CASE TYPE: GERMLINE MUTATIONS OR FAMILIAL SYNDROMES PREDISPOSING TO MYELOID OR LYMPHOID NEOPLASMS.

Mild Megakaryocyte Atypia in a Patient with Presumed Germline GATA2 Mutation, and Active Mycobacterial Infection.

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Clinical:

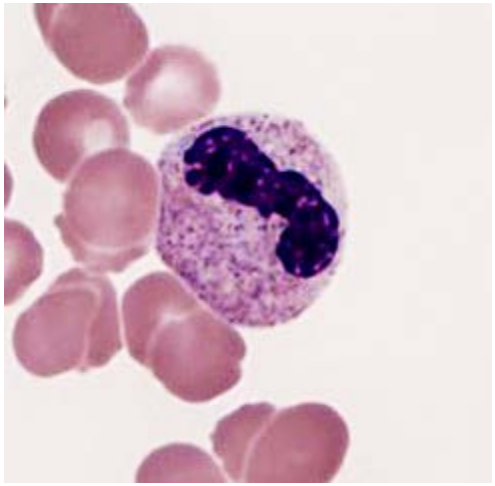
- 44 year old woman transferred for management of invasive pulmonary aspergillosis, disseminated *Mycobacterium avium* infection and likely secondary HLH (meets 6/8 criteria).

- History:
  - Antiphospholipid antibody syndrome/Mixed connective tissue disease
  - Liver biopsy showed granulomatous inflammation with acid fast bacilli and microbiology reported *mycobacterium avium*.
  - Peripheral blood GATA gene sequencing: Heterozyogote pathogenic variant identified in GATA2 p.R398W.
  - No history of CIN.

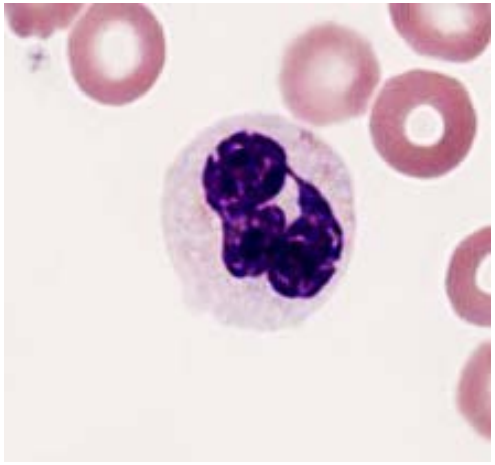
- Family History:
  - Sister died age 36 years of recurrent fevers and infections
  - Mother died at 31 years of colon cancer.

Peripheral blood:

- CBC:
  - WBC, 3.23 K/ul; HGB, 7.7 g/dl; MCV, 84 fl; PLT, 49 K/ul.
  - Diff: Neutrophils, 92%; Lymphocytes, 4%; Monocytes, 1%; Eosinophils, 2%; Basophils, 1%.



- Peripheral blood morphology:
  - WBC:Leukopenia. Mild neutrophilic left shift. Occasional neutrophilic forms show hypogranular cytoplasm (fewer than 10%), no circulating blasts identified.
  - RBC: Normocytic anemia. Rare nucleated red blood cells identified.
  - Platelets: Thrombocytopenia.



Bone marrow aspirate

Megakaryocyte atypia:

(approaching 10%).

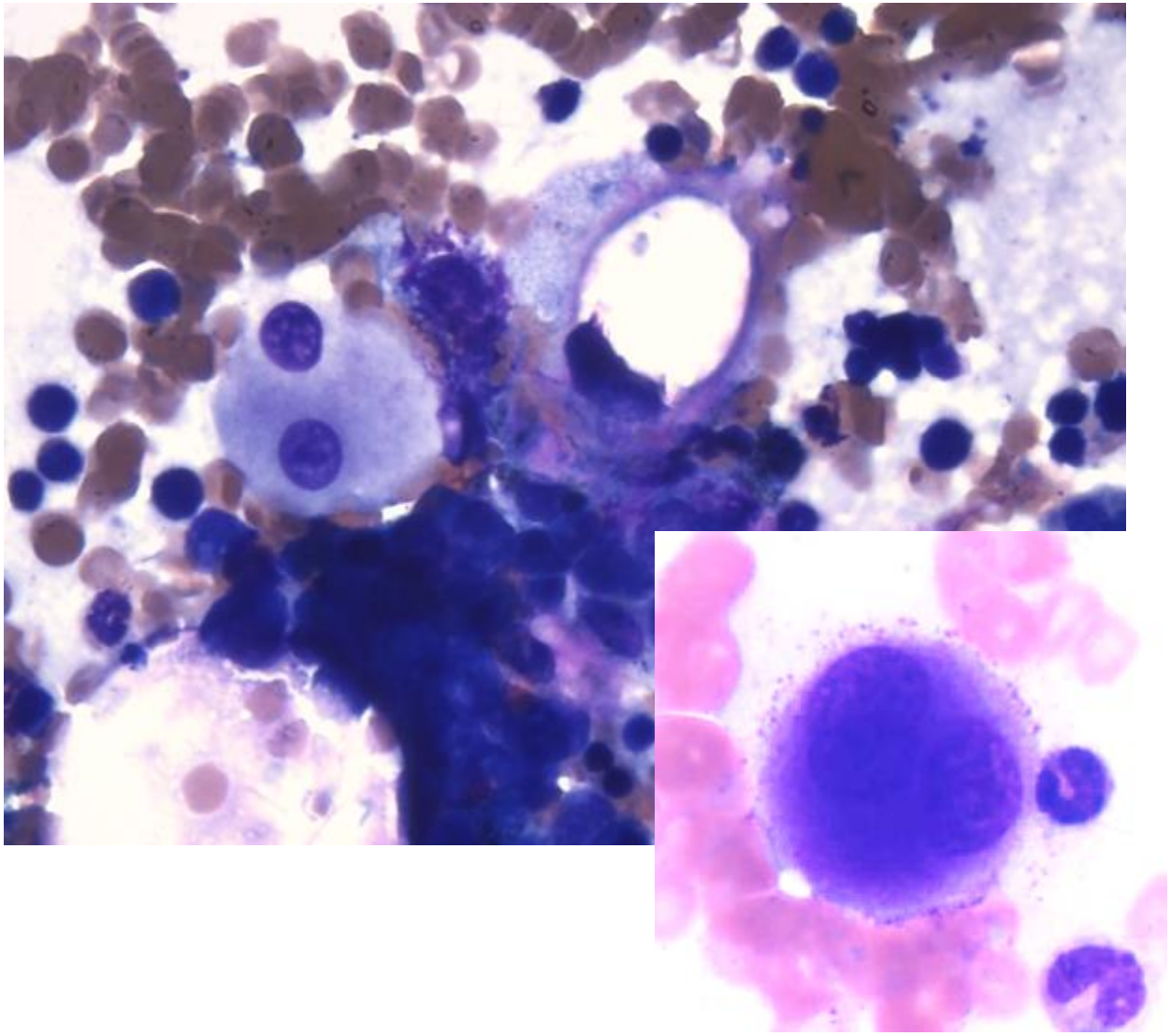
Widely spaced nuclear lobes

Small hypolobated nuclei

Differential count:
Myeloids, 73.5%;
Erythroids, 23.5%;
Blasts, 0%;
Lymphocytes, 1%;

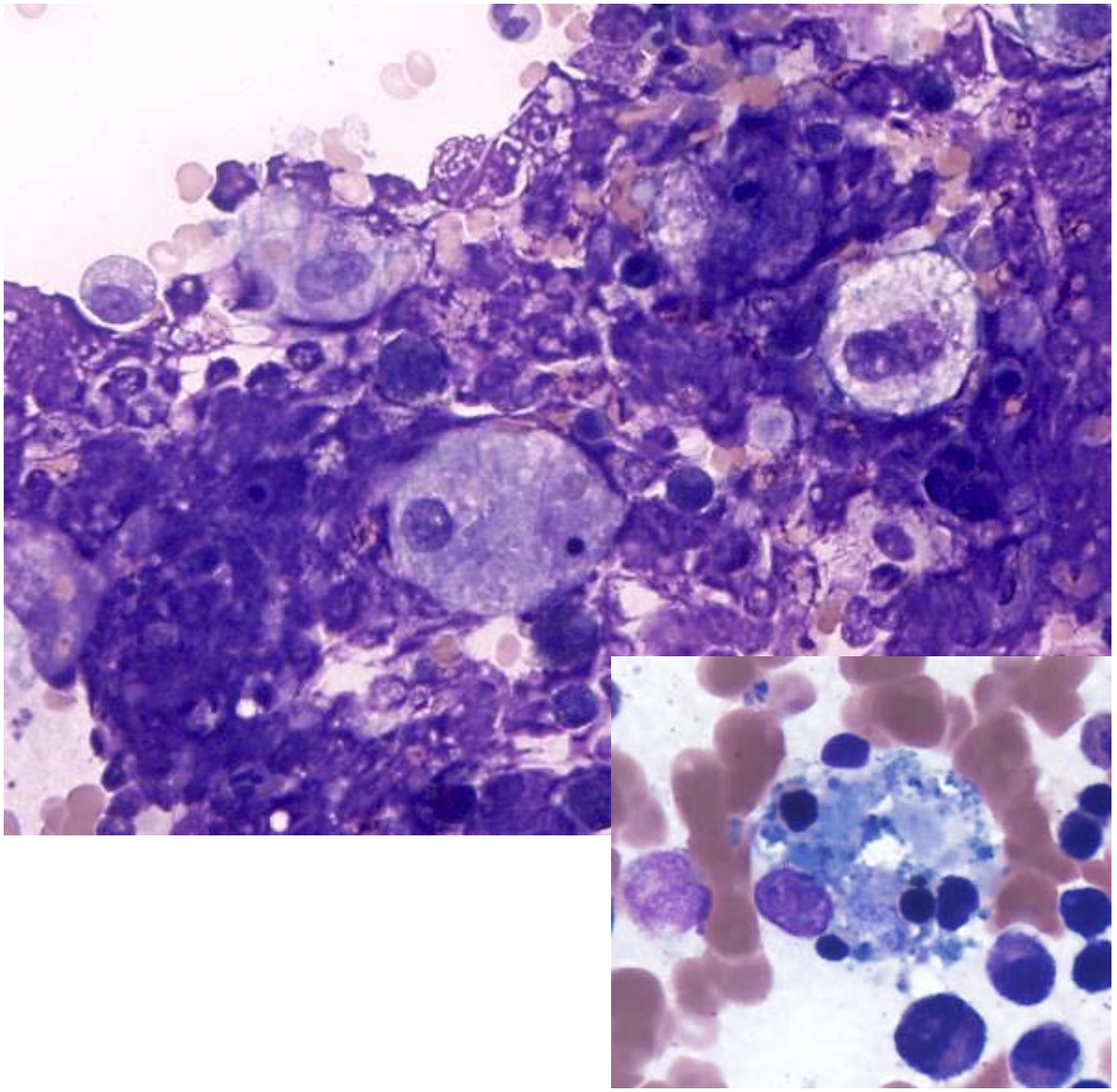
Plasma cells, 2%.

M:E Ratio: 3:1



Bone marrow aspirate

Histiocytes increased and show hemophagocytosis



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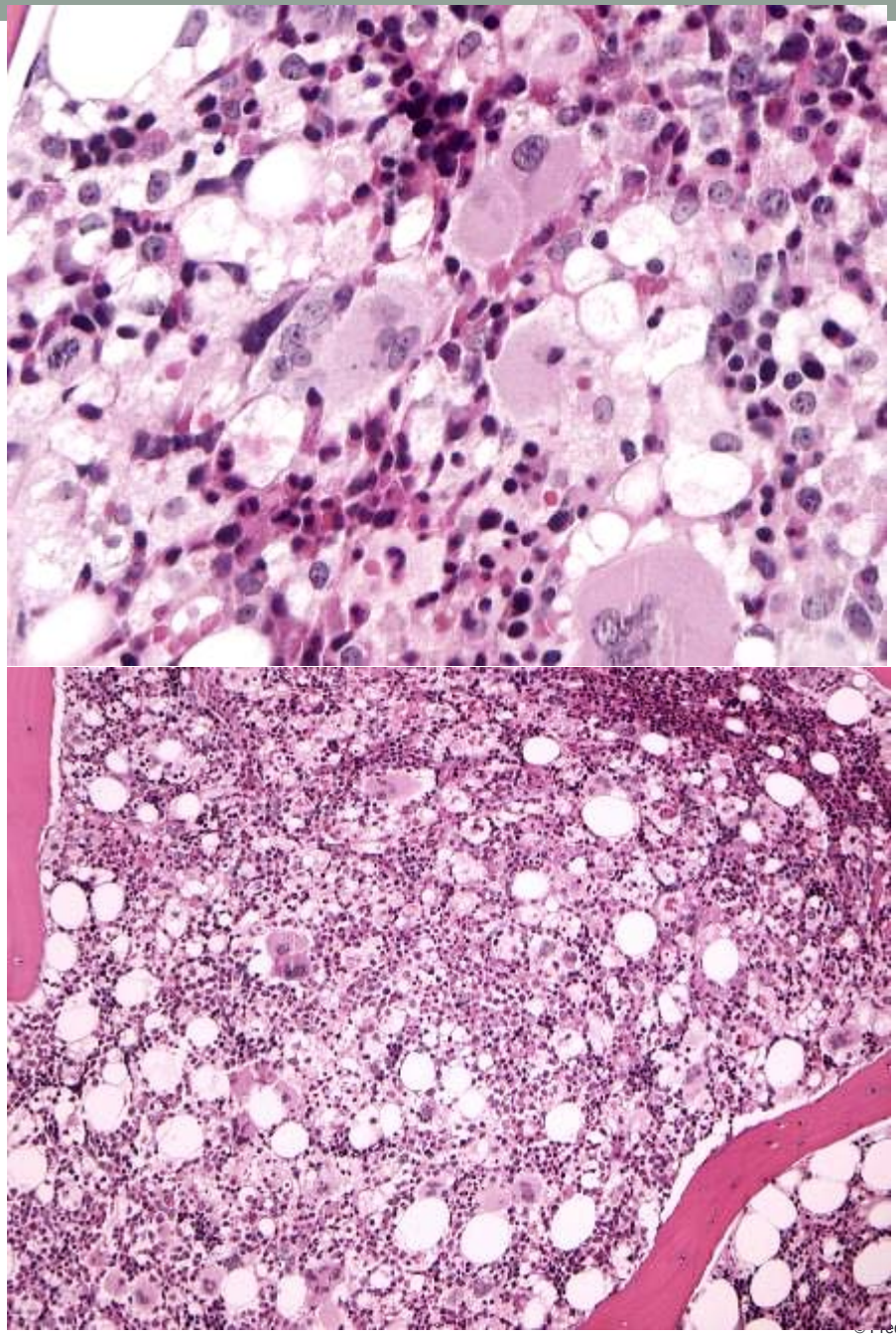
Bone marrow core biopsy

Cellularity: ~80%

Trilineage hyperplasia.

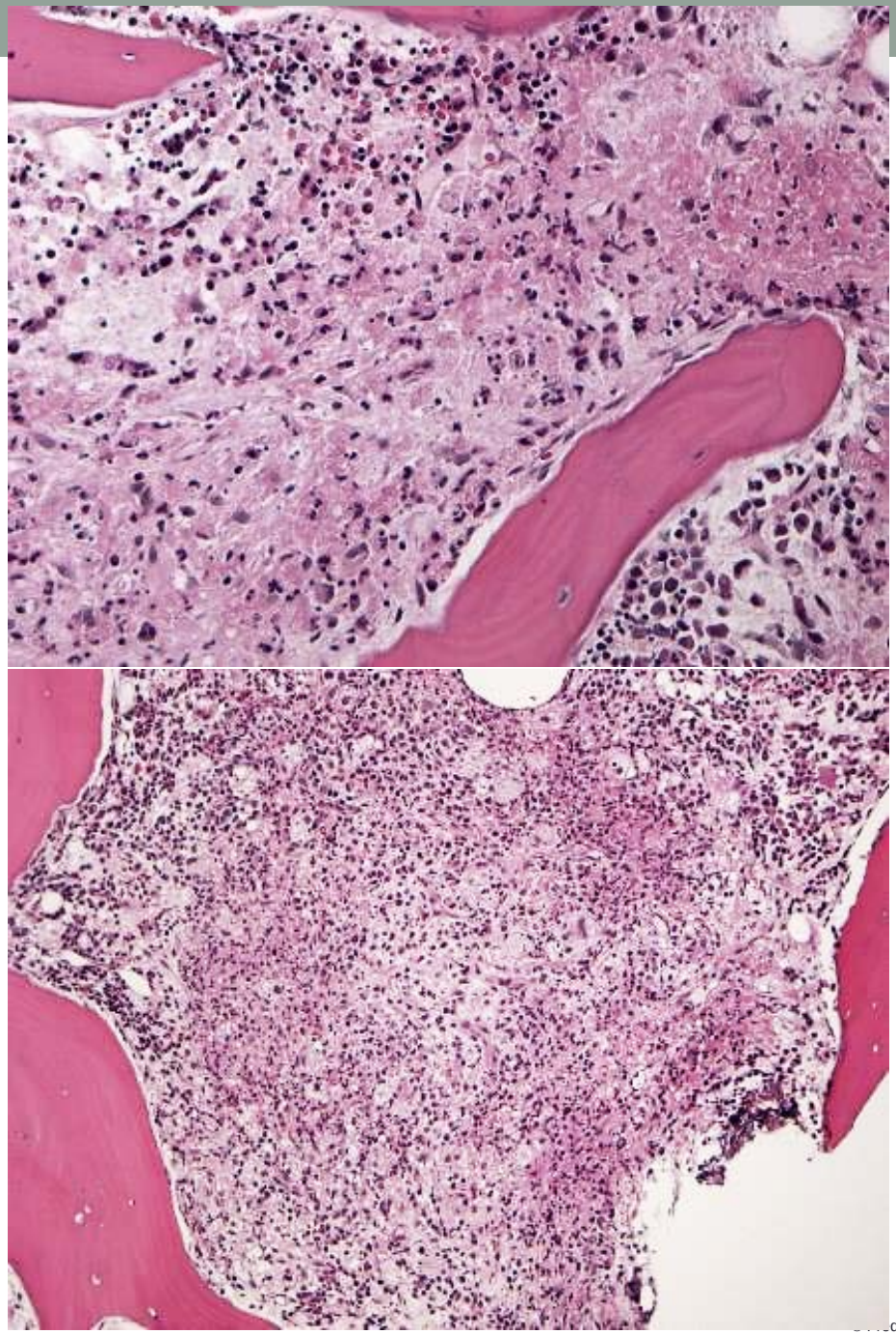
Megakaryocytes with focal clustering

CD163 positive histiocytes are markedly increased and include forms with ingested cells (hemophagocytosis).



Bone marrow core biopsy

Histiocytes increased and form loose granulomas. Many with central necrosis.



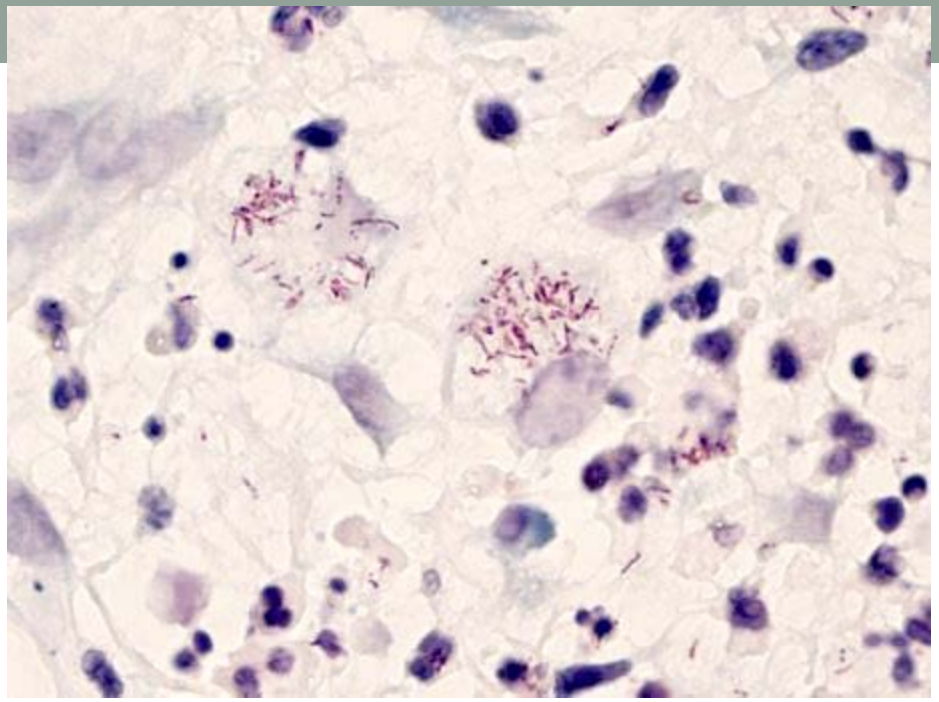
Bone marrow core biopsy

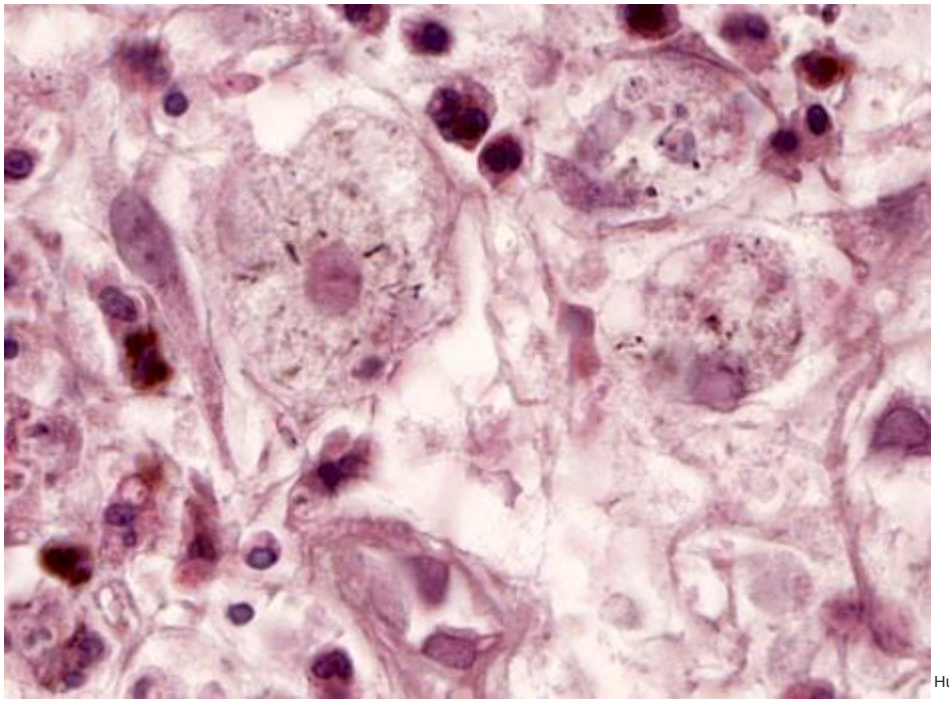
AFB (top right): Numerous Acid fast organisms within the necrotic granulomas

MS (bottom right): highlight numerous organisms within the necrotic granulomas.

Confirmed by culture and sequncing

1. *Mycobacterium avium*
2. No fungal elements identified by stain or culture.





Ancillary studies, bone marrow aspirate:

Flow Cytometry

1. No abnormal myeloid blast, monocyte, or myeloid population identified (see comment).
2. No abnormal B or T cell population identified (see comment).

** B cell (0.0000756% of WBC) and NK cell (0.000153% of WBC) lymphopenia

Cytogenetics

Karyotype:

- 46,XX[20]

Interphase FISH:

- nuc ish(EGR1,D5S23)x2[200],(D7Z1,D7S486)x2[200],(D8Z2x2)[200],(D20S108x2)[200].

Neoplasia SNP Microarray Analysis:

- arr(1-22,X)x2

Molecular, bone marrow aspirate:

Method: Test performed by targeted capture for listed genes followed by next-generation sequencing with Illumina technology.

Tier 1: Currently Actionable	*ABL1*	*ALK*	*BCR*	*BCL2L11*	*BRAF*	*RET*	*CDK4*	*CEBPA*	*KIF5B*
	DDR2	*EML4*	*EGFR*	*ERBB2*	*FLT3*	*IDH1*	*IDH2*	*JAK2*	*RARA*
	KIT	*KRAS*	*MPL*	*NPM1*	*NF2*	*NRAS*	*PDGFRA*	*PML*	
	RICTOR	*ROS1*	*TSC1*	*TSC2*					
Tier 2: Actionable in the Near Future	*ABL2*	*AKT1*	*AKT2*	*AKT3*	*ASXL1*	*ATM*	*AURKA*	*AURKB*	*BAP1*
	BCOR	*CBL*	*CBLB*	*CDK6*	*CDK8*	*CHEK1*	*CHEK2*	*DNMT3A*	*EPHB2*
	ERBB3	*ERBB4*	*FGFR1*	*FGFR2*	*FGFR4*	*FLT1*	*FLT4*	*GATA2*	*GNA11*
	GNAQ	*GRM3*	*HDAC4*	*HIF1A*	*HRAS*	*IGF1R*	*JAK3*	*KDM6A*	*KDR*
	MAP2K1	*MAP2K2*	*MAPK1*	*MC1R*	*MCL1*	*MEN1*	*MET*	*MLH1*	*MLL*
	MRE11A	*MSH2*	*MSH6*	*MYC*	*MYCN*	*NOTCH1*	*PAX5*	*PDGFRB*	*PIK3CA*
	PIK3R1	*PMS2*	*PTEN*	*RAF1*	*NKX2-1*	*SMO*	*SRSF2*	*SUZ12*	*TET2*
	TYR	*VHL*	*MITF*	*ERCC2*					
Tier 3: Frequently Mutated	*APC*	*BAK1*	*BCL2*	*CCND1*	*CCNE1*	*CDH1*	*CDKN2A*	*CREBBP*	*CRLF2*
	CSF1R	*CTNNB1*	*EPHA3*	*EPHA5*	*EPHB6*	*ETV6*	*EZH2*	*FBXW7*	*FGFR3*
	GAB2	*GATA1*	*GNAS*	*GRIN2A*	*HNF1A*	*IKZF1*	*IL7R*	*JAK1*	*MAP2K4*
	MDM2	*MDM4*	*MUTYH*	*MYCL1*	*NF1*	*STK11*	*NOTCH2*	*PBRM1*	*PRPF40B*
	PTCH1	*PTPN11*	*PTPRD*	*RB1*	*RPS14*	*RUNX1*	*SF1*	*SF3B1*	*SMAD2*
	SMAD3	*SMAD4*	*SMARCA4*	*SMARCB1*	*SPRY4*	*SRC*	*TFG*		*TGFBR2*
	TP53	*TRRAP*	*U2AF1*	*U2AF65*	*WT1*	*ZRSR2*			
Germline Pharmacogenomics	*ABCB1*	*ABCC2*	*ABCC4*	*ABCG2*	*C1orf144*	*COMT*	*CYP1B1*	*CYP2C19*	*CYP2C8*
	CYP2D6	*CYP3A4*	*CYP3A5*	*DPYD*	*EIF3A*	*ESR1*	*ESR2*	*FCGR1A*	*UMPS*
	FCGR2A	*FCGR3A*	*GSTP1*	*GUCY1A2*	*ITPA*	*LRP2*	*MAN1B1*	*MTHFR*	*NQO1*
	NRP2	*SLC19A1*	*SLC22A2*	*SLCO1B3*	*SOD2*	*SULT1A1*	*TPMT*	*TYMS*	*UGT1A1*

Result: POSITIVE for mutations in GATA2 p.R398W (see next slide) and STAG2 p.R614X.

- Two alterations were identified of uncertain significance including:
  1. a splice alteration in PALB2 (NM_024675.3:c.2834 plus 4 T to C), and
  2. a possible, but low level ASXL1 frameshift alteration (p.G646Wfs*12, NM_015338.5:c.1934dup).
- No definite mutations, gene amplifications, or gene fusions were otherwise detected in the panel tested.

Pritchard;J Mol Diagn. 2014 Jan;16(1):56-67

Molecular, bone marrow aspirate, GATA2:

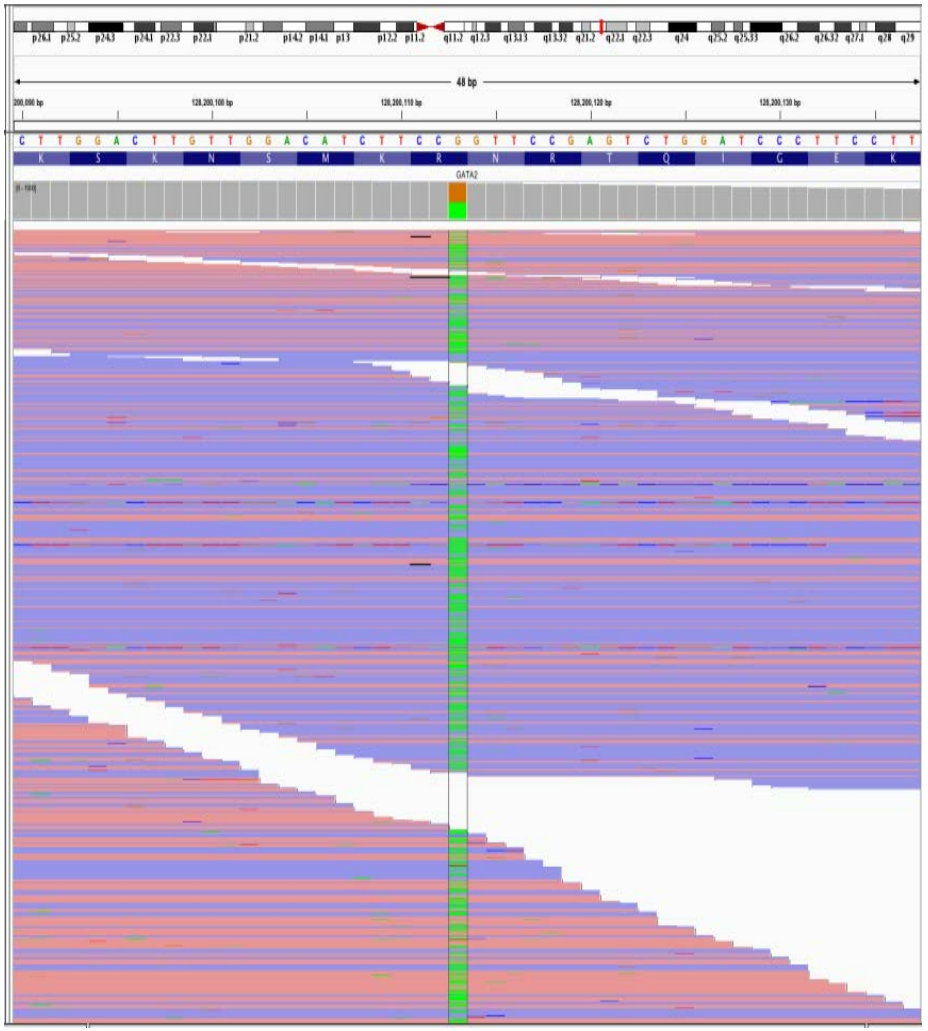
GATA2 (p.R398W, NM_032638.4:c.1192C>T)

hg19 coordinates: chr3:128200113 G>A

reference reads = 484

variant reads = 386

allelic fraction = 0.44



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Molecular, bone marrow aspirate, STAG2:

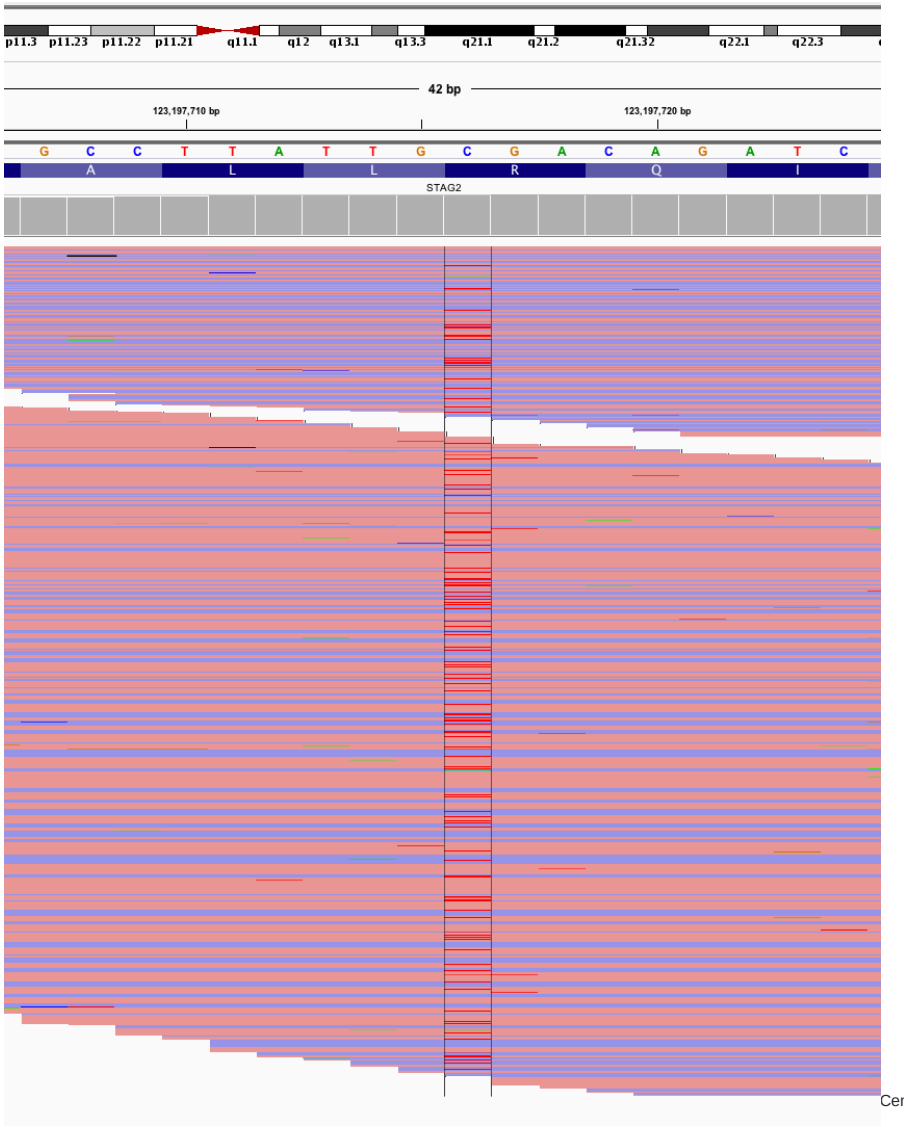
STAG2 (p.R614X NM_006603.4:c.1840C>T)

hg19 coordinates: chrX:123197716 C>T

reference reads = 599

variant reads = 104

allelic fraction = 0.15



Summary of findings:

- Morphology:
  1. Hypercellular marrow with trilineage hyperplasia
  2. Granulomas with necrosis and organisms highlighted by Kinyon AFB and Mahan silver stains.
  3. Hemophagocytosis.
  4. Monocytopenia, lymphopenia and thrombocytopenia.
  5. Mild megakaryocyte atypia (not sufficient for dysplasia).
- Flow cytometry:
  1. No abnormal myeloid blast, monocyte, or myeloid population identified.
  2. No abnormal B or T cell population identified.
- Cytogenetics:
  1. Normal Female karyotype
  2. Normal Female by Neoplasia SNP Microarray Analysis
  3. No evidence of abnormality of 5, 7, 8, and 20 was found by IFISH interphase fluorescence in situ hybridization.
- Molecular:
  1. POSITIVE for mutations in GATA2 p.R398W and STAG2 p.R614X
- Microbiology:
  1. *Mycobacterium avium* isolated from mycobacterial broth: identification by sequence analysis.
  2. No fungal elements identified by stain or culture.

Proposed diagnosis

- **Bone marrow with *Mycobacterium avium* infection and immunodeficiency disorder with germline GATA2 mutation (MonoMac)**

Panel diagnosis

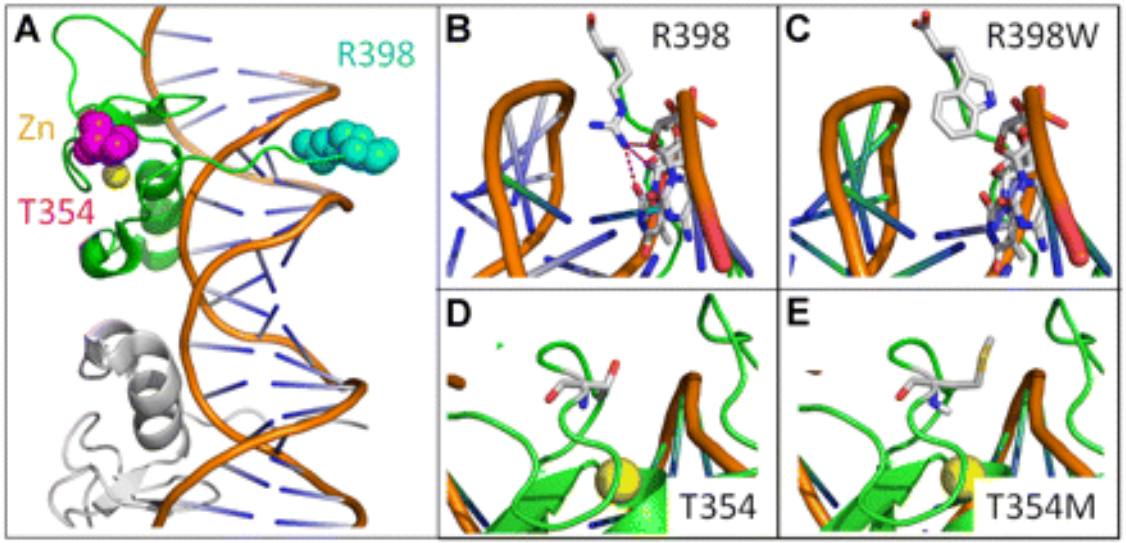
- **Immunodeficiency disorder with germline GATA2 mutation (MonoMac)**

Case discussion points

- GATA2 deficiency are at high risk of developing myeloid stem cell neoplasms such as MDS/AML or CMML
- May show very abnormal marrows with evidence of infection …ie “monoMAC”
- GATA2 deficiency should be in the differential diagnosis of patients presenting with disseminated MAC, HPV, or other opportunistic infections, history of warts, abnormal marrow cytogenetics, and/or a family history of MDS/AML/CMML.

Mutations in monoMAC

- GATA2 : nuclear regulatory protein
  - regulate the expression of multiple target genes
  - binding to the consensus DNA sequence T/A(GATA)A/G located in numerous promoters and enhancers



Dickenson et.al. Blood 2011 118:2656-2658
Wlodarski et.al. Semin Hematol. 2017 Apr;54(2):81-86.

Case discussion point

Typical MonoMAC

- Adult onset
- Monocytopenia, B and NK lymphocytopenia
- Opportunistic infections with mycobacterial, viral, and fungal infections and development of malignancy
- Familial history: autosomal dominant (sporadic cases also described)
- Presenting in marrow as aplastic anemia, MDS, or bone marrow failure

Vinh et.al., Blood 2010 115:1519–1529

Features in our case

- Adult onset
- B and NK lymphopenia
- Opportunistic infection with multiple organisms in history and mycobacterium in current marrow
- Familial history in sib
- Hypercellular marrow with atypical megakaryocytes and granulomas

Hsu et.al., Blood. 2011 Sep 8; 118(10): 2653–2655.

Thank You

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