# ASIP 2017 Journal CME Programs

# JMD 2017 CME Program in Molecular Diagnostics

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

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**CME July Questions # 1-12**

A Review and research article on overgrowth syndromes and a research article and associated Commentary on minimal residual disease monitoring in acute myeloid leukemia were selected for the **July 2017 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 #1-4 are based on: Akgumus G, Chang F, Li MM: Overgrowth syndromes caused by somatic variants in the phosphatidylinositol 3-kinase/AKT/mammalian target of rapamycin pathway. J Mol Diagn 2017, 19:487-497; http://dx.doi.org/10.1016/j.jmoldx.2017.04.001.

Questions #5-6 are based on: Chang F, Liu L, Fang E, Zhang G, Chen T, Cao K, Li Y, Li MM. Molecular diagnosis of mosaic overgrowth syndromes using a custom-designed next-generation sequencing panel. J Mol Diagn 2017, 19:613-624; http://dx.doi.org/10.1016/j.jmoldx.2017.04.006.

Questions #7-12 are based on: Mencia-Trinchant N, Hu Y, Alas MA, Ali F, Wouters BJ, Lee S, Ritchie EK, Desai P, Guzman ML, Roboz GJ, Hassane DC: Minimal residual disease monitoring of acute myeloid leukemia by massively multiplex digital PCR in patients with *NPM1* mutations. J Mol Diagn 2017, 19:537-548; http://dx.doi.org/10.1016/j.jmoldx.2017.03.005 and related Commentary: Wertheim GB, Bagg A: *NPM1* for MRD? Droplet like it's hot! J Mol Diagn 2017, 19:498-501; http://dx.doi.org/10.1016/j.jmoldx.2017.04.008.

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

- Define overgrowth syndromes.
- Understand the basics of the phosphatidylinositol 3-kinase (PI3K)/AKT/mammalian target of rapamycin (mTOR) pathway.
- Describe Proteus syndrome.
- Define CLOVES syndrome.
- Explain the advantages of detecting overgrowth syndromes using deep sequencing.
- Describe the role of the PI3K/AKT/mTOR pathway in overgrowth syndromes.
- Define acute myeloid leukemia (AML).
- Understand the consequence of minimal residual disease (MRD) in AML.
- Explain the presence of the *NPM1*mut transcript in AML patients.
- Define digital PCR (dPCR) assays.
- Understand that the *NPM1* locus and its AML-associated mutations make it a particularly attractive target for MRD assays.

**1. Overgrowth is the primary manifestation of many genetic syndromes. Based on the referenced Review, select the ONE best TRUE statement:** [See J Mol Diagn 2017, 19:487-497.]

a. All overgrowth syndromes are hereditary.
b. Disease-causing variants always occur during meiosis.
c. Disease-causing variants may occur during mitotic cell division.
d. Cancer is always the inevitable consequence of somatic variants.

**2. Postzygotic somatic variants have been identified in cellular pathways that promote growth. Based on the referenced Review , select the ONE best TRUE statement:** [See J Mol Diagn 2017, 19:487-497.]

a. One of the most frequently affected pathways in overgrowth syndromes is the phosphatidylinositol 3-kinase (PI3K)—AKT (V-Akt murine thymoma viral oncogene homolog)—mammalian target of rapamycin (mTOR) pathway.
b. The PI3K complex has three subunits coded by three different genes.
c. PI3K converts phosphatidylinositol (3,4,5)-triphosphate to phosphatidylinositol (3,4)-bisphosphate leading to phosphorylation of 3-phosphoinositide-dependent protein kinase 1.
d. The mTOR is a key protein in the PI3K/AKT/mTOR pathway that is downstream of AKT.

**3. Proteus syndrome is a rare overgrowth syndrome. Based on the referenced Review, select the ONE best TRUE statement:** [See J Mol Diagn 2017, 19:487-497.]

a. Proteus syndrome has an incidence of <1 in 10,000,000.
b. Proteus syndrome was first described as a separate clinical diagnosis in the 18$^{th}$ century.
c. Proteus syndrome was named after the Greek god Proteus, who was capable of changing shape at will.
d. The oldest known case report of Proteus syndrome, that of Joseph Merrick, also known as the Elephant Man, was described in the 17$^{th}$ century.

**4. CLOVES syndrome shares many features with Proteus syndrome but does not meet all of the diagnostic criteria or carry identifiable activating *AKT1* variants. Based on the referenced Review, select the ONE best TRUE statement:** [See J Mol Diagn 2017, 19:487-497.]

a. CLOVES syndrome is distinct from Proteus syndrome in the sense that the latter has a prenatal onset and overgrowth is slightly distorting.
b. Patients with CLOVES syndrome display severely distorted skeletal structures only after a major surgical procedure.
c. CLOVES syndrome has postnatal onset.
d. Patients with CLOVES syndrome are usually identified during adolescence.

**5. The authors of the referenced article developed a next-generation sequencing (NGS) panel that specifically targets mosaic overgrowth syndrome-associated variants in genes of the PI3K/AKT/mTOR pathway. Based on the referenced Review and article, select the ONE best TRUE statement:** [See J Mol Diagn 2017, 19:487-497 and 613-624.]

a. Through deep sequencing, the NGS panel allowed robust determination of both alleles and effectively detected mosaic variants as low as 10%.
b. Among the 50 consecutive clinical cases tested with the targeted NGS panel, a pathogenic variant was identified in 75% of cases.
c. The variant allele frequency ranged from 15% to 45%.
d. Activating variants are only present in the affected tissues in most cases and render mutant cell growth advantages both *in vivo* and *in vitro*.

**6. Every major node of the receptor tyrosine kinase PI3K/AKT/mTOR pathway is frequently mutated or amplified in a wide variety of tumors. Based on the referenced article, select the ONE best TRUE statement:** [See J Mol Diagn 2017, 19:613-624.]

a. PI3Ks are lipid kinases that regulate signaling pathways important for cell growth, proliferation, migration, metabolism, survival, apoptosis, angiogenesis, tumorigenesis, and brain development.
b. The authors of the referenced article studied 24 consecutive clinical cases suspected of mosaic overgrowth syndromes using a custom-designed NGS panel.
c. The authors of the referenced article found an association of the *PIK3CA* D913N variant with MCAP syndrome.
d. Somatic alterations of *PIK3CA*, *PIK3R2*, and *AKT* are well known to be associated with lung cancer and uterine cancer.

**7. Acute myeloid leukemia (AML) is a fatal disease with dismal outcomes. Based on the referenced article and related Commentary, select the ONE best TRUE statement:** [See J Mol Diagn 2017, 19**:**537-548 and 498-501.]

a. Even after achieving initial remission, 50% of patients relapse and ultimately succumb to their disease.
b. Although AML is genetically diverse across the patient population, AML cells within a single patient are homogeneous.
c. Flow cytometric methods of MRD detection are effective but require multiantibody panels and high levels of user expertise in the flow cytometric identification of rare cell populations.
d. Worldwide, there is only one accepted standard for the identification and quantification of MRD in AML.

**8. The presence of MRD is widely recognized as a powerful predictor of therapeutic outcome in AML. Based on the referenced article and related Commentary, select the ONE best TRUE statement:** [See J Mol Diagn 2017, 19:537-548 and 498-501.]

a. PCR-based detection of MRD, in the form of fusion transcripts, can be accomplished in approximately 60% of patients with AML with abnormal cytogenetics.
b. More than half of patients with AML have normal cytogenetics, and 20% to 30% of these patients, in turn, have *NPM1* mutations (*NPM1*muts).
c. Post-therapy monitoring of MRD in patients with *NPM1*mut AML using NGS has been evaluated in several clinical trials.
d. The levels of *NPM1*mut transcript were associated with patient outcome that pointed to *NPM1* as a stable marker for disease progression.

**9. Current methods for determination of MRD in AML involve detection of genotypic aberrations that are specific to leukemic blasts or analysis of phenotypic differences that distinguish leukemic from normal cells. Based on the referenced article and related Commentary, select the ONE best TRUE statement:** [See J Mol Diagn 2017, 19:537-548 and 498-501.]

a. The presence of the *NPM1*mut transcript is associated with high risk of relapse and a lower survival rate compared with patients with MRD.
b. A decrease in *NPM1*mut transcript copies correlated with hematologic remission, and half of the patients achieving complete remission presented <200 copies of the mutant transcript.
c. *NPM1*mut levels >10% after treatment and >20% after allogeneic transplantation were associated with poor overall and disease-free survival.
d. A threshold of 100 *NPM1*mut per $10^4$ *ABL1* copies was proposed for early detection of relapse after therapy.

**10. There is an urgent, unmet need for robust and sensitive assays that can be readily adopted as real-time tools for disease monitoring. Based on the referenced article and related Commentary, select the ONE best TRUE statement:** [See J Mol Diagn 2017, 19:537-548 and 498-501.]

a. Approximately 75% of *NPM1*muts consist of four nucleotide insertions in exon 10 at the 863 position.
b. The most common *NPM1*mut insertion is type B, found in approximately 95% of patients with *NPM1*mut AML.
c. An additional 5% of *NPM1*mut AML patients comprise both type A and type D mutations.
d. More than 50 frameshift insertion mutations have been reported, and at least hundreds are theoretically possible.

**11. MRD testing in patients with *NPM1*mut AML currently requires prior DNA sequencing to identify the specific insertion sequence to match the patient to an appropriate allele-specific qPCR test. Based on the referenced article and related Commentary, select the ONE best TRUE statement:** [See J Mol Diagn 2017, 19:537-548 and 498-501.]

a. Quantitative *NPM1* MRD testing requires maintenance of qPCR assays and plasmid standards for each mutation, with commercial plasmid standards being widely available for the top five mutations.
b. Digital PCR (dPCR) assays in which the standard type-specific qPCR assays for *NPM1*mut are directly adapted individually to dPCR format have been reported to circumvent the need for plasmid standards while demonstrating excellent agreement with qPCR for the detection of rare *NPM1*muts on clinical validation.
c. The excellent concordance between dPCR and qPCR detection of *NPM1*mts on clinical validation obviate the need for custom tests for every new *NPM1*mut that is encountered.
d. Multiparametric flow cytometry has been used for MRD detection in AML and can reliably detect leukemic cells as low as 0.5% (1 in 500) of total cells.

**12. A number of characteristics of the *NPM1* locus and its AML-associated mutations make it a particularly attractive target for MRD assays. Based on the referenced article and related Commentary, select the ONE best TRUE statement:** [See J Mol Diagn 2017, 19:537-548 and 498-501.]

a. *NPM1* mutations are stable throughout the disease, so false-negative results caused by mutation loss never occur.
b. Mutation of *NPM1* is typically present in preleukemic clones.
c. The detection of *NPM1* mutation does not predict relapse.
d. PCR primers designed against insertions in *NPM1* can be highly specific for mutant alleles and will theoretically have minimal amplification of unmutated sequence compared with primers against single-nucleotide alterations.