PRECIPIO EXPECT MORE
203-787-1717
203-901-1289

COMPREHENSIVE REPORT

Patient: John A. Doe
DOB/Gender: 10/10/61 (58 yrs) - Male
Patient ID/MRN: 123456
Date Collected: 04/09/2019

Case# /Status: X19-00257 - Final

Report Category:
Neoplastic

Provider: John Doe, M.D.
Hematology Oncology Associates
Tel: 800-123-4567
Fax: 800-765-4321

DIAGNOSIS:

CALR-positive myeloproliferative neoplasm - most consistent with essential thrombocythemia - see comment

COMMENT

While the differential diagnosis includes essential thrombocythemia (ET) and pre-fibrotic primary myelofibrosis (PMF), the megakaryocytic morphology favors ET. Correlation with clinical findings is advised.

All myeloid and lymphoid neoplasms are now classified and named in accordance with the newly revised 2017 version of the WHO Classification of Tumours of Haematopoietic and Lymphoid Tissues.

COMPONENT DIAGNOSES

Biopsy: Normocellular marrow with numerous large abnormal megakaryocytes
Aspirate: Hypocellular aspirate smears with corresponding biopsy sample showing features diagnostic of Myeloproliferative Neoplasm.
Flow Cytometry: Normal flow study
Karyotyping: No evidence of an acquired clonal abnormality, see interpretation below.
FISH: No evidence of an acquired clonal abnormality, see interpretation below.
Molecular: HemeScreen™ results reveal:
- Negative for JAK2 V617F point mutation
- Negative for JAK2 exon 12 mutations
- Negative for MPL W515L/K point mutations
- The specimen tested positive (mutated; 52bp deletion) for CALR mutations

CLINICAL DATA

ICD-10: D47.3, D46.9, D47.1, Z13.79. ET. MDS/MPD. New diagnosis.

Received CBC, reported on 03/27/2019: WBC 7.5; RBC 5.17; HGB 14.4; HCT 45.1; MCV 87.3; MCH 27.8; MCHC 31.9; RDW 14.1%; PLT 698; MPV 8.1; LYM 21.3%; MON 6.9%; NEU NP; EOS NP; BAS NP (NP = not provided)

Electronically Signed By: Frank Bauer, MD (04/12/19 17:39)

DIAGNOSIS:

Bone marrow, core biopsy: Normocellular marrow with numerous large abnormal megakaryocytes.

MICROSCOPIC DESCRIPTION

Specimen Adequacy: Adequate
Marrow Cellularity: 50%
Megakaryocytes: Increased large hyperlobulated megakaryocytes, some in clusters.
Myeloid Maturation: Complete
Erythroid Maturation: Complete
Lymphoid Aggregates: Not seen
Granulomas: Not seen
Marrow Trabeculae: Normal
Iron Stain: Negative
Marrow Reticulin: Mild focal increase
PAS / Giemsa: Evaluated
Special Stains: Giemsa, Iron, PAS, Reticulin
The purpose for these ancillary tests is to rule out / evaluate ET; MDS/MPD

Electronically Signed By: Frank Bauer, MD (04/12/19 17:37)

GROSS DESCRIPTION:
The specimen is received in formalin labeled with the patient's initials and requisition number, and consists of 1 piece of bone marrow core measuring 2.0 x 0.2 x 0.2 cm. The specimen is submitted in 1 cassette after decalcification.

DIAGNOSIS:

Bone marrow, aspirate: Hypocellular aspirate smears with corresponding biopsy sample showing features diagnostic of Myeloproliferative Neoplasm.

COMMENT

See biopsy comment.

SMEAR REVIEW

The marrow aspirate smear is hypocellular for diagnostic evaluation. Megakaryocytes are reduced in number with normal morphology. Erythroid maturation is normoblastic. Myeloid maturation is sequential. No increase in immature cells is noted. No ring sideroblasts are detected on iron stain of the marrow aspirate.

Number of cells counted: 300

Cell Type	Percent	Ref. Range
Blasts	1 %	0.3 - 3.0 %
Immature myeloid	8 % ↓	12.0 - 21.0 %
Mature myeloid	64 % ↑	35.0 - 55.0 %
Eosinophils	3 %	1.0 - 3.0 %
Basophils	2 % ↑	0.0 - 1.0 %
Lymphocytes	8 % ↓	10.0 - 15.0 %
Plasma cells	1 %	0.0 - 1.0 %
Monocytes	5 % ↑	0.0 - 1.0 %
Erythroid	8 % ↓	15.0 - 25.0 %

Electronically Signed By: Frank Bauer, MD (04/10/19 17:37)

DIAGNOSIS:

Bone marrow, aspirate: Normal flow study.

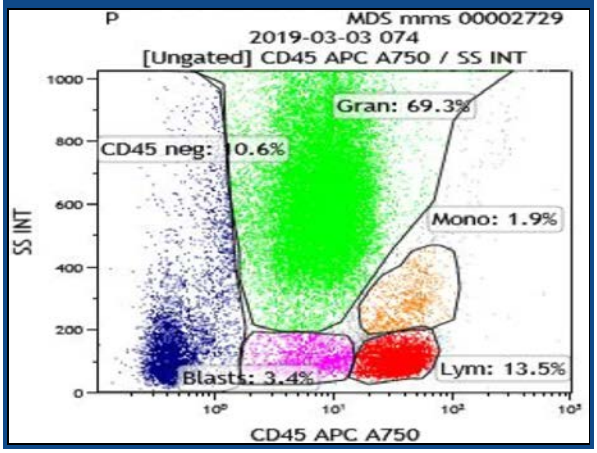
INTERPRETATION

No aberrant myeloid antigen expression suggestive of dysmyelopoiesis is identified. The granulocytes exhibit a normal pattern of expression of CD11b, CD13 and CD16 in bone marrow. There is a total of 1% CD34+ cells identified. The lymphocytes (14%) include 11% polyclonal B-cells, 70% mature T-cells with a normal CD4/CD8 ratio, and 13% natural killer (NK) cells. The CD138+ plasma cells (<1%) have a polyclonal kappa/lambda phenotype.

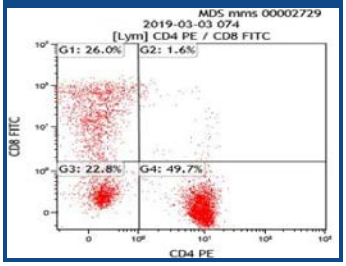
RESULT

Analysis Time: 4/09/19 13:04 Viability: 94% (Normal > 80%) Specimen: BM, Green-top tube

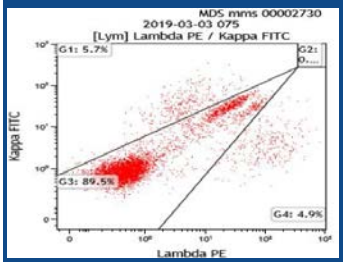
Flow Differential



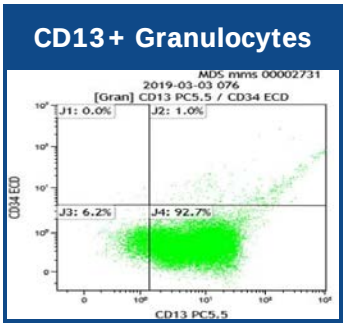
Normal CD4/CD8



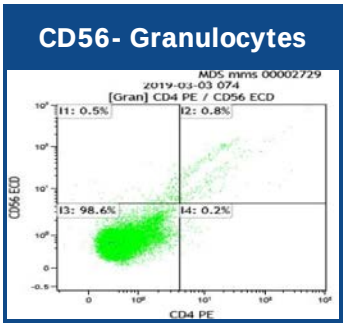
Polyclonal B-Lymphs



CD13+ Granulocytes



CD56- Granulocytes



Flow Cytometry Differential

Lymphocytes:	14%
Monocytes:	2%
Granulocytes:	69%
Plasma Cells:	<1%
Blasts:	3%
nRBC & Debris:	11%

Granulocytes

Marker	%
CD4	<1
CD10	43
CD11b	98
CD13	94
CD14	54
CD15	99
CD16	50
CD19	2
CD33	94
CD34	1
CD45	100
CD56	1
CD61	47
CD64	32
CD71	14
CD117	2
HLA-DR	2

Lymphocytes

Marker	%
CD2	75
CD3	70
CD4	50
CD5	73
CD7	71
CD8	26
CD10	1
CD19	11
CD20	10
CD38	8
CD45	100
CD56	13
Kappa	6
Lambda	5

Plasma Cells

Marker	%
CD45	100
CD138	100
cKappa	36
cLambda	64

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

DIAGNOSIS:

Bone marrow, aspirate: No evidence of an acquired clonal abnormality, see interpretation below.

INTERPRETATION

KARYOTYPE "ISCN": 46,XY[20]; Normal Male Karyotype

Conventional cytogenetic analysis performed to detect and characterize chromosomal aberrations to aid histopathological and clinical diagnosis, which revealed a male karyotype with no evidence of an acquired clonal abnormality.

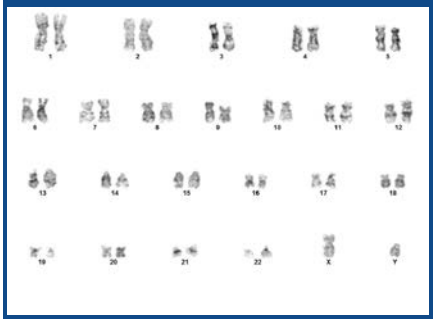
This does not exclude the possibility of an abnormality that cannot be detected at the chromosomal level or exists at a low residual level.

Interpretation of this specimen's cytogenetic results should be made in conjunction with morphologic, immunophenotypic, and clinical findings. The results of this analysis do not exclude the possibility of genetic alterations below the band-resolution of this test or abnormalities due to other etiologies.

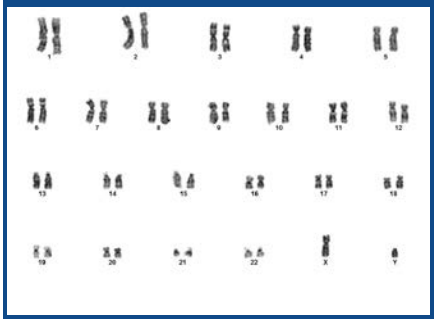
Analysis

Cells Counted:	**20**
Cells Analyzed:	**20**
Cells Imaged:	**3**
Cells Karyotyped:	**3**
Band Level:	**450**
Banding Type:	**G-Banding**
Indication:	**MDS**

Normal Karyotype



Normal Karyotype



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DIAGNOSIS:

Bone marrow, aspirate: No evidence of an acquired clonal abnormality, see interpretation below.

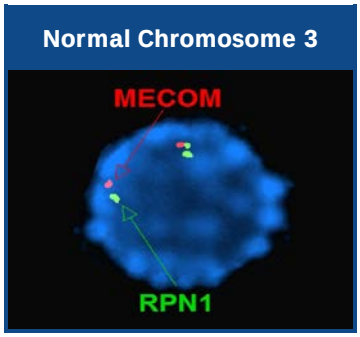
INTERPRETATION

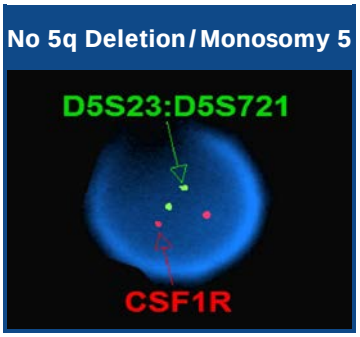
FISH "ISCN": nuc ish (EVIx2)[200],(EGR1x2,CSF1Rx2)[200],(CUXx2,CUL1x2)[200],(CEP8x2,MAFBx2)[200],(MLLx2)[200],(ETV6x2)[200],(RARAx2)[200],(ZNF443x2,ERCC1x2)[200]

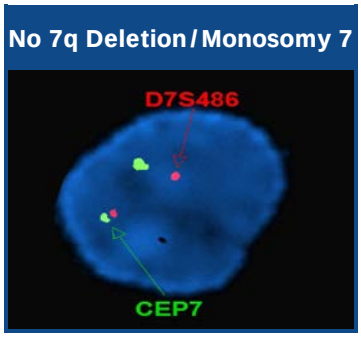
Fluorescence in situ hybridization (FISH) with a panel of probes specific for detection of recurring chromosome abnormalities in MDS was performed on uncultured bone marrow cells.

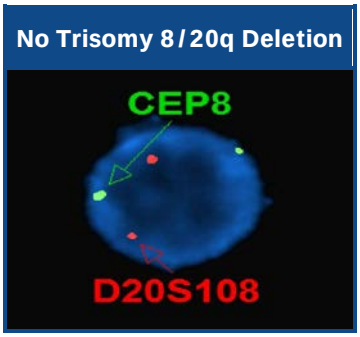
The regions/loci represented in these probe mixes were:

1. EVI/MECOM dual color break apart probe, used to detect rearrangements of chromosome 3 at q26 region, reveal a hybridization pattern within normal limits in 200 analyzed nuclei.
2. EGR1 (5q31), CSF1R (5q33~34) and D5S721:D5S23 (5p15.3), used to detect copy number abnormalities/deletion of chromosome 5, reveal a hybridization pattern within normal limits in 200 analyzed nuclei.
3. CUX1 (7q22), CUL1 (7q36) and a centromere probe to chromosome 7 (CEP 7), used to detect copy number abnormalities/deletion of chromosome 7, reveal a hybridization pattern within normal limits in 200 analyzed nuclei.
4. CEP8 (centromere probe to chromosome 8), used to detect copy number abnormalities of chromosome 8, reveals a hybridization pattern within normal limits in 200 analyzed nuclei.
5. MLL dual color break apart probe, used to detect rearrangement/deletion at 11q23 region, reveals a hybridization pattern within normal limits in 200 analyzed nuclei.
6. RARA, used to detect rearrangement /deletion of 17q21.1 region, reveal a hybridization pattern within normal limits in 200 analyzed nuclei.
7. ETV6 dual color break apart probe, used to detect rearrangement/deletion at 12p13.2, reveals a hybridization pattern within normal limits in 200 analyzed nuclei.
8. ZNF443 (19p13) and ERCC1 (19q13) used to detect copy number abnormalities of chromosome 19, reveal a hybridization pattern within normal limits in 200 analyzed nuclei.
9. MAFB (20q12), used to detect deletion/copy number abnormalities of chromosome 20, reveals a hybridization pattern within normal limits in 200 analyzed nuclei.

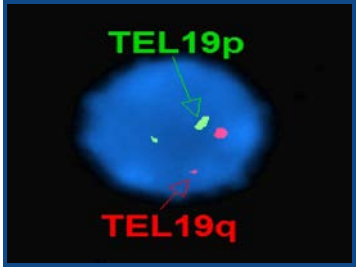




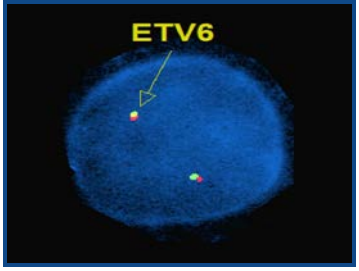




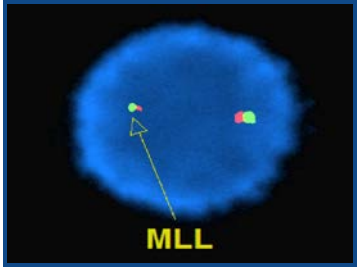
No Trisomy 19



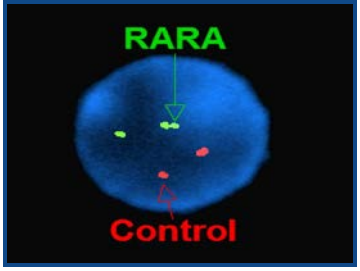
No ETV6 Rearrangement



No MLL Rearrangement



No RARA Rearrangement



Electronically Signed By: Frank Bauer, MD (04/12/19 17:38)

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DIAGNOSIS:

Bone marrow, aspirate:
HemeScreen™ results reveal:
- Negative for JAK2 V617F point mutation
- Negative for JAK2 exon 12 mutations
- Negative for MPL W515L/K point mutations
- The specimen tested positive (mutated; 52bp deletion) for CALR mutations

INTERPRETATION

(HemeScreen): The V617F mutation of the JAK2 (Janus kinase 2) gene has been described in polycythemia vera (PV), essential thrombocythemia (ET) and primary myelofibrosis (PMF) cases.[1-2] Identification of the V617F JAK2 point mutation in myeloproliferative neoplasms (MPN) is indicated in diagnosis, classification and monitoring.

Mutations in the JAK2 exon 12 (Janus kinase 2) gene are rare in ET or PMF, and their occurrence in PV is almost always associated with the absence of JAK2V617F and the presence of a subnormal serum erythropoietin level.[3] The identification of the JAK2 exon 12 mutations in myeloproliferative neoplasms (MPN) may be useful to assist diagnosis, classification and monitoring.

MPL (W515L/K) mutations of the juxtamembrane region of the thrombopoietin receptor MPL (myeloproliferative leukemia virus oncogene homology) have been described in JAK2 V617F-negative primary myelofibrosis (PMF) and essential thrombocythemia (ET).[4] The identification of W515 L/K point mutations in myeloproliferative neoplasms (MPN) may be useful to assist diagnosis, classification and monitoring.

REFERENCES:
1. Baxter et al. The Lancet 2005: 1054 - 1061
2. Levine et al. Cancer Cell 2005: 387-397
3. Pardanani et al Leukemia 21: 2007; Pietra et al Blood 111: 2008
4. Pancrazzi et al. JMD 2008: 435 - 441

(CALR): The specimen tested positive for a 52 bp deletion mutation of calreticulin (CALR) exon 9.

This mutation most likely represents the CALR type 1 mutation: c.1092_1143 del p.L367fs.2-3

The gene encoding calreticulin (CALR) is mutated in the majority (~70-85%) of essential thrombocythemia (ET) and primary myelofibrosis (PMF) cases with non-mutated JAK2.[1,2] CALR mutations are the second most frequent genetic mutation in myeloproliferative neoplasms (MPN) after JAK2.[1,2] Mutations in CALR have been reported to be mutually exclusive with mutations in both JAK2 and MPL and are not reported in polycythemia vera (PV).[2-3] The identification of CALR Exon 9 insertion/deletion mutations in myeloproliferative neoplasms (MPN) may be useful to assist diagnosis, classification, and monitoring.[1-3] In addition, the presence of CALR mutations has been associated with a more benign clinical course in comparison to corresponding disorders associated with JAK2 or MPL mutations.[2-4]

The CALR exon 9 type 1 deletion mutation, as observed in this specimen, has been associated with better overall survival in patients with PMF compared to type 2 insertion mutations.[1] In ET, the CALR type 1 mutation is associated with a myelofibrosis phenotype and an elevated risk of progression from ET to MF.[6]

REFERENCES:
1. Tefferi et al. Blood 2014.
2. Nangalia et al. N Engl J Med. 2013
3. Klampfl et al. N Engl J Med. 2013
4. Rumi et al. Blood.2013
5. Rotunno et al. Blood.2013.
6. Pietra et al. Leukemia. 2016

METHOD:

(Hemescreen): 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 5~10% 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.

(CALR): Polymerase chain reaction (PCR) amplification in combination with fluorescence-based capillary electrophoresis is utilized to detect and monitor insertion /deletion mutations in CALR Exon 9. The CALR Exon 9 amplification yields a wild- type PCR product of 261 base pairs. The presence of an insert mutation in CALR Exon 9 will result in a mutant PCR product >261 base pairs, a deletion mutation yields a PCR product < 261 base pairs. This assay can detect mutations in CALR Exon 9 with a minimum sensitivity of 10% 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.

DISCLAIMERS:

(Hemescreen): 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.

(CALR): This test was developed and its performance characteristics determined by HematoLogics, Inc. It has not been cleared or approved by the US Food and Drug Administration..

Electronically Signed By: Frank Bauer, MD (04/12/19 16:36)

Patient: John A. Doe
Received: 04/09/19 11:04
Case #: X19-00633
Reported: 04/12/19 17:43
Received Information: 2 Formalin containers, 5 smears, 5 touch preps, 2 green-top tubes, 1 lavender-top tube