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An article and associated Commentary on identification of recurrent gene rearrangements in hematological malignancies and research articles on cystic fibrosis and acute myeloid leukemia were selected for the **January 2019 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: Kim B, Lee H, Shin S, Lee S-T, Choi JR: Clinical Evaluation of Massively Parallel RNA Sequencing for Detecting Recurrent Gene Fusions in Hematologic Malignancies. J Mol Diagn 2019, 21:163-170; https://doi.org/10.1016/j.jmoldx.2018.09.002 and associated Commentary by Sabath DE: The Next Generation in Detection of Leukemia-Associated Translocations. J Mol Diagn 2019, 21: 16-18; https://doi.org/10.1016/j.jmoldx.2018.08.009.

Questions #7-9 are based on: Kerschner JL, Ghosh S, Paranjapye A, Cosme WR, Audrézet M-P, Nakakuki M, Ishiguro H, Férec C, Rommens J, Harris A: Screening for Regulatory Variants in 460 kb Encompassing the *CFTR* Locus in Cystic Fibrosis Patients. J Mol Diagn 2019, 21: 70-80; https://doi.org/10.1016/j.jmoldx.2018.08.011.

Questions #10-12 are based on Malmberg ED, Rehammar A, Pereira MB, Abrahamsson J, Samuelsson T, Ståhlman S, Asp J, Tierens A, Palmqvist L, Kristiansson E, Fogelstrand L: Accurate and Sensitive Analysis of Minimal Residual Disease in Acute Myeloid Leukemia Using Deep Sequencing of Single Nucleotide Variations. J Mol Diagn 2019, 21: 149-162; https://doi.org/10.1016/j.jmoldx.2018.08.004.

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

- Discuss various techniques used for identifying recurrent gene rearrangements in hematological malignancies.
- Understand the strengths of using next-generation sequencing (NGS) RNA fusion panels for fusion detection.
- Define cystic fibrosis.
- Describe the functions of *CFTR* gene.
- Define acute myeloid leukemia (AML).
- Define minimum residual disease.
- Describe multi-parameter flow cytometry.

1. **The identification of recurrent gene rearrangements is critical in the diagnosis and choice of treatment for patients with acute leukemia. Based on the referenced article and associated commentary, select the ONE best TRUE statement:** [See J Mol Diagn 2019, 21: 163-170 and See J Mol Diagn 2019, 21: 16-18.]

   a. Chromosome analysis and fluorescence *in situ* hybridization (FISH) lack sensitivity to identify gene rearrangements.
   b. Gene rearrangements which have more than 100 partner genes involving numerous different chromosome breakpoints are fully covered even by the limited number of primer sets used in reverse transcription-PCR.
   c. t(12;21)(p13;q22) with *ETV6-RUNX1* is always missed in the analysis of chromosomes and translocations involving *KMT2A* (*MLL*).
   d. Next-generation sequencing (NGS) technologies represent unbiased methods for detection and identification of the diverse fusion genes that characterize hematologic malignancies without the need for prior knowledge of the nature of chromosomal alterations present in a specific patient.

2. **Given the multitude of diverse gene fusions found in hematologic malignancies as a result of various chromosomal rearrangements, new approaches for screening patient samples to identify actionable gene fusions are required before this molecular analysis becomes practical. Based on the referenced article and associated commentary, select the ONE best TRUE statement:** [See J Mol Diagn 2019, 21: 163-170 and See J Mol Diagn 2019, 21: 16-18.]

    a. The TruSight RNA fusion panel is based upon a hybrid capture method that uses probes targeting 507 partner genes involved in recurrent translocation events in various cancers, enabling simultaneous screening for >500 gene fusions and a practical approach for evaluation of patients with hematologic malignancy.
    b. FusionPlex Pan-Heme panel is based upon an amplicon-based method using gene-specific primers for 199 genes, enabling simultaneous screening for nearly 200 gene fusions and a practical approach for evaluation of patients with hematologic malignancy.
    c. Gene fusions like *ETV-RUNX1* resulting from the t(12;21)(p13;q22) translocation can be detected using traditional reverse transcription-PCR and approximately 100 sets of primers, providing a practical approach for evaluation of this specific gene fusion in patients with hematologic malignancy.
    d. Both the TruSight RNA fusion panel and the FusionPlex Pan-Heme panel represent practical molecular approaches based upon NGS for simultaneous evaluation of hundreds of gene fusions in patients with hematologic malignancies.

3. **Multiplex reverse transcription-PCR can detect recurrent translocations in the majority of samples from patients with hematologic malignancies. Based on the referenced article and associated commentary, select the ONE best TRUE statement:** [See J Mol Diagn 2019, 21: 163-170 and See J Mol Diagn 2019, 21: 16-18.]

    a. Multiplex reverse transcription-PCR is a robust rearrangement method, but can sometimes miss identifying *KMT2A* (*MLL*) translocations attributable to unusual chromosomal breakpoints.
    b. TruSight kit can only identify translocations identified by multiplex reverse transcription-PCR.
    c. Translocations detected by the FusionPlex kit are always missed by the TruSight kit.
    d. Both TruSight and FusionPlex kits can identify reciprocal translocations including *ABL1-BCR*, *RARA-PML*, and *RUNX1T1-RUNX1*.

4. **The detection limits of the NGS assay for *BCR-ABL1* e1a2 transcript can be determined by testing a series of diluted samples derived from patients or using commercially available control materials. Based on the referenced article and associated commentary, select the ONE best TRUE statement:** [See J Mol Diagn 2019, 21: 163-170 and See J Mol Diagn 2019, 21: 16-18.]

    a. The TruSight panel can detect the fusion in samples up to 1.0×10^{-5} dilution.
    b. The FusionPlex panel can detect the fusion in samples up to 1.0×10^{-6} dilution.
    c. The detection sensitivity for TruSight panel can be significantly increased using the Illumina's default algorithm.
    d. Sequencing outputs for the TruSight panel can detect the *BCR-ABL1* transcript up to 1.0×10^{-2} dilution using the Tophat-Fusion algorithm.

5. **Patients with acute leukemia greatly benefit from the identification of gene fusions as specific chromosomal translocations are associated with specific diagnoses and can form the basis for selection of treatment. Based on the referenced article and associated commentary, select the ONE best TRUE statement:** [See J Mol Diagn 2019, 21: 163-170 and See J Mol Diagn 2019, 21: 16-18.]

    a. Targeted molecular drug regimens for treatment of leukemia may be independent of the nature of identified translocations.
    b. Detection of translocations such as *KMT2A* and *BCR-ABL1* may delay stem cell transplantation.
    c. The presence of gene fusions can be detected using cytogenetic studies, but some chromosomal translocations might be missed.
    d. Reverse transcription-PCR tests can be used to detect gene fusions without risk of missing unique chromosomal rearrangements.

6. **NGS RNA panels represent a practical laboratory approach to detecting various gene fusions in hematologic malignancies. Based on the referenced article and associated commentary, select the ONE best TRUE statement:** [See J Mol Diagn 2019, 21: 163-170 and See J Mol Diagn 2019, 21: 16-18.]

    a. Reverse transcription-PCR–based molecular assays have poor sensitivity.
    b. The TruSight RNA fusion panel is based on a hybrid capture method that uses probes targeting over five thousand partner genes for recurrent translocation in various cancers.
    c. The FusionPlex Pan-Heme panel is based on an amplicon-based method using gene-specific primers for over 500 genes for hematologic cancers in combination with an Anchored Multiplex PCR technique.
    d. Both the TruSight and FusionPlex panels are capable of detecting fusions when one of the partner genes is enriched.

7. **Cystic fibrosis (CF) is a common life-shortening autosomal recessive disorder. Based on the referenced article, select the ONE best TRUE statement:** [See J Mol Diagn 2019, 21: 70-80.]

    a. CF is caused by pathogenic variants in the cystic fibrosis transmembrane conductance regulator (*CFTR*) gene.
    b. *CFTR* encodes a cyclic AMP-activated potassium channel.
    c. Newborn screening for CF is uncommon in the majority of European countries due to low occurrence rates.
    d. The majority of documented *CFTR* variants have been designated as disease-causing in the Clinical and Functional Translation of CFTR Database.

8. **The authors used a targeted enrichment approach to deep-sequence the extended *CFTR* locus. Based on the referenced article, select the ONE best TRUE statement:** [See J Mol Diagn 2019, 21: 70-80.]

    a. Five hundred SureSelect biotinylated cRNA probes were used.
    b. The extended region encompassed beyond the -180.1kb and +148.9kb TAD boundaries that demarcate the limits of the *CFTR* locus.
    c. The examined region of the *CFTR* gene includes at least 20 other defined *CFTR* regulatory elements.
    d. Nearly one thousand kb encompassing the extended *CFTR* locus were deep-sequenced.

9. **Among disease-causing variants currently annotated in the *CFTR* gene, only those in the promoter or that disrupt or create splice sites occur in non-coding regions. Based on the referenced article, select the ONE best TRUE statement:** [See J Mol Diagn 2019, 21: 70-80.]

    a. Over 10% of CF patients have unknown molecular lesions.
    b. Additional non-coding variants within the *CFTR* locus likely contribute to the pathogenicity of CF.
    c. Traditional sequencing methods continue to identify novel and/or non-coding variants.
    d. NGS technology has been suboptimal in identifying novel *CFTR* variants.

10. **Acute myeloid leukemia (AML) is the most common form of acute leukemia. Based on the referenced article, select the ONE best TRUE statement:** [See J Mol Diagn 2019, 21: 149-162.]

    a. The persistence of measurable leukemic cells, or minimal residual disease (MRD), after induction therapy is one of the most important risk factors for relapse in both adults and children with AML.
    b. Multi-parameter flow cytometry (MFC) allows assessment of MRD in 99% of patients.
    c. Majority of the leukemic cells carry the leukemia-associated immunophenotype (LAIP) used to identify MRD with MFC.
    d. Antigen expression by leukemic cells is remarkably consistent during treatment.

11. **The sensitivity and negative predictive value of multi-parameter flow cytometry (MFC) for assessment of minimal residual disease (MRD) and relapse prediction in acute leukemia is low. Based on the referenced article, select the ONE best TRUE statement:** [See J Mol Diagn 2019, 21: 149-162.]

    a. Less than 10% of patients that are MFC-MRD negative after end of induction therapy eventually relapse.
    b. A small array of antibodies suffices in the clinical use of MFC for evaluation of MRD.
    c. In cases where a leukemia-specific recurrent fusion gene or mutation (such as in *NPM1*) is present, reverse transcription followed by quantitative PCR (RT-qPCR) can be used for MRD monitoring with higher sensitivity than MRD analysis using MFC.
    d. A substantial percentage of leukemia patients harbor fusion genes or recurrent mutations that can be monitored with RT-qPCR.

12. **Due to the genetically heterogeneous nature of AML, non-recurrent mutations are numerous. Based on the referenced article, select the ONE best TRUE statement:** [See J Mol Diagn 2019, 21: 149-162.]

    a. Among non-recurrent mutations observed in AML, single nucleotide variations (SNVs) are the most common.
    b. Until cost-effective and reliable benchtop sequencers become available, the use of NGS in the clinical setting will remain impractical.
    c. Deep sequencing for minimal residual disease analysis has only been described for chronic lymphocytic leukemia.
    d. Deep sequencing for quantification of mutations has yet to be reported for *NPM1* and *RUNX1*.