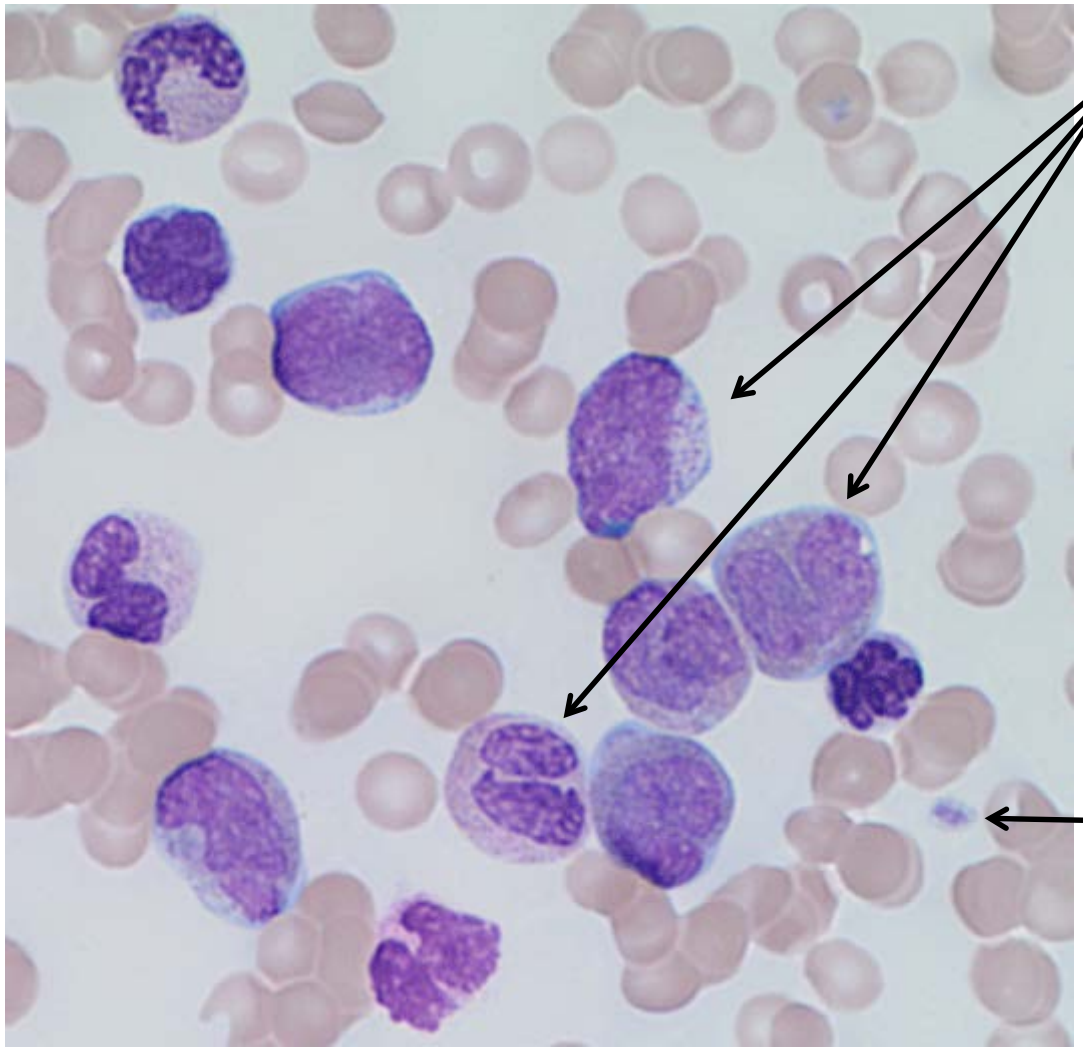
CASE PRESENTATION SH2017-0267

Dr. Larissa Liontos, MD, PhD
Department of Laboratory Medicine & Pathobiology
The University of Toronto
PGY4 Hematological Pathology Resident
Dr. Hubert Tsui, MD, PhD, FRCPC
Department of Laboratory Medicine and Pathobiology
University Health Network, University of Toronto, Toronto, ON, Canada

Clinical Information

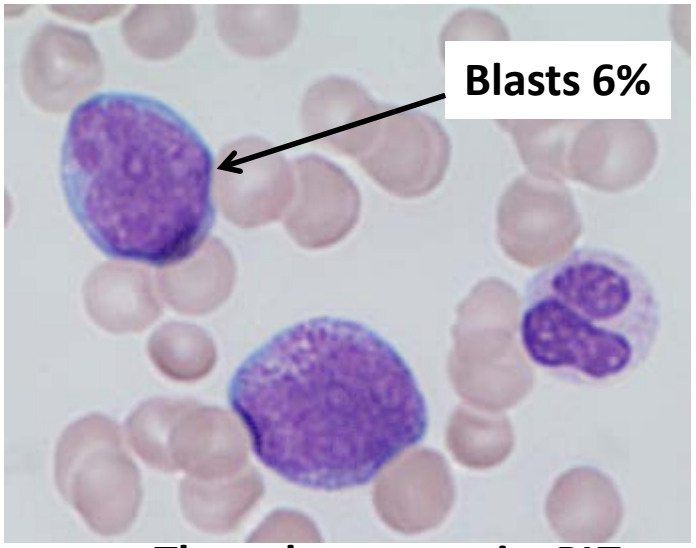
- 65 yo F presented to the hospital with shortness of breath, cough and weakness, placed on antibiotics for presumed pneumonia
- Only significant past medical hx: thymic radiation at age 5
- CBC at presentation: Hb 4.5 g/dL, WBC: 174x10e9/L with left-shift and circulating blasts, Platelets: 55
- Other labs: Retic: 10, LDH 809 U/L
- CT chest-bilateral lung infiltrates (bronchoalveolar lavage showed: immature granulocytes)
- Abdomen Ultrasound: No hepatosplenomegaly

Peripheral blood findings



Left-shift with dysplasia
WBC=125 x10e9/L

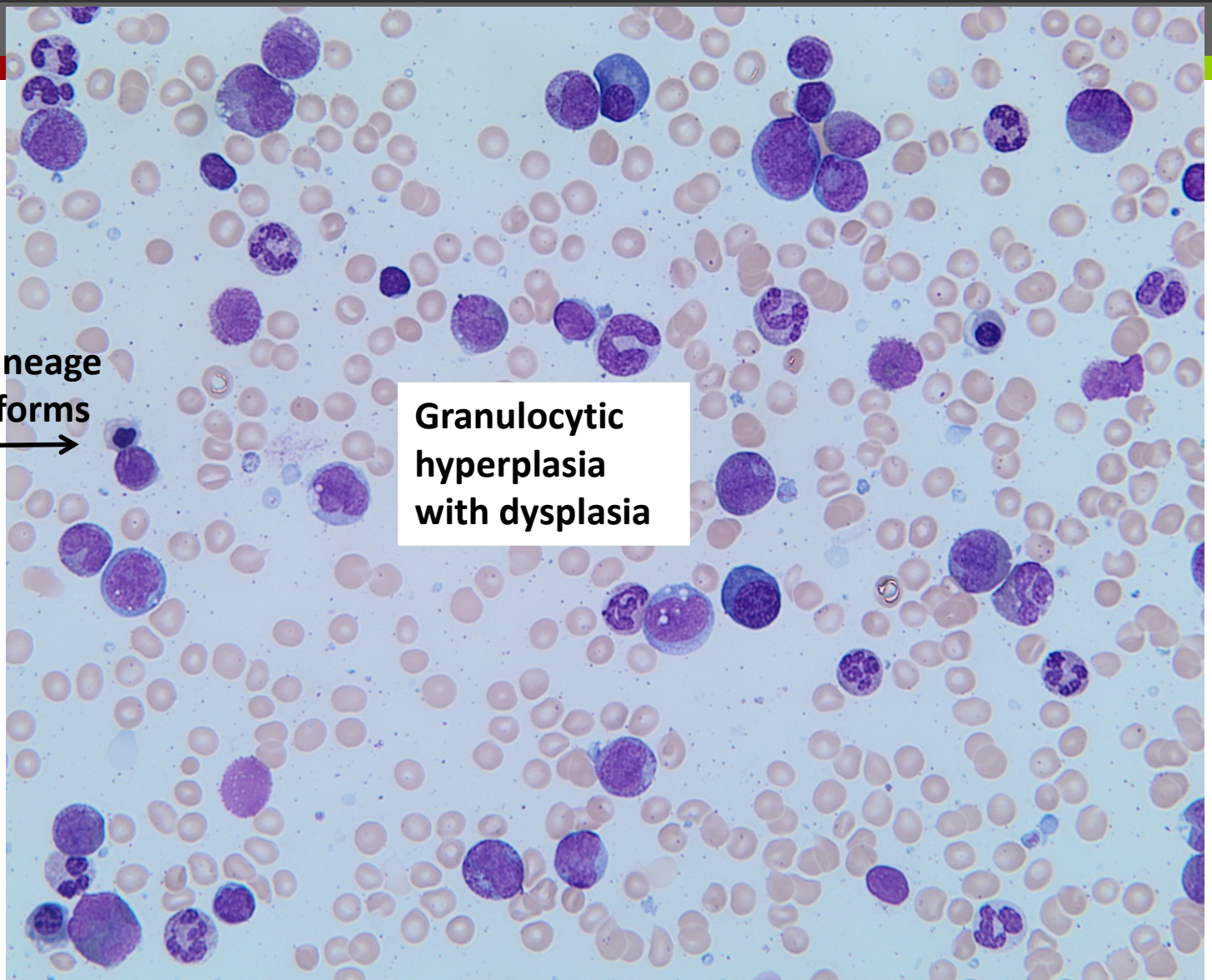
Anemia Hb 7.8 g/dL

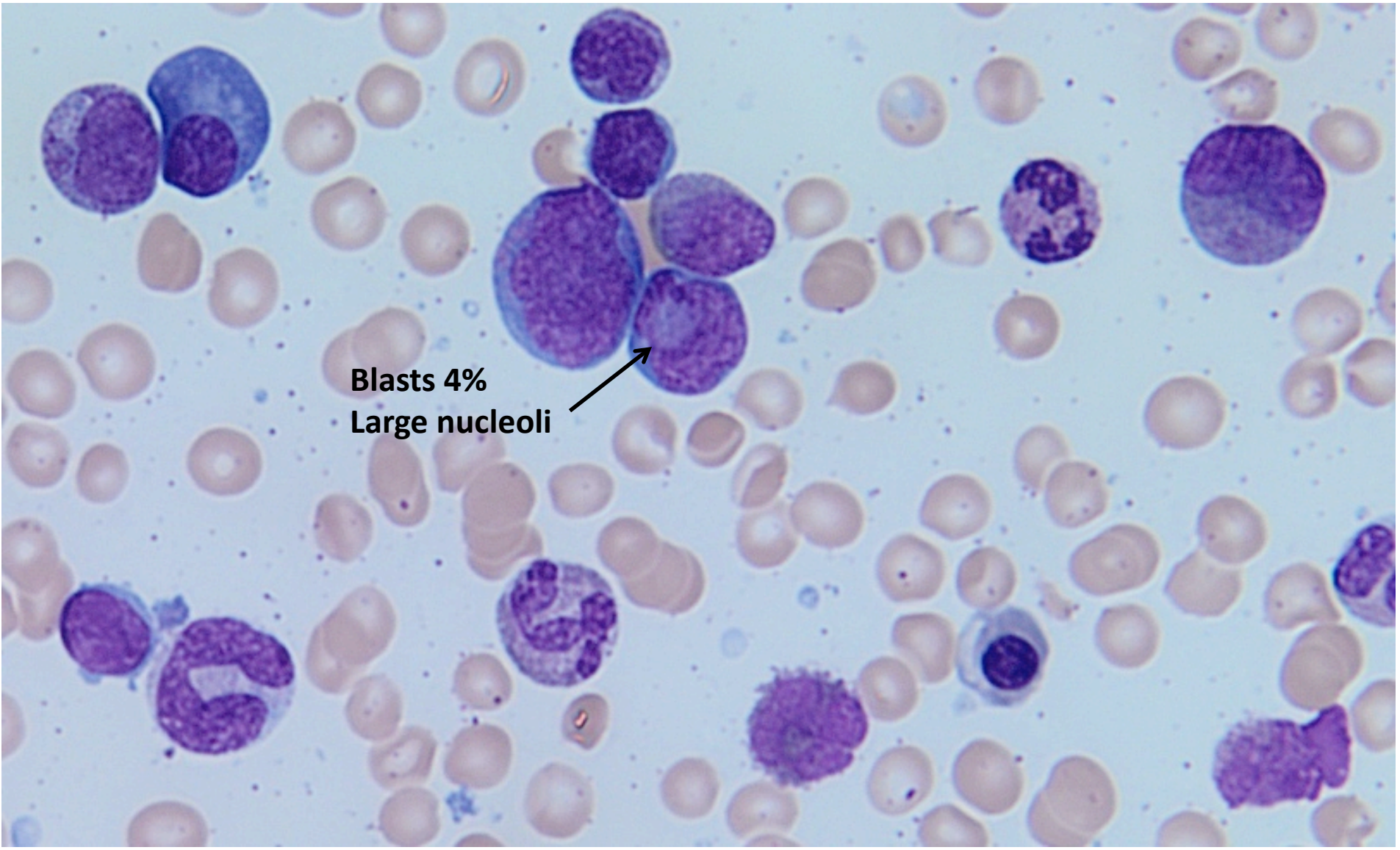


Thrombocytopenia, PLT= 28 x 10e9/L

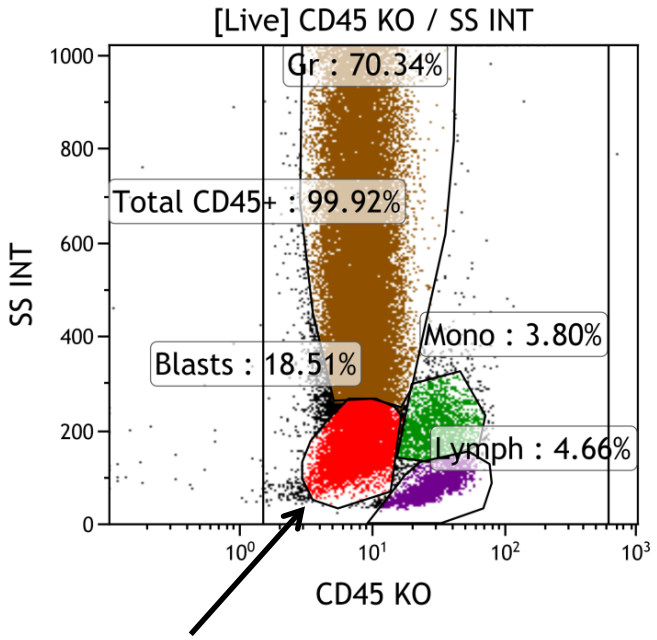
Eosinophils & Basophils within reference range.
Monocytes 3.26 x10e9/L

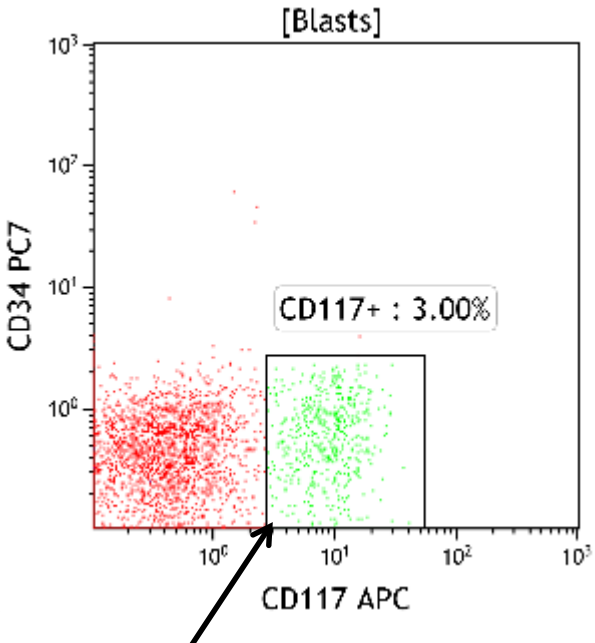
Bone Marrow Aspirate Findings

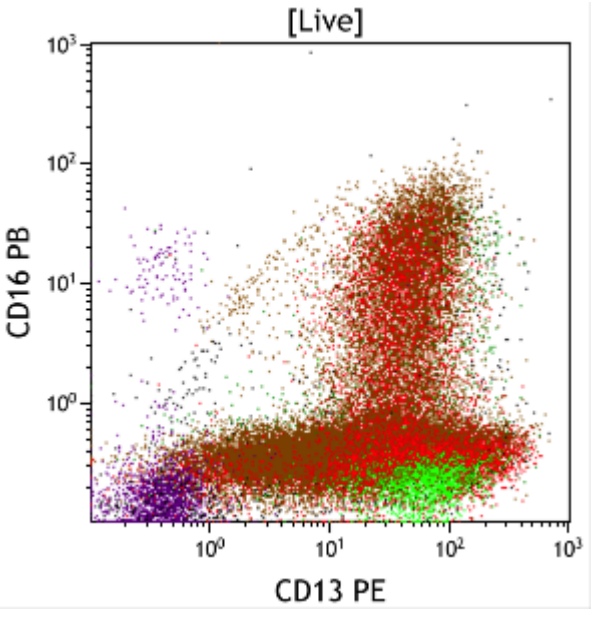




Megakaryocytes (not shown) had normal morphology







Gating of blasts difficult due to increased immature, dysplastic myeloid precursors

Blasts were CD34- & CD117+ and ~3% of total CD33 and CD13 bright

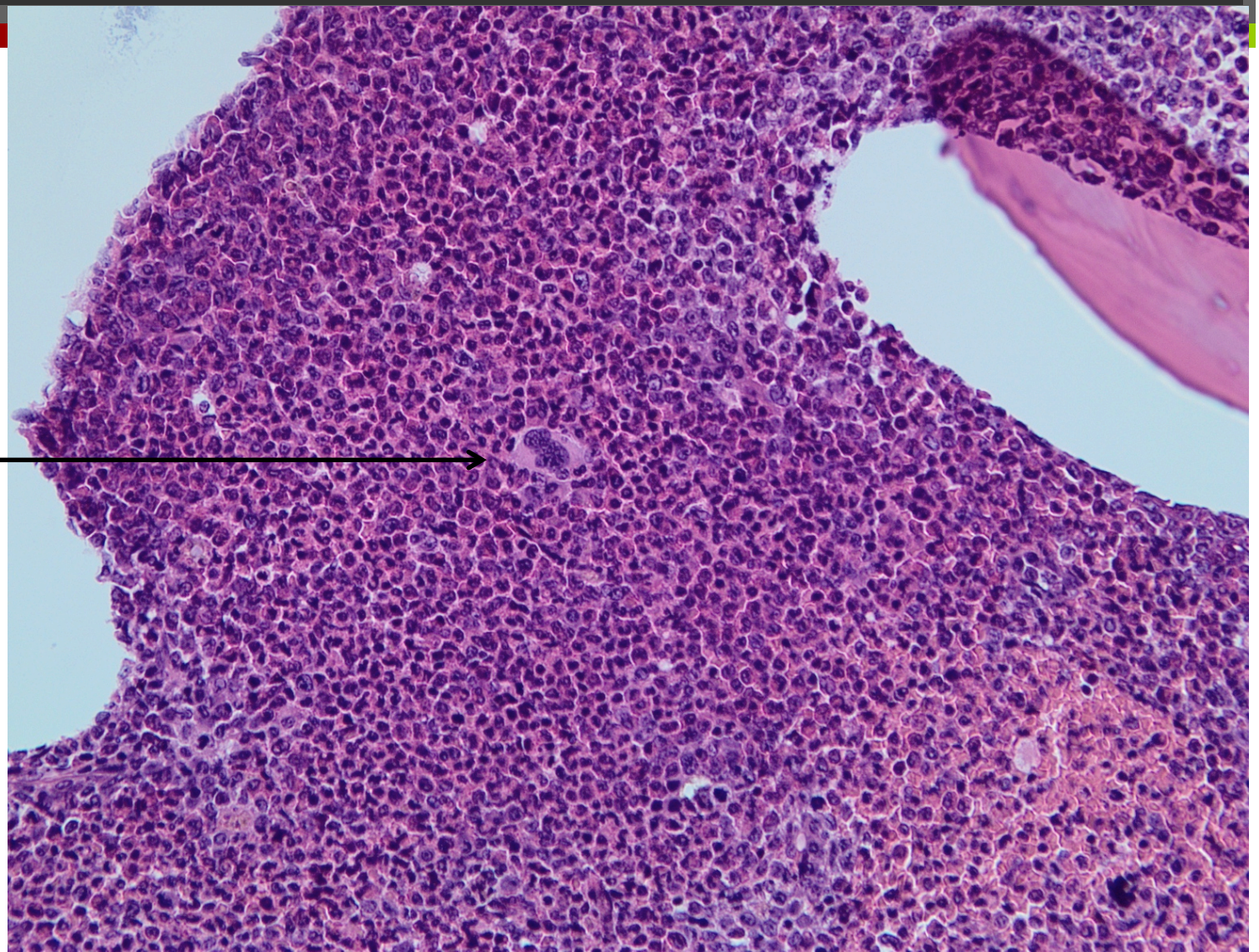
Altered granulocyte maturation pattern, showing "granulocytes" in brown, "myeloid precursors" in red and "blasts" in green

Flow cytometry- Peripheral Blood

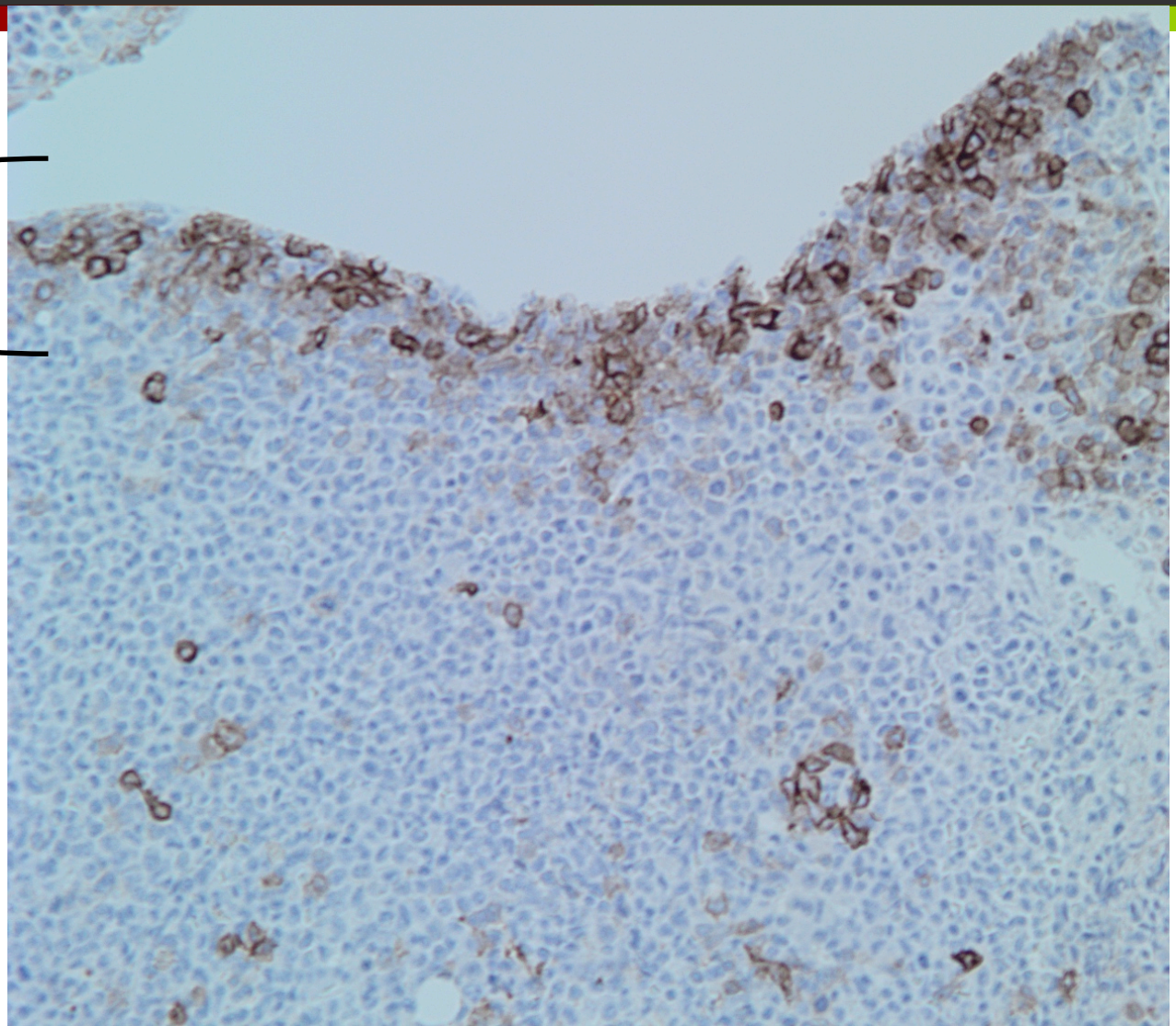
Trephine Biopsy Findings

Hypercellular biopsy

Megakaryocyte with normal morphology



CD117 immunohistochemistry



Expanded paratrabecular cuffs of immature cells

Preliminary diagnosis

- **Favors Atypical Chronic Myeloid Leukemia, chronic phase. Molecular and cytogenetics is pending.**

2016 WHO Definition of Atypical CML

- PB leukocytosis due to increased numbers of neutrophils & their precursors comprising >10%
- Dysgranulopoiesis, which may include chromatin clumping
- No or minimal absolute basophilia; basophils <2%
- No or minimal absolute monocytosis; monocytes <10%
- Hypercellular BM with granulocytic proliferation & dysplasia with or without dysplasia in erythroid & mega lineages
- <20% blasts in PB and BM
- No evidence of PDGFRA, PDGFRB, or FGFR1 or PCM1-JAK2
- Not meeting criteria for BCR-ABL+ CML, PMF, PV, ET

Cytogenetics & Molecular

- Cytogenetics: 46XX [20]
- PCR: Negative for BCR-ABL1 (p190 and p210), Negative for FLT3-ITD, **Positive for NPM1 mutation**
- **NGS panel (53 additional genes) pending**

Reference	Genes mutated in order of frequency	Gene mutations not detected	CMML	CNL
Wang et al. 2014 Targeted sequencing N=65 aCML	**NRAS** JAK2 V617F FLT3 CEBPA	CSF3R CALR MPL KIT IDH1/IDH2 **NPM1**	N/A	N/A
Piazza et al. 2013 Targeted sequencing N=61 aCML	TET2 ASXL1 **SETBP1** EZH2 **NRAS** CBL IDH2 CEBPA	**NPM1** JAK2 FLT3 DNMT3A IDH1	**SETBP1**	**SETBP1**
Meggendorfer et al. 2013 Targeted sanger sequencing N=60 aCML	ASXL1 **SETBP1** CBL	JAK2 exon 12	**SETBP1**	
Gambacorti-Passerini et al. 2015 Whole-exome sequencing N=15 aCML	**SETBP1** **NRAS** EZH2 ASXL1 **ETNK1** U2AF1		**ETNK1**	
Patnaik et al. 2017 Targeted panel N=25 aCML	ASXL1 TET2 **NRAS-16%** **SETBP1-12%** RUNX1 **ETNK1-8%** PTPN11	TP53 CALR MPL IDH1 **NPM1** CEBPA CBL		

Next generation sequencing

Gene	NM id	Variant (cDNA)	Variant (AA)	Variant Allele frequency	Classification
NRAS	NM_002524.3	c.35G>A	p.Gly12Asp (G12D)	46.8	1
DNMT3A	NM_022552.4	c.2645G>A	p.Arg882His (R882H)	47	1 or 2
IDH1	NM_005896.3	c.395G>A	p.Arg132His (R132H)	45.7	1 or 2
NPM1	NM_002520.6	c.860_863dupTCTG	p.Trp288Cysfs*12	40.3	1 or 2
ASXL1*	NM_015338.5	c.4247A>G	p.Glu1416Gly (E1416G)	49.9*	3B or 4B

Sequencing was performed using the Illumina TruSight Myeloid Sequencing Panel (54 genes) *reported as an extremely rare SNP

Molecular Summary

- PCR: Negative for BCR-ABL1 (p190 and p210), Negative for FLT3-ITD, **Positive for NPM1 mutation**
- **NGS panel (53 additional genes):**
  - **5 variants identified (4 with suspected pathogenic effects)**
  - **NPM1 mutation confirmed**
  - **SETBP1 mutation germline**
  - **ETNK1 mutation not on NGS panel and not available at our institution**

Role of NPM1 in AML & MDS/MPN

- NPM1 is one of the most frequently mutated genes in AML (1/3 of cases) & is associated with good prognosis in cases with normal karyotype and absence of FLT3-ITD mutation.
- Falini et al. 2010: 85% of NPM1-mutated AML cases show normal karyotype
- NPM1 mutations are most often seen in *de novo* AML, however, several studies have now shown NPM1 mutation in MDS or MDS/MPN prior to blast counts reaching the threshold for AML diagnosis (Schnittger et al. 2011; Lin et al. 2015)
- Caudill et al. 2006: Shorter time to progression to AML in patients harboring NPM1 mutations in CMML (no studies on prognosis/significance in aCML)

aCML-Diagnostic & Prognostic dilemmas

- **Diagnostic**-
  - No mutation completely characteristic of aCML- SETBP1 & ETNK1 are only seen in a small fraction of cases.
  - The most common mutations ASXL1, DNMT3A, TET2 are not specific for aCML or even MDS and can be present in clonal hematopoiesis of indeterminate significance (CHIP)
- **Prognostic**-
  - aCML is a rare disease with studies to date only including small samples of patients; the largest, a multicenter study, had 65 patients
  - Typically aCML has a poor prognosis with overall survival between 14-30 months and a rate of progression to AML of 40%
  - ASXL1 & SETBP1 mutations may be associated with worse prognosis
  - The prognostic significance of a NPM1 mutation is unclear since it is found so infrequently in MDS/MPNs. In CMML, it may represent accelerated disease with shorter progression to AML.
- **Discussion point-**
  - Should NPM1 mutation be AML disease defining regardless of blast count (and therefore part of the exclusionary criteria for aCML)?

Discussion points

- **How to best classify this case?**
  - Atypical CML (BCR-ABL1-negative)
    - Morphology is consistent, genetic alterations (SETBP1 not present, ETNK1 not in NGS panel)
    - 40% of cases have N-RAS mutations, although none have been reported to be NPM1-mutated
  - Chronic myelomonocytic Leukemia (CMML)
    - Monocytosis present, but less than 10% in PB
    - Neutrophilia is marked in aspirate & biopsy
    - Genetic alterations consistent with this diagnosis
  - MDS/MPN-unclassifiable
  - Are any of these genetic alterations sufficient for a diagnosis of AML with <20% blasts?

Final Diagnosis

- Our Diagnosis: Hypercellular bone marrow with features of MDS/MPN and NPM1 mutation
- Panel Diagnosis: **Atypical Chronic Myeloid Leukemia**

Follow-up

- Patient received on hydroxyurea and followed up at their community hospital 2 months later
- A bone marrow was sent to our institution for consultation
- Consultant diagnosis: AML with myelodysplasia-related changes
  - Blasts with monocytic features/promonocytes greater than 30%
  - Eight months since initial diagnosis, patient is alive on palliative azacytidine

Acknowledgements

- UHN Toronto General Hospital Hematopathology Group
  - Dr. Graeme Quest
  - Dr. Rashmi Goswami
  - Dr. Anne Tierens
  - Dr. David Barth
- Dr. Michael Rauh (KGH/Queen's University)
- UHN advanced molecular diagnostics group
  - Dr. Tracey Stockley
  - Nisha Kanwar

UNIVERSITY OF TORONTO

1. Caudill, J. S. C. *et al.* C-terminal nucleophosmin mutations are uncommon in chronic myeloid disorders. *BJH.* 638–641 (2006).

2. Dao, K. T. & Tyner, J. W. What's different about atypical CML and chronic neutrophilic leukemia? *ASH Education Book.* 264–271. (2015).

3. Gambacorti-passerini, C. B. *et al.* Recurrent ETNK1 mutations in atypical chronic myeloid leukemia. *Blood.* **125,** 499–504 (2017).

4. Lin, P. & Falini, B. Acute Myeloid Leukemia With Recurrent Genetic Abnormalities Other Than Translocations. *AJCP.* 19–28 (2015).

5. Meggendorfer, M. *et al.* SETBP1 mutations occur in 9 % of MDS / MPN and in 4 % of MPN cases and are strongly associated with atypical CML , monosomy 7 , isochromosome i(17)(q10), ASXL1 and CBL mutations. *Leukemia.* **27,** 1852–1860 (2013).

6. Pardanani, A. *et al.* CSF3R T618I is a highly prevalent and specific mutation in chronic neutrophilic leukemia. *Leukemia.* **27,** 1870–1873 (2013).

7. Patel, J. L. *et al.* Coexisting and cooperating mutations in NPM1 -mutated acute myeloid leukemia. *Leuk. Res.* **56,** 7–12 (2017).

8. Patnaik, M. M. *et al.* Targeted next generation sequencing and identification of risk factors in World Health Organization defined atypical chronic myeloid leukemia. *Am. J. Hematology.* 542–548 (2017).

9. Piazza, R. *et al.* Recurrent SETBP1 mutations in atypical chronic myeloid leukemia. *Nature Genetics* **45,** 18–24 (2013).

10. Schnittger, S. *et al.* Characterization of NPM1 -mutated AML with a history of myelodysplastic syndromes or myeloproliferative neoplasms. *Leukemia* **25,** 615–621 (2011).

11. Steensma, D. P. *et al.* Perspectives Clonal hematopoiesis of indeterminate potential and its distinction from myelodysplastic syndromes. *Blood* **126,** 9–17 (2017).

12. sukhai, M. A. *et al.* A classification system for clinical relevance of somatic variants identified in molecular profiling of cancer. *Genetics in Medicine* **18,** (2016).]

13. Wang, S. A. *et al.* Atypical chronic myeloid leukemia is clinically distinct from unclassifiable myelodysplastic / myeloproliferative neoplasms. *Blood* **123,** 2645–2652 (2017).

SH2017-0267

Dr. Liontos

Atypical chronic myeloid leukemia, BCR-ABL1-negative