity tests, and hematopathology (including clonality, translocations, and point mutations);
3. discuss the research underway and/or current molecular approaches to the diagnosis and prognosis of solid and soft tissue tumors;
4. discuss the research underway and/or current molecular approaches to the diagnosis of infectious diseases (including bacterial, fungal, viral, and parasitic pathogens);
5. discuss the research underway and/or current molecular approaches to the diagnosis and prognosis of acquired diseases spanning systems biology;
6. demonstrate a gained level of knowledge of the molecular methods and techniques being used by researchers and practitioners.

Disclosure Policy: The Federation requires that participants in FASEB-sponsored educational programs be informed of the organizers' and the presenters' (speaker, faculty, author, or contributor) academic and professional affiliation, and the existence of any relevant financial relationship an organizer or a presenter has with any proprietary entity producing health care goods or services consumed by, or used on patients, with the exemption of non-profit or government organizations and non-health care related companies. The intent of this disclosure is not to prevent a presenter from providing educational content but allows the participant to be fully knowledgeable in evaluating the information being presented.

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None of the organizers of this educational activity disclosed a relevant financial relationship. Relevant financial relationships of the authors of selected articles in this journal CME program will be disclosed in a footnote to the article and in each examination.

This Answer Booklet includes answer sheets for each of the six bimonthly exams. For each question, the correct answer is highlighted in red and an explanation of the correct answer is included as a "Rationale."

ASIP 2011 Journal CME Programs

ASIP 2011 JMD CME Program in Molecular Diagnostics

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ANSWERS for CME Questions # 1-10 **1d, 2a, 3b, 4b, 5c, 6d, 7c, 8b, 9c, 10a**

1. Laboratorians must be confident that samples used for prenatal analysis are of purely fetal origin. Based on the referenced Special Article, select the ONE statement that is NOT true: [See J Mol Diagn 2011, 13:7-11; DOI: 10.1016/j.jmoldx.2010.11.013; Narasimhan Nagan and Nicole Faulkner are employed by and have stock options with Genzyme Corporation; the other authors of the referenced article did not disclose any relevant financial relationships.]

- a. Maternal cell contamination (MCC) analysis is critically important in the prenatal diagnosis of inherited molecular, cytogenetic, and metabolic disorders and also can serve as an internal quality assurance measure to ensure that the biological mother is matched with her concordant fetus(es), thereby minimizing the possibility of sample mix-up within a prenatal setting.
- b. Contaminating maternal blood can be visualized in 1% to 2% of amniotic fluid (AF) samples and in up to 38% of pelleted amniocytes following centrifugation.
- c. Using highly sensitive molecular testing methods, MCC has been identified in 9.1% of direct or cultured fetal cell preparations, almost one-fifth of which had no visible evidence of maternal blood.
- d. **MCC occurs at a significantly higher rate among AF cultures than in direct (uncultured) AF samples.**

Rationale: MCC occurs at a significantly lower rate among AF cultures than in direct AF samples because culture conditions favor the growth of amniocytes and reduce or eliminate maternal blood cells. In contrast, in chorionic villus samplings (CVS) and abortus samples, the culturing process increases the risk of detectable MCC given the co-localization of maternal and fetal cell lineages in the placenta.

2. Professional organizations have developed standards and guidelines for cytogenetic and molecular genetic testing that recommend MCC testing for prenatal diagnosis; however, surveys of prenatal diagnostic practices in the United States illustrate a lack of standardization. Based on the referenced Special Article, select the ONE statement that is NOT true: [See J Mol Diagn 2011, 13:7-11; DOI: 10.1016/j.jmoldx.2010.11.013; Narasimhan Nagan and Nicole Faulkner are employed by and have stock options with Genzyme Corporation; the other authors of the referenced article did not disclose any relevant financial relationships.]

- a. **Maternal peripheral blood samples are required for MCC testing; maternal buccal samples are not helpful.**
- b. Acceptable specimen types for prenatal analysis include directly obtained, uncultured AF and CVS, cultured AF and CVS, cord blood, peripheral umbilical blood specimens, and products of conception.
- c. Laboratories should have procedures to ensure that every CVS specimen is thoroughly examined under a dissecting microscope to remove any contaminating maternal decidua prior to preparing both direct and long-term CVS cultures.
- d. Only one prenatal test sample should be handled at any one time to ensure accuracy and to prevent a potential mix-up of samples.

Rationale: Both maternal buccal and peripheral blood samples may be used in MCC testing. Paternal samples are not helpful for MCC analysis.

3. Technical guidelines recommend that MCC testing should be performed on DNA extracted from the same sample or culture that was used for concurrent clinical diagnostic testing. Based on the referenced Special Article, select the ONE statement that is NOT true: [See J Mol Diagn 2011, 13:7-11; DOI:

10.1016/j.jmoldx.2010.11.013; Narasimhan Nagan and Nicole Faulkner are employed by and have stock options with Genzyme Corporation; the other authors of the referenced article did not disclose any relevant financial relationships.]

- a. The MCC analysis should utilize a sufficient number of markers to accurately exclude MCC at the level of sensitivity previously determined by the laboratory during its initial MCC assay validation process.
- b. Dinucleotide markers are highly recommended monitors of MCC as opposed to larger repeat markers.
- c. When using capillary electrophoresis technology, peak height and signal-to-noise ratio thresholds should be monitored within the testing laboratory to reliably assess percentage MCC detectable following extraction and molecular amplification.
- d. Identical allelic markers between mother and fetus are uninformative for MCC.

Rationale: Tetranucleotide/pentanucleotide markers are preferable monitors of MCC over smaller repeat markers due to their superior fidelity, robustness of PCR amplification, accurate measurement of repeat units by fragment analysis, distinguishable alleles with a high discriminative capacity, and intergenerational stability among individuals in the general population. In general, MCC assays that include a larger number of polymorphic markers are preferable because of the increased likelihood of having included multiple informative markers that clearly demonstrate an unshared allele between mother and fetus. In addition, markers can differ in their informativeness when the level of MCC is at or near the assay's limit of detection. It is recommended that two to three informative microsatellite markers reflecting clearly definable, separate maternal and fetal genotypes from among a panel of approximately seven to 10 markers be employed to assess the presence of MCC in a prenatal sample.

4. Prenatal diagnostic results should not be released before the MCC analysis is completed. Based on the referenced Special Article, select the ONE statement that is NOT true: [See J Mol Diagn 2011, 13:7-11; DOI:

10.1016/j.jmoldx.2010.11.013; Narasimhan Nagan and Nicole Faulkner are employed by and have stock options with Genzyme Corporation; the other authors of the referenced article did not disclose any relevant financial relationships.]

- a. The MCC report should acknowledge that the absence or presence of MCC in the tested specimen does not rule in/out the presence or absence of contamination in another specimen or subspecimen obtained from the fetus under consideration.
- b. The presence of aneuploidy or copy number variation should be included in the MCC report when MCC assays incidentally identify the presence of chromosomal abnormalities.
- c. The sensitivity of the MCC assay should be indicated on all issued MCC reports.
- d. Multiple gestation pregnancies should be acknowledged on the MCC report along with a caveat regarding the potential impact of fetal sampling on the accuracy of MCC results.

Rationale: The MCC assay should not be used to report the presence of aneuploidy or copy number variation without confirmation by a second method validated specifically for this purpose.

5. Accurate identification of *Candida* yeast infections is essential for clinical management of invasive mycoses.

Based on the referenced article, select the ONE statement that is NOT true: [See J Mol Diagn 2011, 13:12-22; DOI: 10.1016/j.jmoldx.2010.11.014; the authors of the referenced article did not disclose any relevant financial relationships.]

- a. Recent epidemiologic series of invasive mycoses have highlighted a shift from *C. albicans* to other *Candida* species.
- b. *Candida* species other than *C. albicans* are either innately resistant or show decreased susceptibility patterns to azoles, amphotericin B, or echinocandins.
- c. Like bacteria, yeasts transfer resistance between cells, and acquisition of resistance is widely observed in clinical settings.
- d. *C. guilliermondii* strains have decreased susceptibility to echinocandins compounds.

Rationale: There is no transfer of resistance between yeast cells. Acquisition of resistance is mainly observed in restricted clinical settings such as allogeneic blood marrow transplant or AIDS patients under sustained prolonged azole treatment. In a large cohort of 2,019 patients, less than half of the invasive mycosis isolates were *C. albicans*. The decreased susceptibility of *C. guilliermondii* strains to echinocandins compounds has a significant clinical impact since the latter are recommended for first-line therapy of candidiasis.

6. Molecular diagnostic assays for fungal infections may improve species characterization, particularly in cases of closely related *Candida* species. Based on the referenced article, select the ONE statement that is NOT true: [See J Mol Diagn 2011, 13:12-22; DOI: 10.1016/j.jmoldx.2010.11.014; the authors of the referenced article did not disclose any relevant financial relationships.]

- a. Current methods for yeast identification in clinical practice, which are based on phenotypic features and carbohydrate assimilation tests, require at least 2 days.
- b. The authors used a molecular fingerprinting method involving PCR and restriction fragment length polymorphism (RFLP) analysis to discriminate closely related yeast species.
- c. Molecular identification of *C. albicans*, *C. dubliniensis*, *C. glabrata*, *C. bracarensis*, *C. nivariensis*, and *C. tropicalis* was accomplished using selected primers for partial amplification of the intergenic spacer (IGS) of rDNA, whereas amplification of the complete IGS domain was necessary to discriminate other closely related yeast species.
- d. *C. krusei* was reliably identified using primers for amplification of the complete IGS domain.

Rationale: *C. krusei* accounts for less than 4% of *Candida* isolates, although it occurs in 24% of invasive candidiasis cases in patients with hematological malignancies. Its rapid identification is critical since it is intrinsically resistant to azoles compounds. However, *C. krusei* was not successfully amplified using the sets of PCR primers in the referenced study. *C. krusei* can be reliably and rapidly recognized using presumptive identification tests, such as surface pellicle formation and color of the colonies on special agar plates, and can be confirmed within 15 minutes with a latex-agglutination test.

7. The epidermal growth factor receptor (EGFR) signaling pathway is commonly up-regulated in colorectal carcinoma (CRC). Based on the referenced article, select the ONE statement that is NOT true: [See J Mol Diagn 2011, 13:64-73; DOI: 10.1016/j.jmoldx.2010.11.005; the authors of the referenced article did not disclose any relevant financial relationships.]

- a. The broad use of EGFR-targeting monoclonal antibodies has shown a significant clinical benefit in less than 25% of CRC patients.
- b. The presence of *KRAS* mutations is consistently correlated with lack of response to anti-EGFR monoclonal antibodies.
- c. The vast majority of *KRAS* mutations occur in exon 4.
- d. CRC patients with mutations in *BRAF* exhibit resistance to anti-EGFR monoclonal antibodies.

Rationale: Mutations in the small G protein RAS have been reported in 30% to 40% of CRC patients and are the most common predictors of resistance to EGFR-targeting agents. The vast majority of *KRAS* mutations occur in codons 12 and 13 of exon 2. The remainder are predominantly located in codons 61 and 146. Mutations in *KRAS* and *BRAF* are mutually exclusive.

8. Recently published guidelines by the American Society of Clinical Oncology (ASCO) recommend *KRAS* mutation testing in all patients being considered for anti-EGFR monoclonal antibody treatment. Based on the referenced article, select the ONE statement that is NOT true: [See J Mol Diagn 2011, 13:64-73; DOI: 10.1016/j.jmoldx.2010.11.005; the authors of the referenced article did not disclose any relevant financial relationships.]

- a. Sanger sequencing of PCR-amplified DNA is the classic and most widely employed method of mutation detection; however, it is labor-intensive, has suboptimal sensitivity, and is a low-throughput technique.
- b. The Sanger sequencing method is a reasonable approach for the routine testing of *KRAS* and *BRAF* point mutations in lesions with greater than 6% tumor.
- c. When locked nucleic acid (LNA) probes complementary to the wild-type sequences of *KRAS* and *BRAF* are added to the PCR amplification step, they hybridize with high affinity to wild-type *KRAS* and *BRAF*, effectively suppressing the amplification of nonmutant, wild-type DNA, and leading to preferential amplification of the mutant allele.
- d. LNA-PCR requires the concurrent performance of standard PCR for quality control.

Rationale: The Sanger sequencing method is a very reasonable approach for the routine testing of point mutations in both *KRAS* and *BRAF*, provided that testing is based on lesions with greater than 50% tumor. This is a tumor percentage that can be easily obtained from an untreated primary lesion or a well-defined metastasis without treatment or extensive inflammatory infiltrate. Microdissection may be necessary to improve the percentage of tumor cells. The addition of LNA probes can improve the detection sensitivity of *KRAS* and *BRAF* mutations to below 1%. LNA probes are nucleic acid analogs that contain a 2'-O to 4'-C methylene bridge locking the ribose group into a C3'-endo conformation. Introduction of LNA monomers into oligonucleotides increases the melting temperature of DNA heteroduplexes; these modified oligonucleotides bind to complementary sequences with high affinity and can be used for mutant enrichment. LNA-PCR should be performed concurrently with standard PCR for quality control. In the absence of a mutation, the LNA may completely suppress amplification of wild-type DNA, generating no PCR product, which may be indistinguishable from a PCR failure for other reasons.

9. Lynch syndrome is an autosomal dominant predisposition syndrome in which patients have a propensity to develop a broad spectrum of neoplasms, including colorectal adenocarcinomas. Based on the referenced article, select the ONE statement that is NOT true: [See J Mol Diagn 2011, 13:93-99; DOI: 10.1016/j.jmoldx.2010.11.011; the authors of the referenced article did not disclose any relevant financial relationships.]

- a. The underlying genetic basis for Lynch syndrome is the presence of a mutation in one of the DNA mismatch repair (MMR) genes: *MLH1*, *MSH2*, *MSH6*, or *PMS2*.
- b. The defining phenotype of tumors from Lynch syndrome patients is the presence of tumor microsatellite instability (MSI) and loss of protein expression of the affected enzyme in the tumor nuclei as detected by immunohistochemical staining.
- c. Germline deletions involving the 3' end of *EPCAM*, located approximately 16 kb downstream of *MSH2*, result in hypomethylation of the *MSH2* promoter region and subsequent loss of *MSH2* expression from the affected allele.
- d. Approximately 20% to 25% of cases suspected of having a mutation in *MSH2* in which a germline mutation is not detected can be accounted for by germline deletions in *EPCAM*.

Rationale: *EPCAM* is also known as *TACSTD1*. *EPCAM/TACSTD1* is located approximately 16 kb upstream of *MSH2*. Germline deletions involving the 3' end of *EPCAM/TACSTD1* result in hypermethylation of the *MSH2* promoter region. Hypermethylation of the *MSH2* promoter results in loss of expression from the affected allele.

10. Chromosomal translocations and small insertion/deletions occur in specific hematological malignancies and solid tumors. Based on the referenced article, select the ONE statement that is NOT true: [See J Mol Diagn 2011, 13:93-99; DOI: 10.1016/j.jmoldx.2010.11.011; the authors of the referenced article did not disclose any relevant financial relationships.]

- a. The fusion transcript in follicular lymphoma (*BCL2-IGH*) can be detected with high sensitivity and specificity at the RNA level by RT-PCR across the involved exon junction.
- b. PCR detection of chromosomal translocations and small insertion/deletion mutations is challenging when potential amplicon size varies greatly, such as with internal tandem duplication mutation of the *FLT3* gene in leukemia, where breakpoints are widely distributed.
- c. Δ -PCR is a strategy that uses two forward primers and one reverse primer simultaneously to detect translocations and insertion/deletion mutations. The internal forward primer functions as a probe with a defined distance (delta) from the external primer.
- d. Multiplex Δ -PCR can detect and simultaneously distinguish the small insertion/deletion mutations of the *FLT3*, *GATA1*, and *WT1* genes of a leukemic clone.

Rationale: The molecular mechanism in many lymphoid malignancies involves the approximation of an oncogene and an actively transcribed gene resulting in overproduction of the oncogene without a chimeric mRNA or protein. This is the case for follicular lymphoma (*BCL2-IGH*). The translocation must be detected at the DNA level by PCR because of the lack of a fusion transcript.

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ANSWERS for CME Questions March # 1-8

1c, 2b, 3a, 4d, 5b, 6d, 7c, 8a

1. Neurofibromatosis type 1 (NF1) is caused by mutations in the tumor suppressor gene neurofibromin (NF1). Based on the referenced Technical Advance article, select the ONE statement that is NOT true: [See J Mol Diagn 2011, 13:113-122; DOI: 10.1016/j.jmoldx.2010.09.002; the authors of the referenced article did not disclose any relevant financial relationships.]

- a. NF1 is an autosomal dominant disorder with an incidence of 1 in 3,500 live births.
- b. Symptoms of patients with NF1 include freckling, cutaneous neurofibrosis, and an increased risk for malignant tumors.
- c. **NF1 is a small gene, composed of 3 exons.**
- d. NF1 is fully penetrant but has highly variable expression, even within the same family.

Rationale: NF1 is a large gene, spanning more than 350 kb of genomic DNA. It is comprised of 57 constitutive exons and three alternatively spliced exons, encoding an 11-13 kb ubiquitous transcript.

2. Identifying lesions that cause NF1 is difficult due to the complexity of the NF1 gene. Based on the referenced Technical Advance article, select the ONE statement that is NOT true: [See J Mol Diagn 2011, 13:113-122; DOI: 10.1016/j.jmoldx.2010.09.002; the authors of the referenced article did not disclose any relevant financial relationships.]

- a. NF1 lacks mutation hotspots, preventing directed mutation screening.
- b. **Few NF1 mutations (~5% to 10%) are small lesions; most mutations are large deletions encompassing NF1 and its neighboring genes (~85% to 90%).**
- c. Although one germline mutation of the NF1 gene may not be sufficient to cause cancer development, NF1 has a very high mutation rate, and a second somatic hit produces a tumor.
- d. Approximately 50% of all NF1 patients are sporadic cases.

Rationale: Most NF1 mutations (~85% to 90%) are small lesions, such as single base substitutions, insertions, or deletions. Other mutations are single or multi-exon deletions or duplications (~2%), while the remaining mutations are microdeletions encompassing NF1 and its neighboring genes (~5% to 10%). The NF1 gene is subject to one of the highest mutation rates known for human genes.

3. Diverse techniques have been used to identify NF1 mutations. Based on the referenced Technical Advance article, select the ONE statement that is NOT true: [See J Mol Diagn 2011, 13:113-122; DOI: 10.1016/j.jmoldx.2010.09.002; the authors of the referenced article did not disclose any potential conflicts of interest.]

- a. **Variations in entire-gene deletions and/or intragenic copy number can be identified with the protein truncation test (PTT) and single strand conformation polymorphisms (SSCP).**
- b. Entire-gene deletions can be tested by fluorescence *in situ* hybridization (FISH), Southern blotting, array comparative genomic hybridization (aCGH), and multiplex ligation-dependent probe amplification (MLPA).
- c. Screening for NF1 mutations revealed a high proportion of mutations affecting the correct splicing of the gene, emphasizing the importance of mRNA studies.
- d. The authors analyzed the entire NF1 coding region using an original RNA-based assay by complementing denaturing high-performance liquid chromatography (DHPLC) to carry out a mutational screening of NF1 cDNA with MLPA to identify microdeletions and alterations in intragenic copy number.

Rationale: FISH, Southern blotting, aCGH, and MLPA are used to identify entire-gene deletions and/or variation in intragenic copy number. PTT and SSCP are not used to study variation in intragenic copy number or entire-

gene deletions. Intragenic lesions have been identified with diverse techniques including PTT, SSCP, denaturing gradient gel electrophoresis (DGGE), temperature gradient gel electrophoresis (TGGE), DHPLC, and cDNA sequencing. Each method possesses varying degrees of sensitivity.

4. Fibrous dysplasia (FD) is a benign fibro-osseous lesion of the medullary cavity. Based on the referenced article, select the ONE statement that is NOT true: [See J Mol Diagn 2011, 13:137-142; DOI: 10.1016/j.jmoldx.2010.10.003; the authors of the referenced article did not disclose any relevant financial relationships.]

- a. FD and other benign fibro-osseous lesions frequently have overlapping histologic features, making differentiation of FD and other benign fibro-osseous lesions difficult.
- b. A mutation in the α subunit of G protein ($GS\alpha$) has been identified in FD but not in other benign fibro-osseous lesions.
- c. Point mutations in codon 201 of $GS\alpha$ result in replacement of Arg with another amino acid, primarily His or Cys.
- d. **The mutated $GS\alpha$ (Arg→His) is not a functional protein.**

Rationale: The mutated $GS\alpha$ (G→A mutation for Arg→His as well as other point mutations) is constitutively activated, leading to cAMP activation and downstream physiological responses in affected tissues, including bone, skin, endocrine glands, and other tissues.

5. Arg201 detection requires an extremely sensitive and quantitative assay. Based on the referenced article, select the ONE statement that is NOT true: [See J Mol Diagn 2011, 13:137-142; DOI: 10.1016/j.jmoldx.2010.10.003; the authors of the referenced article did not disclose any relevant financial relationships.]

- a. The Arg201 mutation results in a mosaic distribution of mutated to normal cells, which may affect the severity of phenotypic expression.
- b. **The percentage of mutant stem cells increases as a lesion ages.**
- c. The percentage of mutated cells within an FD lesion decreases with age, potentially resulting in false negative results in older patients.
- d. Direct sequencing and restriction fragment length polymorphism (RFLP) are the two most widely used methods to identify Arg201 mutations.

Rationale: The percentage of mutant stem cells in an FD lesion decreases as a lesion progresses, resulting in “normalization” of FD of bone.

6. Pyrosequencing can provide an alternative for sequence analysis, especially for targeted mutation identification, as is the case for FD-associated mutations. Based on the referenced article, select the ONE statement that is NOT true: [See J Mol Diagn 2011, 13:137-142; DOI: 10.1016/j.jmoldx.2010.10.003; the authors of the referenced article did not disclose any relevant financial relationships.]

- a. Peptide nucleic acid clamping during direct sequencing enables detection when the mutant contents are below analytical sensitivity, but this technique is not meant to measure the true percentage of mutant species in the lesion.
- b. RFLP cannot detect novel mutation types and usually requires more than one restriction enzyme if the mutation type needs to be specified.
- c. The pyrosequencing approach of Liang et al has a detection sensitivity of approximately 5% of affected cells admixed with normal tissue.
- d. **The pyrosequencing methodology used by Liang et al sequentially identified the two most common types of mutation in FD in separate reactions.**

Rationale: The pyrosequencing methodology identified not only the two most common types of FD mutation (Arg→His and Arg→Cys) simultaneously in a single reaction, but also other mutations.

7. Early detection of tumors and metastasis is critical for improving treatment strategies and patient outcome. Based on the referenced article, select the ONE statement that is NOT true: [See J Mol Diagn 2011, 13:189-198; DOI: 10.1016/j.jmoldx.2010.10.002; the authors of the referenced article did not disclose any relevant financial relationships.]

- a. About 90% of all cancer deaths arise from the metastatic dissemination of primary tumors.
- b. Primary colon carcinomas cannot be sufficiently distinguished by clinical outcome parameters, such as local recurrence and metastasis, via conventional clinical and histopathological/immunohistochemical examination.

- c. The lymph node-metastasis classification represents the main tool for identifying prognostic differences among patients with early-stage colorectal cancer.
- d. There is a clear need to develop molecular markers that can be used clinically for detection, prognosis, and therapeutic efficacy monitoring for cancer.

Rationale: The tumor-node-metastasis (TNM) classification represents the main tool for identifying prognostic differences among patients with early-stage colorectal cancer. This histological staging system is also used for gastric cancer.

8. Circulating nucleic acids can be detected in plasma, permitting plasma-based molecular expression profiling. Based on the referenced article, select the ONE statement that is NOT true: [See J Mol Diagn 2011, 13:189-198; DOI: 10.1016/j.jmoldx.2010.10.002; the authors of the referenced article did not disclose any relevant financial relationships.]

- a. Extracellular plasma DNA from colon cancer patients is confined in a vesicle-like structure and is enriched in mitochondrial DNA (mt-DNA).
- b. The quantitative detection of tumor-derived nucleic acids in blood might allow the identification of occult tumors and metastases in apparently healthy individuals.
- c. Blood-based diagnostics are not only useful for snap shots like tumor marker determination in patients' biopsies, but they also allow monitoring of disease progression and therapeutic efficacy and response.
- d. Stein et al provide a reliable and simple plasma-based assay for transcript quantification of the metastasis-promoting gene *S100A4*.

Rationale: Extracellular plasma RNA from colon cancer patients is confined in a vesicle-like structure and is mRNA-enriched.

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ANSWERS for CME Questions May # 1-8 1a, 2d, 3b, 4d, 5b, 6b, 7a, 8c

1. Osteosarcoma is the most common bone malignancy in humans. Based on the referenced article, select the ONE statement that is NOT true: [See J Mol Diagn 2011, 13:289-296; DOI: 10.1016/j.jmoldx.2010.11.020; the authors did not disclose any potential conflicts of interest.]

- a. Osteosarcoma lesions most frequently occur during early bone development in infants.
- b. Patients with recurrent or metastatic osteosarcoma have a less than 20% long-term survival rate.
- c. Overall, 5-year survival is approximately 65%.
- d. Osteosarcoma patients frequently need debilitating surgery in addition to chemotherapy.

Rationale: Osteosarcoma lesions most commonly occur in long bones as a high-grade metaphyseal lesion during the adolescent growth spurt.

2. Osteosarcoma typically has a heterogeneous pattern of unbalanced, complex chromosomal abnormalities reflecting genomic instability. Based on the referenced article, select the ONE statement that is NOT true: [See J Mol Diagn 2011, 13:289-296; DOI: 10.1016/j.jmoldx.2010.11.020; the authors did not disclose any potential conflicts of interest.]

- a. Unlike osteosarcoma, soft tissue sarcomas are classically associated with singular reproducible cytogenetic aberrations.
- b. The genomic complexity of osteosarcoma includes karyotypic gains and losses.
- c. Current prognosis for osteosarcoma relies on the presence or absence of metastasis at presentation and the percentage of tumor necrosis following neo-adjuvant chemotherapy, as estimated by the Huvos grading system.
- d. Early reports implicate copy number changes in chromosome 4 in the prognosis of osteosarcoma.

Rationale: Early reports implicate copy number changes in chromosome 19 in the prognosis of osteosarcoma.

3. Lockwood et al used comparative genomic hybridization (CGH) to evaluate DNA copy number changes in osteosarcoma tumor samples. Based on the referenced article, select the ONE statement that is NOT true: [See J Mol Diagn 2011, 13:289-296; DOI: 10.1016/j.jmoldx.2010.11.020; the authors did not disclose any potential conflicts of interest.]

- a. A whole-genome tiling path array was used for CGH analysis.
- b. 62 genes were over-expressed in cancer as compared with normal tissues.
- c. The list of amplified genes included previously identified targets such as MYC.
- d. The novel target Cyclin E1 was amplified in osteosarcoma.

Rationale: Review of the expression profile of osteosarcoma xenograft models showed that 43 genes were over-expressed in cancer as compared to normal tissue samples.

4. Next generation sequencing (NGS) describes high-throughput, massively paralleled DNA sequencing methods. Based on the referenced article, select the ONE statement that is NOT true: [See J Mol Diagn 2011, 13:325-333; DOI: 10.1016/j.jmoldx.2011.01.006; the authors did not disclose any potential conflicts of interest.]

- a. NGS provides massive amounts of sequencing data for relatively little cost.
- b. NGS studies of tumor genomes/exomes have changed our understanding of these disease processes.
- c. Tumor genome studies have been performed for acute myeloid leukemia, prostate cancer, and breast cancer.
- d. The cost of sequencing is rapidly decreasing, costing \$2,000 per genome in January 2011.

Rationale: The cost of sequencing is rapidly decreasing, costing less than \$20,000 per genome in January 2011 as compared with \$2.7 billion for the initial human genome sequence.

5. The source of tissue specimens greatly influences the quality of NGS data. Based on the referenced article, select the ONE statement that is NOT true: [See J Mol Diagn 2011, 13:325-333; DOI: 10.1016/j.jmoldx.2011.01.006; the authors did not disclose any potential conflicts of interest.]

- a. In routine clinical practice, the most common tissue samples available are formalin-fixed paraffin-embedded (FFPE) specimens.
- b. Most targeted NGS approaches were developed and optimized for high-quality DNA extracted from FFPE tissues.
- c. Fresh tissue is available for laboratory testing in many inherited diseases, hematolymphoid malignancies, and some solid tumors.
- d. Banked frozen tissue for many rare tumors is scarce, making it difficult to perform retrospective studies of these malignancies.

Rationale: Nearly all NGS approaches were developed and optimized for high quality DNA extracted from fresh tissue, which is less readily available than FFPE specimens.

6. Recent technical advances have made it possible to target defined regions of the genome using NGS methodologies. Based on the referenced article, select the ONE statement that is NOT true: [See J Mol Diagn 2011, 13:325-333; DOI: 10.1016/j.jmoldx.2011.01.006; the authors did not disclose any potential conflicts of interest.]

- a. Genome-partitioning approaches allow performance of deep-sequencing analysis of the exome or of groups of genes correlated with specific diseases.
- b. Targeted NGS methodologies require a low initial investment (often <\$1,000), which varies based on the number of genes or region to be evaluated.
- c. Duncavage et al report the development of a laboratory-generated hybrid-capture enrichment method that is optimized for DNA extracted from FFPE tissue.
- d. Duncavage et al reproducibly detected viral genome deletions and insertion sites from Merkel cell polyomavirus anywhere within the human genome.

Rationale: Targeted NGS methodologies require a high initial investment (often >\$10,000), depending on the number of genes or regions to be evaluated.

7. β -arrestin-1 is an adaptor protein that regulates signaling and trafficking of G-protein-coupled receptors. Based on the referenced article, select the ONE statement that is NOT true: [See J Mol Diagn 2011, 13:340-351; DOI: 10.1016/j.jmoldx.2011.01.009; the authors did not disclose any potential conflicts of interest.]

- a. β -arrestin-1 (also termed arrestin 2) is exclusively expressed in retinal photoreceptors.
- b. The β -arrestin adaptor proteins are activated and phosphorylated by G-protein-coupled receptor kinases.
- c. β -arrestins function as scaffold proteins that interact with a number of different signaling molecules.
- d. Signaling pathways reported to be modulated by β -arrestins include the TGF- β , IFG-1R, PI3K, and MAPK pathways.

Rationale: β -arrestin-1 (also termed arrestin 2), like β -arrestin-2 (also termed arrestin 3), is expressed in virtually all tissues. Arrestins 1 and 4 are exclusively expressed in retinal photoreceptors.

8. β -arrestins have been implicated in multiple types of cancer. Based on the referenced article, select the ONE statement that is NOT true: [See J Mol Diagn 2011, 13:340-351; DOI: 10.1016/j.jmoldx.2011.01.009; the authors did not disclose any potential conflicts of interest.]

- a. In colorectal cancer, the interaction between β -arrestin-1 and proto-oncogene SRC (alias c-Src) is critical for cell migration *in vitro* and metastatic spread to the liver *in vivo*.
- b. Murine liver cancer and lymphoma cells inoculated into β -arrestin -1 transgenic mice formed tumors more rapidly than in both β -arrestin-2 transgenic and wild-type mice.
- c. Migration of an invasive breast cancer cell line has been shown to be increased following knockdown of the β -arrestins.
- d. The authors link high expression of stromal β -arrestin -1 to reduced patient survival, and patients with low or moderate levels of stromal β -arrestin-1 did not benefit from tamoxifen.

Rationale: Migration of the invasive breast cancer cell line MDA-MB-231 has been shown to be reduced following knockdown of β -arrestins. β -arrestin-2 has in addition been implicated in cell death in MCF-7 and MDA-MB-231 cells by blocking morphine-induced cell death through the pro-apoptotic caspase-8 pathway.

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ANSWERS for CME Questions July # 1-8 **1c, 2d, 3b, 4c, 5b, 6d, 7a, 8d**

1. DNA from formalin-fixed, paraffin-embedded (FFPE) tissues can be utilized for human papillomavirus (HPV) testing, but potential technical problems have limited the use of FFPE specimens in retrospective epidemiology studies. Based on the referenced Technical Advance, select the ONE statement that is NOT true: [See J Mol Diagn 2011, 13:377-381; DOI: 10.1016/j.jmoldx.2011.03.007; the authors did not disclose any relevant financial relationships.]

- a. Fragmentation and DNA-protein cross-linking by formaldehyde exposure, as well as the presence of paraffin, can negatively affect both DNA yield and amplification efficiency.
- b. Labor intensity, chemical toxicity, and the risk of accidentally removing small tissue fractions are disadvantages of the use of xylene and ethanol to physically remove paraffin wax during pretreatment.
- c. **Tissue sections may occasionally stick to the upper walls of sample tubes subjected to the traditional xylene wash step, resulting in less than optimal contact of the specimen with the buffer.**
- d. Aggressive heat treatment (120°C) results in higher DNA yields and increased sensitivity for HPV testing, demonstrating an advantage over traditional xylene purification protocols..

Rationale: Tissue sections occasionally stick to the upper walls of the tube during the high heat incubation step such that consistent temperature of and buffer contact with the specimen may be less than optimal. Therefore, it is recommended that tubes be gently shaken or finger-flicked and that there be visual verification that tissue fractions are floating in the lysis buffer shortly after paraffin has melted. Disadvantages of the traditional xylene wash step include the manual pipetting labor and time effort, the tedious separation of liquids from tissue fragments, and the danger of accidentally removing small tissue fractions by pipetting.

2. Drug-resistant *Mycobacterium tuberculosis* (MTB) strains and nontuberculous mycobacterium (NTM) have emerged recently. Based on the referenced article, select the ONE statement that is NOT true: [See J Mol Diagn 2011, 13:390-394; DOI: 10.1016/j.jmoldx.2011.02.004; the authors did not disclose any relevant financial relationships.]

- a. The gold standard method of identifying mycobacteria is a mycobacterial culture; however, surgically resected tissues are usually fixed in formalin and are consequently not amenable to culture.
- b. As an alternative rapid diagnostic tool, conventional MTB PCR, targeting *IS6110*, *TRC4*, or *GCRS*, has less sensitivity and specificity when using FFPE specimens.
- c. In FFPE specimens, the sensitivity of real-time MTB PCR (targeting the *senX3-regX3* intergenic region) was significantly higher than that of nested MTB PCR.
- d. **The positive predictive value of nested MTB PCR in FFPE specimens was 54.5% compared with acid-fast-bacilli (AFB) culture results of fresh specimens from the same patients.**

Rationale: The sensitivity, specificity, positive predictive value, and negative predictive value of nested MTB PCR were 68.3%, 98.5%, 97.7%, and 76.5%, respectively, compared with AFB culture results.

3. NTM is an increasingly important cause of infections, especially in patients with underlying chronic diseases, immunosuppression, and HIV infection. Based on the referenced article, select the ONE statement that is NOT true: [See J Mol Diagn 2011, 13:390-394; DOI: 10.1016/j.jmoldx.2011.02.004; the authors did not disclose any relevant financial relationships.]

- a. Of 73 cases with histological features of tuberculosis, four were positive by AFB staining, negative by nested or real-time PCR, and positive for growth in AFB cultures.
- b. Although the spectrum of pathological changes that are characteristic of NTM infection is generally similar to that seen in ordinary MTB infection, caseous necrosis was a definitive diagnostic feature that distinguished NTM from MTB infection.
- c. NTM infection should be included in the differential diagnosis of suspected MTB cases that are positive by AFB staining but negative by nested or real-time PCR.
- d. Liquid media support the growth of MTB and NTM better than solid media.

Rationale: The spectrum of pathological changes that are characteristic of NTM infection is similar to that seen in ordinary MTB infection. There is no definitive diagnostic feature for NTM infections. However, some characteristics, including defined granulomas and lack of significant caseation, have been reported to help in differentiating NTM from MTB infections.

4. *Clostridium difficile* has been associated with antibiotic-induced diarrhea and pseudomembranous colitis for over thirty years. Based on the referenced article, select the ONE statement that is NOT true: [See J Mol Diagn 2011, 13:395-400; DOI: 10.1016/j.jmoldx.2011.03.004; reagent kits for the BD GeneOhm Cdiff assay were provided by Becton, Dickinson and Co., and the Xpert *C. difficile* test was provided by Prodesse, Inc.; the authors did not disclose any other relevant financial relationships.]

- a. *C. difficile* is an anaerobic, spore-forming, gram-positive bacillus.
- b. Toxigenic strains of *C. difficile* are transmitted via the fecal-oral route.
- c. Annually, there are more reported deaths from *C. difficile* infection (CDI) acquired in hospitals than from CDI acquired in nursing homes.
- d. Although CDI was historically associated primarily with antibiotic treatment, community-acquired CDI is now recognized.

Rationale: According to annual data from the Centers for Disease Control and Prevention, there are 9,000 deaths from hospital-acquired CDI, 3,000 post-discharge deaths, and 16,500 deaths from nursing home-acquired CDI.

5. Both non-molecular and molecular methods are currently in use for detection of CDI. Based on the referenced article, select the ONE statement that is NOT true: [See J Mol Diagn 2011, 13:395-400; DOI: 10.1016/j.jmoldx.2011.03.004; reagent kits for the BD GeneOhm Cdiff assay were provided by Becton, Dickinson and Co., and the Xpert *C. difficile* test was provided by Prodesse, Inc.; the authors did not disclose any other relevant financial relationships.]

- a. The molecular assays for CDI center on detecting the *tcdB* gene.
- b. The three molecular methods used in this study have not yet been FDA-cleared for the detection of the *tcdB* gene.
- c. The glutamate dehydrogenase (GDH) antigen/toxin A/B enzyme immunoassay was the least sensitive of the tested methods.
- d. The most costly molecular assay had the advantage of rapid results and limited processing requirements.

Rationale: All three molecular tests have been FDA-cleared for detection of the *tcdB* gene.

6. Pendred syndrome and DFNB4 (nonsyndromic congenital deafness, locus 4) are associated with congenital sensorineural hearing loss. Based on the referenced article, select the ONE statement that is NOT true: [See J Mol Diagn 2011, 13:416-426; DOI: 10.1016/j.jmoldx.2011.03.003; the authors did not disclose any relevant financial relationships.]

- a. Pendred syndrome and DFNB4 are autosomal recessive disorders associated with mutations in the *SLC26A4* and *FOXI1* genes.
- b. Pendrin, the protein product of *SLC26A4*, is involved in the anion exchange of chloride, iodide, and bicarbonate and is necessary for inner ear function.
- c. Clinically, both Pendred syndrome and DFNB4 are associated with temporal bone abnormalities.
- d. An abnormal perchlorate discharge test and/or euthyroid goiter can be present in both Pendred syndrome patients and DFNB4 patients.

Rationale: An abnormal perchlorate discharge test and/or euthyroid goiter can be present in patients with Pendred syndrome but not in those with DFNB4. Allelic heterogeneity is a possible cause of the different phenotypes due to differences in residual activity of the protein products.

7. Currently, clinical testing is available for **SLC26A4** and not for **FOXI1**. Based on the referenced article, select the **ONE** statement that is **NOT** true: [See J Mol Diagn 2011, 13:416-426; DOI: 10.1016/j.jmoldx.2011.03.003; the authors did not disclose any relevant financial relationships.]

- a. Numerous different mutations have been identified in all 18 exons of **SLC26A4**.
- b. **FOXI1** is a member of the forkhead family of winged helix transcription regulators and is a transcription activator of **SLC26A4**.
- c. Mutations in both the **FOXI1** gene itself and at the **FOXI1** binding site in the promoter of the **SLC26A4** gene have been described in both Pendred syndrome and DFNB4.
- d. While up to 50% of the multiplex families with Pendred syndrome or DFNB4 have been reported to carry **SLC26A4** mutations, **FOXI1** gene mutations are only detected in approximately 1% of probands.

Rationale: SLC26A4 is composed of 21 exons, and mutations have been identified in 20 exons. More than 180 variants have been identified throughout the gene, including in introns and splice sites.

8. High resolution melting (HRM) is a mutation screening method with potential for cost-effective high-throughput clinical testing. Based on the referenced article, select the **ONE** statement that is **NOT** true: [See J Mol Diagn 2011, 13:416-426; DOI: 10.1016/j.jmoldx.2011.03.003; the authors did not disclose any relevant financial relationships.]

- a. HRM can detect both heterozygous and homozygous sequence changes by monitoring the changes in melting temperature and melting curve shape compared to a reference sample.
- b. Denaturing high performance liquid chromatography (dHPLC) is an example of a mutation scanning method that is accurate and reliable; however, dHPLC inherently cannot distinguish homozygous mutations from wild-type sequences without the mixing of PCR products derived from wild-type DNA with those generated from a patient sample to form heteroduplexes.
- c. With HRM analysis, Sanger sequencing is only necessary for amplicons in which aberrant melt curves are observed.
- d. **HRM, in general, has a reported analytical sensitivity for variant detection of 2%.**

Rationale: HRM, in general, has a reported analytical sensitivity for variant detection of 5%. The SLC26A4 HRM assay was developed to identify variants in Pendred syndrome or DFNB4, inherited genetic disorders that would have 0% (no variants), 50%, or 100% variants in the sample for a wild-type, heterozygous, or homozygous genotype, respectively. As long as the variant generates adequate melt curves for HRM analysis, the HRM method with an analytical sensitivity of 5% should detect variants with a level of 50% or 100% in the actual test samples.

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ANSWERS for CME Questions September # 1-8

1a, 2c, 3c, 4b, 5b, 6a, 7a, 8b

1. Multiple mutations are associated with *BCR-ABL1*-negative myeloproliferative neoplasms (MPNs). Based on the referenced Review, select the ONE statement that is NOT TRUE: [See J Mol Diagn 2011, 13:461-466; DOI: 10.1016/j.jmoldx.2011.05.007; the authors of the referenced article did not disclose any relevant financial relationships.]

- a. The clonal hierarchy of tumors that possess more than one genetic mutation associated with MPNs can be easily determined through genetic technology-based assays.
- b. The disease-initiating mutation in *BCR-ABL1*-negative MPN is unknown.
- c. The *JAK2V617F* mutation is found in a majority of *BCR-ABL1*-negative MPN patients.
- d. A minority of *BCR-ABL1*-negative MPN patients have mutations in the *MPL*, *LNK*, *CBL*, or *DNMT3A* genes.

Rationale: Clonal hierarchy is not easily predicted in tumors that possess more than one MPN-associated genetic mutation.

2. Specific mutations associated with MPNs are linked to specific leukemic disorders. Based on the referenced Review, select the ONE statement that is NOT TRUE: [See J Mol Diagn 2011, 13:461-466; DOI: 10.1016/j.jmoldx.2011.05.007; the authors of the referenced article did not disclose any relevant financial relationships.]

- a. *JAK2V617F* contributes to abnormal myeloproliferation in MPN.
- b. *JAK2* exon 12 mutations tend to contribute to erythroid lineage-weighted disorders.
- c. *MPL* mutations usually involve exon 8 and contribute primarily to megakaryocytic myeloproliferation.
- d. *LNK* mutations mapping to exon 2 are more prevalent in blast-phase MPN rather than chronic-phase disease.

Rationale: Although *MPL* mutations primarily contribute to megakaryocytic myeloproliferation, the most commonly associated mutation occurs in exon 10.

3. Multiple factors should be considered with respect to routine screening of *JAK2* or *MPL* in clinical practice. Based on the referenced Review, select the ONE statement that is NOT TRUE: [See J Mol Diagn 2011, 13:461-466; DOI: 10.1016/j.jmoldx.2011.05.007; the authors of the referenced article did not disclose any relevant financial relationships.]

- a. The authors conclude that the practice of *en bloc* screening for *JAK2V617F*, *JAK2* exon 12, and *MPL* mutations is not justified by the scientific evidence and is economically irresponsible.
- b. *JAK2V617F* studies using either peripheral blood or bone marrow are concordant; therefore, either specimen source may be analyzed.
- c. Surprisingly, an appreciable fraction of control samples will contain mutations in *JAK2* or *MPL*.
- d. *JAK2* exon 12 mutation screening is only indicated in the presence of *JAK2V617F*-negative erythrocytosis.

Rationale: Mutations in *JAK2* or *MPL*, as borne out by prodigious amounts of screening, do not appear readily in normal samples.

4. Critical diagnosis and treatment options rely strongly on the measurement of percentage mutant allele burden from specimen tissue. Based on the referenced Review, select the ONE statement that is NOT TRUE: [See J Mol Diagn 2011, 13:461-466; DOI: 10.1016/j.jmoldx.2011.05.007; the authors of the referenced article did not disclose any relevant financial relationships.]

- a. When measuring mutated allele burdens in specimens, most commercial assays use a $\geq 1\%$ cut-off to minimize false-positive readouts.
- b. **Mutant allele burden assays are only reliable before treatment is begun, as post-treatment measurements are confounded by mixed populations of cells.**
- c. A mutant allele burden measured as $<50\%$ may still contain cells in the specimen that are homozygously mutated.
- d. In primary myelofibrosis, a lower mutant allele burden equates with poorer survival.

Rationale: Quantitative assays are very useful in monitoring treatment response, including residual disease after allogeneic stem cell transplantation.

5. *PI3KCA*-associated tumors may require the development of complex therapeutic strategies. Based on the referenced article, select the ONE statement that is NOT TRUE: [See J Mol Diagn 2011, 13:504-513; DOI: 10.1016/j.jmoldx.2011.04.003; this study was supported in part with funding from a commercial research grant from Novartis Pharmaceuticals and Christopher L. Corless was a consultant/advisory board member to Novartis Pharmaceuticals and on the Sequenom speakers bureau; the other authors of the referenced article did not disclose any relevant financial relationships.]

- a. *PI3KCA* mutations frequently appear in conjunction with mutations in other human oncogenes; therefore, multiple chemotherapeutics, each with a different target, may need to be co-administered to be effective.
- b. **The quaternary structure of p110- α , the protein encoded by *PI3KCA*, renders it a poor target for drug treatment; therefore, multiple drugs must be designed to deactivate the protein.**
- c. Multiple tumorigenic mutations have been found within *PI3KCA*, which require different chemotherapeutics, as one treatment modality is unlikely to target all possible effects of all the mutations.
- d. Although the same *PI3KCA* mutation may be found in tumors from different organs, chemotherapeutic responses may be cell context-specific.

Rationale: The structure of p110- α does not render it highly resistant to inactivation.

6. Cystic fibrosis is caused by mutations in the *CFTR* gene encoding the cystic fibrosis transmembrane conductance regulator. Based on the referenced article, select the ONE statement that is NOT TRUE: [See J Mol Diagn 2011, 13:520-527; DOI: 10.1016/j.jmoldx.2011.05.004; the authors of the referenced article did not disclose any relevant financial relationships.]

- a. ***CFTR* is a member of the G protein-coupled receptor superfamily.**
- b. The incidence of cystic fibrosis is $\sim 1:3,500$ in the Caucasian population.
- c. Approximately 1,900 different mutations have been identified within the *CFTR* gene sequence.
- d. The most common *CFTR* mutation is F508del.

Rationale: *CFTR* is a member of the ATP-binding cassette (ABC) transporter family. *CFTR* contains two repeated units composed of a membrane-spanning domain (MSD) and a nucleotide binding domain (NBD) harboring an ATP binding and hydrolysis site.

7. Mutations in the *CFTR* gene may be classified into distinct groups according to the mechanisms that disrupt *CFTR* function. Based on the referenced article, select the ONE statement regarding a relevant pathophysiologic mechanism of *CFTR* dysfunction that is NOT TRUE: [See J Mol Diagn 2011, 13:520-527; DOI: 10.1016/j.jmoldx.2011.05.004; the authors of the referenced article did not disclose any relevant financial relationships.]

- a. **Mislocalization of *CFTR* to the basal membrane of epithelial cells.**
- b. Impaired protein maturation leading to degradation of *CFTR* protein.
- c. Defective regulation of *CFTR* channel activity.
- d. Altered ionic selectivity and conductance.

Rationale: Normally, *CFTR* localizes to the apical membrane of epithelial cells, but mutations have not been observed that redirect *CFTR* to the basal membrane.

8. Three assays were used to characterize novel *CFTR* mutations and to establish a correlation between genotype and patient phenotype. Based on the referenced article, select the ONE statement regarding a relevant *CFTR* mutation assay that is NOT TRUE: [See J Mol Diagn 2011, 13:520-527; DOI: 10.1016/j.jmoldx.2011.05.004; the authors of the referenced article did not disclose any relevant financial relationships.]

- a. Automated iodide efflux assay to determine activity of the transporter.
- b. ⁸⁶Rb⁺ uptake assay to characterize the removal of Na⁺ by the transporter.
- c. Immunoblotting to determine protein maturation.
- d. Green fluorescent protein (GFP)-tagged expression in cell culture to determine protein localization.

Rationale: The ⁸⁶Rb⁺ uptake assay is designed to test inhibitors of the Na⁺/K⁺ ATPase and is not a relevant assay for *CFTR* mutation analysis

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ANSWERS for CME Questions November # 1-8

1d, 2c, 3b, 4d, 5d, 6a, 7a, 8b

1. Mutations and polymorphisms in isocitrate dehydrogenase 1 (*IDH1*) and *IDH2* are found in a subset of gliomas and acute myeloid leukemia (AML). Based on the referenced article and related Commentary, select the ONE statement that is NOT TRUE: [See J Mol Diagn 2011, 13:678-686; DOI: 10.1016/j.jmoldx.2011.06.004 and related Commentary J Mol Diagn 2011, 13:605-608; DOI: 10.1016/j.jmoldx.2011.08.001; the authors of the referenced articles did not disclose any relevant financial relationships.]

- a. AML is not a single disease entity, and clinical outcome of AML patients is associated with specific mutations, each with distinct pathogenesis and clinical behavior.
- b. *IDH1* and *IDH2* are metabolic enzymes involved in the citric acid cycle and participate in cytosolic NADPH production necessary for the regeneration of reduced glutathione, a major antioxidant in mammalian cells.
- c. In AML, the most common mutations in *IDH1* and *IDH2* include *IDH1*R132 and *IDH2*R172.
- d. Four evolutionarily conserved arginine residues are encoded in exon 1 of both *IDH1* and *IDH2*.

Rationale: Three evolutionarily conserved arginine residues are encoded in exon 4 of each gene: R100, R109, and R132 of *IDH1* and R140, R149, and R172 of *IDH2*. Mutations affecting the two *IDH* enzymes share common features. The mutations affect an arginine residue leading to reorganization of the active site, result in loss of enzymatic function for oxidative decarboxylation of isocitrate, and convey a gain of function for NADPH-dependent reduction of α -ketoglutarate.

2. Tumor-associated mutations in *IDH1* and *IDH2* confer an adverse prognosis for AML cases with normal cytogenetics. Based on the referenced article and related Commentary, select the ONE statement that is NOT TRUE: [See J Mol Diagn 2011, 13:678-686; DOI: 10.1016/j.jmoldx.2011.06.004 and related Commentary J Mol Diagn 2011, 13:605-608; DOI: 10.1016/j.jmoldx.2011.08.001; the authors of the referenced articles did not disclose any relevant financial relationships.]

- a. Mutations in the *IDH1* and *IDH2* genes result in neomorphic enzymatic activity capable of converting α -ketoglutarate to R(-)-2-hydroxyglutarate (2-HG).
- b. Levels of 2-HG are increased in *IDH*-mutant tumors and inhibit the activity of TET2.
- c. Tumors with mutations in *IDH1* and *IDH2* show DNA hypomethylation.
- d. Mutations in *IDH1*, *IDH2*, and *TET2* tend to be mutually exclusive, ie, AML with a mutation in one of these genes rarely has a mutation at either of the other two loci.

Rationale: Tumors with mutations in *IDH1* and *IDH2* are hypermethylated and have specific DNA methylation profiles. The hypermethylation is likely influenced by the overproduction of 2-HG through mutant *IDH1* or *IDH2* or by mutant *TET2*. Increased levels of 2-HG inhibit the activity of *TET2*, an enzyme that converts 5-methylcytosine to 5-hydroxymethylcytosine at CpG residues of mammalian DNA, thereby influencing the epigenetic state of the cell. It should be noted that the *TET2* gene is frequently mutated in AML. By overproducing 2-HG through mutant *IDH1*, *IDH2*, or *TET2*, neoplastic cells may alter the epigenetic control of growth regulatory genes.

3. Screening assays are designed to have high sensitivity and a negligible false-negative rate as long as the false-positive rate is acceptably low. Based on the referenced article and related Commentary, select the ONE statement that is NOT TRUE: [See J Mol Diagn 2011, 13:678-686; DOI: 10.1016/j.jmoldx.2011.06.004 and related Commentary J Mol Diagn 2011, 13:605-608; DOI: 10.1016/j.jmoldx.2011.08.001; the authors of the referenced articles did not disclose any relevant financial relationships.]

- a. High-resolution melting (HRM) curve analysis offers a rapid, closed-system method for genotyping that relies on differences in the melting properties of the wild-type and mutant sequences.
- b. Of the various reported *IDH1* and *IDH2* genotyping methods, pyrosequencing is considered the “gold standard” and is widely used.
- c. A clinically validated HRM assay for detecting *IDH1R132* and *IDH2R172* mutations in gliomas has been described.
- d. Patel et al (DOI: 10.1016/j.jmoldx.2011.06.004) took advantage of the fact that *IDH1* and *IDH2* mutations occur in defined regions of the coding sequence and used standard PCR to amplify the regions of interest, followed by HRM curve analysis of the duplex DNA from their PCR products.

Rationale: Sanger sequencing is considered the “gold standard” method for *IDH* genotyping in AML. However, Sanger sequencing assays to detect *IDH* mutations are labor intensive and not cost effective for clinical testing of low-frequency mutations. In addition to direct methods such as Sanger sequencing and pyrosequencing, methods of *IDH* mutation detection that have been reported include restriction endonuclease digestion, single-stranded confirmation polymorphism, HRM analysis, and immunohistochemistry.

4. Osteogenesis imperfecta (OI) represents a group of diseases involving connective tissue that are characterized by bone fragility. Based on the referenced article, select the ONE statement that is NOT TRUE: [See J Mol Diagn 2011, 13:648-656; DOI: 10.1016/j.jmoldx.2011.06.006; the authors of the referenced article did not disclose any relevant financial relationships.]

- a. Approximately 90% of patients with OI exhibit dominant mutations in the *COL1A1* and *COL1A2* genes, which encode $\alpha 1$ and $\alpha 2$ type I collagen chains, respectively.
- b. Type I OI demonstrates a milder clinical presentation and is frequently associated with impaired synthesis of structurally normal collagen due to class I mutations such as premature stop codons in the coding sequence of *COL1A1*.
- c. Class II mutations involve glycine substitutions in the triple-helical domain of collagen and are often associated with more severe clinical presentation.
- d. *De novo* mutations, in particular in *COL1A1*, are often observed in patients with the mildest clinical presentation (types I and IV).

Rationale: OI is traditionally classified into types I to IV on the basis of phenotype and outcome, although other types have recently been described. *De novo* mutations are often observed in patients with the most severe OI, ie, types II and III, whereas types I and IV are usually observed in familial clusters.

5. The increasing availability of methods for scanning whole genes has increased the demand for the molecular diagnosis of OI. Based on the referenced article, select the ONE statement that is NOT TRUE: [See J Mol Diagn 2011, 13:648-656; DOI: 10.1016/j.jmoldx.2011.06.006; the authors of the referenced article did not disclose any relevant financial relationships.]

- a. OI is traditionally diagnosed on the basis of the typical phenotype and is confirmed at radiography.
- b. Molecular analysis can confirm the diagnosis of OI, particularly in patients without well-defined phenotypes.
- c. Molecular diagnosis of OI is limited by the dispersion of causative mutations in all exons of the affected collagen genes (51 exons in *COL1A1* and 52 exons in *COL1A2*).
- d. Splice mutations within intron-exon boundaries are rare in OI and account for less than 10% of cases.

Rationale: 20% to 30% of OI cases involve splice mutations within intron-exon boundaries.

6. Denaturing high-performance liquid chromatography (DHPLC) is an alternative methodology for gene scanning analysis. Based on the referenced article, select the ONE statement that is NOT TRUE: [See J Mol Diagn 2011, 13:648-656; DOI: 10.1016/j.jmoldx.2011.06.006; the authors of the referenced article did not disclose any relevant financial relationships.]

- a. Denaturing gradient gel electrophoresis (DGGE) and single-strand conformation polymorphism (SSCP) methods are more sensitive than DHPLC.
- b. An advantage of DHPLC is that it is an inexpensive method with high analytical sensitivity.
- c. Since a few OI cases are due to mutations in the *LEPRE1*, *CRTAP*, *PPIB*, *FKPB65*, and *SERPINH1* genes, it is important to exclude with high predictivity mutations in *COL1A1* and *COL1A2* before analyzing a wider series of potentially causative genes.
- d. With the use of their DHPLC procedure, the authors of the referenced article identified four novel polymorphisms in *COL1A1* and two novel polymorphisms in *COL1A2*.

Rationale: Both DGGE and SSCP are less sensitive than DHPLC.

7. Tissue specimens available for molecular analysis are often formalin fixed and paraffin embedded (FFPE), but the RNA isolated from FFPE tissues is often of poor quality and quantity. Based on the referenced article, select the ONE statement that is NOT TRUE: [See J Mol Diagn 2011, 13:687-694; DOI: 10.1016/j.jmoldx.2011.06.007; the authors of the referenced article did not disclose any relevant financial relationships.]

- a. The isolated RNA from FFPE tissues is fragmented to an average length of 350 bases.
- b. RT-PCR of FFPE-derived RNA is generally inefficient for amplicons longer than 120 to 200 bases and cannot detect low-level transcripts.
- c. Tissue fixation and storage methods such as HOPE (HEPES-glutamic acid buffer-mediated organic solvent protection effect) may preserve RNA for molecular analysis that is superior to that extracted from FFPE tissues.
- d. The HOPE method fixes in alcohol, rather than formalin, and uses low-melting paraffin.

Rationale: The isolated RNA from FFPE tissues is fragmented to an average length of 100 to 200 bases.

8. Understanding the chemical changes in RNA that occur during fixation, processing, and embedding may lead to better strategies for preventing or reversing degradation. Based on the referenced article, select the ONE statement regarding a relevant CFTR mutation assay that is NOT TRUE: [See J Mol Diagn 2011, 13:520-527; DOI: 10.1016/j.jmoldx.2011.05.004; the authors of the referenced article did not disclose any relevant financial relationships.]

- a. It was previously demonstrated that common formaldehyde-RNA adducts were methyl(hydroxymethyl) groups, ethyl methyl ethers, and methylene bridge cross-links on the exocyclic amine of the adenine base.
- b. Formalin fixation resulted in a loss of only 10% of bound RNA when mRNA was released from oligo(dT)25-mers on paramagnetic beads.
- c. Heating formalin-fixed RNA in either xylene or paraffin (hydrocarbon solvents) produced substantially reversible cross-linking and substantially irreversible adducts.
- d. The data suggest that paraffin embedding causes significant RNA damage in warm nonpolar solvents after formaldehyde fixation and dehydration.

Rationale: Formalin fixation resulted in a loss of approximately two-thirds of bound RNA, possibly because substantial modification of A bases decreased their binding to T in the oligo(dT) primers used for mRNA binding to paramagnetic beads.



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