Society of Hematopathology 2017 Workshop
Session 1 summary
Germline Predisposition Syndromes

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Until every child is well™

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TEACHING HOSPITAL

Myeloid Neoplasms with Germline Predispositions

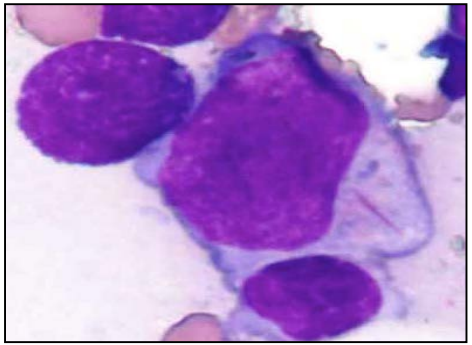
new recognition in the revised WHO classification

- **Without a pre-exisiting disorder or organ dysfunction**
  - AML with germline CEBPA mutation **(2 cases)**
  - Myeloid neoplasms with germline DDX41 mutation **(1 case)**
- **Pre-existing platelet disorder**
  - Germline RUNX1 **(10 cases)**
  - Germline ANKRD26 **(1 case)**
  - Germline ETV6 mutation
- **Other organ dysfunctions**
  - Germline GATA2 **(16 cases)**
  - Bone marrow failure syndromes
  - JMML associated with NF, Noonan's or Noonan-like disorders **(3 cases)**
  - Down syndrome

AML with germline *CEBPA* mutation

- Biallelic *CEBPA* mutations
  - Encodes a granulocyte differentiation factor on chromosome 19
  - Germline mutation at 5’ end of gene
  - Somatic mutation at 3’ end of the other allele
    - Acquired at the time of progression to AML
- Morphologic, immunophenotypic and cytogenetic features similar to sporadic AML with *CEBPA* mutations

Case 230 P. Khattar:
39 yo with strong family



Myeloid neoplasms with germline *DDX41* mutations

- Inherited mutations in the gene on chromosome 5 encoding the DEAD box RNA helicase DDX41
  - Major subset DDX41 mutation is biallelic (one mutation is germline)
- Prevalence is unclear – *DDX41* mutations found in 1.5% of myeloid neoplasms
- Long latency –presentation in 60s

  - **CASE 318 H. Kurt**:

    67 year-old man who had been having slowly decreasing white blood cell and platelet counts for the last 7 years
    - Presented with **AML**, normal karyotype, no dysplasia
    - Received transplant from his brother
  - After 4 months from stem cell transplant, the patient accepted skin biopsy for further genetic testing.
    - DDX41 NM 016222.2(DDX41): c.3G>A p.M1?

Myeloid Neoplasms with Germline Predispositions AND pre-existing platelet disorders

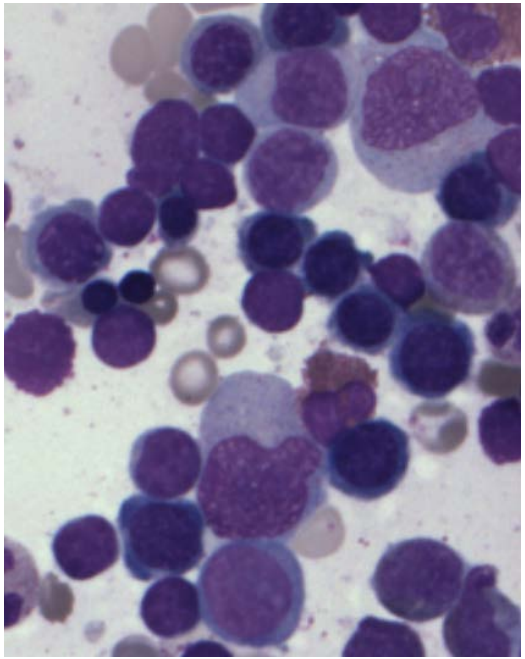
- **Germline mutations in *RUNX1* gene**
  - gene on chromosome band 21q22
  - encodes one subunit of the core binding transcription factor that regulates expression of several genes essential for hematopoiesis.
  - Somatic RUNX1 mutations are associated with poor prognosis in AML/MDS
- Mild to moderate thrombocytopenia
- Functional platelet defects -> prolonged bleeding
- Increased risk of developing MDS, AML or T-ALL

Cases with germline *RUNX1* mutations

Familial platelet disorder with predisposition to AML

Case Number	Submitter	Age	Diagnosis	Interesting aspects
219	Geyer	37	Thrombocytopenia	Variant of unknown signficance
271	Mosse	3	Thrombocytopenia	Extensive family h/o AML
309	Kanagal-Shamanna	13	Thrombocytopenia	46,XX,inv(9)(p12q13)[20]
364	Reddy	25	Thrombocytopenia	Incidental presentation

Case 271- extensive family history of AML



*Diagnostic criteria for myeloid neoplasm in this setting is the same as for sporadic cases

- Presence of germline *RUNX1* mutations does not place case into category of myeloid neoplasm with germline predisposition syndrome category

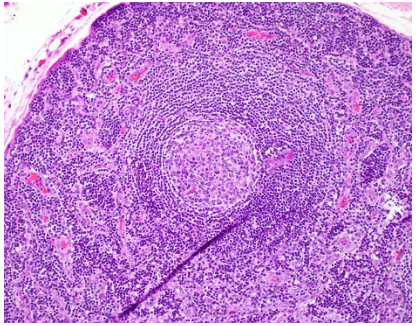
- **Thrombocytopenia with germline *RUNX1* mutation**

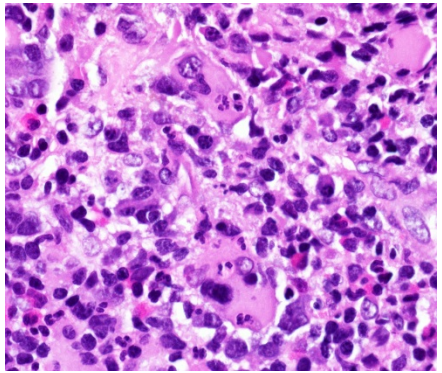
case 200 Xiao

TAFRO – Variant of idiopathic multicentric Castleman disease

Thrombocytopenia, Anasarca, Fever, Renal dysfunction/reticulin fibrosis and Organomegaly

- 3 y/o male with no known family history presented with fever, generalized lymphadenopathy, hepatosplenomegaly, pleural effusions, ascites, thrombocytopenia and anemia.
- WBC 17.2, Hgb 7.8, platelets 14, BUN 85, Cr 0.7, Cystatin C 3.45, ALP 108, CRP 15, ESR 118, Albumin 2, and LDH 481. He also had elevated IL-6
- RUNX1 exon4 p.G87C (c.259G>T) (VAF 35%)
  - Germline analysis confirmed that the RUNX1 mutation is present in DNA from nail, lymph node and bone marrow at 50% VAF.





MDS/AML with germline *RUNX1* mutations

- Risk of transformation to MDS/AML is estimated to be ~30-40%
- Progression to MDS/AML likely requires additional mutations
  - may account for some of the variation in penetrance of MDS/AML as well as the variable neoplasm phenotypes that develop

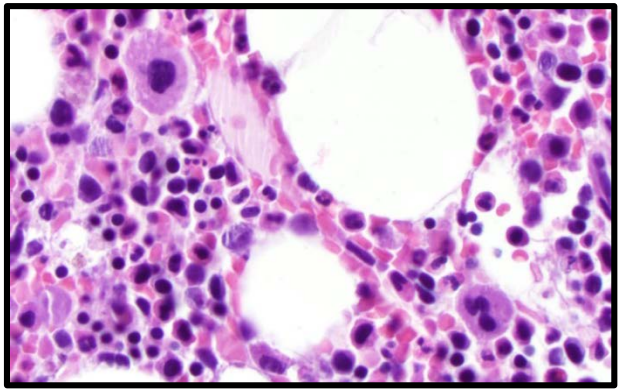
Case #	Submitter	Age	Diagnosis	Other mutations	Cytogenetic
38	Chisholm	12	**AML**		46,XX,t(2;11)(q31;p15)[20]
339	Kanagal-Shamanna	7	**MDS-MLD**		del(5)(q31q34)
284	Bailey	32	**AML**	NRAS, BCOR	t(2;21)(q23;q22)

Myeloid Neoplasms with Germline Predispositions AND pre-existing platelet disorders

- **Germline mutations in *ANKRD26* gene**
  - located on chromosome band 10p12.1
- Mutations occur within the 5' untranslated region of the gene
  - disrupt the assembly of RUNX1 and FLI1 on the ANKRD26 promoter

- **Case 268 Neppalli**
- 43 yo with long standing history of thrombocytopenia (and family history of thrombocytopenia)
  - Normocellular marrow but with decreased megakaryocytes and frequent hypolobated forms

➢ Thrombocytopenia with germline ANKRD26 mutation

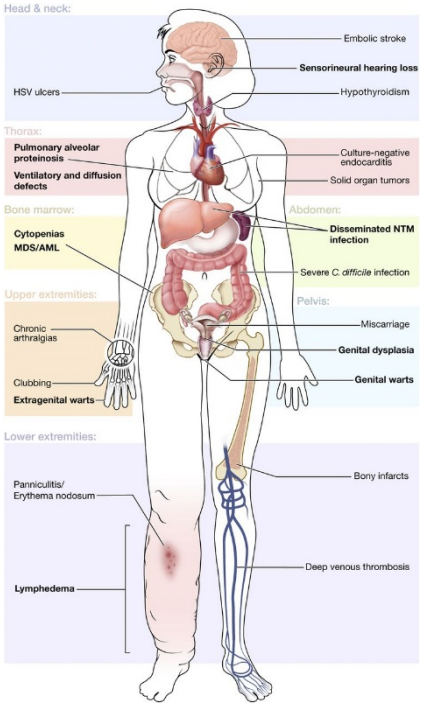


Myeloid neoplasms with germline predispositions AND other organ dysfunctions

- Germline *GATA2* mutations
- Bone marrow failure syndromes
- JMML associated with NF, Noonan's or Noonan-like disorders
- Down syndromes

Myeloid neoplasms with germline *GATA2*

- Four separate syndromes
  - MonoMAC syndrome
    - monocytopenia and non-tuberculous mycobacterial infection
  - Dedritic cell, monocyte B- and NK lymphoid (DCML) deficiency with vulnerability to viral infectons
  - Familial MDS/AML
  - Emberger syndrome
    - Primary lymphadema, warts, predisposition to MDS/AML



Michael A. Spinner et al. Blood 2014;123:809-821

AML with germline *GATA2* mutations

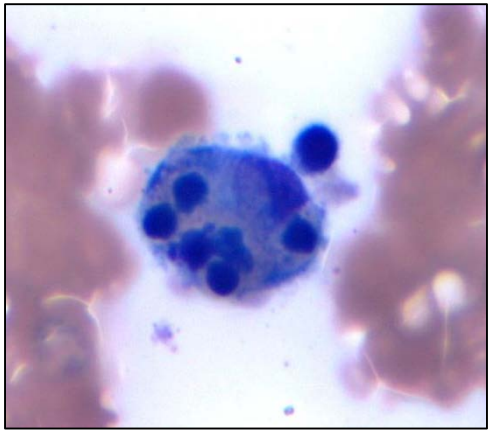
Case Number	Age	Name	Diagnosis	Other mutations	Cytogenetics
20	18	Scordino	AML-MRC	-	Complex karyotype
48	6	Siegele	AML-MRC	WT1, JAK2, CSF3R, KRAS	Monosomy 7
236	30	Boyer	AML-MRC	-	Complex karyotype
266	16	Batdorf	AML-MRC	-	Normal

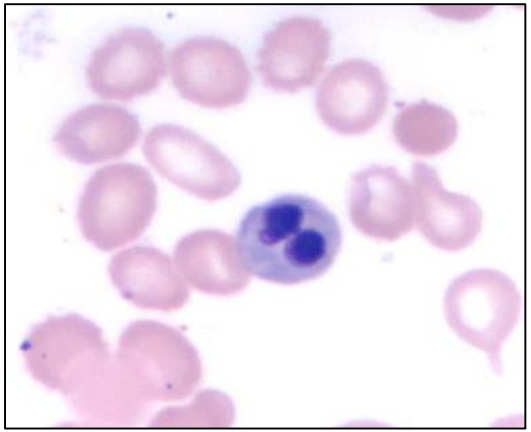
MDS with germline *GATA2* mutation

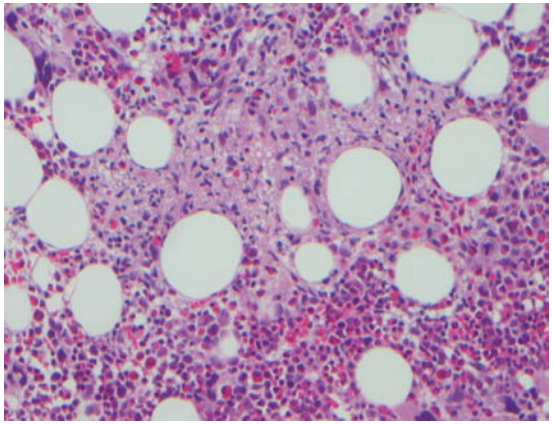
Case Number	Name	Age	Diagnosis	Other Mutations	Cytogenetics
105	Williams	57	MDS-MLD	-	Normal
138	Malek	13	MDS-MLD	-	Normal
157	Crane	45	MDS-MLD	-	Trisomy 21 & 8
337	Balakrishna	31	MDS-MLD	-	Trisomy 8
176	Koo	10	MDS-EB1	NRAS, PTN11, SETBP1, ASXL1	Monosomy 7
52	Moore	22	MDS-EB2	-	Monosomy 7
87	Chiu	5	RCC	ASXL1	Deletion 7q, trisomy 8
381	Wang	17	RCC	-	Trisomy 8
40	Chisholm	17	CMML-1	KRAS, NF1, SETBP1, STAT3, WT1	Monosomy 7

Other disorders with germline *GATA2* mutations

- Case 258 Hussein: 62 yo with FUO and 10 year history of lymphopenia and monocytopenia of unclear etiology





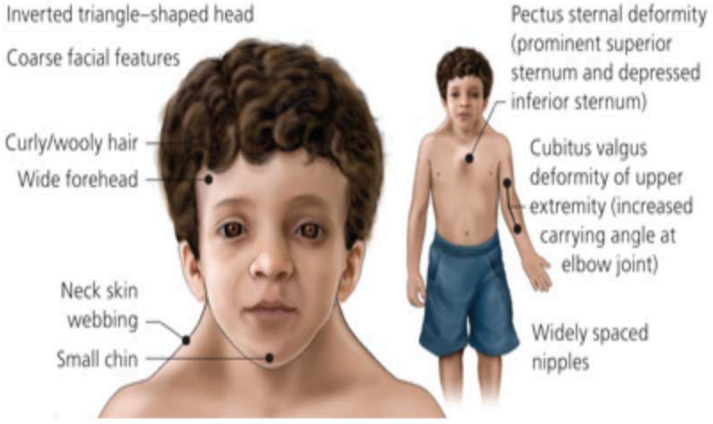


➢ HLH in a patient with bone marrow deficiency (MonoMAC) with germline *GATA2*

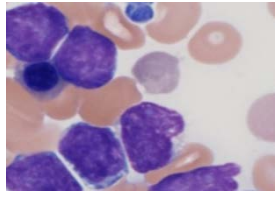
Myeloid Neoplasms with Germline Predispositions and organ dysfunction

Noonan's syndrome

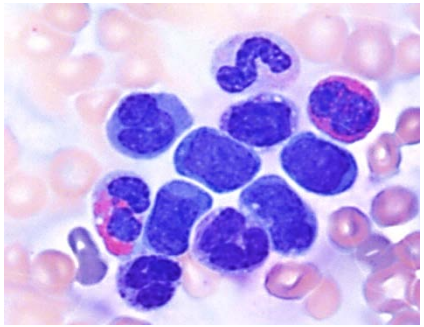
- relatively common (1/2000 births) developmental disorder
  - Characteristic appearance and congenital heart defects
  - Associated with mutations in genes that are part of the RAS/RAF/MEK/ERK signal transduction pathway
    - Variants in PTPN11 (50%), SOS1, RAF1, KRAS, NRAS, BRAF, MAPK1
- Increased risk of malignancy
  - JMML, ALL, rhabdomyosarcoma, neuroblastoma, glioma



Case 99 Knez – B lymphoblastic leukemia in 19 months old with Noonan's syndrome and *SHOC2* gene mutation



JMML



Mandatory

- Monocyte count > 1x109
- Blast % in PB and BM <20%
- Splenomegaly
- Absence of BCR-ABL

Oncogenics

- Somatic mutation in PTPN11, KRAS or NRAS
- Clinical diagnosis of NF-1 or germline NF1
- Germline CBL mutation and loss of
- heterozygosity of CBL

If negative for oncogenics, 2 need to be met

- Monosomy 7
- HbF increased for age
- Myeloid precursors in PB
- Spontaneous growth or GM-CSF hypersensitivity
- Hyperphosphorylation of STAT5

Case 292 Nguyen: 49 day old with dysmorphic features c/w Noonan's presents with splenomegaly and leukocytosis (50-97k/uL)

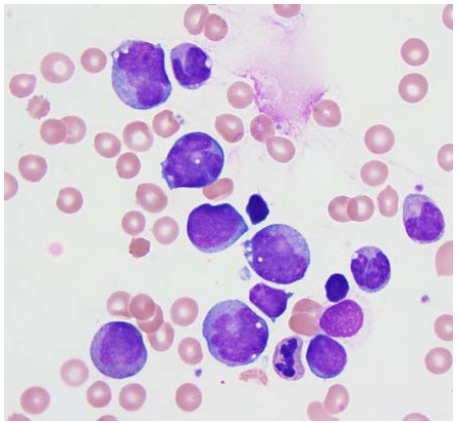
- PTPN11 c.218C>T (p.Thr73Ile) missense mutation

Case 320 Curry: Newborn with prenatal diagnosis of Noonan's

- Persistent thrombocytopenia, leukocytosis, and borderline high Hgb F, but no hepatosplenomegaly
- De novo heterozygous pathogenic variant in the PTPN11 gene (p.S502L).
- Resolved at one year follow up
  - ➢ **Transient Myeloproliferative Disorder in a patient with germline *PTPN11* (Noonan's syndrome)**

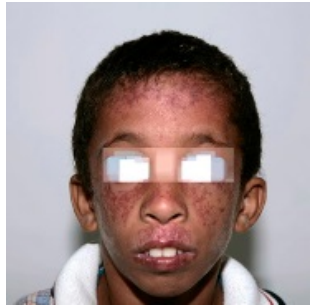
Case 55 Bayerl: 4 month-old asymptomatic girl was found to have splenomegaly, hepatomegaly, leukocytosis and anemia

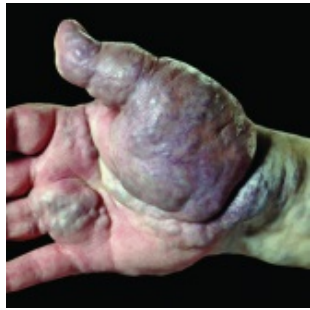
- PB 9.5% blasts***, BM 29% blast***/blast equivalents
- Normal karyotype*, NF1* c.1139T>G (90%)
- After diagnosis of her leukemia, she was found to have >6 café au lait macules.
  - ➢ **AML-NOS with germline *NF1* mutation**



AML with other inherited conditions

Case #	Submitter	Age	Diagnosis	Mutation	Cytogenetic	Syndrome
253	Leeman-Neil	23	AML-MRC	WT1 & NF1 (somatic) **BLM (germ)**	Complex Karyotype	**Bloom**
225	Batdorf	18 months	Therapy-related AML	Germline PTCH TGRB1 microdeletion of unknown significance	t(8;16)(p11:2;p 13.3);KAT6A-CREBBP	-
264	Leeman-Neil	18	AML-MRC	IDH1, NRAS, WT1	Complex	**Mafucci**
234	Meyerson	53	AML	RUNX1, STAG2	Inv(3) and germline t(8;21)	-

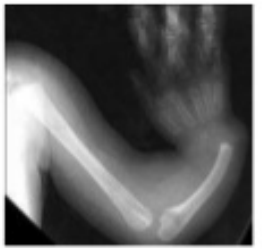




MDS with other inherited syndromes

Case #	Submitter	Age	Diagnosis	Mutation	Cytogenetic
170	Gong	11	MDS-EB2 & LCH	RBM8A	Normal
196	Malek	11	MDS-MLD	G6PC3	Normal
80	Klco	3,4 and 14 months	RCC MIRAGE*	SAMD9 x3	Monosomy 7x3
273	Judd	5 months	MDS/MPN, unclassifiable MIRAGE*	SAMD9	Monosomy 7

TAR syndrome



***MIRAGE**
myelodysplasia, infections, restriction of growth, adrenal hypoplasia, enteropathy

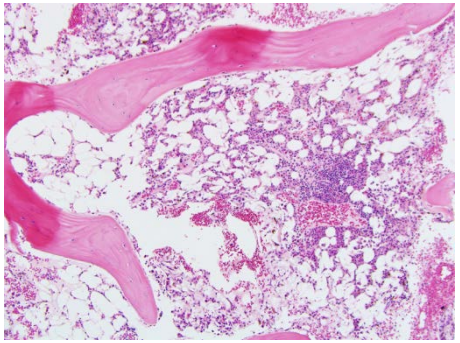
Lymphoid neoplasms

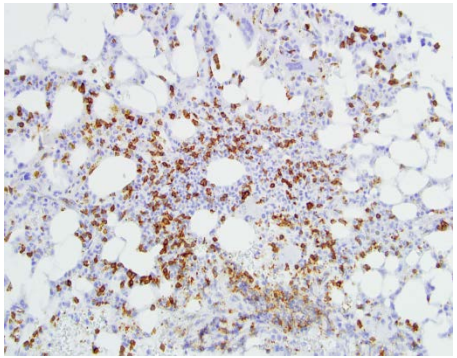
Case	Submitter	Age	Diagnosis	Cytogenetics	Mutation
101	Raciti	18 months	B lymphoblastic leukemia	46,XX,i(9)(q10)[2]/46,XX[21]	Heterozygous PAX5 mutation
194	Baker	1	B lymphoblastic leukemia	Monosomy 7	ELANE mutation
346	Pullarkat	19	Classical Hodgkin lymphoma	Normal	CSF3R (variant of undetermined significance)
342	Wake	40	T-LGL and PRCA	Normal	CTLA4

T-cell LGL and pure red cell aplasia

case 342

- 40 yr old with PMH of recurrent respiratory and GI infections presented lymphocytic colitis
- Hypocellular bone marrow with diffuse, interstitial pattern and multiple non-paratrabecular lymphoid aggregates
- Erythroid precursors were absent
- Flow identified an abnormal expanded gamma delta T-cell population expressing CD3, CD8, CD57, CD2, CD7, TCR γδ, and heterogeneous CD5
- Tcell clonality was positive
- Mutation in the gene ***CTLA4*** **(151C>T; R51X)**, confirmed by Sanger sequencing
  - Also present in daughter

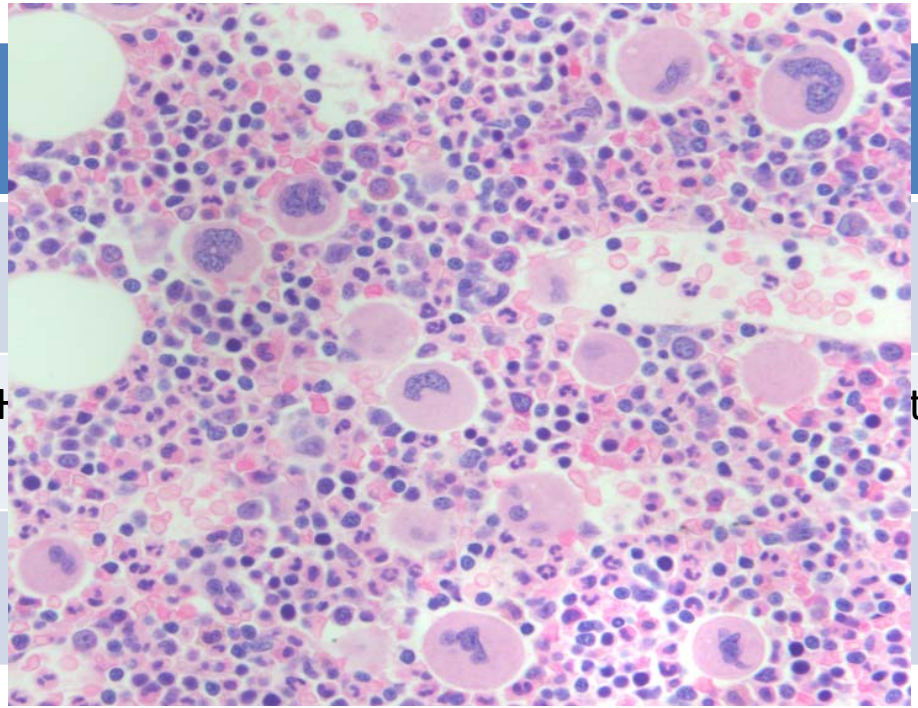




CD8 stain

Incidental findings

Case	Submitter	Age
333	Coberly	56
97	Raciti	6
209	Velu	38



Summary from Session 1

- 51 cases submitted in this category
- Most common cases submitted included germline mutations in *GATA2* (n=16) and *RUNX1* (n=10) genes
- Mostly myeloid neoplasms (MDS/AML) (n=42) but lymphoid neoplasms (including B-ALL, FL, T-cell LGL) were also submitted
- No specific clinical features
  - germline mutations are associated with non-neoplastic hematological disorders, organ dysfunction, or inherited syndromic disorders

Myeloid neoplasms with germline predisposition

- Morphology of the neoplasm depends on its subtype
- Presence of a genetic predisposition does not in itself place a case into the category of a myeloid neoplasm
- Diagnostic criteria for the germline predisposition disorders are the *same* as those for sporadic cases
  - diagnosis of MDS may be challenging in some cases
    - Early dysplastic features may not progress to MDS or AML for decades
    - Increased blasts, increasing marrow cellularity, increasing cytopenias, and/or the presence of additional cytogenetic or molecular genetic abnormalities

Rise of NGS testing in clinical setting

- **Somatic or germline mutations?**
- Standard sequencing cannot distinguish, but could give clues…
  - Near heterozygous (40-60%) or near homozygous (≥90%) allelic frequency
  - ‘Threshold’ allelic frequency to warrant germline testing is not standarized
  - Multiple mutations in *CEBPA, RUNX1* or *GATA2* genes
- Counseling and germline testing are next steps

Germline testing

- Cultured skin fibroblasts are preferred tissue
  - Can take 3-6 weeks for cultures to yield sufficient DNA
  - DNA from epithelial cells of hair follicles is more readily available
- Buccal swabs or saliva are frequently contaminated with hematopoietic cells and should be avoided
- Other sources include nail clippings or mesenchymal cells from bone marrow aspirate smears

Scenarios when genetic testing is advised in newly diagnosed patients with MDS/AML

- Somatic testing identified a mutation associated with germline predisposition syndrome (*CEBPA, GATA2, RUNX1*)
- Hematologic or cytogenetic characteristic of MDS/AML suggestive of germline predisposition
- Genetic syndrome known to predispose to cancer
- Previous malignancy, family history cancer
- Cytopenias, immune deficiency, atypical infections, lymphadema or organ-system manifestation

Thank you!