Patient: Jane A. Doe

Case No: P11-00352

DOB/Gender: xx/xx/xxxx (66 yrs.) - Female
SSN: xxx-xx-xxxx
MRN/ID: 123456

Provider: John Doe, MD
Account: Hematology Oncology Assoc
Phone: 800-123-4567 **Fax:** 800-123-4444
Copy: Joe Smith, MD

Precipio Diagnostics
4 Science Park, 3rd Floor, New Haven, CT 06511
Phone: 203-787-7888 Fax: 203-823-4882
www.precipiodx.com

Collected: xx/xx/xx
Received: xx/xx/xx
Reported: xx/xx/xx

Alert Status: Routine
Report Status: Final
Report Category: Neoplastic

Clinical information: ICD-9 273.10. Monoclonal paraproteinemia.

Received information: 2 Formalin containers, 10 smears, 2 green-top tubes, 1 lavender-top tube

COMPREHENSIVE DIAGNOSIS

Professional Services Provided By Yale SCHOOL OF MEDICINE

FINAL DIAGNOSIS:

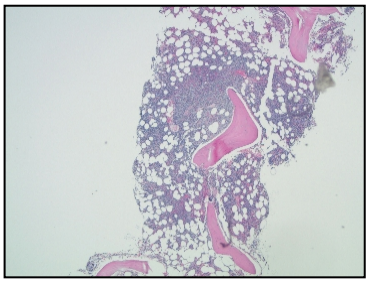
Lymphoplasmacytic lymphoma (see comment)

COMMENT:
The overall findings are consistent with involvement by a LOW GRADE NON-HODGKIN B-CELL LYMPHOMA. Although the plasma cell component is morphologically limited and a clonal population of plasma cells was not definitively detected by flow cytometry or immunohistochemistry, in the setting of a monoclonal IgM of approximately 2 g/dL, the presence of a MYD88 mutation is most consistent with involvement by a LYMPHOPLASMACYTIC LYMPHOMA. Correlation with clinical and laboratory findings is advised.

Electronically signed by: Demetrios Braddock, MD, PhD
Department of Pathology, School of Medicine
Yale University

RESULTS SUMMARY:

Biopsy:	Non-Hodgkin B-cell lymphoma (see comment)
Aspirate:	Maturing trilineage hematopoiesis with frequent small lymphocytes
Flow Cytometry:	Suspicious for a non-Hodgkin B-cell lymphoproliferative disorder (see comment)
Karyotyping:	Normal female karyotype
FISH:	No Clonal Abnormalities Detected with probes specific for recurrent abnormalities in Plasma Cell Myeloma (see interpretation for probes tested)
Molecular:	MYD88 mutation(s) detected: P.L265P (see comment)


Lymphoid Infiltrate

BONE MARROW BIOPSY

Professional Services Provided By
Yale SCHOOL OF MEDICINE

DIAGNOSIS:

Bone marrow, core & clot biopsies: non-Hodgkin B-cell lymphoma (see comment)

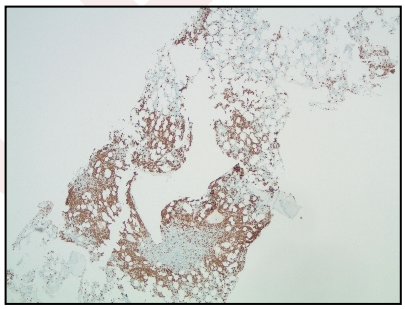
COMMENT:

The findings are consistent with involvement by a NON-HODGKIN B-CELL LYMPHOMA with a differential that includes a marginal zone lymphoma and lymphoplasmacytic lymphoma. Correlation with clinical and laboratory findings is advised.

Electronically signed by: Demetrios Braddock, MD, PhD
Department of Pathology, School of Medicine
Yale University

MICROSCOPIC DESCRIPTION:

Marrow Cellularity:	Mildly hypercellular (60%)
Infiltrate:	Approximately 30% of the cellularity is comprised of an interstitial infiltrate of lymphocytes that are small sized with round nuclei, condensed chromatin, indistinct nucleoli and small amounts of cytoplasm occurring singly and in one large aggregate.
Myeloid Maturation:	Normal
Erythroid Maturation:	Normal
Myeloid/Erythroid Ratio:	Mildly increased
Megakaryocytes:	Normal in number and morphology
Granulomas:	Not identified
Iron Stain:	No stainable iron is seen in this decalcified specimen
Marrow Reticulin:	Mild increase in fibrosis is noted in association with the lymphoid aggregate
Marrow Trabeculae:	Normal
Clot preparation:	Similar findings to the core biopsy
PAS / Giemsa:	Examined
Special Stains:	Giemsa, Iron, PAS, Reticulin
Immunostains:	CD20, CD79a and Pax-5 highlight numerous lymphocytes. An aggregate of CD3 positive T cells is seen. CD138 positive plasma cells account for less than 5% of the marrow cellularity and are polyclonal for immunoglobulin kappa and lambda light chains.



CD79a

ADDITIONAL STUDIES:

Stain	Result
CD3 (MRQ-39)	See microscopic description
CD20 (L26)	See microscopic description
CD79a (SP18)	See microscopic description
CD138/syndecan-1 (B-A38)	See microscopic description
Kappa (L1C1)	See microscopic description
Lambda (Lamb14)	See microscopic description
PAX5 (SP34)	See microscopic description

GROSS DESCRIPTION:

1. 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 1.0 x 0.2 x 0.2 cm. The specimen is submitted in 1 cassette after decalcification.

2. The specimen is received in formalin labeled with patient's initials and requisition number, and consists of 1 piece of bone marrow clot measuring 0.5 x 1.4 x 1.4 cm. The specimen is submitted in 1 cassette.

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 Diagnostics, LLC, 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".

BONE MARROW ASPIRATE

Professional Services Provided By
Yale SCHOOL OF MEDICINE

DIAGNOSIS:

Bone marrow, aspirate: Maturing trilineage hematopoiesis with frequent small lymphocytes

Electronically signed by: Demetrios Braddock, MD, PhD
Department of Pathology, School of Medicine
Yale University

SMEAR REVIEW:

The marrow aspirate smear is spicular and cellular with maturing trilineage hematopoiesis and scattered small lymphocytes. Megakaryocytes are normal in number and morphology. The myeloid : erythroid (M:E) ratio is approximately 8:1. Erythroid maturation is normal. Myeloid maturation is normal. No increase in immature cells is noted. Scant, focal iron is seen without ring sideroblasts on iron stain of the marrow aspirate.

Number of cells counted: 222

Cell Type	Percent	Ref. Range
Blasts	0 %	**0.3 - 3.0 %**
Immature Myeloid	9 %	**12.0 - 21.0 %**
Mature Myeloid	63 %	**35.0 - 55.0 %**
Eosinophils	3 %	**1.0 - 3.0 %**
Basophils	0 %	**0.0 - 1.0 %**
Lymphocytes	16 %	**10.0 - 15.0 %**
Plasma Cells	1 %	**0.0 - 1.0 %**
Monocytes	0 %	**0.0 - 1.0 %**
Erythroid	8 %	**15.0 - 25.0 %**
M:E ratio	**8:1**	**2 - 4:1**

FLOW CYTOMETRY

Professional Services Provided By
Yale SCHOOL OF MEDICINE

DIAGNOSIS:

Bone marrow, aspirate: Suspicious for a non-Hodgkin B-cell lymphoproliferative disorder (see comment)

COMMENT:

The differential diagnosis includes lymphoplasmacytic lymphoma and a marginal zone lymphoma. Most cases of chronic lymphocytic lymphoma, mantle cell lymphoma and follicular lymphoma will express either CD5 or CD10. Correlation with the concurrent bone marrow core and aspirate morphology and cytogenetic findings is advised.

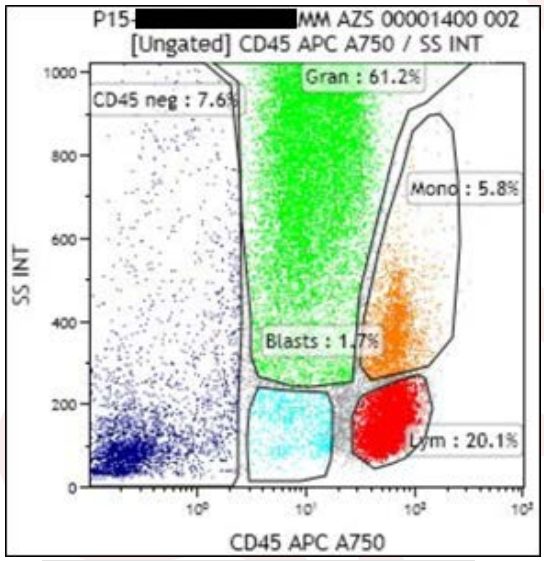
Electronically Signed By: Demetrios Braddock, MD, PhD
Department of Pathology, School of Medicine
Yale University

INTERPRETATION:

The lymphocytes (20%) include 17% B-cells with an excess of immunoglobulin kappa to lambda light chains (5:1), but are negative for CD5 and CD10. Seventy-four percent (74%) of the lymphocytes are mature T-cells with a normal CD4/CD8 ratio, and 10% natural killer (NK) cells. Less than 1% of the cellularity are plasma cells. Although excess cytoplasmic kappa light chain is detected, no cohesive population of cells is observed.

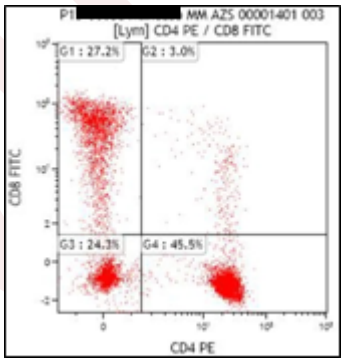
RESULT:

Analysis Time: xx/xx/xx 1536 hr **Viability:** 98% (Normal > 80%) **Specimen:** BM, Lavender-top tube

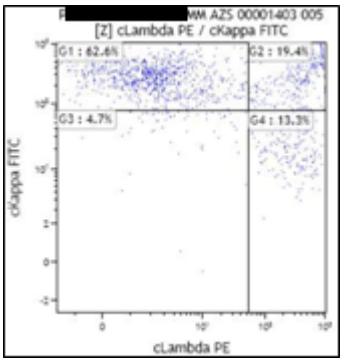


Flow Cytometry Differential

Lymphocytes:	20%
Monocytes:	6%
Granulocytes:	61%
Plasma Cells:	<1%
Blasts:	2%
nRBC & Debris:	8%



Normal CD4/CD8 Ratio



Kappa/Lambda B-Lymphocytes

Lymphocytes Gated Population (CD45 and side scatter)

T/NK-cell	%	B-Cell	%	Other	%
CD8	25	Kappa	13	CD45	100
CD4	48	Lambda	6		
CD56	10	CD20	17		
CD3	74	CD38	13		
CD2	82	CD10	2		
CD5	68	CD19	17		
CD7	81				

Plasma Cells Gated Population (CD45 and side scatter)

T/NK-cell	%	Plasma Cells	%
CD56 (Plasma Cells)	17	IgA	19
		IgG	47
		IgM	87
		cKappa	54
		cLambda	4
		CD19 (Plasma Cells)	93

Intensity: B = bright D = dim M = moderate

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CYTOGENETICS, KARYOTYPING

Professional Services Provided By
Yale SCHOOL OF MEDICINE

DIAGNOSIS:

Bone marrow, aspirate: Normal female karyotype

Electronically signed by: Demetrios Braddock, MD, PhD
Department of Pathology, School of Medicine
Yale University

INDICATION:

Monoclonal paraproteinemia

KARYOTYPE "ISCN":

46,XX[20]; Normal Female Karyotype

INTERPRETATION:

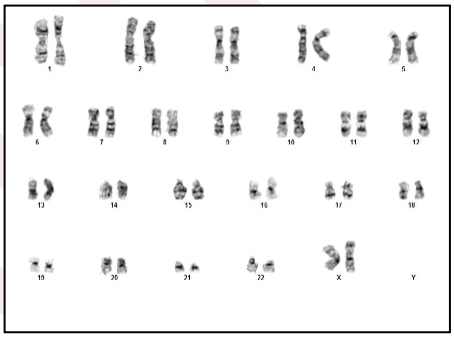
Conventional cytogenetic analysis shows a female karyotype with no evidence of an acquired clonal abnormality.
This does not exclude the possibility of an abnormality that cannot be detected at the chromosomal level or exists at a low residual level.

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.

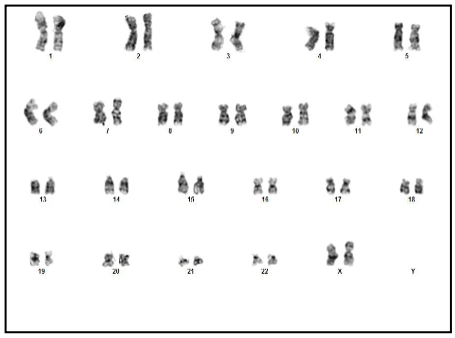
FISH studies are recommended and much more sensitive than G-band analysis for cases of plasma cell neoplasia because plasma cells have a very low proliferation rate in culture. FISH studies are recommended for clinically-significant abnormalities.
(NCCN Guidelines, ver 2.2013, Multiple Myeloma, National Comprehensive Cancer Network, nccn.org)

RESULT:

Analysis	
Cells Counted:	20
Cells Analyzed:	20
Cells Imaged:	3
Cells Karyotyped:	3
Banding Type:	G-Banding
Band Level:	450



Normal Karyotype



Normal Karyotype

DIAGNOSIS:

Bone marrow, aspirate: No Clonal Abnormalities Detected with probes specific for recurrent abnormalities in Plasma Cell Myeloma (see interpretation for probes tested)

Electronically signed by: Demetrios Braddock, MD, PhD
Department of Pathology, School of Medicine
Yale University

FISH "ISCN":

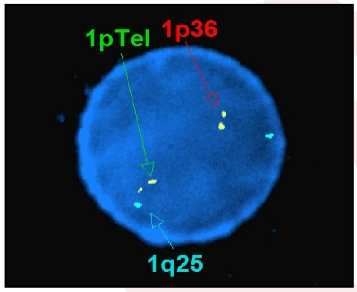
nuc ish (p58x2,1q25x2)[200],(CEP9x2)[200],(CCND1x2,IGHx2)[200],(D13S319x2,13q34x2)[200],(p53x2)[200]

INTERPRETATION:

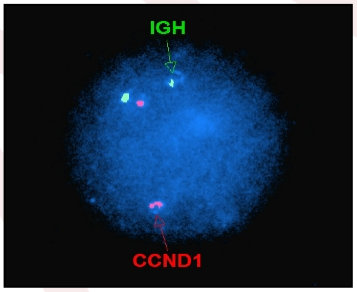
Fluorescence in situ hybridization (FISH) with a panel of probes specific for detection of recurring chromosome abnormalities in plasma dysplasia was performed on uncultured bone marrow cells.

The regions/loci represented in these probe mixes were:

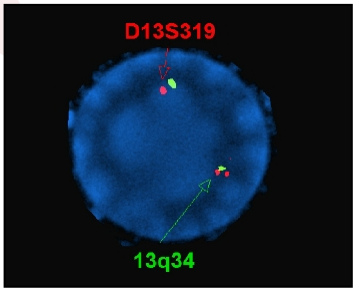
1. IGH/CCND1 dual color, dual translocation probes to 11q13 & 14q32 regions respectively, reveal a hybridization pattern within normal limits in 200 analyzed nuclei.
2. P53 (17p13.1), used to detect deletion/copy number abnormalities of chromosome 17p, reveals a hybridization pattern within normal limits in 200 analyzed nuclei.
3. 1p36 Microdeletion Region Probe - LSI p58 (1p36) /TelVysion 1p/LSI 1q25, used to detect copy number abnormalities of chromosome 1, reveal a hybridization pattern within normal limits in 200 analyzed nuclei.
4. CEP9 (centromere probe to chromosome 9), used to detect copy number abnormalities of chromosome 9, reveals a hybridization pattern within normal limits in 200 analyzed nuclei.
5. D13S319 (13q14.3) and 13q34, used to detect copy number abnormalities/deletion of chromosome 13, reveal a hybridization pattern within normal limits in 200 analyzed nuclei.



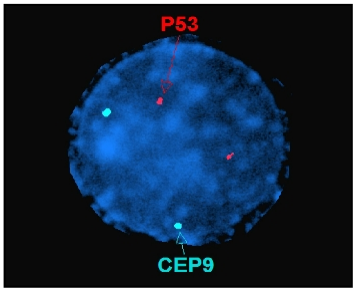
Normal Chromosome 1



No IGH/CCND1 Translocation



No 13q14.3 Deletion



No P53 Deletion/Trisomy 9

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DIAGNOSIS:

Bone marrow, aspirate: MYD88 mutation(S) detected: P.L265P (see comment)

Electronically Signed By: Demetrios Braddock, MD, PhD

INTERPRETATION:

MYD88 mutation is the most frequent genomic abnormality in diffuse large cell lymphoma (DLBCL) activated B-cell-like (ABC) subtype, detected in 40% of cases. MYD88 is rarely mutated in the germinal center B-cell-like (GCB) DLBL, therefore, it can be used to differentiate between the two subtypes. MYD88 mutation is detected in approximately 90% of cases of Waldenstrom macroglobulinemia/lymphoplasmacytic lymphoma. MYD88 mutation analysis can be a useful prognostic tool for patients with IgM-MGUS since the L265P mutation is associated with a higher risk of disease progression and a greater disease burden. MYD88 mutation has also been reported to be common (40%) in central nervous system lymphoma.

METHODOLOGY:

Total nucleic acid was extracted from patient's plasma, PB/BM cells or paraffin-embedded tissues (FFPE). Bi-directional Sanger sequencing of exon 5 of MYD88 was performed, including the L265P mutation hot spot. This is a sequencing-based assay which has a typical sensitivity of 10-15% for detecting MYD88 mutations in a wild-type background. Various factors including quantity and quality of nucleic acid, sample preparation, and sample age can affect assay performance.

REFERENCES:

1. Trøen G, Warsame A, Delabie J. CD79B and MYD88 Mutations in Splenic Marginal Zone Lymphoma. ISRN Oncol. 2013;2013:252318.
2. Xu L, et al. MYD88 L265P in Waldenstrom's macroblobulinemia, IgM monoclonal gammopathy, and other B-cell lymphoproliferative disorders using conventional and quantitative allele-specific PCR. Blood. 2013;121(11):2051-2058.

The performance characteristics of this test have been determined by the laboratory. This test has not been approved by the FDA. The FDA has determined such clearance or approval is not necessary. The laboratory is CLIA certified to perform high complexity clinical testing.

END OF REPORT