

2017 Society for Hematopathology Workshop Case
Presentation:

**A 72 year old female with Waldenstrom macroglobulinemia
and a rising white blood cell count**

Case # SH2017-0108

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Clinical presentation

- 72 year-old female with no significant past medical history who was diagnosed with an IgM kappa monoclonal gammopathy of undetermined significance in 2007 and followed expectantly.
- Laboratory studies in March 2016 showed the following:

Test	Result
WBC	2.87 K/ μ L
Differential	43% neutrophils 57% lymphocytes
Hematocrit	30.9%
Platelets	196 K/ μ L
Serum IgM	1,321 mg/dL
Serum electrophoresis/immunofixation	IgM kappa (0.66 g/dL)

- Due to worsening anemia, she underwent a bone marrow biopsy, with findings consistent with lymphoplasmacytic lymphoma (LPL).
 - Molecular analysis was positive for an *MYD88* mutation (c.794T>C; p.L265P).

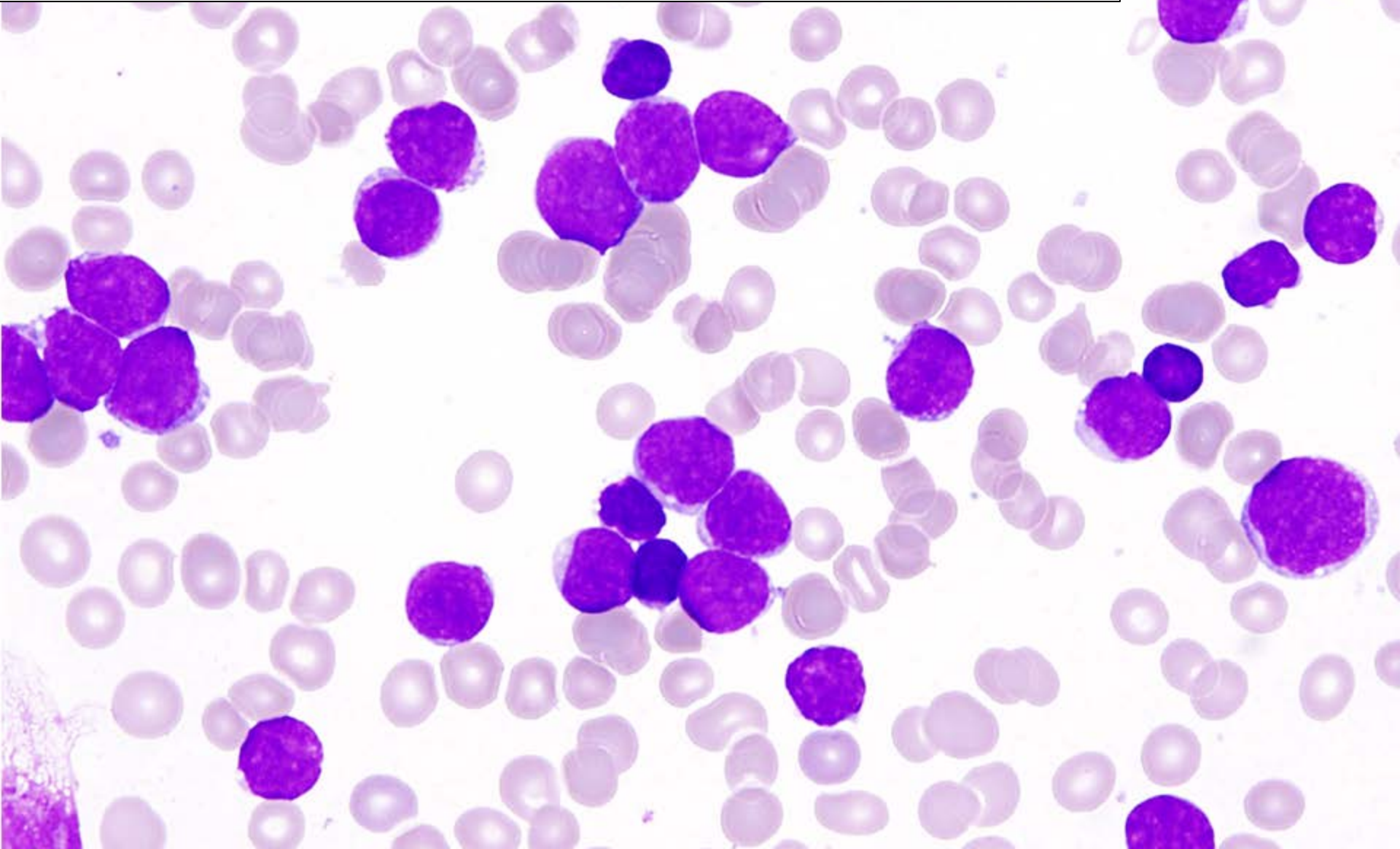
Clinical presentation

- The patient presented six weeks later with acutely increased dyspnea on exertion and night sweats.
- A CBC at that time showed:

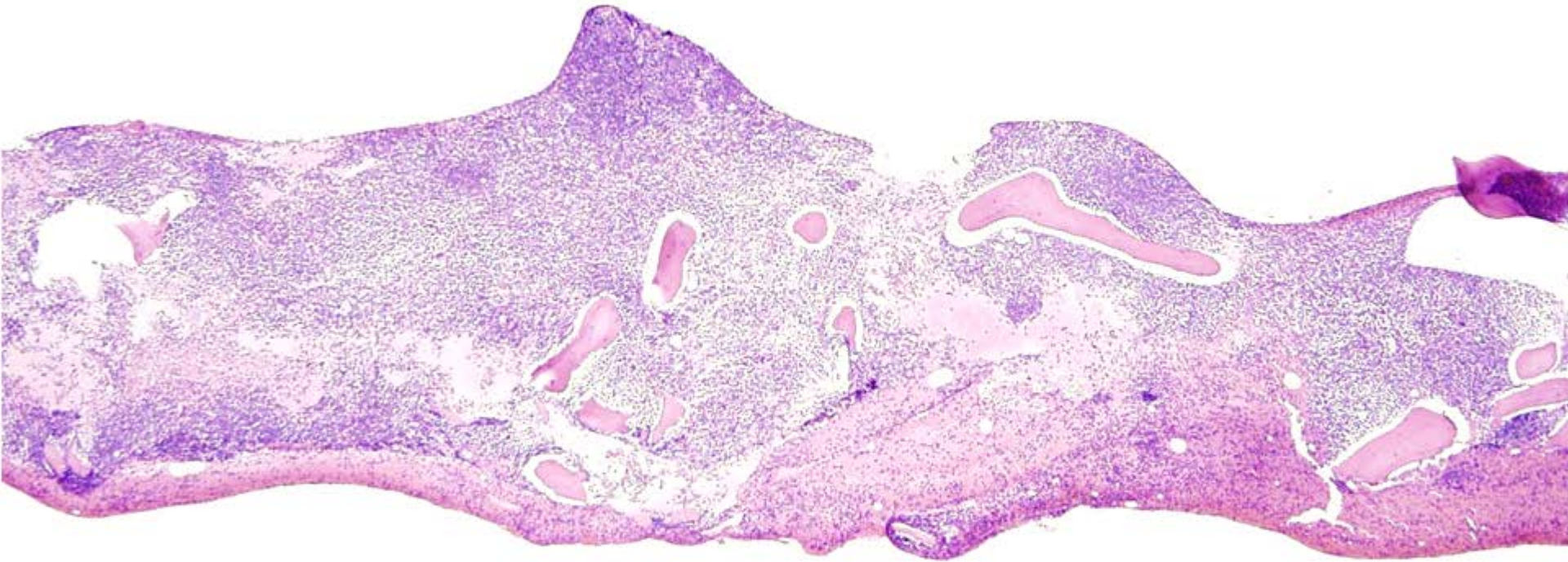
Test	Result
WBC	108 K/ μ L
Differential	5% neutrophils 33% lymphocytes 62% others (immature-appearing cells)
Hematocrit	28.5%
Platelets	66 K/ μ L

- Clinical concern was for progression of the patient's known LPL.
- However, peripheral blood flow cytometry showed a population of CD45-dim, CD19-positive, CD20-negative cells; along with peripheral smear morphology, the possibility of acute leukemia was raised.
- A repeat bone marrow biopsy was performed.

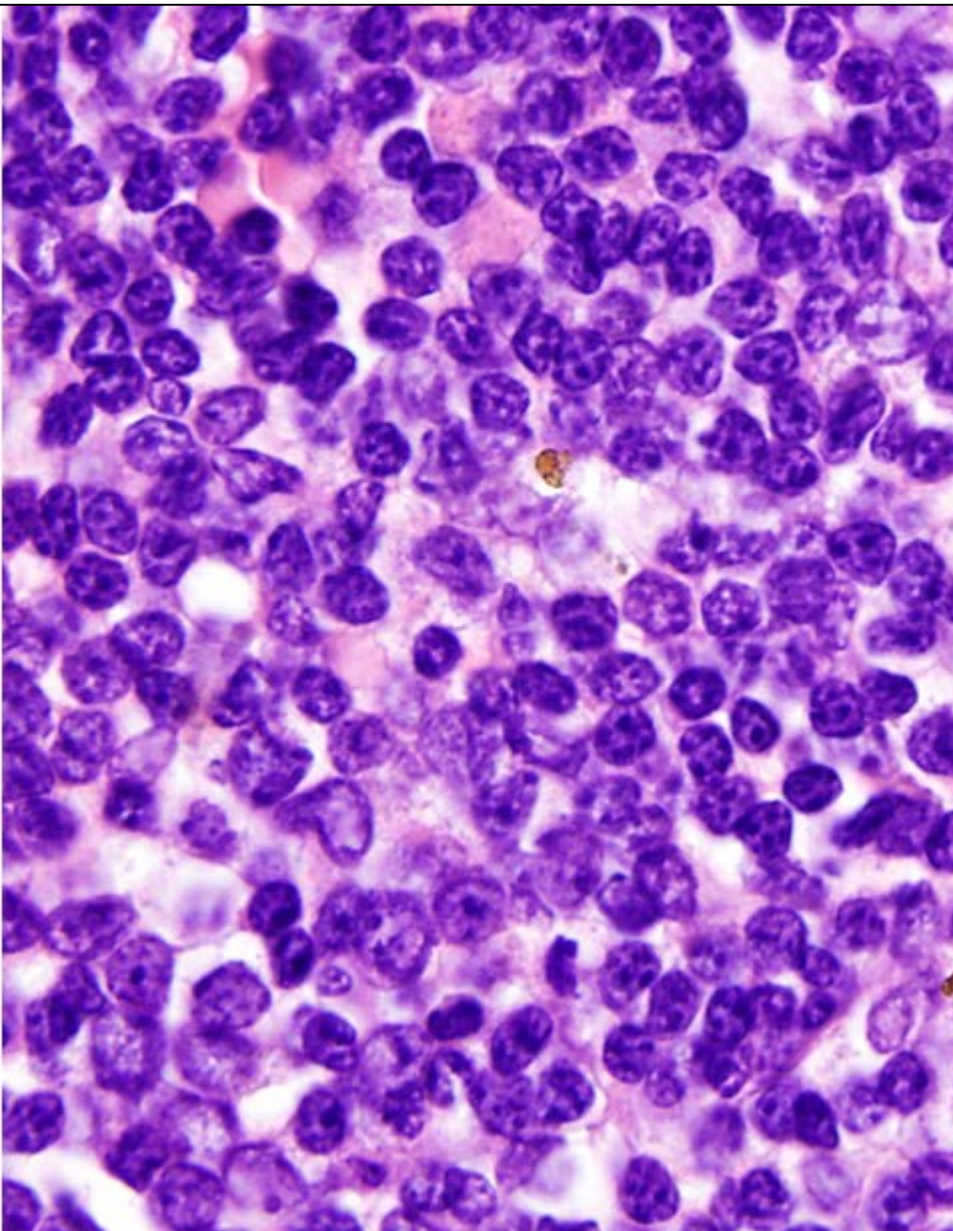
Aspirate smear: 95% lymphoid cells with small to intermediate sized mature lymphocytes and lymphoplasmacytoid forms as well as a population of intermediate to large sized, more immature-appearing cells with dispersed chromatin and distinct nucleoli.



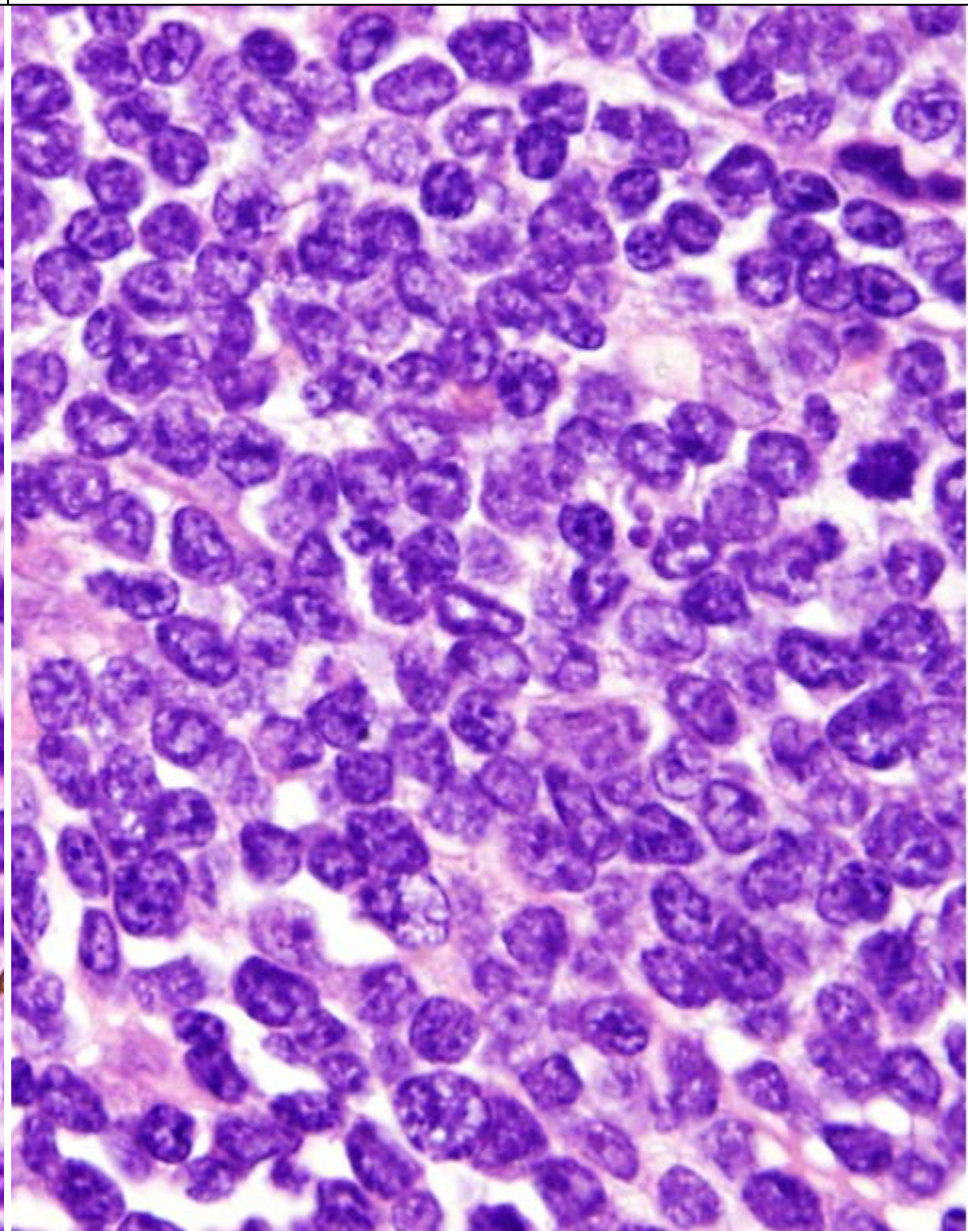
Hypercellular bone marrow biopsy (95% cellular) with a diffuse infiltrate of small to intermediate sized lymphoid cells with round to irregular nuclei and variably condensed chromatin



Small to intermediate sized mature lymphocytes with condensed/clumped chromatin.

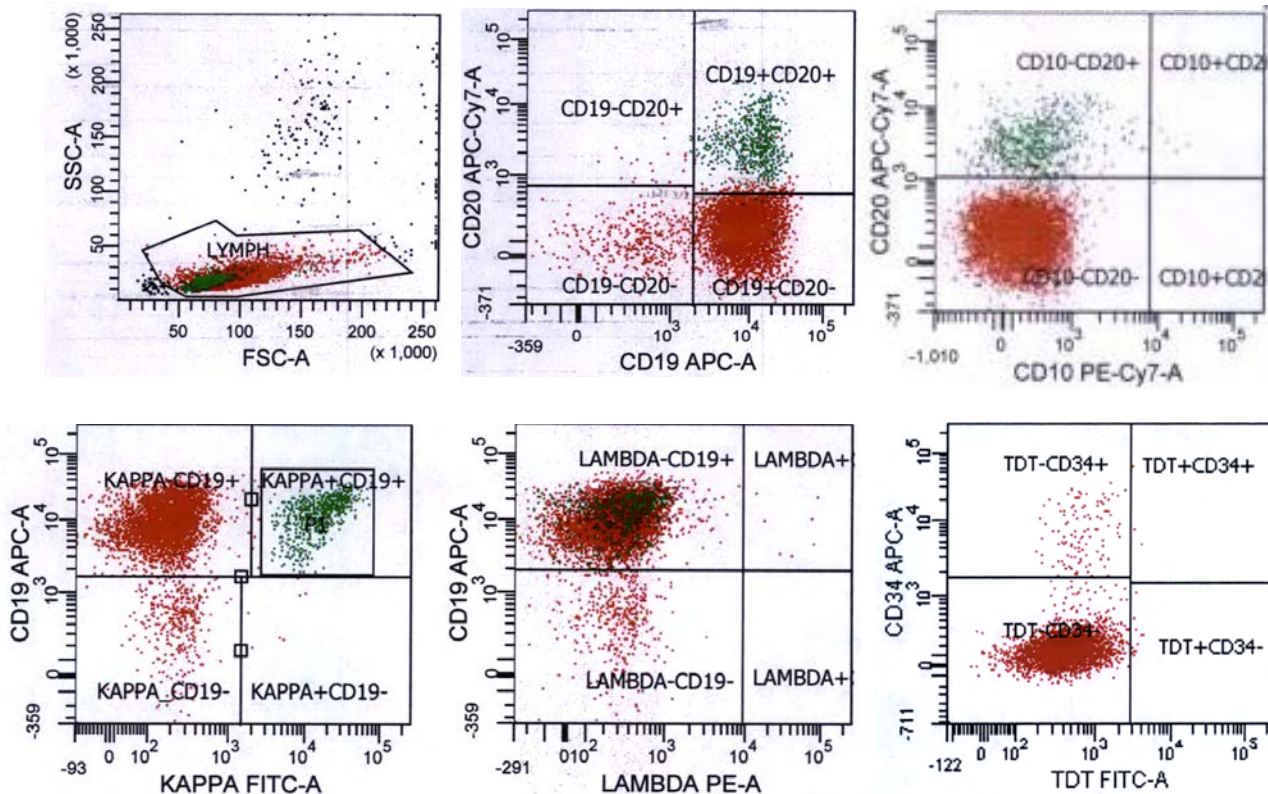


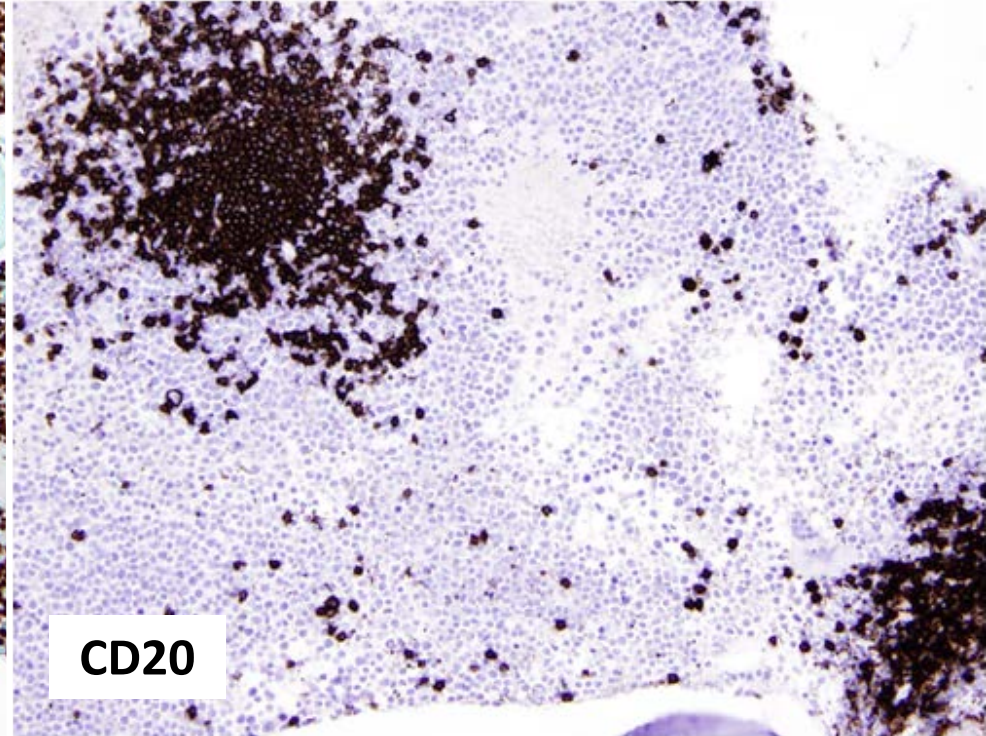
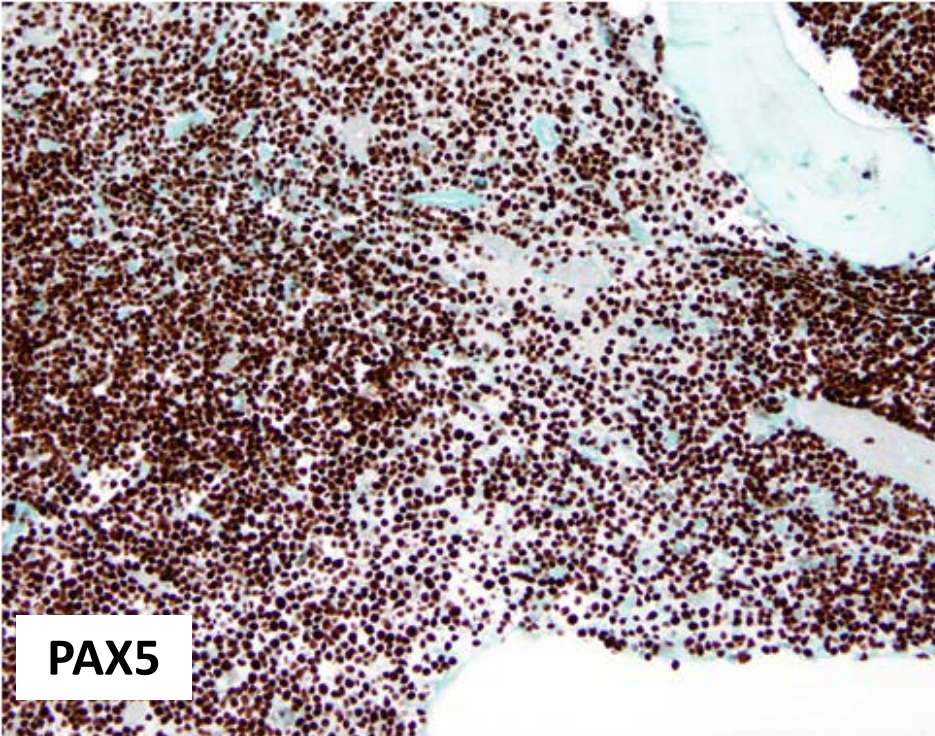
Intermediate to large sized, immature-appearing cells with more dispersed chromatin and distinct nucleoli.



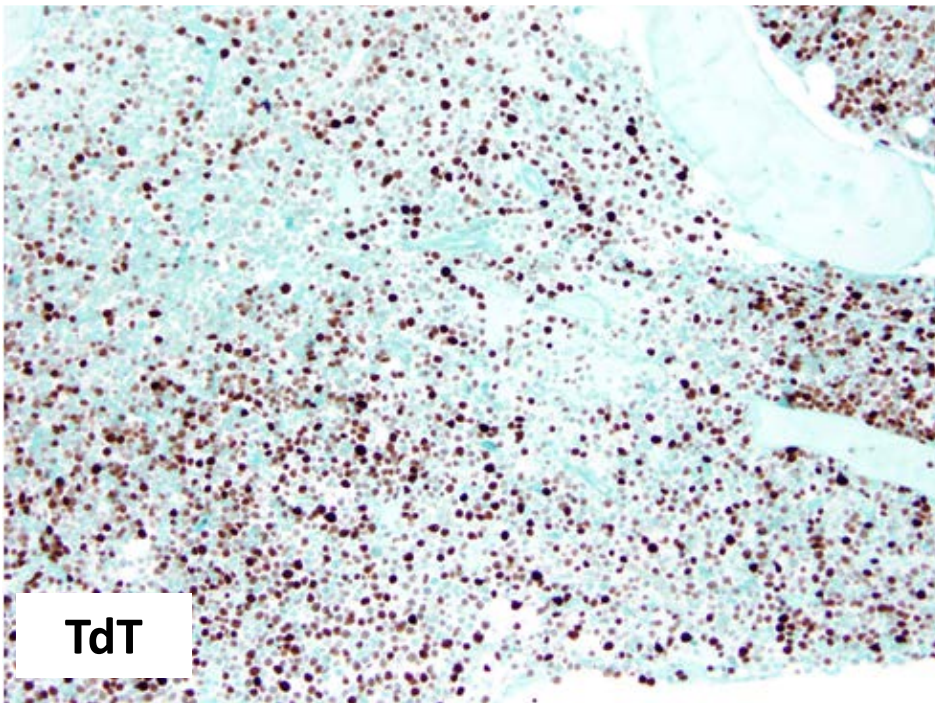
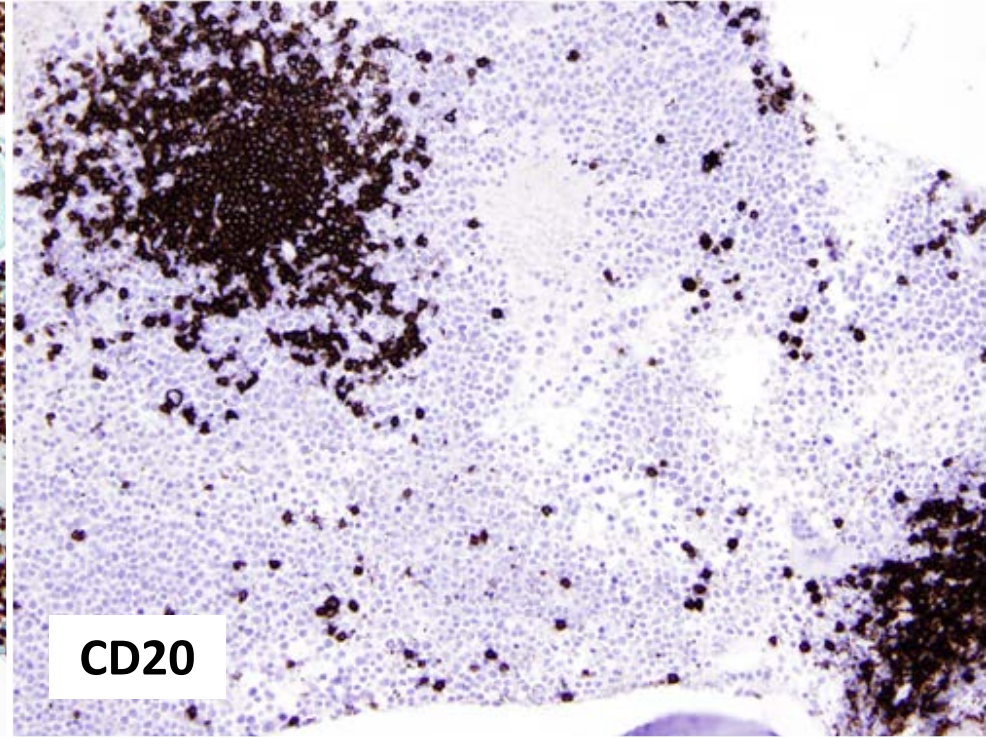
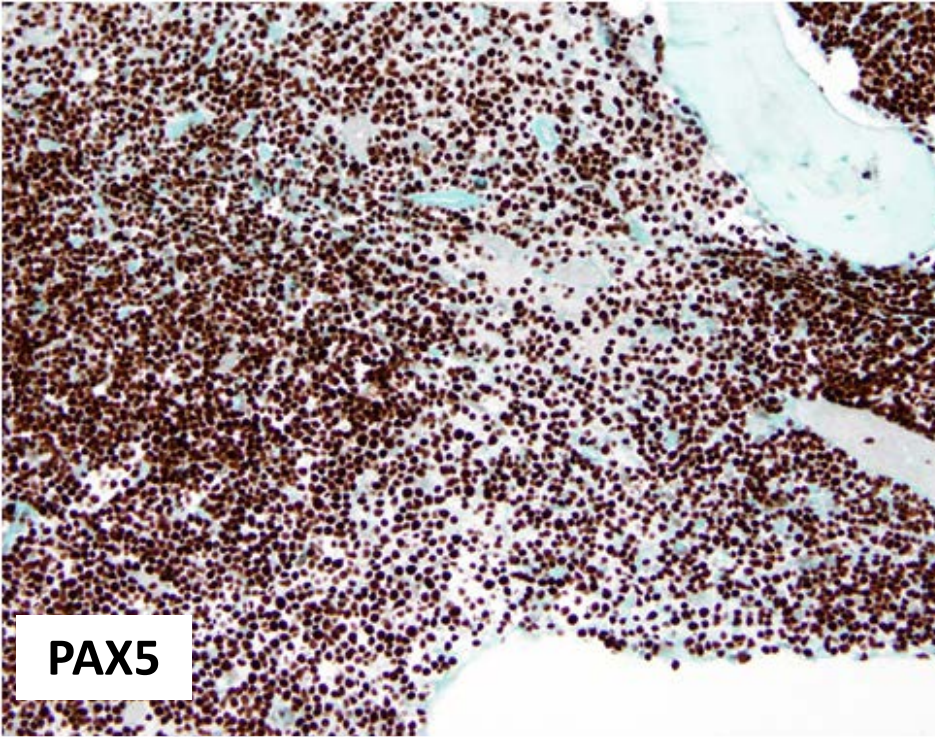
Flow cytometry identified two abnormal B-cell populations:

1. One population (9% of total events) was composed of small-sized cells that expressed CD19 and CD20, exhibited monotypic surface kappa light chain staining, and were negative for CD5, CD10, CD11c, CD34, and TdT.
2. The second population (77% of total events) was composed of cells ranging in size from small to large that showed dim CD45 expression, were positive for CD19, showed no demonstrable surface light chain expression, and were negative for CD20, CD5, CD10, CD34, and TdT.



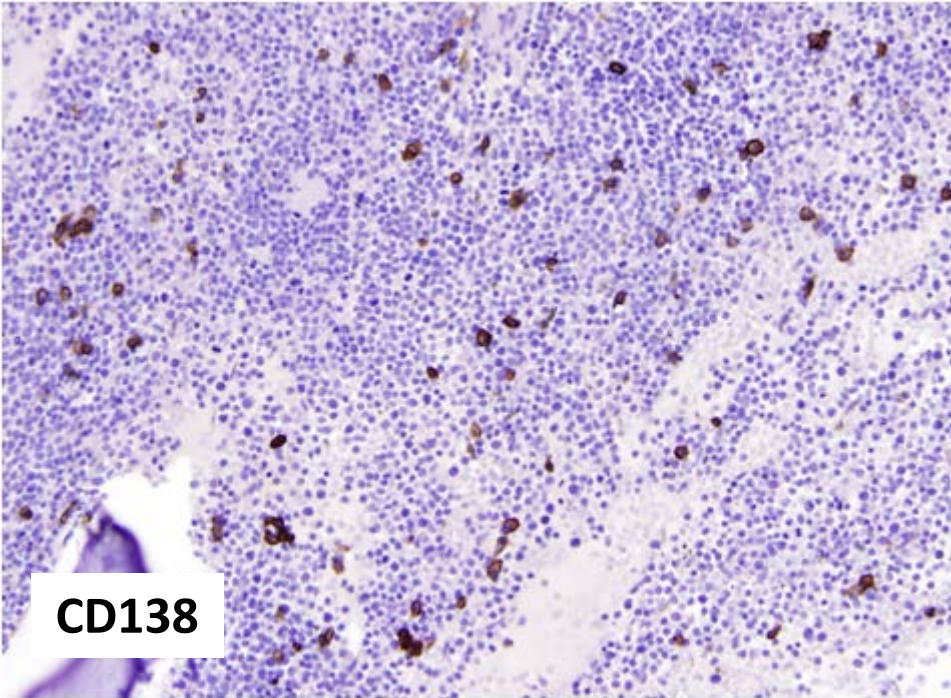


Immunohistochemical studies showed that PAX5 was diffusely positive in all cells. CD20 was positive only in the small, mature lymphocytes.



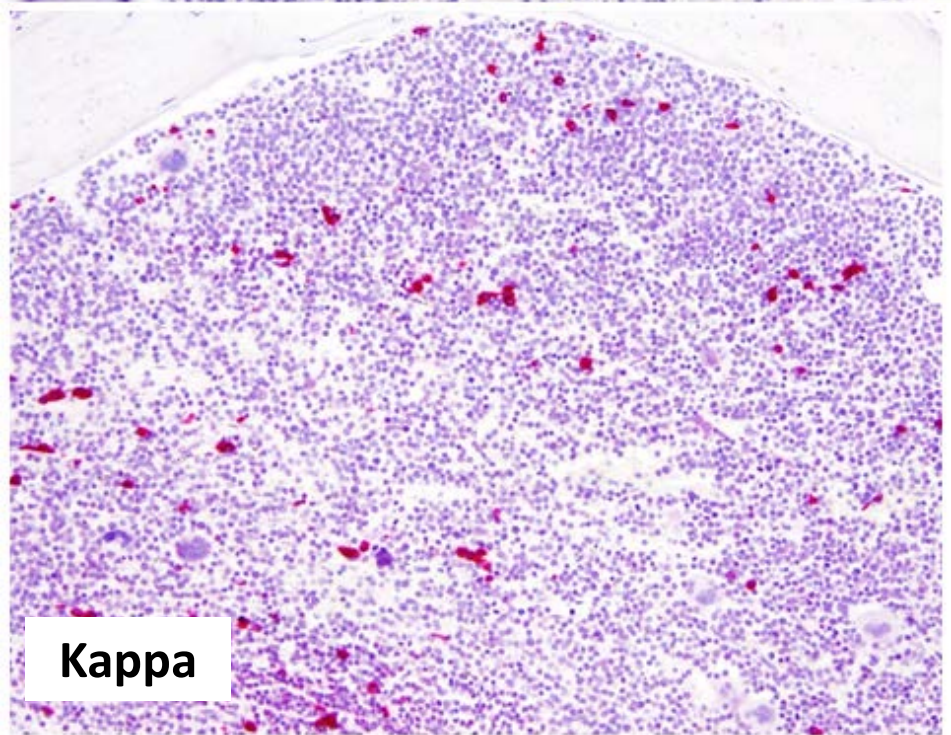
Immunohistochemical studies showed that PAX5 was diffusely positive in all cells. CD20 was positive only in the small, mature lymphocytes.

A TdT stain performed after cytogenetic results were available was positive only in the immature-appearing cells.

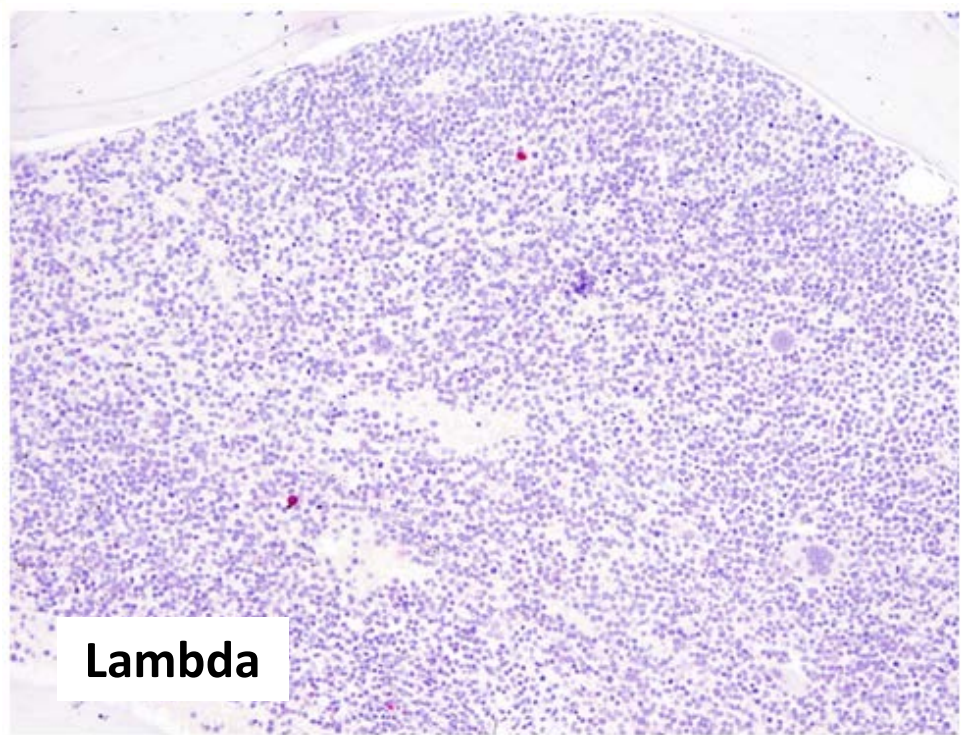


CD138

Immunohistochemical and in situ hybridization studies highlighted scattered CD138-positive plasma cells, which showed monotypic expression of immunoglobulin kappa light chain.



Kappa



Lambda

Cytogenetic results

- Two distinct karyotypes were detected (K1, unstimulated; K2, CpG-stimulated):
 - **K1: 46,XX,t(4;11)(q21;q23)[10]/46,XX[5].ish t(4;11)(5'MLL+;3'MLL+)[5]**
 - **K2: 46,XX,der(9)t(4;9)(q?21;q34)[4]/46,XX[6]**
- 15 metaphases were analyzed from an unstimulated bone marrow specimen, of which 10 metaphases contained a classic t(4;11) translocation. *KMT2A (MLL)* rearrangement was confirmed by FISH.
- 10 metaphases were analyzed from a culture stimulated with CpG, a mitogen for mature B-cells but not B-lymphoblasts, of which 4 metaphases contained an unbalanced der(9)t(4;9).

Next generation sequencing results

- Targeted next generation sequencing of 95 genes commonly mutated in hematologic disorders was performed via paired end sequencing using an Illumina MiSeq (Illumina, Inc. San Diego, CA, USA). A TruSeq Custom Amplicon Kit was used for library preparation

Pathogenic Single Nucleotide Variants and Small Insertions/Deletions:

MYD88 NM_002468 c.794T>C p.L265P; allele frequency (AF) of 14.8%

Other Variants of Unknown Significance (VUS):

CSF3R NM_156039 c.2092C>T p.R698C; AF of 45.3%

TP53 NM_000546 c.847_847insGGG p.282_283insG; AF of 32.3%

Follow up

- The overall findings were interpreted as involvement by B-lymphoblastic leukemia with a *KMT2A* (*MLL*) rearrangement and persistent involvement by LPL.
- Due to a delay in treatment initiation, repeat bone marrow analysis was performed prior to the start of induction therapy for B-lymphoblastic leukemia.

	Diagnostic bone marrow biopsy	Repeat bone marrow biopsy
Flow cytometry: CD45-dim, CD20-negative population lacking surface light chain expression	77% of total events	92% of total events
Cytogenetics	K1: 46,XX,t(4;11)(q21;q23)[10]/ 46,XX[5] K2: 46,XX,der(9)t(4;9)(q?21;q34)[4]/ 46,XX[6]	K1:46,XX,t(4;11)(q21;q23)[8]/ 46,XX[5] K2: not obtained
Molecular analysis		
MYD88 p.L265P	14.8%	Not detected
CSF3R p.R698C (VUS)	45.3%	51.5%
TP53 p.282_283insG (VUS)	32.3%	53.9%

Contribution of cytogenetic findings to diagnosis

- Marrow involvement by two morphologically and phenotypically distinct B cell populations could be consistent with progression of LPL.
- However, the cytogenetic and molecular findings argue against this.
 - *KMT2A* rearrangements have rarely been reported in B cell lymphomas (6 cases in the literature¹).
 - One case of large cell transformation of splenic marginal zone lymphoma.
 - To our knowledge, *KMT2A* rearrangements have not been reported in progression of LPL.
 - Cytogenetic analysis of the CpG-stimulated culture (likely representing the mature B cell LPL clone) showed a der(9)t(4;9) without a *KMT2A* rearrangement.
 - Identification of the *KMT2A* rearrangement in the unstimulated sample and in the absence of the der(9)t(4;9) suggests that the *KMT2A* rearrangement occurred in a distinct clone.

Contribution of molecular findings to diagnosis

- On sequential sequencing analysis, the *TP53* VUS AF increased from 32.3% to 53.9%.
- The *MYD88* mutation was below the assay limit of detection on the second sequencing analysis.
- Both the initial *TP53* VUS AF and the change in AF on subsequent analysis suggest that this variant is somatic rather than germline.
- The particular *TP53* variant seen here has not been reported in publically available databases (COSMIC or cBioPortal); however, it occurs at a known “hotspot” in the DNA binding domain (Arg282) and has been reported in the literature in two cases of ALL².

Along with morphologic and flow cytometric results, the overall cytogenetic and molecular findings are consistent with expansion of a *TP53*-mutated B-ALL clone and contraction of an *MYD88*-mutated LPL clone.

Panel diagnosis: 1. Lymphoplasmacytic lymphoma; 2. B-lymphoblastic leukemia with t(v;11q23.3), *KMT2A*-rearranged.

Additional thoughts

- Could the patient's B-ALL represent a therapy-related malignancy?
 - Although *KMT2A* rearrangements have been reported to occur at increased frequency in therapy-related B-ALL³, the patient had received no prior therapy for her LPL.
- How common are *TP53* mutations in B-ALL?
 - Reported to occur in approximately 6-15% of adult B-ALL cases^{2,4,5}.
 - Very common in low hypodiploid cases (approximately 90%)^{6,7}.
 - Mutations in Kinase-Ras pathway components reported in ~50% of *KMT2A*-rearranged B-ALL⁸.
 - *TP53* mutations reported in 16% of *KMT2A*-rearranged B-ALL².
- How commonly does B-ALL arise in patients with LPL?
 - Patients with LPL are at increased risk for DLBCL and MDS/AML⁹.
 - B-ALL arising in a patient with LPL has been reported only twice¹⁰; here, we present a third such case.

References

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Final panel diagnosis: 1. Lymphoplasmacytic lymphoma; 2. B-lymphoblastic leukemia with t(v;11q23.3), KMT2A-rearranged.

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