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MOLECULAR

**Patient:** John A. Doe
**DOB/Gender:** 10/10/44 (74 yrs) - Male
**Patient ID/MRN:** 123456
**Date Collected:** 01/02/2023

**Case# P23-00323**
**Status: Final**
**Report Category:**
**Not Detected**

**Provider:** Jane Smith, M.D.
Hematology Oncology Associates
Tel: 800-123-4567
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## DIAGNOSIS:

Peripheral blood:
HemeScreen™ Cytopenia results reveal:
- Negative for ASXL1 mutations
- Positive for WT1 mutations* see comments
- Negative for DNMT3A mutation
- Negative for RUNX1 mutations
- Negative for SF3B1 mutations

## COMMENT

WT1 exon 8 variant of unknown significance detected. Base change c.1107A>G. Protein change p.R369=.

## INTERPRETATION

ASXL1 (additional sex combs like 1) mutations can be seen in MDS, MPN, CMML, refractory anemia and AML. When present, the mutation is associated with poor prognosis and more aggressive disease. ASXL1 mutation occurs in CMML often (~45% of cases), and is rarely seen in PV. ASXL1 is often associated with RUNX1 cytogenetic abnormalities in both MDS and AML, as well as CEBPA in AML. Poor prognosis and aggressive disease is often paired with ASXL1 mutation.

Research has shown WT1 (Wilms' Tumor 1) can lead to higher relapse rate and poor prognosis in patients with AML. WT1 mutation can be seen in 6-15% of AML cases, often with patients who have FLT3-ITD and/or CEBPA mutations. Induction chemotherapy resistance has been associated with WT1 mutation. WT1 mutation can be used as a tool in determining MRD.

DNMT3A (DNA methyltransferase 3A) mutation can be seen in AML and MDS. Patients who have MDS with DNMT3A mutation have an increased chance of disease transformation to AML. DNMT3A mutation has poor prognosis for AML patients, and can be used to monitor treatment. DNMT3A mutation is often seen with IDH2 and SF3B1 mutations.

RUNX1 (runt-related transcription factor 1) mutation is commonly seen in chemotherapy-related MDS, but is also seen in MDS, AML, as well as AML after MDS. Recently RUNX1 mutation has been discovered in CML patients. RUNX1 mutation is also associated with abnormal cytogenetics, including monosomy 7, trisomy 21 and trisomy 13. RUNX1 and monosomy 7 can lead to rapid progression of AML when the patient is being treated for MDS. CEBPA and NPM1 are not commonly associated with mutated RUNX1. Whereas, FLT3 mutation is more commonly associated with RUNX1 mutation, approximately 30% in FLT3-ITD cases and 11% in FLT3-TKD cases. RUNX1 mutation has a poor prognosis regardless of cytogenetic abnormalities and should be considered in the diagnosis of AML.

SF3B1 (Splicing Factor 3b Subunit 1) is involved in DNA repair; mutation of this gene causes dysregulation of the maintenance of DNA. SF3B1 mutation is commonly associated with unmutated IgVH, fludarabine-resistance, and concurrent TP53 mutation. This mutation is associated with poor outcome, <10 yr survival. Studies have shown SF3B1 mutations are later events in disease progression.

**REFERENCES:**
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2. Rampal R, Figueroa ME. Wilms tumor 1 mutations in the pathogenesis of acute myeloid leukemia. Haematologica. 2016;101(6):672-679. doi:10.3324/haematol.2015.141796
3. Lin ME, Hou HA, Tsai CH, et al. Dynamics of DNMT3A mutation and prognostic relevance in patients with primary myelodysplastic syndrome. Clin Epigenetics. 2018;10:42. Published 2018 Apr 2. doi:10.1186/s13148-018-0476-1
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5. Albert, Charna. "Integrating NGS into the Cytopenia Workup." CAP TODAY, 20 May 2022, https://www.captodayonline.com/integrating-ngs-into-the-cytopenia-workup/.
6. Xia Y, Fan L, Wang L, Gale RP, Wang M, Tian T, Wu W, Yu L, Chen YY, Xu W, Li JY. Frequencies of SF3B1, NOTCH1, MYD88, BIRC3 and IGHV mutations and TP53 disruptions in Chinese with chronic lymphocytic leukemia: disparities with Europeans. Oncotarget. 2015 Mar 10;6(7):5426-34. doi: 10.18632/oncotarget.3101. PMID: 25605254; PMCID: PMC4467158. https://pubmed.ncbi.nlm.nih.gov/25605254/
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**METHOD:**
Melting curve analysis in combination with real-time PCR is a natural extension of continuously monitored PCR within each cycle. During high resolution DNA melting analysis (HRM or HRMA), melting curves are produced using dyes that fluoresce in the presence of double-stranded DNA (dsDNA). Using specialized instruments designed to monitor fluorescence during heating; as the temperature increases, the fluorescence decreases, producing a characteristic melting profile.

This assay can detect mutations with a minimum sensitivity of 2% depending on the wild type background in the specimen. Although molecular testing is highly accurate rarely false-positive and false-negative diagnostic errors may occur.

HRM analysis was performed using HRM v3.1 Thermo Fisher software to discriminate DNA sequences based on their composition, length, GC content, or strand complementarities.

The somatic mutations are being confirmed by Sanger sequencing bi-directional method. This assay has a sensitivity of 5~10% for detecting mutant in wild-type background. Various factors including quantity and quality of nucleic acid, sample preparation and sample age can affect assay performance.

**Electronically Signed By:** Frank Bauer, MD, Precipio, Inc. (01/06/2023 11:00)

Disclaimer: The adequacy of staining is verified by the appropriate LSI controls. The reagents used for these assays are for research use only (RUO). Their performance characteristics have been initiated by Precipio, Inc., New Haven, CT. They have not been reviewed by the FDA. The FDA has deemed that such approval is unwarranted for clinical use. These assays should be viewed as experimental and/or research use only.

## CLINICAL DATA

ICD-10: E85.9, D64.9, D75.9. Amyloidosis, unspecified. Anemia, unspecified. New diagnosis.

Received CBC, reported on 12/15/2022: WBC 3.9; RBC 3.73; HGB 11.3; HCT 34.8; MCV 93.0; MCH 30.2; MCHC 32.4; RDW 14.9%; PLT 231; MPV 6.6; LYM 17.3%; GRAN 78.4%; MID NP; MON 4.3%; NEU NP; EOS NP; BAS NP; (NP = not provided)

**Patient:** John A. Doe
**Case #:** P23-00323
**Received Information:** 1 green-top tube(s), 2 lavender-top tube(s)
**Received:** 01/02/2023 10:39
**Reported:** 01/06/2023 11:30