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

CME July Questions # 1-12

A Review of molecular monitoring of minimal residual disease in acute myeloid leukemia, a Technical Advance describing PCR assays for the detection of rare somatic mutations, and an article on next-generation sequencing strategies for diagnosis of myopathies and muscular dystrophies were selected for the **July 2018 JMD CME Program in Molecular Diagnostics**. The authors of the referenced articles, the planning committee members, and staff have no relevant financial relationships with commercial interests to disclose.

Questions #1-5 are based on: Selim A, Moore AS: Molecular minimal residual disease monitoring in acute myeloid leukemia. J Mol Diagn 2018, 20:389-397; http://dx.doi.org/10.1016/j.jmoldx.2018.03.005.

Questions #6-8 are based on: Vargas DY, Marras SAE, Tyagi S, Kramer FR: Suppression of wild-type amplification by selectively enhancing agents in PCR assays that utilize SuperSelective primers for the detection of rare somatic mutations. J Mol Diagn 2018, 20:415-427; http://dx.doi.org/10.1016/j.jmoldx.2018.03.004.

Questions #9-12 are based on: Zenagui R, Lacourt D, Pegeot H, Yauy K, Morales RJ, Theze C, Rivier F, Cances C, Sole G, Renard D, Walther-Louvier U, Ferrer-Monasterio X, Espil C, Arné-Bes M-C, Cintas P, Uro-Coste E, Negrier M-LM, Rigau V, Bieth E, Goizet C, Claustres M, Koenig M, Cosée M: A reliable targeted next-generation sequencing strategy for diagnosis of myopathies and muscular dystrophies, especially for the giant titin and nebulin genes. J Mol Diagn 2018, 20:533-549; http://dx.doi.org/10.1016/j.jmoldx.2018.04.001

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

- Discuss the importance of minimal residual disease (MRD) monitoring in acute myeloid leukemia (AML).
- Describe RNA- and DNA-based quantitative PCR (qPCR) AML MRD strategies.
- Define the advantages and disadvantages of RNA and genomic DNA in AML MRD detection.
- Describe gene fusions in AML and their role in MRD monitoring.
- Describe SuperSelect primers and how they improve specificity for rare somatic mutations.
- Discuss how Hofmeister salts can improve specificity of qPCR.
- Describe genetic causes of myopathies and myelodystrophies (M-MDs).
- Describe the nebulin (*NEB*) and titin (*TTN*) genes and their repeat tandem sequences.
- Describe new genetic variants in M-MDs detected by next-generation sequencing strategies.

1. Acute myeloid leukemia (AML) is a heterogeneous disease characterized by diverse genetic abnormalities and variable morphology, immunophenotypes, and clinical outcomes. Based on the referenced Review, select the ONE best TRUE statement: [See J Mol Diagn 2018, 20:389-397.]

a. Clonal composition of AML varies because subclones are subjected to natural and treatment-induced selection pressures, presenting challenges when selecting genetic abnormalities as markers of post-treatment minimal residual disease (MRD).
b. A lack of sensitivity of a MRD marker will produce false-positives.
c. A lack of specificity of a MRD marker will produce false-negatives.
d. A commonly accepted benchmark in limit of detection (LOD) in AML MRD assays is 10^{-2} (meaning 1 leukemic cell in 100 cells measured).

2. The mainstay of molecular AML MRD monitoring at present are real-time quantitative PCR (qPCR)—based methods. Based on the referenced Review, select the ONE best TRUE statement: [See J Mol Diagn 2018, 20:389-397.]

a. PCR-based assays of fusion genes and *NPM1* mutations have the potential to collectively monitor approximately 40% of AML presenting in children and younger adults.
b. PCR-based assays of fusion genes and *NPM1* mutations have the potential to collectively monitor approximately 60% of AML presenting in adults over the age of 60 years.
c. *NPM1* mutations ($NPM1^{mut}$) are present in 45% of all AML cases.
d. Only one recurrent mutation of the *NPM1* gene has emerged as a suitable MRD marker in AML.

3. The nucleic acid source for molecular AML MRD assays may be genomic DNA or RNA. Based on the referenced Review, select the ONE best TRUE statement: [See J Mol Diagn 2018, 20:389-397.]

a. DNA-based qPCR monitoring predominates in routine practice.
b. Sample quality is particularly important for DNA compared to RNA.
c. Compared with multiparametric flow cytometry—based MRD, most molecular MRD tests must be batched to be economically viable.
d. RNA provides an absolute quantitation of the number of leukemic cells in a sample.

4. MRD monitoring of fusion genes is possible in AML cases characterized by recurrent chromosomal rearrangement. Based on the referenced Review, select the ONE best TRUE statement: [See J Mol Diagn 2018, 20:389-397.]

a. Rearrangements have provided robust molecular MRD markers and have allowed monitoring for approximately 30% of children and 50% of young adults.
b. Assay sensitivities of AML MRD monitoring of fusion genes approach 10^{-5}.
c. There is only a single type of *CBFB-MYH11* fusion transcript, greatly simplifying its screening.
d. Three different reverse primers are required to identify the three commonest break points in *RUNX1-RUNX1T1* transcripts.

5. The prospect of using next-generation sequencing (NGS) for both identification and monitoring of AML MRD targets is promising; however, the selection of the correct platform and the correct mutation(s) to monitor is pivotal. Based on the referenced Review, select the ONE best TRUE statement: [See J Mol Diagn 2018, 20:389-397.]

a. Whole-genome sequencing will provide greater coverage than whole-exome sequencing if the intention is to detect single nucleotide polymorphisms and small insertions/deletions in coding regions.
b. If fusion genes are the primary target, whole-exome sequencing will provide greater coverage than whole-genome sequencing.
c. A shortcoming of NGS-based MRD is that common mutations often include those that are present in pre-leukemic clones, rendering them unsuitable as MRD markers.
d. Mutations in epigenetic regulators are among the commonest mutations in childhood AML.

6. There is great interest in developing techniques that can detect rare somatic mutations by using routine blood samples. Based on the referenced Technical Advance, select the ONE best TRUE statement: [See J Mol Diagn 2018, 20:415-427.]

a. SuperSelective primers are PCR primers that selectively initiate synthesis on mutant target sequences, but are unlikely to initiate synthesis on related wild-type sequences.
b. SuperSelective primers consist of a unique 5'-tag sequence that is complementary to the sequence of the DNA target fragment.
c. SujeprSelective primers consist of a bridge sequence that is complementary to the corresponding intervening sequence of the DNA target fragment.
d. SuperSelective primers consist of a long 3'-foot sequence that is perfectly complementary to the related wild-type DNA fragment.

7. The main purpose of SuperSelective primer design is to cause the initiation of amplicon synthesis to be dependent on the transitory presence of the hybrid formed by the foot sequence. Based on the referenced Technical Advance, select the ONE best TRUE statement: [See J Mol Diagn 2018, 20:415-427.]

a. Weaker mismatched wild-type foot hybrids persist for only a few seconds.
b. Perfectly complementary mutant foot hybrids might persist for a few hundred milliseconds.
c. It was previously found that in real-time PCR assays using SuperSelective primers, as few as 5 mutant DNA fragments in a sample that also includes one million closely related wild-type DNA fragments give rise to a real-time amplification signal that occurs a few thermal cycles earlier than a sample that contains only a million closely related wild-type DNA fragments.
d. The inherent reliable sensitivity of SuperSelective PCR assays is currently limited to approximately 5 mutant target DNA fragments in the sample being tested.

8. The inclusion of Hofmeister salts in the PCR assay increases selectivity in SuperSelective primer-based PCR assays. Based on the referenced Technical Advance, select the ONE best TRUE statement: [See J Mol Diagn 2018, 20:415-427.]

a. The optimal concentration of tetramethylammonium chloride (TMAC) is independent of the design of the SuperSeletive primers that are used.
b. TMAC was most effective when the interrogating nucleotide of the SuperSelective primer was located at the 5' end of its foot sequence.
c. Longer feet and smaller bubbles require less TMAC to achieve an optimal effect.
d. The optimal TMAC concentration depended on the length of the foot sequence and the size of the bubble that the SuperSelective primer formed with its target DNA fragment.

9. Myopathies and muscular dystrophies (M-MDs) are a set of phenotypically and genetically heterogeneous diseases. Based on the referenced article, select the ONE best TRUE statement: [See J Mol Diagn 2018, 20:533-549.]

a. Thus far, >150 M-MD genes have been identified.
b. In a study of 177 unsolved cases of myopathies, next-generation sequencing identified 10% to 15% additional variants compared to whole-exome sequencing.
c. Implementation of targeted NGS strategy in a diagnostic context requires an in-depth development step to ensure capture of regions of interest in an exhaustive manner.
d. Insertions/deletions (INDELs) are the third most common type of genomic variants.

10. Until recently, some M-MD genes were not analyzed because of their large size. Based on the referenced article, select the ONE best TRUE statement: [See J Mol Diagn 2018, 20:533-549.]

a. The titin gene (*TTN*) has 363 exons.
b. The *TTN* gene has two tandem repeats that share 98% DNA sequence homology.
c. GC-rich sequences are more prevalent at the 3' end of the gene of the *TTN* gene.
d. *In silico* bioinformatics prediction tools are very reliable for the interpretation of the functional impact of variants in the *TTN* gene.

11. The authors of the referenced article used a relaxed probe set design to analyze the repeated exons of *TTN* and the nebulin gene (*NEB*). Based on the referenced article, select the ONE best TRUE statement: [See J Mol Diagn 2018, 20:533-549.]

a. The *NEB* gene has 176 exons.
b. In *NEB*, the triplicated region of 27 exons is arranged in three sets of nine perfectly repeated exons.
c. Within the triplicated repeat regions of *NEB*, exons 89 and 105 are identical.
d. Difficulties in detecting mutations in the repeated exons of *NEB* probably result from the combination of capture failure, incorrect mapping of sequences, and INDEL underdetection within triplicated exons.

12. NGS data from 128 patients affected by M-MDs without identified genetic etiology were analyzed. Based on the referenced article, select the ONE best TRUE statement: [See J Mol Diagn 2018, 20:533-549.]

a. Overall, 74 pathogenic or likely pathogenic mutations were identified.
b. 52 substitutions were identified.
c. Five copy number variants were identified.
d. 17 INDELs were identified.