

HYPOCELLULAR BONE MARROW...

WHAT'S NEXT?

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Let's start with a case

- 26-year-old male presents with septic shock

CBC

WBC 0.5 K/uL

RBC 2.27 M/uL

HGB 7.0 g/dL

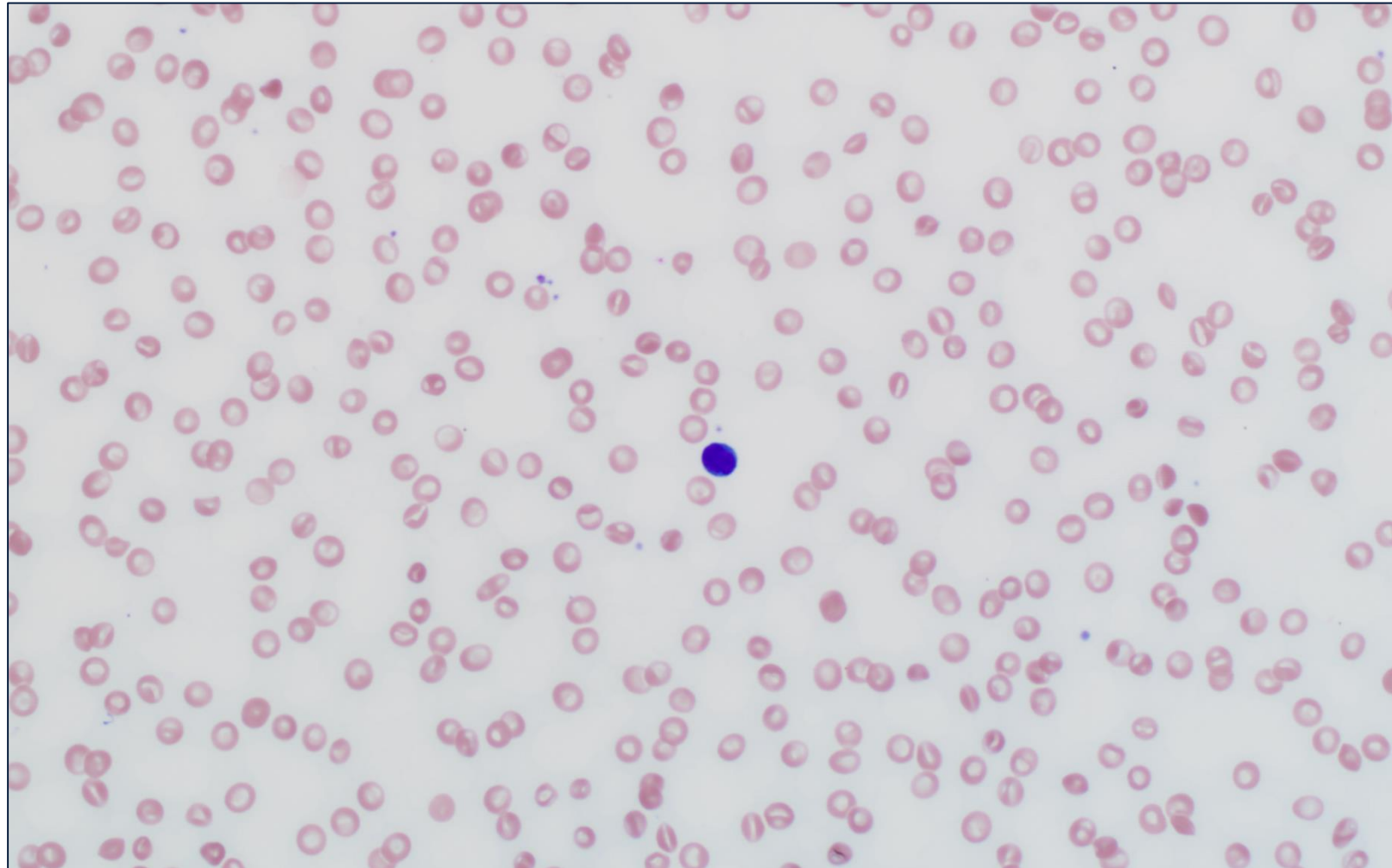
PLAT 21 K/uL

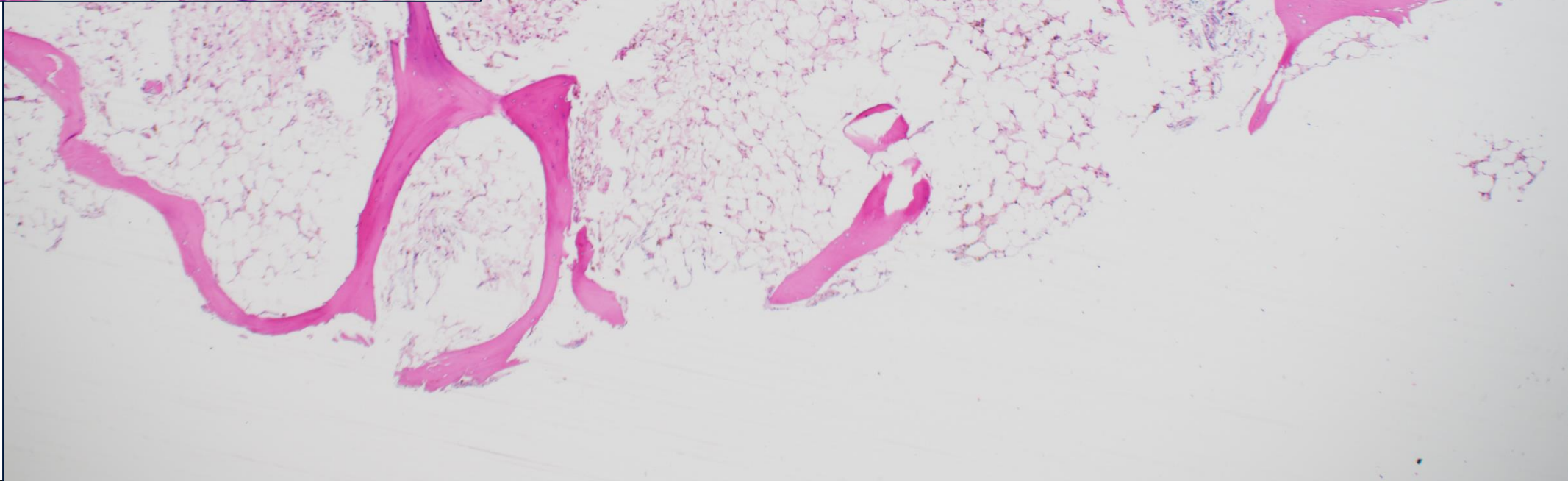
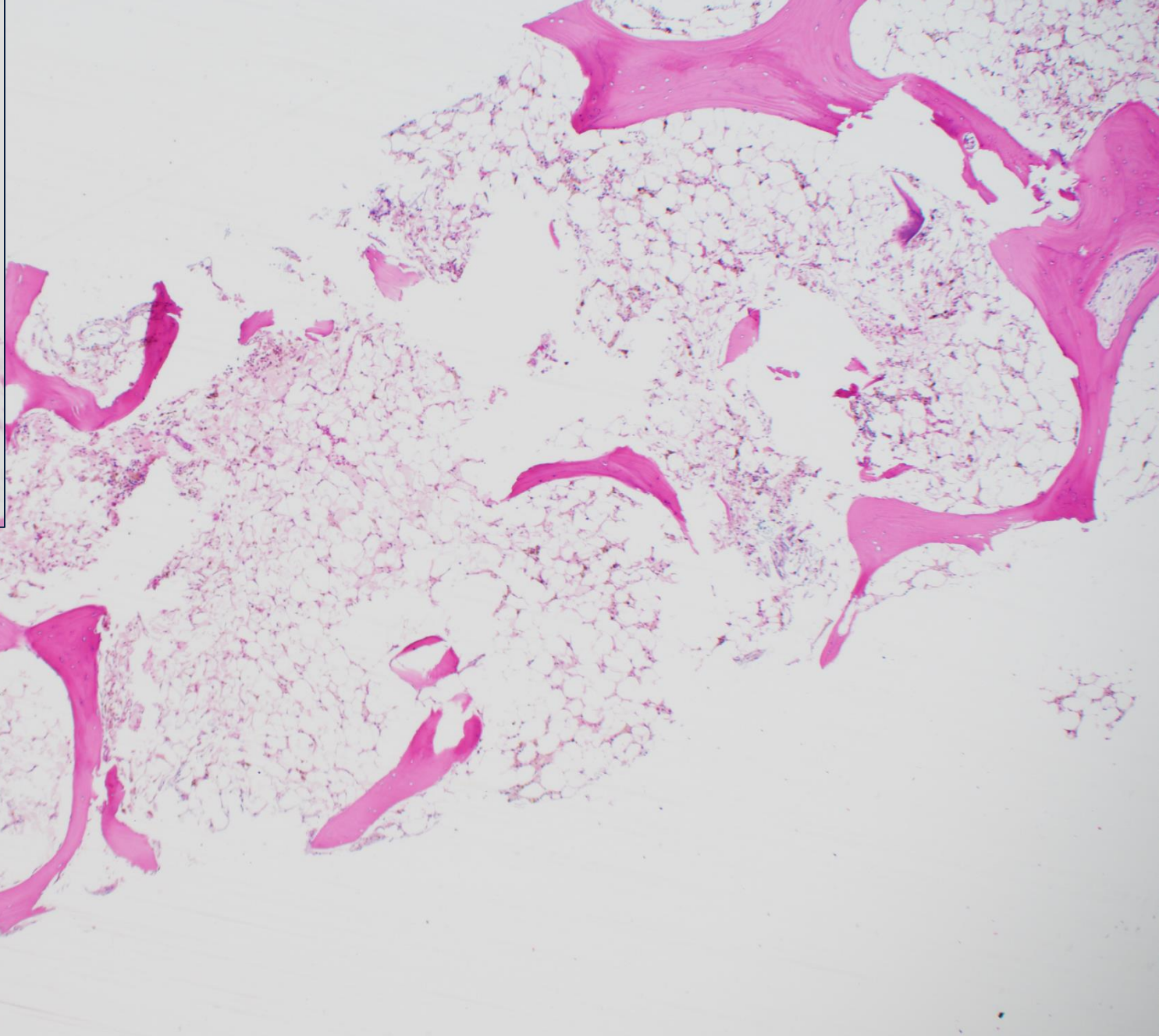
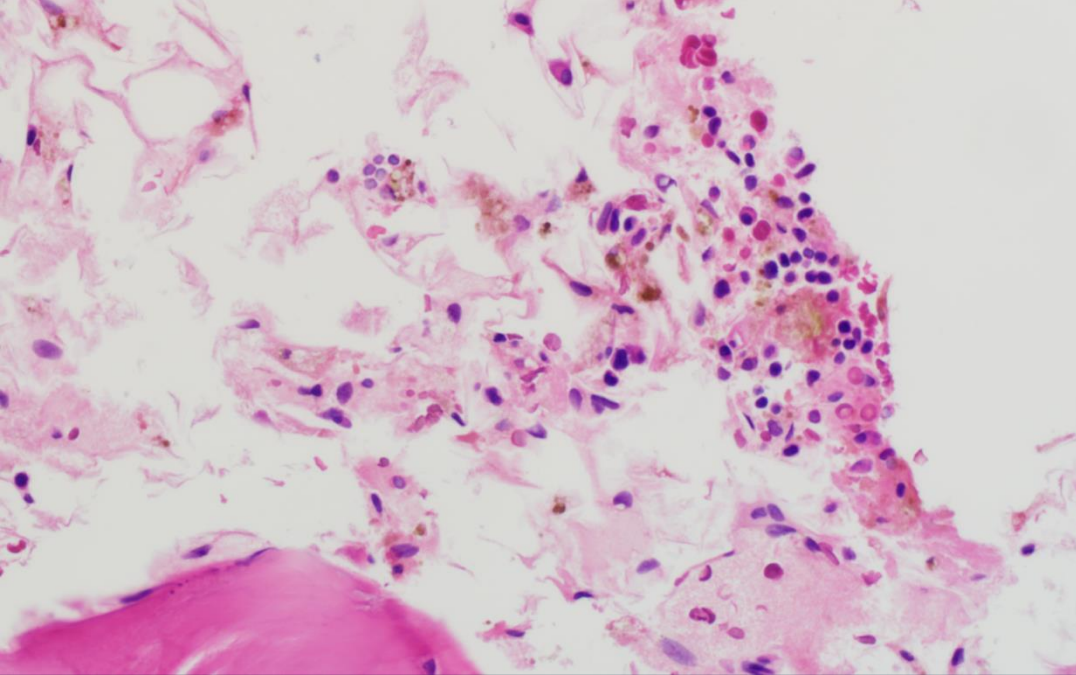
WBC differential

Neutrophils 2%

Lymphocytes 97%

Monocytes 1%





Ancillary studies

- Flow cytometry: unremarkable
- Karyotype: no metaphase cells

DIAGNOSIS:

Markedly hypocellular marrow with no morphologic or flow cytometric evidence of malignancy

Is there anything else to be done?

Definitions

- Bone marrow failure (BMF) – sustained inability of the bone marrow to produce adequate numbers of blood forming elements
 - Unilineage aplasia (red cell aplasia, neutropenia, thrombocytopenia)
 - Trilineage aplasia = aplastic anemia
- Aplastic anemia (AA) – multiple cytopenias with TRILINEAGE bone marrow failure in absence of secondary bone marrow replacement process (neoplasia, reticulin fibrosis, etc.)
 - Constitutional (constitutional BMF)
 - Acquired AA
 - Secondary
 - Idiopathic – separate entity

APLASTIC ANEMIA: DIAGNOSTIC CRITERIA

All three criteria must be met

I. At least two of the following:

1. Neutropenia – ANC <0.5 K/uL
2. Thrombocytopenia – PLAT <20 K/uL
3. Corrected reticulocyte count <20 K/uL

$$\text{Corrected reticulocyte count} = \text{RETIC (\%)} \times \text{HCT (\%)} / 45$$

II. Bone marrow cellularity:

1. Marked hypoplasia - <25% of normal age-appropriate cellularity, OR
2. Moderate hypoplasia (25-50% of normal age-appropriate cellularity with <30% cells being hematopoietic)

III. ABSENCE of overt malignancy, fibrosis, or abnormal infiltrates replacing marrow

Stratification by severity

Very severe	ANC of 0-0.2 K/uL
Severe	ANC of 0.21-0.5 K/uL
Non-severe	ANC of >0.5 K/uL

Strong predictor of survival

Bone Marrow Cellularity

THE AMERICAN JOURNAL OF CLINICAL PATHOLOGY
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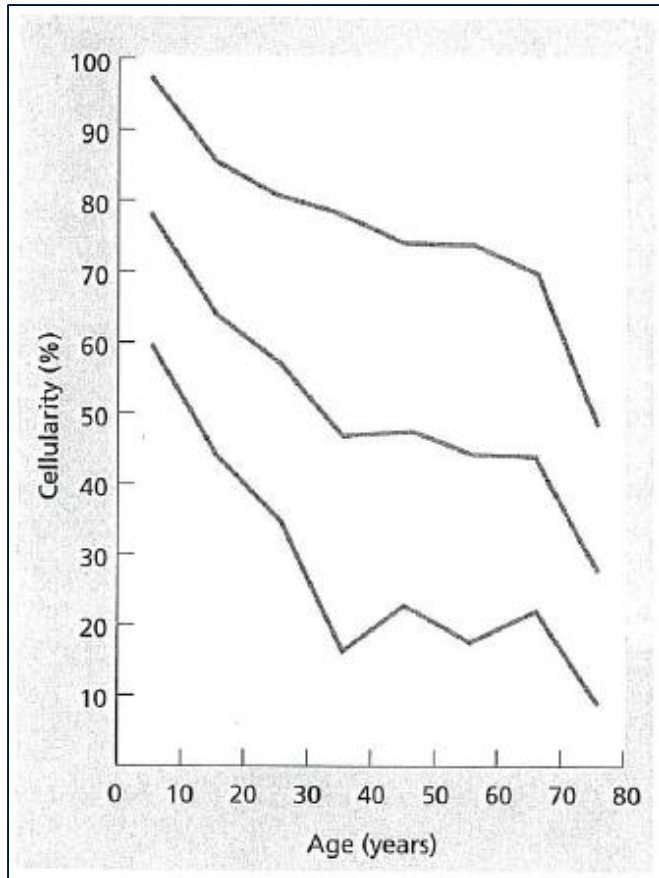
Vol. 43, No. 4
Printed in U.S.A.

NORMAL VARIATIONS WITH AGING OF THE AMOUNT OF HEMATOPOIETIC TISSUE IN BONE MARROW FROM THE ANTERIOR ILIAC CREST

A STUDY MADE FROM 177 CASES OF SUDDEN DEATH EXAMINED BY
NECROPSY

ROBERT J. HARTSOCK, M.D., EDWARD B. SMITH, M.D., AND
CHARLES S. PETTY, M.D.

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D. C. 20305

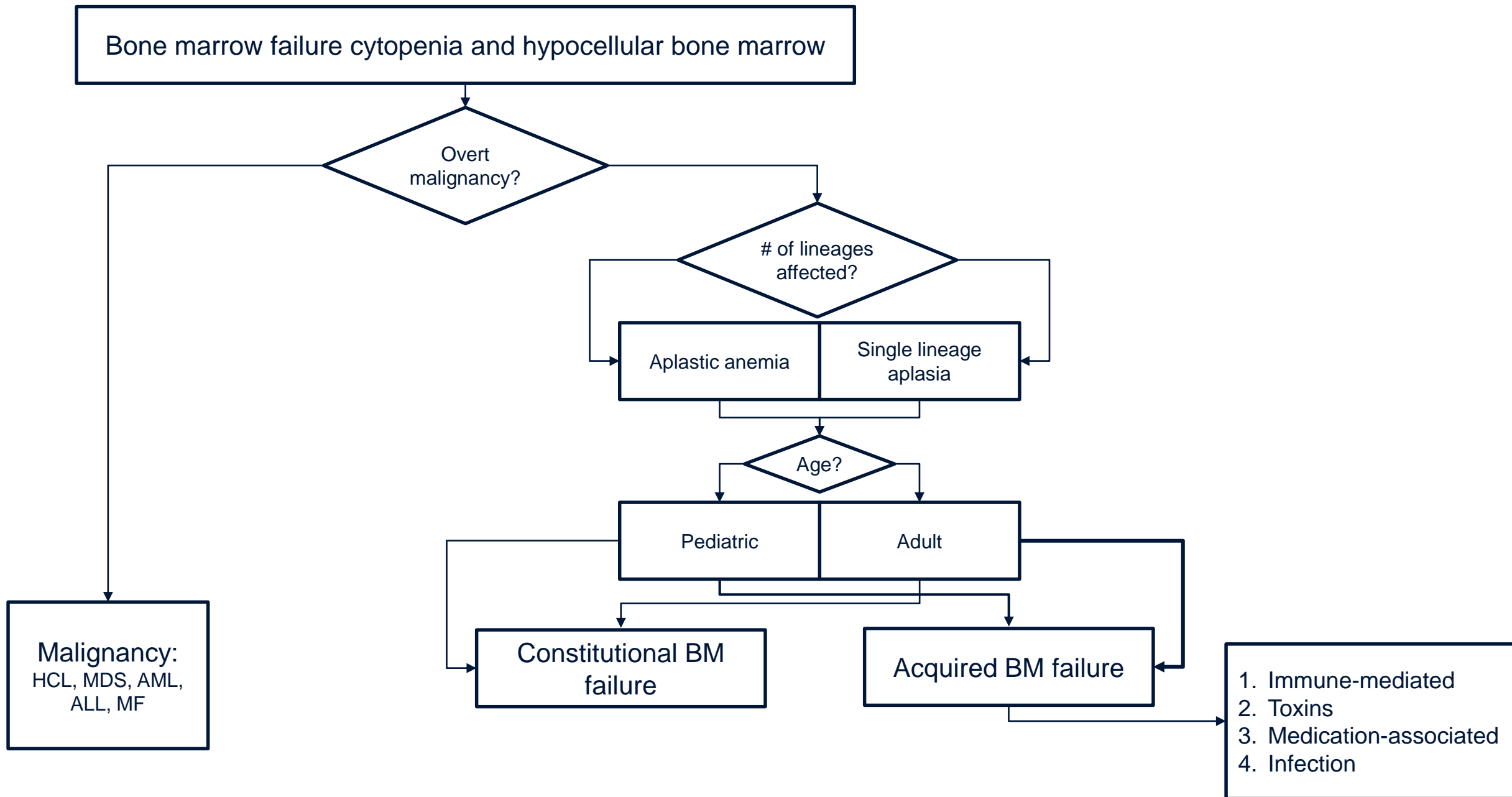


B. Bain Bone Marrow Pathology

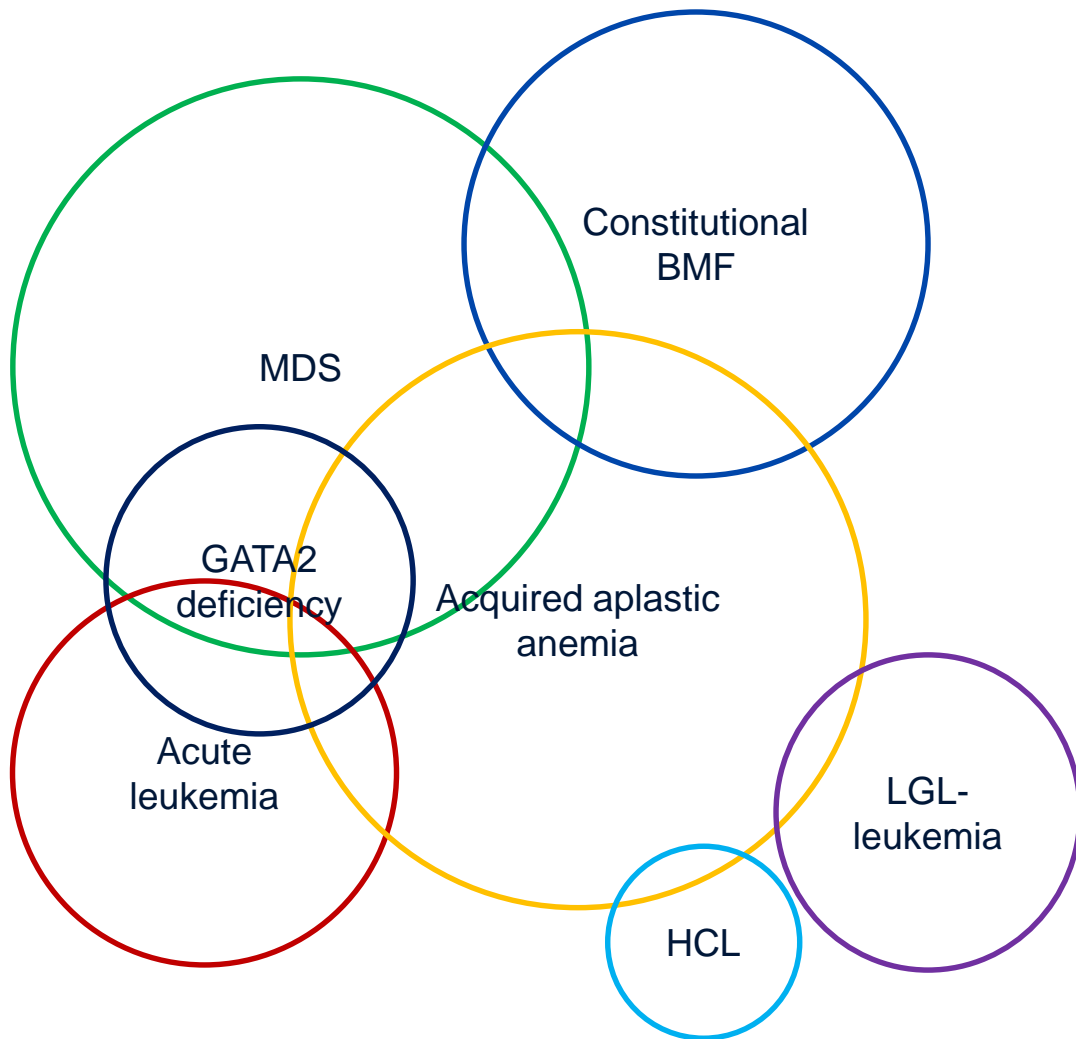
- “... except in extreme old age, cellularity of less than 20% is likely to be abnormal, as is cellularity of more than 80% in those above 20 years of age” (B. Bain)

Age	Cellularity
Newborn	80-100%
1-3 months	80-100%
Child	60-80%
Adult (20-70 years)	40-70%
Elderly (>70 years)	≤25%

Adapted from K. Foucar, Bone Marrow Pathology, 4th Ed



Aplastic anemia: Differential diagnosis



Practical approach

I. Hematologic malignancy

- I. Lymphoid: T-LGL leukemia, HCL
- II. Myeloid: MDS and AML, myeloid neoplasm with germline mutations

II. Constitutional bone marrow failure

- I. Fanconi anemia
- II. Dyskeratosis congenita

III. Acquired aplastic anemia

- I. Secondary AAA
- II. Idiopathic AAA

Case

- 66-year-old male with history of well-controlled rheumatoid arthritis
- Currently on Abatacept
- Evaluated for persistent neutropenia

CBC

WBC 5.76 K/uL

RBC 2.49 M/uL

HGB 9.4 g/dL

PLAT 164 K/uL

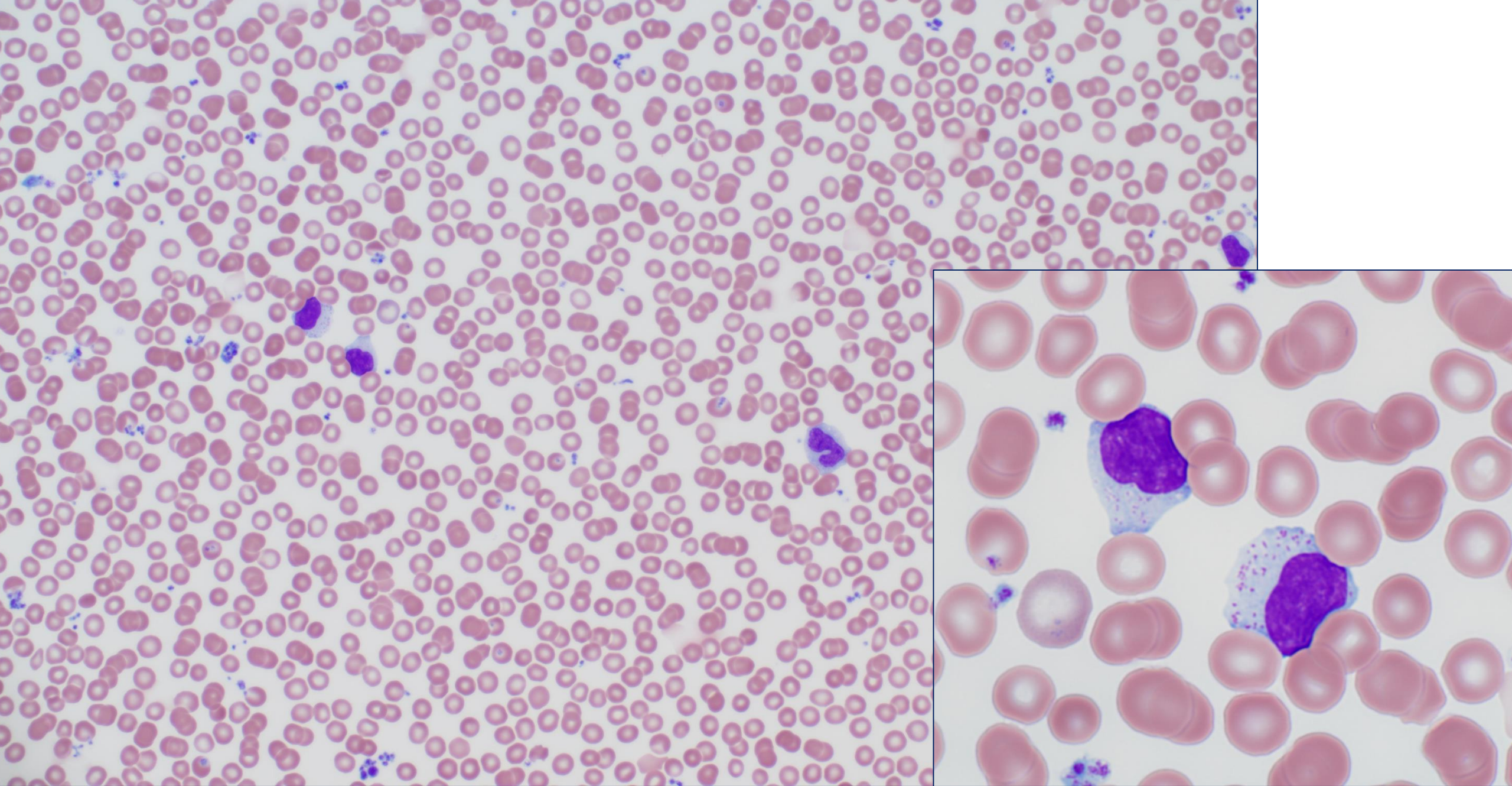
WBC differential

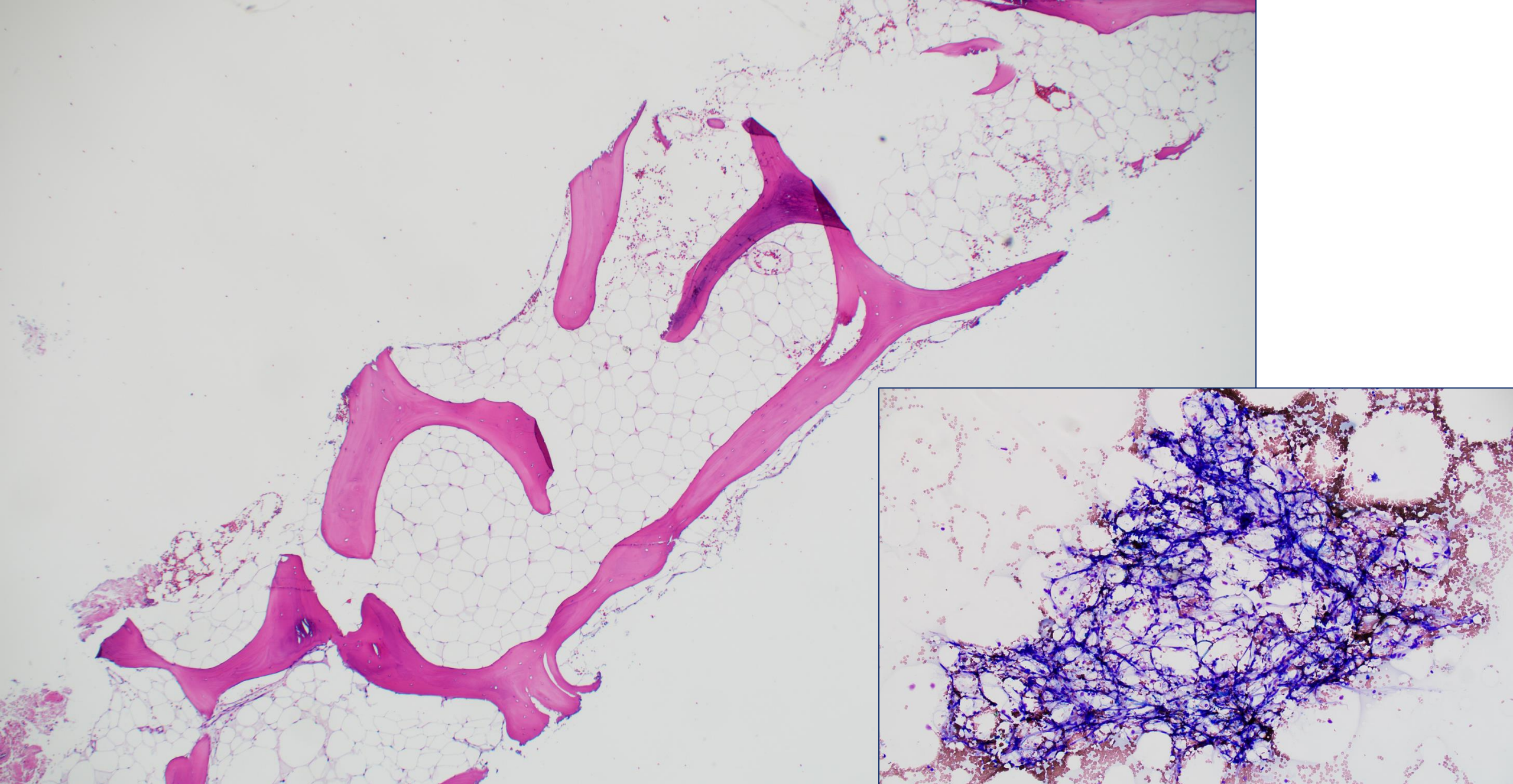
Neutrophils 2% (<0.2 K/uL)

Lymphocytes 89% (5.1 K/uL)

Monocytes 8%

Eosinophils 1%





Ancillary studies

- Flow cytometry
 - Large population of LGL-like T-cells: CD2 weaker than normal, CD3+, CD4-/CD8+, CD5 weaker than normal
- T-cell clonality by PCR: monoclonal pattern
- Karyotype: 46,XY [5]

Diagnostic possibilities

- Issues to be addressed:
 1. Bone marrow failure
 2. Large population of monoclonal immunophenotypically atypical T-LGLs

Differential diagnosis

1. T-LGL leukemia
2. Reactive/autoimmune expansion of T-LGLs
3. Medication-associated or immune-mediated neutropenia

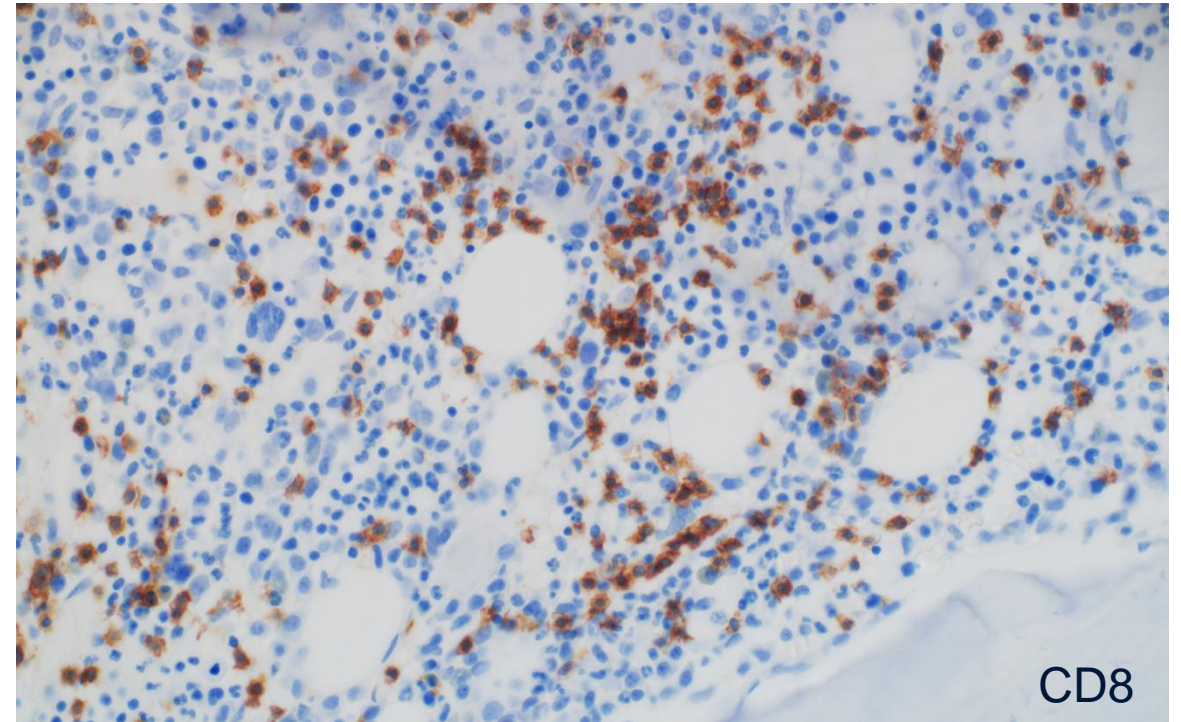
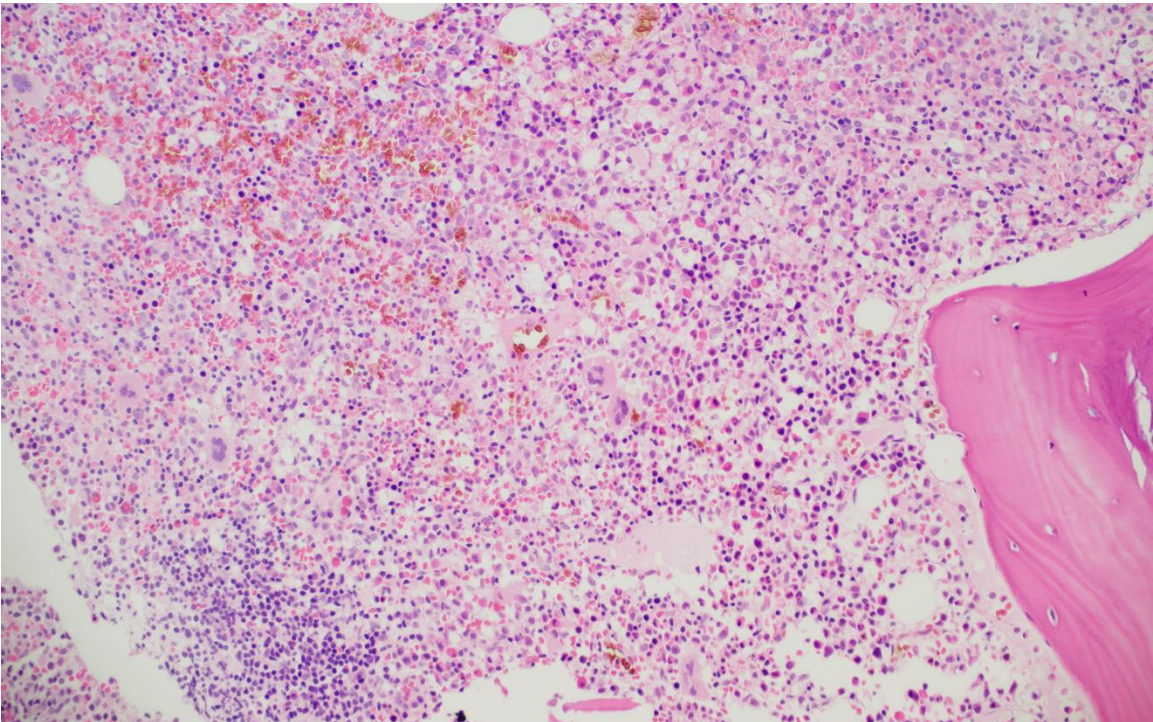
FINAL DIAGNOSIS: T-LGL LEUKEMIA

T-LGL leukemia

- Disease of adults: most cases occur in individuals of 45-75 years old
- Persistent (>6 months) unexplained increase in LGLs, usually >2 K/uL
- Presentation
 - severe neutropenia is common
 - thrombocytopenia is rare
 - common association with rheumatoid arthritis, hypergammaglobulinemia, autoantibodies
- Indolent non-progressive disorder

T-LGL leukemia: findings

- Bone marrow
 - slightly hypercellular (50%), but can also be normo- and hypocellular (50% cases)
 - interstitial/intrasinusoidal increase in LGLs which can be difficult to appreciate on H&E
 - non-neoplastic B-cell-rich lymphoid aggregates are common



T-LGL leukemia: findings

- Flow cytometry
 - In most cases, the immunophenotype is of mature alpha/beta-positive cytotoxic T-cells: CD2+, CD3+, CD4-/CD8+, CD57+, frequently CD16+ and CD56+
 - Common downregulation/loss of CD5 and/or CD7
- TCR clonality studies positive

Caveat: the presence of oligoclonal/monoclonal T-cell population(s) should always be interpreted in a clinical and morphologic context. Positive clonality does not mean lymphoma.

Case

- 56-year-old female with recent history of weakness, fatigue, and weight loss
- PMH: diabetes, hypertension, thyroid disease

CBC

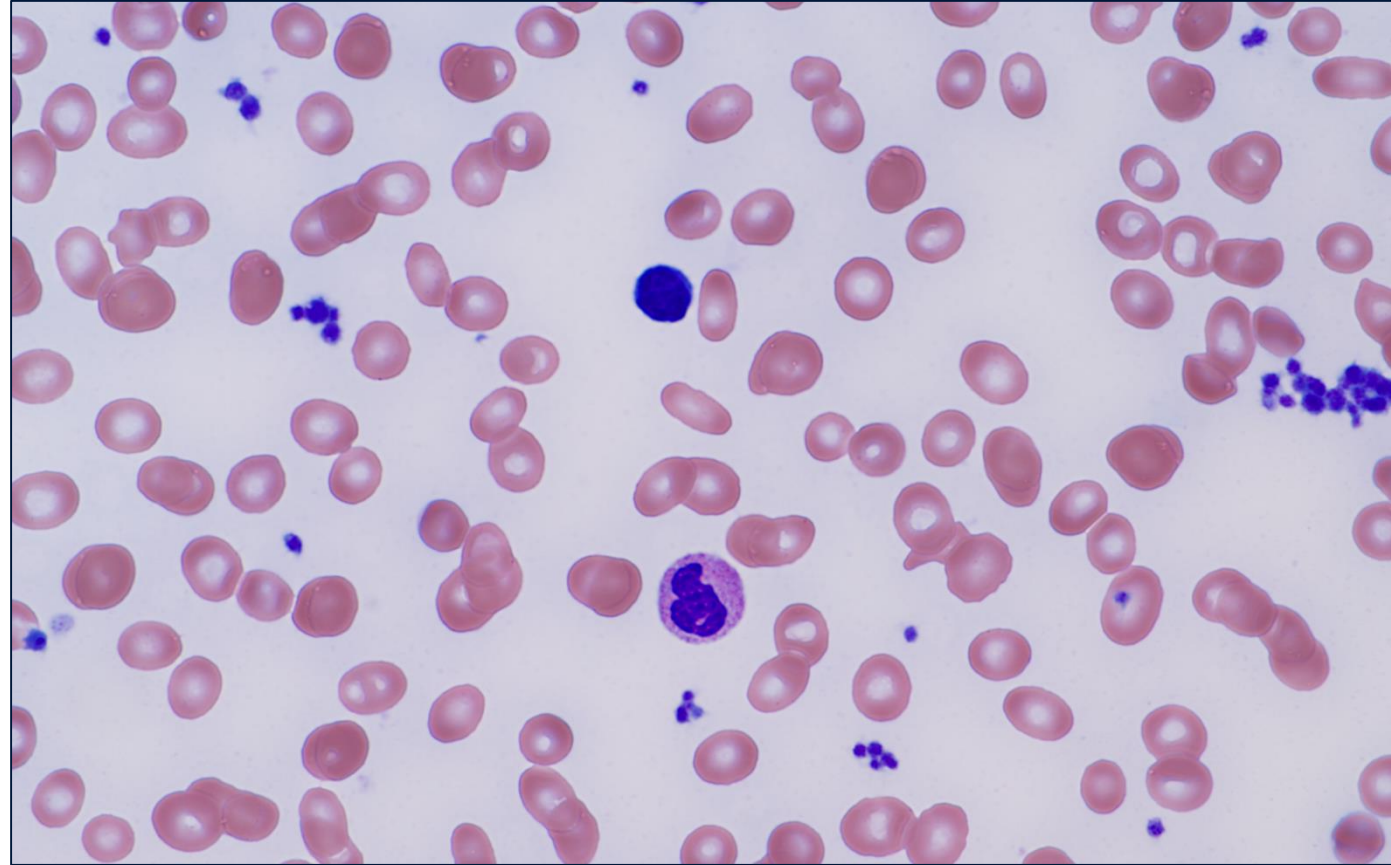
WBC 3.61 K/uL

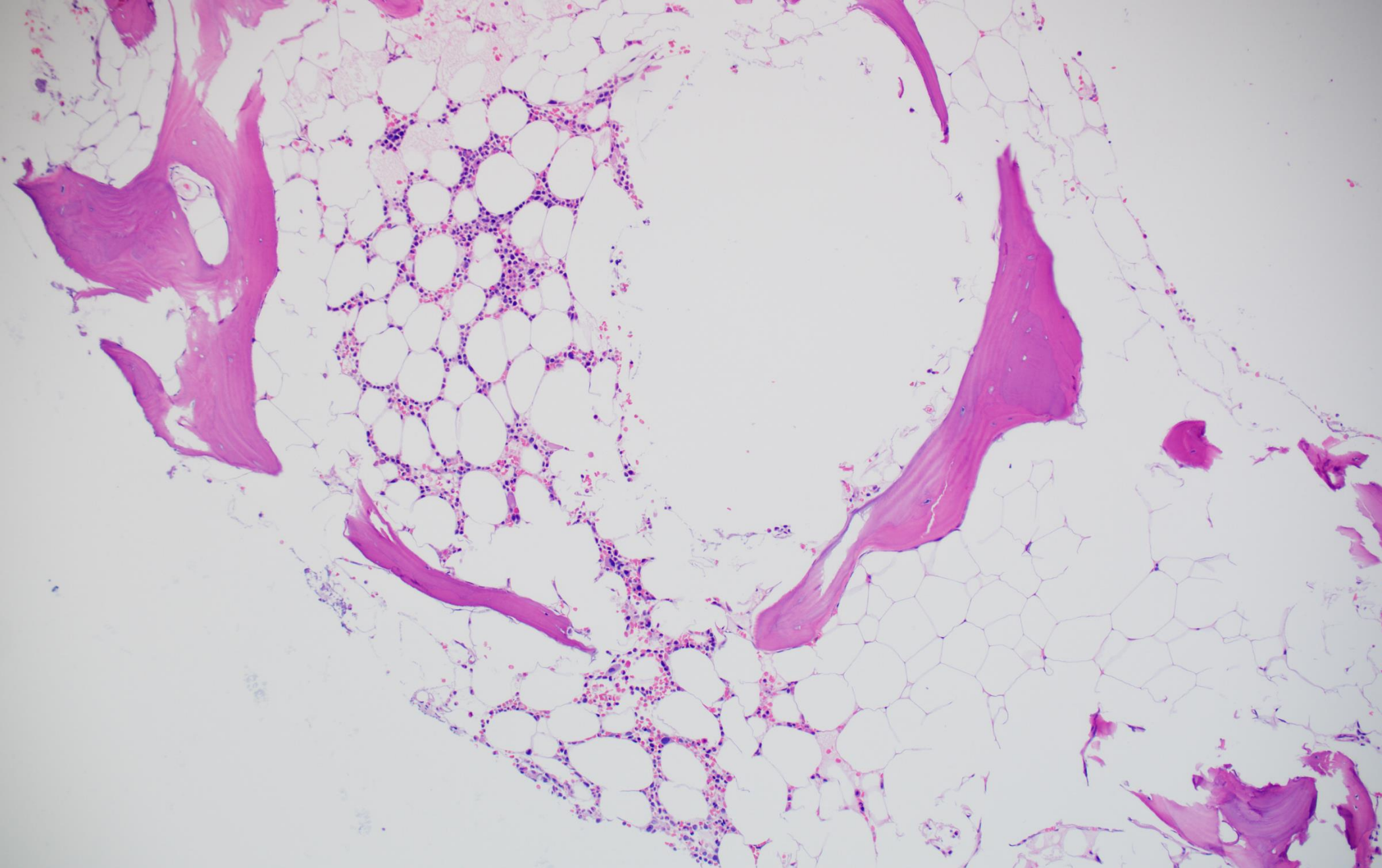
RBC 2.73 M/uL

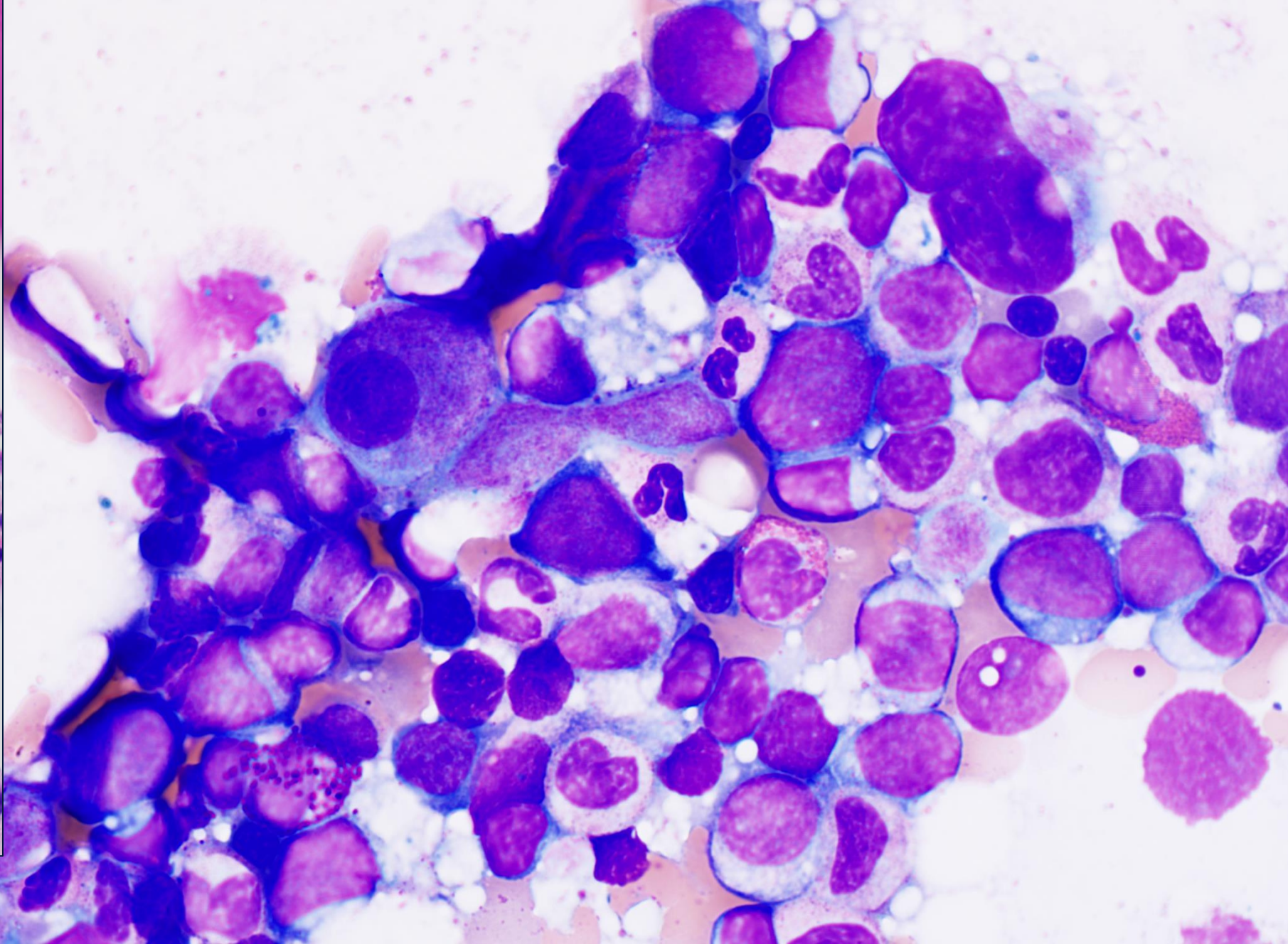
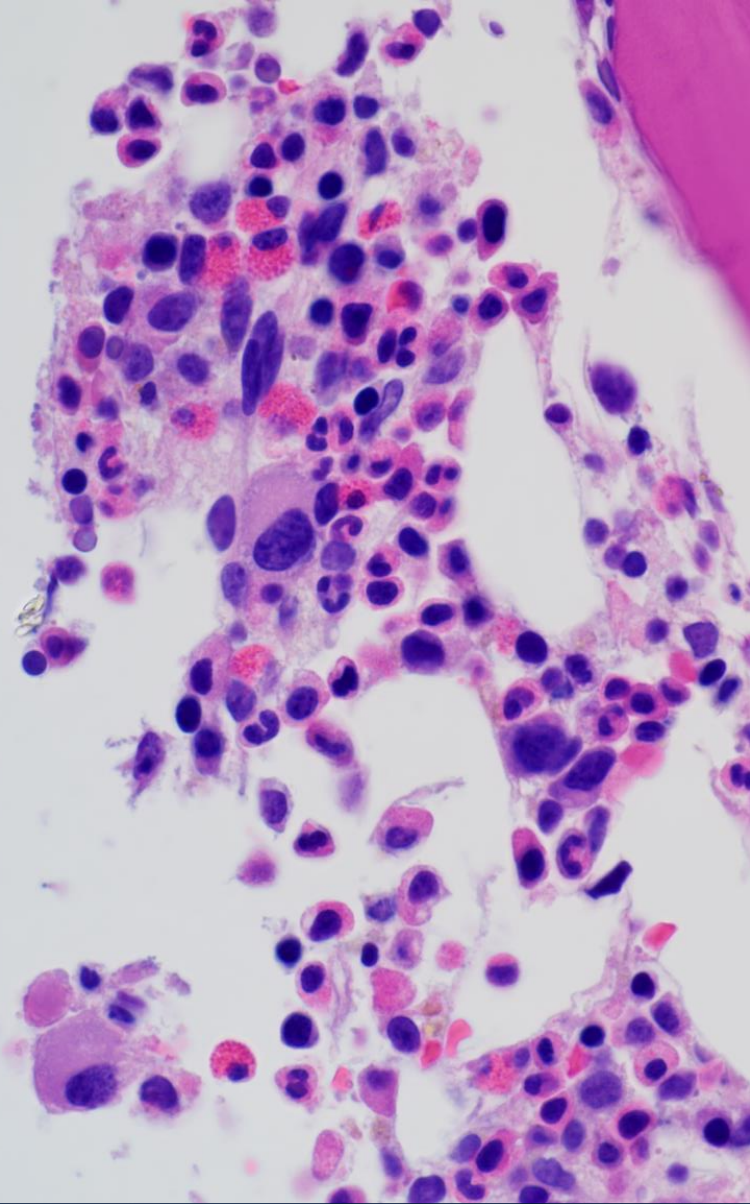
HGB 8.5 g/dL

MCV 112.5 fL

PLAT 261 K/uL







Ancillary studies

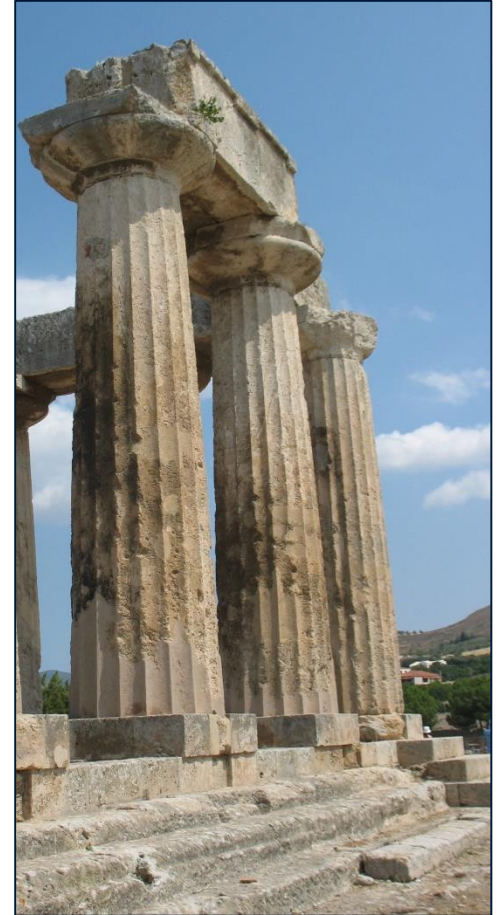
- Flow cytometry: unremarkable
- MDS FISH: 5q31 (EGR1) deletion detected
- Karyotype: 46,XX,del(5)(q13q33) [20]
- NGS:
 - *TET2* p.Ser214fs, VAF of 31.9%
 - *IDH2* p.Arg140Leu, VAF of 2.2%
 - *SF3B1* p.Arg625Cys, VAF of 2.1%
 - No *TP53* mutations

FINAL DIAGNOSIS: **MDS with isolated del(5q)**

Hypoplastic myelodysplastic syndrome (h-MDS)

- Subset of MDS, characterized by BM hypocellularity
 - <30% in adults younger than 60 years
 - <20% in those older than 70 years
- Comprise 10-15% of all MDS
- Does not represent a specific MDS subtype/entity
- Pathogenesis differs from non-h-MDS – significant immune/autoimmune dysregulation similar to i-AA
- Other diagnostic criteria are the same as for non-h-MDS

1. Cytopenia
2. Morphologic dysplasia
3. Genetic abnormalities

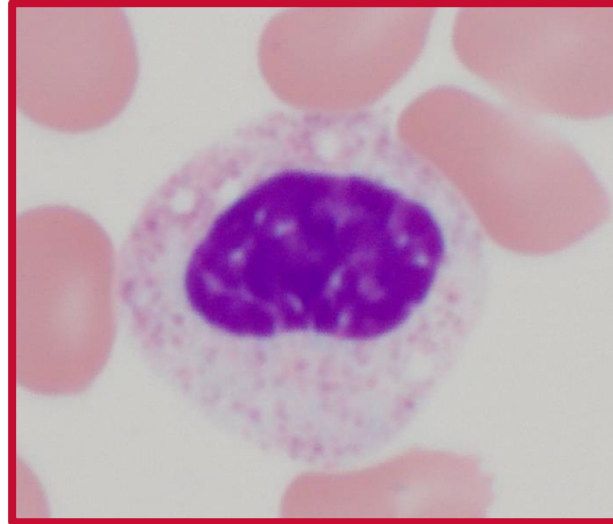


Dysgranulopoiesis

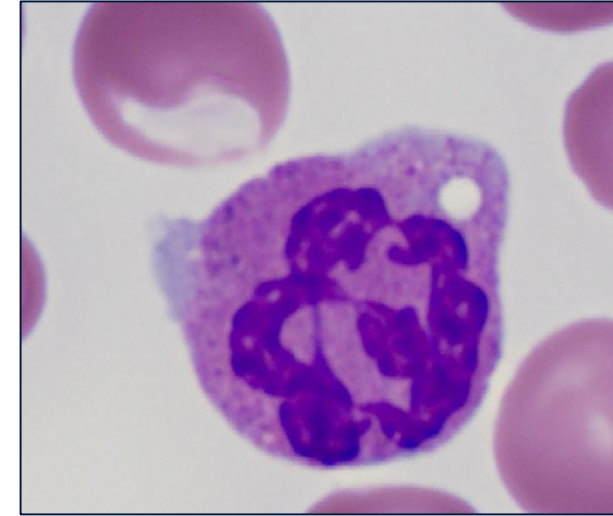
NORMAL NEUTROPHIL



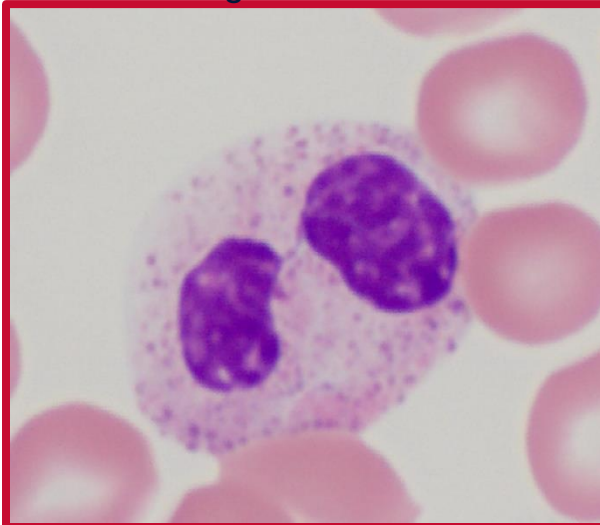
Stodtmeister cell



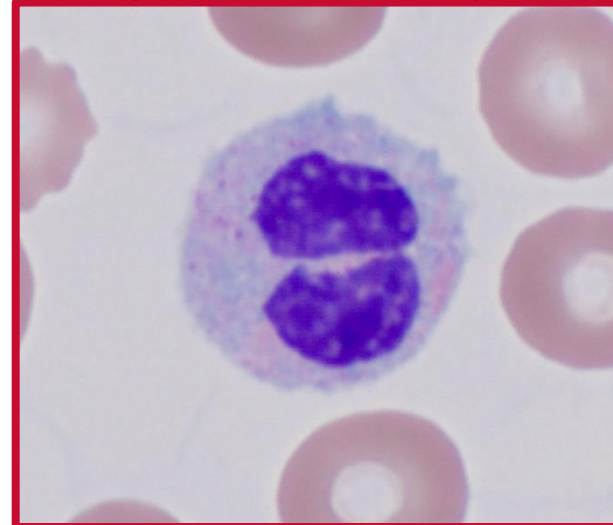
Hypersegmented nucleus



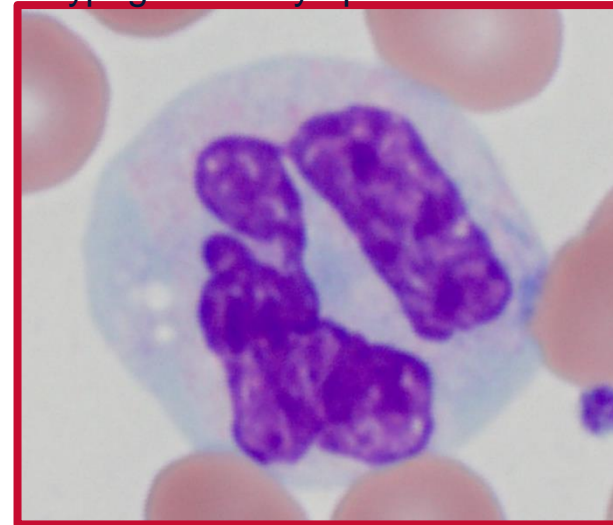
Pseudo-Pelger-Huet



Hypogranular Pseudo-Pelger-Huet

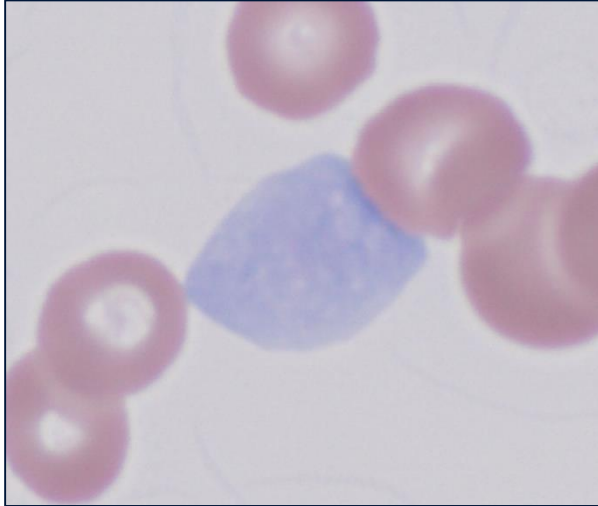


Hypogranular cytoplasm

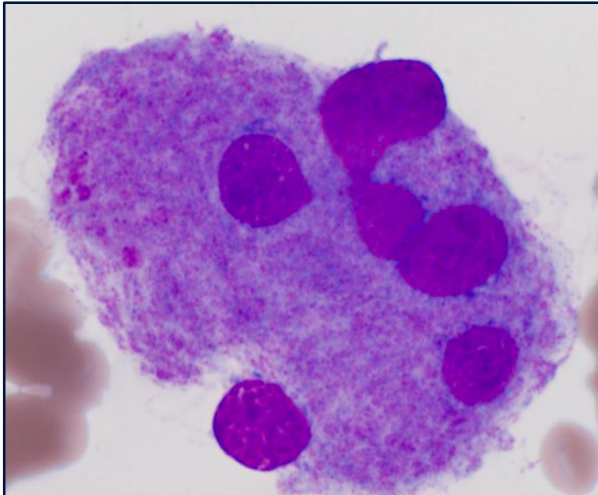


Dysmegakaryopoiesis

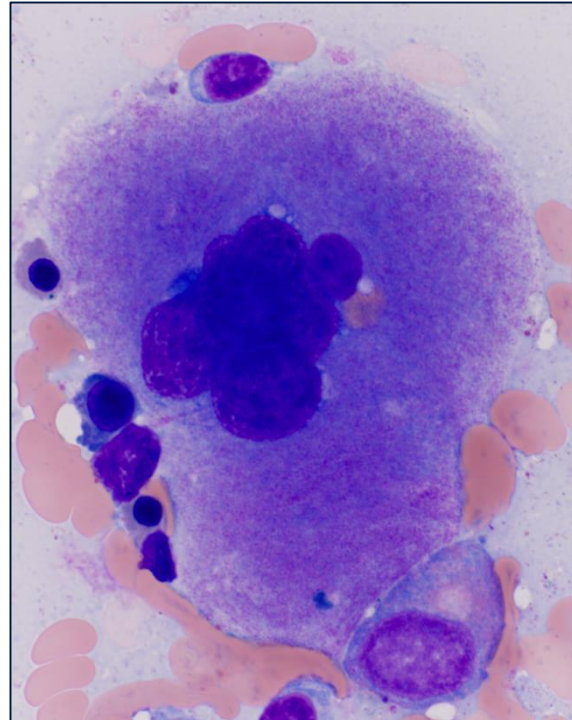
Giant hypogranular platelet



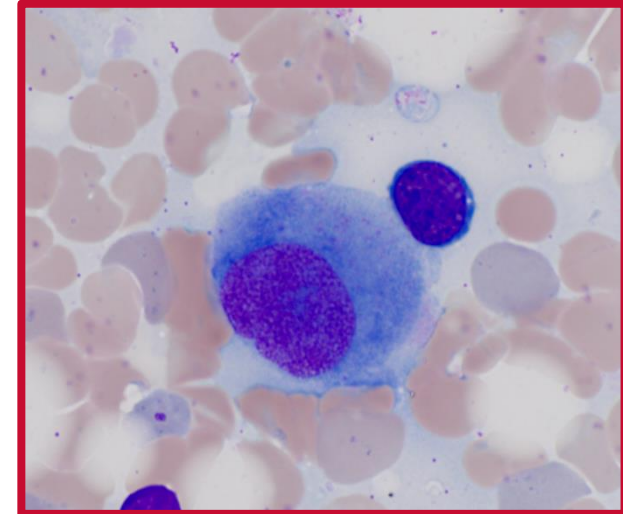
Separated nuclear lobes/
multinucleation



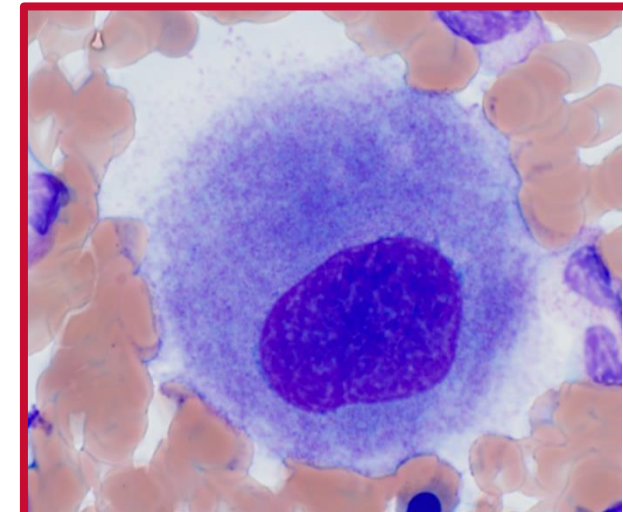
NORMAL MEGAKARYOCYTE



Micromegakaryocyte

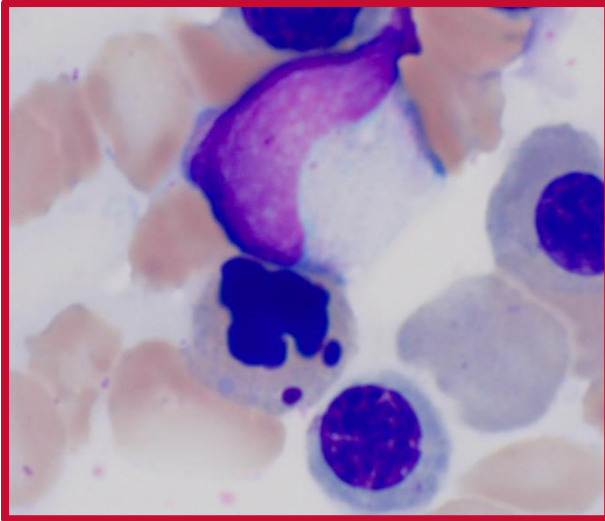


Hypolobated nucleus

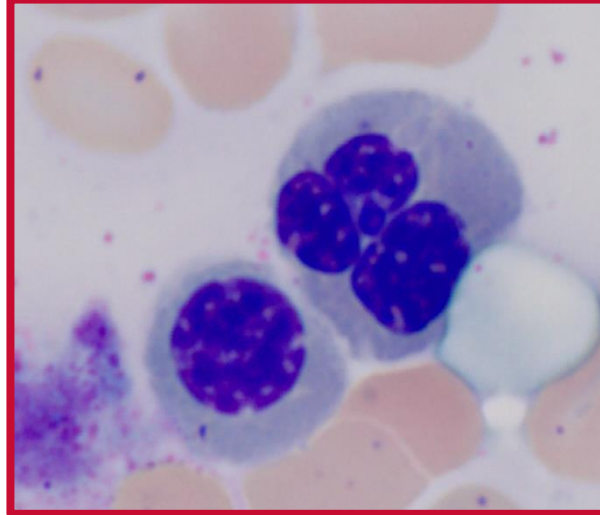


Dyserythropoiesis

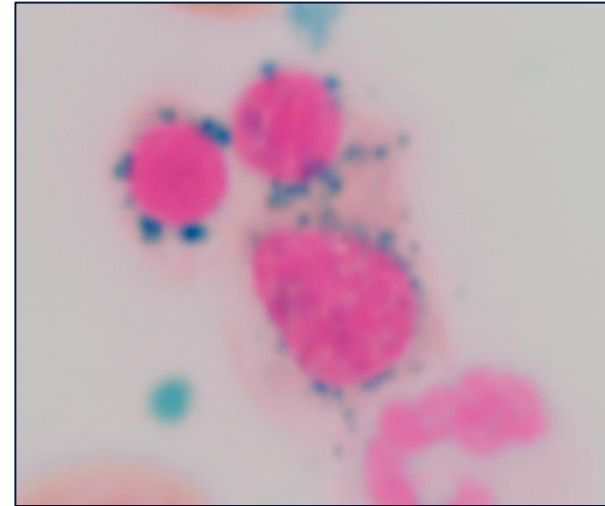
Karyorrhexis



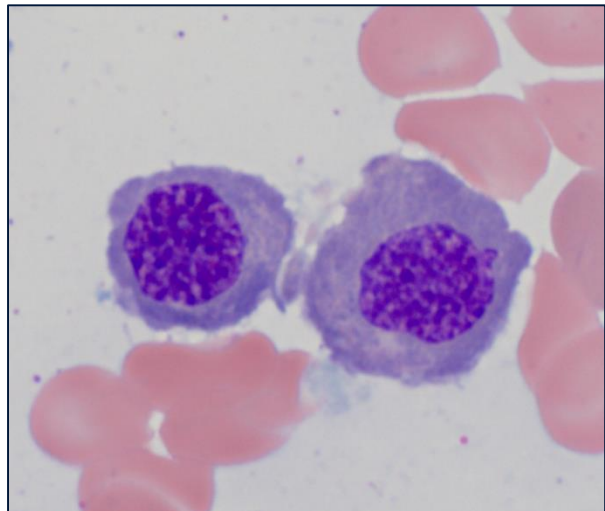
Multinucleation



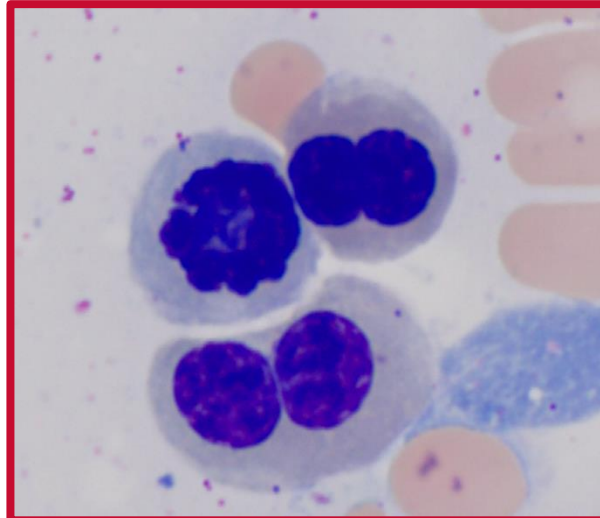
Ring sideroblasts



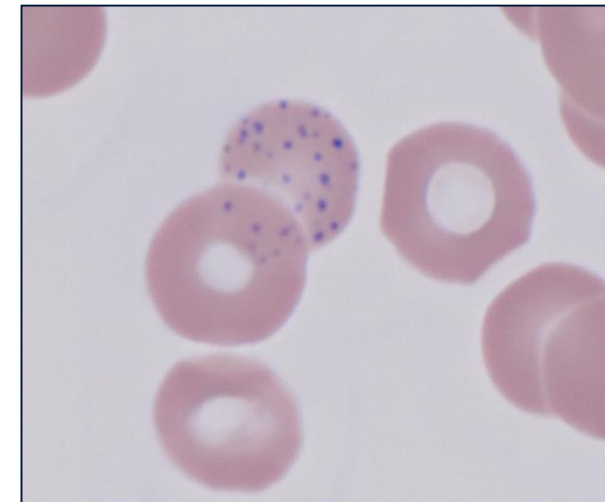
N/C dysynchrony



Binucleation

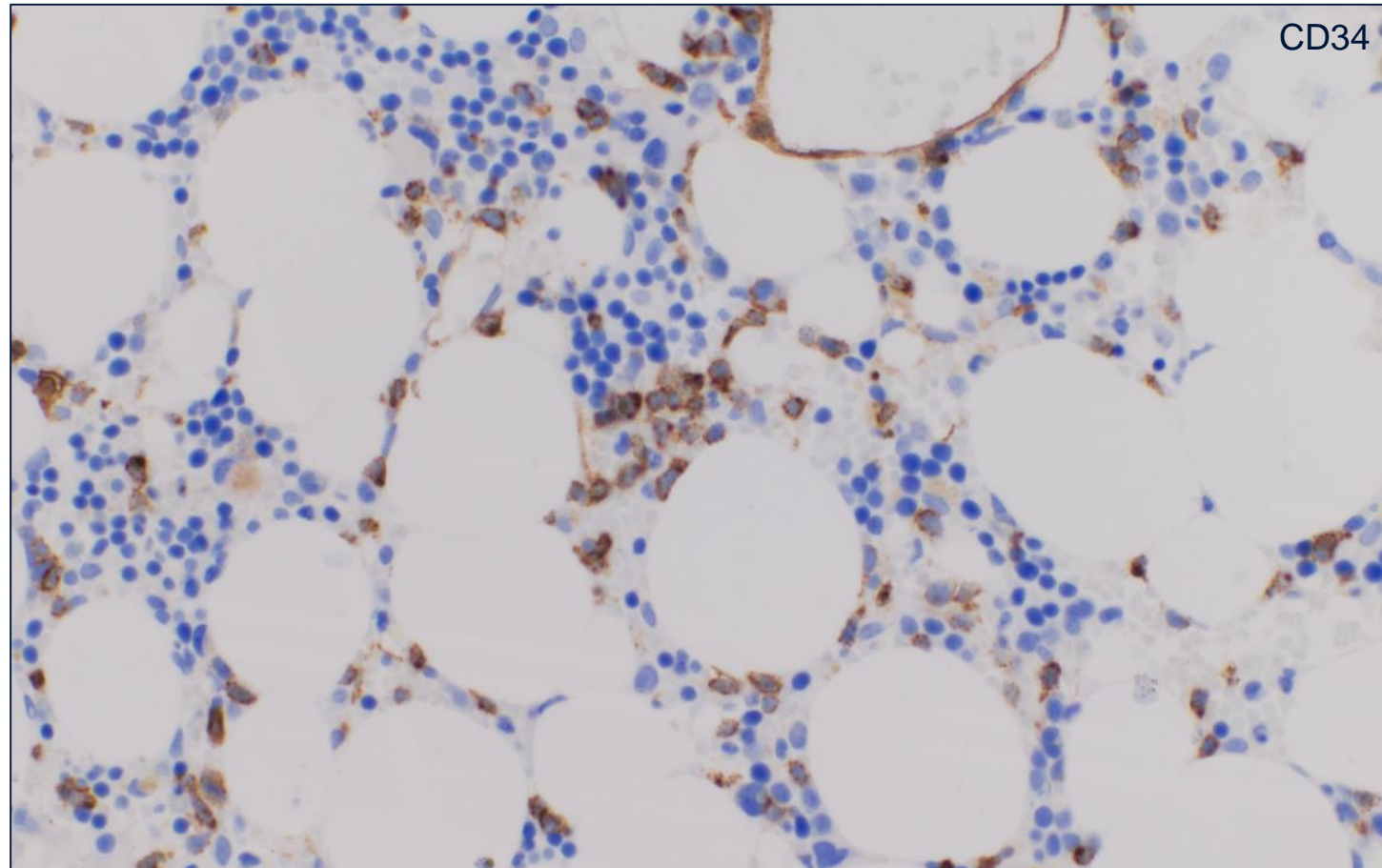


Basophilic stippling



h-MDS

- Atypical localization of immature precursors (ALIP)
- Aggregates (3-5 cells) or clusters (>5 cells) of immature cells



h-MDS Flow Cytometry

Although not necessary for diagnosis of MDS, but may be helpful

- Increased CD34+ blasts
- Abnormal maturation patterns
 - altered CD13 and/or CD16 expression
- Aberrant immunoprofile
 - CD56 and/or CD7 expression on granulocytes, monocytes, or blasts
- Decreased side scatter on granulocytes

h-MDS: Cytogenetics

Box 45-1 Recurrent Cytogenetic Abnormalities in Myelodysplastic Syndrome

Gain or loss of chromosomal material (relatively common)

- 7 or del(7q)
- 5 or del(5q)
- +8*
- +21, -21
- 17, i(17q), or unbalanced translocations at 17p
- 20 or del(20q)*
- del(11q)
- Y*
- del(9q)
- +6
- del(12p) or unbalanced translocations at 12p
- 13 or del(13q)

Other translocations and inversions (relatively uncommon)

- t(3;3)(q21;q26), inv3(q21q26), t(3;21)(q26;q22), and other 3q21 and 3q26 translocations
- t(1;7)(p11;p11)
- t(2;11)(p21;q23)
- t(11;16)(q23;p13)
- t(6;9)(p23;q34)
- t(2;11)(p21;q23)

R Hasserjian and D Head, Hematopathology, 2016

- In a setting of PERSISTENT CYTOPENIA of undetermined origin, the presence of these cytogenetic abnormalities are considered **presumptive evidence of MDS**
- **Caveat:** del(20q), trisomy 8, and -Y are **NOT** MDS-defining and cannot be used in isolation to make a diagnosis of MDS

MDS: Molecular Picture

- RNA splicing machinery
 - SF3B1, SRSF2, ZRSR2, and U2AF1 genes
 - **Most common mutations in MDS**
- Epigenetic machinery
 - TET2, DNMT3A, IDH1/2, EZH2, ASXL1
 - **Second most common mutations in MDS**
- DNA damage response
 - TP53
- Transcriptional regulation
 - RUNX1, BCOR, ETV6
- Signal transduction
 - CBL, NRAS, JAK2



Pediatric MDS

- A very uncommon condition
- Likely to present with neutropenia and thrombocytopenia, and not anemia
- Bone marrow hypocellularity is more commonly observed
- MDS-EB in children usually has relatively **stable slowly-progressing** course

Refractory Cytopenia of Childhood (RCC)

- Provisional entity
- Clonal stem cell defect
- Most common type of MDS in children (50%-90% of all MDS in children)
- Diagnostic criteria:
 1. Persistent cytopenia: neutropenia and thrombocytopenia are most common
 2. No excess blasts: $<5\%$ on the BM AND $<2\%$ in PB
 3. Dysplasia: ≥ 2 lineages AND $\geq 10\%$ cells in each affected lineage
- 80% cases demonstrate hypocellular marrow

Refractory Cytopenia of Childhood (RCC): Bone Marrow findings

- Hypocellularity is very common

Minimal histological criteria

1. Erythropoiesis

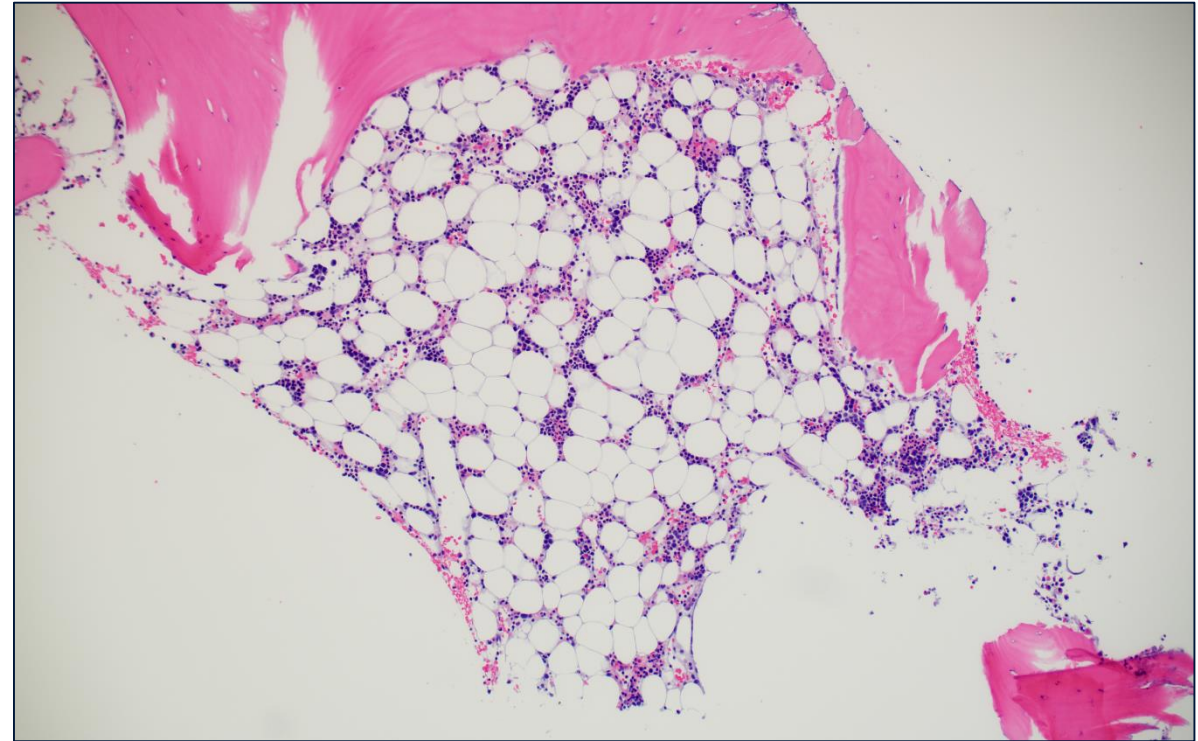
- a few clusters of >20 erythroid precursors
- arrest of maturation with increased number of pronormoblasts
- increased number of mitoses

2. Granulopoiesis

- no minimal diagnostic criteria

3. Megakaryopoiesis

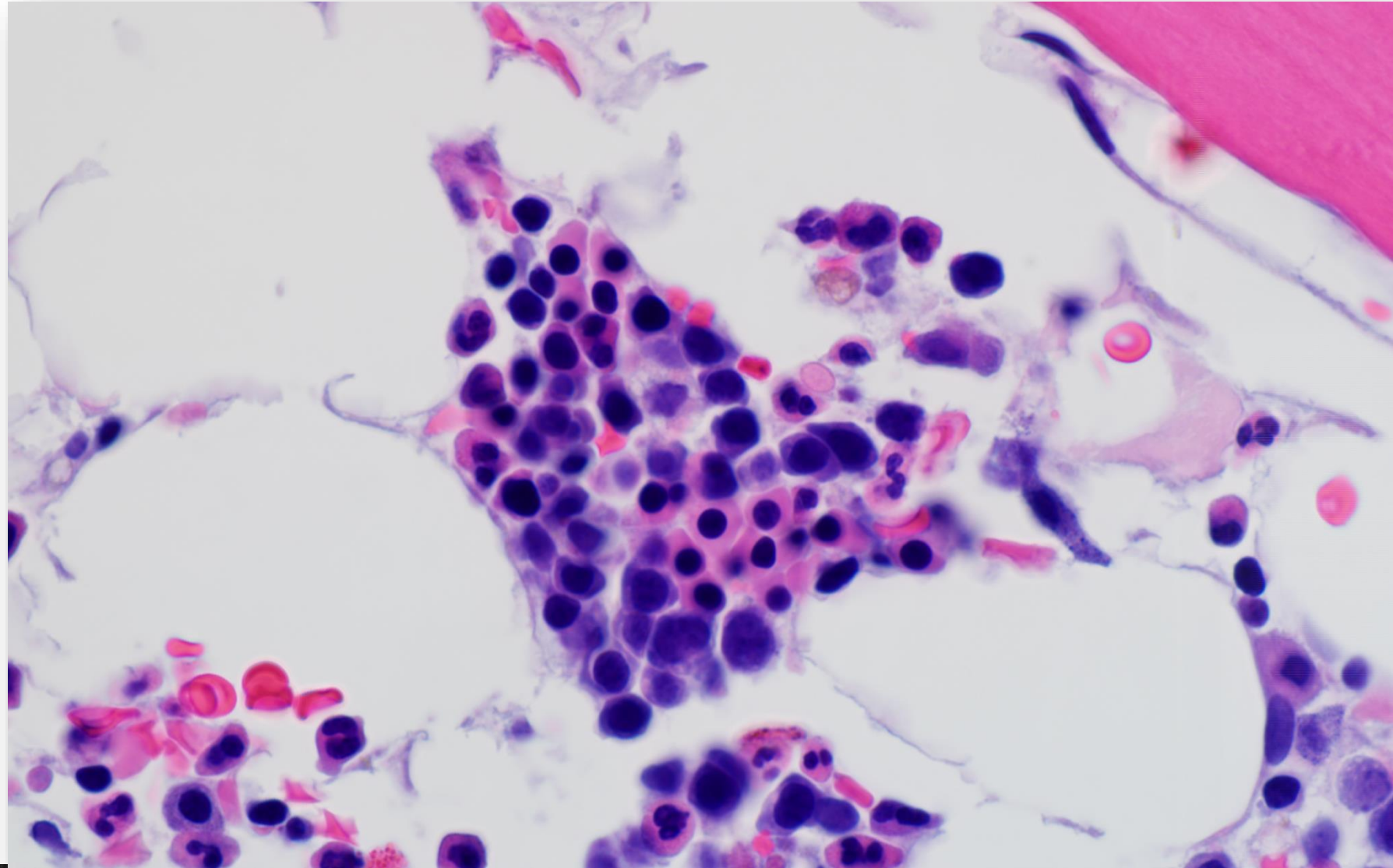
- UNEQUIVOCAL micromegakaryocytes
- IHC for megakaryocytes is obligatory (CD61, CD41, CD42b)



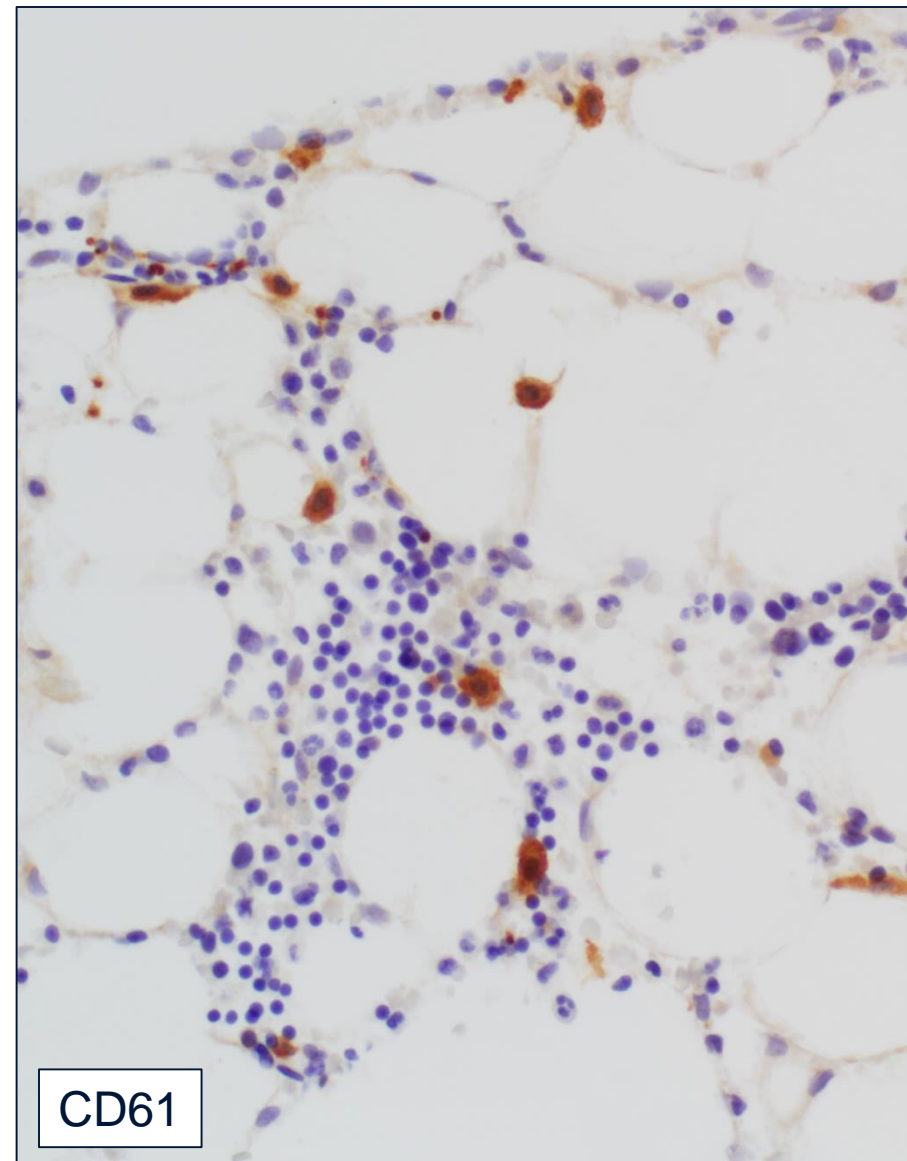
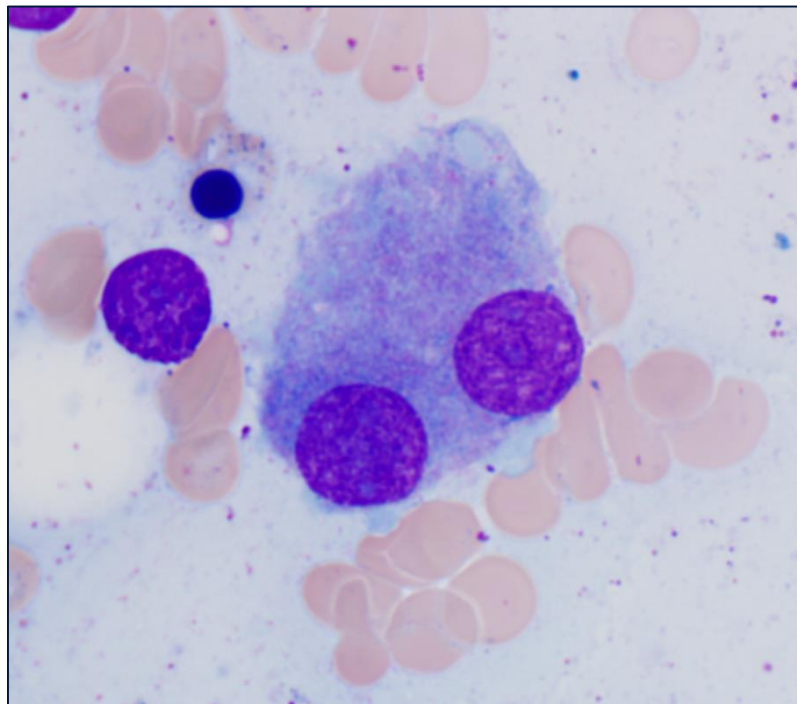
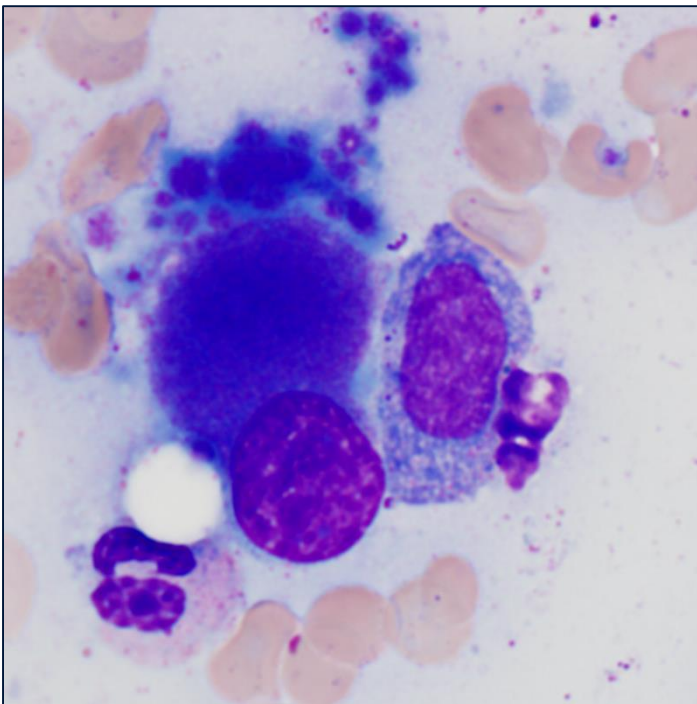
- Because overall morphology can closely resemble AAA, serial biopsies at least 2 weeks apart may be warranted

RCC: Erythropoiesis

1. A few clusters of >20 erythroid precursors
2. Arrest of maturation with increased number of pronormoblasts
3. Increased number of mitoses



RCC: Megakaryopoiesis



CD61

Hypocellular marrow in pediatric patients

	RCC	Constitutional BMF	AAA
Peripheral cytopenia	Milder than in AAA	Significant overlap between RCC and AAA	Significant
Erythroid islands	Significant, >20 cells Patchy foci Abnormal localization Increased immature erythroid precursors Increased mitoses Significant dyserythropoiesis		Rare, 10 cells or less Adequate maturation No significant dyserythropoiesis
Myeloid cells	Markedly decreased (more severely than in AAA) Left shifted Dysgranulopoiesis		Markedly decreased Adequate maturation No significant dyspoiesis
Megakaryopoiesis	Markedly decreased Significant dysplasia with micromegakaryocytes		Markedly decreased No significant dysplasia, no micromegakaryocytes No abnormal localization
CD34-positive blasts	Not increased No clusters/ALIP		Not increased
Caveats			After immunosuppressive therapy, the histologic pattern cannot be reliably distinguished from RCC
Common cytogenetic/molecular findings	Monosomy 7 (8%-48%) – higher risk of progression Trisomy 8		

Case

- 58-year-old male with recent history of weakness and fatigue

CBC

WBC 1.29 K/uL

RBC 3.73 M/uL

HGB 13.3 g/dL

PLAT 187 K/uL

WBC differential

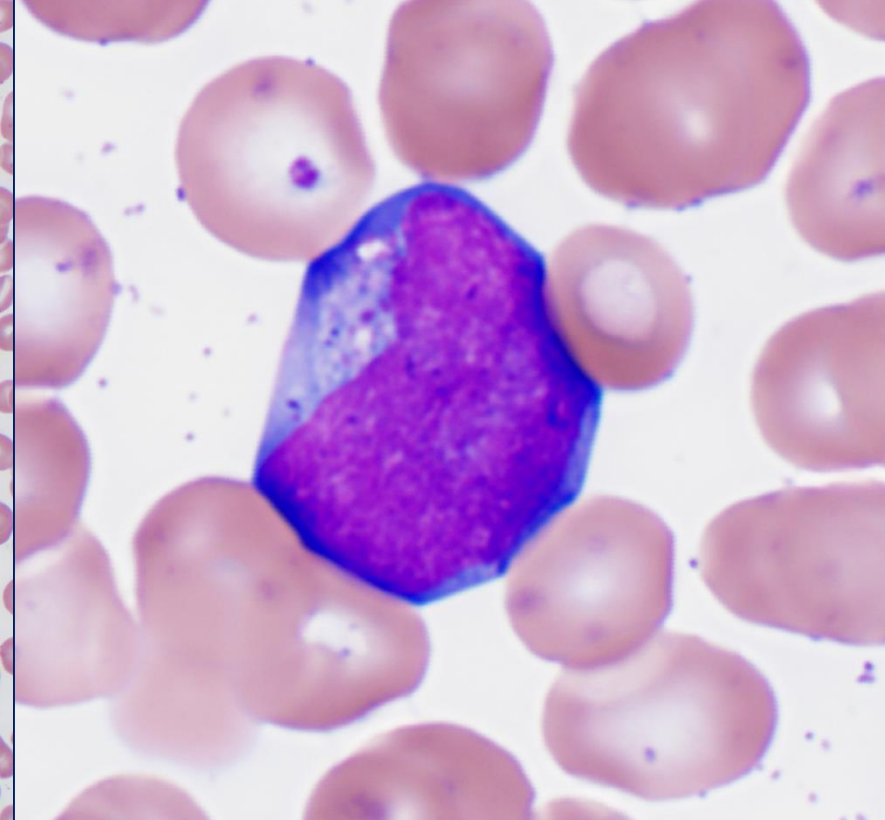
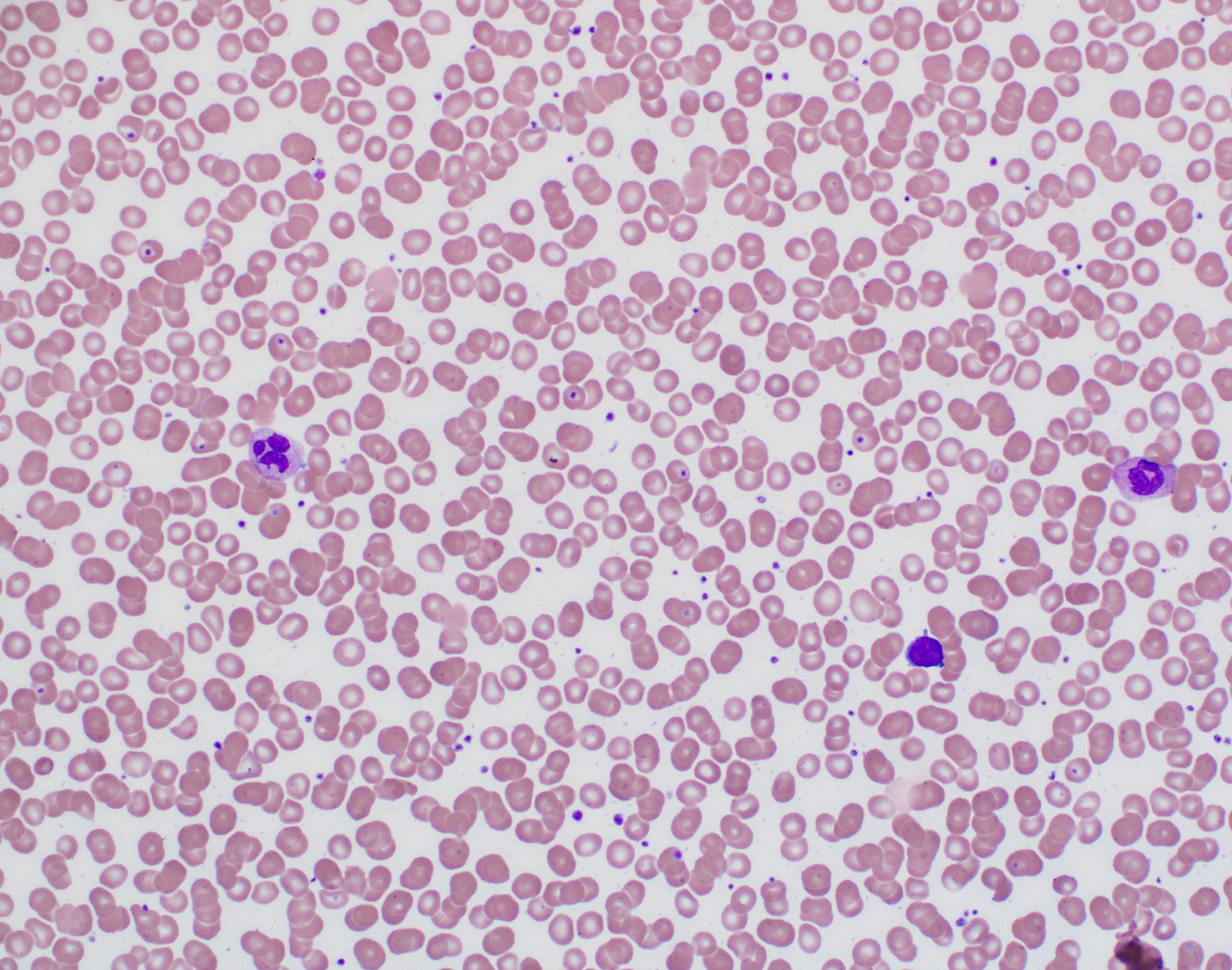
Neutrophils 32%

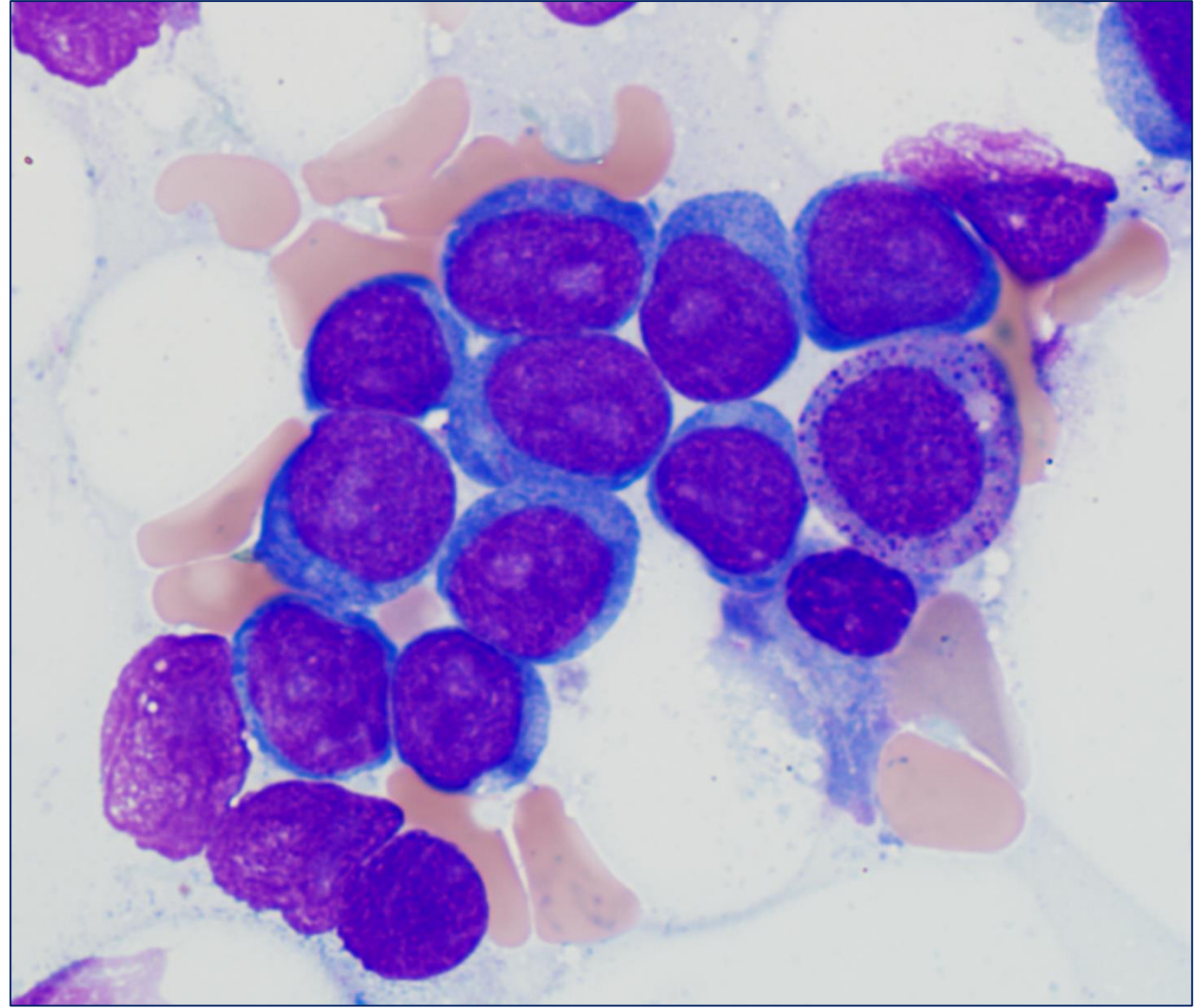
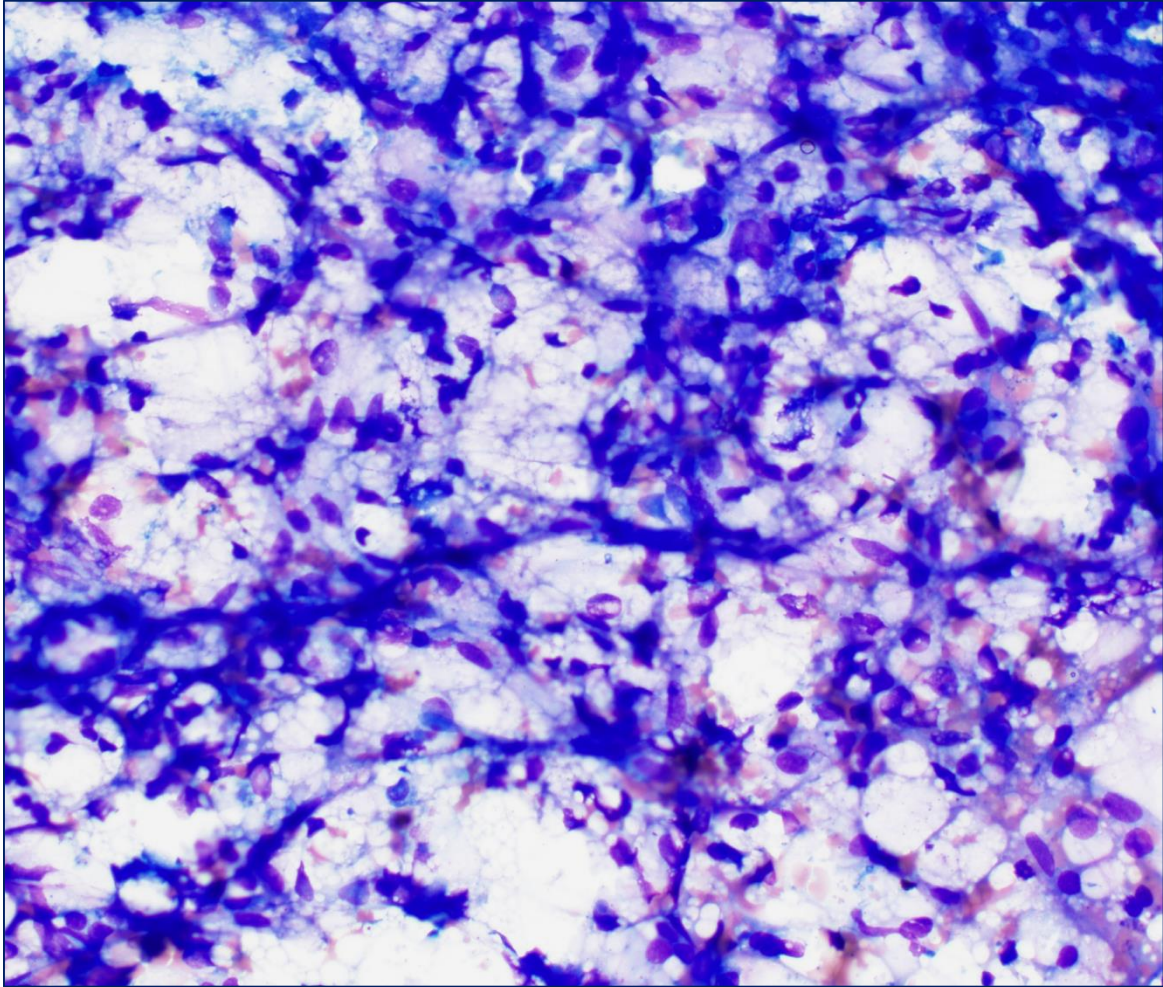
Lymphocytes 58%

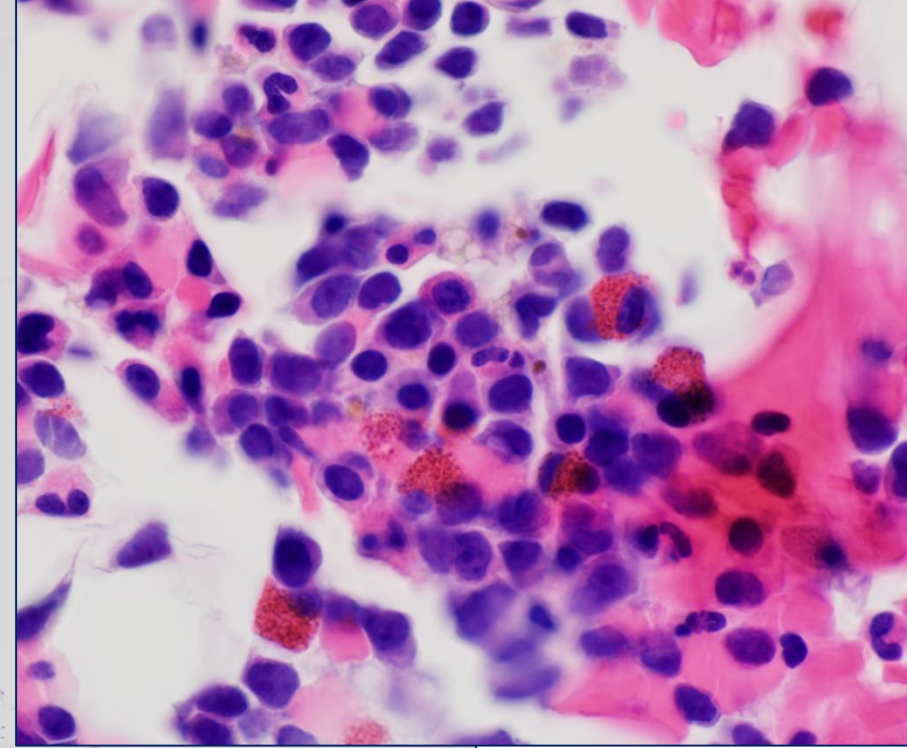
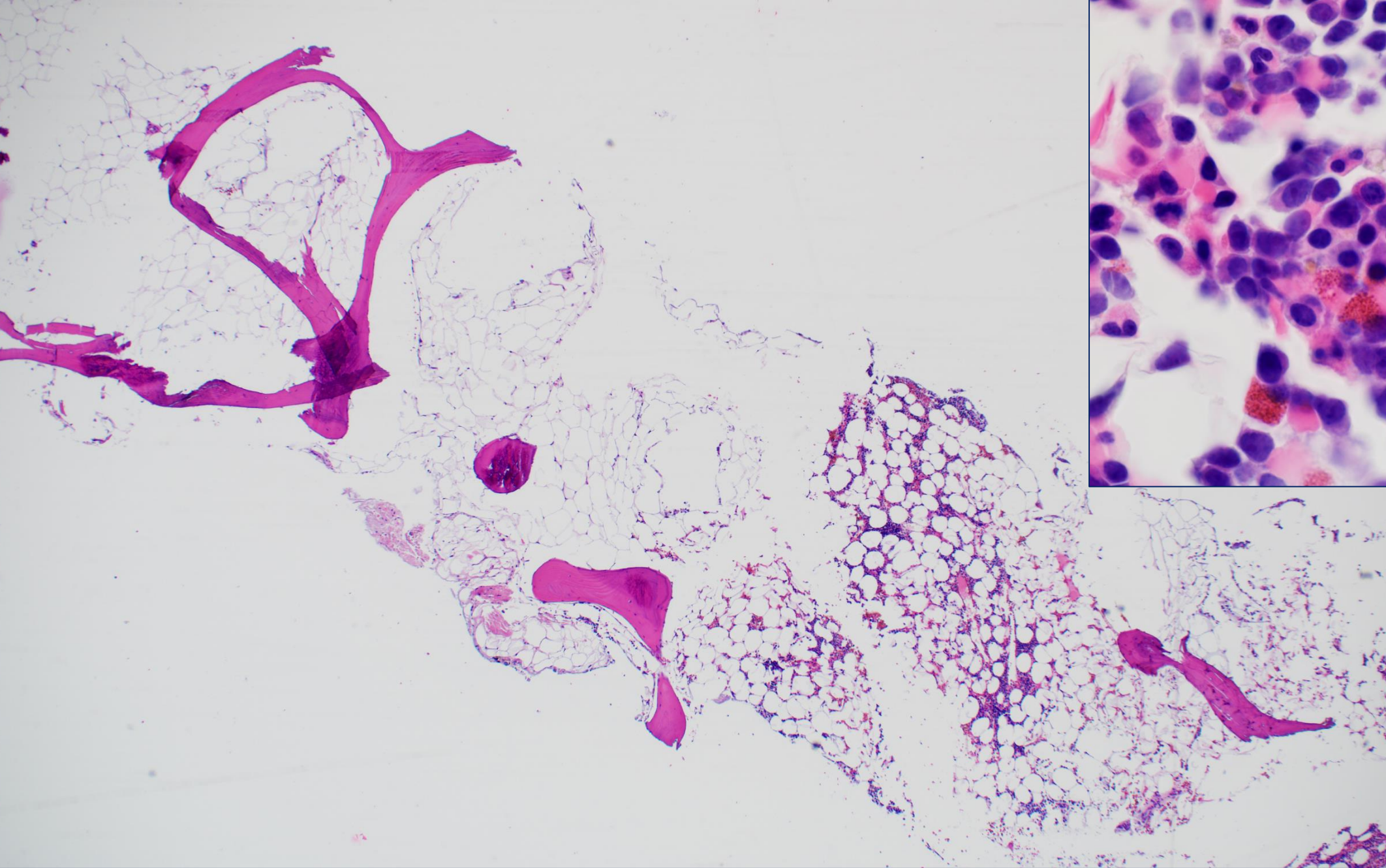
Monocytes 3%

Eosinophils 4%

Basophils 1%







Ancillary studies

- Flow cytometry
 - CD34+ myeloblasts comprise 28% of the leukocytes
- AML/MDS FISH: negative
- Karyotype: 46,XY [20]
- NGS: negative

FINAL DIAGNOSIS: **ACUTE MYELOID LEUKEMIA, NOS**

Hypoplastic Acute Leukemia

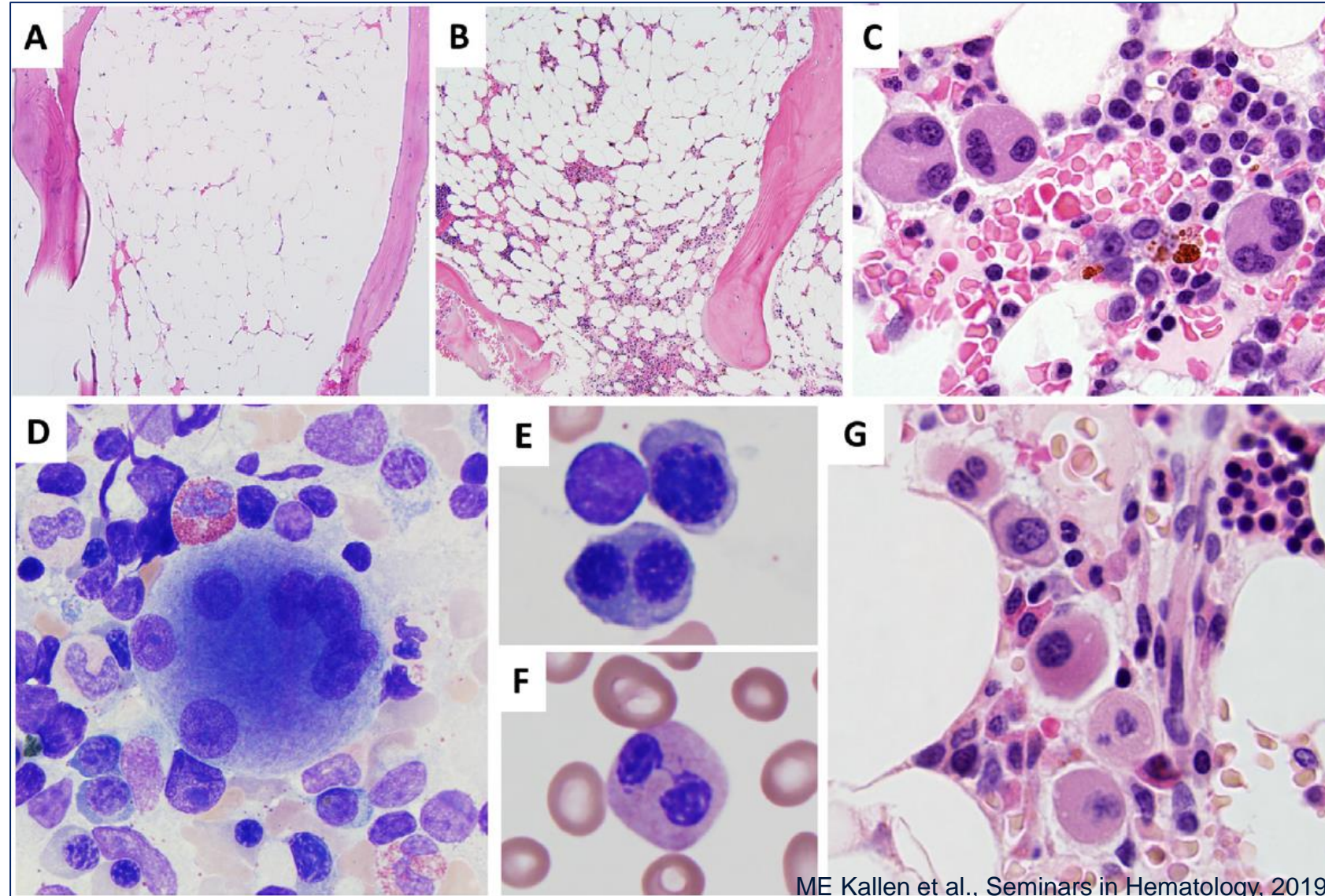
- Infrequent presentation
- Hypoplastic ALL
 - more common
 - occur mostly in pediatric patients
- Hypoplastic AML
 - rare
 - mostly in elderly adults
- Circulating blasts are uncommon, or rare if present

Myeloid neoplasms with germline GATA2 mutation

- Germline mutation in GATA2 gene
- AD disorder presenting in late childhood, adolescence, and adulthood
- Spectrum of overlapping phenotypes:
 - DCML (dendritic cells, monocytes, B-cell, and NK-cells) deficiency
 - MonoMAC syndrome: monocytopenia and non-TB mycobacterial infection
 - Familial MDS or AML
 - pulmonary proteinosis, warts, and sensorineural hearing loss
- 70% of affected individuals develop MDS/AML at median age of 29 yo

Myeloid neoplasms with germline GATA2 mutation: Morphology

- BM hypocellularity is characteristic
- Trilineage dysplasia
- Prominent dysmegakaryopoiesis
- Increased reticulin fibrosis



ME Kallen et al., Seminars in Hematology, 2019

Myeloid neoplasms with germline GATA2 mutation: other clues

- Flow cytometry shows
 - Abnormal granulocytic maturation
 - **Disproportionately and markedly reduced monocytes, B-cells (including hematogones), and NK-cells**
 - useful finding to distinguish from AAA
 - Plasma cells may be increased and abnormally express CD56
 - Commonly increased T-cell, particularly LGL T-cells; can be immunophenotypically atypical
- Most common cytogenetic abnormalities are monosomy 7 and trisomy 8

Constitutional bone marrow failure disorders

- More commonly in pediatric population
- Later-age onset, especially in DC
- Two most common are Fanconi anemia (FA) and dyskeratosis congenita (DC)

Fanconi anemia

Genomic instability disorder characterized by

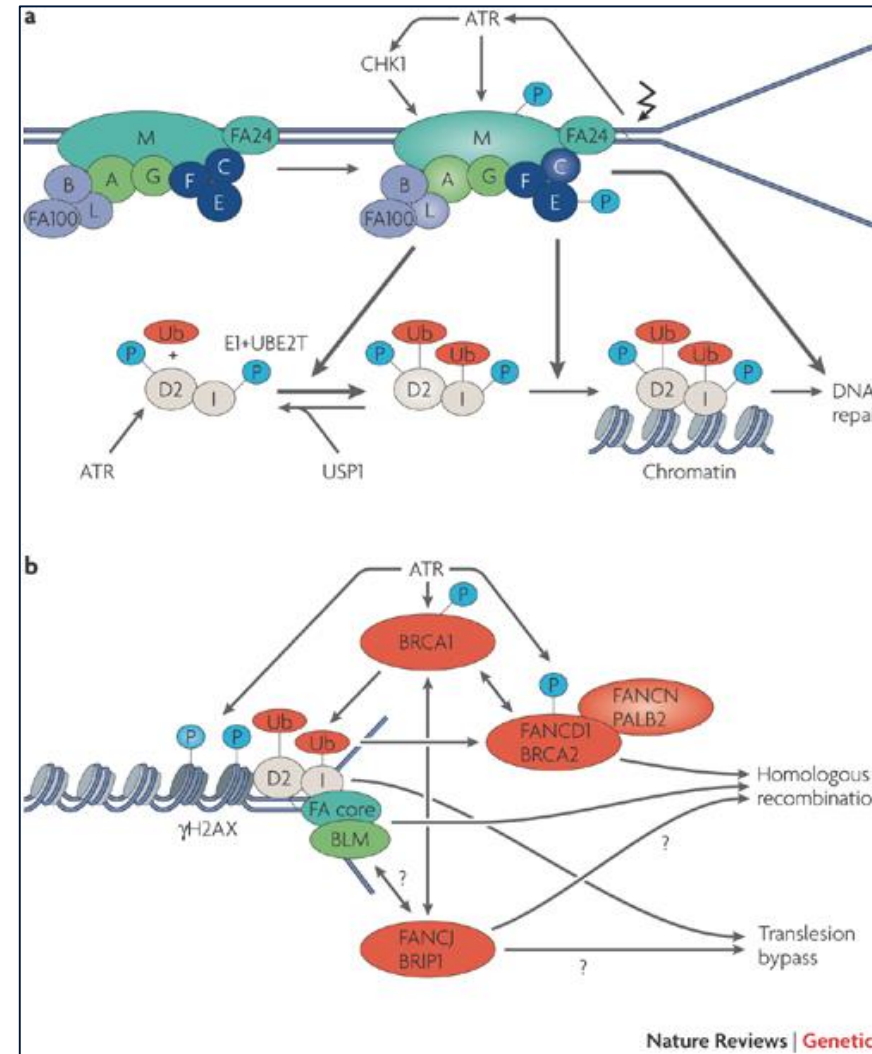
- chromosomal fragility and breakage,
 - progressive BM failure, peripheral cytopenias,
 - developmental anomalies, and
 - a strong propensity for hematologic and solid tumor cancers:
 - Median patient age 16 yo
 - Crude risk of cancer: acute leukemia 5-10%, MDS 5%, solid tumors (SSC of head/neck and upper GI) 5-10%
-
- Prevalence 1-5 per 1,000,000
 - All racial groups, Spanish gypsies carrier frequency 1 in 64-70
 - Median age at diagnosis is 6.5 years, but can be 0-49 years

Fanconi anemia: Molecular aspects

DNA damage response pathway

1. CORE COMPLEX: 8 wild type proteins 'FANC' (A,B,C,E,F,G,L,M) and 4 additional proteins FAAP (16,20,24,100)
2. ID COMPLEX: two downstream proteins FANC I and D2
3. DOWNSTREAM EFFECTOR COMPLEX: 5 other proteins: FANCD1, BRCA2, FANCI, BACH1, and BRIP1

21 known FA subtypes



1

- Mutation of DNA repair pathway

2

- Increased sensitivity to oxygen
- Defective DNA repair

3

- Inability to repair interstrand links

4

- Shortened cell survival
- Increased apoptosis

Fanconi anemia: Findings

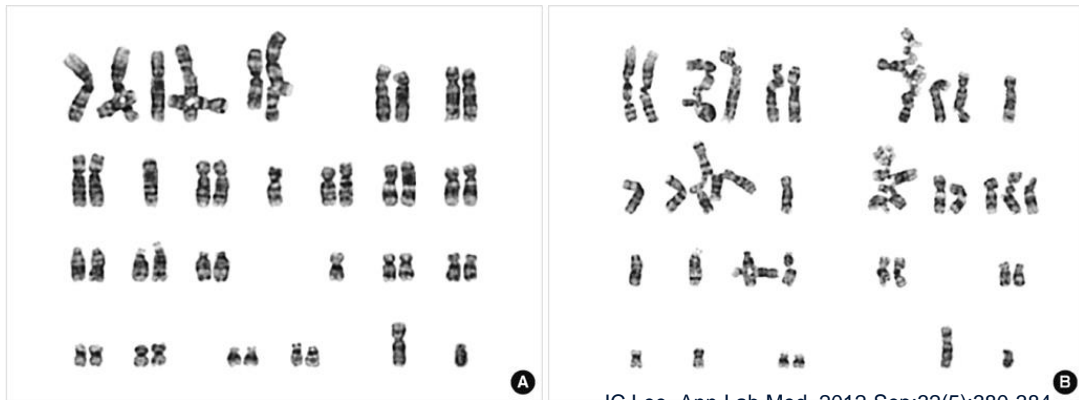
- Characteristic congenital physical anomalies (but only 70% of patients) : skin pigmentation and café-au-lait spots 40%, short stature 40%, upper limb anomalies 35%, hypogonadism 27%, eye, eyelid or epicanthal anomalies 20%
- Gradual onset of BM failure in the 1st decade of life
 - Initial macrocytosis and thrombocytopenia, followed by neutropenia and anemia
 - Initially erythroid hyperplasia, +/- dysplasia, may be similar to pediatric MDS
 - Progression results in hypocellularity

Fanconi anemia: Testing

Chromosome fragility

Increased chromosomal breakage in the presence of DNA-crosslinking agent

Metaphases of lymphocytes from PB



False negative results in case of clonal somatic reversion

Recommended for patients younger 50 yo evaluated for AA

Molecular

NGS panels including FA genes

85-90% of FA cases have mutations in *FANCA*, *FANCC*, or *FANCG*

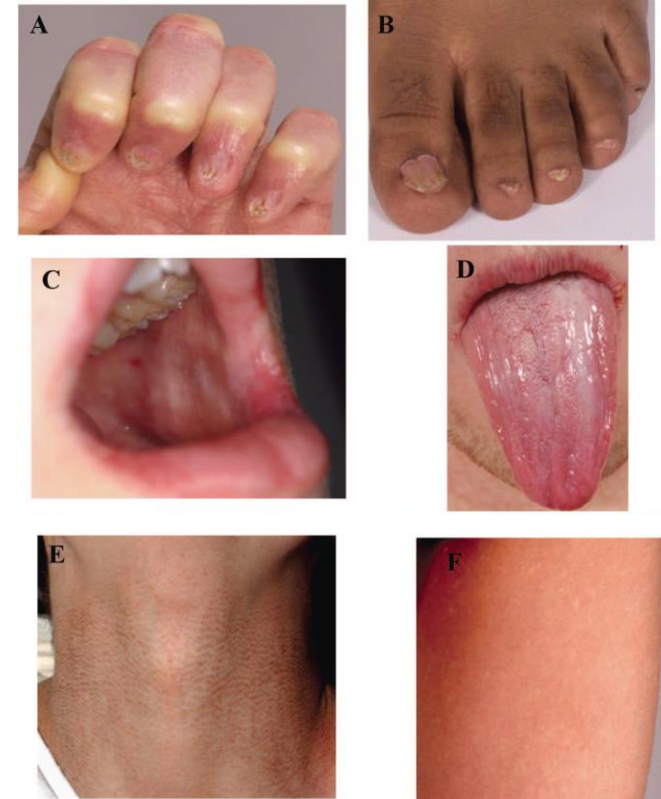
Sensitivity depends on the number of genes in the panel

Dyskeratosis congenita

Inherited multisystem disorder of the mucocutaneous and hematopoietic systems and a wide variety of other somatic abnormalities

Part of '**TELOMERE BIOLOGY DISORDERS**'

- Incidence 4 per 1,000,000/year
- Classic triad (full triad present in less than ½ of patients)
 - nail dystrophy
 - oral leukoplakia
 - lacy reticular pigmentation of neck and upper chest
- Bone marrow failure in 90%
- 10-15% patients will develop cancer
 - MDS/AML very common (40% cumulative risk at 50 yo), SSC/adenocarcinoma of oropharynx and upper GI

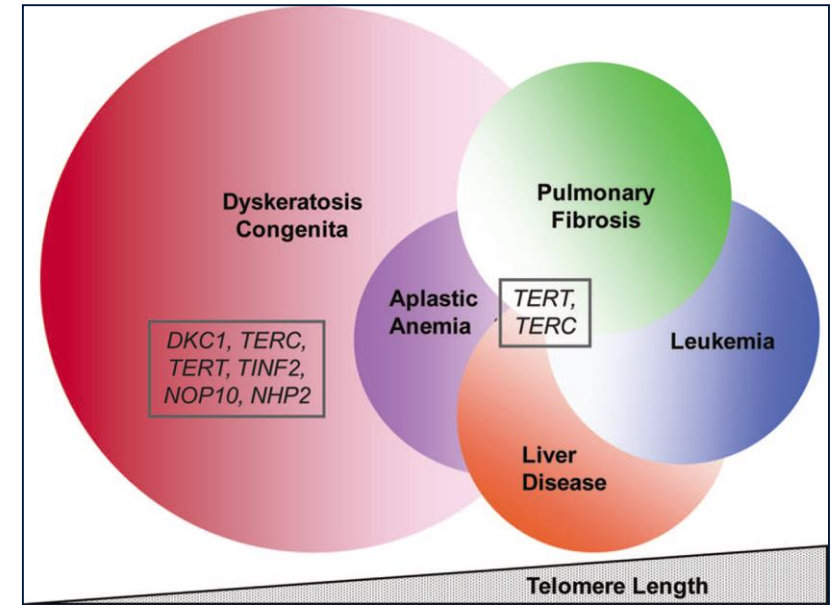


SA Savage, Genetics in Medicine, 2010

Telomere biology disorders

Telomerase

- Synthesis and extension of terminal telomeric DNA
- Developmental regulation
 - expression in most human tissues only during the 1st week of embryogenesis
 - otherwise, maintained in highly proliferative tissues (skin, intestine, bone marrow, ovaries, testes)
 - upregulated in neoplastic cells
- Two core components: TERT and TERC along with other factors, including Dyskerin



SA Savage, Genetics in Medicine, 2010

Dyskeratosis congenita: Findings

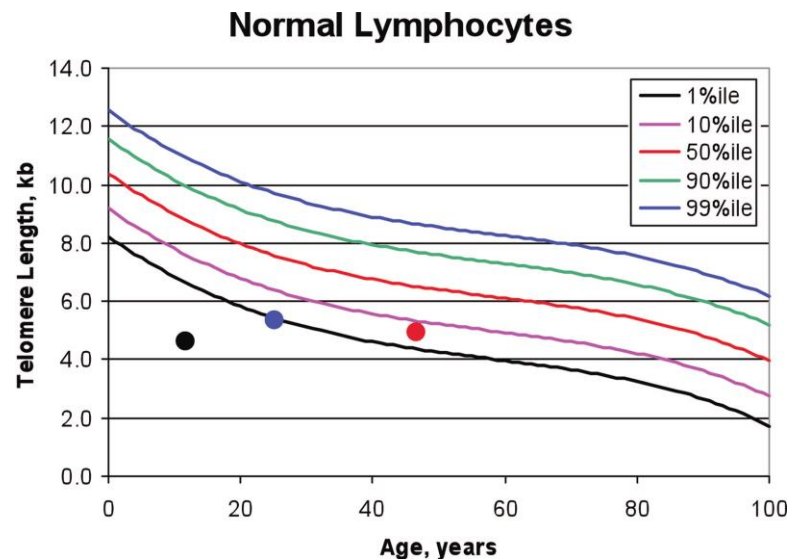
- Usually during childhood, but late onset is not uncommon
- Skin changes appear first, followed by mucosal changes and BM failure
- Other manifestations and important family history: palmar hyperhydrosis, hair loss, eye abnormalities, dental decay, osteoporosis, hypoplastic testes, urethral stenosis, **idiopathic pulmonary fibrosis, liver disease**

Dyskeratosis congenita: Testing

Telomere length measurement

Screening test by flow cytometry combined with FISH

- Measures telomere length on different WBC populations
- Considerable interindividual variability
- Careful interpretation within the clinical context



SA Savage, Genetics in Medicine, 2010

Molecular

NGS panels including genes involved in DC

Total of 17 genes can be affected

DKC1 is the most common (17-36%) – X-linked recessive

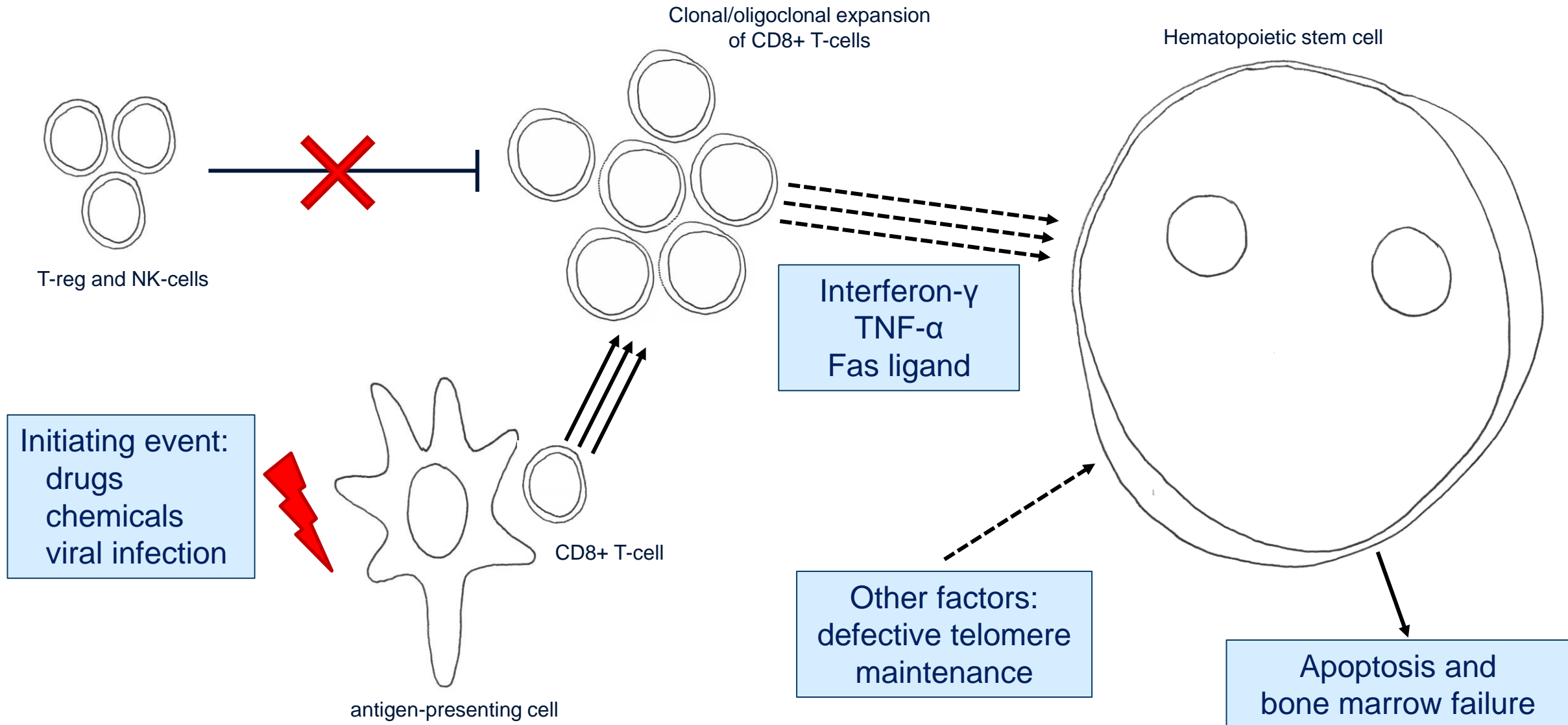
TINF2 (11-24%), *TERC* (6-10%) – AD

RTEL1 (2-8%) - AR

Idiopathic Acquired Aplastic Anemia

- Non-neoplastic bone marrow failure caused by an autoimmune T-lymphocyte-mediated attack on hematopoietic stem and progenitor cells [NS Young, Am Soc Hematol Educ Program, 2013]
- Incidence
 - 2-3 per million/year in North America and Europe
 - 4-8 per million/year in Asia
- Sex predilection M:F = 1:1
- Age of onset: bimodal
 - Young adulthood – 20 yo
 - Elderly – 60 yo

Idiopathic Acquired Aplastic Anemia: Pathogenesis



Idiopathic Acquired Aplastic Anemia: Clinical Presentation

- Relatively recent onset of cytopenia-related features
 - anemia-related symptoms are very common
 - platelet type bleeding and petechiae
 - infections due to neutropenia are not common
- Close attention to possible dysmorphic features

Idiopathic Acquired Aplastic Anemia: Bone marrow

- Trilineage hypoplasia
 - Diffuse hypocellularity
 - Hematopoiesis is usually erythroid-predominant
 - Mild dyspoiesis can be seen
 - Dyserythropoiesis indicative of “stress hematopoiesis”
 - Mild dysmegakaryopoiesis, <10%, no atypical localization: order IHC
 - Blasts are not increased, usually extremely rare: order IHC
- Ancillary studies
 - Flow cytometry: no increased blasts, no atypical immunophenotypic findings
 - Chromosome analysis
 - Next-generation sequencing

Idiopathic Acquired Aplastic Anemia and Clonal Hematopoiesis

- Well known linkage between AAA and clonal hematopoiesis
- Acquisition of mutations in hematopoietic cells is a part of aging
 - 10% of individuals 65 yo and older have clonal hematopoiesis [Genoveese et al., NEJM, 2014]
 - By molecular techniques most commonly mutated genes are DNMT3A, TET2, and ASXL1
 - By chromosome analysis most common alterations are loss of chromosome Y
- In context of myeloid malignancies, a spectrum of conditions is recognized

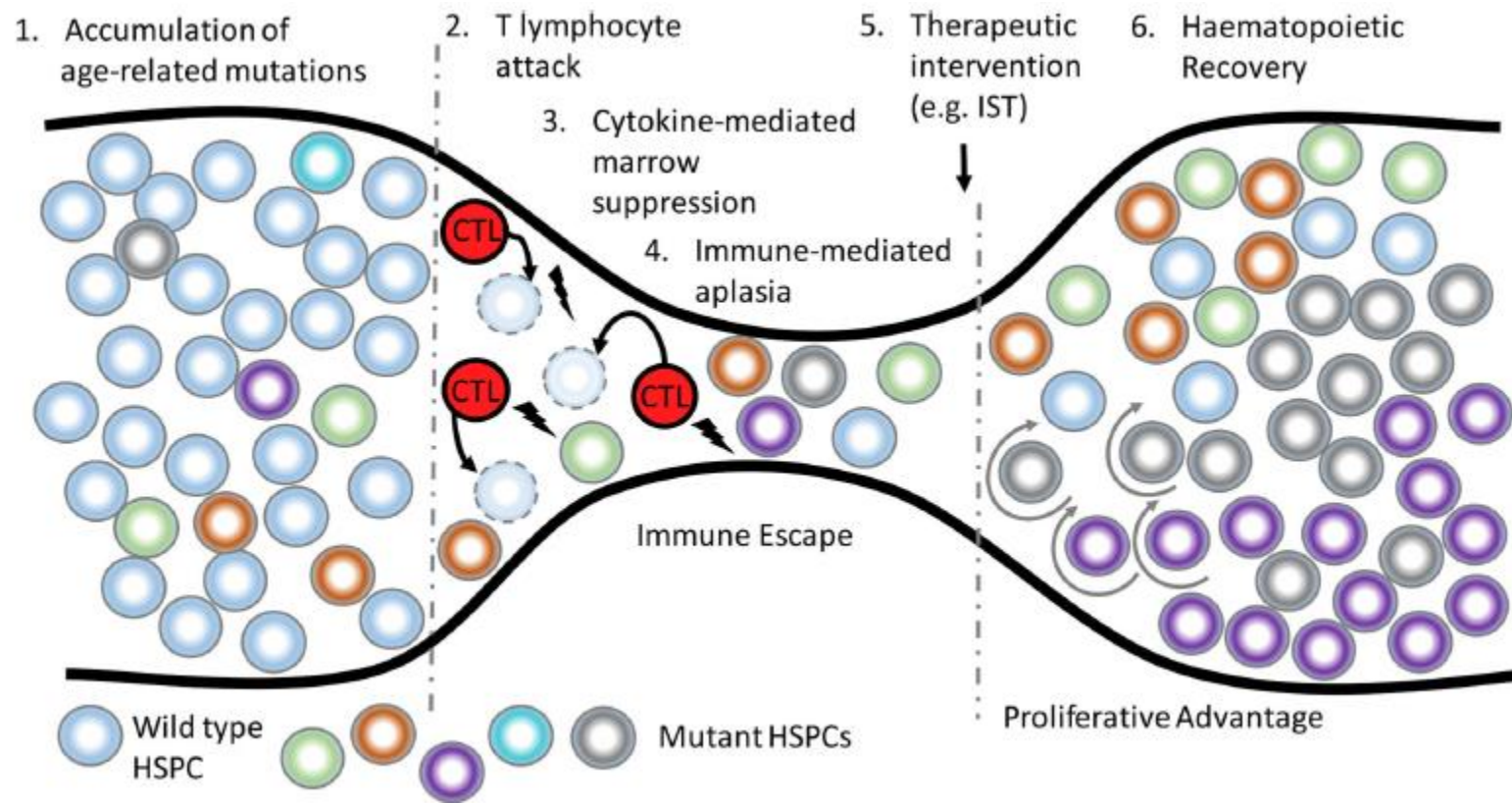
	Traditional ICUS			MDS by WHO 2008	
	'Non-clonal' ICUS	CHIP	CCUS	Lower Risk MDS	Higher Risk MDS
Clonality	–	+	+	+	+
Dysplasia	–	–	–	+	+
Cytopenias	+	–	+	+	+
BM Blast %	< 5%	< 5%	< 5%	< 5%	< 19%
Overall Risk	Very Low	Very Low	Low (?)	Low	High
Treatments	Obs/BSC	Observation	Obs/BSC/GF	Obs/BSC/GF IMiD/IST	HMA/HCST
			Clonal Cytopenias		

ICUS: idiopathic cytopenia of undetermined significance
CHIP: clonal hematopoiesis of indeterminate potential
CCUS: clonal cytopenia of undetermined significance

Steensma et al. *BLOOD*, 2015

Idiopathic Acquired Aplastic Anemia and Clonal Hematopoiesis

- Pre-existing age-related mutations serve as a substrate for clonal selection
 - Clones with less-immunogenic properties
 - Clones more resistant to T-cell mediated death/cytokine-mediated suppression
- Altogether, clonal hematopoiesis is detected in 70-85% of the AA patients
 - 60% of cases with childhood onset
 - virtually 100% in adults



Stanley et al. Br J Haematol, 2017

Idiopathic Acquired Aplastic Anemia and Clonal Hematopoiesis

Cytogenetic/Chromosomal alterations

Seen in 25% of patient with AAA

- -7/del(7) – associated with worse prognosis in AAA
- Trisomy 8 – if present with del(13q), is associated with better response to IST
- Other: del(13q), trisomy 6, trisomy 15, and trisomy 21

Caveat: -7/del7 is also considered “presumptive evidence of MDS”

- Copy-neutral loss of heterozygosity (CN-LOR) of 6p at the site of MHC locus
 - 10-13% of patients
 - Decreased immunogenic features
 - Relatively specific for AAA (very rare in MDS and general population)

Molecular

Most commonly mutated genes in AAA:

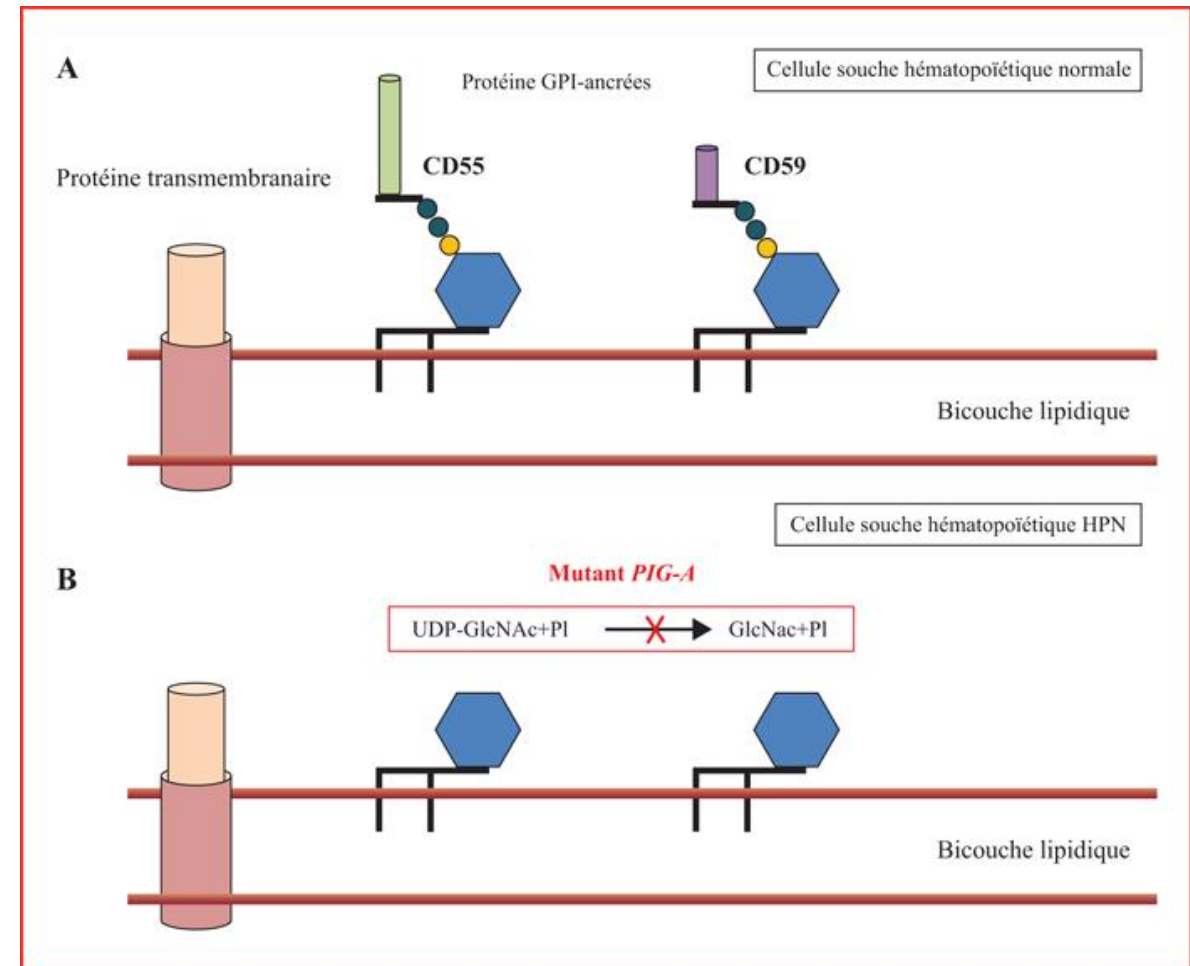
- *PIGA*
- *ASXL1*
- *BCOR*
- *DNMT3A*

Mutated *ASXL1*, *DNMT3A*, and *RUNX1* are associated with higher rates of malignant transformation

Mutated *BCOR* or *BCORL1* are associated with better response to IST

PNH clones

- PNH clones are present in 50% of AAA cases
- Somatic mutation in PIG-A gene (Xp) resulting in abnormal GPI protein processing and expression
- Confers survival and proliferative advantage to the mutated cells in context of AAA
- Usually small size: >0.1% on granulocytes and >0.2% on RBC; very rarely >10%
- **Associated with better prognosis in AAA**

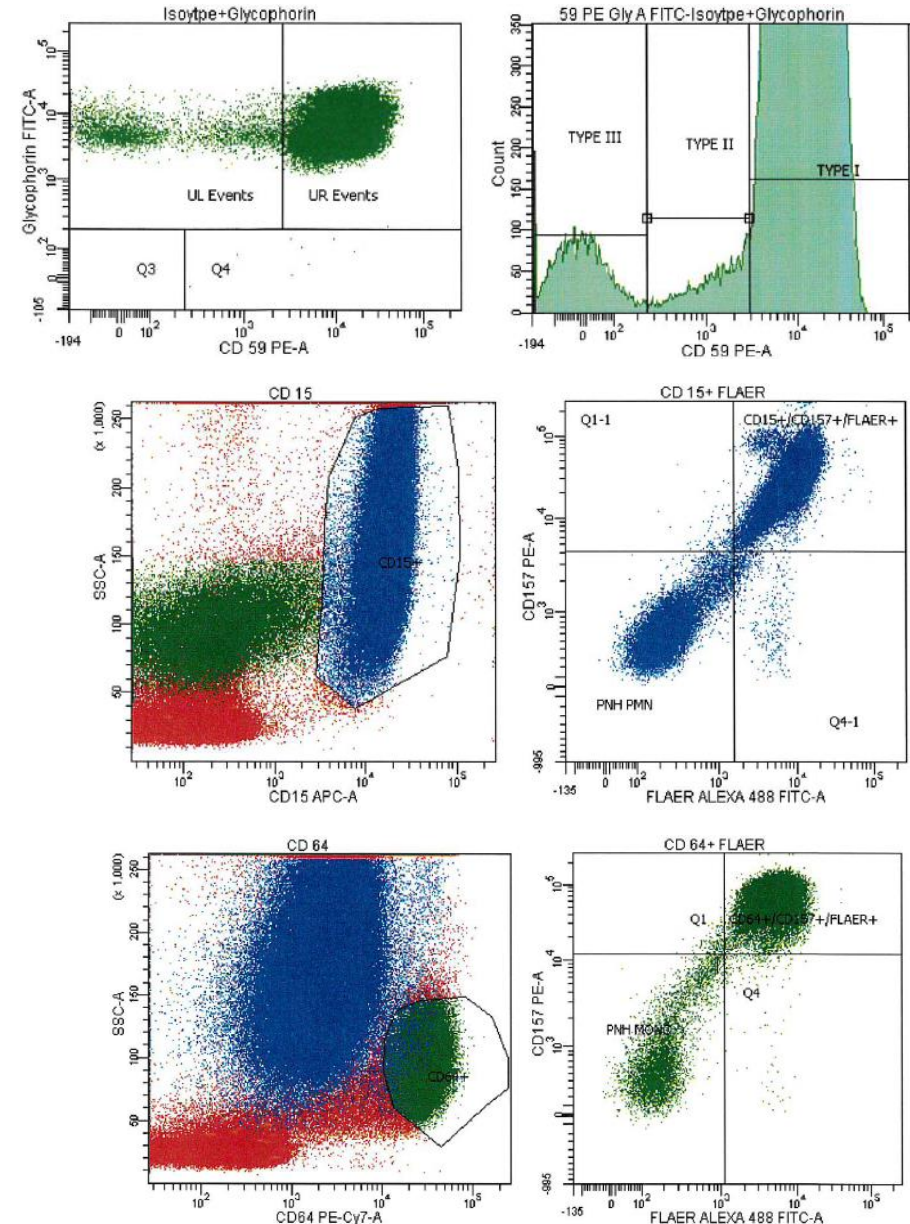


N Gendron, et al. Sang Thrombose Vaisseaux, 2018

PNH clone: Testing strategy

FLOW CYTOMETRY

- Gold standard
- Measures percentage of WBCs and RBCs that lack GPI-linked membrane proteins
 - CD59 and CD55 on RBCs
 - CD157-FLAER on WBCs
- High sensitivity: 0.005% for RBC/GRANs and 0.02% for MONOs



Back to the case

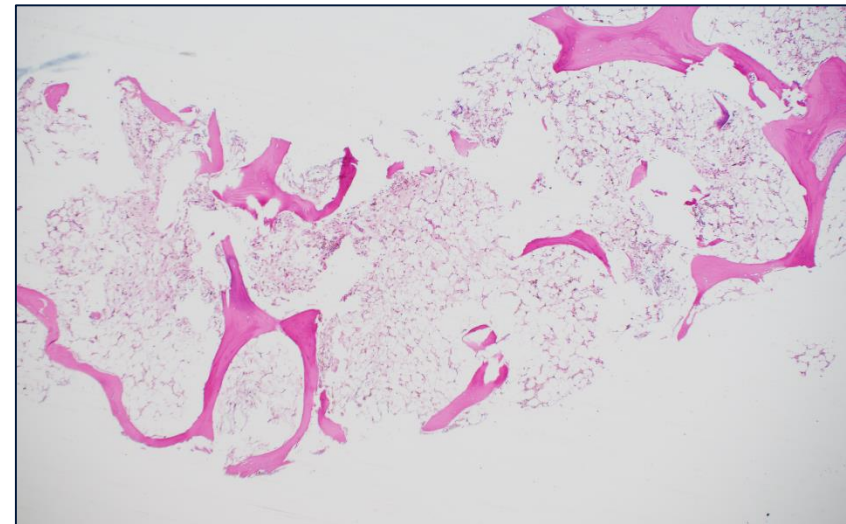
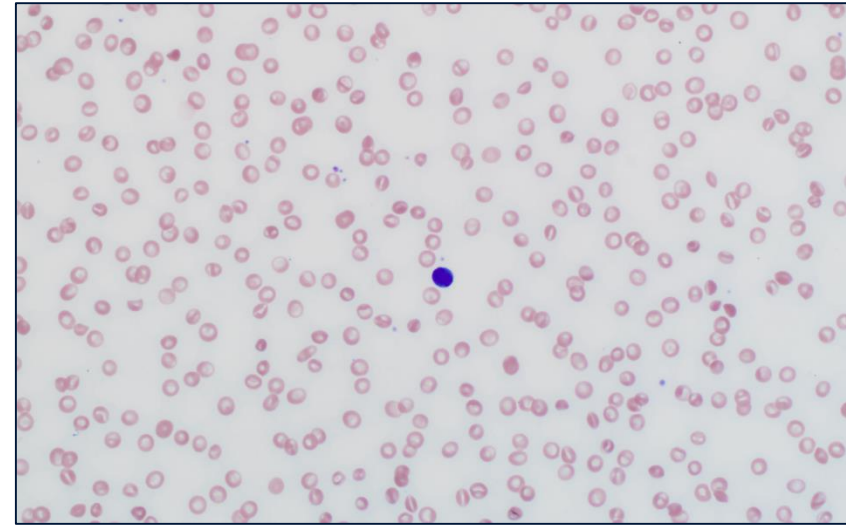
- 26-year-old male presents with septic shock
- SNP-microarray: male complement (XY), no DNA copy number changes or copy-neutral long stretches of homozygosity
- T-cell clonality: not detected
- Myeloid NGS panel (57 genes): no mutations detected
- PNH testing: small subclinical PNH clone
- FA chromosome breakage: negative
- Telomere length: shortening at 10th percentile
- DC panel: negative

DIAGNOSIS:

Markedly hypocellular marrow with no morphologic or flow cytometric evidence of malignancy

Most consistent with acquired aplastic anemia

Follow-up: the patient underwent HSCT and is currently doing well



Hypocellular Bone Marrow: What's Next? – Take home points

1

- Confirm **BMF** using morphologic and laboratory criteria

2

- Address **secondary aplastic anemia**
- Tests to consider: vitamin B₁₂, folic acid, autoantibodies, HepA/B/C serology, LFTs, thorough personal history, exposure to medications/drugs/infections

3

- Address **BM replacement disorder or overt malignancy**
- Tests to consider: flow cytometry, special stains (reticulin, trichrome, iron), IHC (CD34, TdT, CD61, CD41, CD42, CD3, CD20), chromosome analysis, NGS

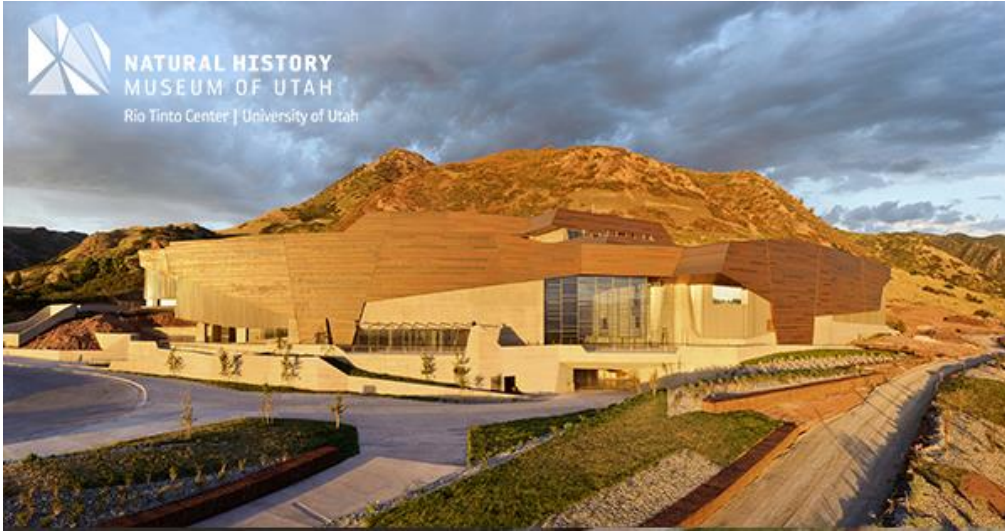
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- Address **constitutional BMF**
- Tests to consider: thorough physical examination for dysmorphisms, telomere length measurement, chromosomal breakage analysis, mutation analysis for Fanconi anemia, dyskeratosis congenita, etc.

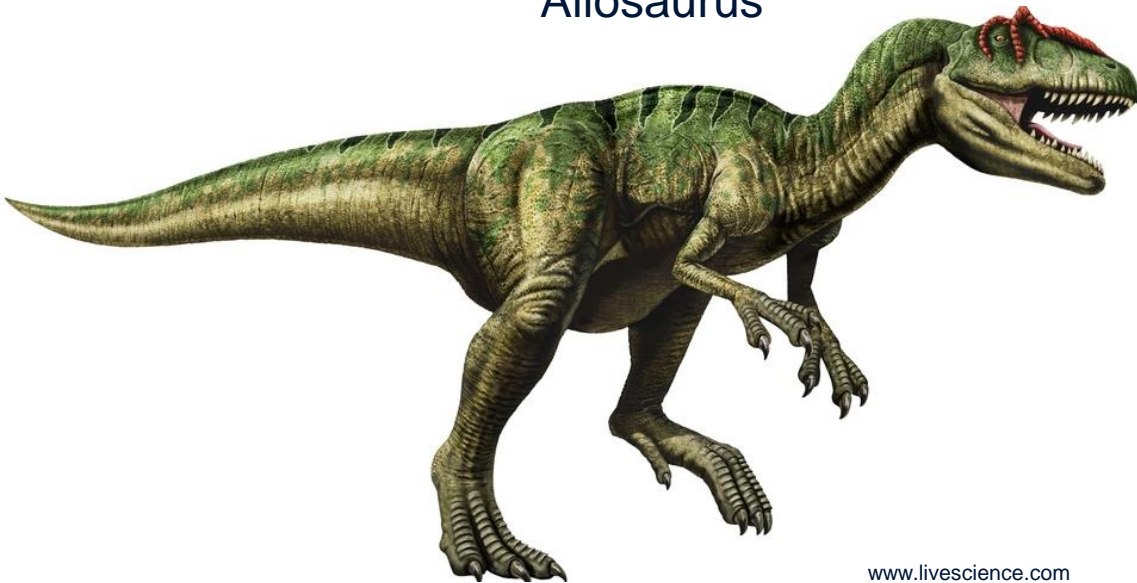
5

- Address **idiopathic acquired aplastic anemia**
- Tests to consider: PNH evaluation, NGS, SNP-array

Hypocellular marrow



Allosaurus



www.livescience.com



Photograph by Carolyn Levitt-Bussian
Courtesy of Natural History Museum of Utah, UMNH VP 21351

THANK YOU