Chronic Myelomonocytic Leukemia with molecular abnormalities SH2017-0351

Madhu P. Menon MD,PhD, Juan Gomez MD, Kedar V. Inamdar MD,PhD and Kristin Karner MD

Madhu P Menon, MD, PhD
Henry Ford Hospital

Patient History

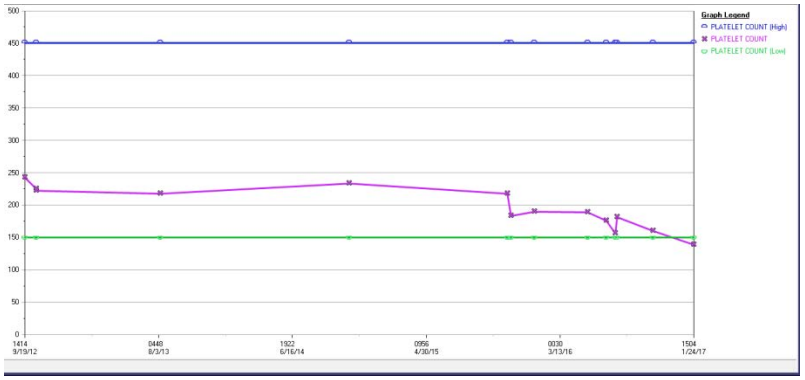
- A 68 year old male with normocytic normochromic anemia for 5 years without a specific etiology (Normal iron and Vit B12/Folate studies)
- NO fatigue, night sweats, splenomegaly etc.
- A bone marrow biopsy 7 months back revealed a hypercellular bone marrow with minimal dysplasia not meeting criteria for MDS/MPN.
- A new bone marrow biopsy was performed and NGS Myeloid panel (targeted sequencing) was ordered on the bone marrow

Hemoglobin (g/dl)

g/dl

Most recent bone marrow biopsy

Platelet count (K/ul)



K/ul

Most recent bone marrow biopsy

	Ref Range & Units	1/24/17 3:04 PM
WBC Count	3.8 - 10.6 K/uL	4.6
RBC Count	4.40 - 6.00 M/uL	3.97 (L)
Hemoglobin	13.5 - 17.0 g/dL	12.0 (L)
Hematocrit	41 - 53 %	36.1 (L)
MCV	80 - 100 fl	90.9
MCH	26 - 34 pg	30.2
MCHC	31 - 37 g/dL	33.3
RDW	<14.5 %	19.1 (H)
Platelet Count	150 - 450 K/uL	139 (L)

DIFFERENTIAL COUNT

Neutrophil,%: 57

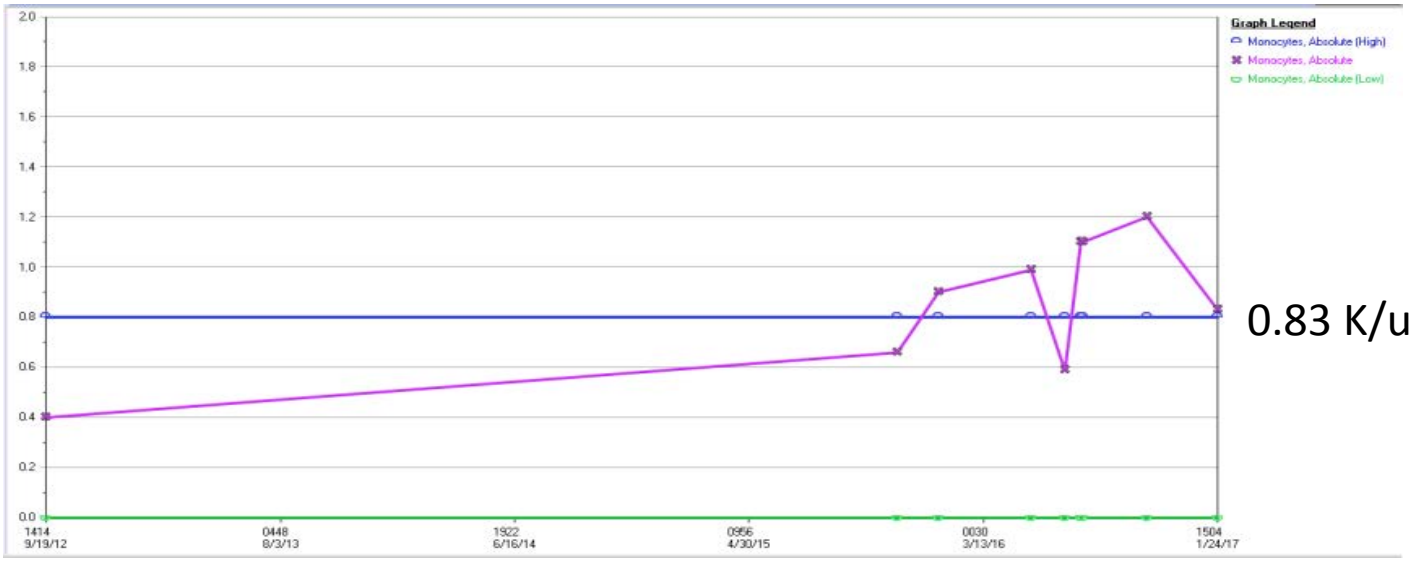
Lymphocyte,%: 22

Monocyte,%: 18

Eosinophil,%: 2

Basophil,%: 1

Monocyte count (normal range, 0-0.8 K/ul)



0.83 K/ul

K/ul

Most recent bone marrow biopsy

Intermittent monocytosis for 5 years ; sustained monocytosis for 3 months above 1k/ul was seen before dropping to the most recent monocyte count of 0.83k/ul.

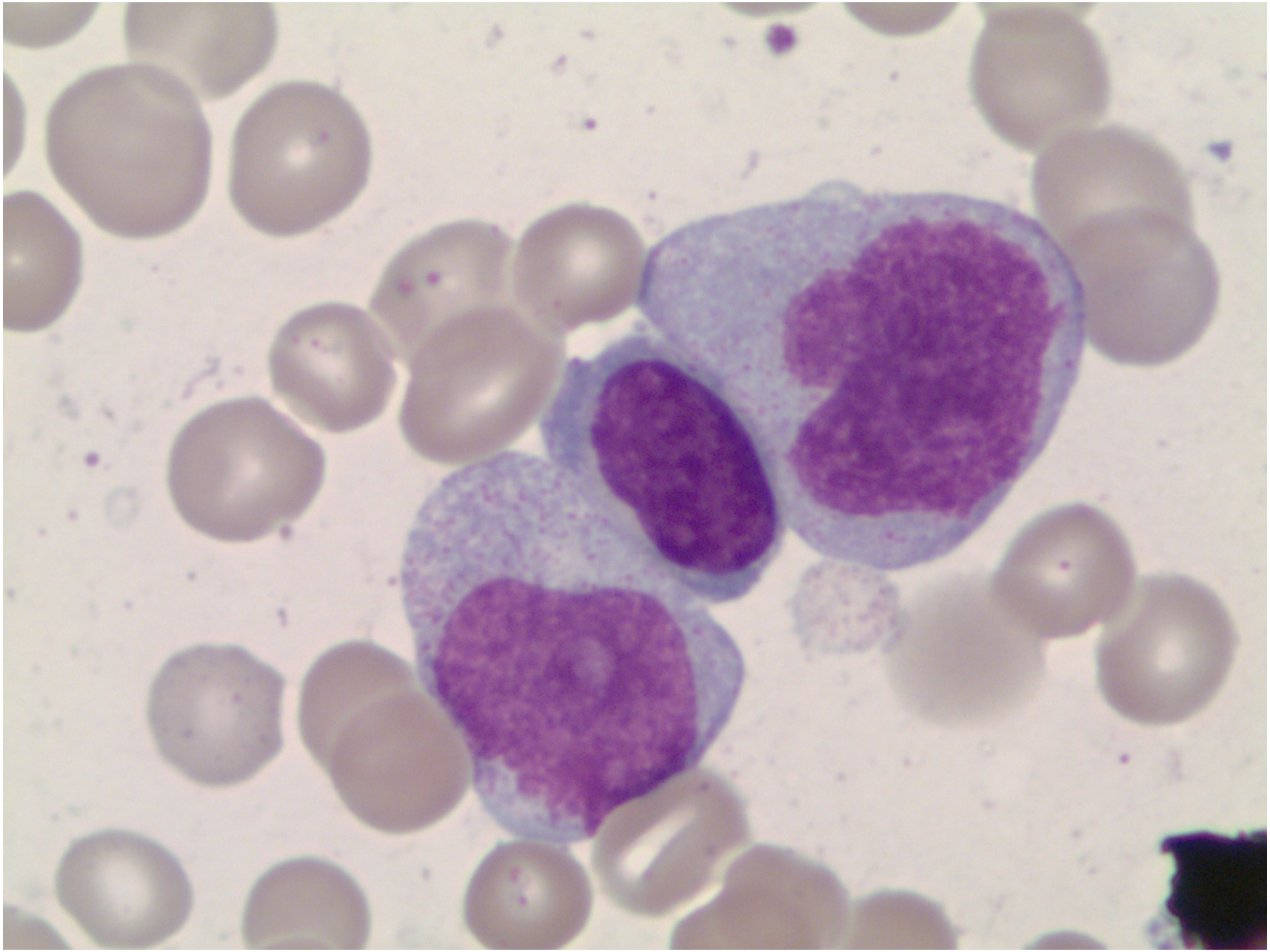
Bone marrow aspirate count (1000 cells)

Myeloblast	**2%**	**Promonocytes**	**2 %**	Lymphoblast	%
Promyelocyte	6%	**Monocytes**	**11%**	Prolymphocyte	%
Myelocyte	7%	Erythroid	37%	Lymphocyte	2%
Metamyelocyte	4%			Atypical Lymph	%
Bands	15%			Plasmablast	%
PMN:	13%			Proplasmacyte	%
Eosinophils	1%			plasma Cell:	1%
Basophils	%				

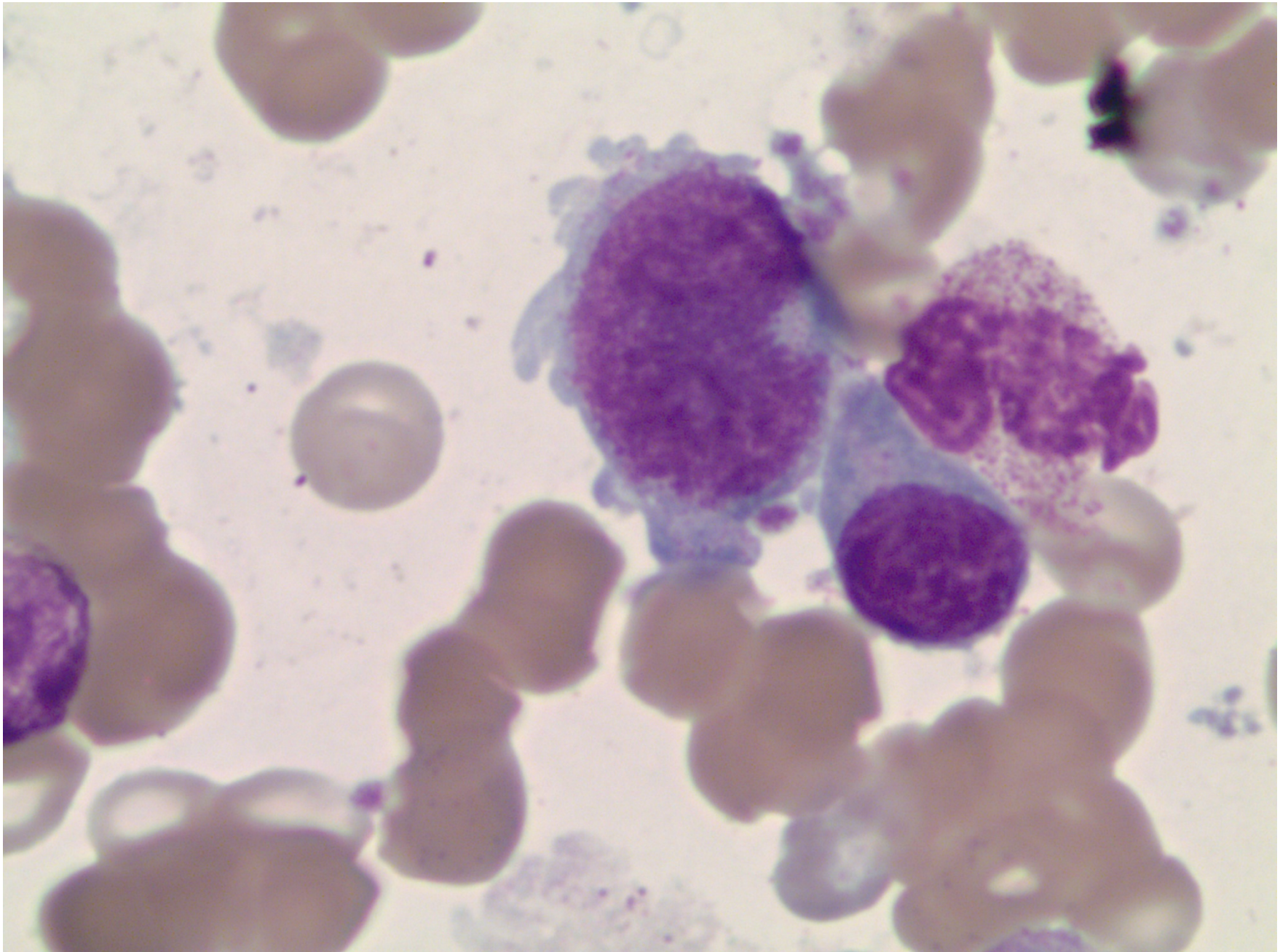
M:E ratio, **1.6:1**

Rare ringed sideroblasts noted

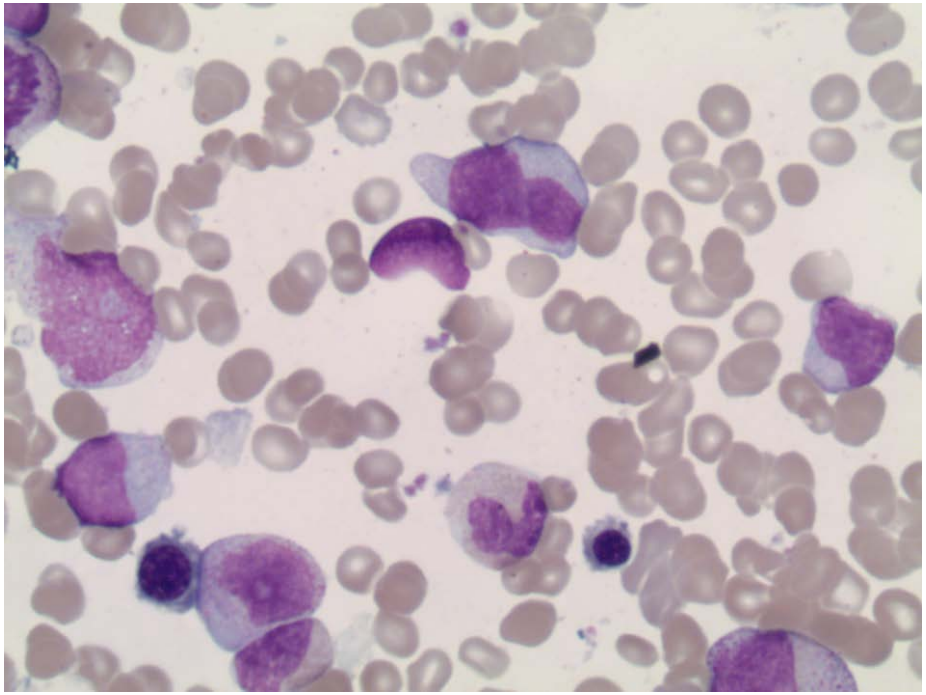
Monoblasts and Promonocytes, 1000x

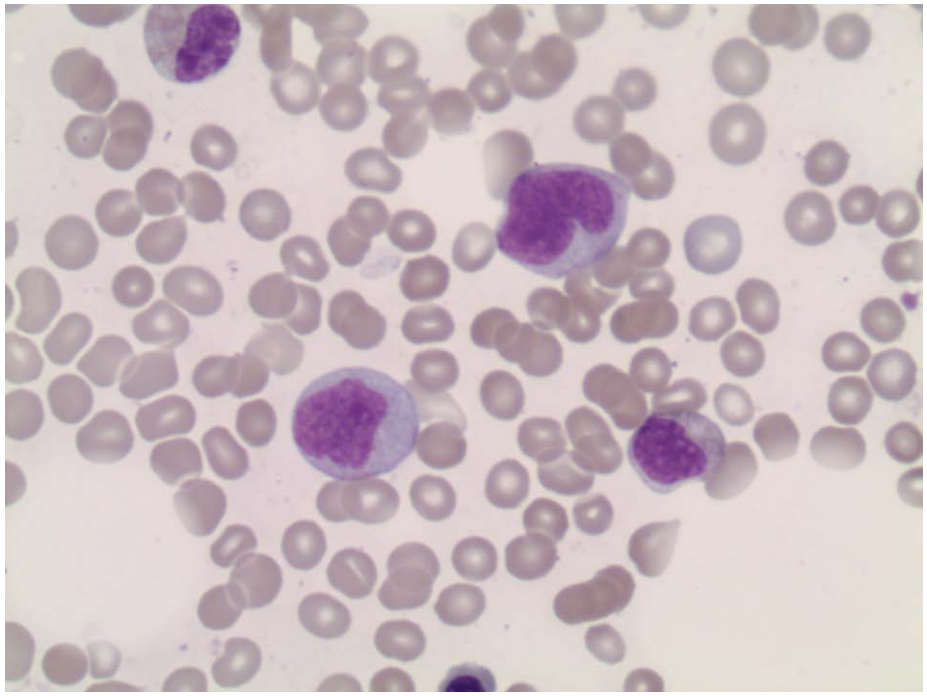


Promonocytes, 1000x

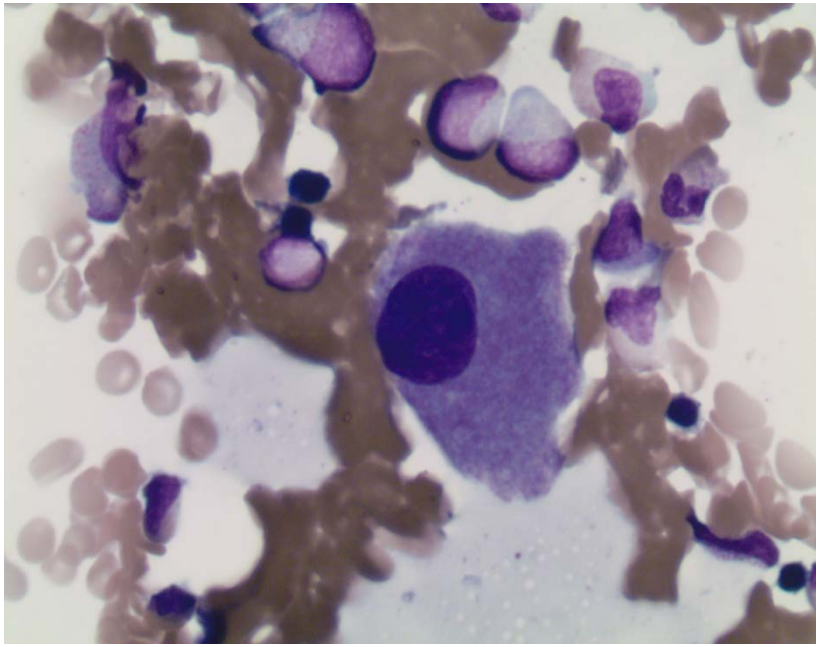


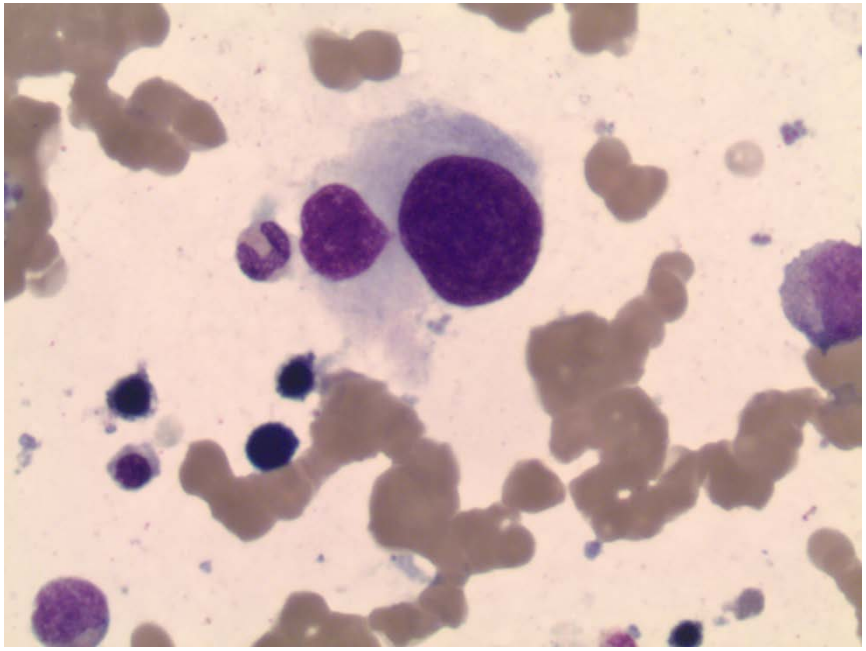
Atypical and immature monocytes, 500x

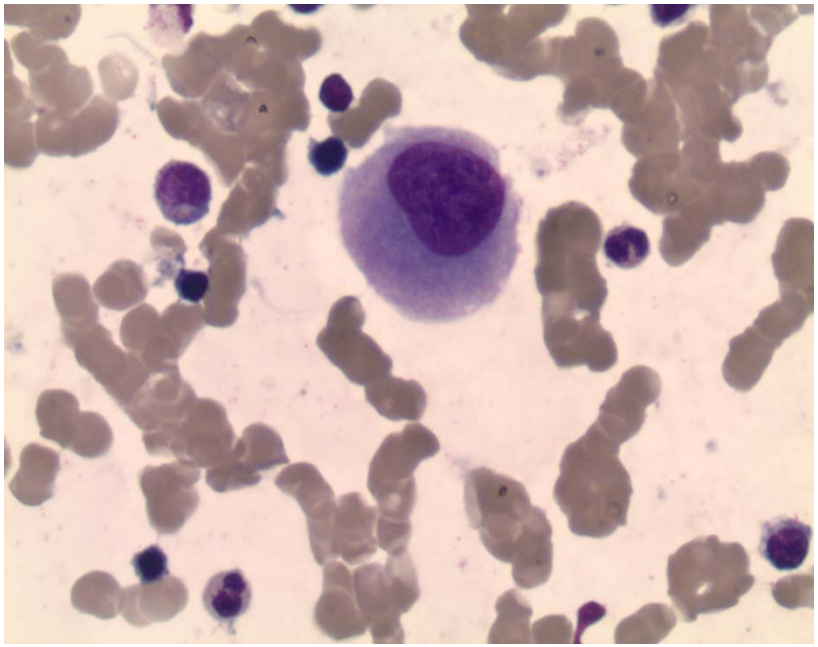




Dysplastic megakaryocytes, 500x



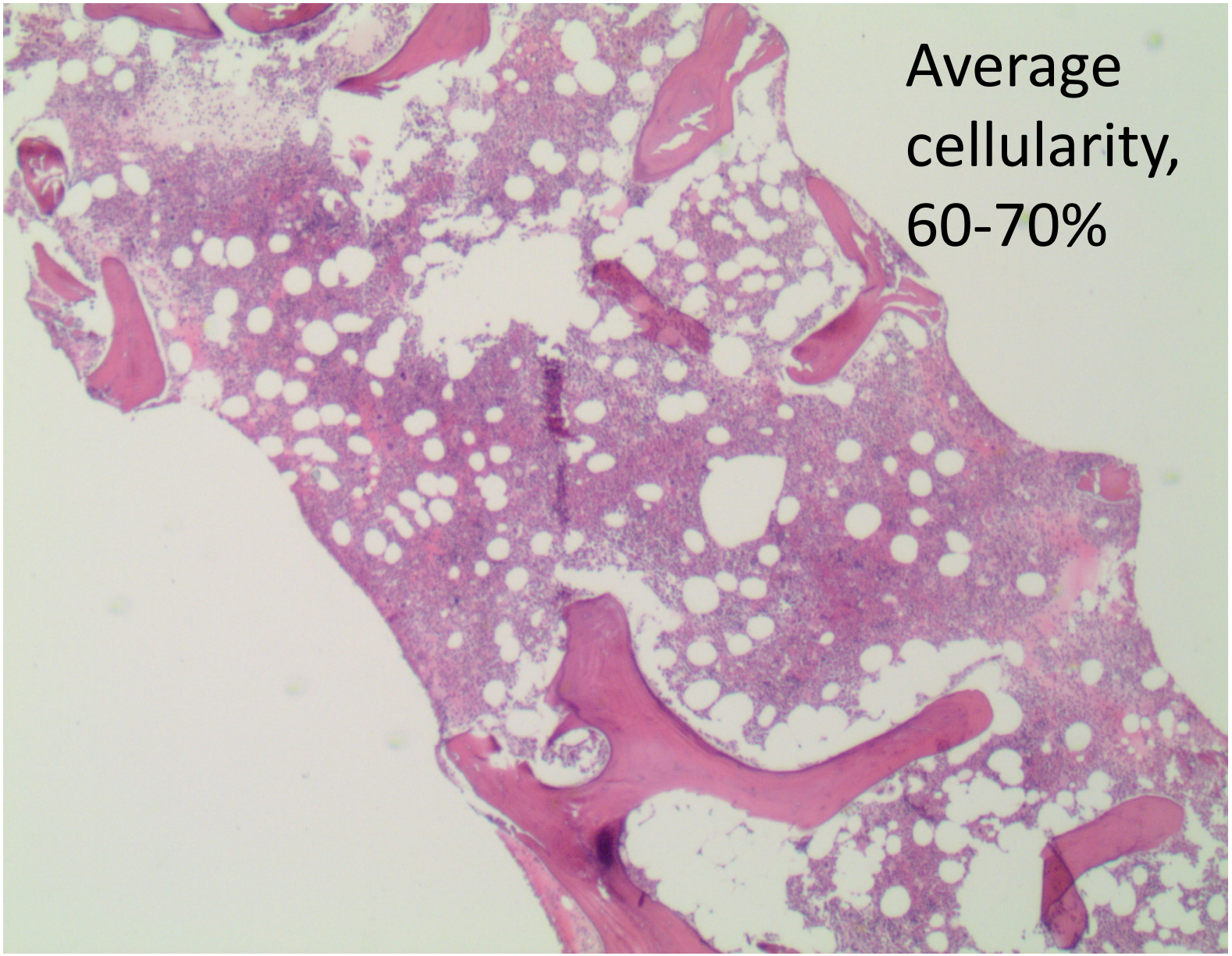




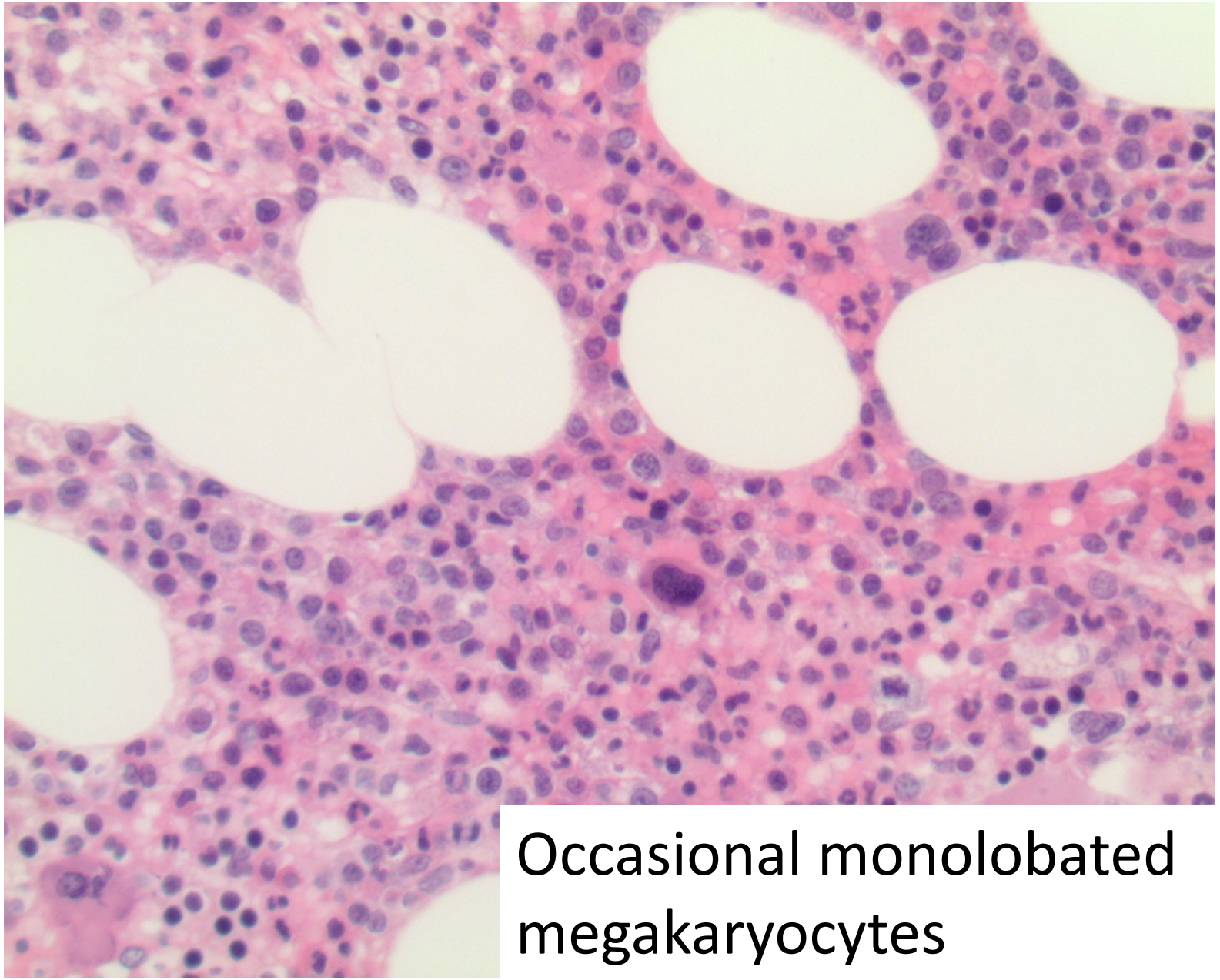
> 10% Dysplastic megakaryocytes

Rare dyspoietic erythroid cells and rare hypogranular neutrophils (less than 10%)

Bone marrow biopsy, 20x

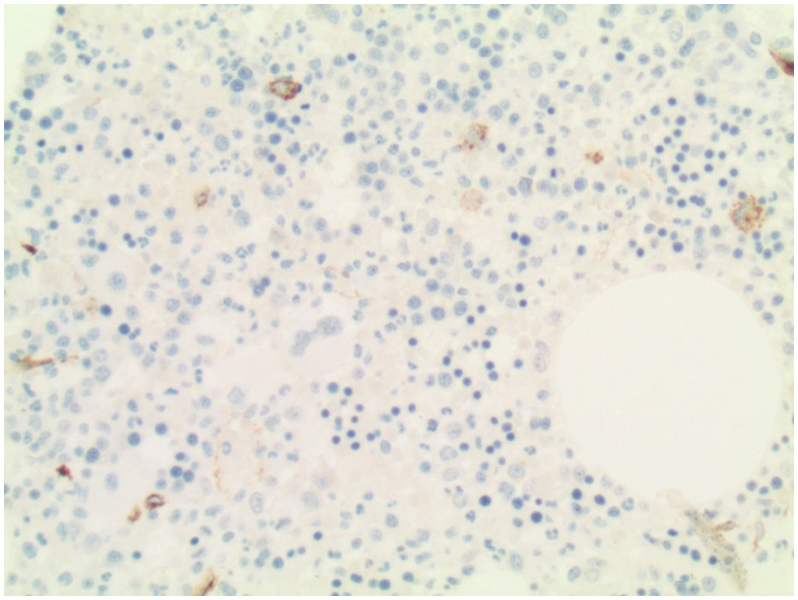


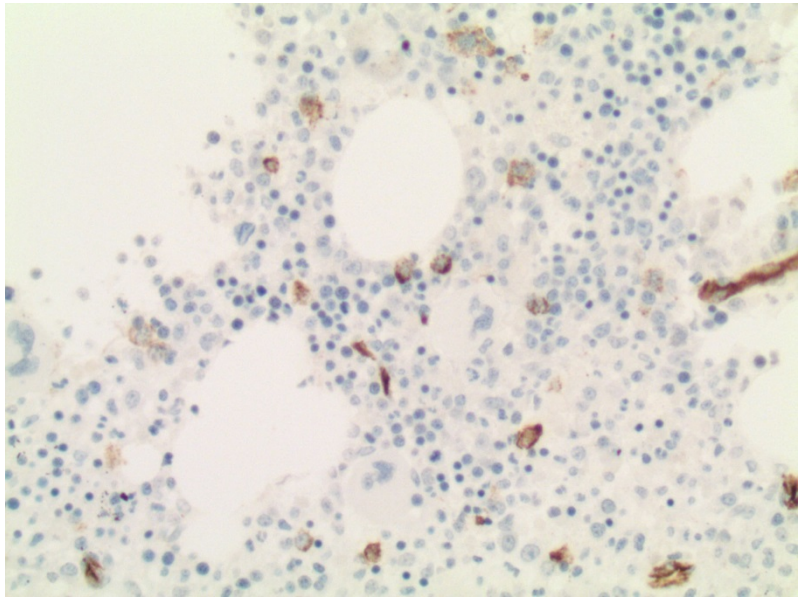
Bone marrow biopsy, 200x



Occasional monolobated megakaryocytes

CD34 staining





Reticulin staining- NO fibrosis (MF grade 0/3)

- **Chromosomal analysis:** 46, XY
- **FISH for MDS:** NO abnormalities detected
- **NGS**- Trusight myeloid panel from Illumina. PCR products sequenced on MiSeqDx sequencer (Technical sensitivity of 5%).
- Annotation was done using Cartagenia Bench Lab

NGS mutations (Dr. Juan Gomez)

1. **SRSF2** c.284C>A p.P95H
Variant Allele fraction 53.4%

2. **TET2** c.3195_3202dupGACTAGAC p.Q1068Rfs*17
Variant Allele fraction 72.1%

3. **TET2** c.3972_3973dupTC p.H1325Lfs*39
Variant allele fraction 11.5%

4. **ASXL1** c.2290delC p.L764Yfs*8
Variant Allele fraction 48.15%

Proposed Diagnosis

Chronic Myelomonocytic Leukemia-0

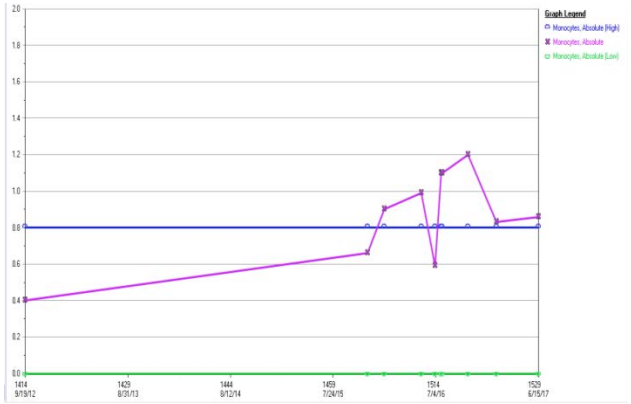
Panel Diagnosis

Chronic Myelomonocytic Leukemia-0.
The mutational
Spectrum is characteristic of CMML

Patient Followup

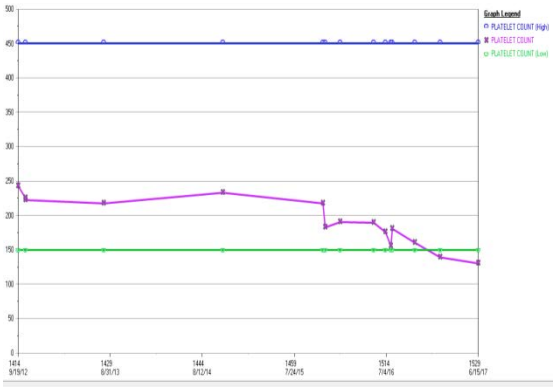
- NO active treatment; observation only
- Absolute monocyte count up from 0.83 to 0.86 K/ul
- HgB down from 12 g/dl to 11.4 g/dl
- Platelet count down from 139 K/ul to 130 K/ul

Monocyte count (K/ul)



Hemoglobin (g/dl)

Platelet count (K/ul)



Chronic Myelomonocytic Leukemia

Table 11. Diagnostic criteria for CMML

CMML diagnostic criteria
• Persistent PB monocytosis ≥1 × 10^9/L, with monocytes accounting for ≥10% of the WBC count
• Not meeting WHO criteria for *BCR-ABL1*$^+$ CML, PMF, PV, or ET*
• No evidence of *PDGFRA*, *PDGFRB*, or *FGFR1* rearrangement or *PCM1-JAK2* (should be specifically excluded in cases with eosinophilia)
• <20% blasts in the blood and BM†
• Dysplasia in 1 or more myeloid lineages. If myelodysplasia is absent or minimal, the diagnosis of CMML may still be made if the other requirements are met and
• An acquired clonal cytogenetic or molecular genetic abnormality is present in hemopoietic cells‡
or
• The monocytosis (as previously defined) has persisted for at least 3 mo and
• All other causes of monocytosis have been excluded

Arber et al. Blood 2016

Chronic Myelomonocytic Leukemia

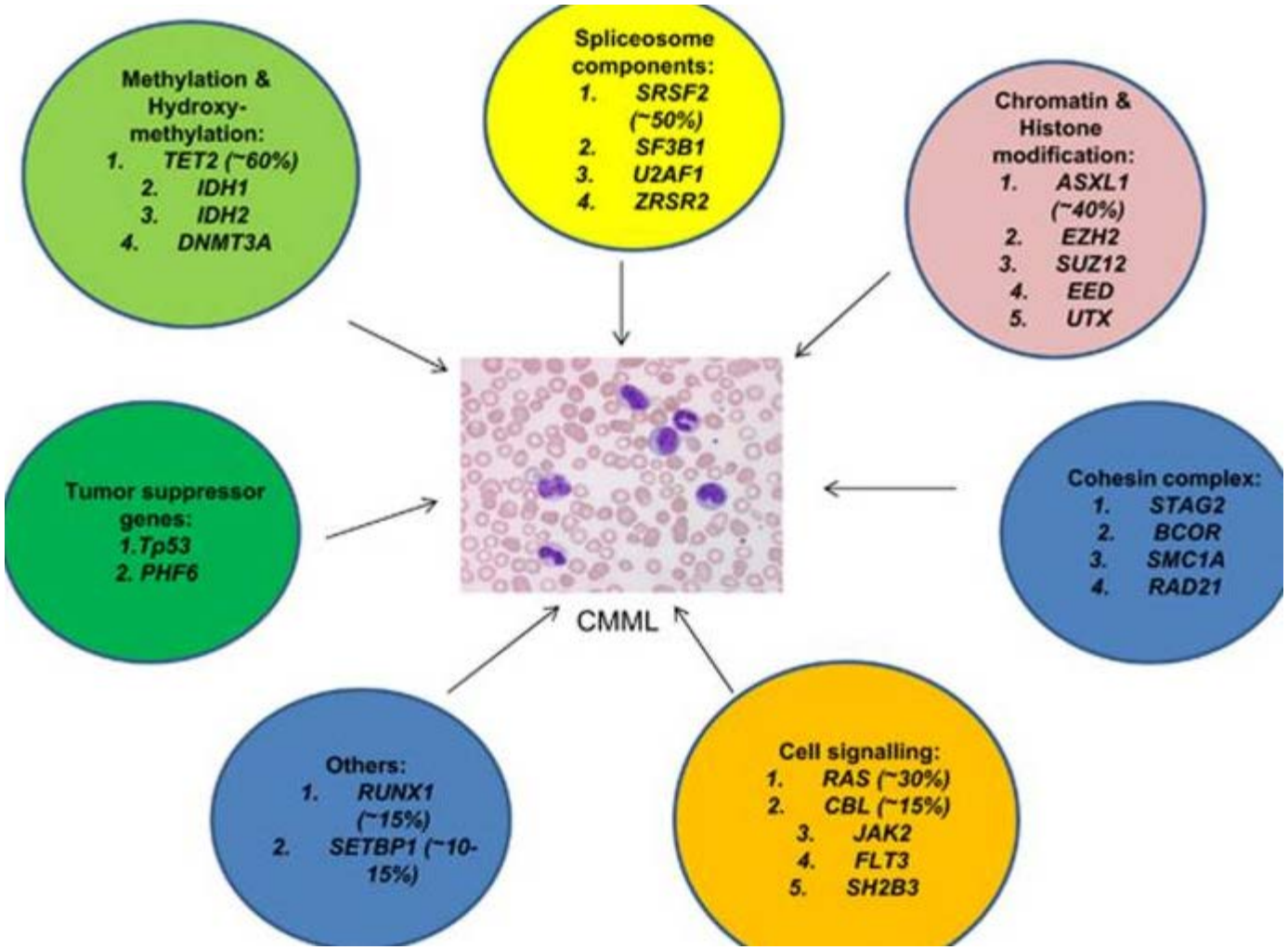
- CMML: **proliferative type** (WBC≥13K/ul) vs. **dysplastic type** (WBC <13K/ul) *(Cervera et al. Am J Hematol 2014)*

- Better prognostication: **CMML-0** (<2% blasts in PB and <5% blasts in BM), **CMML-1** (2-4% blasts in PB and/or 5-9% blasts in BM) and **CMML-2** (5-19% blasts in PB and/or 10-19% blasts in BM) *(Schuler et al. Leukemia Res 2014, Cervera et al. Am J Hematol 2014)*

- Clonal cytogenetic abnormality seen in **30%** of cases (trisomy 8, -Y, monosomy 7 and del7q, trisomy 21 and complex karyotypes)

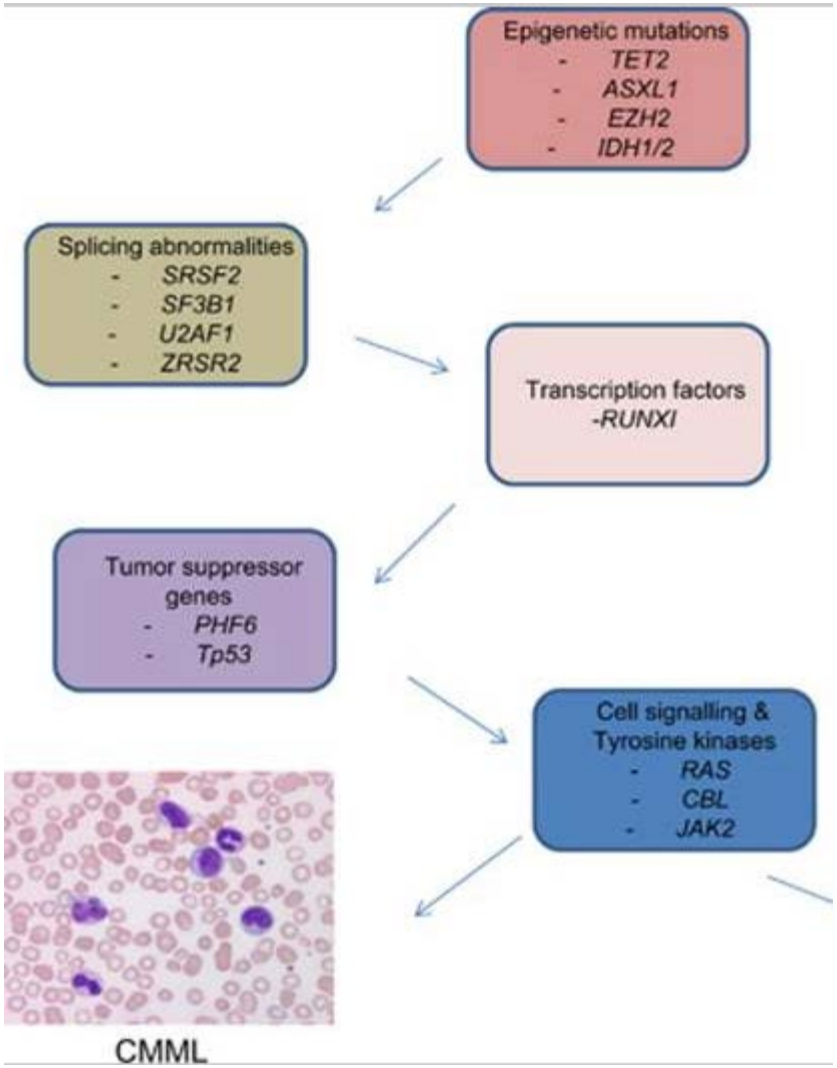
- Gene mutations seen in **>90%** of CMML cases (*Patnaik and Tefferi, Blood Cancer Journal 2016*)

CMML mutation spectrum



Patnaik and Tefferi, Blood Cancer Journal, 2016

Mutational evolution and clonal hierarchy in CMML



Patnaik and Tefferi, Blood Cancer Journal, 2016

SRSF2 mutations

- Spliceosome component mutations which affects pre-mRNA splicing

- MDS (15-20%), CMML (50%), primary myelofibrosis (15-20%) and AML

- CMML: association with increased age and diploid karyotype

(Yoshida K et al. Nature 2011 , Lasho TL et al. Blood 2012, Papemmanuil et al. NEJM 2011, Aribi A et al. Cancer 2007, Wu et al. Blood 2012, Patnaik et al. Am J Hematol 2013)

ASXL1 mutations

- Epigenetic regulator gene which regulates chromatin by interacting with polycomb group repressive complex proteins (PRC1 and PRC2)

- MDS (15-20%), CMML (40-50%), primary myelofibrosis (20-35%) and AML (5-10%)

- ASXL mutations associated with a proliferative phenotype (higher WBC, absolute monocyte count and immature myeloid cells)

- Multivariate analyses, ASXL1 mutations(frameshift and nonsense) impact survival (Molecular Mayo model and Groupe Francais des Myelodysplasies (GFM) model)

(Patnaik et al. Leukemia 2014 , Patnaik et al. Leukemia 2013, Itzykson R et al. JCO 2013, Abdel-Wahab et al. Leukemia 2011, Boultwood J et al. Leukemia 2010)

TET2 mutations

- Epigenetic regulator gene which impacts DNA methylation and hydroxy-methylation

- MDS (15%), CMML (60%), polycythemia vera (15%), primary myelofibrosis (15%) and AML (20%) and systemic mastocytosis (30%)

- No clear impact on either OS or leukemia free survival in CMML (Itzykson T et al. JCO 2013)

(Delhommeau F et al. NEJM 2009, Tefferi A et al. Leukemia 2009 and Abdel-Wahab O et al. Blood 2009)

Differential diagnoses

- **Reactive monocytosis** with a non-neoplastic cause of the megakaryocytic dyspoiesis (especially considering that most recent monocyte count is less than 1 K/ul and monocytosis is intermittent)

  -Detection of characteristic mutations help in establishing CMML diagnosis in this case (i.e. clonal nature of disease)

  -Caveat: Context, Context and Context considering that these mutations can be seen in healthy older individuals (CHIP) (Jaiswal et al. NEJM 2014 and Genovese et al. NEJM 2014)

- **MDS with monocytosis (MDS criteria, PB monocytes < 1 K/ul)**

  -Is there a mutational spectrum that differentiates MDS from CMML?

  -Higher mutation burden in CMML as compared to MDS (Reinig et al. AJCP, 2016, Malcovati et al. Blood 2014)

  -In the context of dysplasia, combination of TET2 and SRSF2 is highly specific for CMML disease phenotype (specificity, 97.6%) (Malcovati et al. Blood 2014)

- **MDS/MPN unclassifiable**

Modern Pathology (2017) 30, 1213–1222

Oligomonocytic chronic myelomonocytic leukemia (chronic myelomonocytic leukemia without absolute monocytosis) displays a similar clinicopathologic and mutational profile to classical chronic myelomonocytic leukemia

Julia T Geyer[1], Wayne Tam[1], Yen-Chun Liu[1], Zhengming Chen[2], Sa A Wang[3], Carlos Bueso-Ramos[3], Jean Oak[4], Daniel A Arber[5], Eric Hsi[6], Heesun J Rogers[6], Katherine Levinson[7], Adam Bagg[7], Duane C Hassane[1], Robert P Hasserjian[8] and Attilio Orazi[1]

[1]Department of Pathology and Laboratory Medicine, Weill Cornell Medical College, New York, NY, USA; [2]Division of Biostatistics and Epidemiology, Department of Healthcare Policy & Research, Weill Cornell Medical College, New York, NY, USA; [3]Department of Hematopathology, the University of Texas MD Anderson Cancer Center, Houston, TX, USA; [4]Department of Pathology, Stanford University, Stanford, CA, USA; [5]Department of Pathology, University of Chicago, Chicago, IL, USA; [6]Department of Laboratory Medicine, Cleveland Clinic, Cleveland, OH, USA; [7]Department of Pathology and Laboratory Medicine, University of Pennsylvania, Philadelphia, PA, USA and [8]Department of Pathology, Massachusetts General Hospital, Boston, MA, USA

Oligomonocytic CMML vs. CMML (Geyer et al. Mod Path 2017)

- Oligomonocytic CMML, 44 cases (relative monocytosis ≥ 10%, Absolute monocyte 0.5-1K/ul)
- Lower WBC count and ANC but similar monocyte percentage, HgB and Plt count
- M:E ratio normal or decreased vs. CMML
- 38% progressed to CMML (median: 12 months)
- Percentage of mutations lower as compared to CMML
- TET2, ASXL1 and SRSF2 mutations most common
- CBL mutations exclusive to CMML group
- A subset of oligomonocytic CMML represent early phase of “dysplastic” CMML

Conclusion

- NGS can be helpful in cases where all criteria are not fulfilled for CMML diagnosis (to establish clonal nature of the disease)
- NGS can help prognosticate CMML (ASXL1 mutations)
- Not all mutations are created equal (ASXL1 frameshift and nonsense mutations are prognostically important)

Questions?

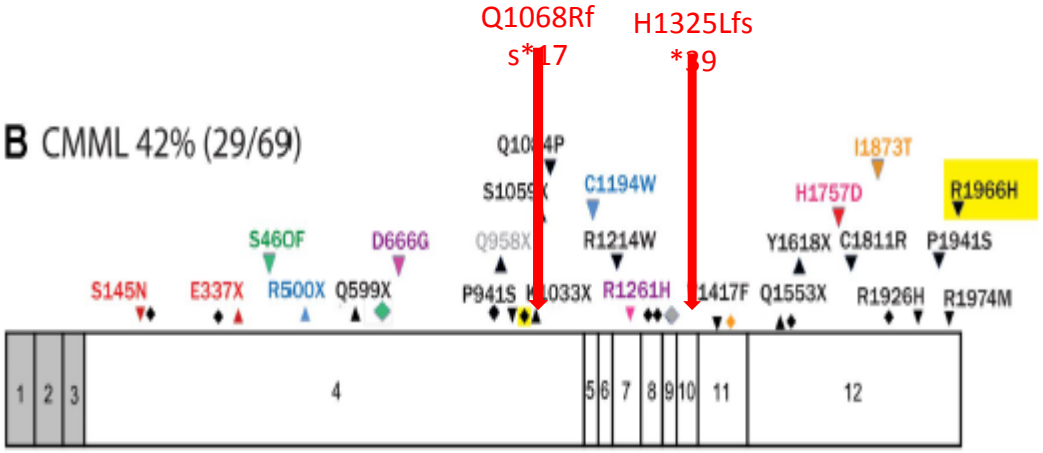
Panel Diagnosis

Chronic Myelomonocytic Leukemia-0.
The mutational
Spectrum is characteristic of CMML

Table 1: Gene Regions Assessed by the TruSight Myeloid Sequencing Panel

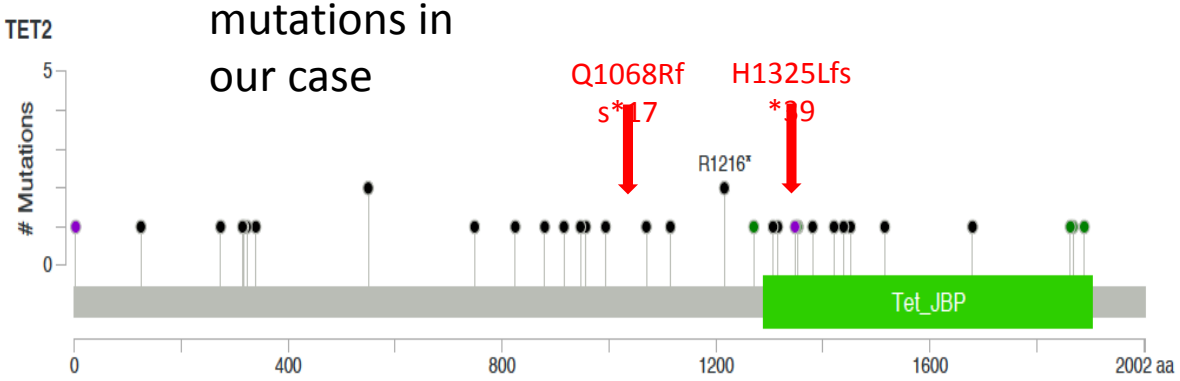
Gene	Target Region (exon)	Gene	Target Region (exon)	Gene	Target Region (exon)	Gene	Target Region (exon)
ABL1	4–8	*DNMT3A*	full	*KDM5A*	full	*RAD21*	full
ASXL1	12	*ETV6/TEL*	full	*KIT*	2, 8–11, 13 + 17	*RUNX1*	full
ATRX	8-10 and 17–31	*EZH2*	full	*KRAS*	2 + 3	*SETBP1*	4 (partial)
BCOR	full	*FBXW7*	9 + 10 + 11	*MLL*	5–8	*SF3B1*	13–16
BCORL1	full	*FLT3*	14 + 15 + 20	*MPL*	10	*SMC1A*	2, 11, 16 + 17
BRAF	15	*GATA1*	2	*MYD88*	3–5	*SMC3*	10, 13, 19, 23, 25 + 28
CALR	9	*GATA2*	2–6	*NOTCH1*	26–28 + 34	*SRSF2*	1
CBL	8 + 9	*GNAS*	8 + 9	*NPM1*	12	*STAG2*	full
CBLB	9, 10	*HRAS*	2 + 3	*NRAS*	2 + 3	*TET2*	3–11
CBLC	9, 10	*IDH1*	4	*PDGFRA*	12, 14, 18	*TP53*	2–11
CDKN2A	full	*IDH2*	4	*PHF6*	full	*U2AF1*	2 + 6
CEBPA	full	*IKZF1*	full	*PTEN*	5 + 7	*WT1*	7 + 9
CSF3R	14–17	*JAK2*	12 + 14	*PTPN11*	3 + 13	*ZRSR2*	full
CUX1	full	*JAK3*	13				

- 1. SRSF2 c.284C>A p.P95H
- Additional information:
- 1-2. 17q25.1
- 3. HG19
- 4. NM_003016.4
- 7. 3502 reads
- 8. 3051 reads
- 9. 3502/6553=53.4%
-
- 2. TET2 c.3195_3202dupGACTAGAC p.Q1068Rfs*17
- Additional information:
- 1-2. 4q24
- 3. HG19
- 4. NM_001127208.2
- 7. 6333 reads
- 8. 2450 reads
- 9. 6333/8783=72.1%
-
- 3. TET2 c.3972_3973dupTC p.H1325Lfs*39
- Additional information:
- 1-2. 4q24
- 3. HG19
- 4. NM_001127208.2
- 7. 1634 reads
- 8. 12554 reads
- 9. 1634/14188=11.5%
-
- 4. ASXL1 c.2290delC p.L764Yfs*8
- Additional information:
- 1-2. 20q11.21
- 3. HG19
- 4. NM_015338.5
- 7. 8749 reads
- 8. 9433 reads
- 9. 8749/18182=48.15%

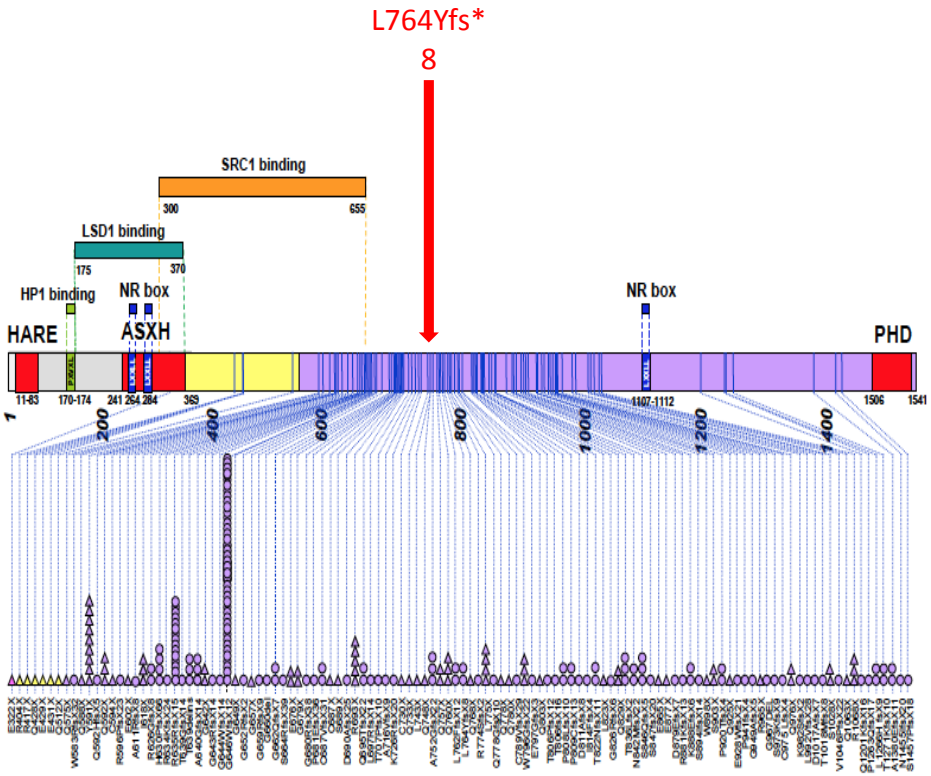


Taken from reference 1. It shows the position of TET2 mutations in myeloid neoplasms

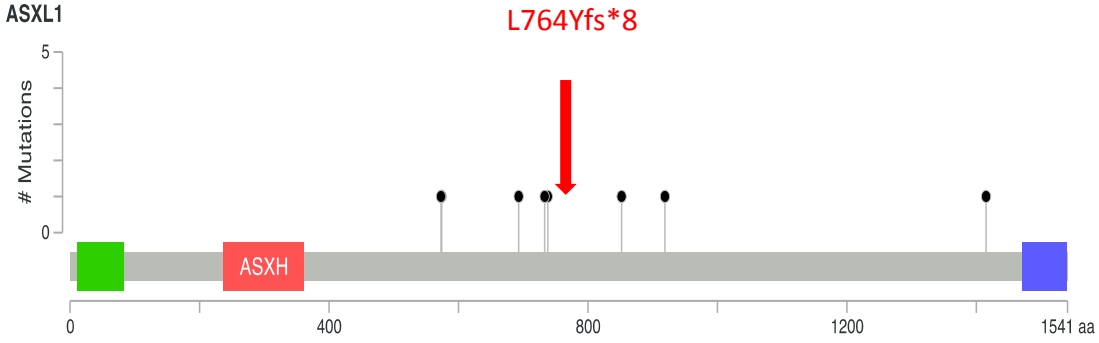
Red arrow is the mutations in our case



Taken from cBioPortal (MSKCC database). It shows the position of TET2 mutations in AML and MDS (this database does not have many myeloid neoplasms yet). The green box is the double stranded B-helix dioxygenase domain which is affected by



Taken from reference 2. It shows the position of ASXL1 mutations in myeloid neoplasms as well as the domains



Taken from cBioPortal (MSKCC database which is publicly available). It shows the position of ASXL1 mutations in AML and MDS. The blue box is the PHD domain which is the one that needs to be lost in order for the protein