Cairo Diagnostics

**Medical Director**
Sherif Ibrahim, M.D. Ph.D.

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**CLIA#** 33D2047314

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White Plains, NY 10604

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**cairodiagnostics.com**

| | | | |
|---|---|---|---|
| **Specimen** | BONE MARROW | **Final Report** | 10/11/2025, 1:00pm |
| **Your No** | 123456 **Requisition** | **Case No.** | **Z25-00178** |
| **Collected** | 10/07/25, 9:00am | **Received** | 10/07/25, 11:00am |

| **Patient** | | | |
|---|---|---|---|
| **Name** | **Doe, Jane** | | |
| **DOB** | 01/01/63 (62 yrs) | **Sex** | Female |
| **ID#** | **Tel.** | | |

| **Physician** | | | | | |
|---|---|---|---|---|---|
| **Facility** | Location123 | | | **Account No.** | 10604-test |
| **Attending:** | John Smith, M.D. | **Tel.** | | **Fax** | |
| **Corresponding** | | **Tel.** | | **Fax** | |

## DIAGNOSIS

BONE MARROW, ASPIRATION, CLOT, AND BIOPSY:

- MARKEDLY HYPERCELLULAR MARROW, TRILINEAGE DYSPLASIA, AND SIGNIFICANT BLAST INCREASE (~15-20%), CONSISTENT WITH ACUTE MYELOID LEUKEMIA WITH NPM1 MUTATION. SEE COMMENT.
- POSITIVE MOLECULAR STUDIES FOR NPM1 TYPE A (AF: 46.96%) AND NRAS (AF: 38.27%) MUTATIONS.
- POSITIVE KARYOTYPE AND FISH STUDIES FOR LOW-LEVEL TRISOMY 8.

**Electronically Signed and Reported by:** Sherif Ibrahim, MD, PhD

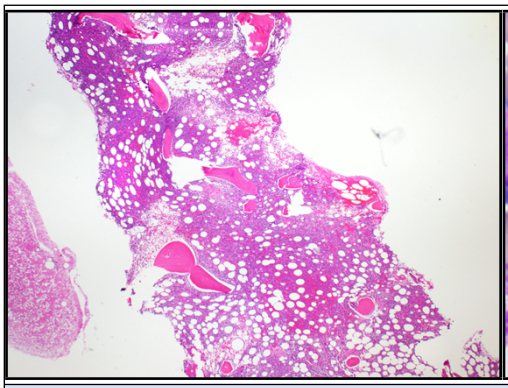
## COMMENT

NPM1 mutation is described in rare cases of aggressive MDS with rapid transformation to AML. WHO (5th edition) suggests that NPM1 mutation is an AML-defining mutation regardless of the number of myeloid blasts.

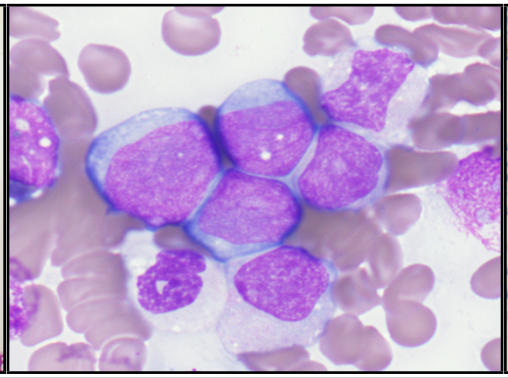
Case discussed with Dr. Smith on 10/07/2025 at 2 PM.

## CLINICAL HISTORY

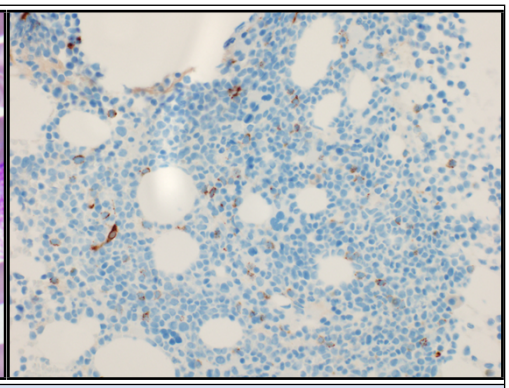
62 year old female with anemia and thrombocytopenia, rule out MDS.
ICD-10: D64.9, D69.6, D46.9



**Markedly Hypercellular Marrow**



**Cluster of Blasts on Aspirate Smear**



**CD 117+ Blasts ~15-20%**

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**Patient Name Doe, Jane** **Case No. Z25-00178**

## GROSS DESCRIPTION

Received in formalin labeled with patient's name is a bone marrow biopsy measuring 2.0cm in length. After decalcification of the bone marrow biopsy, the specimen is submitted entirely for routine histology in one cassette labeled C1.
Received in formalin labeled with patient's name is a bone marrow clot measuring 2.0x 1.0x 0.4cm. A representative section of the specimen is submitted for routine histology in one cassette labeled C2. Aspirate smears were submitted.

## MICROSCOPIC DESCRIPTION

The biopsy is markedly hypercellular for age with patchy cellularity at ~70-80%. Trilineage hyperplasia with progressive maturation is observed. Megakaryocytes are significantly increased in number with pleomorphism (monolobated and multi-nucleated forms with some pyknotic nuclei). Myeloid elements show progressive maturation with some left shift and increase in blasts (~15-20%). Erythroid elements show left shift. A small lymphoid aggregate composed of small mature lymphocytes is noted.

## CLOT SECTION

The sections are reflective of the biopsy and show similar morphologic findings.

## IMMUNOHISTOCHEMICAL STAINS

Immunohistochemical stains were performed on bone marrow biopsy sections for CD34, CD61, CD71, CD117, and myeloperoxidase. CD34 (subset of blasts), CD117 and MPO stained increased myeloid blasts, singly and in small clusters (~15-20%). CD117 also stained a population of left shifted myeloid elements. CD71 stained erythroid precursors. CD61 stained increased megakaryocytes.

## ASPIRATE

The smears are particulate and hypercellular. Trilineage hematopoiesis with dysplasia is observed. Megakaryocytes are increased in number with some monolobated and multinucleated forms. Myeloid elements show progressive maturation to segmented neutrophils with left shift, dysplastic features (hypogranularity and abnormal segmentation) and increase in blasts (~18%). Erythroid elements show progressive maturation with dysplastic features (budding and nuclear cytoplasmic dissociation). Few mature appearing lymphocytes, eosinophils and plasma cells are seen. Monocytes are increased in number.

**Differential Count**

| Cell Type | Result | Ref. Range | Cell Type | Result | Ref. Range |
|---|---|---|---|---|---|
| Red Cell/Precursors | 25 | 20.0 – 40.0% | Blast | 18 | 0 – 3.0% |
| Lymphocyte | 7 | 3.0 – 20.0% | Promyelocyte | 5 | 2.0 – 4.0% |
| Plasma cell | 1 | 0 – 4.0% | Neutrophil/Precursors | 30 | 45.0 – 69.0% |
| Eosinophil | 2 | 1.0 – 5.0% | Monocyte | 12 | 0 – 2.0% |
| Basophil | | 0 – 2.0% | M:E | 3:1 | |

## SPECIAL STAINS

Reticulin stain shows mild to focally moderate increase in reticulin content.
Iron stains show increased storage and sideroblastic iron.

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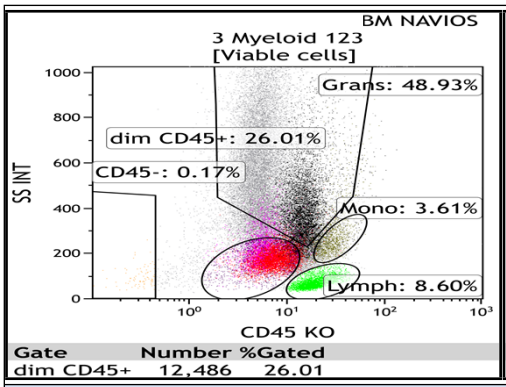
## FLOW CYTOMETRY INTERPRETATION

**BONE MARROW**

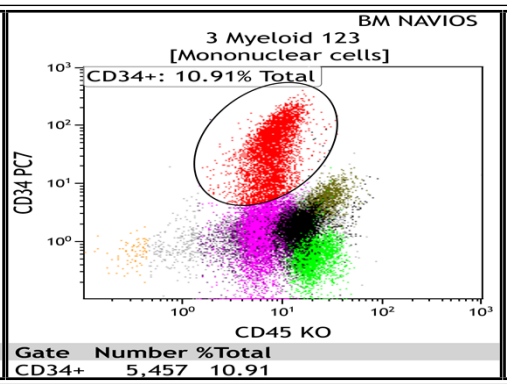
- LARGE POPULATION OF MYELOID BLASTS DETECTED (~11%). SEE BONE MARROW REPORT FOR FURTHER EVALUATION.

## VIABILITY AND ANALYSIS

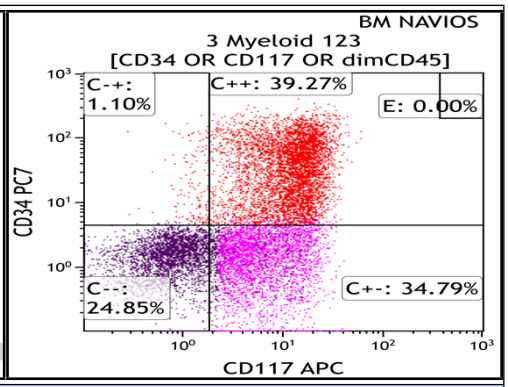
The specimen has viability of ~96%. The above data were generated on a population of cells that cytometrically correspond to lymphoid cells. The gated lymphoid population comprises ~8% of the total cells.




Increase in Blasts/Dim CD45 Population




CD34 Positive Blasts )~11%)




CD117 vs CD34 Positive Blasts

## PHENOTYPE

~11% of the cells examined are detected in the blast gate. These cells are positive for CD34 (subset), CD117, HLA-DR, CD13, CD33, CD56, CD38 and cytoplasmic(c) myeloperoxidase (MPO), and are negative for surface(s) CD3, cCD3, sCD19, sCD10, cCD79a, and cCD22, and the rest of the markers tested. The rest of the neutrophils show normal forward and low-side scatter properties. The B cells are a minor population (~1% of the total). They express the pan B-cell markers CD19, CD20, and CD22 and lack CD5 and CD10. The B cells are polyclonal with regard to light chain determinants. The T-cell population (~2% of the total) expresses the pan T-cell antigens (CD2, CD3, CD5, and CD7) in a non-aberrant fashion. The CD4/CD8 ratio is ~4/1.

## PERIPHERAL BLOOD

A CBC study was submitted (10/07/2025): WBCs 6.26, Hgb 7.9, MCV 93.4, Platelets 59.

| Markers Tested | | | | |
|---|---|---|---|---|
| **B-Cell Markers** | **T-Cell Markers** | **Myeloid Markers** | **Plasma Cell Markers** | **Miscellaneous Markers** |
| **CD20** | **CD7** | **CD33** | | **HLA-DR** |
| **Kappa** | **CD4** | **CD13** | | **CD38** |
| **CD10** | **CD8** | **CD14** | | **CD45** |
| **Lambda** | **CD5** | **CD11b** | | **CD56** |
| **CD22** | **CD2** | **CD16** | | **CD117** |
| **CD19** | **CD3** | **CD71** | | **CD34** |
| **CD79a** | **cCD3** | **CD64** | | **MPO** |
| | **CD36** | | | **Anti-TdT** |
| | | | | **CD235a** |
| | | | | **CD41** |
| | | | | **CD123** |

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**Patient Name Doe, Jane** **Case No. Z25-00178**

## FISH DIAGNOSIS

**BONE MARROW**

AML/MDS FISH study is positive for low-level trisomy 8. See Interpretation

**Director of Cytogenetics:** Sherif Ibrahim, MD, PhD **Hematopathologist:** Sherif Ibrahim, MD, PhD

**RESULT** nuc ish (D5S23,D5S721,EGR1)x2,(D7Z1,D7S522)x2,(D8Z2x3,D20S108x2),(RUNX1T1x3,RUNX1x2),(KMT2A)x2,(ETV6)x2,(PML,RARA)x2,(CBFB)x2,(TP53,CEP17)x2; Abnormal FISH Pattern

| Locus | Probe | Result | Cells Counted |
|---|---|---|---|
| 5p15.2/5q31 | D5S23, D5S721/EGR1 | Negative/ (98%) | 200 |
| 7p11.1-q11.17q31 | D7Z1/D7S522 | Negative/ (98%) | 200 |
| 8p11.1-q11.1 | CEP 8 (D8Z2) | Positive/ Trisomy 8 (7.5%) | 200 |
| 12p13.2 | ETV6 | Negative/ (98%) | 200 |
| TP53.1(p13.1)/CEP 17 (p11.1q11.1) | TP53/CEP 17 | Negative/ (98%) | 200 |
| 20q12 | D20S108 | Negative/ (98%) | 200 |
| 8q21.3/21q22 | RUNX1T1/RUNX1 | Positive/ Negative RUNX1/RUNX1T1 translocation. Positive for trisomy 8 Gain (4.5%) | 200 |
| 11q23 | KMT2A (MLL) | Negative/ (98.5%) | 200 |
| 15q22-24/17q21 | PML/RARA | Negative/ (98%) | 200 |
| 16q22 | CBFB | Negative/ (100%) | 200 |

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## INTERPRETATION

FISH is low level positive for gain of an additional copy of chromosome 8 (trisomy 8) in 7.5% of nuclei. The RUNX1/RUNX1T1 t(8;21) gene probe set shows an abnormal hybridization pattern consistent with trisomy 8.

Trisomy 8 is a recurrent finding in myeloid disorders, including acute myeloid leukemia, myelodysplastic syndrome and myeloproliferative neoplasms. Trisomy 8 is the most frequent numerical aberration in AML, occurring as the sole karyotypic abnormality in 5% of all cytogenetically abnormal AML cases without specificity for any particular disease subgroup. Patients with trisomy 8 generally proceed through a myelodysplastic phase prior to development of overt leukemia.

This FISH study is negative for RUNX1T1/RUNX1 t(8;21), negative for MLL (11q23) translocation or amplification, negative for CBFB [inversion of chromosome 16 or t(16;16)] gene rearrangement, and negative for PML/RARA t(15;17).

This FISH study is also negative for monosomy 5 /5q deletion, negative for monosomy 7 /7q deletion, negative for 20q deletion, negative for ETV6 deletion or translocation, and negative for TP53 /17p13 deletion.

Limitations: Please note that this FISH test detects only the loci specifically targeted by these probes. It does not detect any other numerical nor structural abnormalities of the chromosomes that are not tested. Correlation with other clinical and laboratory findings is recommended.

For any probes tested, cutoff values were determined by analyzing normal cases. If the percentage of abnormal cells is at or below the cutoff values, the probe is considered normal. If the percentage is above the cutoff values, the probe is considered abnormal.

FISH PROBE: NORMAL CUTOFF
EGR1: EGR1 Deletion: >5.1% / Monosomy 5: >1.5% / Others: >5.1%
D7S522: D7S522 Deletion: >4.4% / Monosomy 7: >4.4% / Others: >5.7%
CEP 8: Trisomy 8: >3.8% / Others: >5.1%
RUNX1T1;RUNX1: RUNX1T1;RUNX1 Translocation: >1.5% / Single Fusion: >5.1% / Extra Copy of RUNX1T1: >3.8% / Others: >4.4%
MLL: MLL Rearrangement: >2.3% / Loss of MLL: >5.1% / Extra Copy of MLL: >3.1% / Others: >3.1%
ETV6: ETV6 Rearrangement: >6.3% / Loss of ETV6: >1.5% / Extra Copy of ETV6: 3.8% / Others: >5.7%
PML;RARA: PML;RARA Translocation: >1.5% / Single Fusion: >6.3% / Others: >3.8%
CBFB: CBFB Rearrangement: >2.3% / Loss of CBFB: >5.1% / Extra Copy of CBFB: >2.3% / Others: >2.3%
D20S108: D20S108 Deletion: >3.8% / Others: >2.3%
TP53: TP53 Deletion: >5.1% / Others: >4.4%

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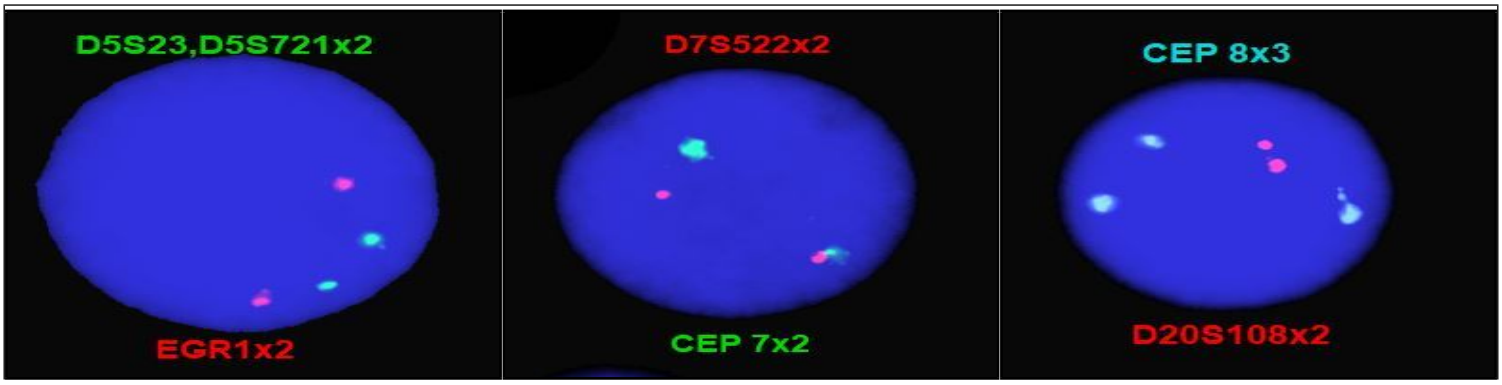
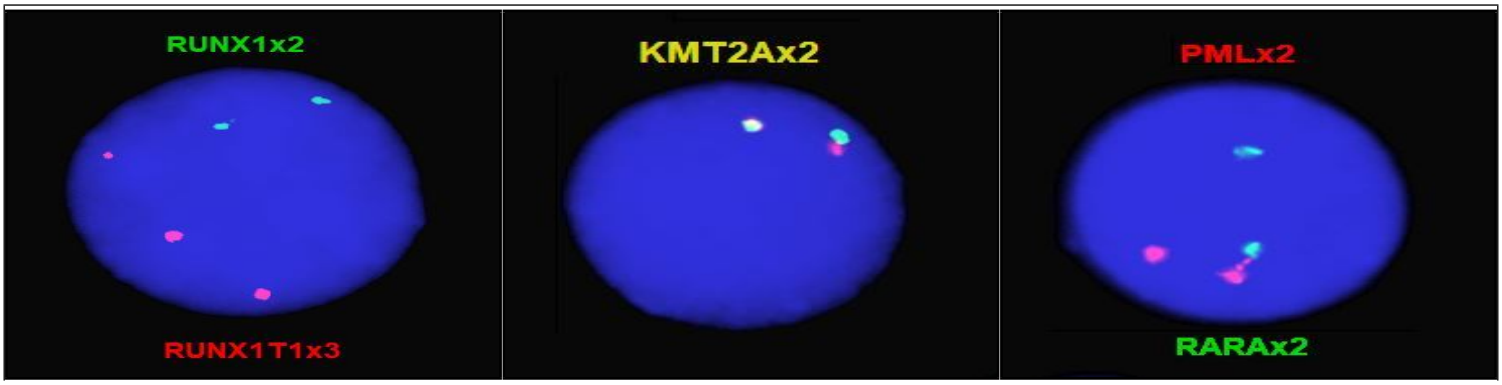
**Patient Name Doe, Jane** **Case No. Z25-00178**

Imaging analysis for all FISH probes are performed manually. No automated imaging for FISH is performed by Cairo Diagnostics, LLC.

The following probes contain 2 or more separate fluorochromes and are considered multiplex probes: ABBOTT MOLECULAR PROBES: 1PTEL/P58/1Q25, CEP 3/MYB, BCL6, FGFR3/IGH, D5S23;D5S721/EGR1, D7Z1/D7S522, CEP 8 (D8Z2)/D20S108, RUNX1T1/RUNX1, MYC, MYC/IGH/CEP 8, ABL1/BCR, CEP 9/CEP 15/TP53, CCND1/IGH, BIRC3/MALT1, ATM/TP53, MLL, CEP 12/D13S319/LAMP1, ETV6, D13S319/LAMP1, IGH, IGH/MAF, IGH/BCL2, PML/RARA, CBFB,TP53/CEP 17 and CYTOCELL FISH PROBES: CHIC2/FIP1L1/PDGFRA, PDGFRB, PCM1/JAK2, FGFR1/CEP 8, IGH/MAFB.



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**Patient Name** Doe, Jane **Case No.** Z25-00178

## KARYOTYPE DIAGNOSIS

**BONE MARROW**

Abnormal Female Karyotype, characterized by gain of an additional copy of chromosome 8 (trisomy 8).
See Interpretation

**Director of Cytogenetics:** Sherif Ibrahim, MD, PhD **Hematopathologist:** Sherif Ibrahim, MD, PhD

## INTERPRETATION

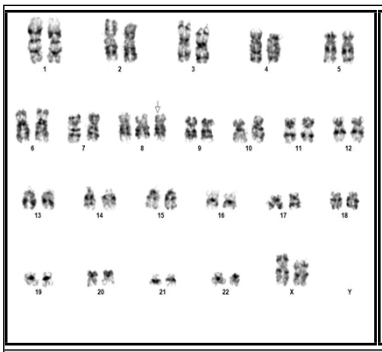
Chromosome analyses of the 24 and 48 hr. cultures show one abnormal cell (out of 20 cells) characterized by gain of an additional copy of chromosome 8 (trisomy 8). No other aberrations were identified, and nineteen normal cells were found.

Trisomy 8 has been reported in myeloid malignancies such as acute myeloid leukemia (AML) and myelodysplastic syndrome (MDS) and myeloproliferative disorders. Correlation with hematopathology is suggested.

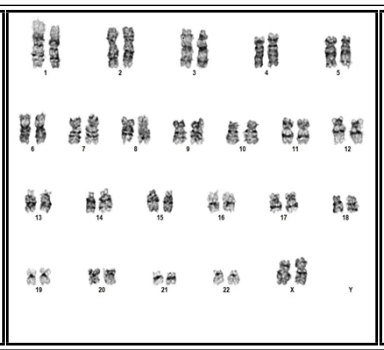
NOTE: concurrent FISH studies showed the presence at low level (7.5% of nuclei) of trisomy 8. Correlation with concurrent NGS sequencing studies is recommended.

**Results:** (G-Banding): 47,XX,+8[1]/46,XX[19]; Abnormal Female Karyotype

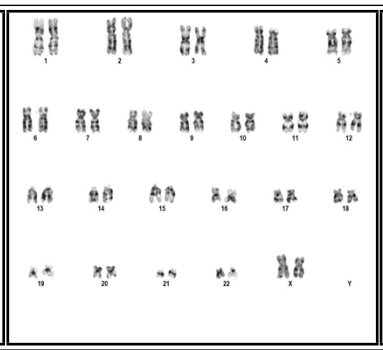
| No. of cells with < 44 / 44 / 45 / 46 / 47 / >47 Chromosomes | | | | Average band resolution | 450 |
|---|---|---|---|---|---|
| | 1 | | 18 1 | | |
| **Cells Counted** | 20 | **Cells Analyzed** | 20 | **Cells Captured** | 20 | **Cells Karyotyped** | 4 |



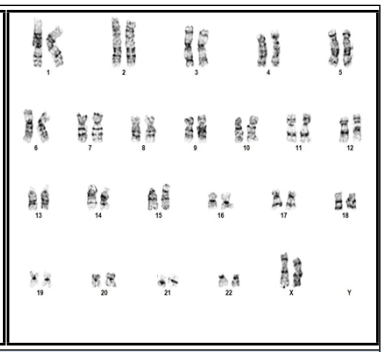
**Abnormal Karyotype (C-1, Cell #3)**



**Normal Karyotype (C-1, Cell #7)**



**Normal Karyotype (C-2, Cell #15)**



**Normal Karyotype (C-2, Cell #16)**

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| **Specimen** | BONE MARROW | **Final Report** | 10/11/2025, 1:00pm |
| **Your No** | 123456 **Requisition** | **Case No.** | **Z25-00178** |
| **Collected** | 10/07/25, 9:00am | **Received** | 10/07/25, 11:00am |

| **Patient** | | | |
|---|---|---|---|
| **Name** | Doe, Jane | | |
| **DOB** | 01/01/63 (62 yrs) | **Sex** | Female |
| **ID#** | | **Tel.** | |

| **Physician** | | | | | |
|---|---|---|---|---|---|
| **Facility** | Location123 | | | **Account No.** | 10604-test |
| **Attending:** | John Smith, M.D. | **Tel.** | | **Fax** | |
| **Corresponding** | | **Tel.** | | **Fax** | |

## MOLECULAR STUDIES

### BONE MARROW

RESULTS: POSITIVE for NGS myeloid panel

1. NPM1 c.863_864insTCTG (p.Trp288CysfsTer12, Tier I) mutation was detected with 46.96% allele frequency.
2. NRAS c.38G>T (p.Gly13Val, Tier II) mutation was detected with 38.27% allele frequency.
3. RNA Fusions: None detected.

The NGS raw data were manually reviewed to rule out low-level FLT3-ITD mutations.

**Interpretation:**

The molecular study is positive for NPM1 type A (c.863_864insTCTG, p.Trp288CysfsTer12, 46.96%) and NRAS (c.38G>T, p.Gly13Val, 38.27%) mutations. These findings support a clonal hematologic process, as the presence of a somatic mutation with a variant allele frequency (VAF) ≥10%, or two or more somatic mutations, is associated with a positive predictive value (PPV) of 0.86 and 0.88, respectively, for diagnosing myeloid neoplasm (PMID: 28424163).

In myelodysplastic syndrome (MDS), NPM1, one of the 16 main effect genes, is included in IPSS-M (Molecular International Prognosis Scoring System for MDS, https://mds-risk-model.com/) for MDS patients (NCCN Guidelines Version 3.2023). NPM1 gene mutations rarely occur in non-acute myeloid neoplasms with <20% blasts. They have been reported in approximately 2% of MDS cases, primarily in those with excess blasts, and in about 3% of MDS/MPN cases, predominantly classified as chronic myelomonocytic leukemia (CMML). These mutations are associated with an aggressive clinical course, relatively rapid progression to overt acute myeloid leukemia (AML), and poor survival outcomes (33255988). Notably, the WHO 5th edition does not require a 20% blast threshold, whereas the ICC still requires at least 10% blasts for the diagnosis of NPM1-mutated AML.
The prognosis of NPM1-mutated AML is influenced by other co-occurring mutations. Mutated NPM1 without FLT3-ITD is categorized as a favorable risk group whereas mutated NPM1 with FLT3-ITD mutation and wild-type NPM1 with FLT3-ITD mutation and no adverse-risk cytogenetic lesions are included in the intermediate risk group. Of note, AML with NPM1 mutation and adverse-risk cytogenetic abnormalities are categorized as adverse-risk (2022 ELN, NCCN Guidelines Version 6.2023).

In myelodysplastic syndrome (MDS), NRAS, one of the 16 main effect genes, is included in IPSS-M (Molecular International Prognosis Scoring System for MDS, https://mds-risk-model.com/) for MDS patients (NCCN Guidelines Version 3.2023).
NRAS mutation is reported 10~13% in AML (16434492, 32699322). Therapeutically predictive or prognostic implications for NRAS mutations are not well established or formally incorporated into clinical algorithms for AML (NCCN Guidelines Version 6.2023).

**Director of Molecular:** Sherif Ibrahim, M.D. , PhD **Hematopathologist:** Sherif Ibrahim, M.D. , PhD

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|---|---|

## COMMENT

**Comprehensive Myeloid Panel**

Our next-generation Comprehensive Myeloid Panel empowers precision in the diagnosis and management of hematologic malignancies by consolidating multiple complex assays into one streamlined test. Designed for maximum clinical impact, this assay delivers broad and actionable genomic insight into the key drivers of myeloid disease.
With an expanded total of 81 genes (52 DNA mutation genes and 29 RNA fusion genes), the panel enables highly sensitive detection of single nucleotide variants, insertions/deletions, and fusion transcripts with a limit of detection of 3%. This integrated approach accelerates turnaround time, enhances workflow efficiency, and provides deeper molecular clarity to guide treatment decisions.

20 Full Genes: ASXL1, BCOR, BCORL1, CALR, CEBPA, DDX41, ETV6, EZH2, IKZF1, NF1, PHF6, PRPF8, RAD21, RB1, RUNX1, SH2B3, STAG2, TET2, TP53, ZRSR2.

32 Hotspot Genes: ANKRD26, ABL1, BRAF, CBL, CSF3R, DNMT3A, ETNK1, FLT3, GATA1, GATA2, HRAS, IDH1, IDH2, JAK2, KIT, KRAS, MPL, MYD88, NPM1, NRAS, PPM1D, PTPN11, SF3B1, SMC1A, SMC3, STAT3, STAT5B, SETBP1, SRSF2, U2AF1, UBA1, WT1.

29 Fusion Driver Genes: ABL1, ALK, BCL2, BRAF, CCND1, CREBBP, ETV6, EGFR, FGFR1, FGFR2, FUS, HMGA2, JAK2, KMT2A (MLL), MECOM, MET, MLLT10, MLLT3, MYBL1, MYH11, NTRK3, NUP214, PDGFRA, PDGFRB, RARA, RBM15, RUNX1,TCF3, TFE3.

This assay is designed to detect specific gene fusions (analytical sensitivity: $10^{-4}$), single nucleotide variants (SNVs), and small insertions and deletions (InDels) with an analytical sensitivity of 3% variant allele frequency (VAF). Very large deletions or insertions may not be detected. The assay has been validated for FLT3 insertions up to 99 bp with a sensitivity of 1.5% VAF.
A negative result does not exclude the presence of a mutation below the assay's limit of detection.
Results should always be interpreted in conjunction with clinical, morphologic, and immunophenotypic findings.
Library preparation is performed using AmpliSeq™ chemistry, and sequencing is carried out on the Ion Torrent S5 Prime system. Bioinformatic analysis is conducted using the Torrent Suite Server (v5.18) and Ion Reporter Software (v5.20).