Is this Lymphocyte

Normal

Reactive

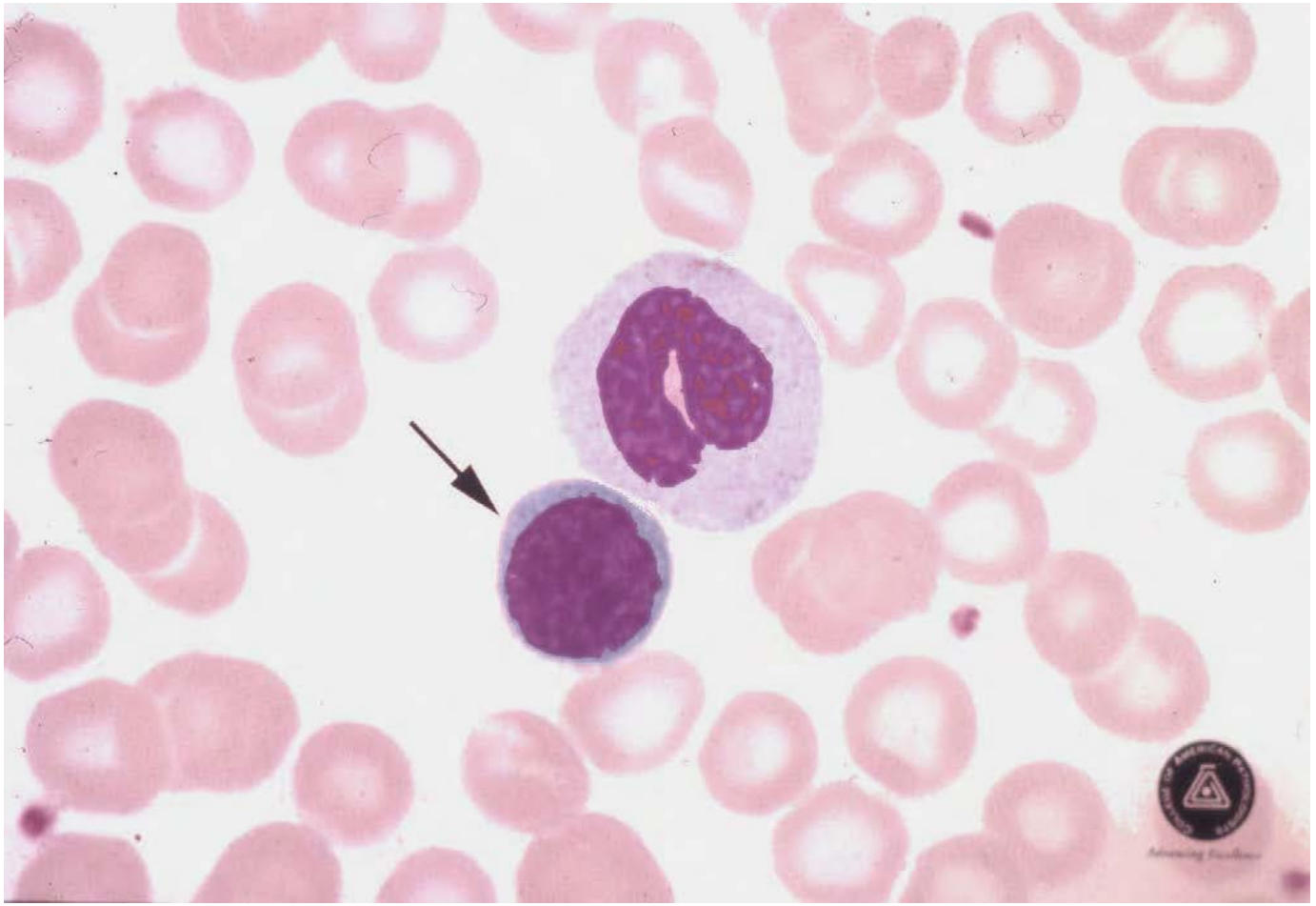
Malignant?

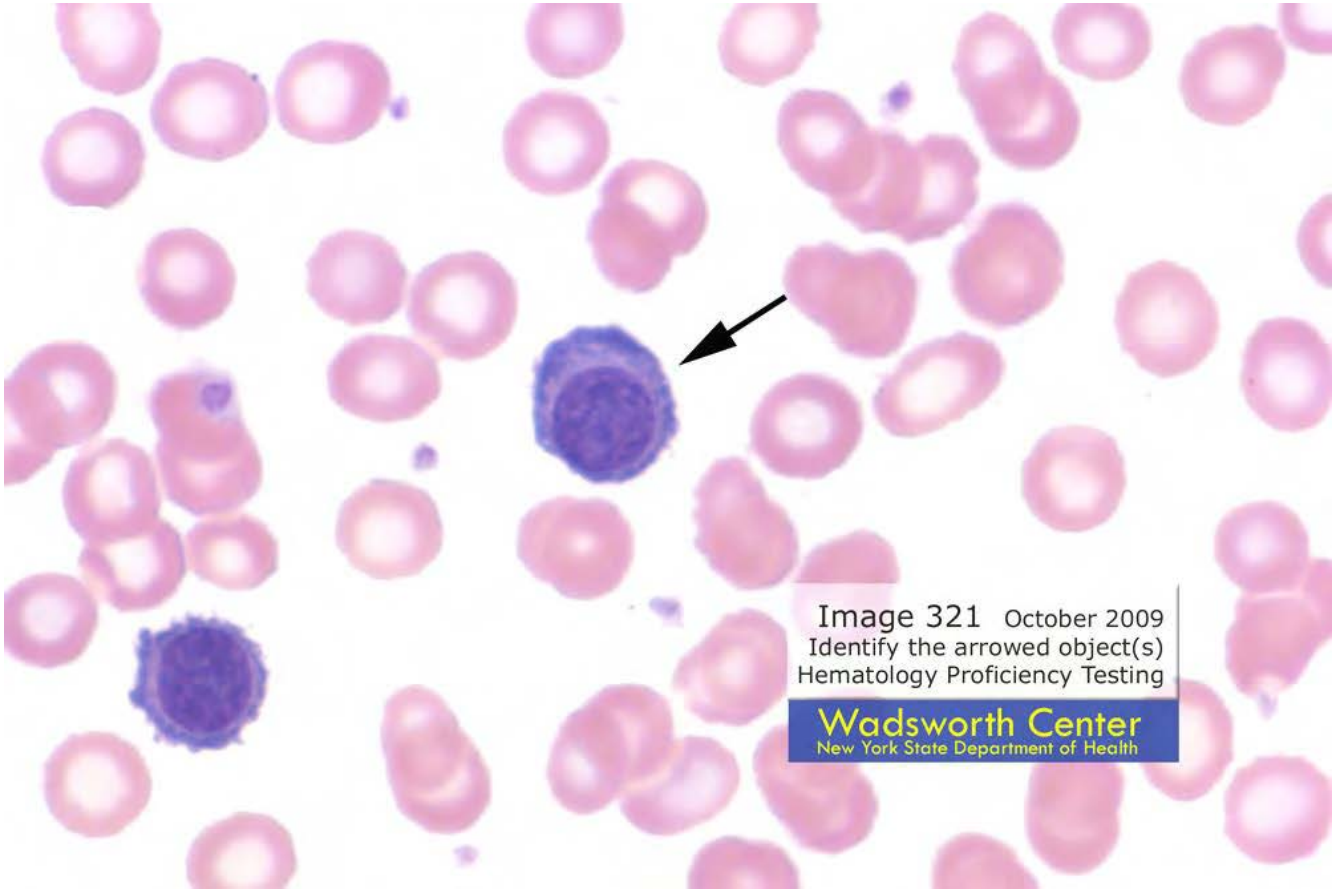
Steve Marionneaux

September 16, 2017

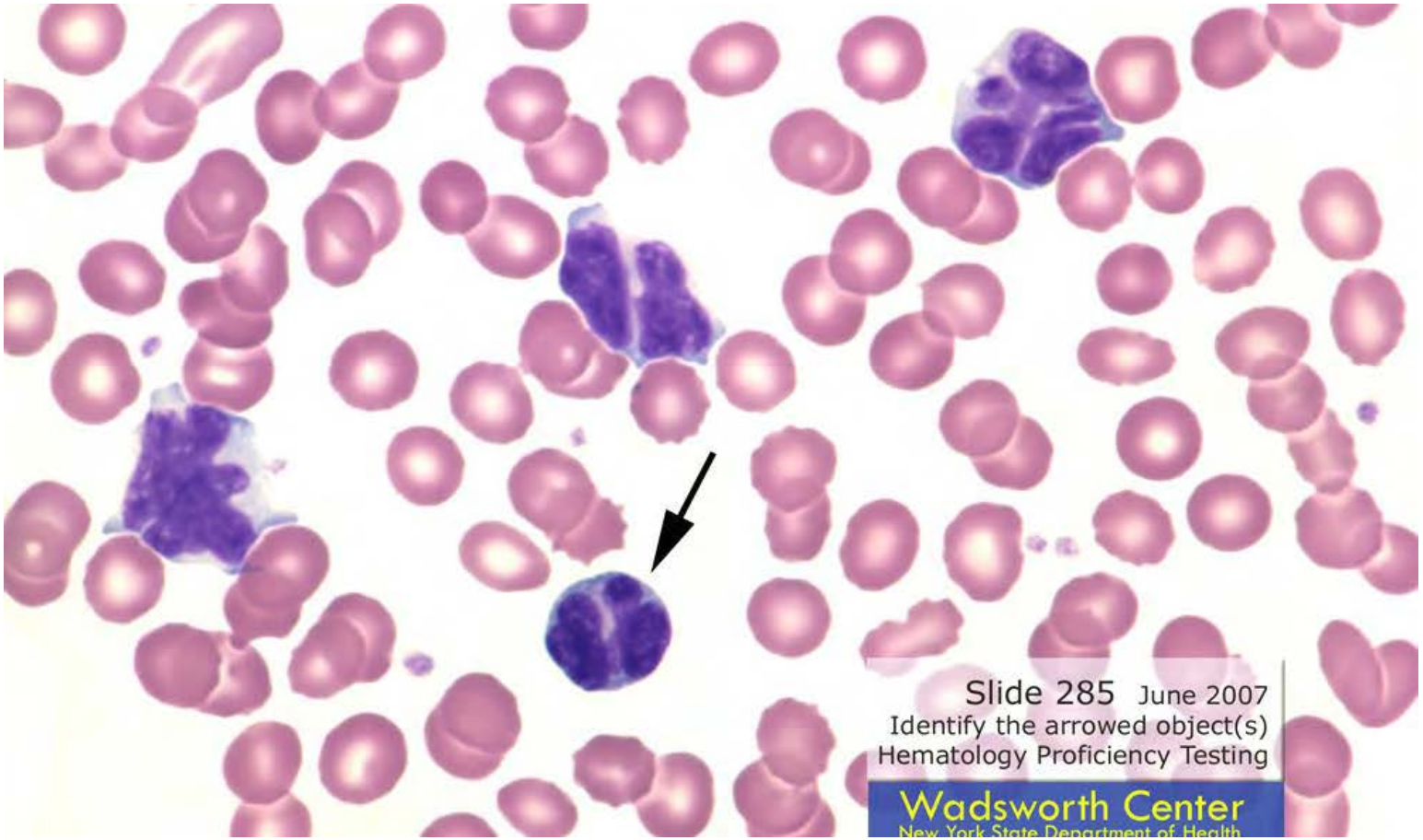
From CAP Survey

Lymphocyte correctly identified by 96.8% of participants.



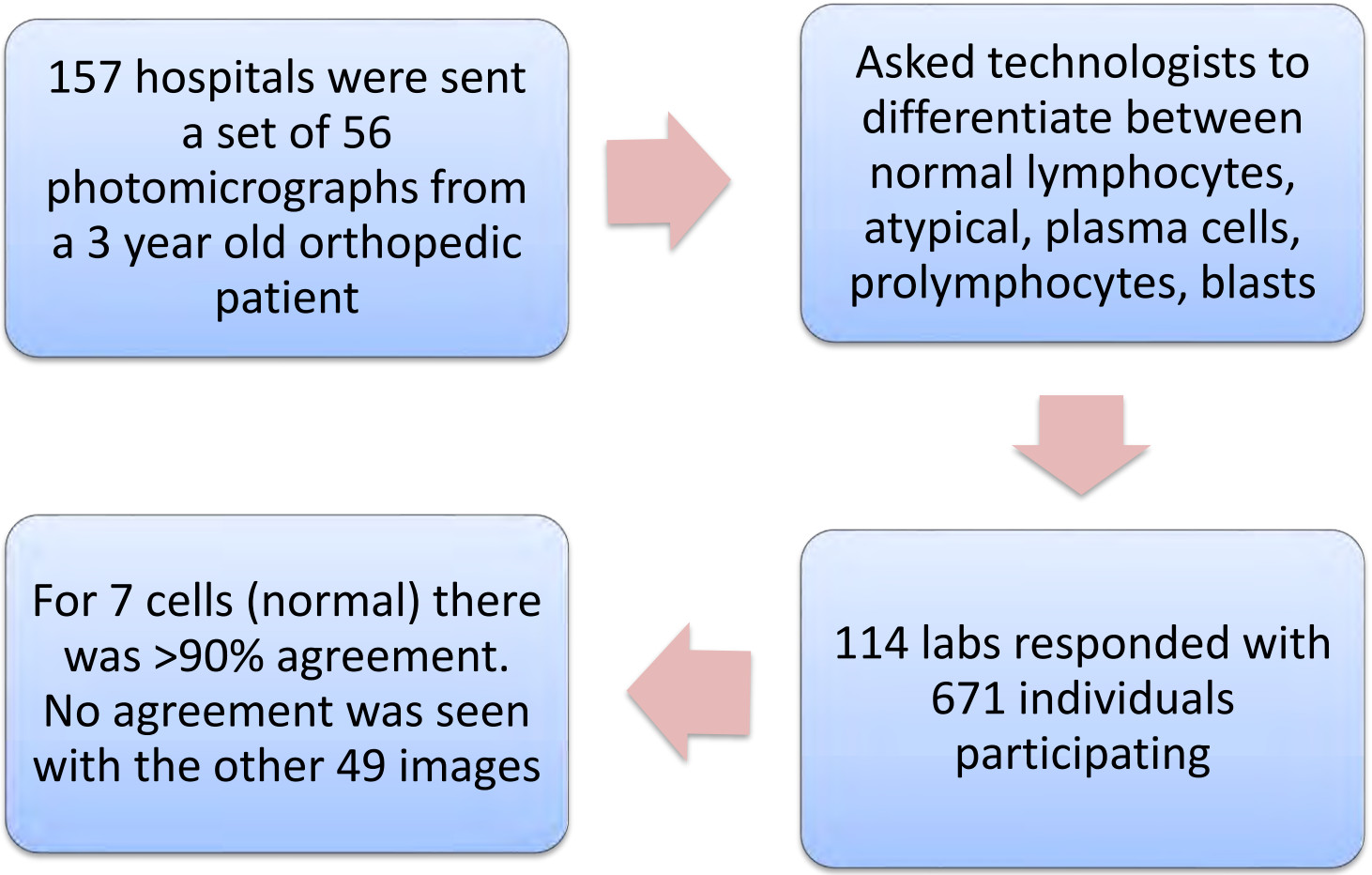


Number of Responses	Percent of Labs	Cell type or finding
228	63.0%	Plasma cell
74	20.4%	Hairy cell
29	8.0%	Nucleated red cell
22	6.1%	Reactive/Atypical lymphocyte
7	1.9%	Normal lymphocyte

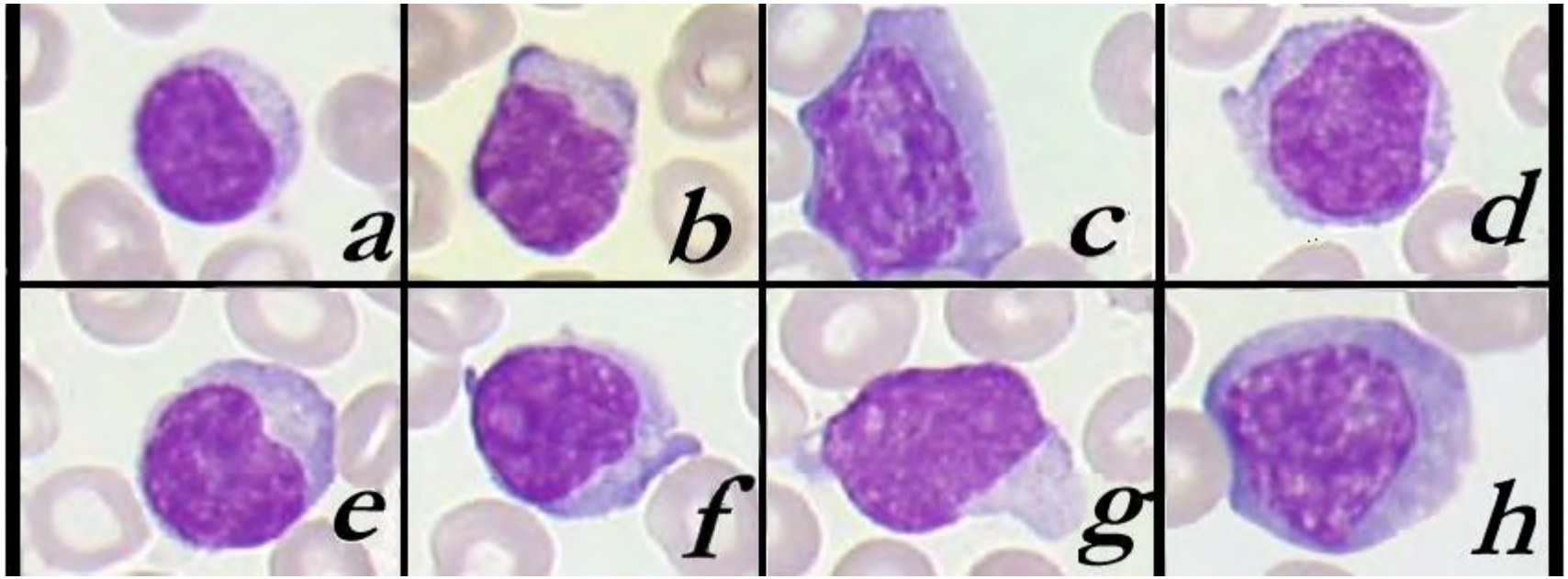


Number of Responses	Percent of Laboratories	Cell Identified
295	73.8%	Lymphoma/Sézary cell
67	16.8%	Atypical lymphocyte
22	5.5%	Monocyte
9	2.3%	Segmented neutrophil
4	1.0%	Normal lymphocyte

Study Which Evaluated Consistency with Morphologic Evaluation of Lymphocytes



van der Meer, W. et al. The divergent morphological classification of variant lymphocytes in blood smears. *J Clin Pathol.* 2007;*60:838-839*



van der Meer, W. et al. The divergent morphological classification of variant lymphocytes in blood smears. *J Clin Pathol.* 2007;*60:838-839*

The Divergent Morphological Classification of Variant Lymphocytes in Blood Smears

Summary of author's conclusions:

- Morphology of lymphocytes is complex; unfortunately a uniform definition of abnormal lymphocytes is lacking
- Confusing terminology is used: variant, atypical, etc
- Proving clinical information at bench should lead to a better interpretation of morphology
- Recognition of abnormal lymphs can contribute to rapid diagnosis of various conditions

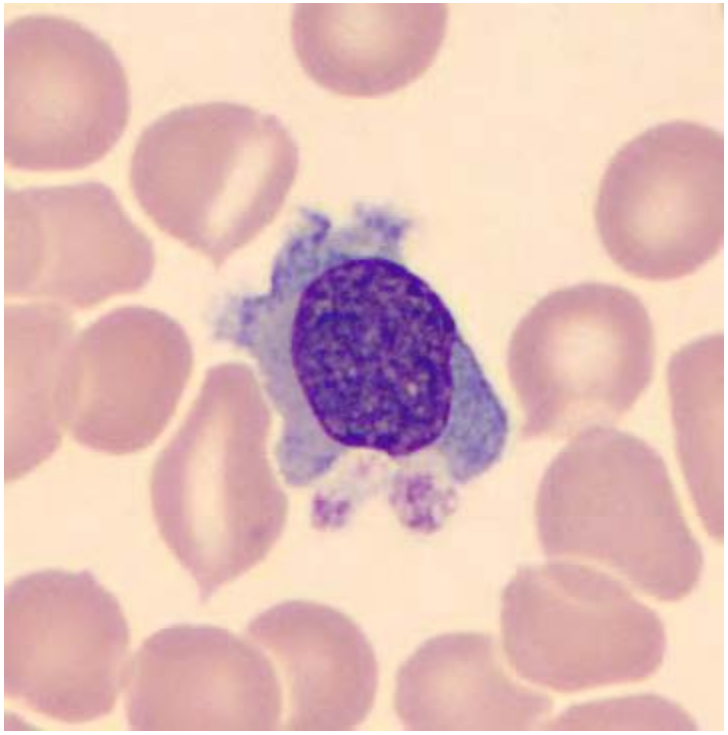
Today's talk will address these issues and hopefully provide useful information on how to recognize and classify the various forms of lymphoid cells that can be encountered on peripheral blood smears

van der Meer, W. et al. The divergent morphological classification of variant lymphocytes in blood smears. *J Clin Pathol.* 2007;*60:838-839*

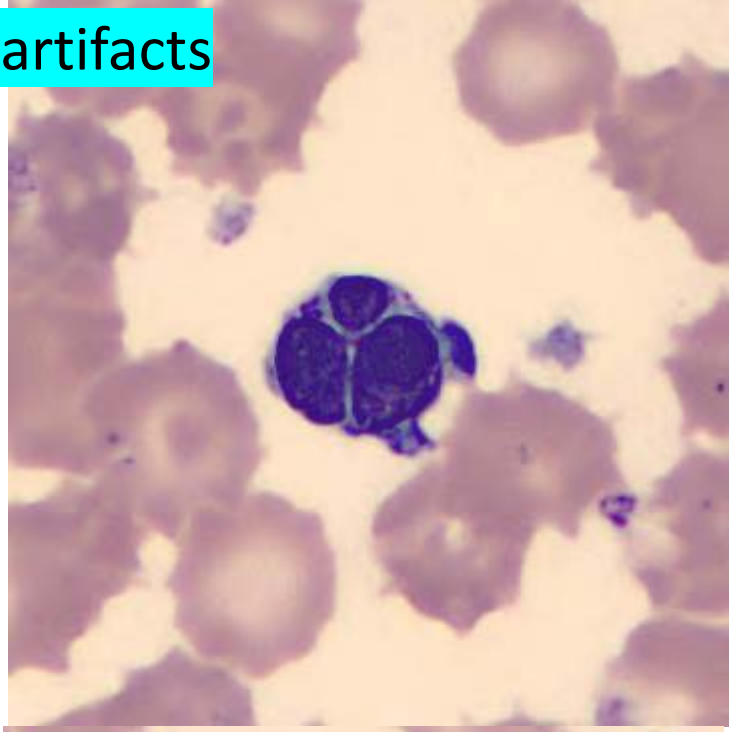
Consider sample age

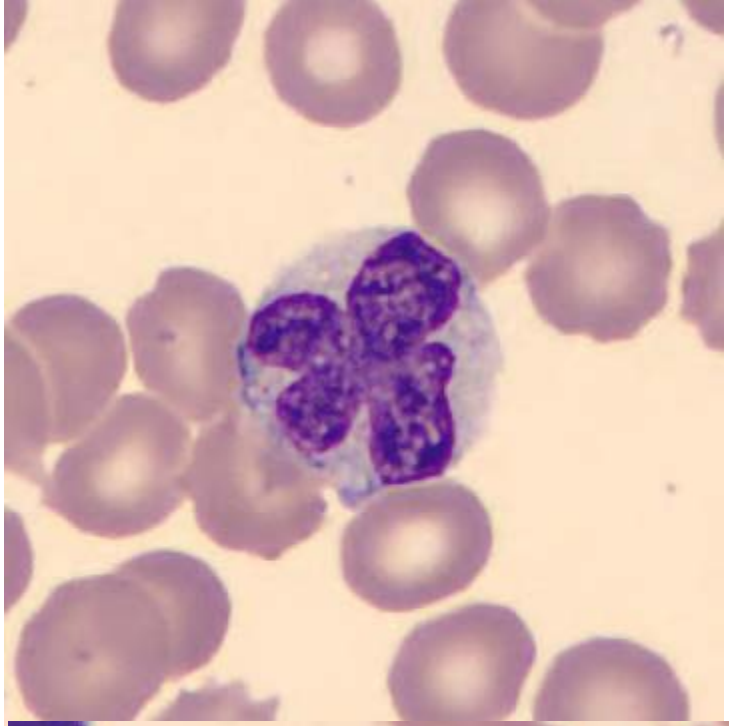
Storage artifacts in lymphocytes

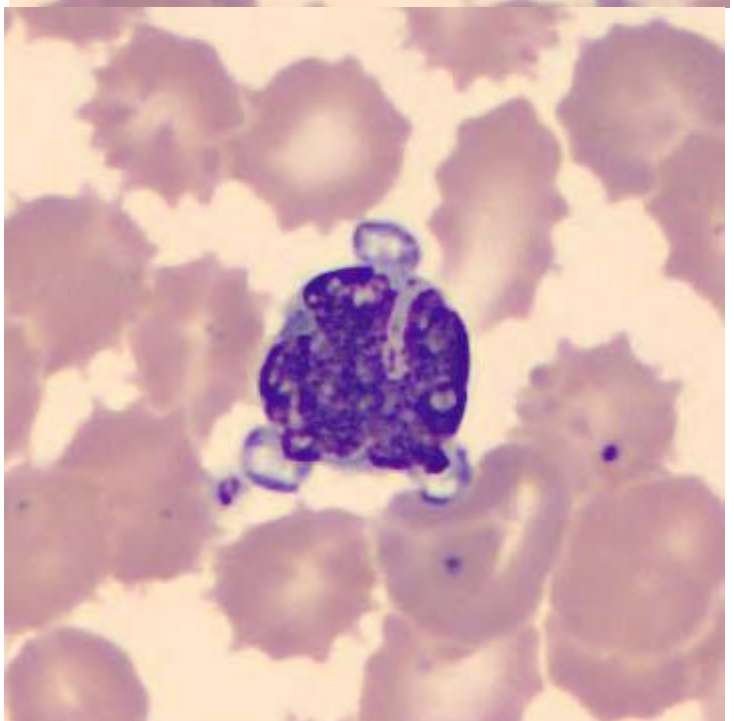
- Hyperchromatic cells
- Vacuolated
- Smudged or necrobiotic
- Nuclear membrane breakage, nucleoplasm leaks mimicking
  - Cytoplasmic inclusions
  - Nuclear lobes
- Cytoplasmic blebbing or projections

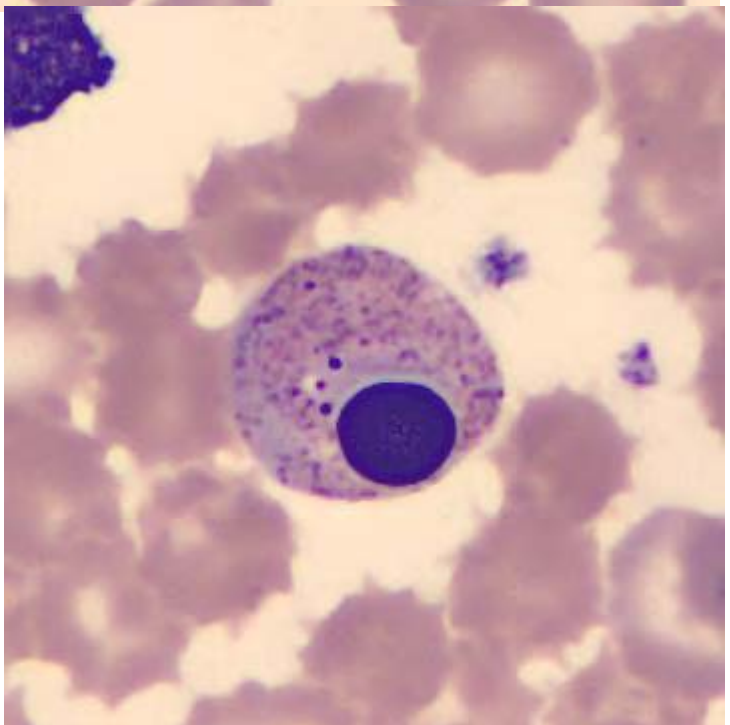


Storage artifacts









Normal Lymphocyte

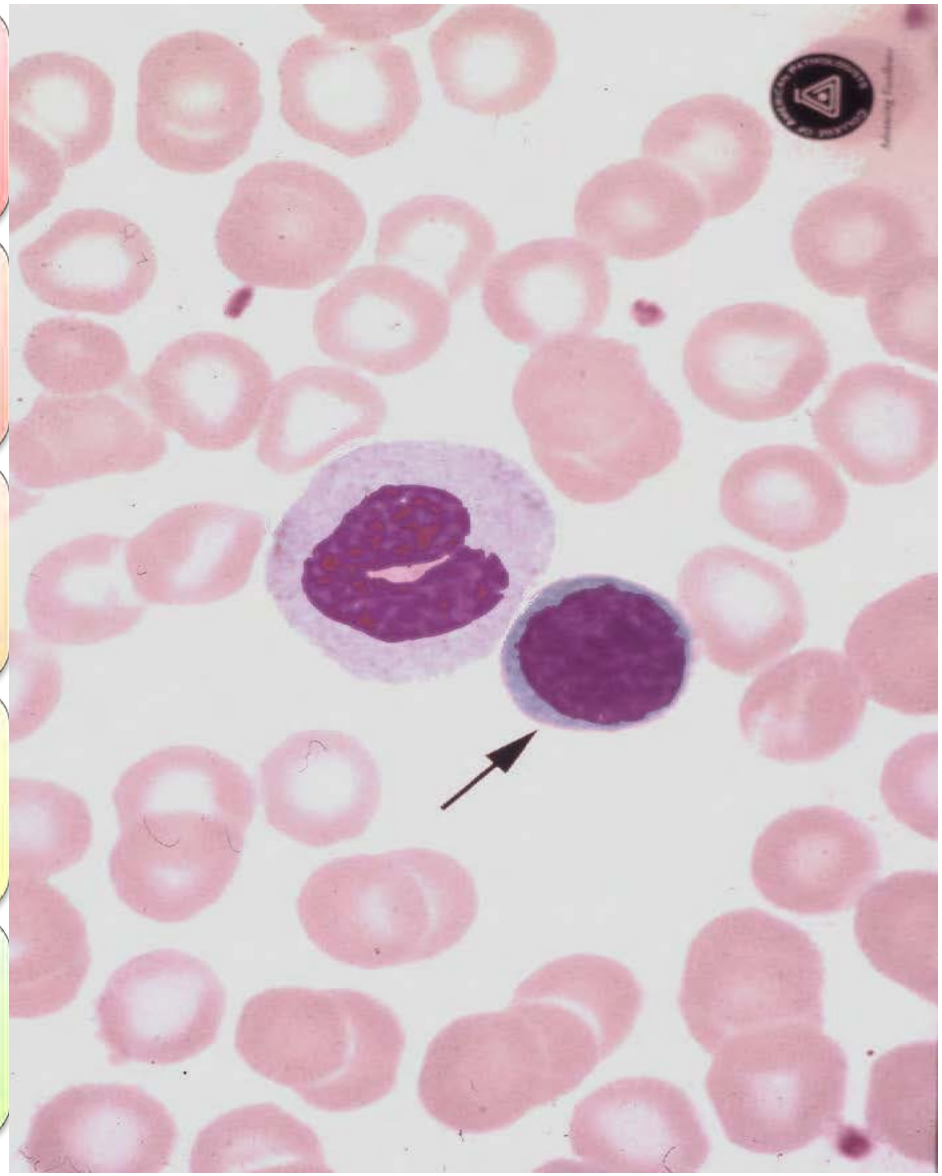
Can be T or B
(cannot distinguish morphologically)

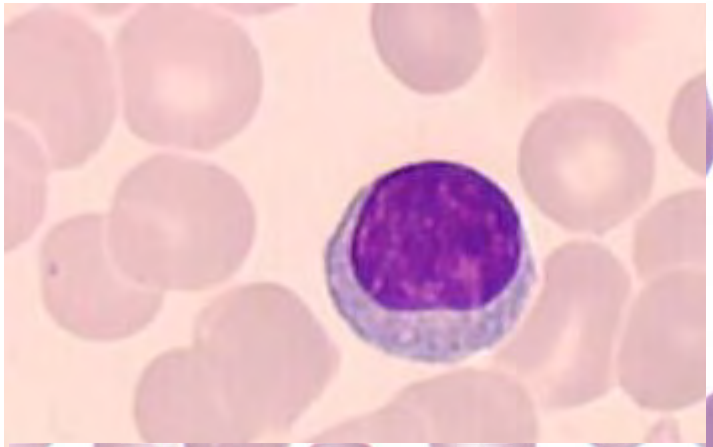
Small, round to ovoid cell,
7 to 15 µm, high N:C ratio.

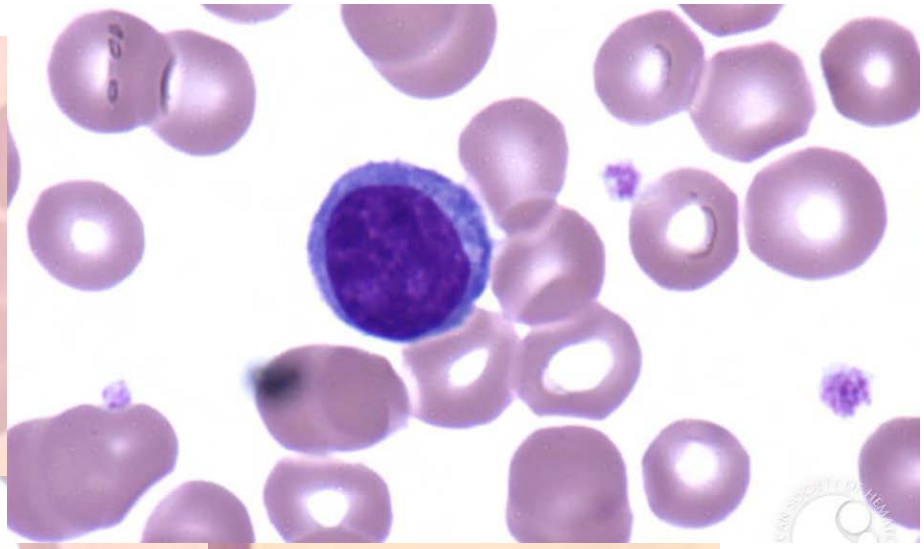
Chromatin is diffusely dense or coarse and clumped

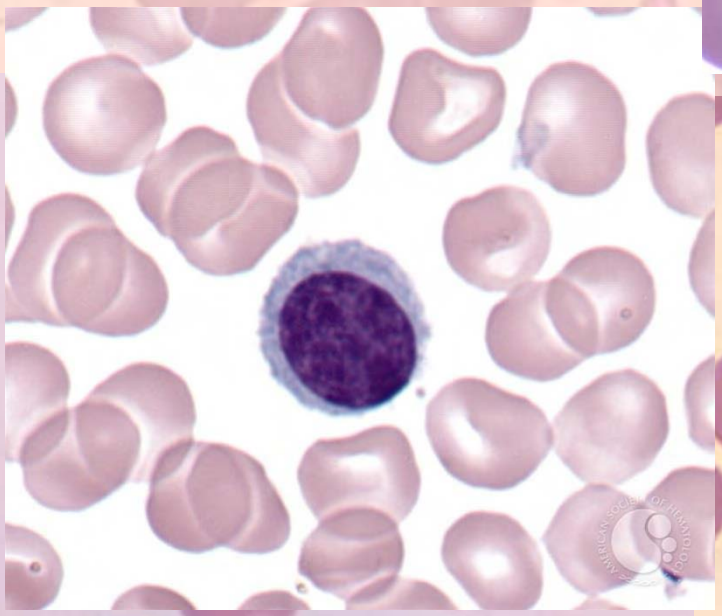
Nucleoli, if present, are small and inconspicuous.

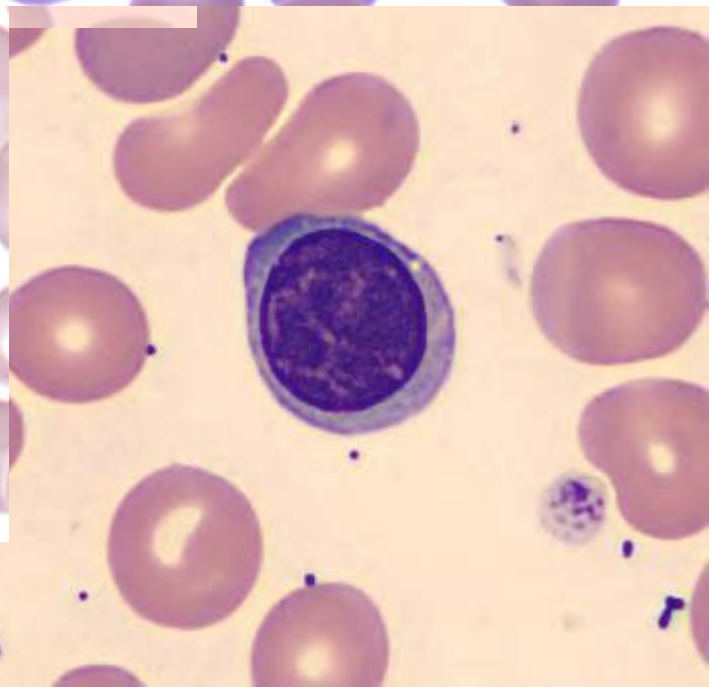
Some normal lymphs are medium-sized due to an increased amount of cytoplasm

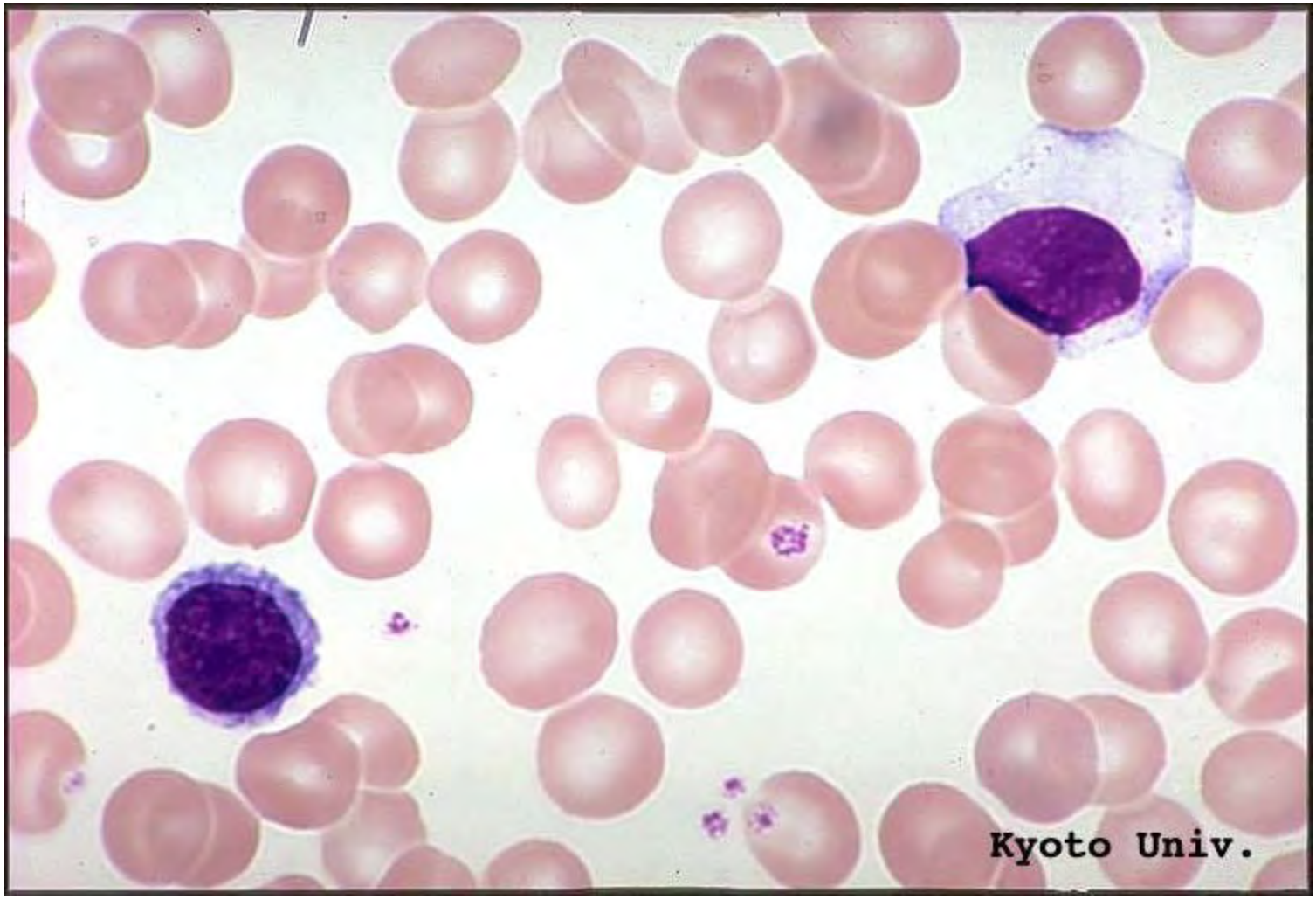




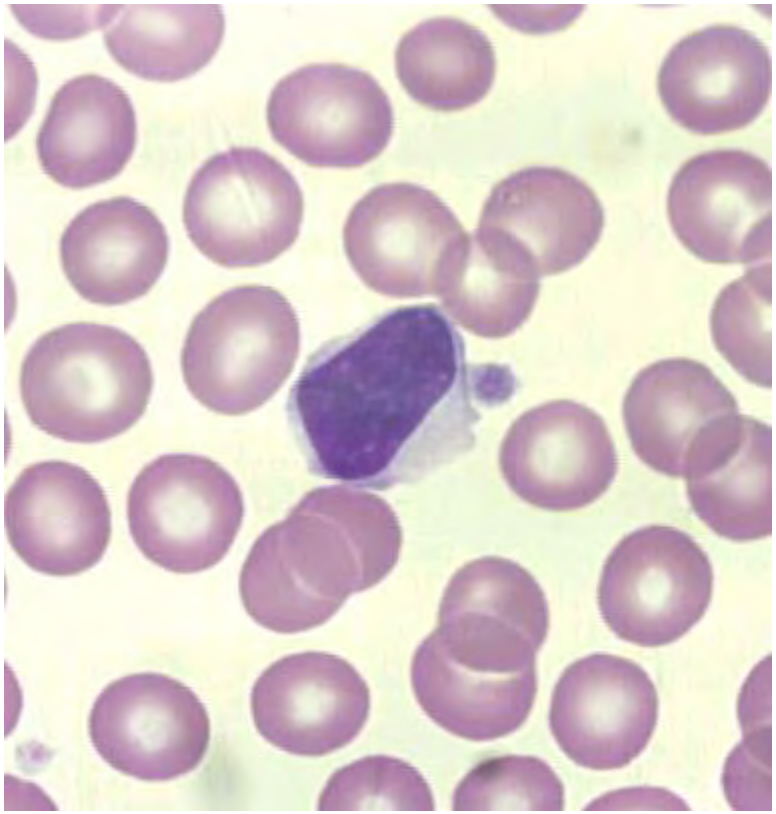






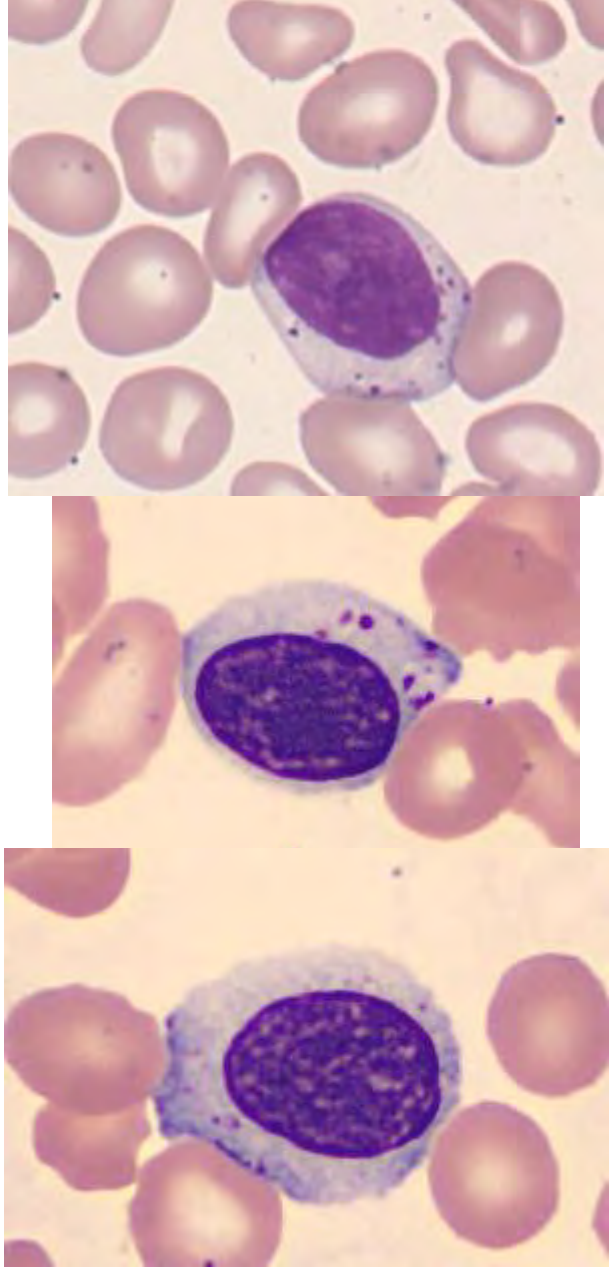

Kyoto Univ.

Normal Medium Sized Lymphocyte



Large Granular Lymphocytes

- Larger lymphocytes: LGLs
- Dense chromatin; pale blue cytoplasm
- Distinct red-purple azurophilic granules
- They are NOT reactive, atypical, or variant
- Normal range: Up to 15% of circulating lymphs (<600/uL).
  Should comment if appear increased



Lymphocytosis: Absolute vs Relative

Should lymphocytosis be defined in terms of absolute or relative numbers?

Consider this patient

- WBC = 2000/uL (5000-10000/uL)
  - Neutrophils = 20% (50-70)
  - Lymphocytes = 80% (20-40) – Relatively increased but……
- Calculate absolute numbers
  - Neutrophils: 2000/uL x .20 = 400/uL (2000–7000/uL)
  - Lymphocytes: 2000/uL x .80 = 1600/uL (1000–4000/uL)
- Absolute neutropenia. The lymphocyte number is actually normal.

Assessment should always be based on absolute number (>4,000/uL)

Smear Review: Lymphocyte Morphology

Reactive Lymphs

Heterogeneous population

Variation in N:C ratio

Variable nuclear shapes

Chromatin smooth - clumped

Nucleoli may be visible

Appearance of Cytoplasm

- Increased amount
- Variable deep basophilia
- May be indented by surrounding cells

Malignant Lymphs

Homogeneous population

High N:C ratio

Variably clumped chromatin

Morphology consistent with type of neoplasm

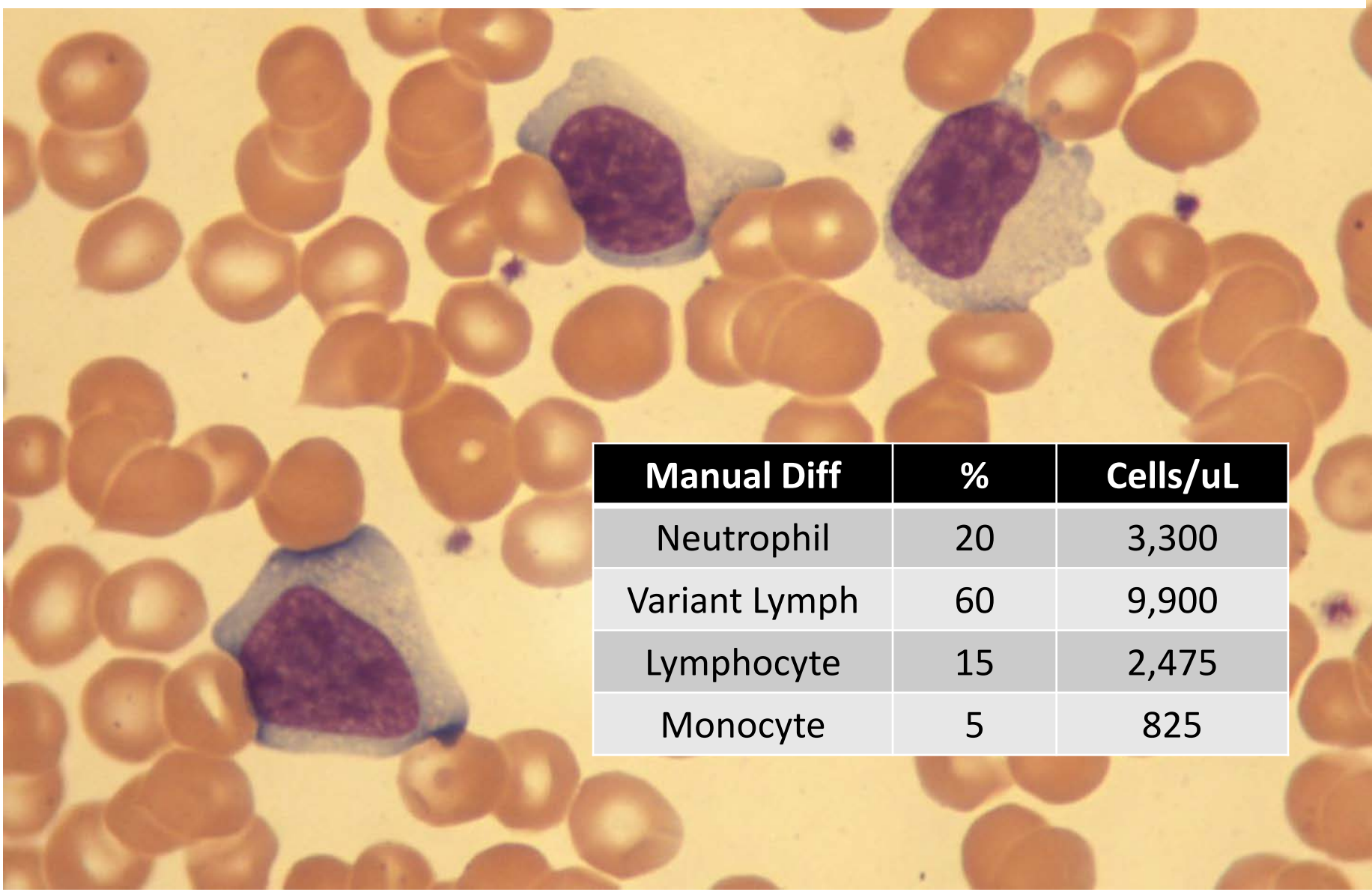
- Frayed cytoplasm
- Single prominent nucleolus
- Cerebriform nuclei
- Irregular nuclear contours; bizarre shapes; clefting

patient case

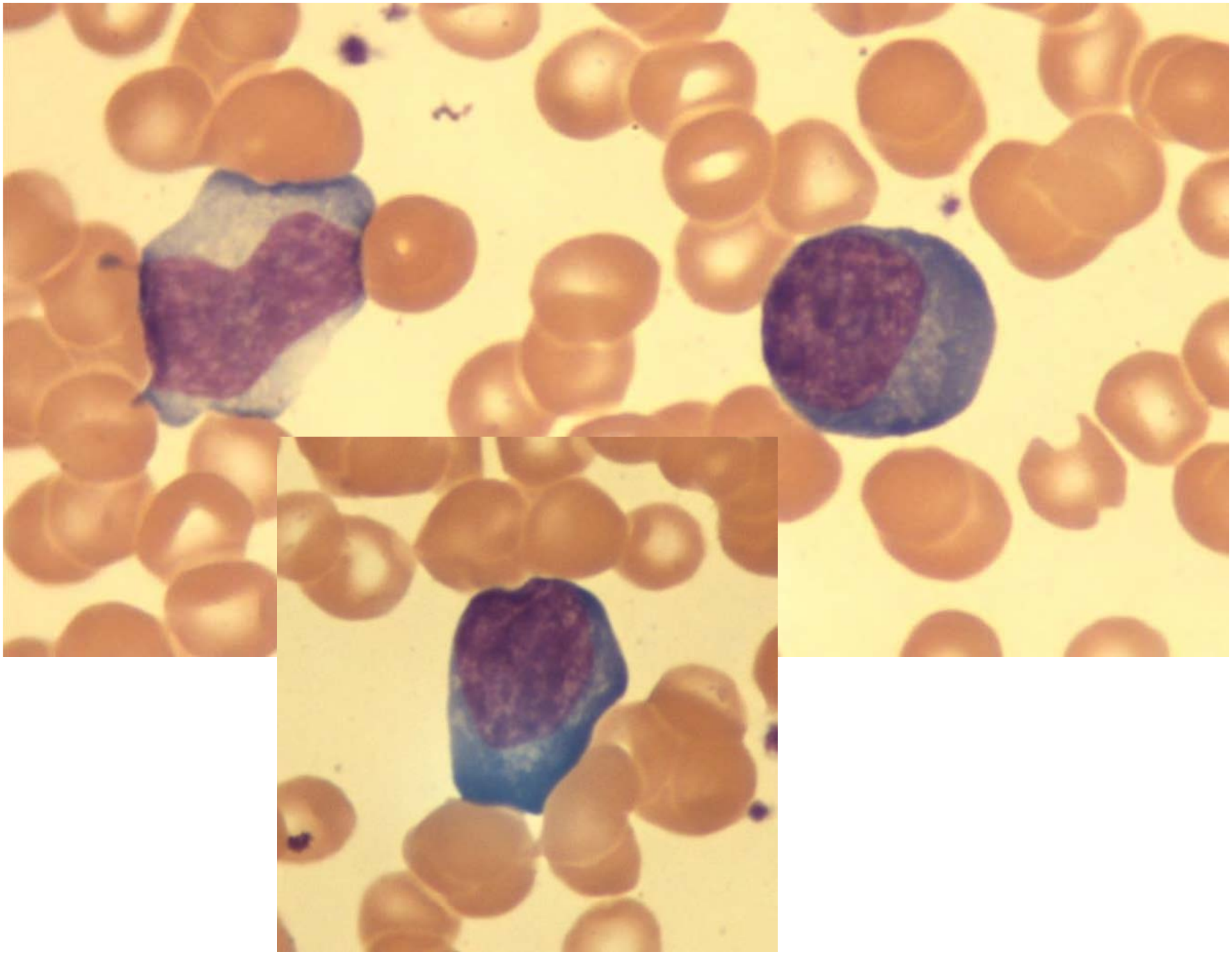
CLINICAL
19 year old male feeling awful for 1 week
Sore throat and fatigue
Fever 100.7^{o}F
Right cervical lymphadenopathy

LABS	PATIENT	REFERENCE
WBC	16,500 /uL	5000 - 10,000 /uL
Absolute Lymphocytes	12,375 /uL	1000 – 4000 uL
Hemoglobin	13.6 g/dL	13.5 – 16.0 g/dL
Platelets	209,000 /uL	150,000 – 400,000/uL
ALT	303	5 – 37 U/L
AST	276	10 – 37 U/L
Throat culture negative for β-Hemolytic Streptococci		

Presence of Atypical Lymph flag triggered review

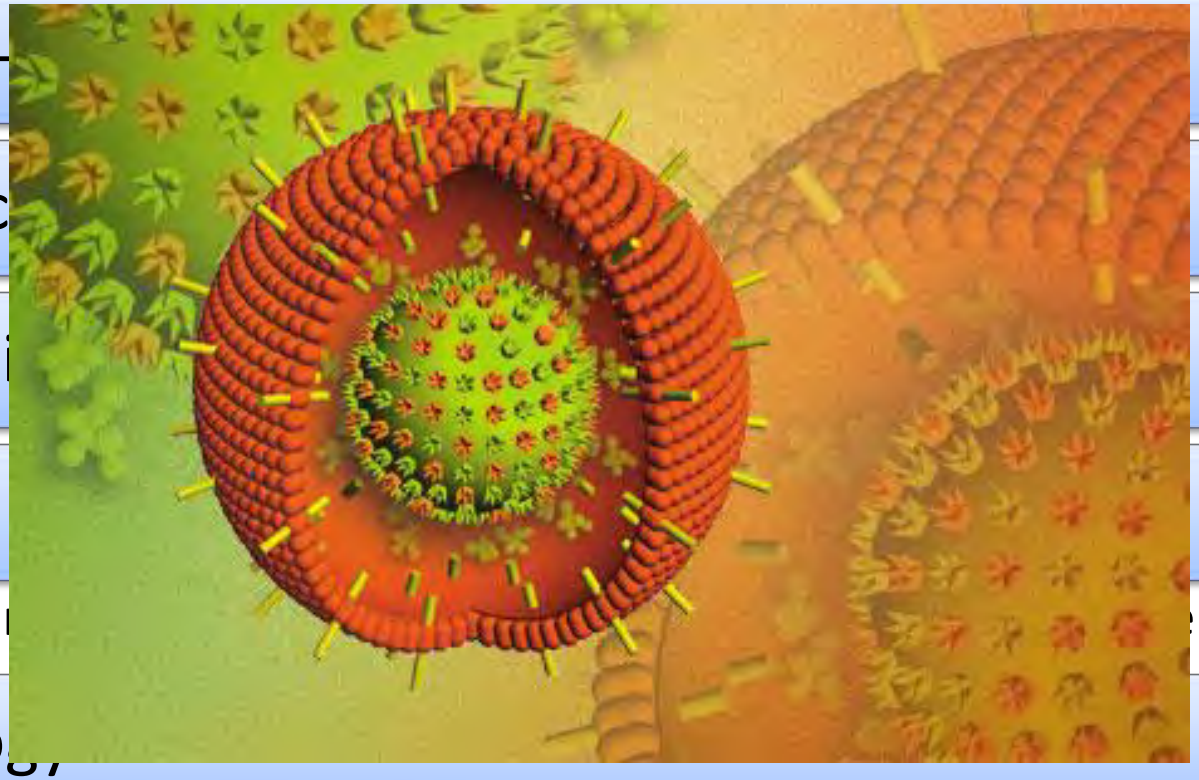


Manual Diff	%	Cells/uL
Neutrophil	20	3,300
Variant Lymph	60	9,900
Lymphocyte	15	2,475
Monocyte	5	825



Infectious Mononucleosis

Epstein-



Adolesc

Self-limi

Clinical

- Fever, r nomegaly

Patholog

- Reactive absolute lymphocytosis (T-cells)
- Infects B lymphocytes and oropharyngeal epithelium. Both share CD 21 receptor

Infectious Mononucleosis

3 criteria for laboratory confirmation

- Absolute lymphocytosis
- Variant (Atypical? Reactive?) lymphocytes on peripheral smear
- a positive serologic test for Epstein-Barr virus (EBV)

Differential Diagnosis

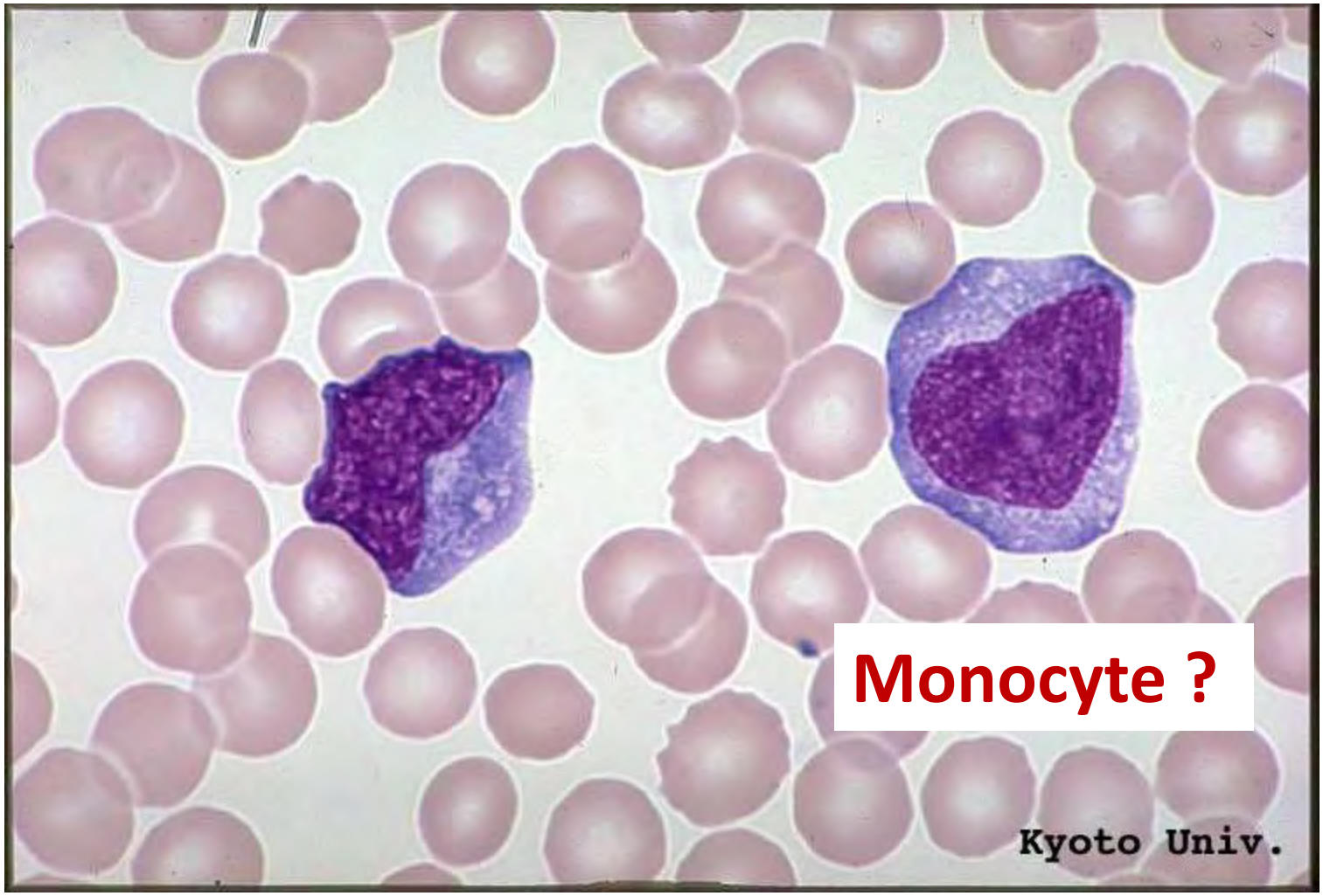
- Rubella
- Diphtheria
- Cytomegalovirus Infection (CMV)
- Hepatitis B
- HIV
- ß-Hemolytic Streptococci
- Toxoplasmosis

Reactive Lymphocytosis: Other Causes

- Toxoplasmosis
- Idiosyncratic drug reactions
  - Phenytoin
  - Other
- Listeria
- Mycoplasma
- Post vaccination
- Autoimmune disease
- Sudden onset of stress from myocardial infarction

Foucar K, Reichard K, Czuchlewski D. *Bone Marrow Pathology.* 3rd ed. ASCP Press; 2010

Reactive Lymphocytes



Plasmacytoid Lymphocyte

Form of reactive lymphocyte

Has some plasma cell features

- Prominent chromatin clumping
- Perinuclear halo
- Eccentric nucleus

Often seen mixed in a heterogeneous population of reactive lymphocytes

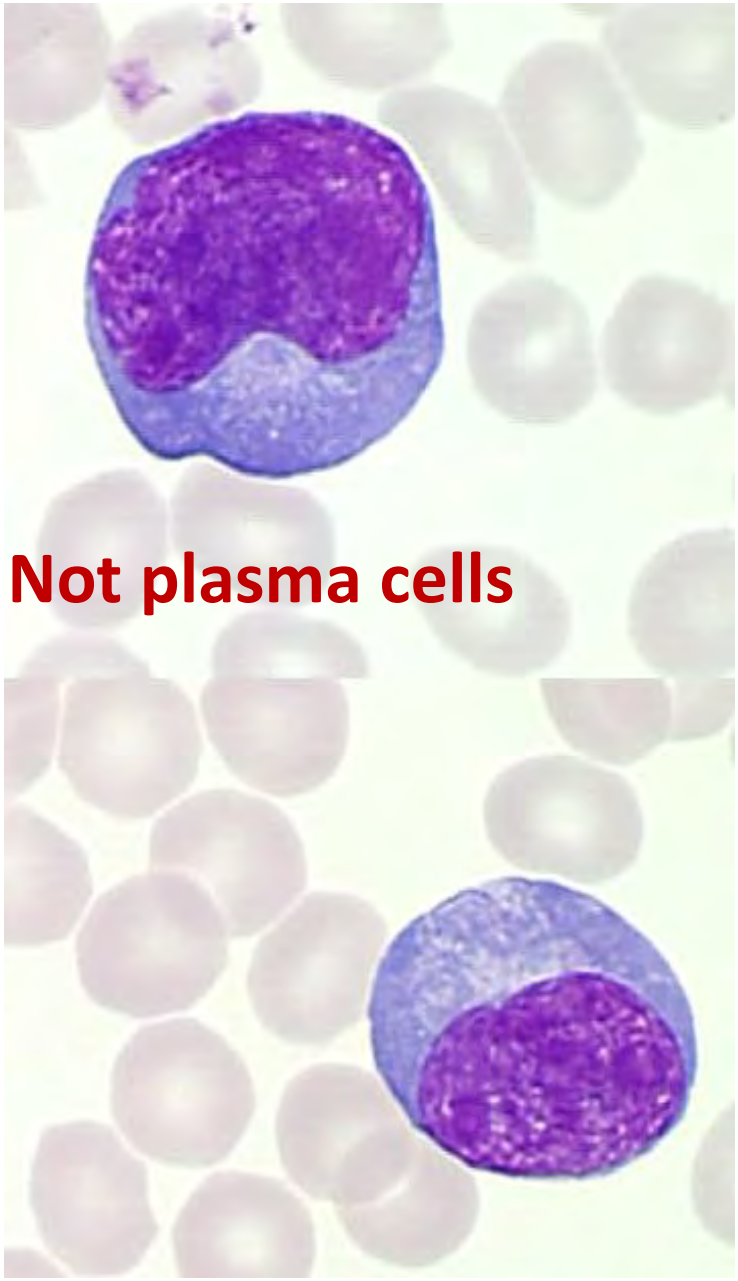
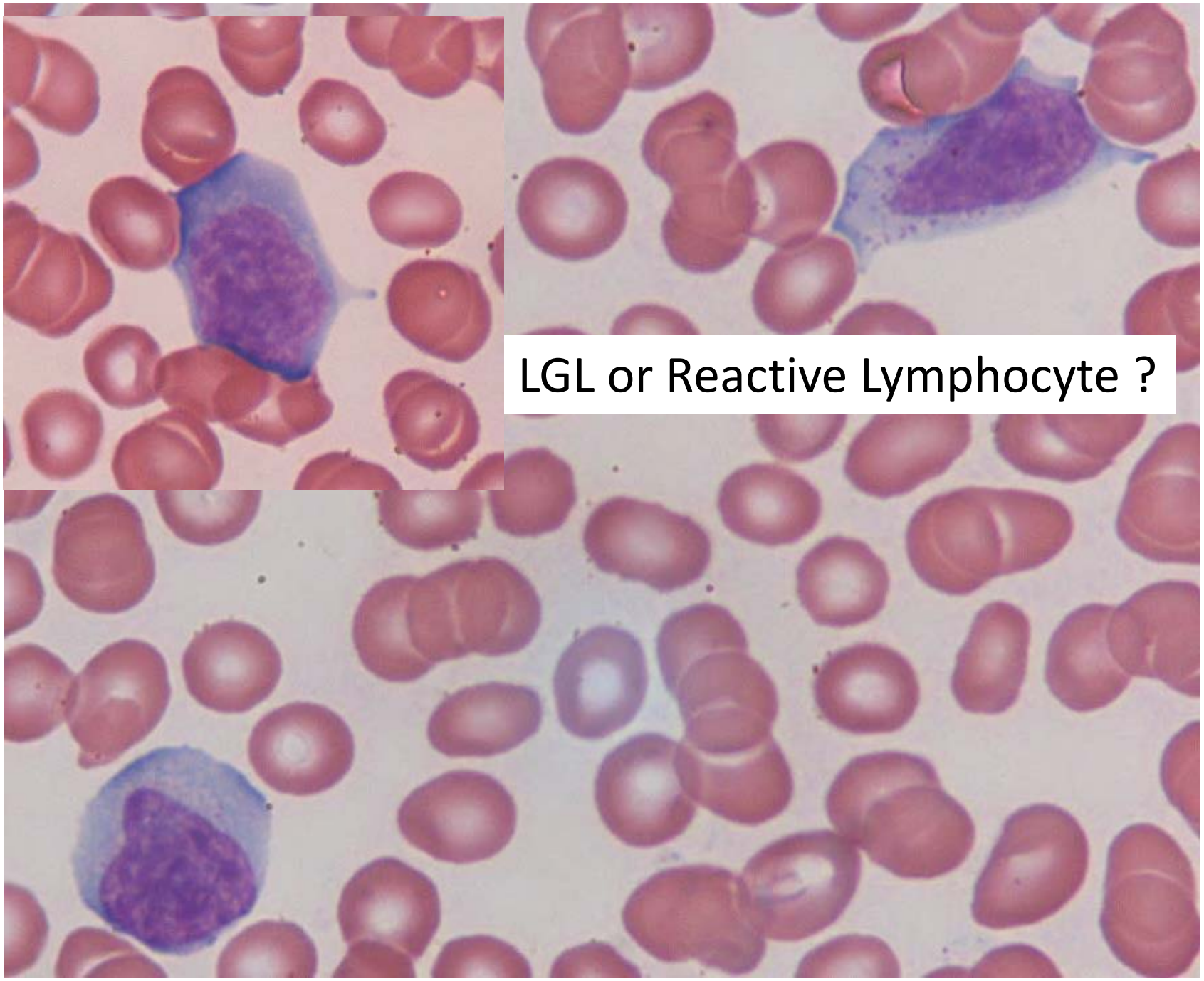
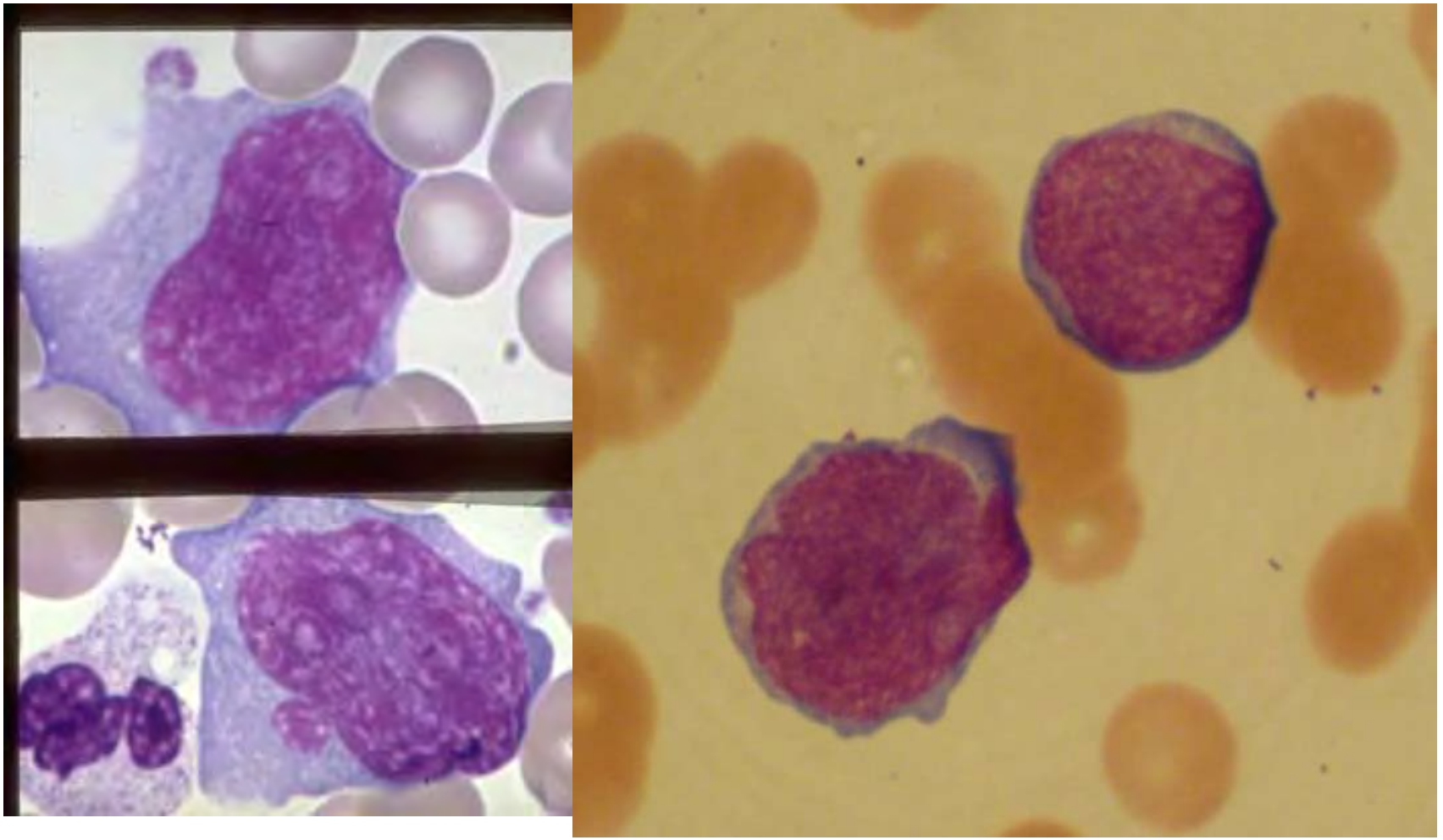
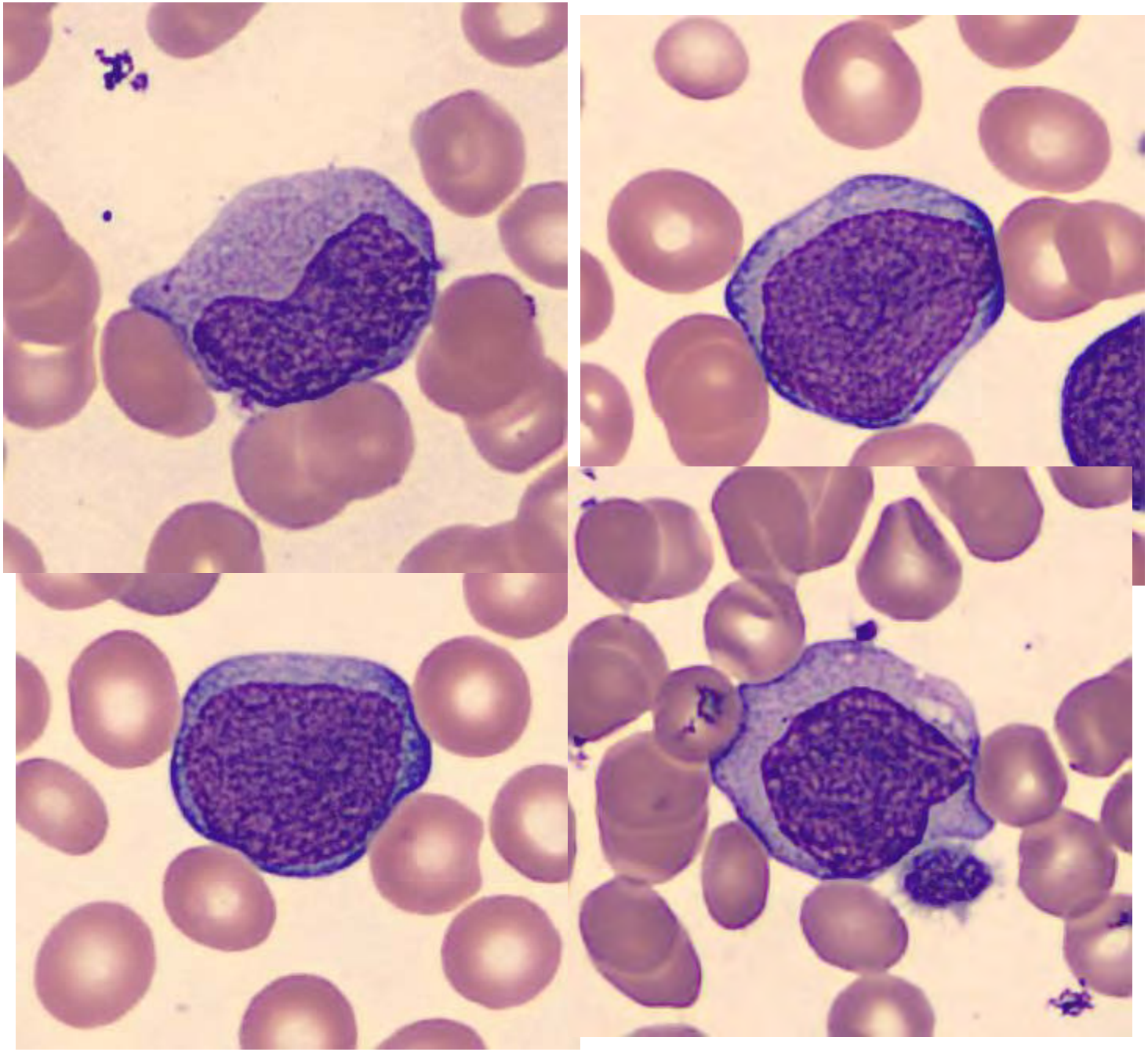


image.bloodline.net


LGL or Reactive Lymphocyte ?



http://www.bloodline.net/external/image-atlas.html



WHO Classification of Hematopoietic Neoplasms

Stratifies hematopoietic neoplasms according to lineage:

- Myeloid
- Lymphoid
- Histiocytic/dendritic

Precursor neoplasms are considered separately from mature

Classification of lymphoid neoplasms is based on pathologic, immunophenotypic, genetic and clinical features

Lymphoid Neoplasms: Introduction

B cell and T/NK neoplasms are clonal tumors of immature and mature B cells, T cells or natural killer (NK) cells at various stages of maturation

B and T cell neoplasms often mimic stages of normal differentiation, so to some extent are classified according to the normal stage

- Not always : hairy cell leukemia

Leukemia/lymphomas share malignant cell types

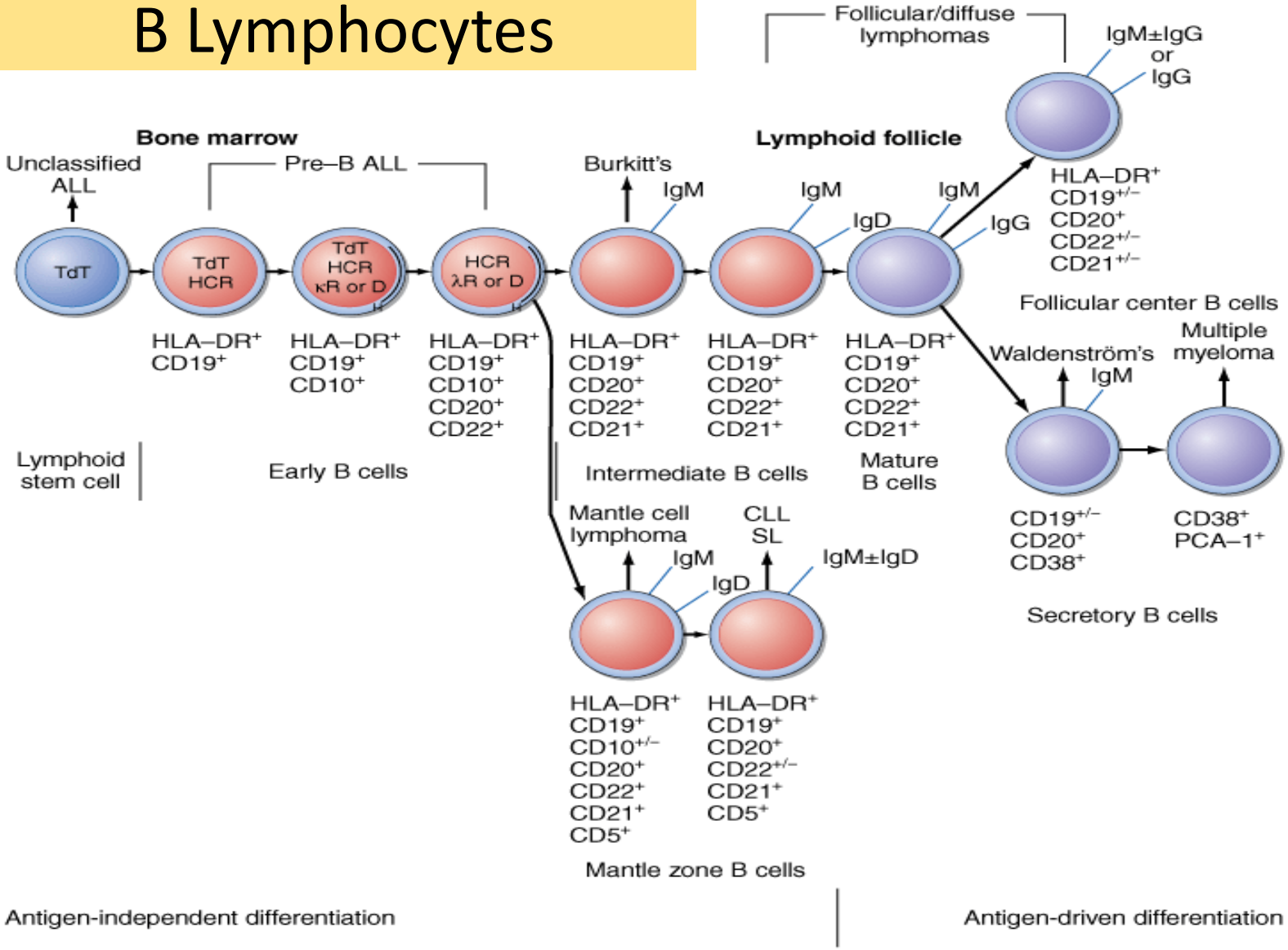
Leukemia and lymphoma differ only in where disease presents:

- Leukemia = mostly marrow (>20%) and blood involvement
- Lymphoma = predominate tissue infiltration with <25% marrow involvement

Lymphoid Neoplasms: Classification

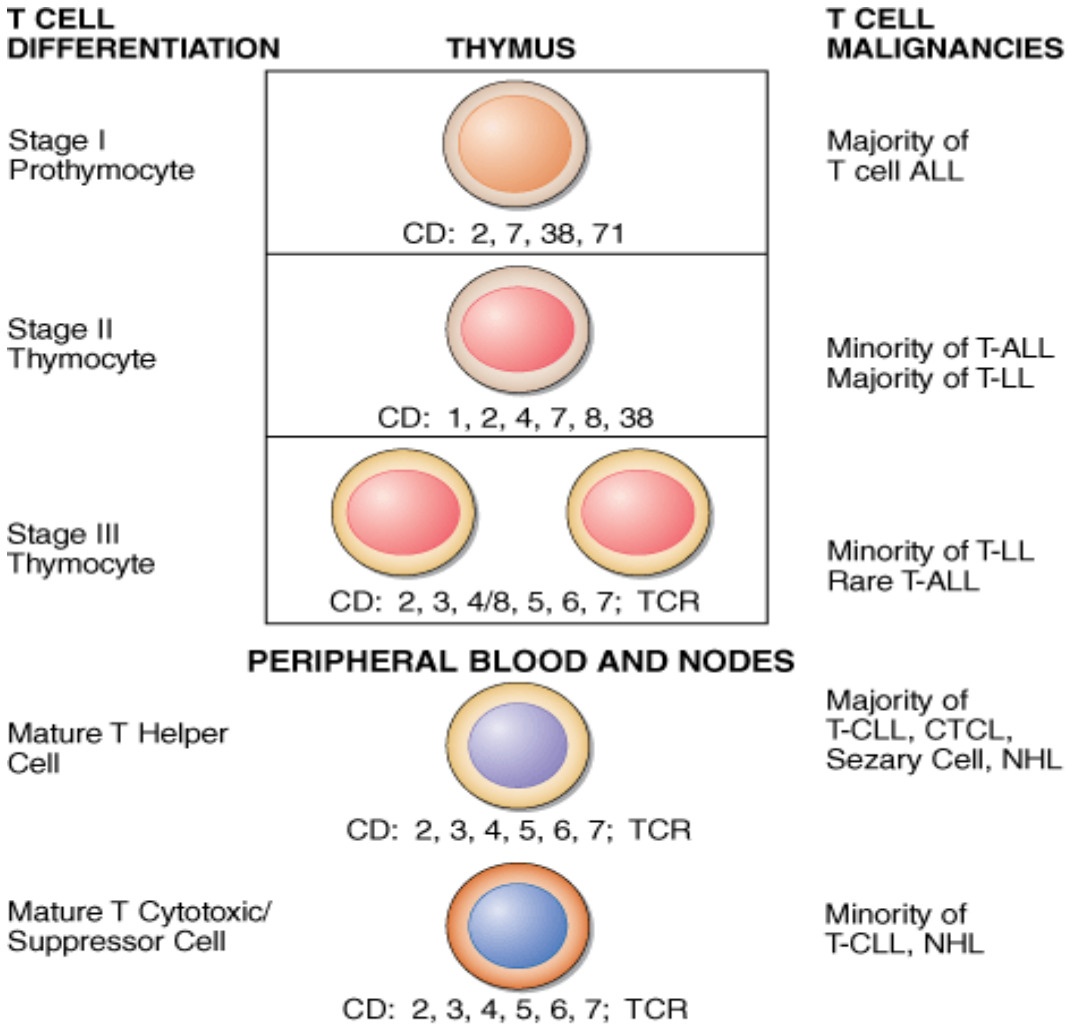
- Lymphoid neoplasms are classified mostly using morphology and immunophenotype
- No one marker (CD) is specific for any neoplasm
- Combination of morphologic features and a panel of antigenic markers is necessary for accurate diagnosis
- Within any given disease entity, variations in antigenic make up (CD markers) can be seen
- Morphology and immunophenotype can change over time as a result of additional genetic mutations and clonal evolution

B Lymphocytes



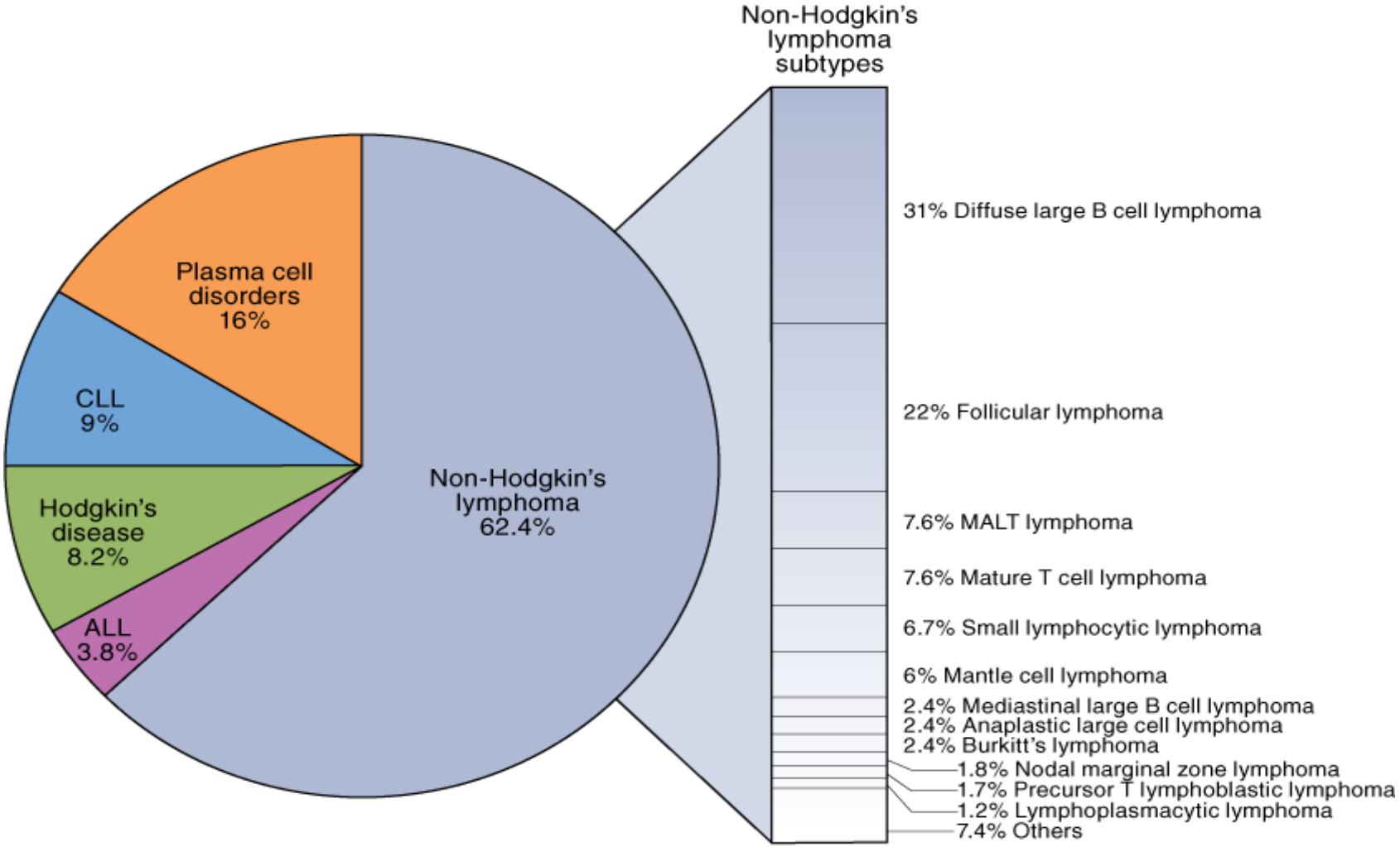
Source: Fauci AS, Kasper DL, Braunwald E, Hauser SL, Longo DL, Jameson JL, Loscalzo J: *Harrison's Principles of Internal Medicine*, 17th Edition: http://www.accessmedicine.com

T Lymphocytes



Source: Fauci AS, Kasper DL, Braunwald E, Hauser SL, Longo DL, Jameson JL, Loscalzo J: *Harrison's Principles of Internal Medicine*, 17th Edition: http://www.accessmedicine.com

Relative Frequency of Lymphoid Malignancies



Source: Fauci AS, Kasper DL, Braunwald E, Hauser SL, Longo DL, Jameson JL, Loscalzo J: *Harrison's Principles of Internal Medicine*, 17th Edition: http://www.accessmedicine.com

CLL: CD19+, CD20+ (CD19>CD20), CD5+, CD23+, CD200+, surface immunoglobulin (dim), CD10-, FMC7 dim or -, CD79b dim or –

PLL: CD19+, CD22+ (bright), surface immunoglobulin (bright), FMC7 +, CD5 +/-, CD10 –T cell: CD4+ or CD4+/CD8+ (dual), TCL1 +, CD2+, CD3+,CD7+, CD52+,Tdt -

HCL: CD20+ (bright), CD25+, CD103+, CD11c+, surface immunoglobulin (bright), CD5 + (dim) or -CD10-

LGL: T cell: CD8+, CD3+, CD57+, CD16+, CD2+, CD5+(dim), CD7 +(dim) or - NK cell: CD56+, CD3-, CD16+, CD2 + (dim) or -, CD7+ (dim) or -, CD57+(dim) or -

ATLL: CD2+, CD5+, CD3+, CD7-, CD4+, CD25+(bright), CD30+ , CCR4+, FOXP3+

BL: CD19+,CD20+, CD22+, CD10+, surface immunoglobulin (bright), Ki67+,Tdt –

FL: CD19+, CD20+ CD10+, surface immunoglobulin (bright) CD5-, CD23-

DLBCL: CD19+, CD20+, CD79b+, CD22+(bright), CD10 +/-

MCL: CD19+, CD20+, CD5+, CD23-, CD200-, FMC7+, CD22+ , CD79b+, CD10 +/-

MZL: CD19+,CD20+, CD22+, CD79b+, surface immunoglobulin (bright), FMC7+, CD5-, CD10-, CD23-

MF/ SS: CD2+ , CD3+,CD5+, CD7-, CD4+, CD26-

MM: CD45 dim or -, CD38+, CD138+, CD56+, CD117+, cytoplasmic light chain

World Health Organization (WHO) classification of lymphoid neoplasms

MATURE B-CELL NEOPLASMS

Chronic lymphocytic leukemia /small lymphocytic lymphoma
Monoclonal B-cell lymphocytosis*
B-cell prolymphocytic leukemia
Splenic marginal zone lymphoma
Hairy cell leukemia
Splenic B-cell lymphoma/leukemia, unclassifiable
    Splenic diffuse red pulp small B-cell lymphoma
    Hairy cell leukemia-variant
Lymphoplasmacytic lymphoma
    Waldenström macroglobulinemia
Monoclonal gammopathy of undetermined significance (MGUS), IgM*
Mu heavy chain disease
Gamma heavy chain disease
Alpha heavy chain disease
Monoclonal gammopathy of undetermined significance (MGUS), IgG/A*
Plasma cell myeloma
Solitary plasmacytoma of bone
Extraosseous plasmacytoma
Monoclonal immunoglobulin deposition diseases*
Extranodal marginal zone lymphoma of mucosa-associated lymphoid tissue (MALT lymphoma)
Nodal marginal zone lymphoma
    Pediatric nodal marginal zone lymphoma

Swerdlow, S. H. The 2016 revision of the World Health Organization (WHO) classification of lymphoid neoplasms
Blood 2016 :blood-2016-01-643569; doi: https://doi.org/10.1182/blood-2016-01-643569

World Health Organization (WHO) classification of lymphoid neoplasms

Follicular lymphoma
- In situ follicular neoplasia*
- Duodenal-type follicular lymphoma*

Pediatric-type follicular lymphoma*

*Large B-cell lymphoma with IRF4 rearrangement**

Primary cutaneous follicle center lymphoma

Mantle cell lymphoma
- In situ mantle cell neoplasia*

Diffuse large B-cell lymphoma (DLBCL), NOS
- Germinal center B-cell type*
- Activated B-cell type*

T cell/histiocyte-rich large B-cell lymphoma

Primary DLBCL of the CNS

Primary cutaneous DLBCL, leg type

EBV positive DLBCL, NOS*

*EBV+ Mucocutaneous ulcer**

DLBCL associated with chronic inflammation

Lymphomatoid granulomatosis

Primary mediastinal (thymic) large B-cell lymphoma

Swerdlow, S. H. The 2016 revision of the World Health Organization (WHO) classification of lymphoid neoplasms Blood 2016 :blood-2016-01-643569; doi: https://doi.org/10.1182/blood-2016-01-643569

World Health Organization (WHO) classification of lymphoid neoplasms

Intravascular large B-cell lymphoma
ALK positive large B-cell lymphoma
Plasmablastic lymphoma
Primary effusion lymphoma
*HHV8 positive DLBCL, NOS**
Burkitt lymphoma
*Burkitt-like lymphoma with 11q aberration**
High grade B-cell lymphoma, with *MYC* and *BCL2* and/or *BCL6* rearrangements*
High grade B-cell lymphoma, NOS*
B-cell lymphoma, unclassifiable, with features intermediate between DLBCL and classical Hodgkin lymphoma

MATURE T-AND NK-NEOPLASMS
T-cell prolymphocytic leukemia
T-cell large granular lymphocytic leukemia
Chronic lymphoproliferative disorder of NK cells
Aggressive NK cell leukemia
Systemic EBV+ T-cell Lymphoma of childhood*
Hydroa vacciniforme-like lymphoproliferative disorder*
Adult T-cell leukemia/lymphoma
Extranodal NK/T-cell lymphoma, nasal type
Enteropathy-associated T-cell lymphoma
Monomorphic epitheliotropic intestinal T-cell lymphoma*
*Indolent T-cell lymphoproliferative disorder of the GI tract **
Hepatosplenic T-cell lymphoma
Subcutaneous panniculitis- like T-cell lymphoma

Swerdlow, S. H. The 2016 revision of the World Health Organization (WHO) classification of lymphoid neoplasms Blood 2016 :blood-2016-01-643569; doi: https://doi.org/10.1182/blood-2016-01-643569

MATURE T-AND NK-NEOPLASMS

World Health Organization (WHO) classification of lymphoid neoplasms

Mycosis fungoides
Sézary syndrome
Primary cutaneous CD30 positive T-cell lymphoproliferative disorders
  Lymphomatoid papulosis
  Primary cutaneous anaplastic large cell lymphoma
Primary cutaneous gamma-delta T-cell lymphoma
Primary cutaneous CD8 positive aggressive epidermotropic cytotoxic T-cell lymphoma
*Primary cutaneous acral CD8+ T-cell lymphoma**
*Primary cutaneous CD4 positive small/medium T-cell lymphoproliferative disorder**
Peripheral T-cell lymphoma, NOS
Angioimmunoblastic T-cell lymphoma
*Follicular T-cell lymphoma**
*Nodal peripheral T-cell lymphoma with TFH phenotype**
Anaplastic large cell lymphoma, ALK positive
Anaplastic large cell lymphoma, ALK negative *
*Breast implant-associated anaplastic large cell lymphoma**

Swerdlow, S. H. The 2016 revision of the World Health Organization (WHO) classification of lymphoid neoplasms Blood 2016 :blood-2016-01-643569; doi: https://doi.org/10.1182/blood-2016-01-643569

Mature Lymphoid Neoplasms

Chronic lymphoproliferative neoplasms are clonal proliferations of morphologically & immunophenotypically mature B or T cells characterized by

- Low proliferation rate (most cases)
- Prolonged cell survival due to failed apoptosis

Leukemia vs Lymphoma: what's the difference?

- Leukemias: primary manifestation in bone marrow and blood; sometimes also involve extramedullary sites
- Lymphoma: presents in lymphoid organs and other tissues; may also involve marrow and blood
  - Hodgkin Lymphoma: not covered today. Reed-Sternberg cell not found in circulation
  - Non-Hodgkin Lymphoma – cells can be found in circulation

72 year old male

Annual physical

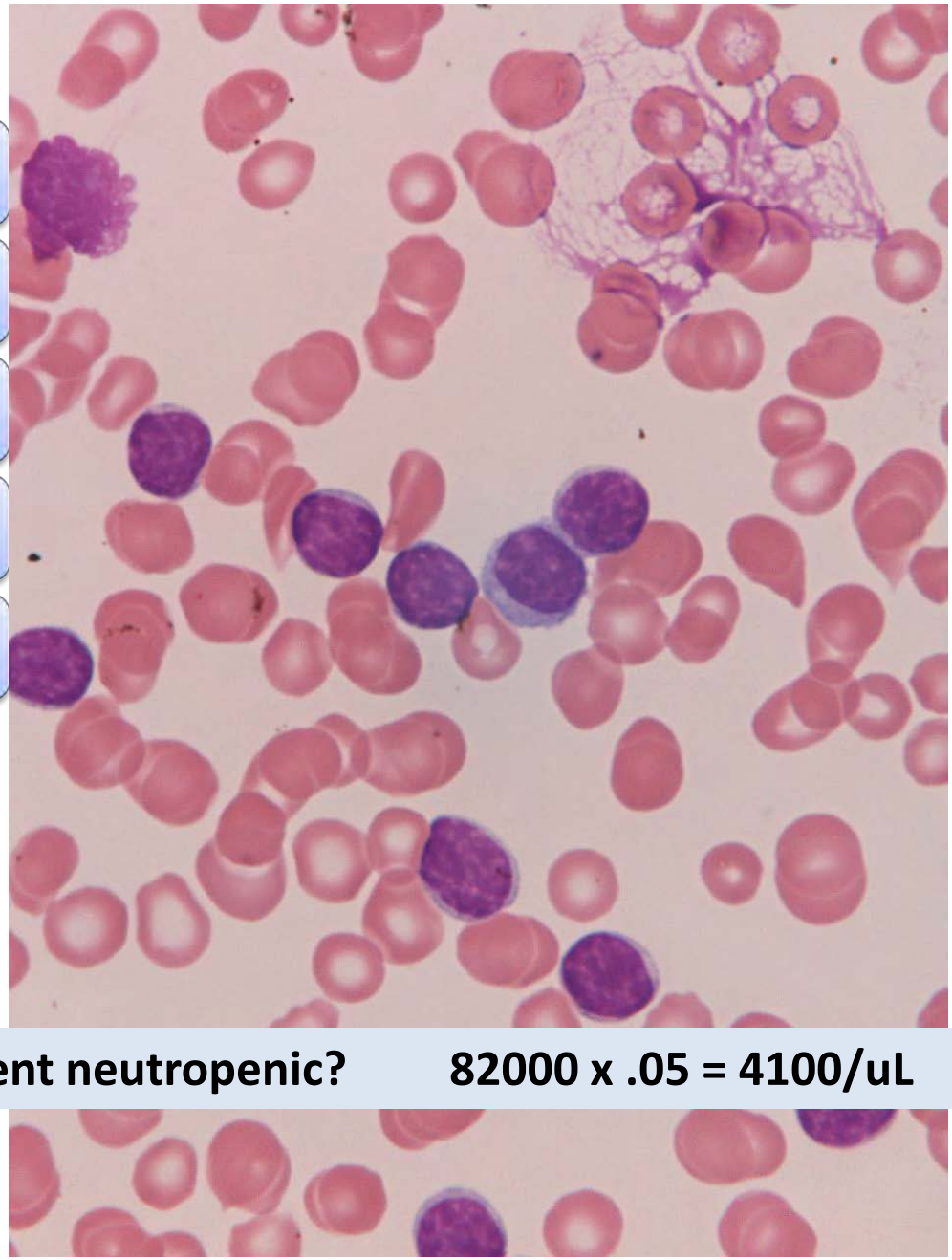
Feels great

No lymphadenopathy

No splenomegaly

CBC

- WBC: 82,000/uL
- HGB: 14.2 g/dL
- Platelets: 231,000/uL
- Manual Diff
  - 95% Lymphs
  - 5% Neutrophils
  - Smudge cells



Is the patient neutropenic? **82000 x .05 = 4100/uL**

Chronic Lymphocytic Leukemia

Accumulation of mature B-Lymphocytes in bone marrow, blood, lymph nodes, spleen and liver

Most common leukemia in the US

- 25% of all leukemia cases diagnosed annually
- 15,500 new cases per year and 4390 death

Older adults, with a higher incidence in males

Family history of CLL is the most important risk factor for the development of CLL; 8-10% of patients with CLL have a family history

Chronic Lymphocytic Leukemia: Clinical

Symptoms - depends on disease progression

- Most patients are asymptomatic at diagnosis; some are detected during a routine physical exam or pre-surgical testing
- Lymphadenopathy in some
- Neutropenia, anemia and thrombocytopenia usually later in disease
- When patients have symptoms, they are non-specific:
  - Weakness
  - fever
  - frequent infections
  - Bleeding
  - night sweats
  - weight loss.

CLL: Diagnostic Criteria

Sustained lymphocytosis > 5000/uL

Immunophenotype

- Specific Pattern of Reactivity
  - Expression of > 1 B cell antigen (CD19, CD20, CD23)
  - Expression of T cell antigen CD5 (without expression of other pan-T cell antigens)
  - Weak or negative expression of sIg (weak/neg)
  - Clonal light chain restriction (only one type of light chain)

CLL Lymphocyte Morphology

Most lymphocytes have scant cytoplasm – high N:C ratio

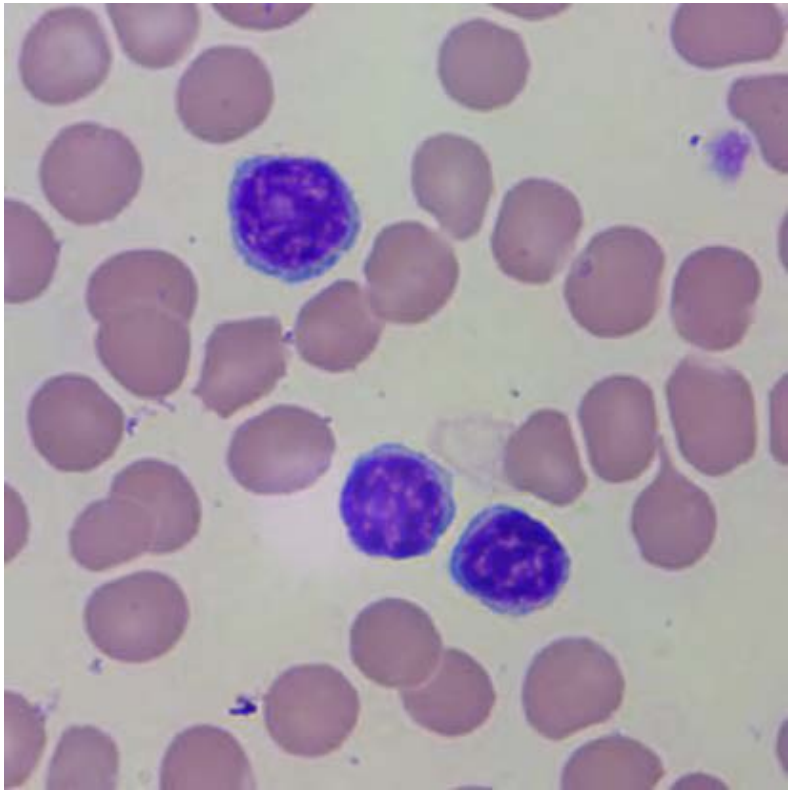
Chromatin pattern is atypically dense with clumps of dark chromatin separated by narrow pale spaces - “soccer ball”

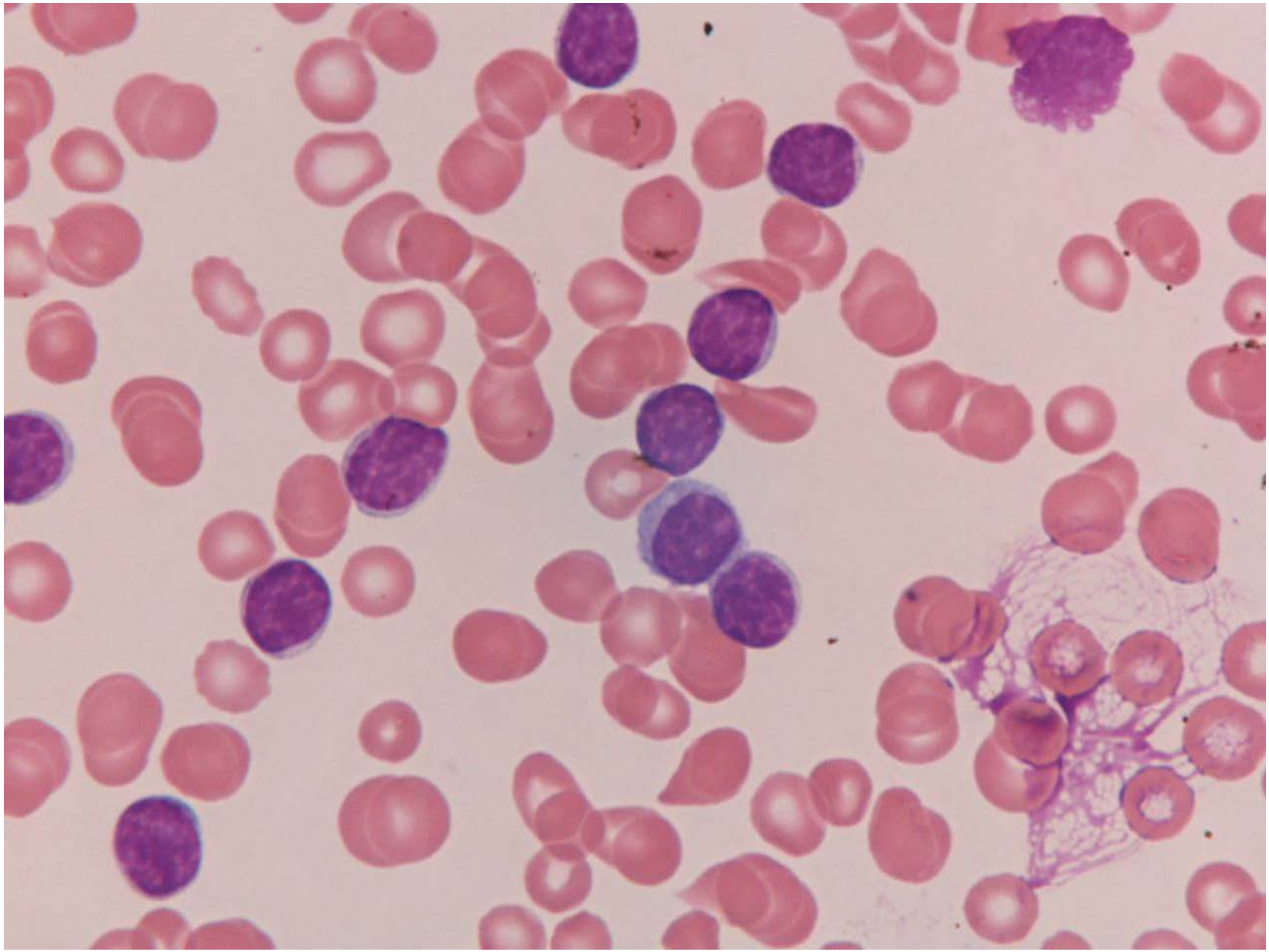
Prolymphocytes are frequently seen but represent less than 10% of the total lymphocyte population

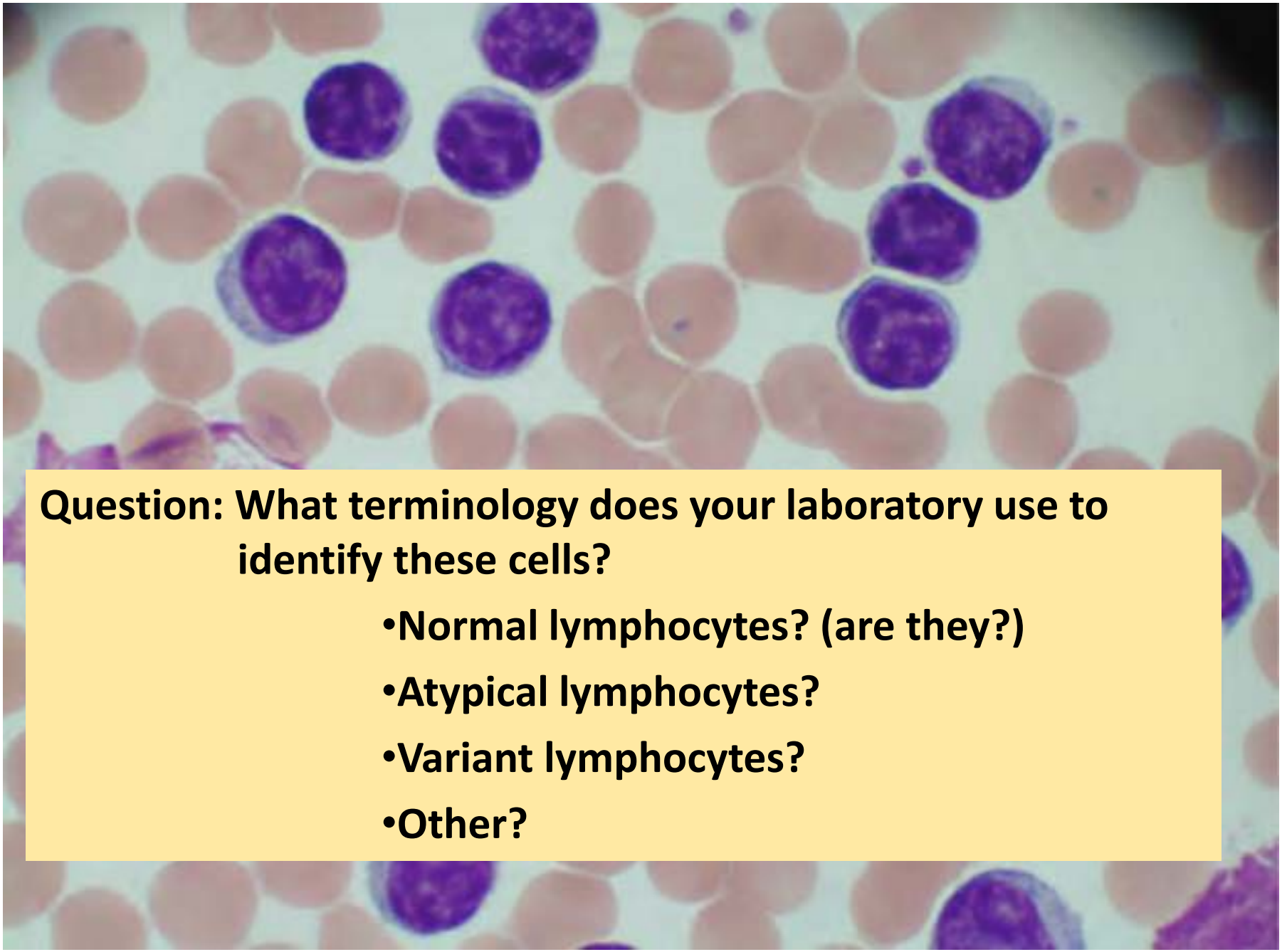
- Larger cell with single prominent nucleolus
- Less condensed chromatin
- Pale blue cytoplasm

Smudged lymphocytes

Characteristic Chromatin Clumping pattern







Question: What terminology does your laboratory use to identify these cells?

- **Normal lymphocytes? (are they?)**
- **Atypical lymphocytes?**
- **Variant lymphocytes?**
- **Other?**

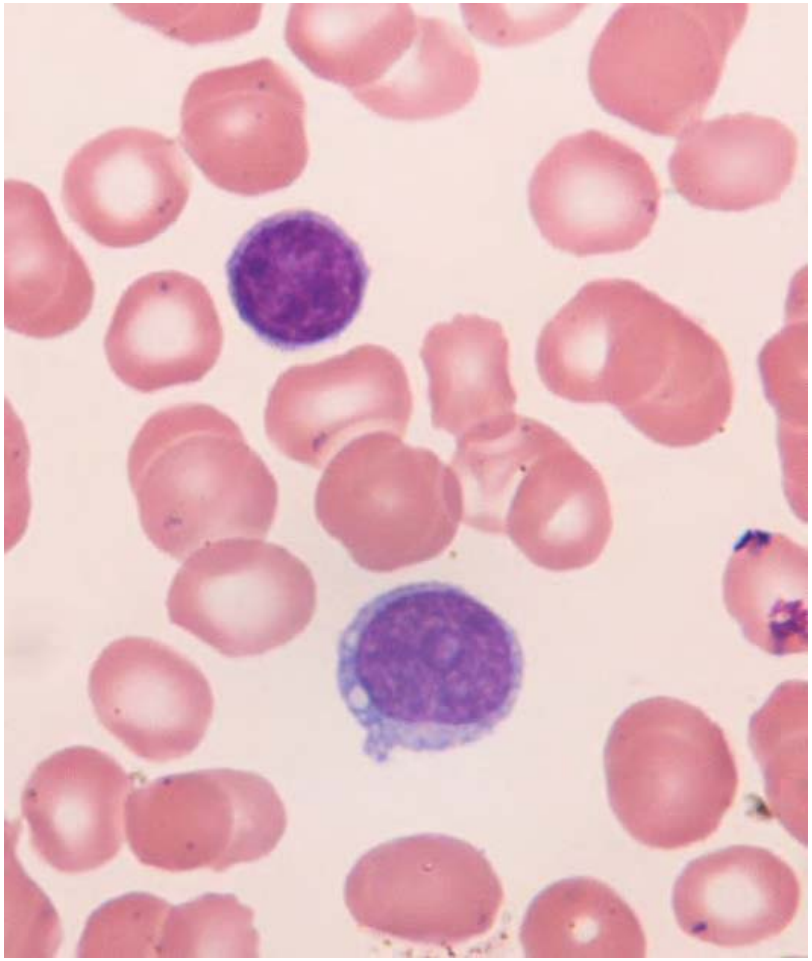
Atypical CLL (15% of cases)

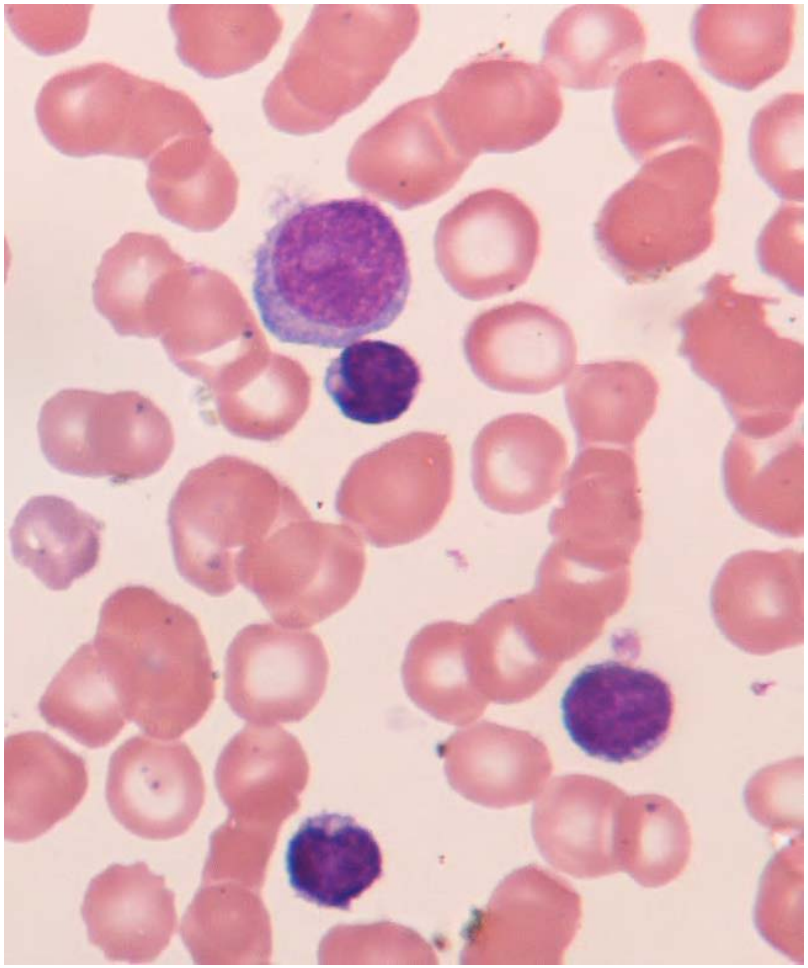
>15% large lymphoplasmacytoid cells and/or cleaved cells.

CLL/PL (prolymphocytes)

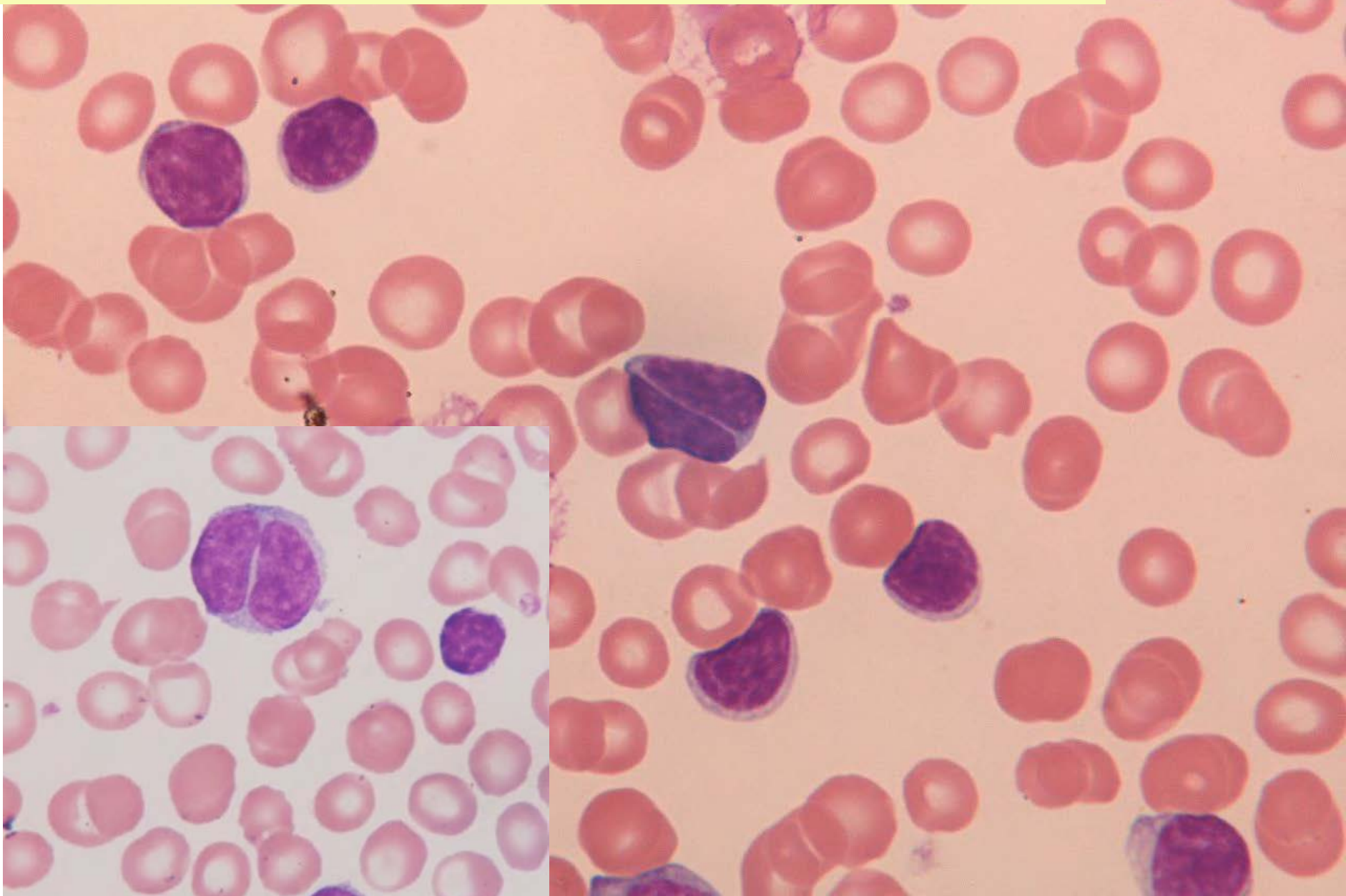
- 10% - 55% circulating prolymphocytes with the rest being small CLL cells.
- May present at initial diagnosis or develop as a transformation following a chronic phase.
- More aggressive disease
- Higher lymphocyte count
- Marked splenomegaly.

CLL/PL

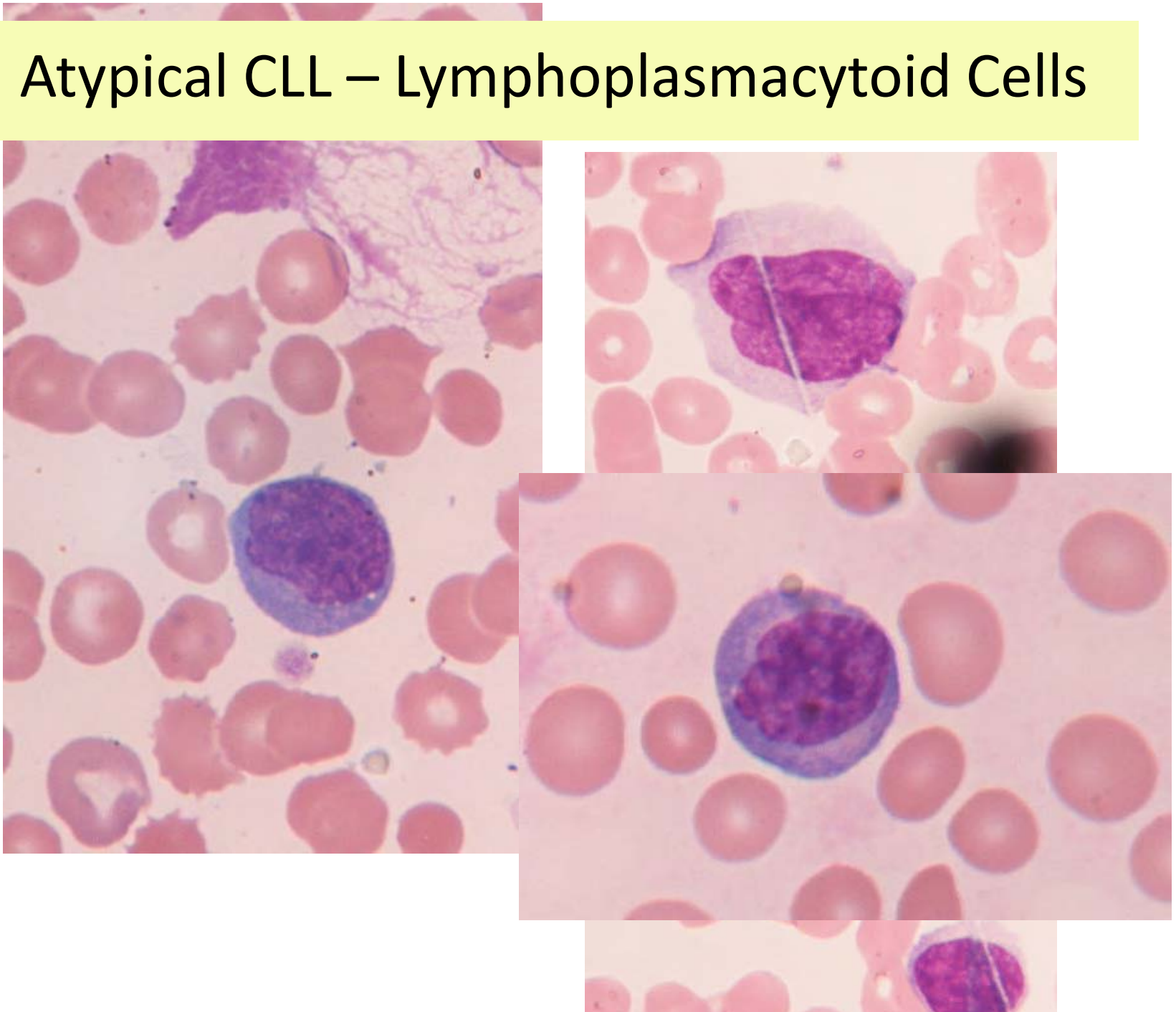




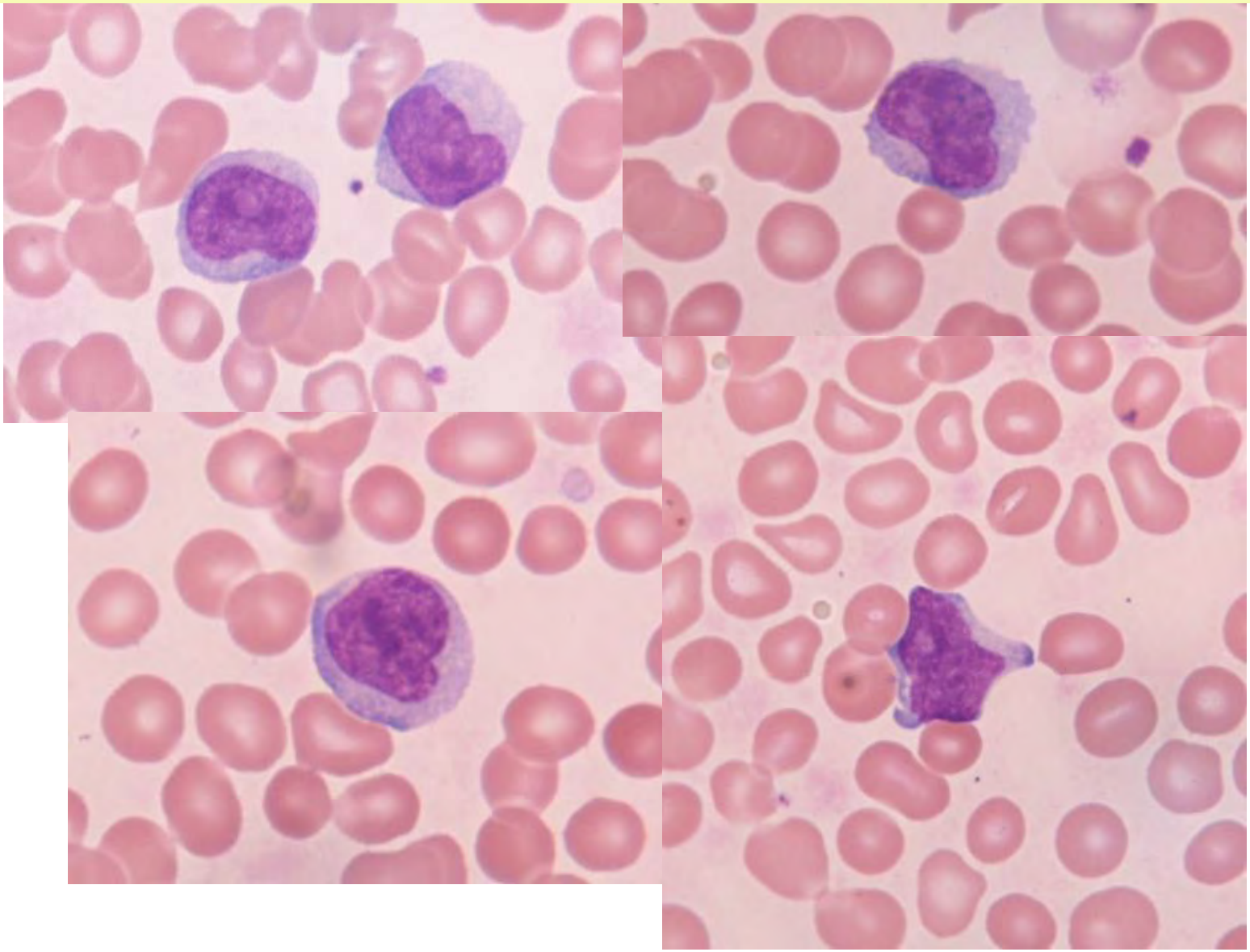
Atypical CLL – Cleaved Forms



Atypical CLL – Lymphoplasmacytoid Cells



Atypical CLL – Lymphoplasmacytoid Cells



Smudge Cells in CLL

Smudge cells can affect accuracy of manual WBC diff if ignored

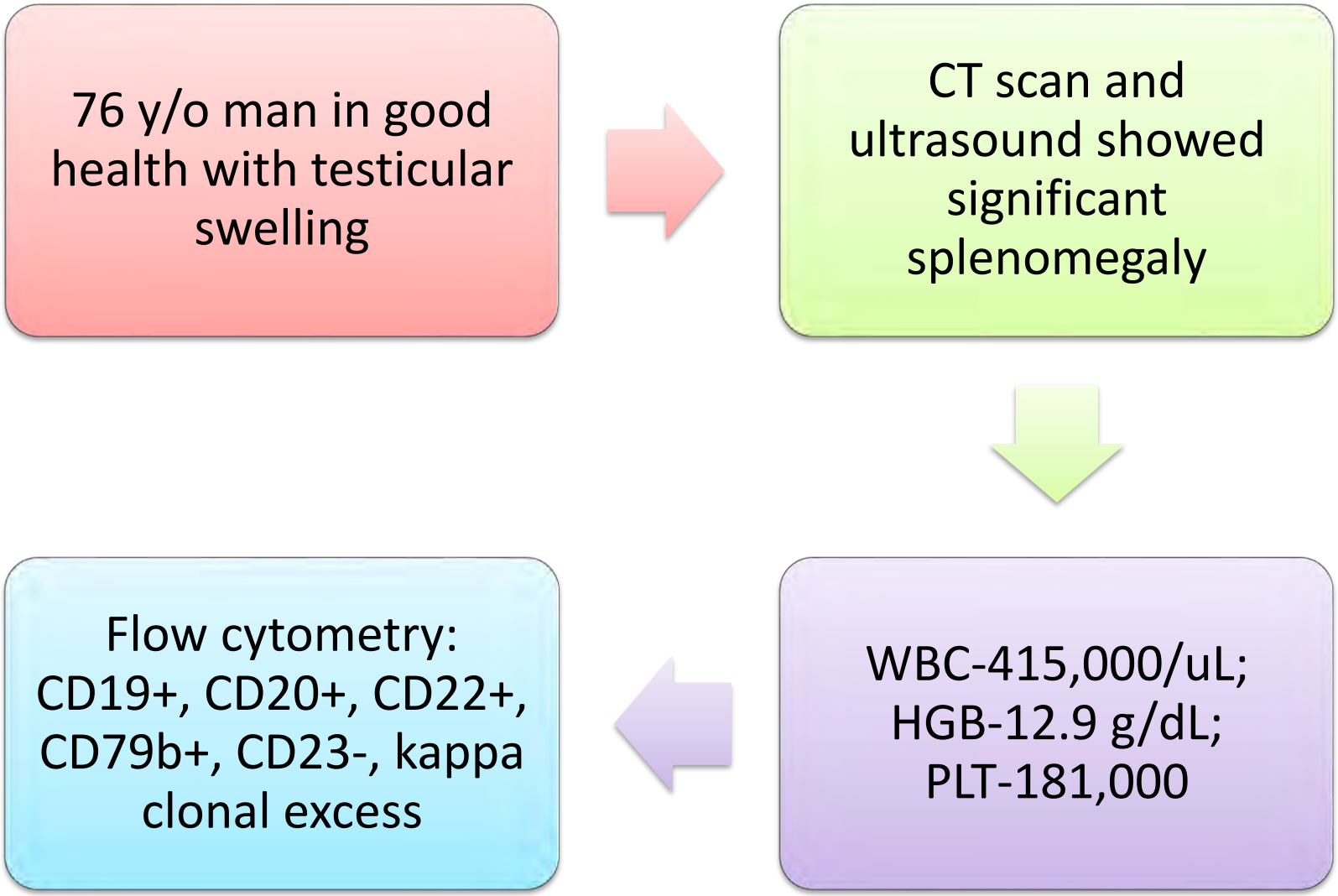
- Falsely decrease count of CLL lymphocytes
- Falsely increase other cell %

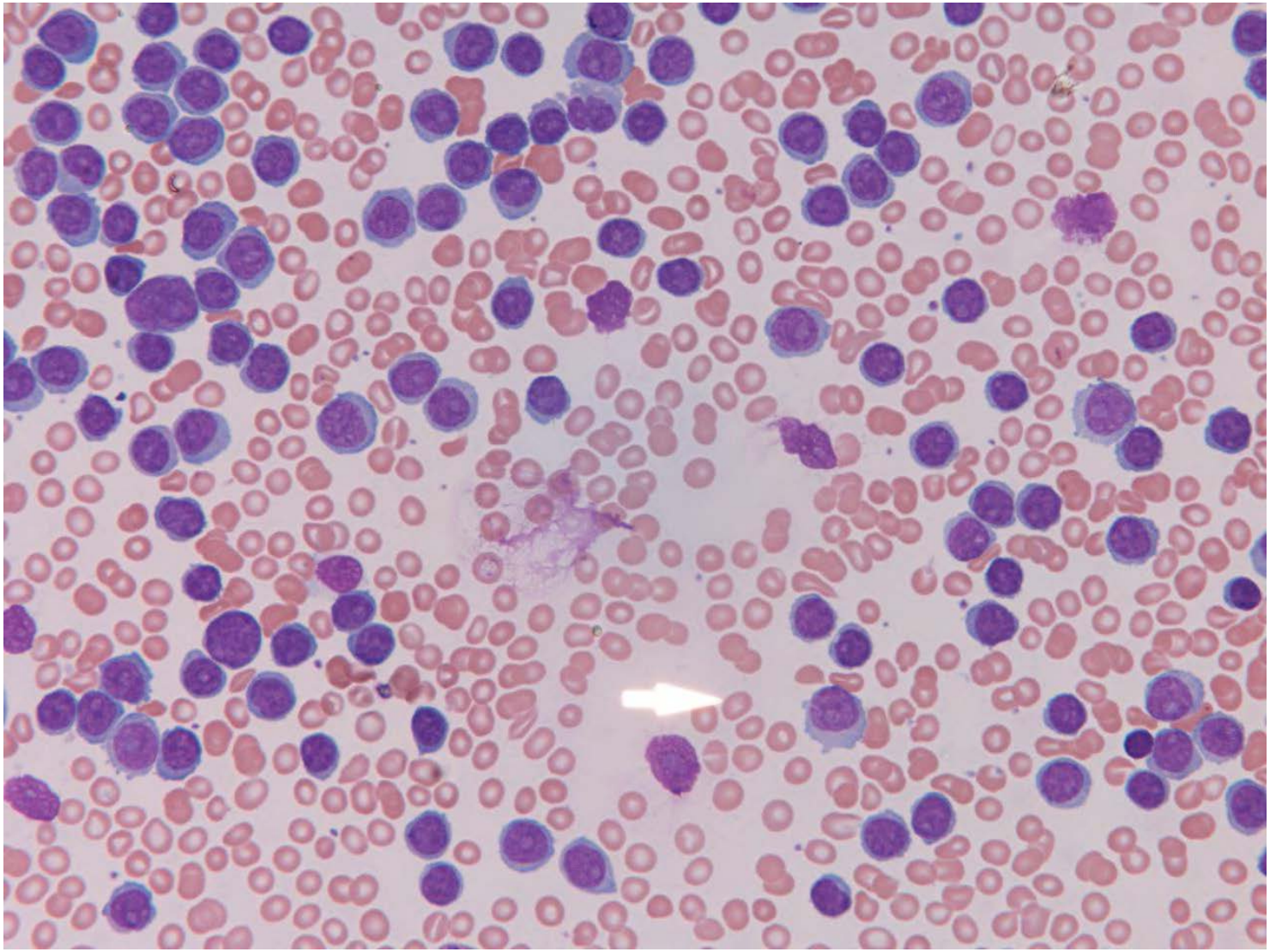
Albumin + EDTA blood decreases smudging

Another option: count smudge cells as lymphs in the manual differential (ONLY in CLL patients)

(New guidelines from ICSH)

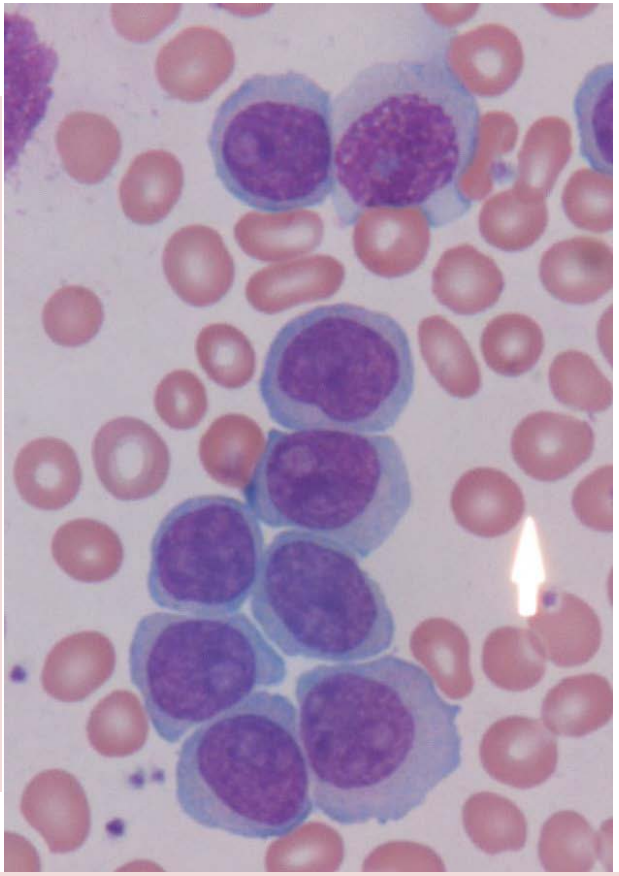
Reference needed


76 y/o man in good health with testicular swelling
CT scan and ultrasound showed significant splenomegaly
WBC-415,000/uL; HGB-12.9 g/dL; PLT-181,000
Flow cytometry: CD19+, CD20+, CD22+, CD79b+, CD23-, kappa clonal excess



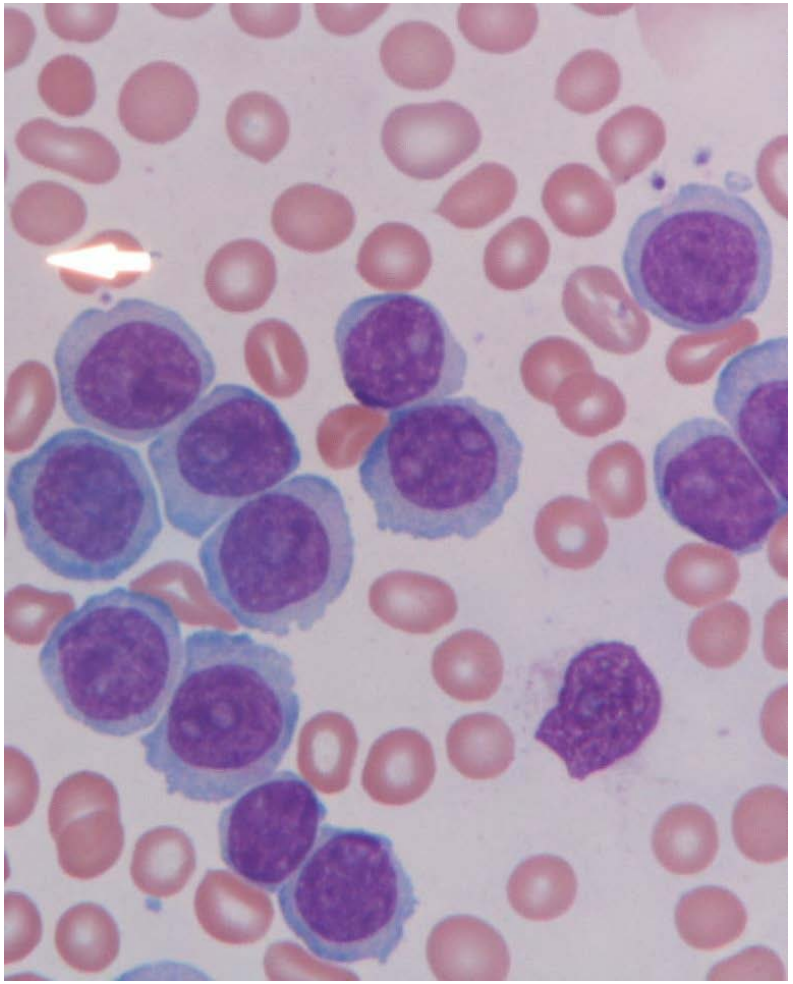
Prolymphocytic Leukemia

- Rare, most patients >60y
- Prolymphocytes are >55% of lymphoid cells
- Marked leukocytosis
- Splenomegaly
- Aggressive disease
- Survival: 30-50 months

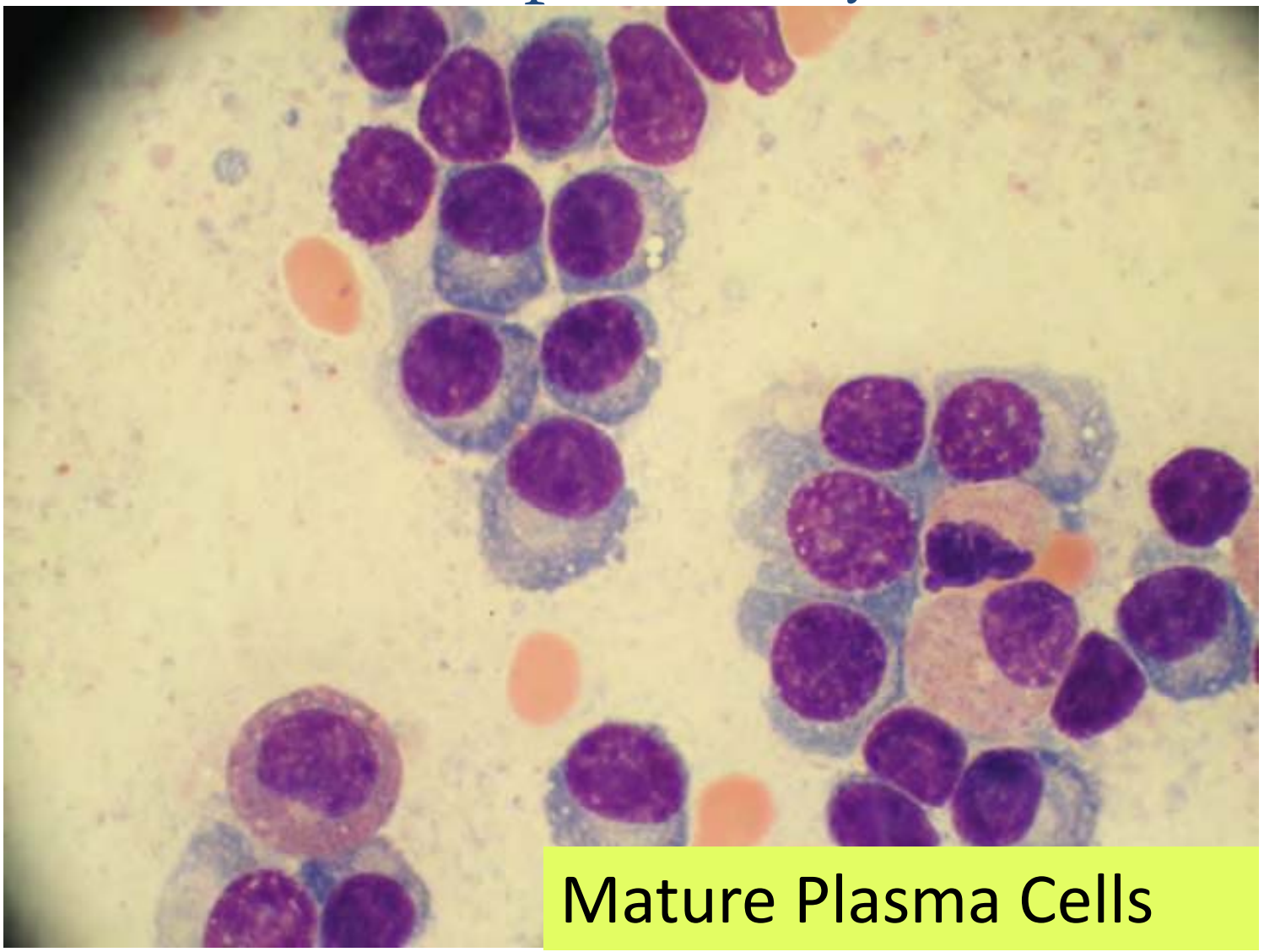


- Genetic testing
  - 17p deletion in 50% - assoc with TP53 gene mutation – likely underlies aggressive course & treatment resistance

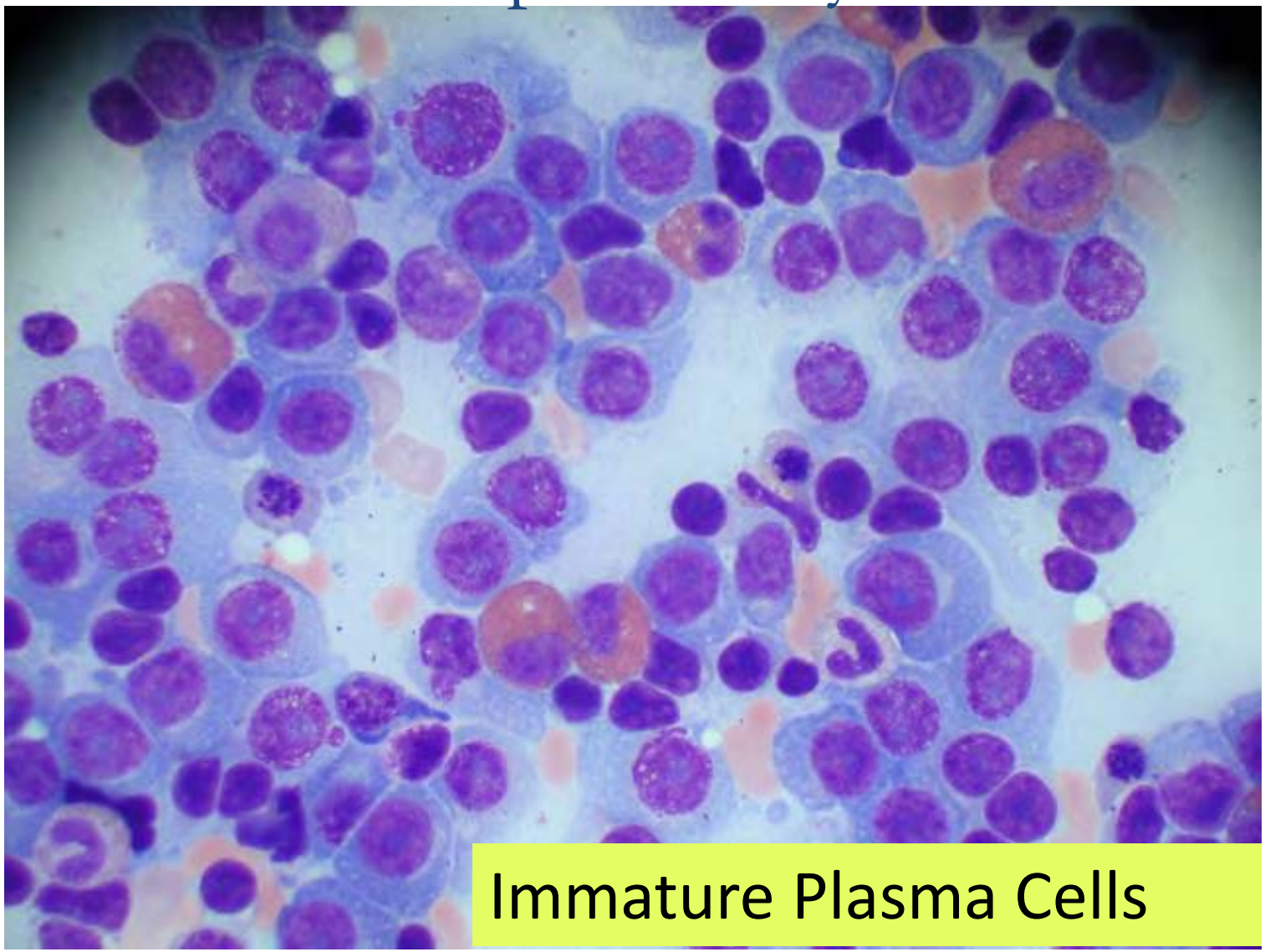
- Prolymphocyte
  - Intermediate stage of lymphocyte maturation
  - Pale blue cytoplasm
  - Moderately clumped chromatin
  - Prominent nucleolus



Bone Marrow Aspirate : Myeloma

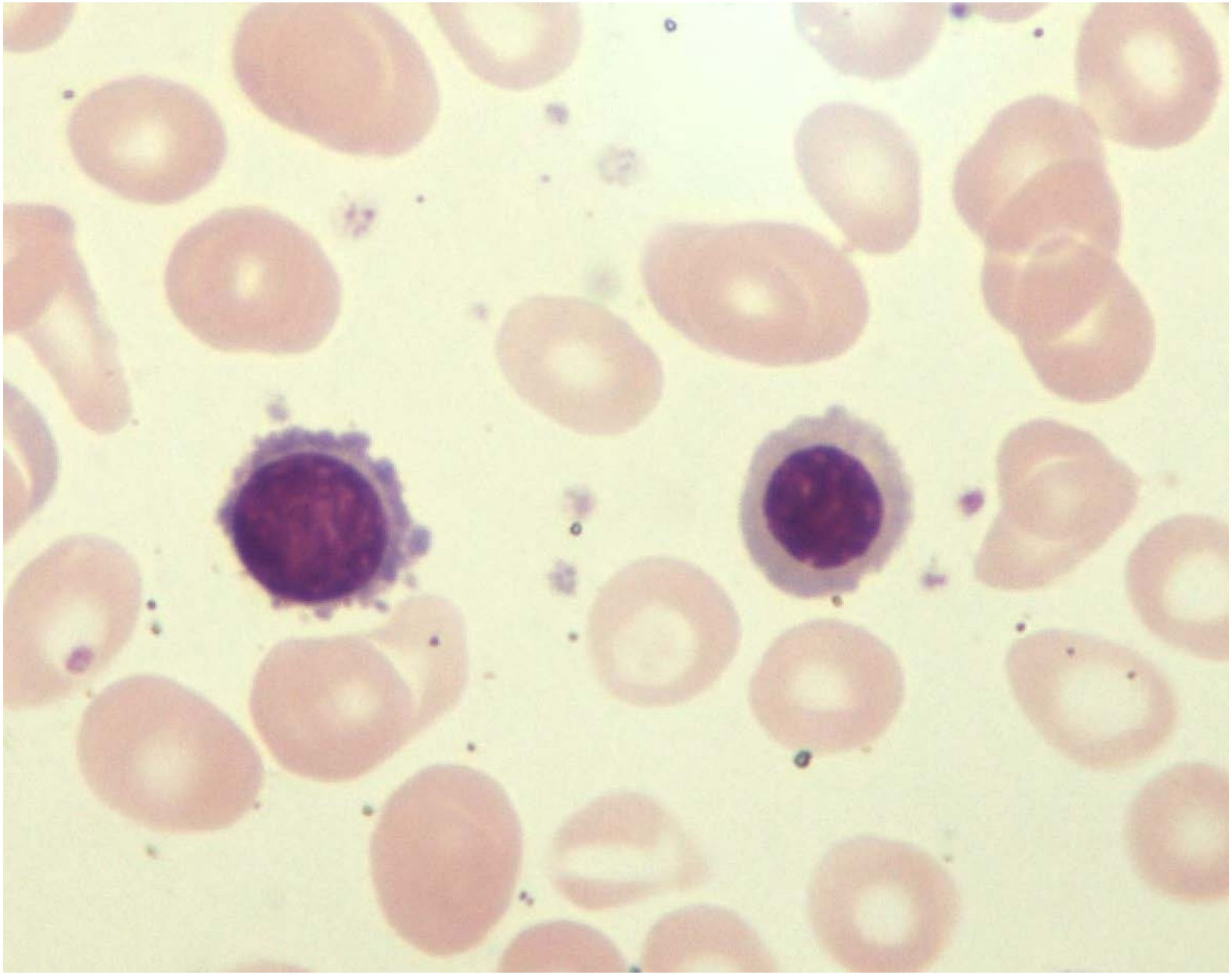


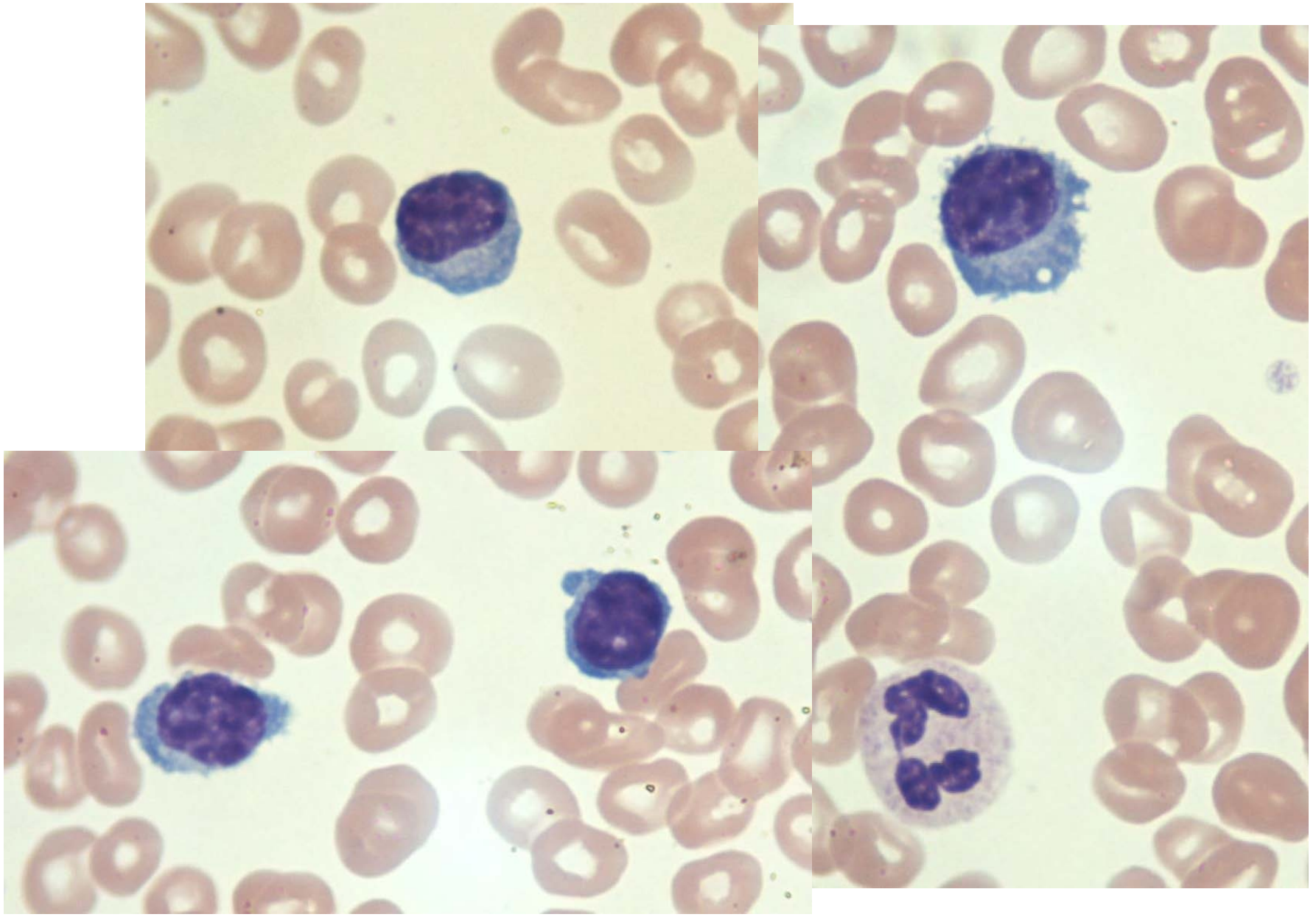
Bone Marrow Aspirate : Myeloma

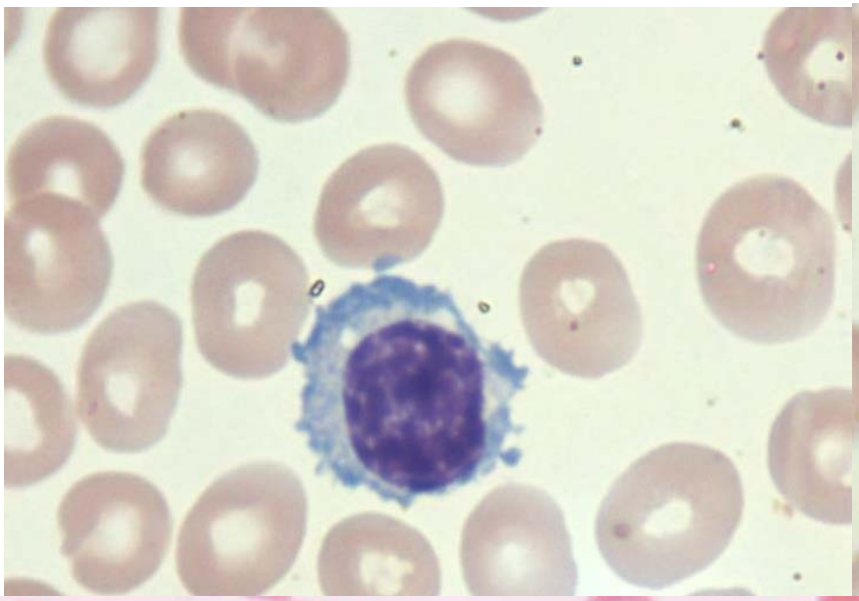
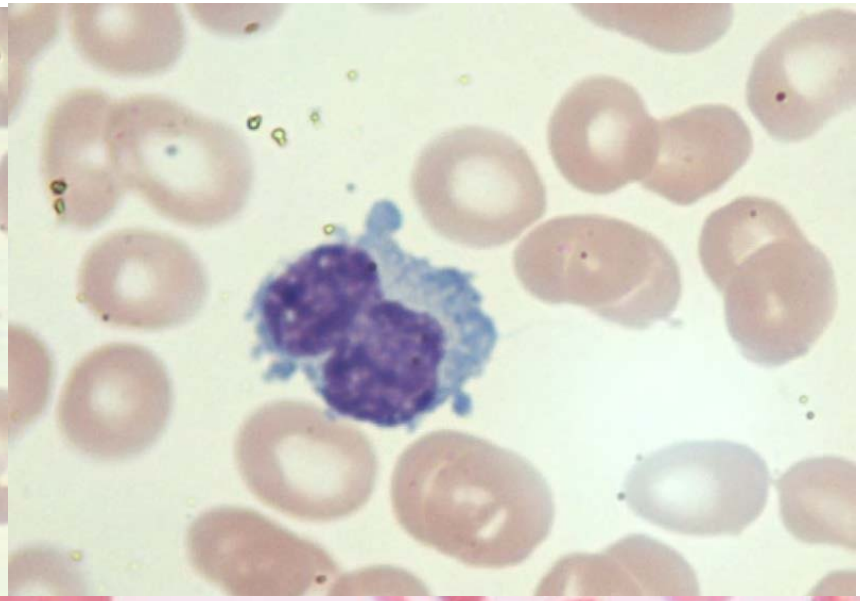
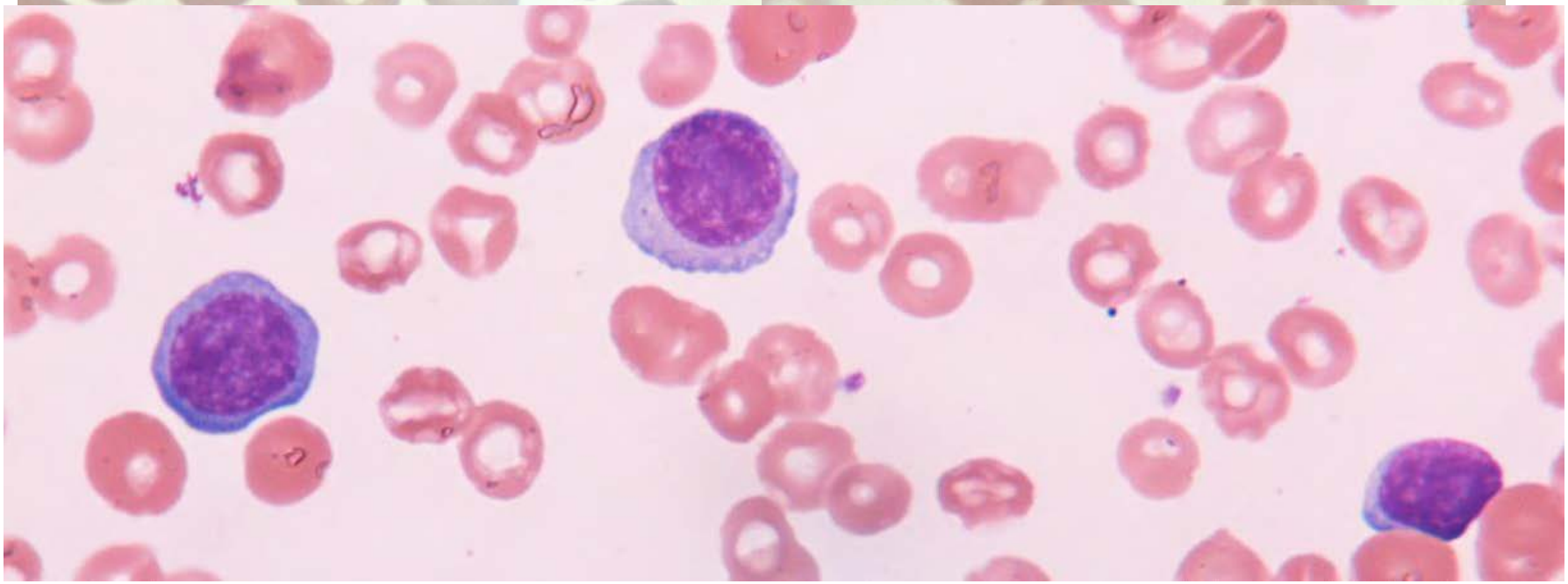


Myeloma plasma cells: morphology

- Broad morphologic spectrum of plasma cells in myeloma
  - Normal
  - Small cell/lymphoid: lymphoplasmacytoid
  - Cleaved/lobulated forms
  - Multinucleated
  - Immature:
    - Proplasmacytes
    - Plasmablasts
  - Flame cells
  - Mott cells





brake

48 year old male

Presented with headaches, abdominal pain, weakness and fatigue

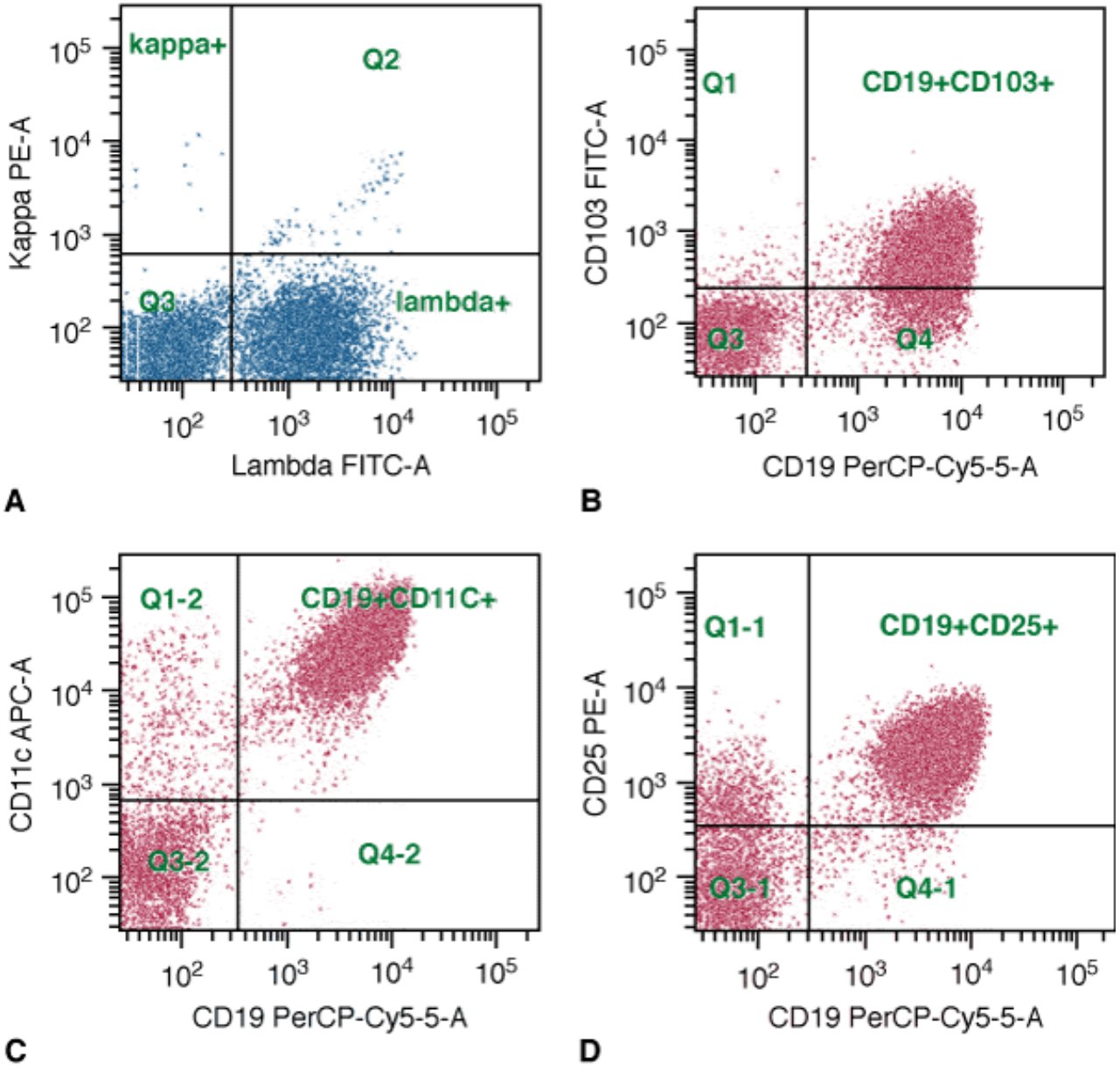
Physical examination found marked splenomegaly

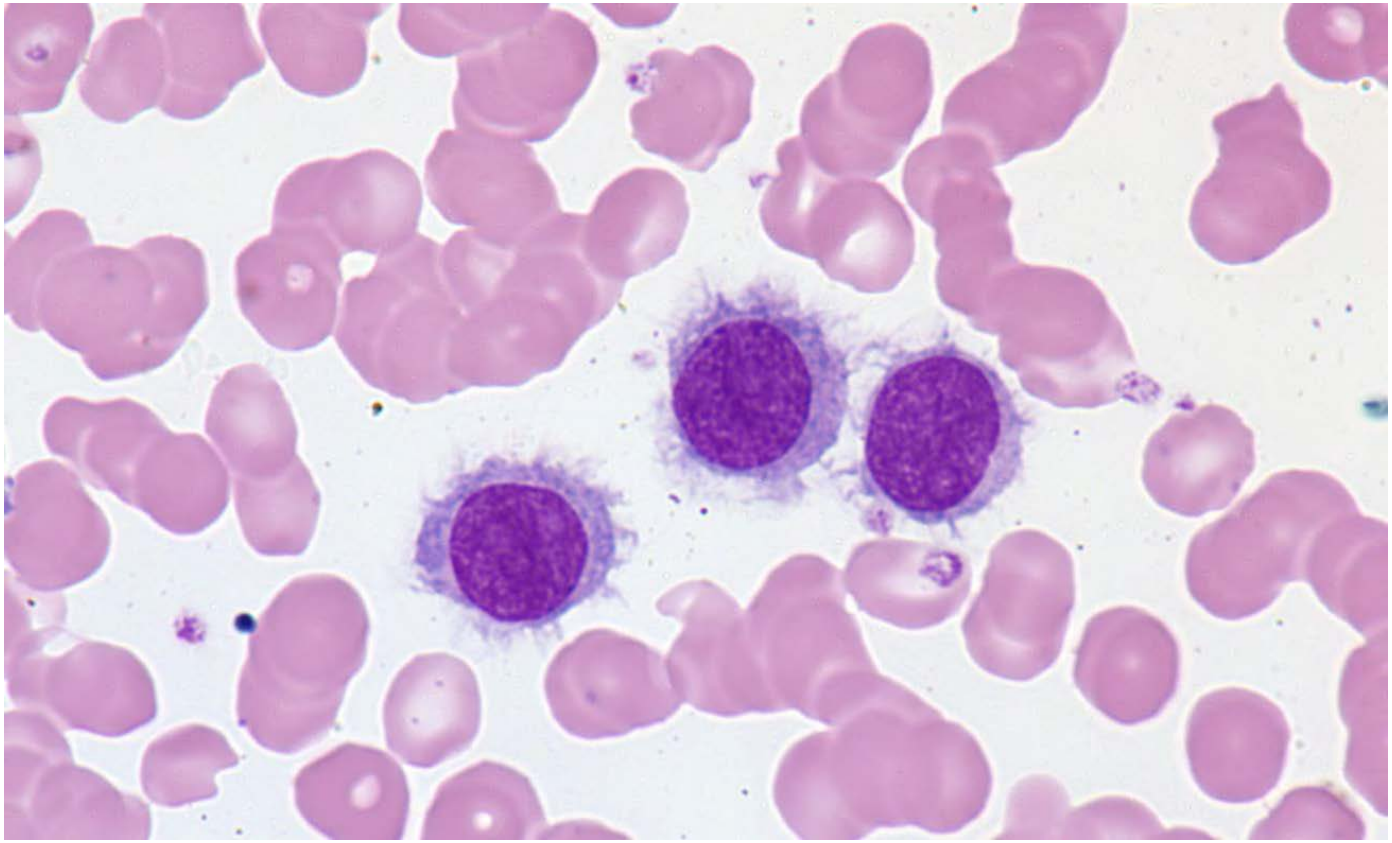
HGB = 8.6 g/dL, PLT = 110,000/uL, WBC = 4000/uL

Bone marrow aspiration for flow cytometry was difficult due to marrow fibrosis

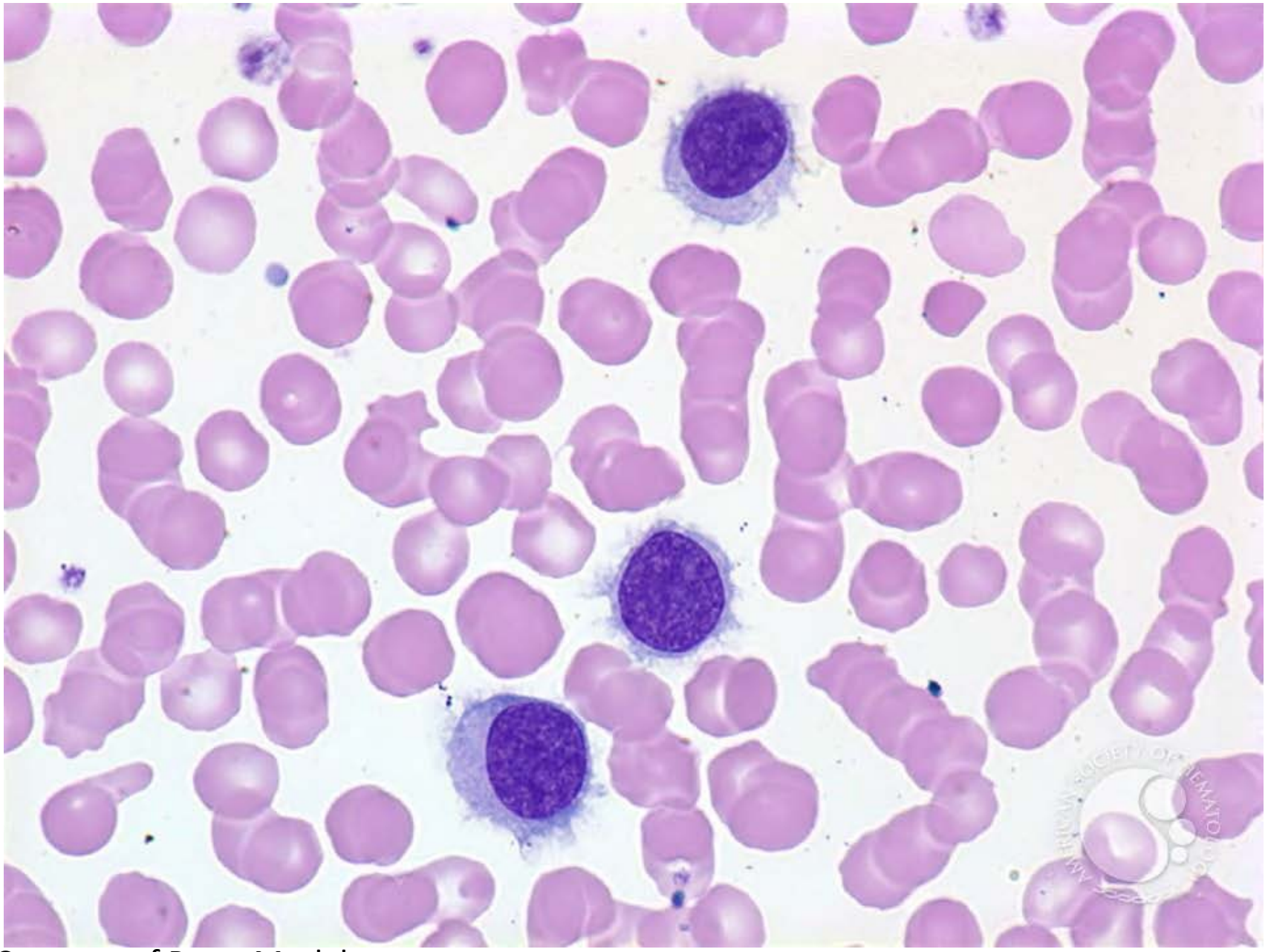
FLOW Cytometry

- Light chain restricted
- CD19
- CD20
- CD22
- CD11c
- CD25
- CD103





Courtesy of Peter Maslak



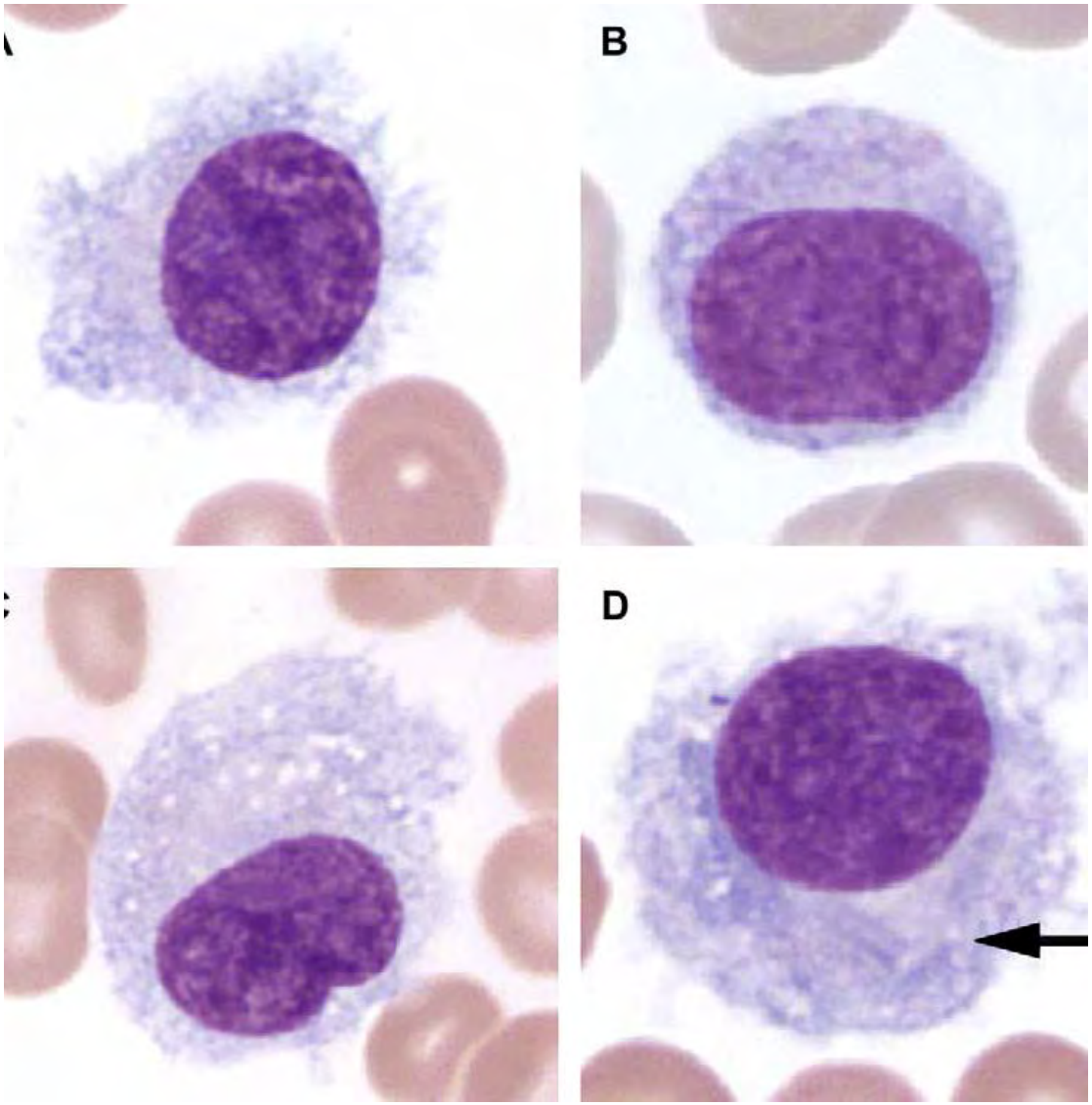
Courtesy of Peter Maslak

Hairy Cell Leukemia

- Indolent mature B-cell neoplasm
- Rare, <5% of leukemias
- Middle age – elderly, usually men (4:1)
- Splenomegaly
- Pancytopenia
  - Including profound monocytopenia
    - May be masked with auto-differentials that classify hairy cells as monocytes

Cytoplasmic border may be serrated, frayed, or wind-blown

Nuclei usually round – oval but may appear as spindled, reniform, horseshoe-shaped, or bi-lobed



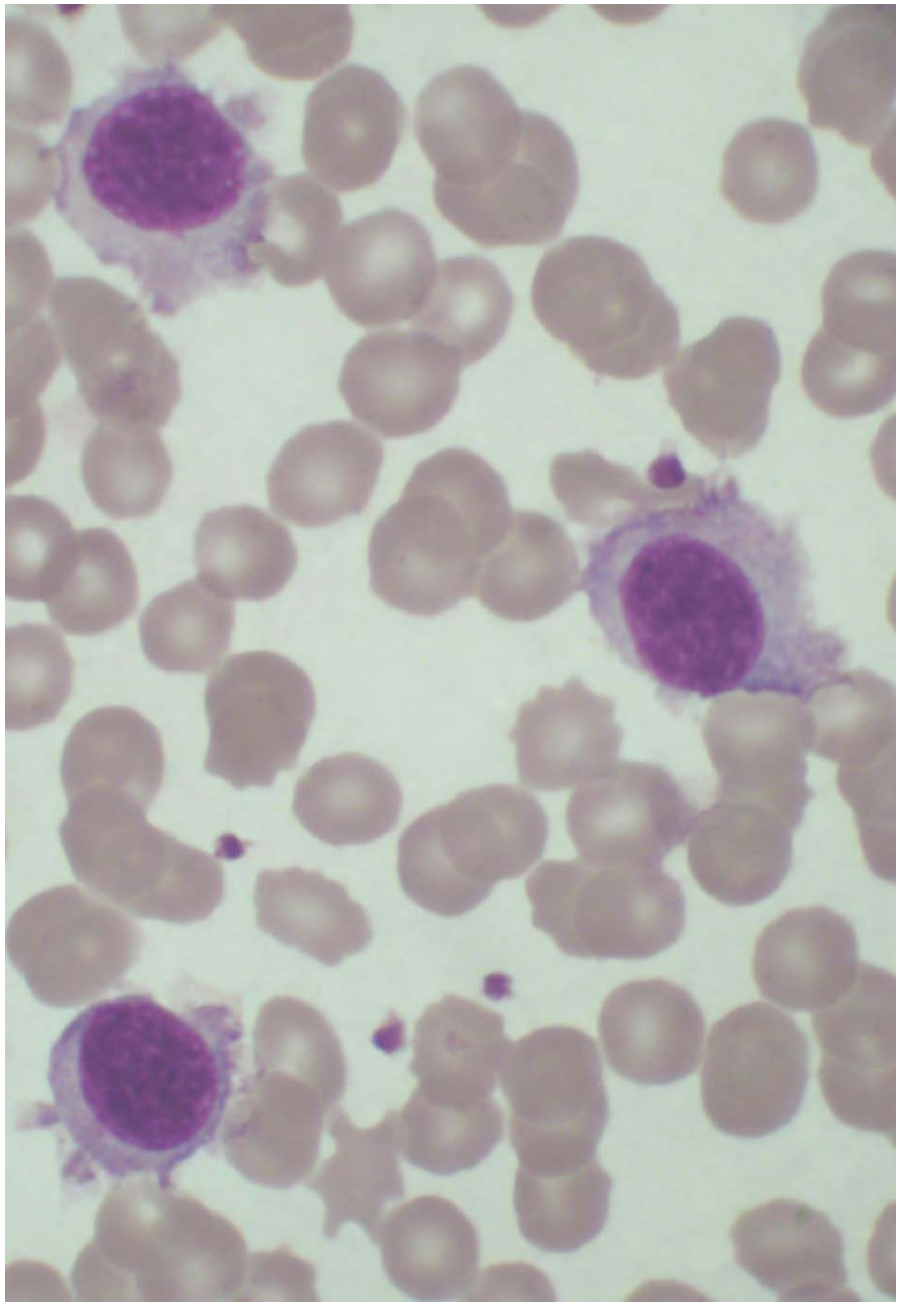
Hematol Oncol Clin N Am 20 (2006) 1023–1049

Hairy Cell Morphology

The cytoplasm stains pale gray-blue with a textured look

Chromatin is partially clumped and granular appearing - intermediate between a mature lymphocyte and a blast.

Nucleoli usually not visible



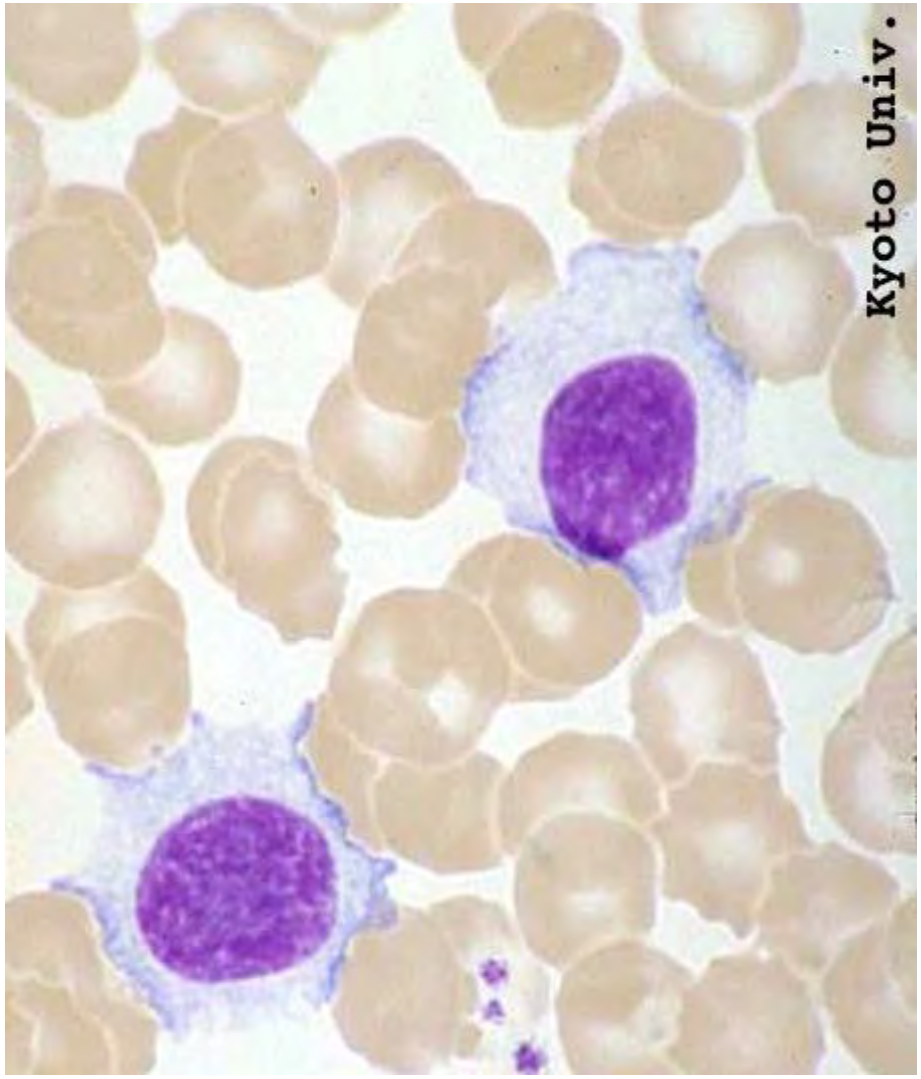
Hairy Cell Morphology

Hairy cells sometimes difficult to find (Look at thicker area and feathered edge of smear)

Many don't have ''textbook'' features – no villous projections

A careful search usually reveals some with typical morphology

"Non-textbook" forms become easier to identify



Hairy Cell Morphology

On smear review, sometimes called

- Monocytes (profound monocytopenia in Hairy cell leukemia)
- Variant lymphocytes
- Large granular lymphocytes
- Normal lymphs

Splenic Marginal Zone Lymphoma

Low grade small B-cell lymphoma

Age at diagnosis: 22-79y; median: 68y

Most patients present with mod – massive splenomegaly

Lymphadenopathy: rare

Anemia (64%); Thrombocytopenia (15%)

Absolute lymphocytosis in 75% - malignant cells usually found in peripheral blood

Splenic Marginal Zone Lymphoma

Immunophenotype

- Surface IgM ++, CD 19+, CD20+, CD79b+
- CD5-, CD10-, CD23- , Bcl-6 neg & cyclin D1 neg.

Cytogenetics

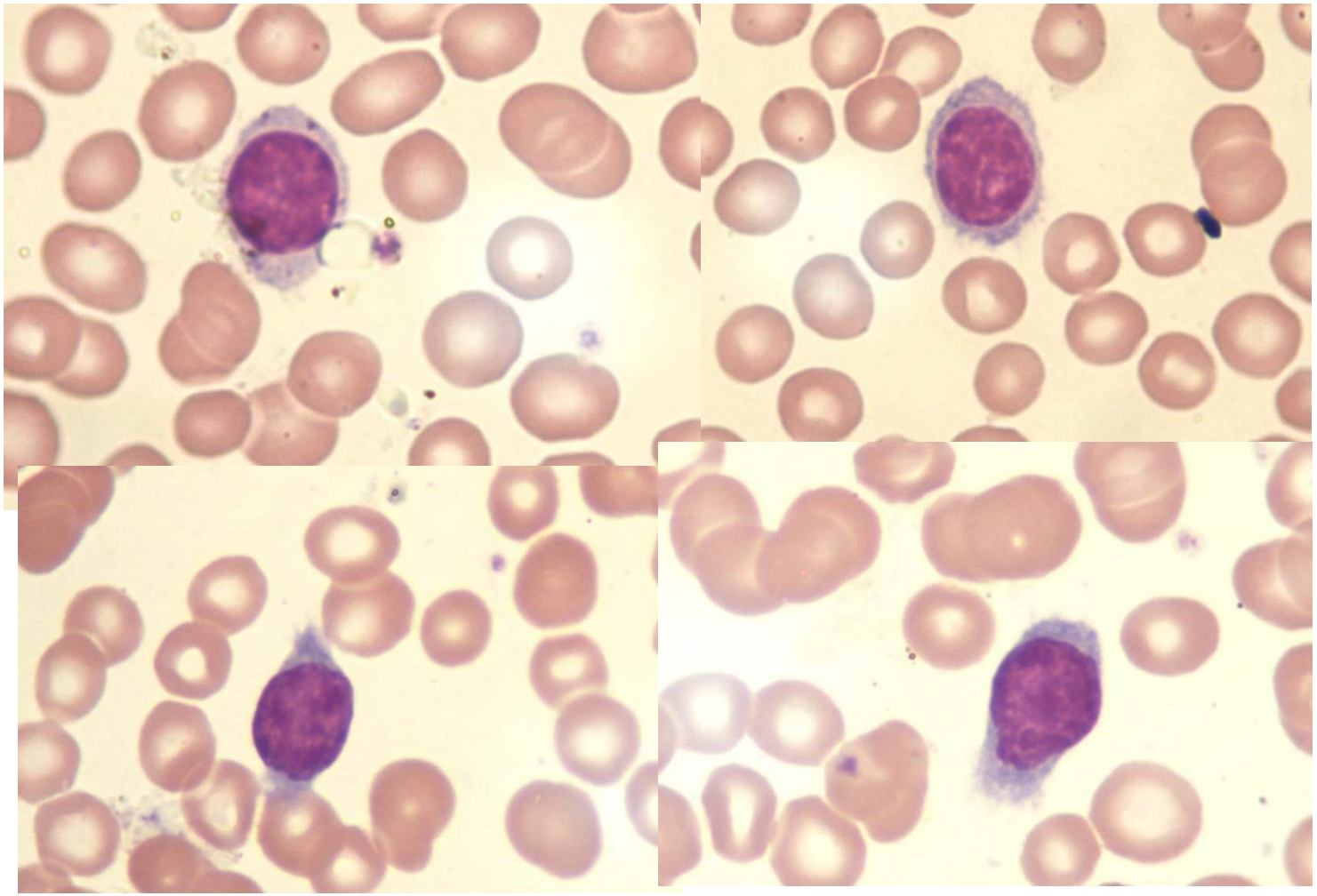
- Complex abnormalities in 80% of cases
- Gains in 3q and 12q
- 7q deletion

Histology

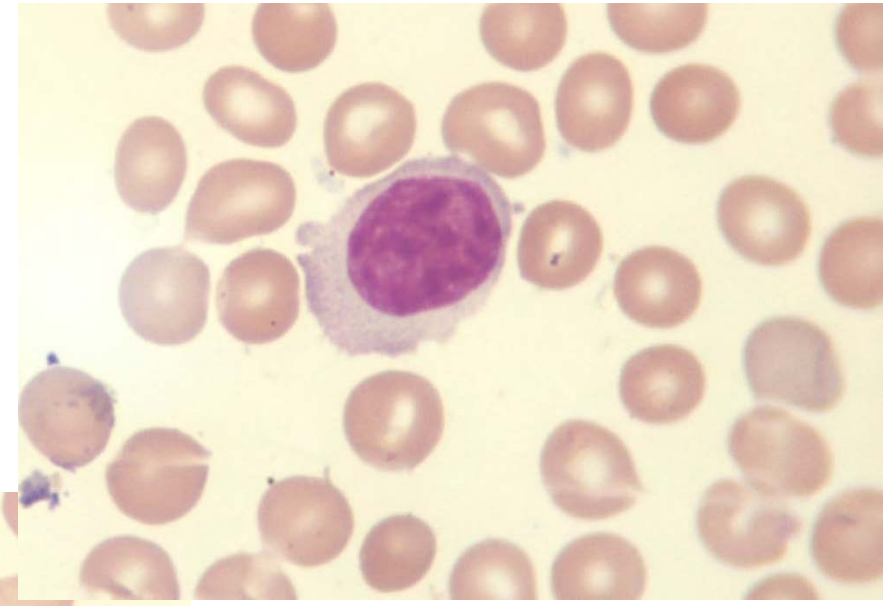
- Spleen & lymph nodes: micronodular lymphoid infiltrate

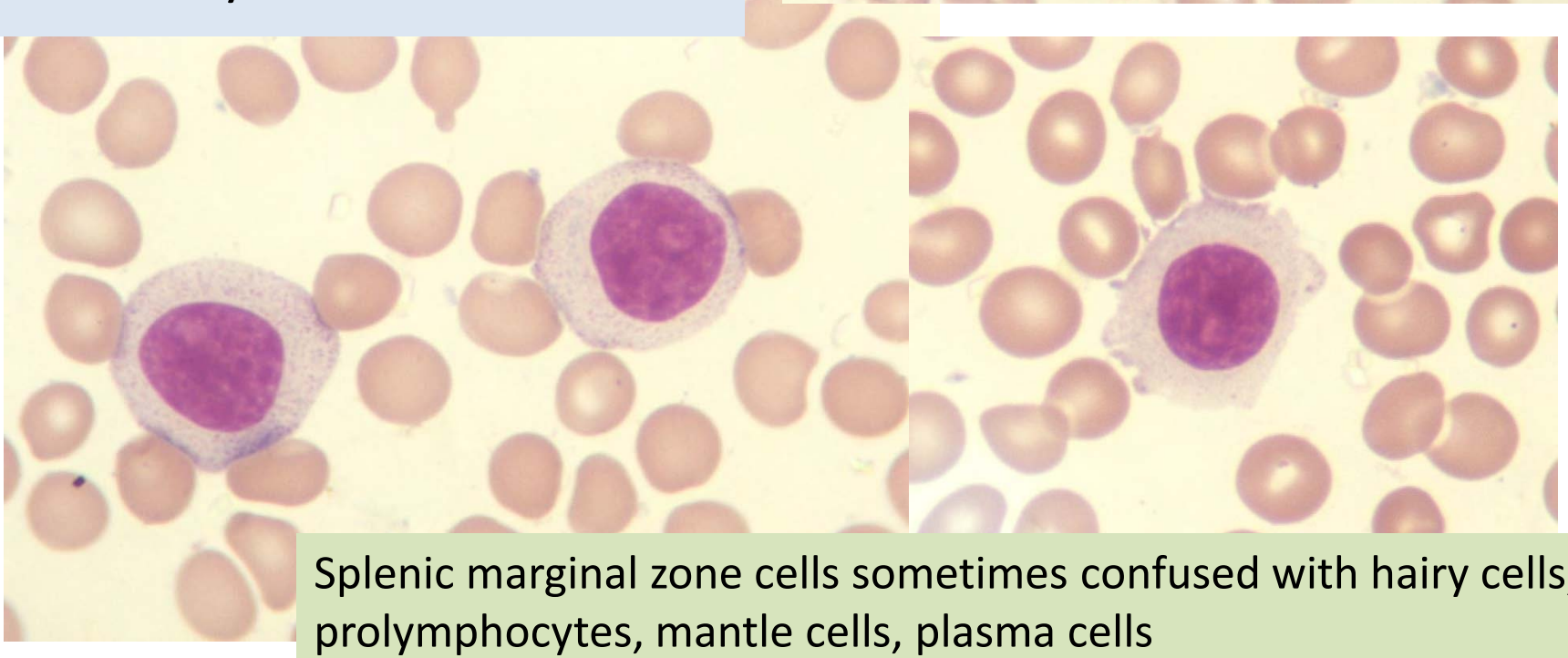
Matutes, E. (2008). Splenic marginal zone lymphoma proposals for a revision of diagnostic, staging and therapeutic criteria. *Leukemia, 22,* 487-495.

- Round-oval nucleus
- Condensed chromatin
- Basophilic cytoplasm with short villi which may be polar



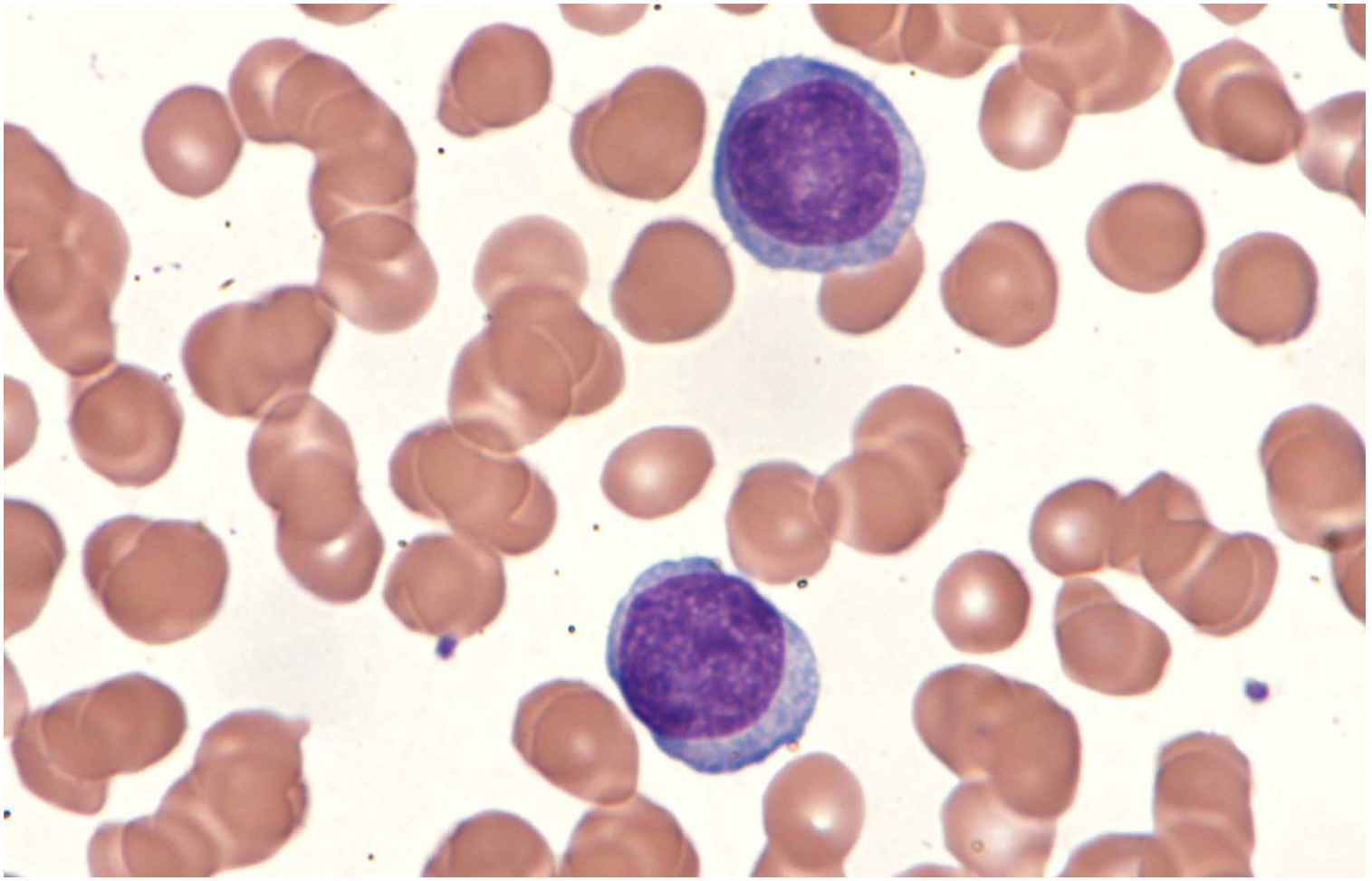
- Can see morphologic heterogeneity
- Lymphoid cells without villous features
- Lymphoplasmacytoid cells
- Resemble prolymphocytes
- Monocytoid cells





Splenic marginal zone cells sometimes confused with hairy cells, prolymphocytes, mantle cells, plasma cells

Marginal Zone Lymphoma



Courtesy of Peter Maslak

41 year old HIV patient

41 yo male presented with ptosis of the left eyelid and right testicular swelling.

Severe headaches, weakness, and weight loss of approximately 50 to 60 pounds in the previous 5 to 6 months

Diagnosed with HIV 2 months ago

- CD4 = 58/uL (600 - 1400/uL)
- HIV viral load = 500,000 copies/mL

On physical examination, the patient was afebrile and cachectic appearing

Infectious Diseases in Clinical Practice: March 2007 - Volume 15 - Issue 2 - pp 116-118

No lymphadenopathy was appreciated in inguinal, cervical, supraclavicular, or axillary areas.

Labs: WBC= $3.2 \times 10^3/\mu L$ with **7% atypical lymphocytes,** HGB=9.1 g/dL, HCT=28%

After 2½ weeks of HAART therapy, CD4+ = 166/µL; viral load = 5600 copies/mL.

Ultrasound revealed enlarged, hyperemic testicles with a diffuse echo texture suggestive of a metastatic neoplasm

MRI of brain showed small mass

Infectious Diseases in Clinical Practice: March 2007 - Volume 15 - Issue 2 - pp 116-118

Lumbar puncture did not reveal any malignant cells

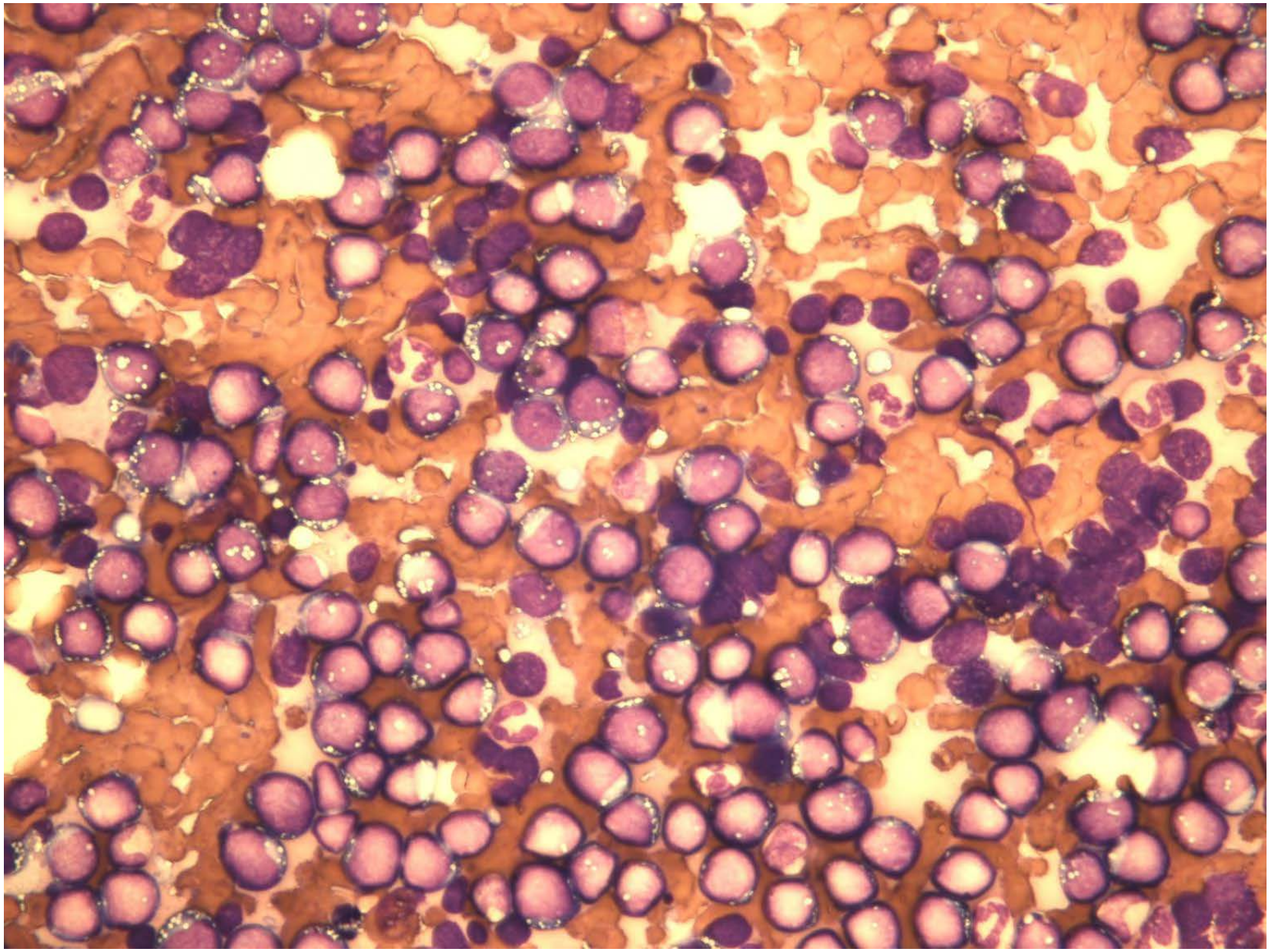
Bone marrow specimen showed atypical, blast-like cells (next slide).

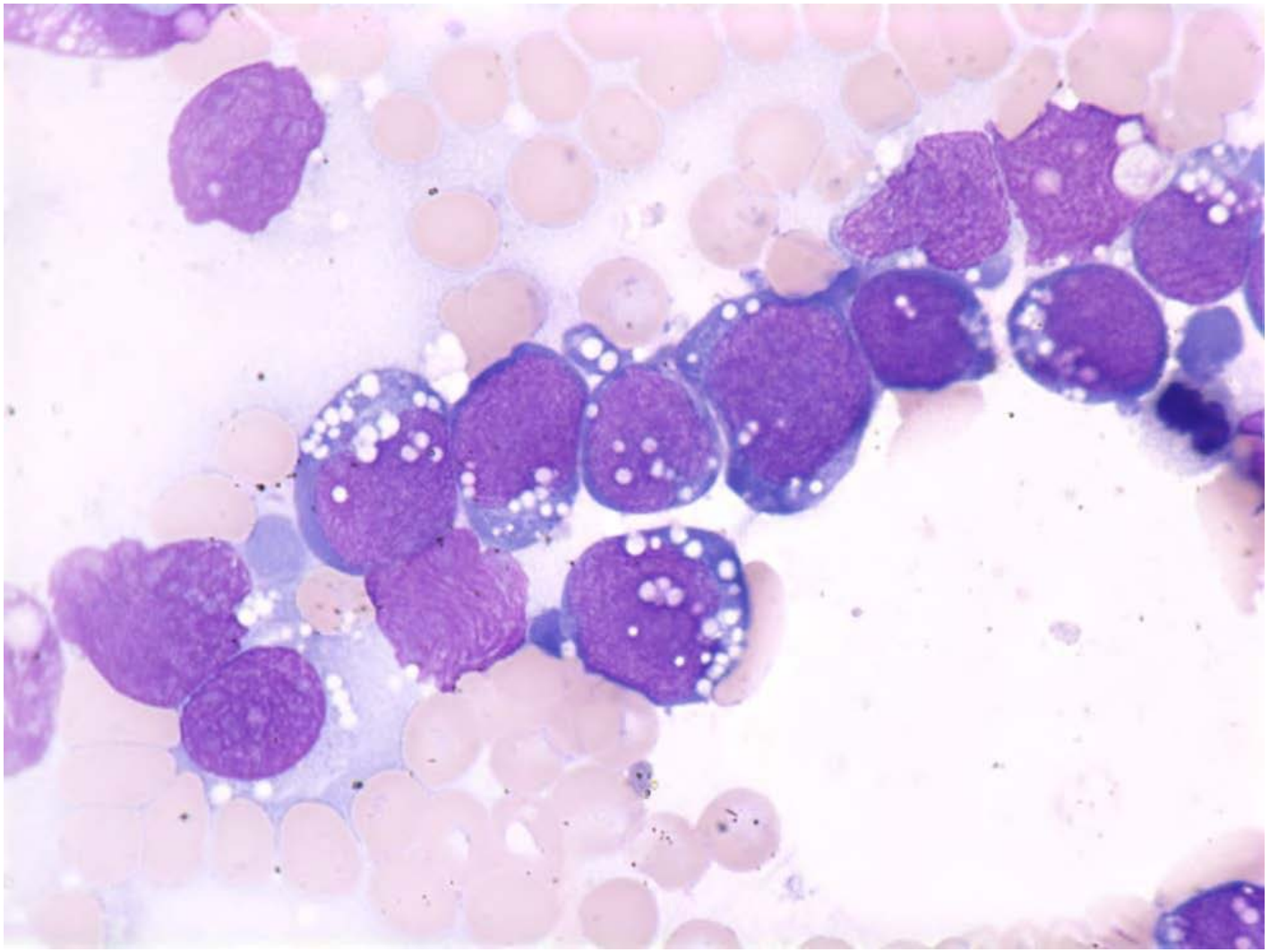
Flow cytometry was positive for CD19, CD20, CD22, CD10, BCL6, CD38, **CD77 and CD43**. Kappa light chain restricted. **TdT negative**

FISH detected t(8;14)

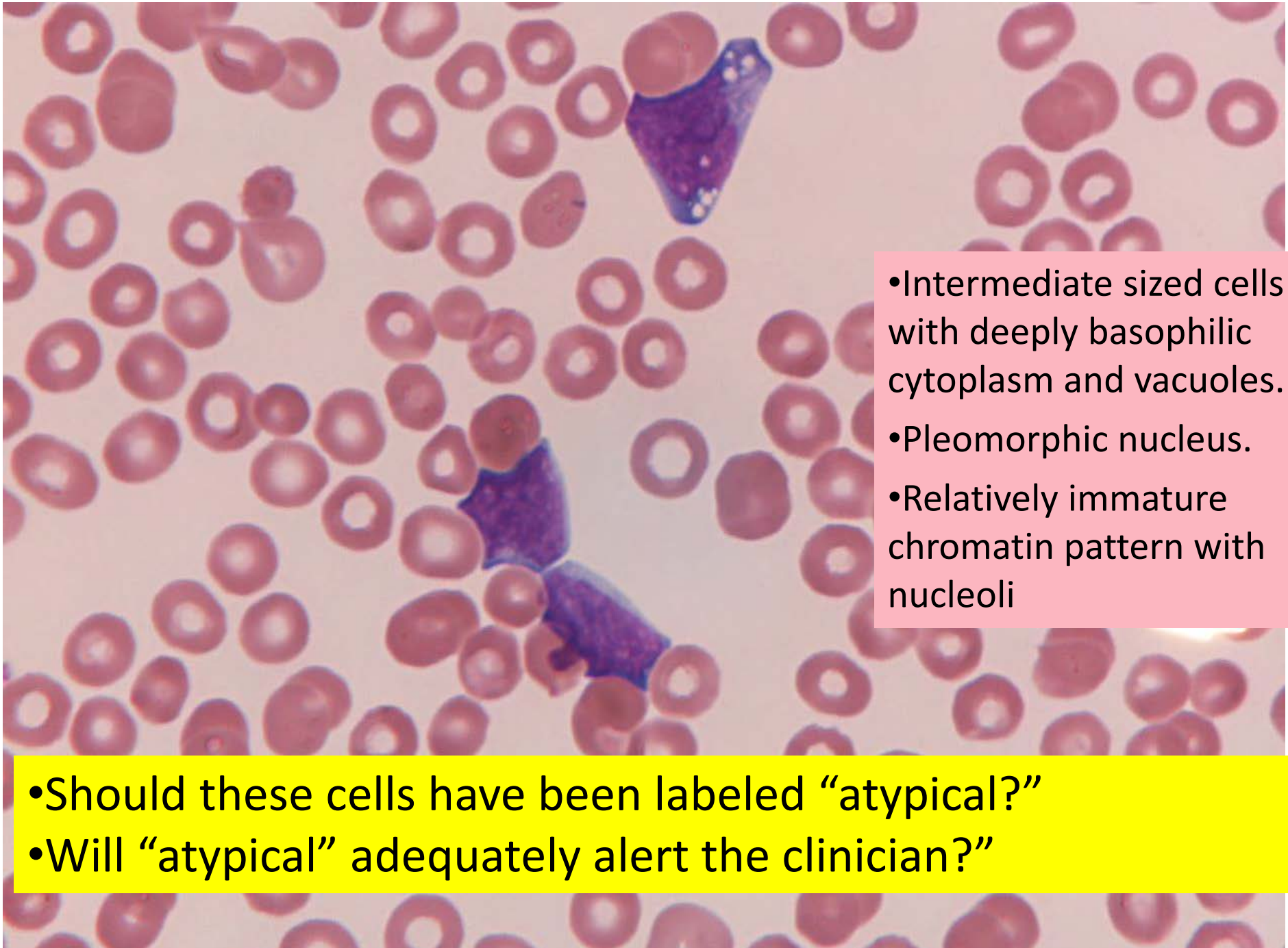
Patient refused chemotherapy for personal reasons and died 4 months later.

Infectious Diseases in Clinical Practice: March 2007 - Volume 15 - Issue 2 - pp 116-118





Courtesy of Peter Maslak



- Intermediate sized cells with deeply basophilic cytoplasm and vacuoles.
- Pleomorphic nucleus.
- Relatively immature chromatin pattern with nucleoli

- Should these cells have been labeled “atypical?”
- Will “atypical” adequately alert the clinician?”

Burkitt Lymphoma

Aggressive B cell neoplasm. Three types:

- Endemic type - tropical Africa, most common malignancy in children)
- Sporadic type – worldwide; children & young adults
- Immunodeficiency associated

Previously classified as ALL L3 (leukemic phase)

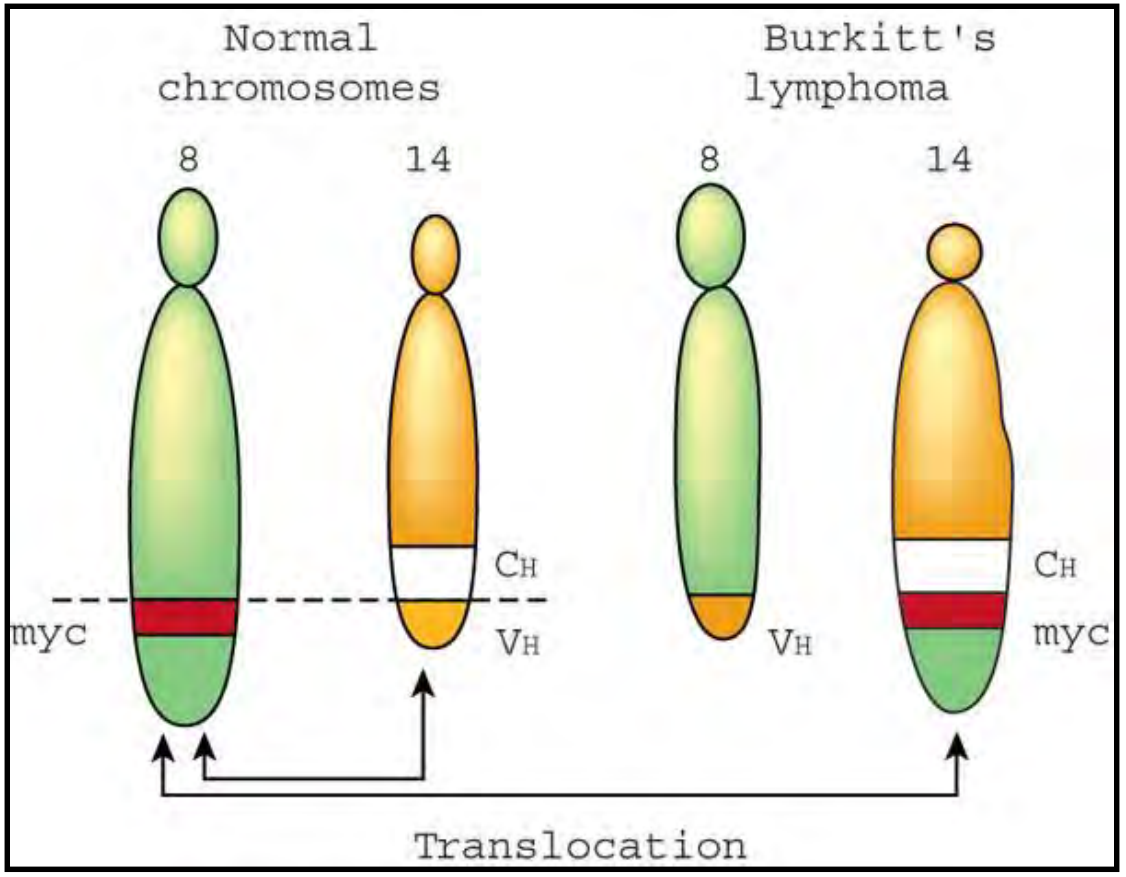
EBV and malaria association

t(8;14) in >90%

Incidence of marrow and blood involvement varies but found more in immunodeficiency related

- IG heavy chain genes located on chromosome 14
- Protooncogene c-*myc moves* from chromosome 8 to 14 close to IG gene
- c-*myc* is now in a region of active gene transcription
- Overproduction of the c-*myc* product (a transcription factor essential for cell division) stimulates lymphocyte mitosis activity and disease!

t(8;14)



Gustav J. V. Nossal. Nature 421, 440-444(23 January 2003)

Follicular lymphoma

Follicular lymphoma accounts for 20% of all lymphomas

Mostly adults; median 6th decade

Most patients have widespread disease at diagnosis

- Peripheral and central (abdominal, thoracic) lymphadenopathy and splenomegaly
- Otherwise asymptomatic
- Marrow involved in 40-70%

“Indolent lymphoma” but not curable (some exceptions)

Biopsy lymph node – cell suspension for flow analysis

- Monoclonal surface Immunoglobulin, pan B-cell antigens

Follicular Lymphoma

B CELLS

SIg+	CD20+	CD79a+	BCL6+	CD5-
CD19+	CD22+	BCL2+	CD10+	CD43-

LYMPHOCYTE MORPHOLOGY

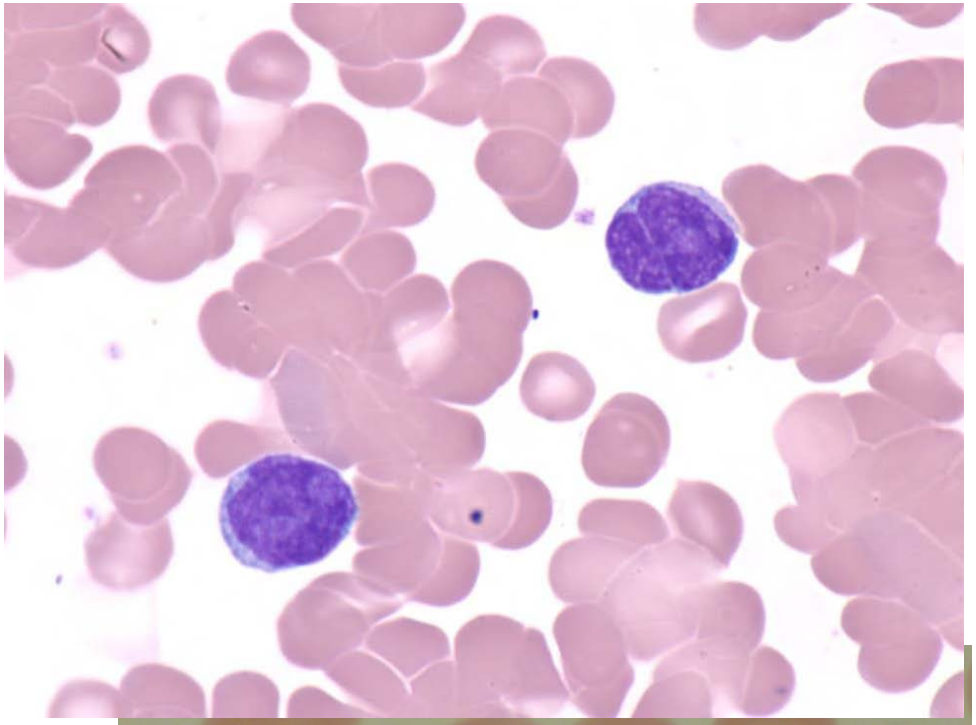
Small – Medium Sized

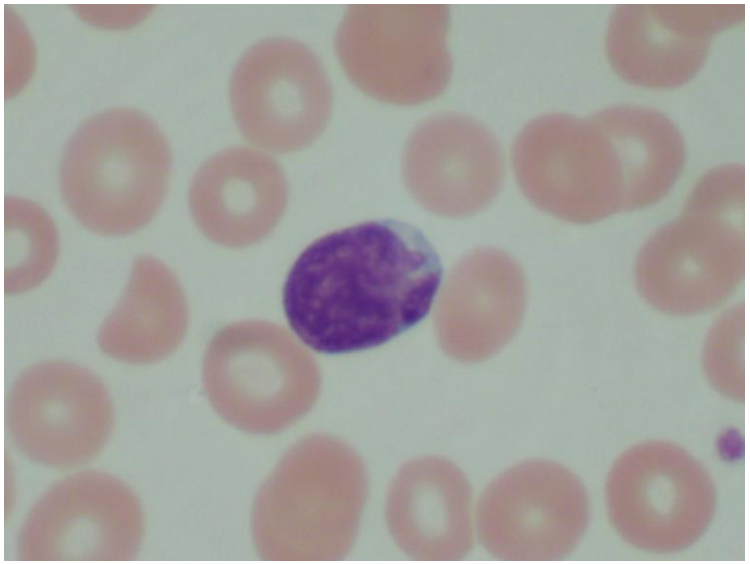
- Angulated, elongated, twisted or cleaved nuclei
- Inconspicuous nuclei
- Scant pale cytoplasm

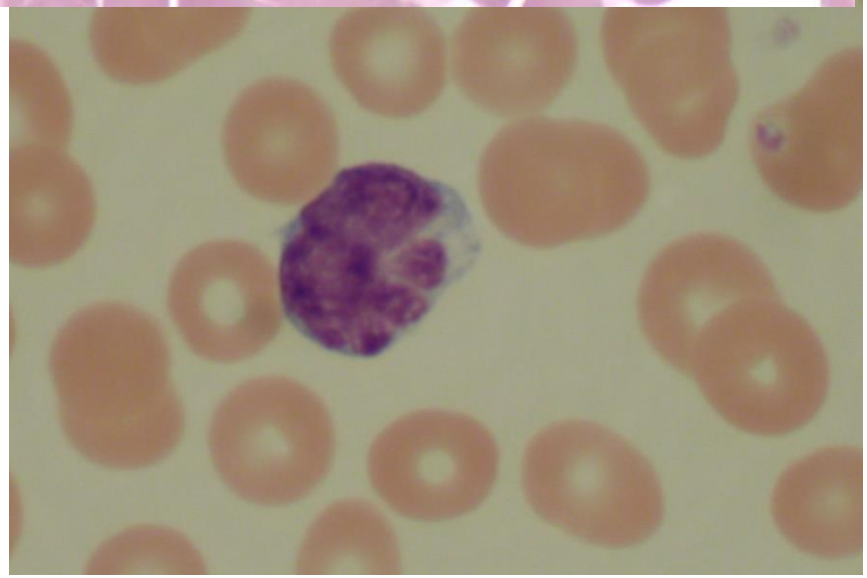
Large Cells

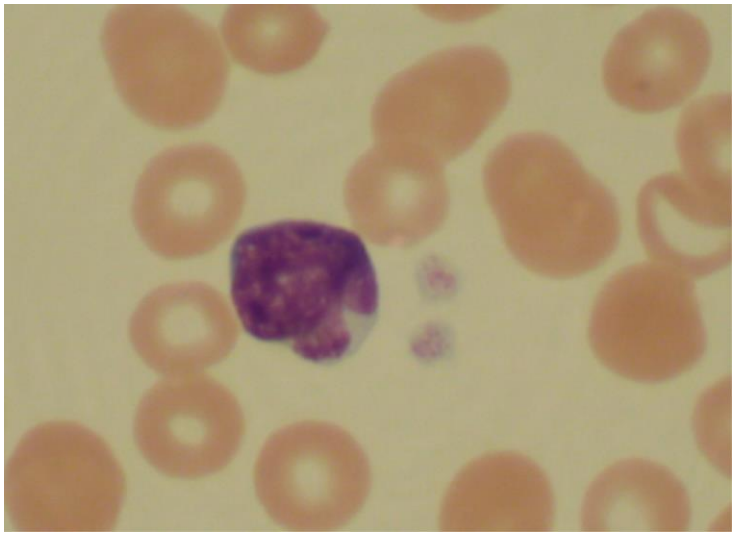
- 3X size of lymphocyte
- Round/oval occasionally dented or multilobulated nuclei
- Vesicular chromatin
- 1-3 nucleoli
- Scant cytoplasm

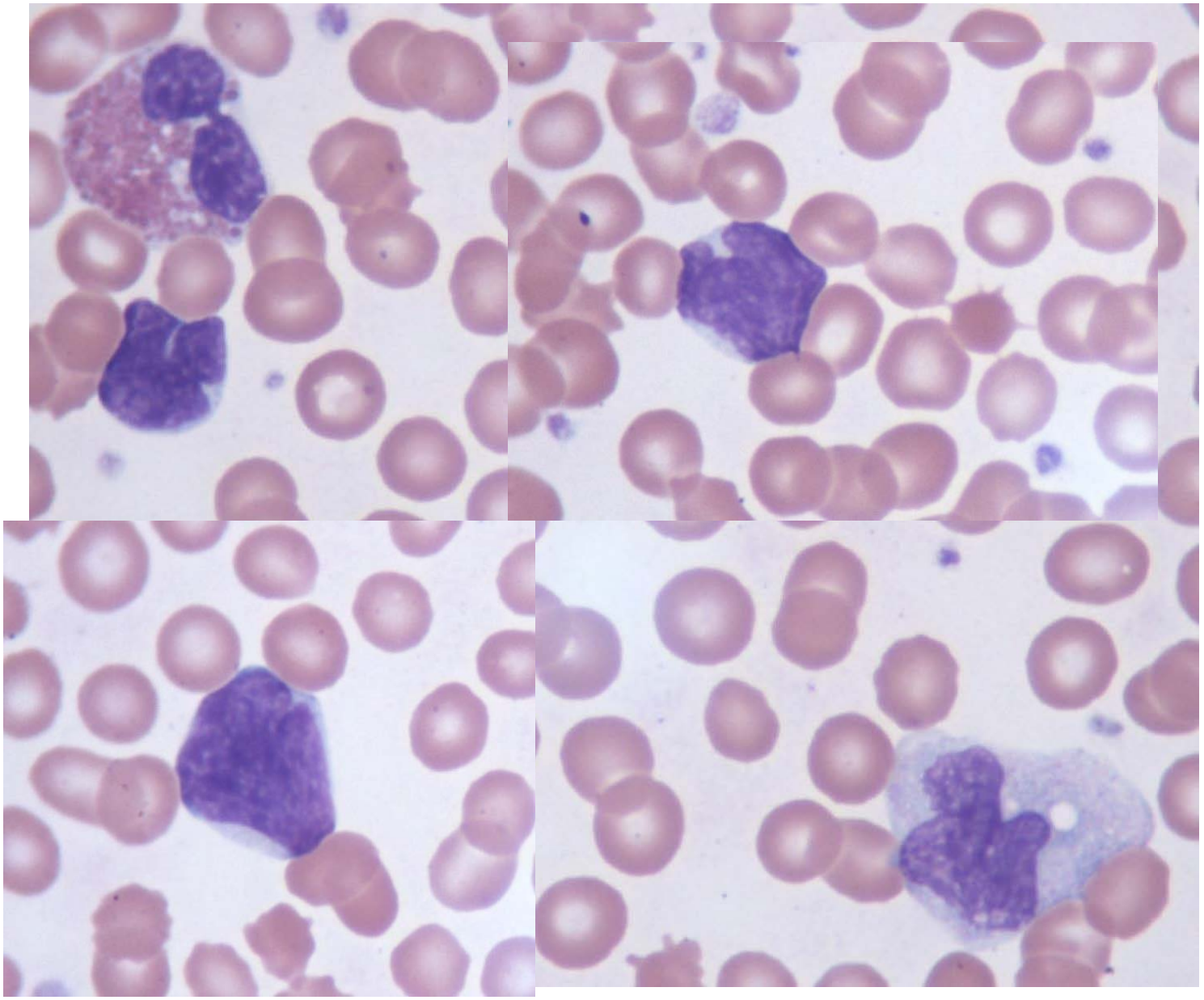
Follicular Lymphoma



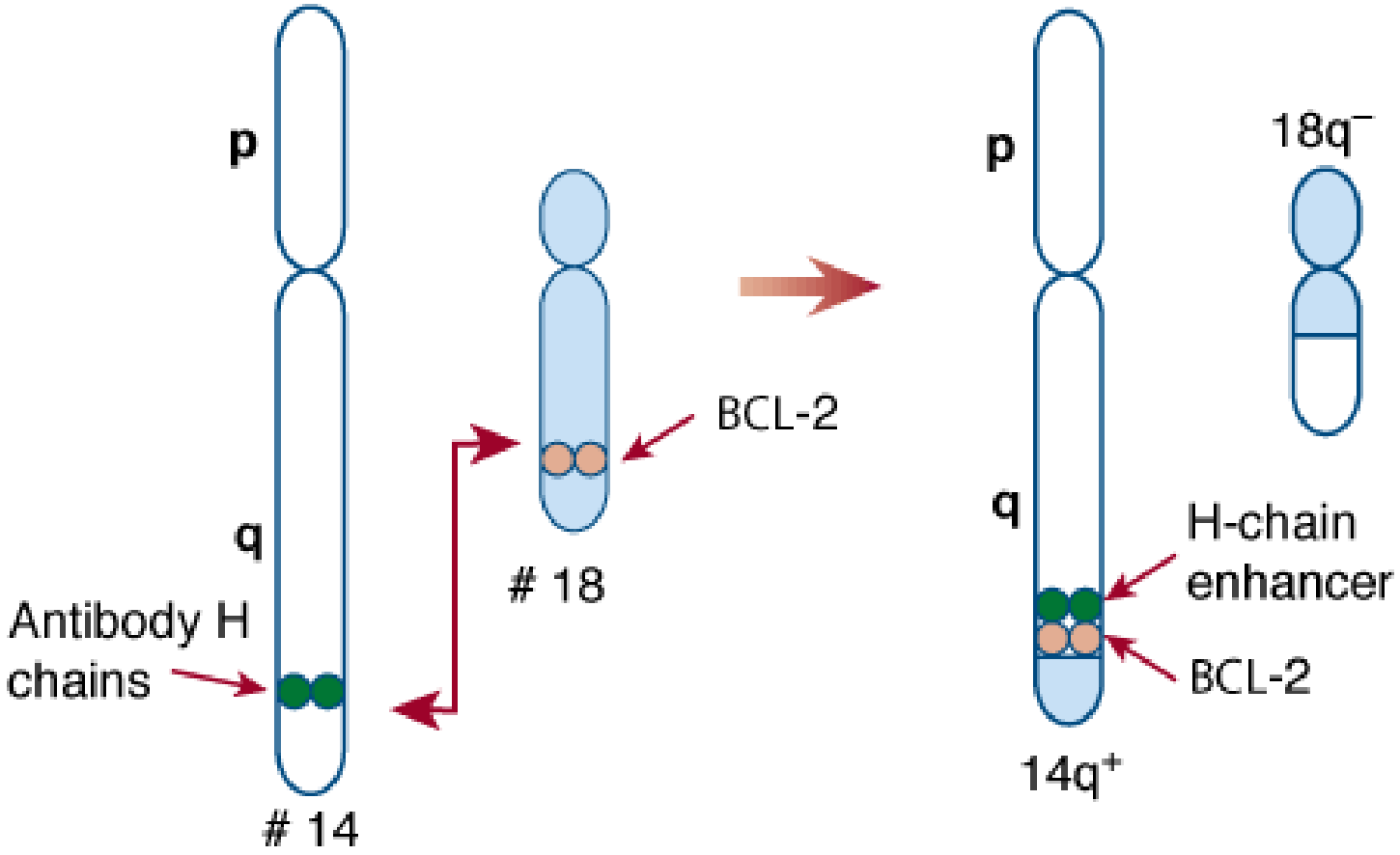






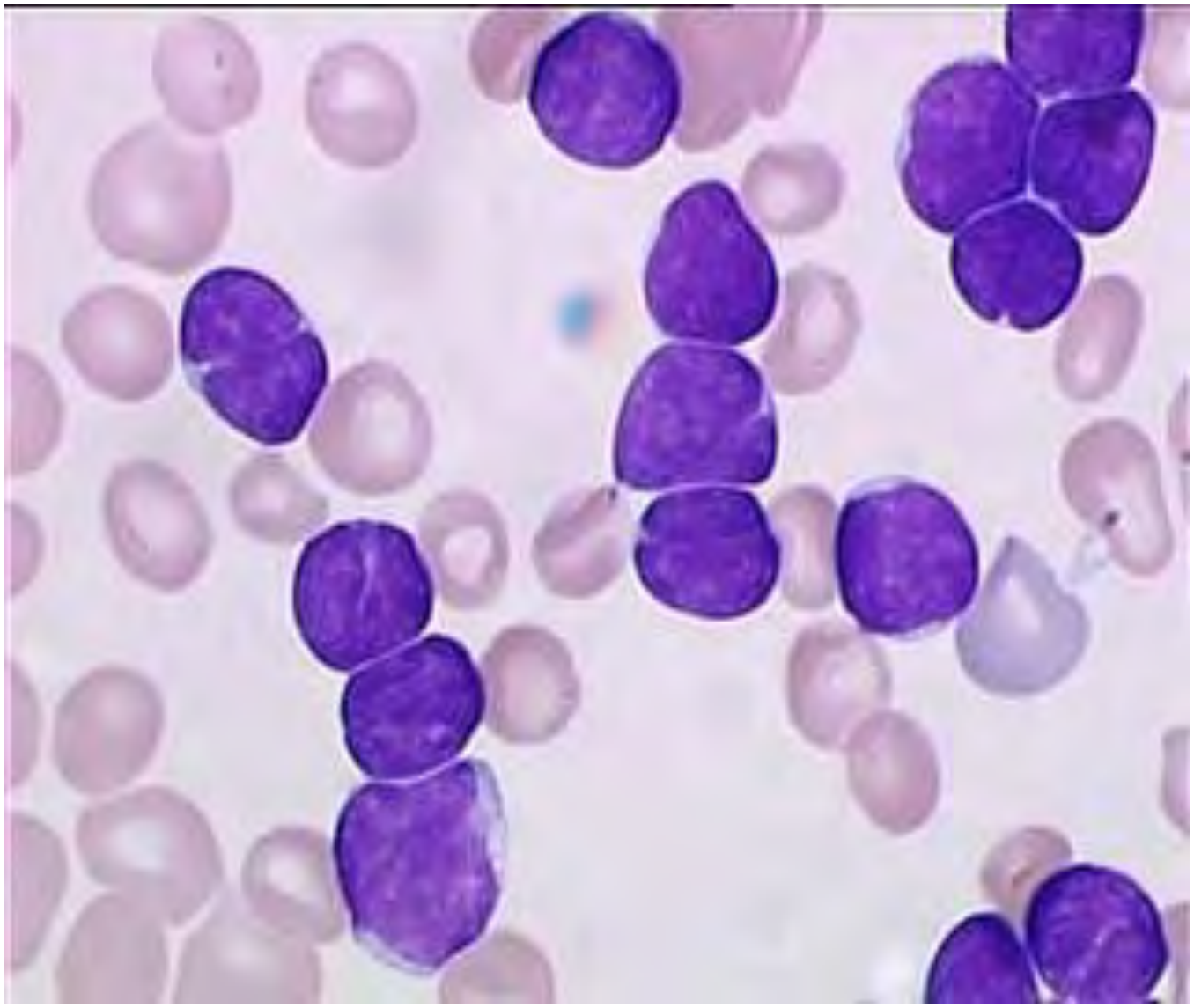


- t(14;18) present in >80% of cases
- BCL-2 gene (from chr 18) juxtaposed with Ig heavy chain gene (chr 14)
- Overexpression of BCL-2 protein
- Inhibition of apoptosis



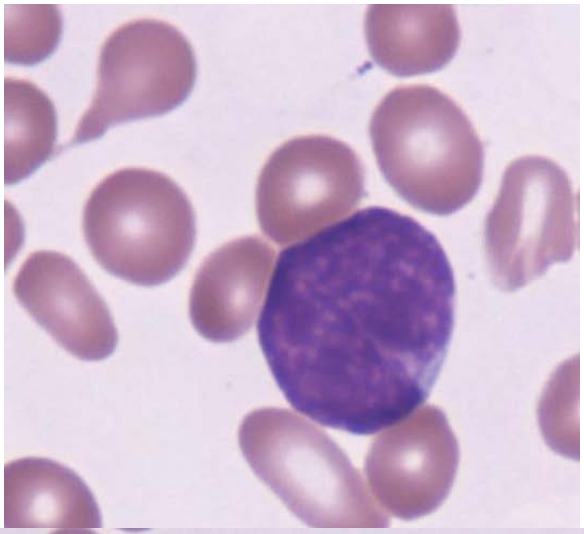
Source: Lichtman MA, Kipps TJ, Seligsohn U, Kaushansky K, Prchal JT: *Williams Hematology, 8th Edition*: http://www.accessmedicine.com

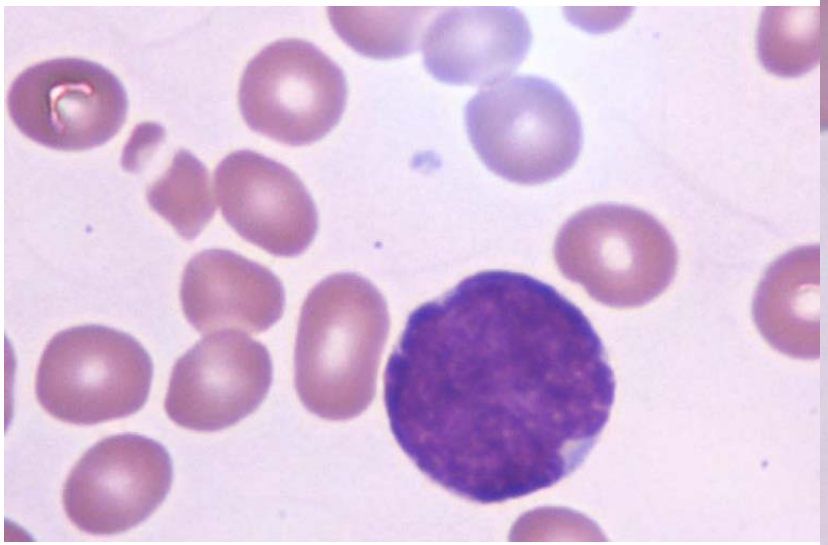
Follicular

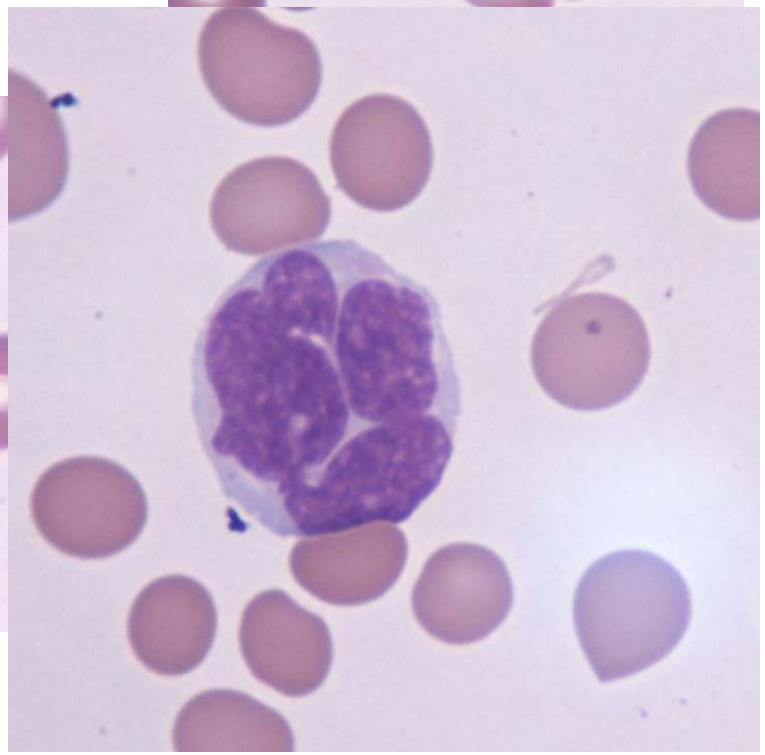


Next Patient

- WBC: 2,600/uL (1800 lymphs)
- HGB: 8.2 g/dL
- PLT: 43,000







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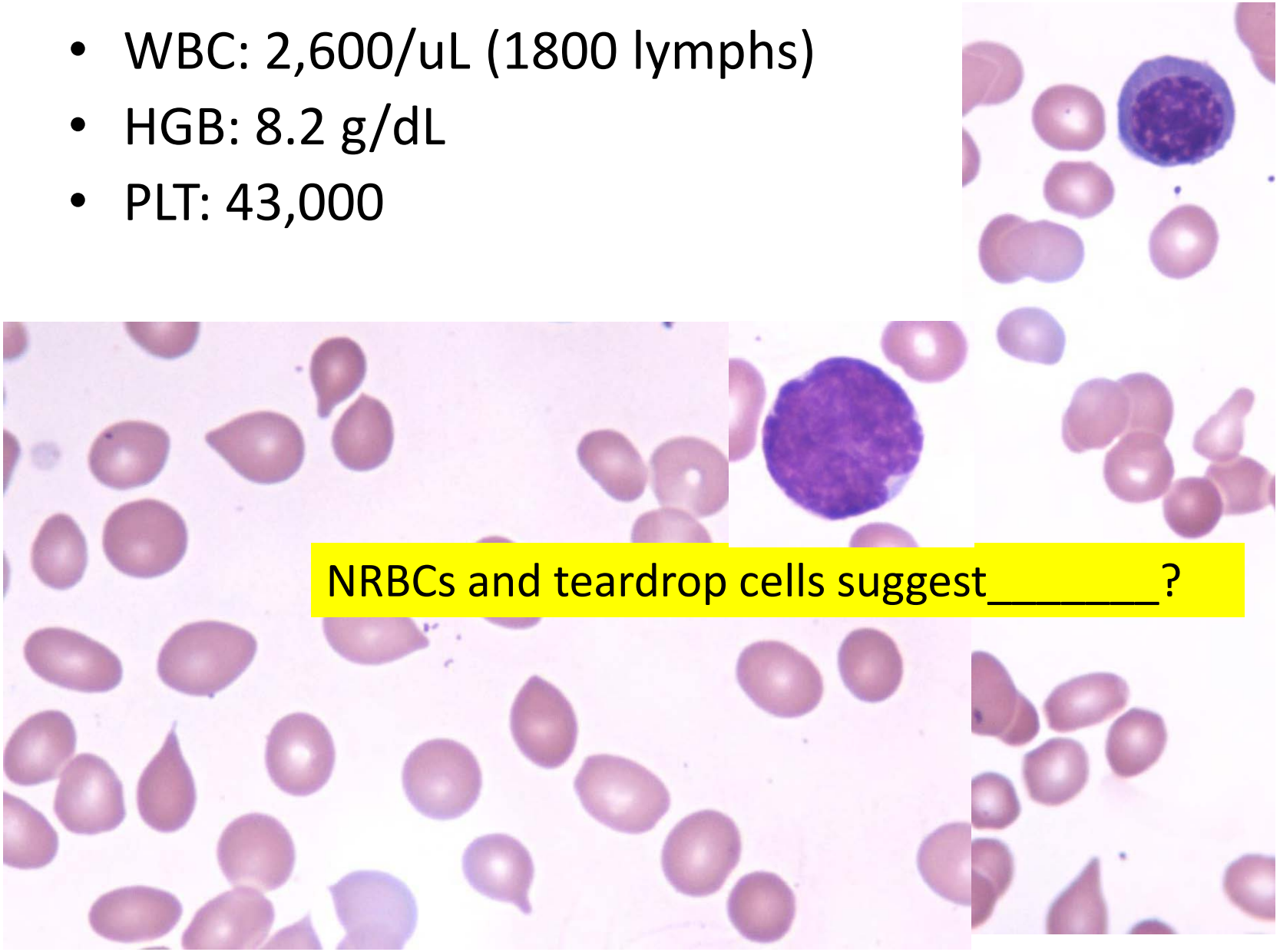
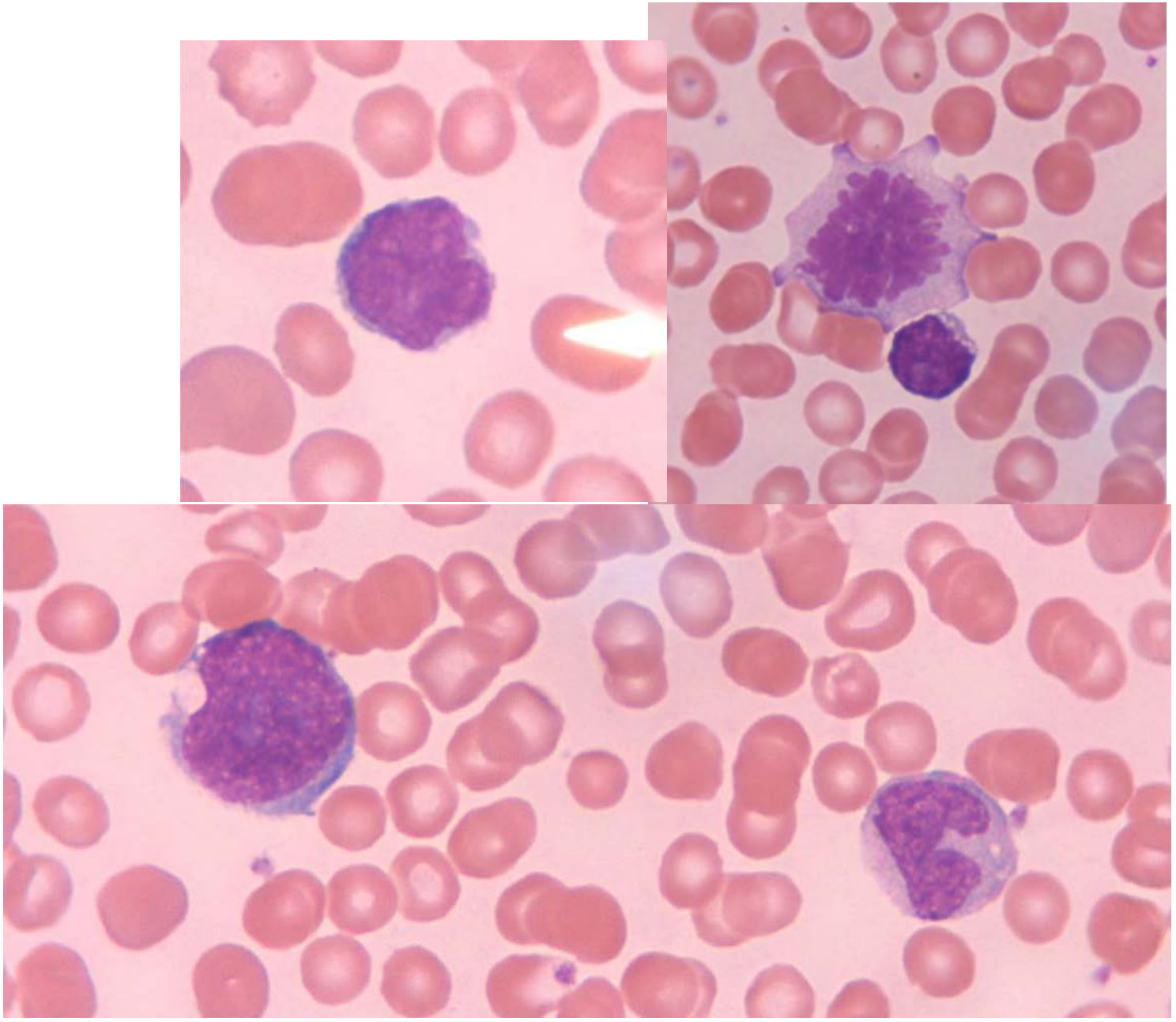
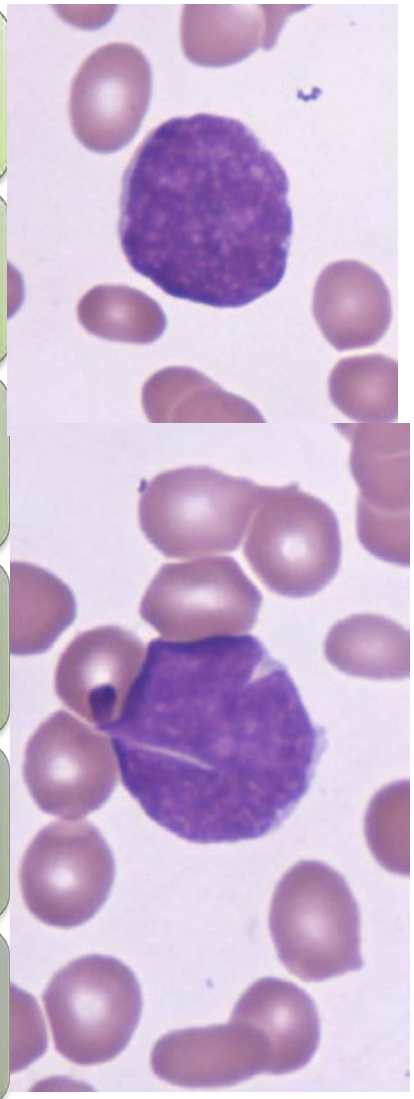




Mantle Cell Lymphoma

- 6% of non-Hodgkin lymphoma diagnosed annually in USA
- M/F =4:1; median=60y
- 70% present with widespread disease at DX
- Lymphadenopathy (90%), Splenomegaly (60%)
- Cytopenias related to marrow involvement
- Absolute lymphocytosis associated with circulating malignant cells



http://emedicine.medscape.com/article/203085

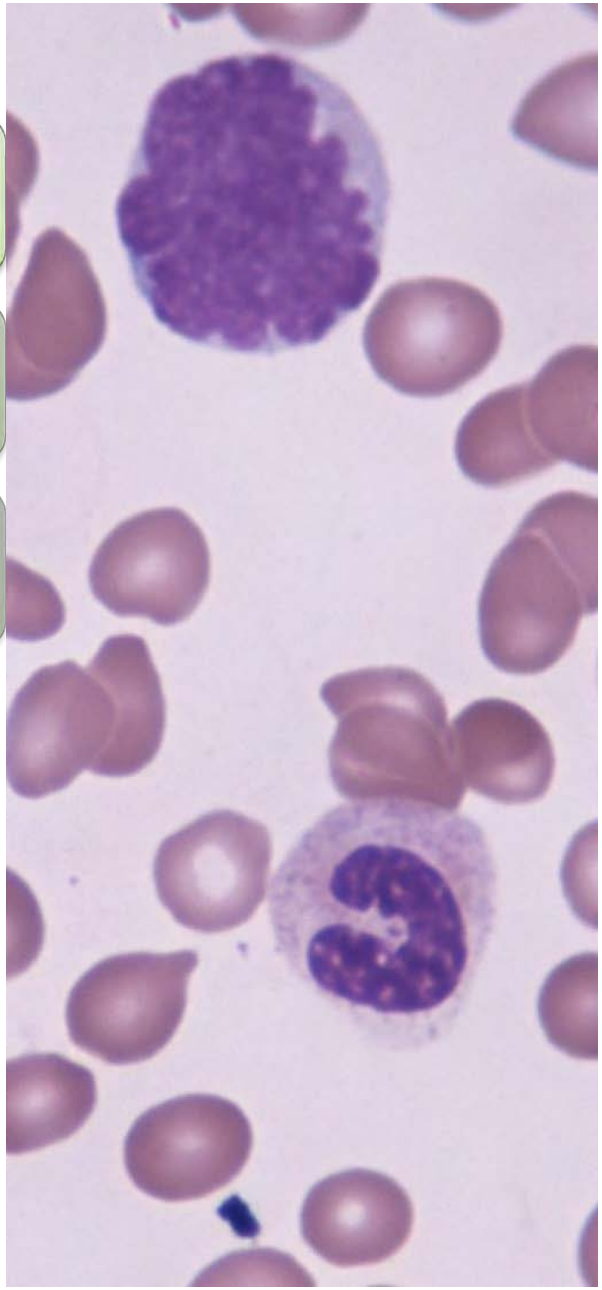
Mantle Cell Lymphoma

May be challenging to diagnose

Overlapping immunophenotypic markers

Highly variable morphology

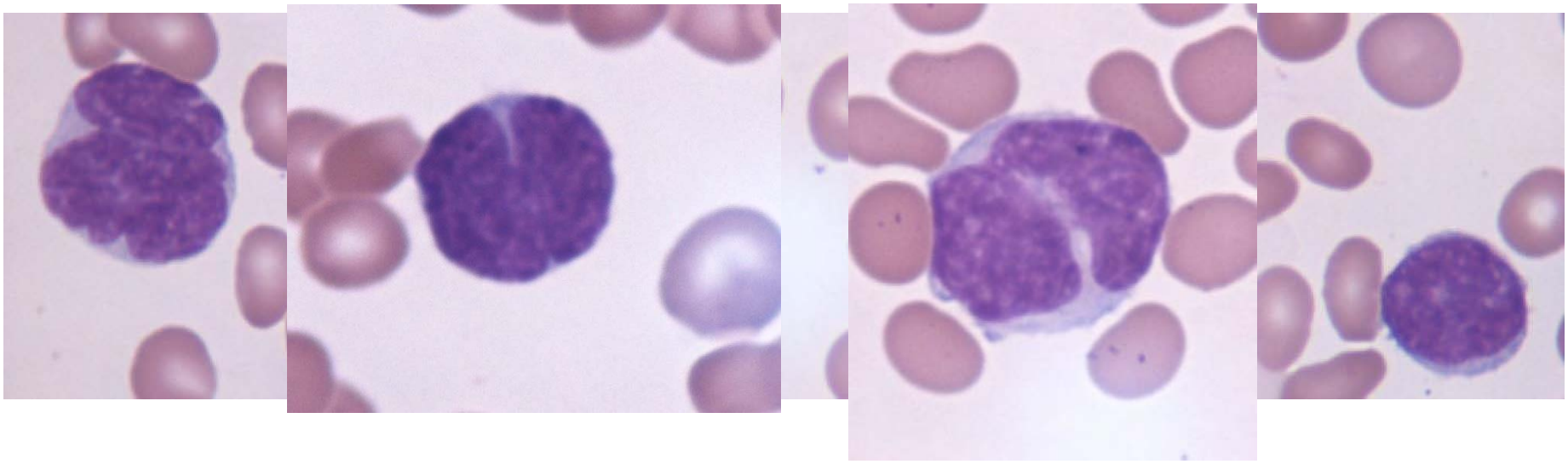
- Blast like resembling ALL
- Mod-large amount cytoplasm mimicking splenic marginal zone or hairy cell
- Prominent nucleoli – resembles prolymphocytic leukemia
- Morphology consistent with CLL



Mantle Cell Lymphoma

t(11;14) results in over expression of the PRAD1/CCND1 gene, encoding the cyclin D1 protein

- Cyclin D1 plays a key role in cell-cycle regulation, activating cyclin-dependent kinases, propelling cells through the G1 checkpoint into the S phase of the cell cycle.
- Cyclin D immunohistochemical stain important for diagnosis



Next……53 year old female

July 2006: Presented with rash over right thumb, index finger, abdomen, posterior thighs, breast, arms and neck

Punch biopsy of rash: atypical dense lymphoid infiltrate consistent with Adult T-cell lymphoma

Oct 2006: PET/CT revealed hypermetabolic activity in abdomen

Bone marrow: 9% small T-cells (CD2, CD3, CD4, CD5)

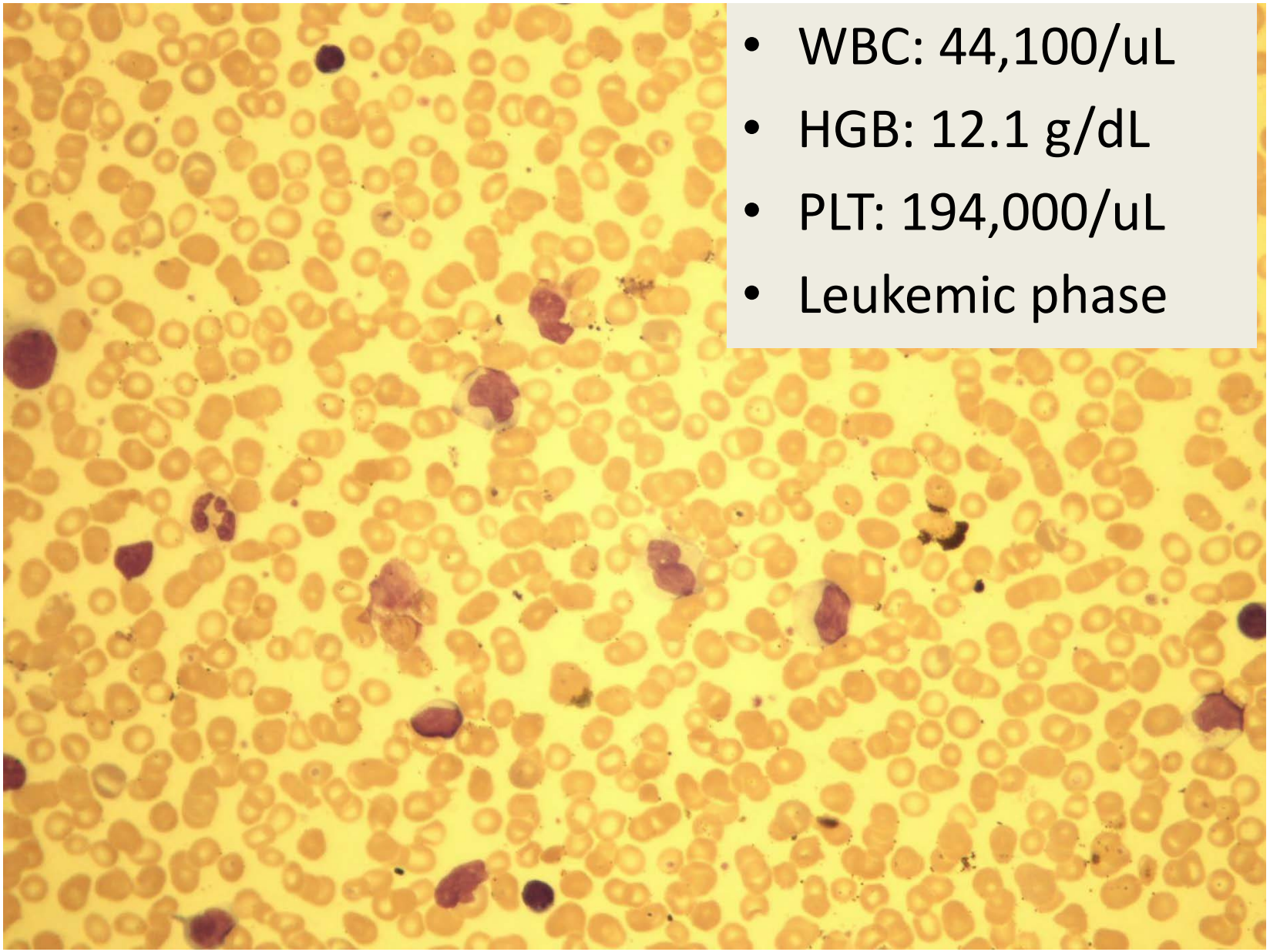
53 year old female

Underwent chemotherapy - clinical trial; no standard of care for initial treatment

Multiple hospitalizations: hypercalcemia

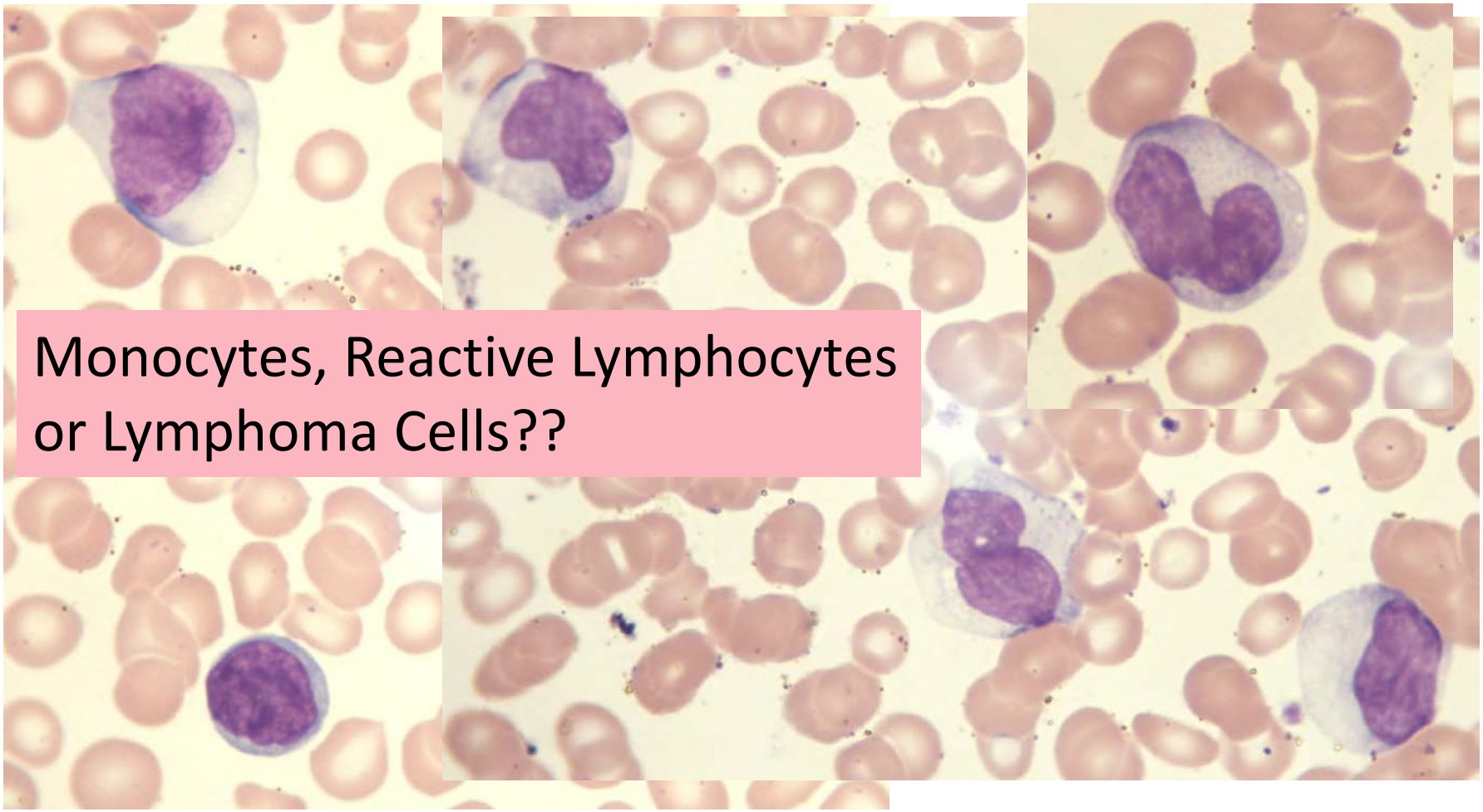
July 2007: PET showed splenic involvement and positive axilla, subpectoral, periaortic, pelvic wall, and inguinal lymph nodes

Oct 2007: patient extremely frail and seen at clinic

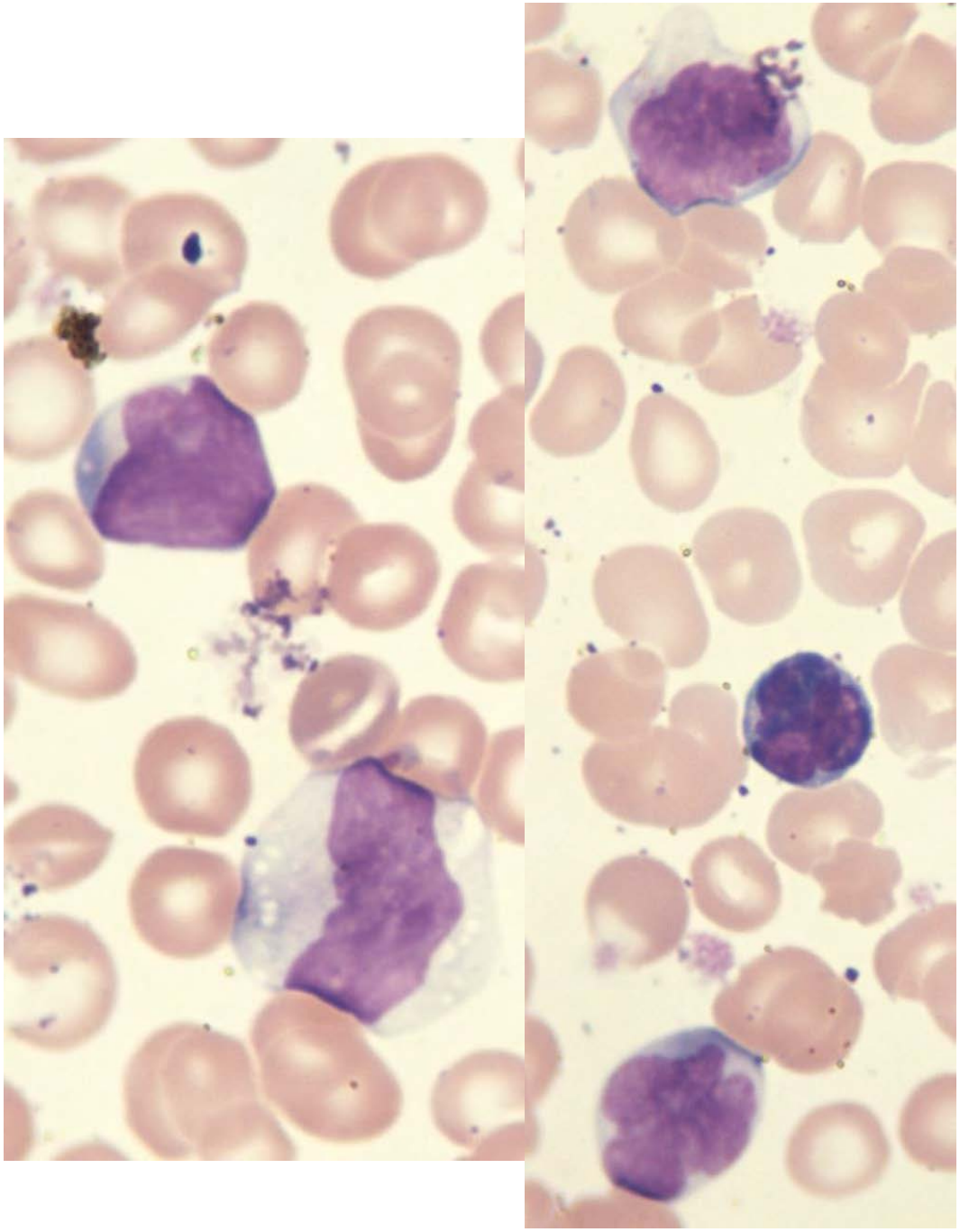


- WBC: 44,100/uL
- HGB: 12.1 g/dL
- PLT: 194,000/uL
- Leukemic phase

T Cell Lymphoma (HTLV-1 Related)

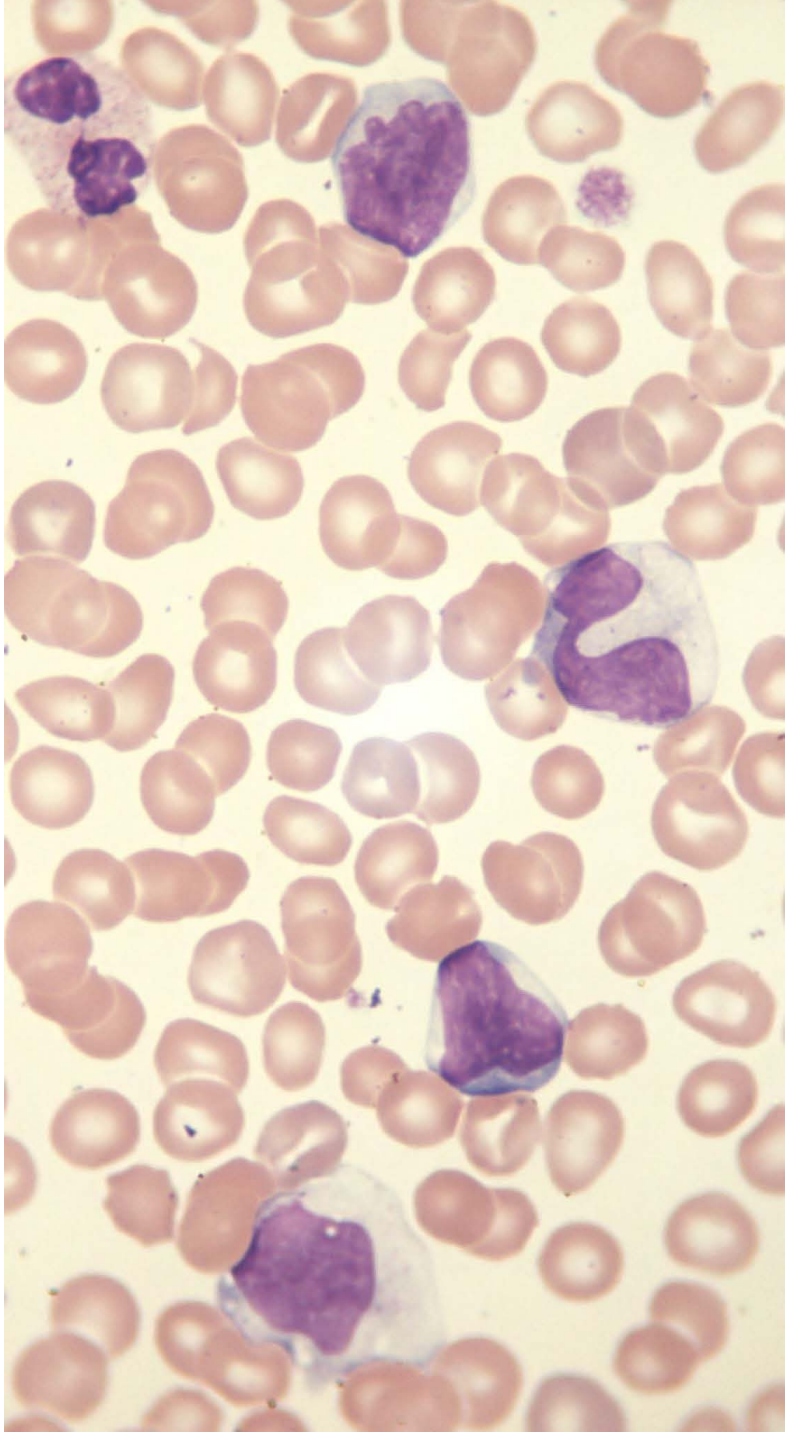


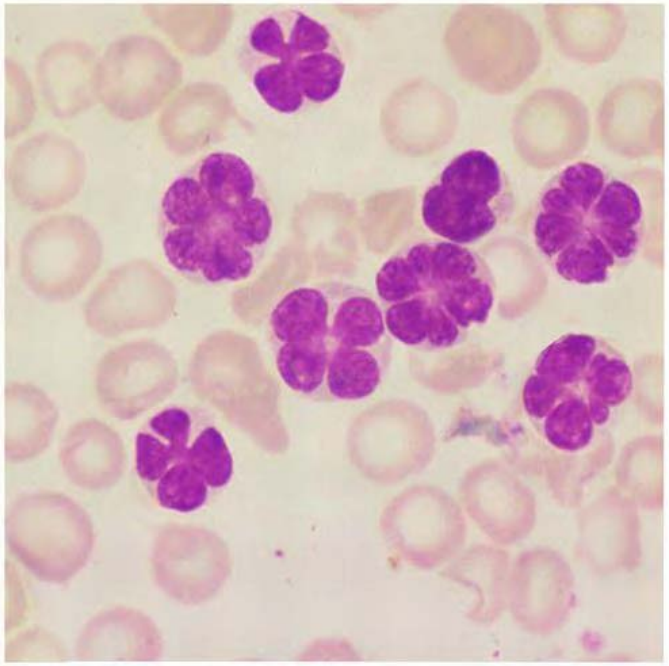
- Somewhat heterogeneous
- Appear reactive?

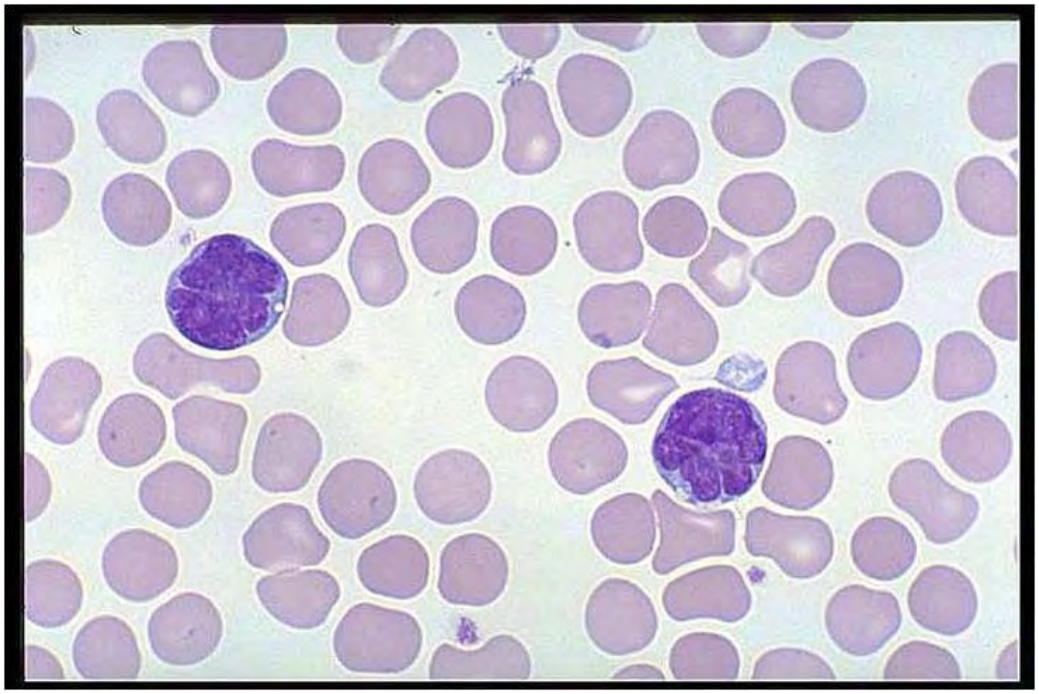


Adult T-Cell lymphoma/Leukemia

- HTLV-1 positive: 2.5 % of carriers will develop lymphoma after long latency
- Very aggressive multisystem disease
- Prominent extramedullary disease
- Skin and bone lesions
- Hypercalcemia
- Short survival times





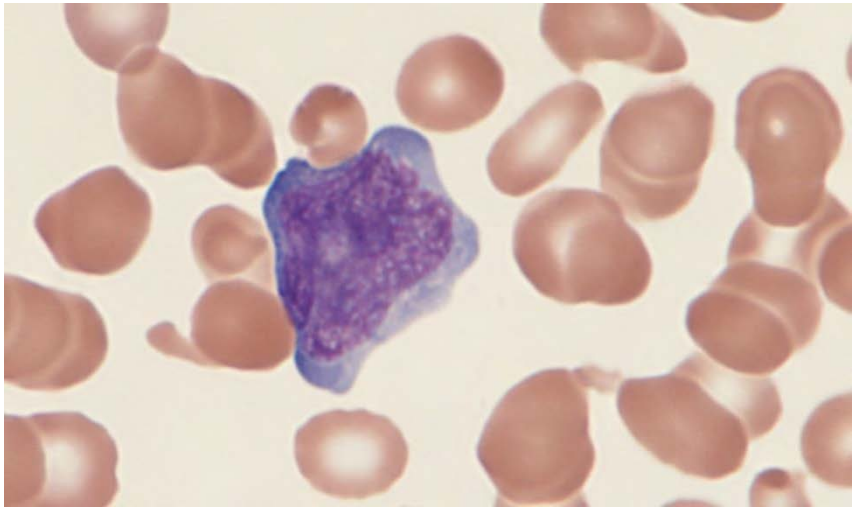


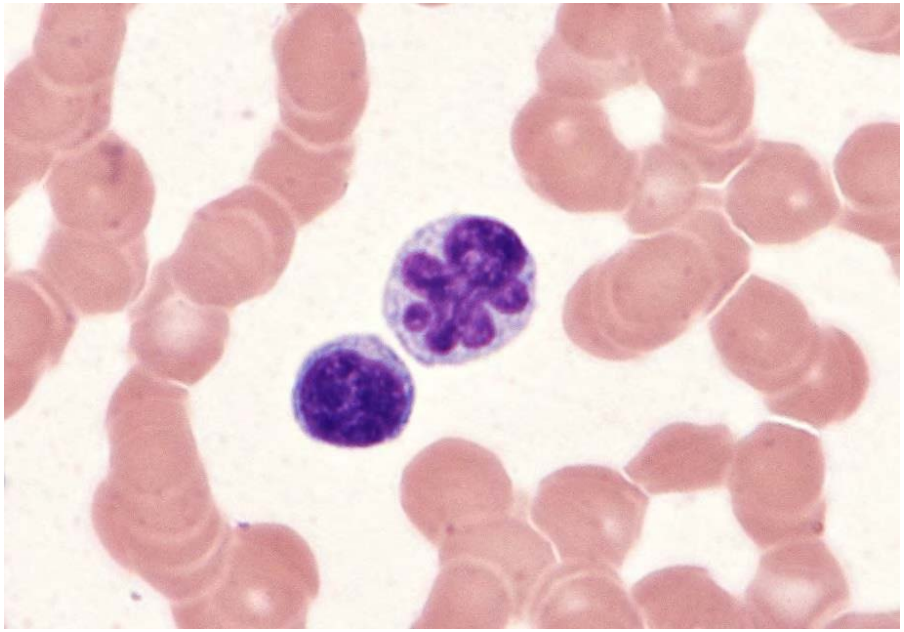
pinterest.com

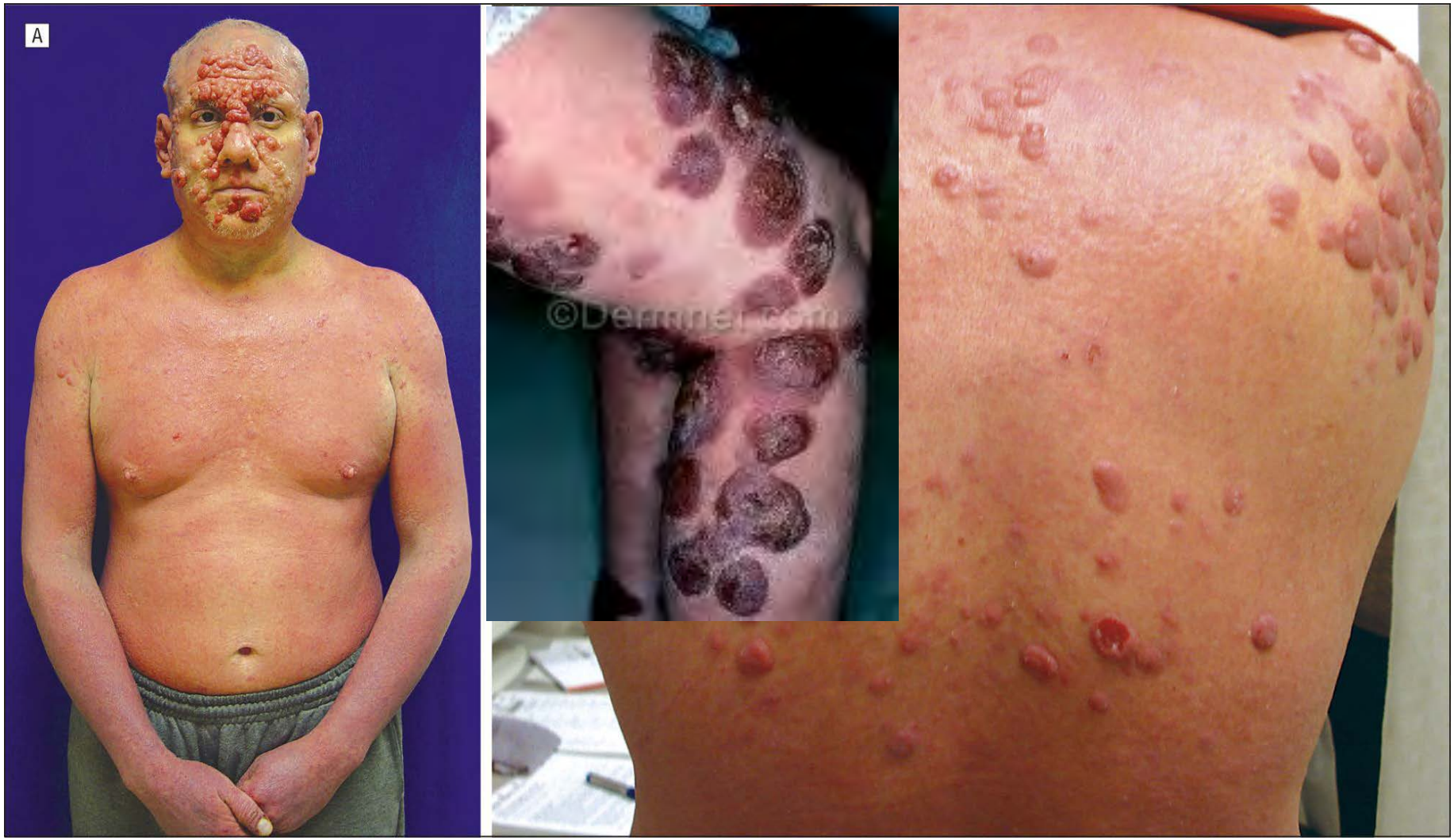
movies-hukenyonbaker.blogspot.com

Acute Adult T-Cell lymphoma/Leukemia

- Striking leukocytosis with abnormal lymphoid cells:
  - Heterogeneity of size and nuclear configuration
  - Pronounced nuclear irregularities:
    - coarse lobulation
    - cloverleaf forms
  - Variably clumped chromatin, variable amount cytoplasm



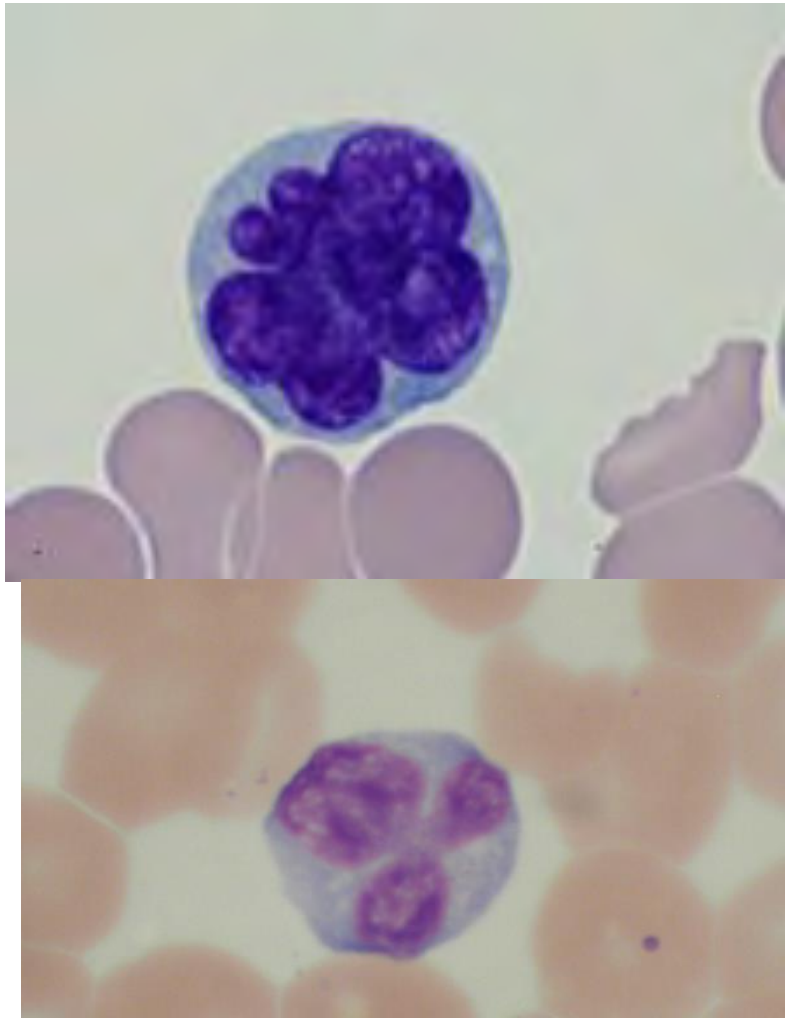




Arch Dermatol. 2009;145(1):92-94

Adult T-cell Lymphoma/Leukemia

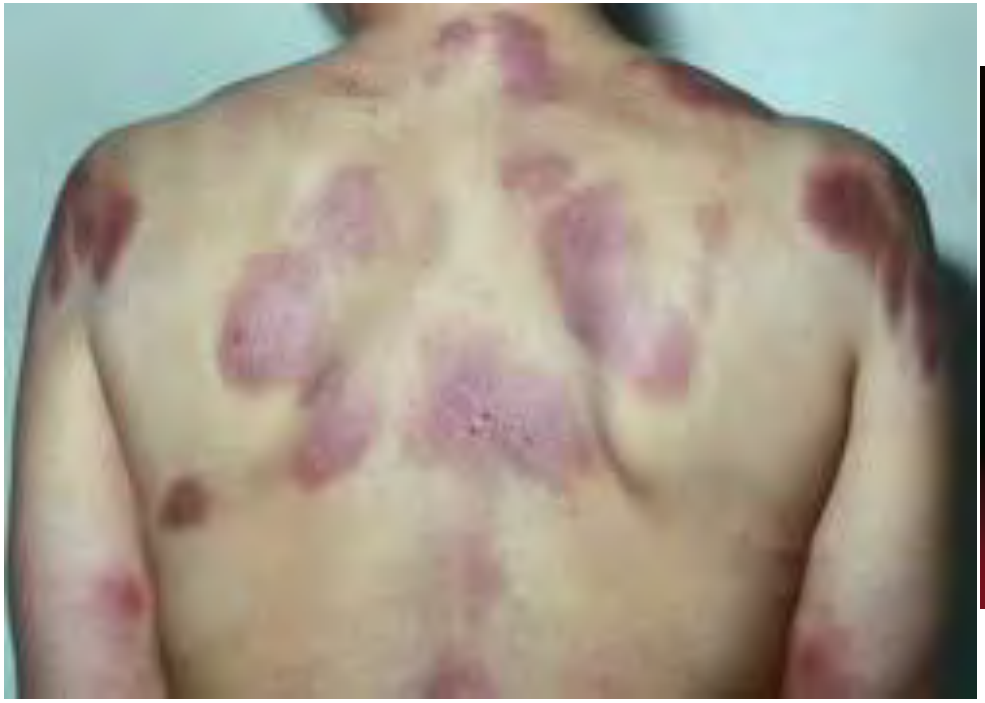
- Immunophenotype
  - TdT –
  - CD2 +
  - CD3 +
  - CD5 +
  - Most cases are CD4 helper phenotype
- Molecular
  - Clonal T cell receptor gene rearrangement

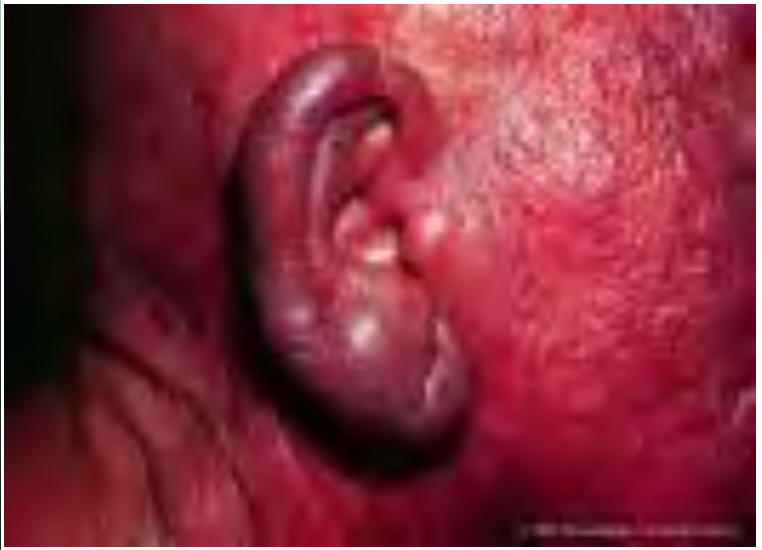


Next T-Cell Disease: Sezary Syndrome

- Closely related to mycosis fungiodes
- Generalized exfoliative erythroderma with characteristic blood involvement
- Mostly males >60 y
- Generalized lymphadenopathy
- Aggressive disease; 5 year survival 10-20%

Mycosis Fungoides





Sezary Syndrome

Clonal T-cells in skin, lymph nodes and blood

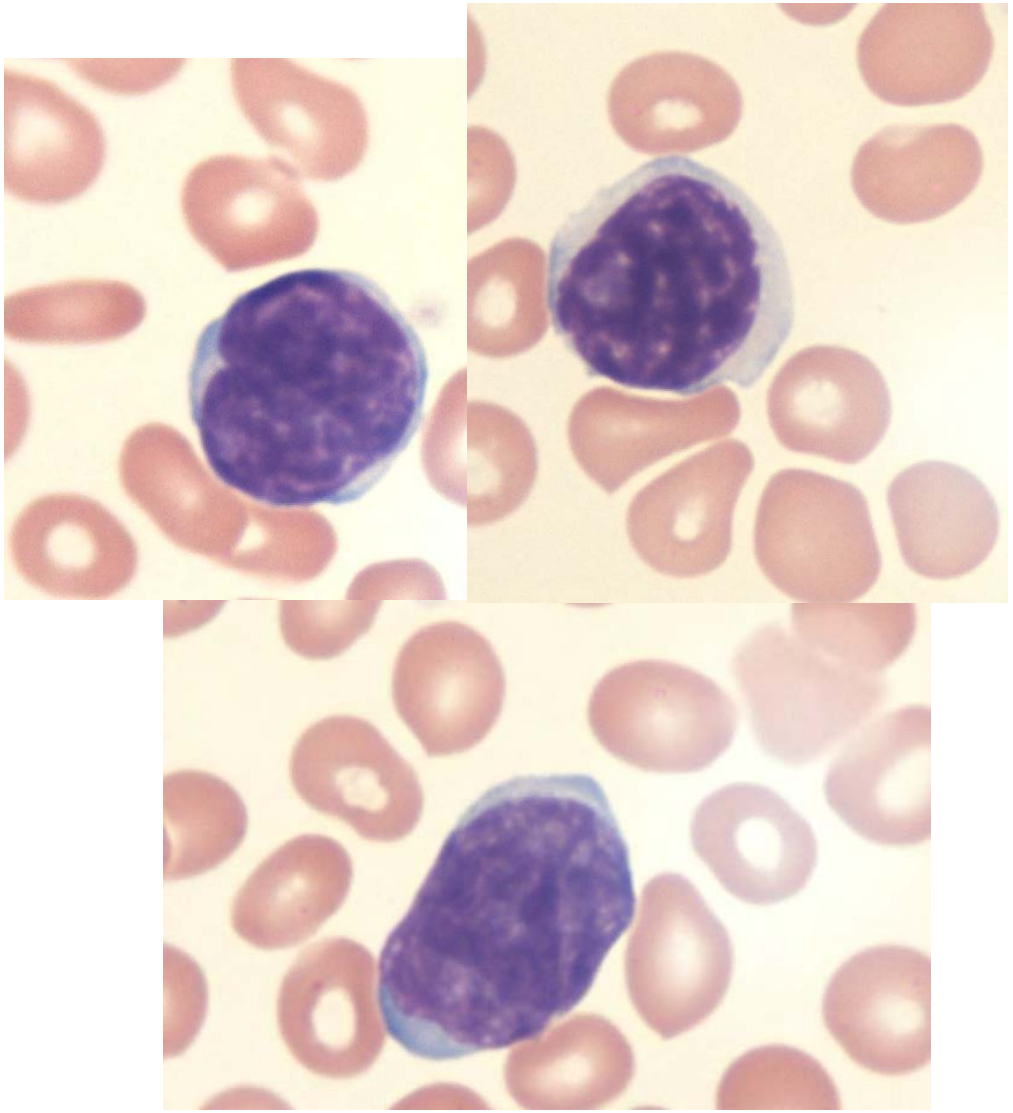
Demonstrate Sezary cells (>1000/uL) in blood by immunophenotype and morphology

- Immunophenotype: mature T helper cell phenotype, CD4/CD8 ratio >10
- <u>Abnormal lymphoid cells in circulation:</u>

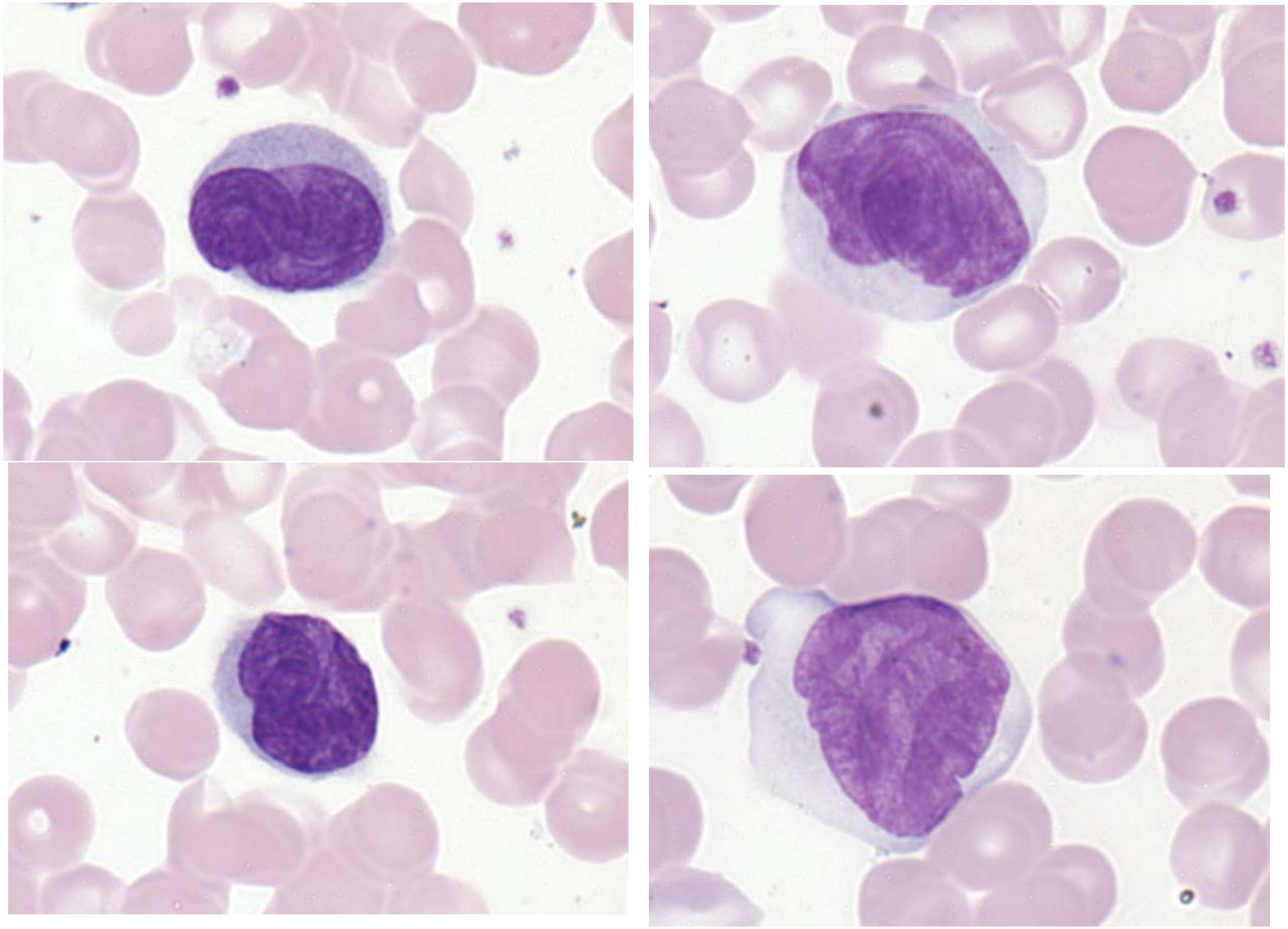
Variable Size	Absent/inconspicuous nucleoli
Cerebriform nuclei	High N:C ratio
Variable/coarse chromatin	Cytoplasm: basophilic & agranular

Sezary Cells

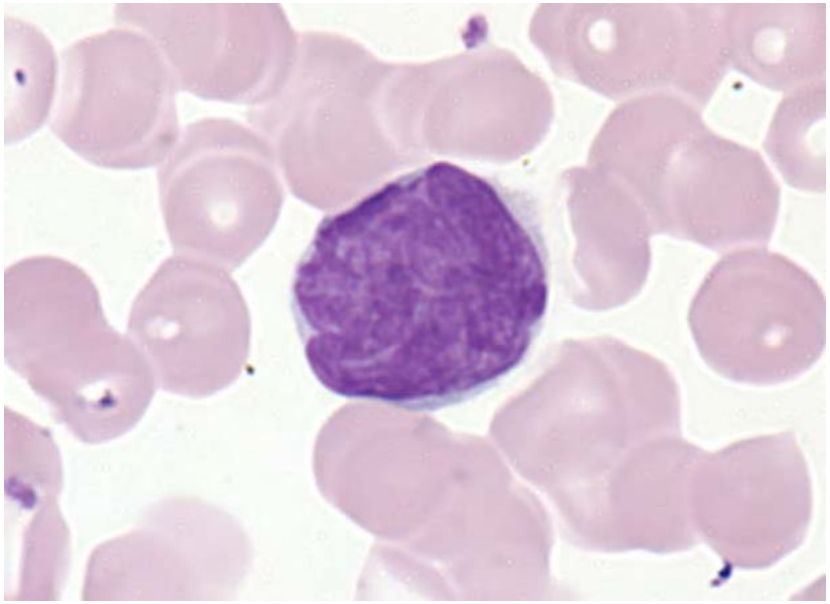
- Large (15-25um)
- Pale blue cytoplasm
- Convoluted cerebriform nuclei
- Coarse chromatin

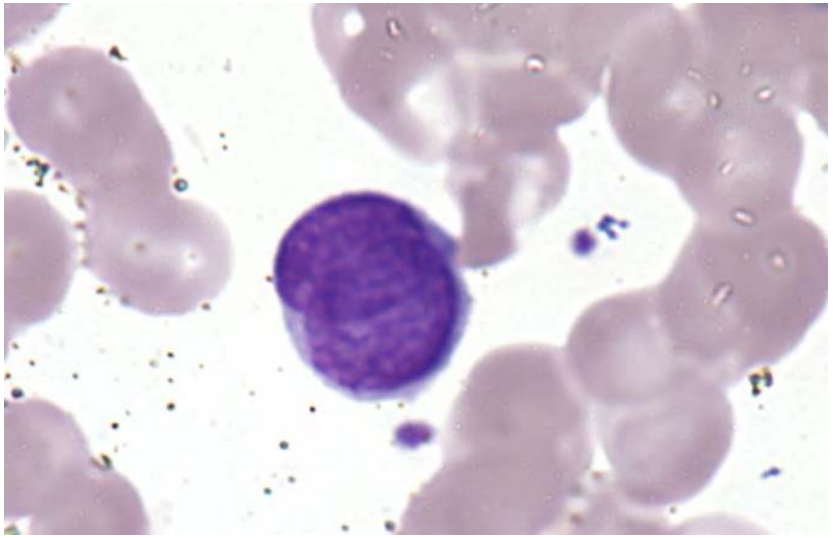


Sezary cells



Courtesy of Peter Maslak





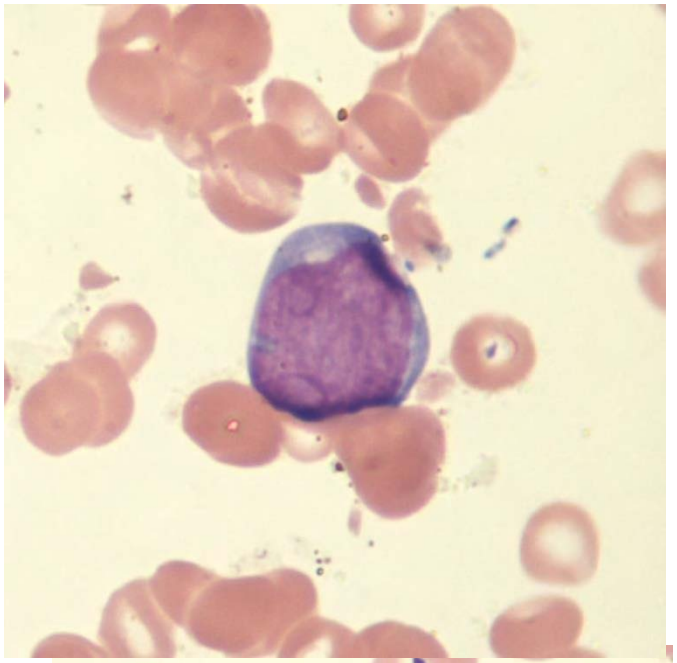
Courtesy of Peter Maslak

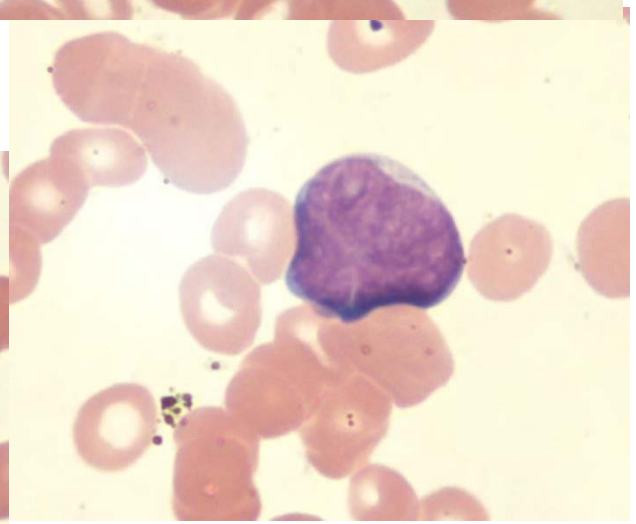
Diffuse Large B-cell Lymphoma

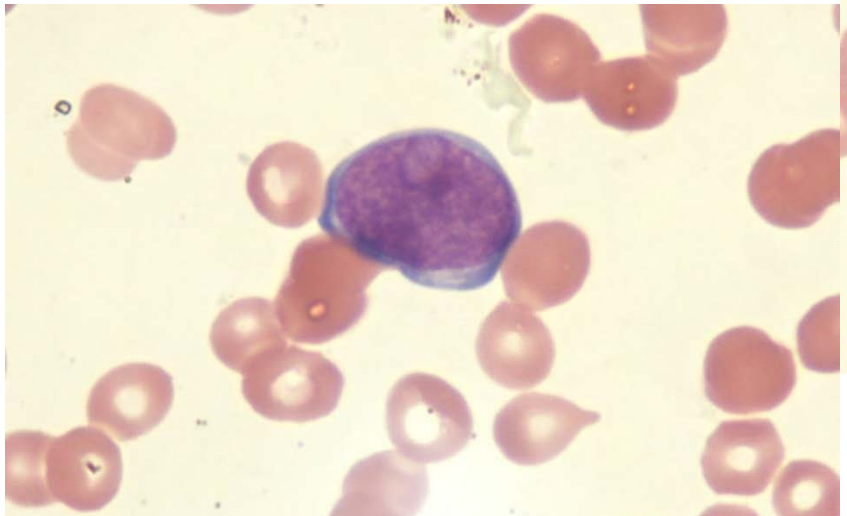
- most common form of aggressive non-Hodgkin lymphoma
- usually symptomatic
- extranodal involvement is common
- cell of origin: germinal center B-cell
- curable in ~ 40%

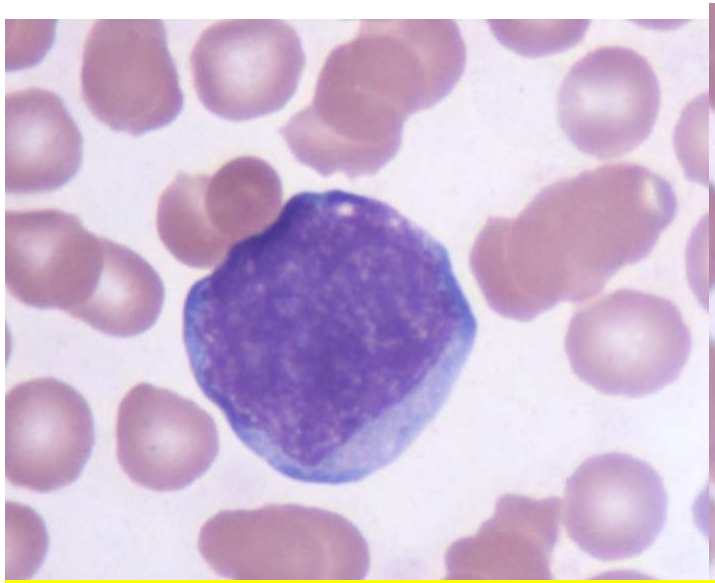
Diffuse Large B-Cell Lymphoma

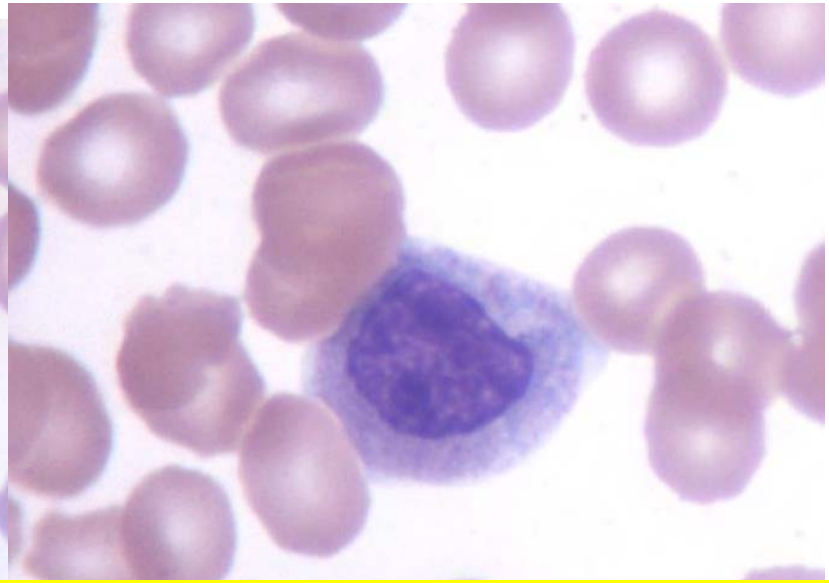
- Large lymphoid cells with variable nuclear features
- May mimic acute leukemia
  - Should they be called BLASTS or LYMPHOMA CELLS or SOMETHING ELSE?



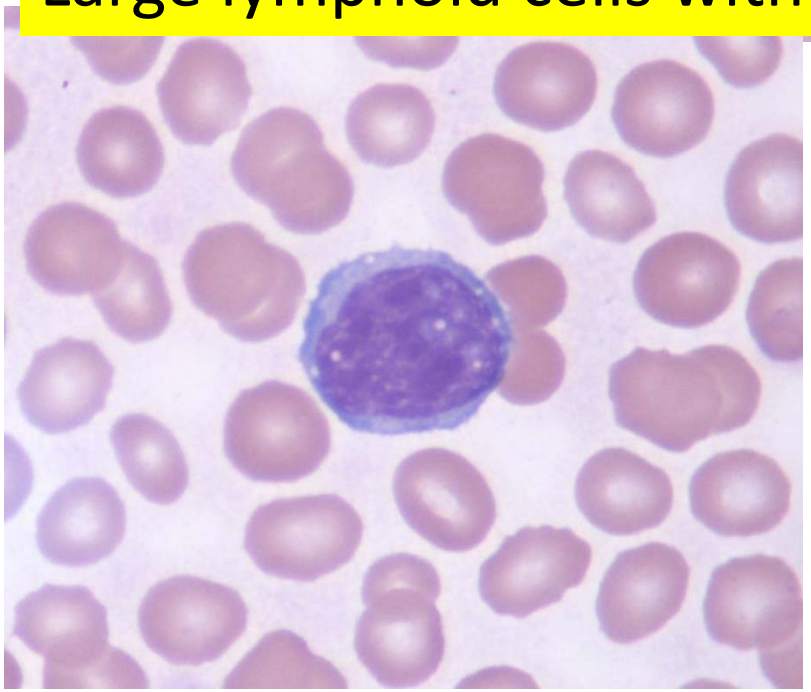


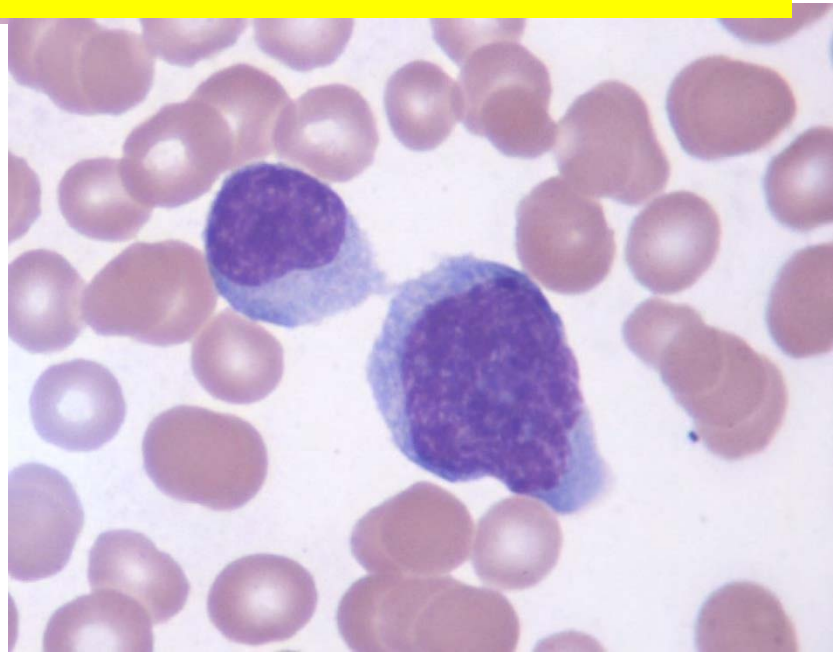




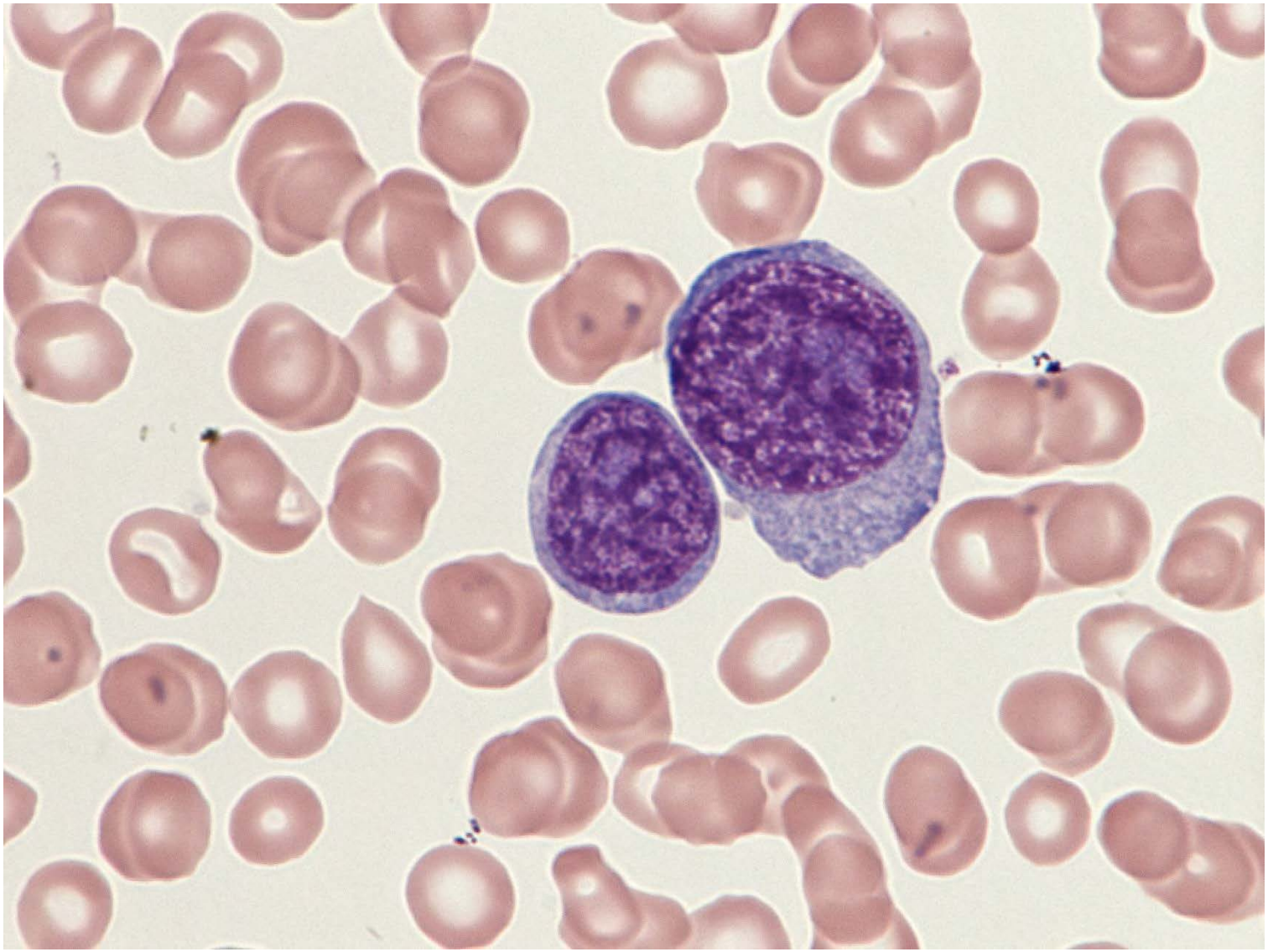


Large lymphoid cells with condensed chromatin





Diffuse large B cell lymphoma



Courtesy of Peter Maslak

my suggestions

terminology and reporting methods

recommended

- Instrument diff was already released
- Smear is reviewed for RBC morph, confirmation of auto diff or other reason
- Variant lymphoid cells (appear reactive or malignant/abnormal) are seen on smear

As long as the smear confirms the instrument lymphocyte count, use a qualitative statement to describe the abnormal population of lymphs such as:

Lymphocytes with reactive features features seen on smear

Abnormal lymphoid cells with blast-like features seen on smear

Abnormal lymphoid cells with irregular nuclear contours seen on smear

Abnormal lymphoid cells with folded/clefted nuclei seen on smear

Abnormal lymphoid cells with villous cytoplasmic projections seen on smear (when Hairy cells are suspected)

Abnormal lymphoid cells with cerebriform nuclei and moderately condensed chromatin seen on smear (when Sezary cells are suspected)

Abnormal lymphoid cells with prominent nucleoli seen on smear (Reserve for cells resembling prolymphocytes)

Abnormal lymphoid cells seen on smear (CLL cases where there is an absolute lymphocytosis and the number of prolymphocytes or atypical forms is <10%)

Increased number of prolymphocytes / large atypical / lymphoid cells with cleaved nuclei seen on smear (Use in CLL patients with >10% of these cells)

Some of the lymphocytes appear variant (or reactive/abnormal)

Variant lymphoid cells (appear reactive or malignant/abnormal) are encountered while performing a manual differential and they cannot be separated from the normal lymphocyte population

use a qualitative statement to describe the abnormal population of lymphs such as “the lymphocyte population includes a population of

- Lymphocytes with reactive features
- Abnormal lymphoid cells with blast-like features
- Abnormal lymphoid cells with irregular nuclear contours
- Abnormal lymphoid cells with folded/clefted nuclei

Abnormal lymphoid cells with villous cytoplasmic projections
(when Hairy cells are suspected)

Abnormal lymphoid cells with cerebriform nuclei and moderately condensed chromatin
(when Sezary cells are suspected)

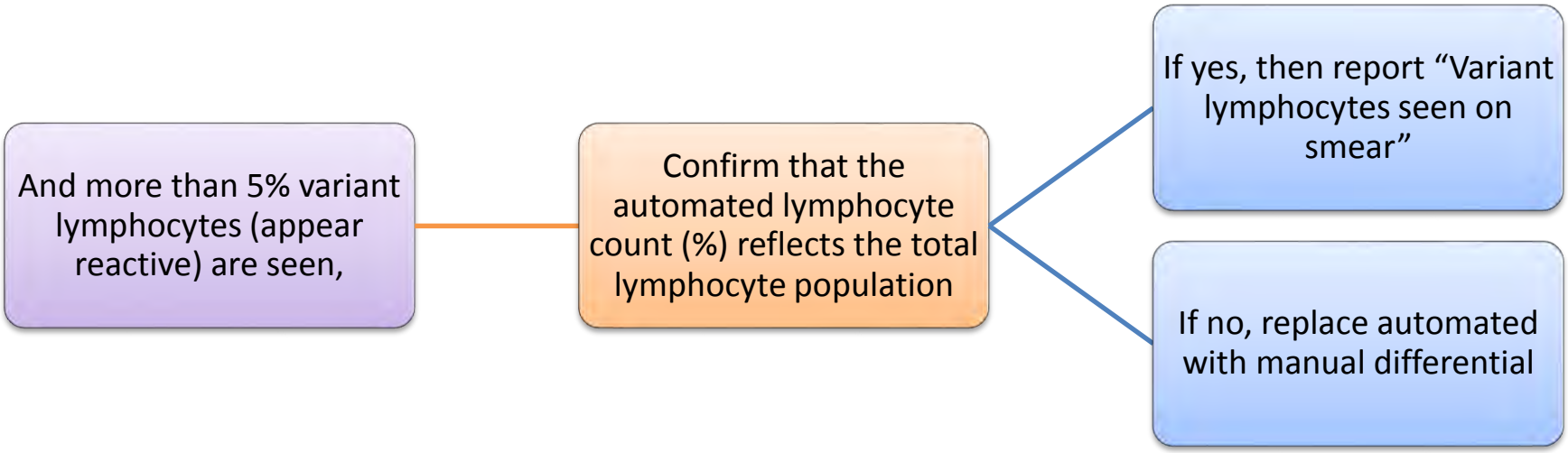
Abnormal lymphoid cells with prominent nucleoli seen on smear (Reserve for cells resembling prolymphocytes)

Abnormal lymphoid cells seen on smear (CLL cases where there is an absolute lymphocytosis and the number of prolymphocytes or atypical forms is <10%)

Increased number of prolymphocytes / large atypical / lymphoid cells with cleaved nuclei seen on smear (Use in CLL patients with >10% of these cells)

Cells with variant (or reactive/abnormal) morphology

Scenario: automated differential was already reported and smear is being reviewed for morphology, confirmation of automated counts , or other reason



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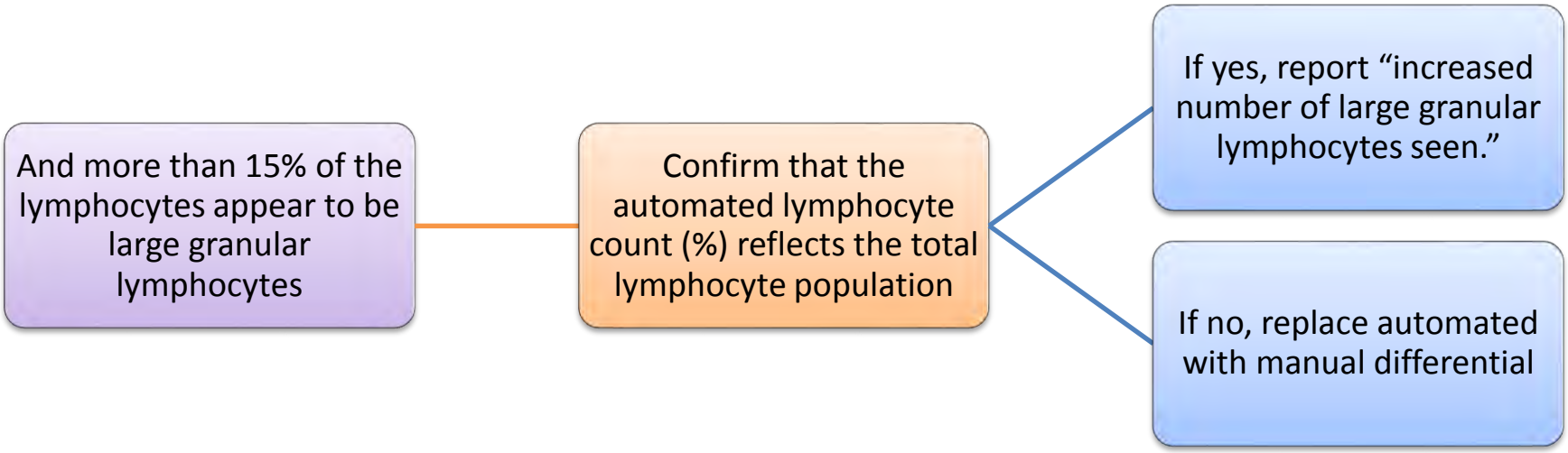
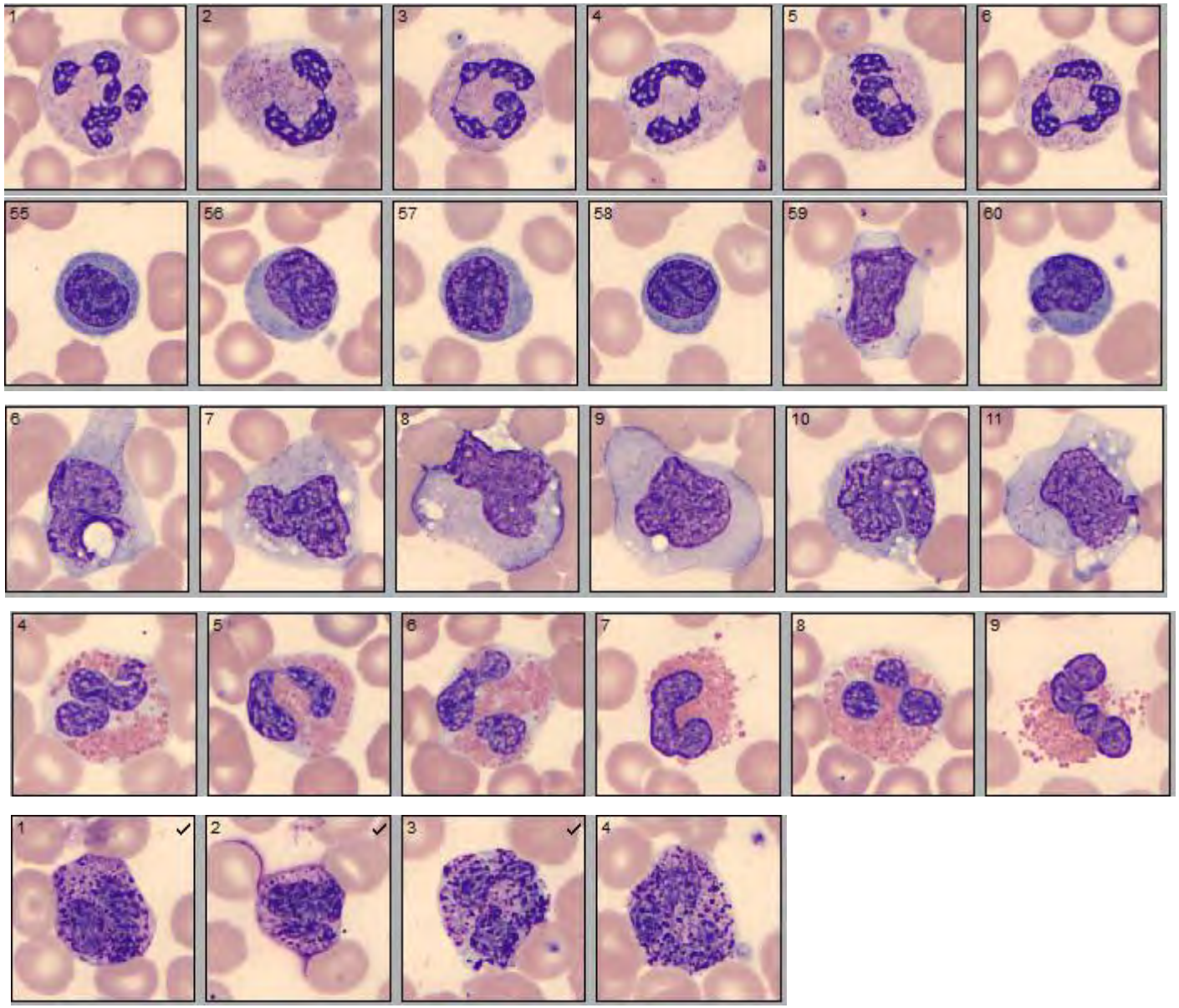


Scenario: More than 15% of lymphocytes appear to be large granular lymphocytes (LGL) on manual differential

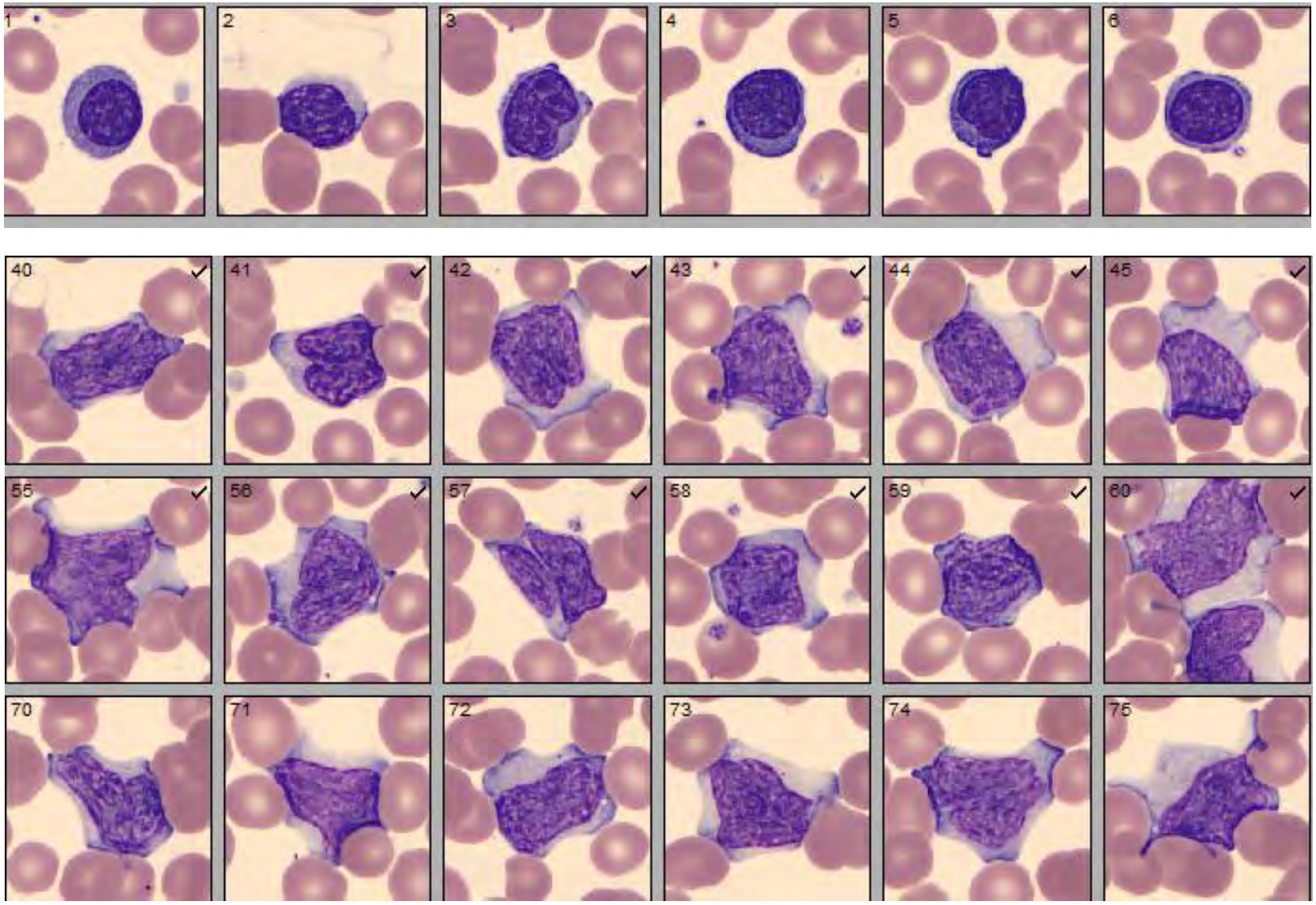
Count the LGLs as lymphocytes

and add comment: “Increased number of large granular lymphocytes seen”

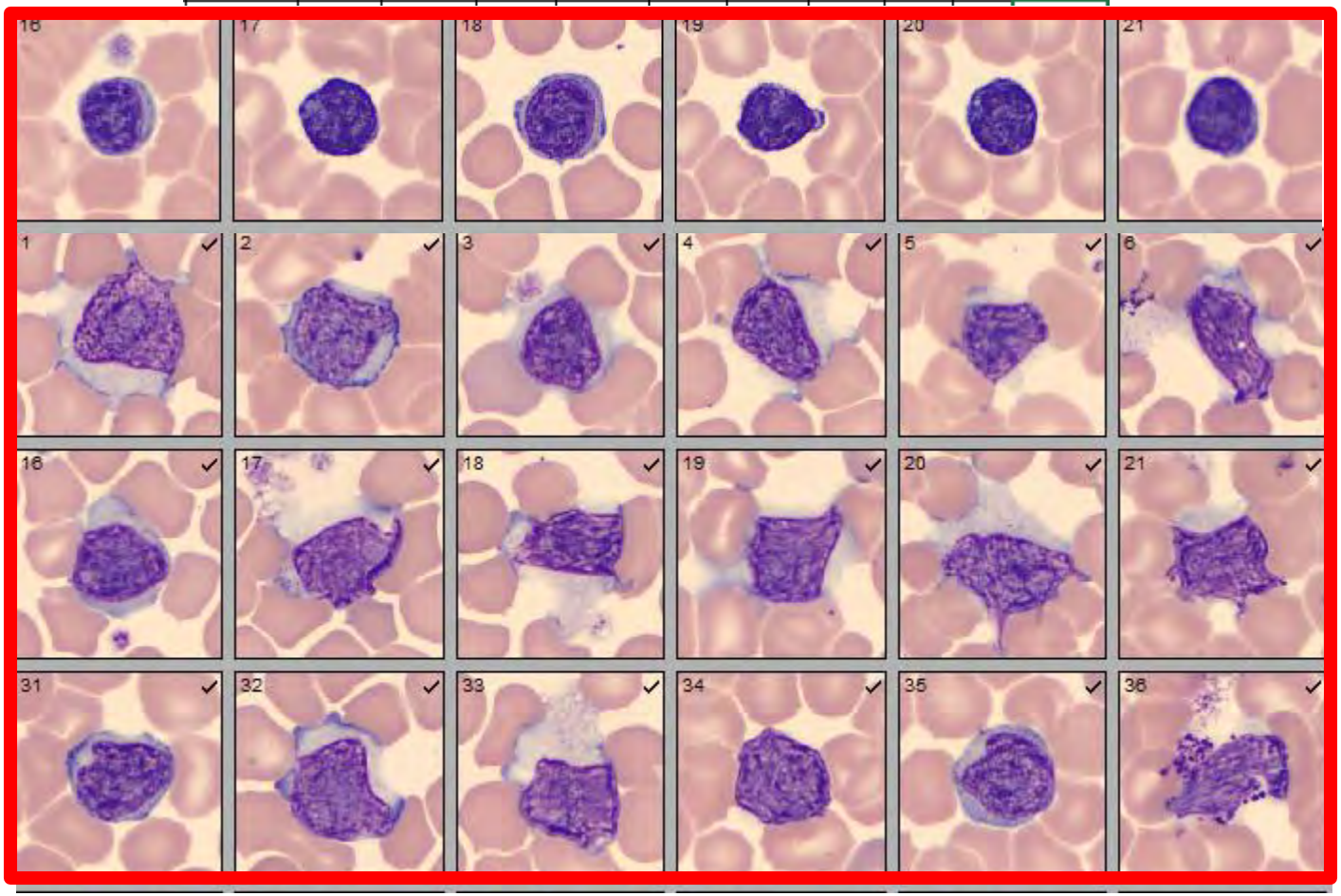
Digital images of normal leukocytes

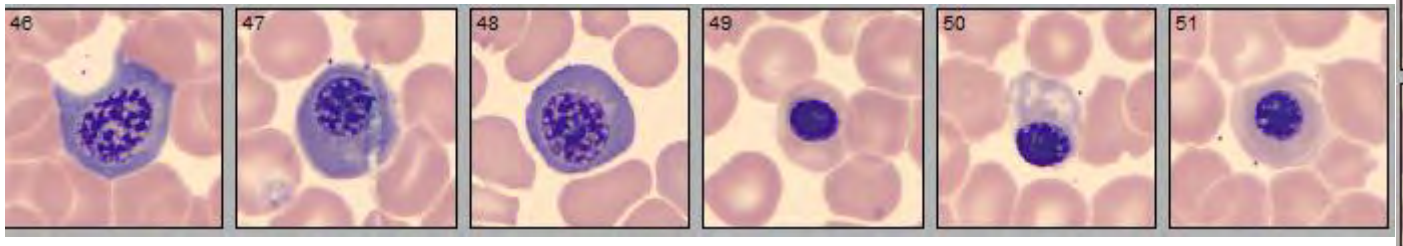


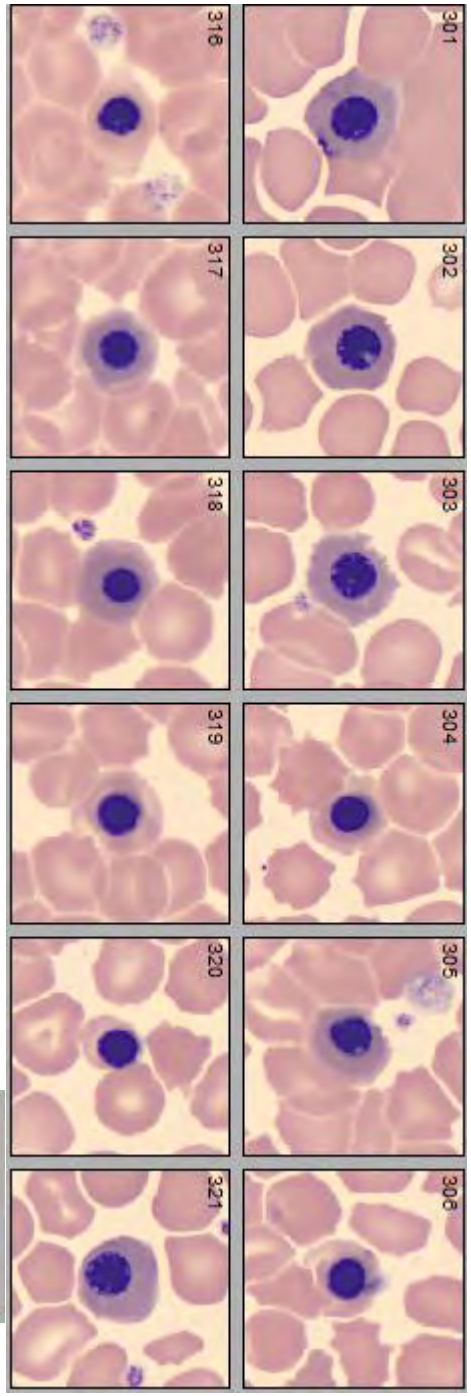
WBC	HGB	MCV	PLT	NRBC	Ne	Ly	Mo	Eo	Ba	NRBC	VLYM
15.25	14.6	96.1	148	0.1	46.3	4.4	7.4	3.4	1.5		36.9



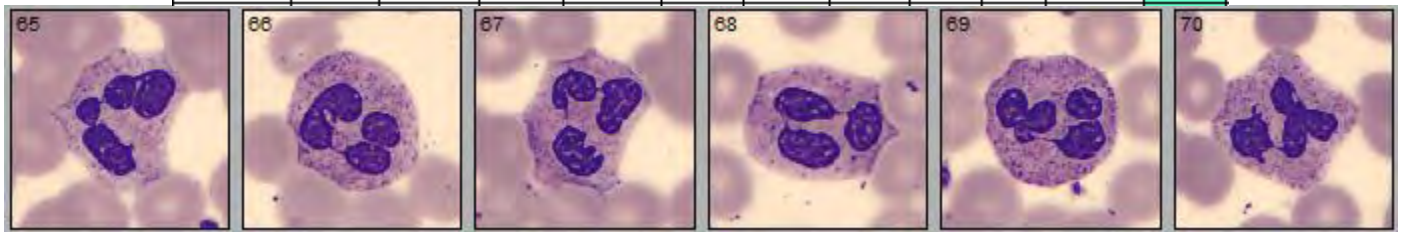
WBC	HGB	MCV	PLTo	PLTi	Ne	Ly	Mo	Eo	Ba	NRBC
7.7	17.9	121.00	221	248	15	68	11.2	5.1	0.5	152.8

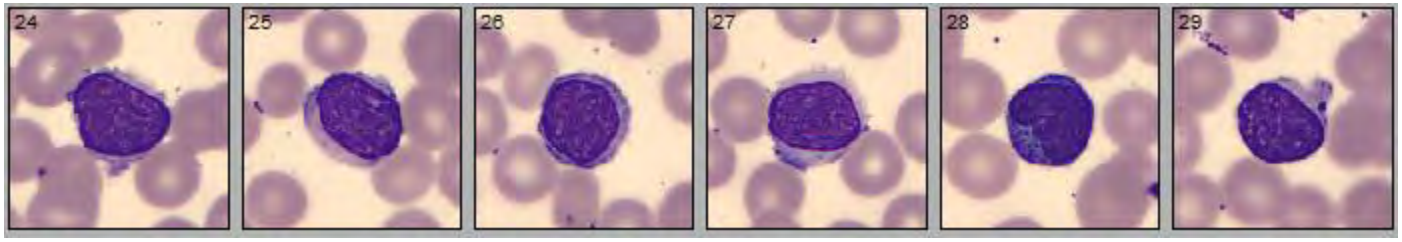


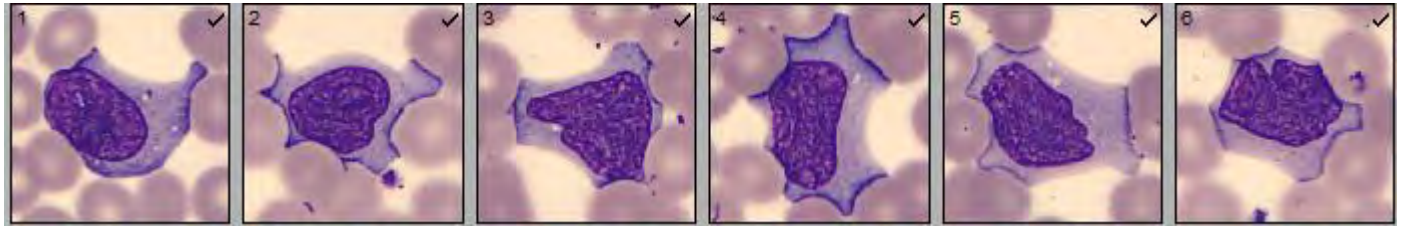


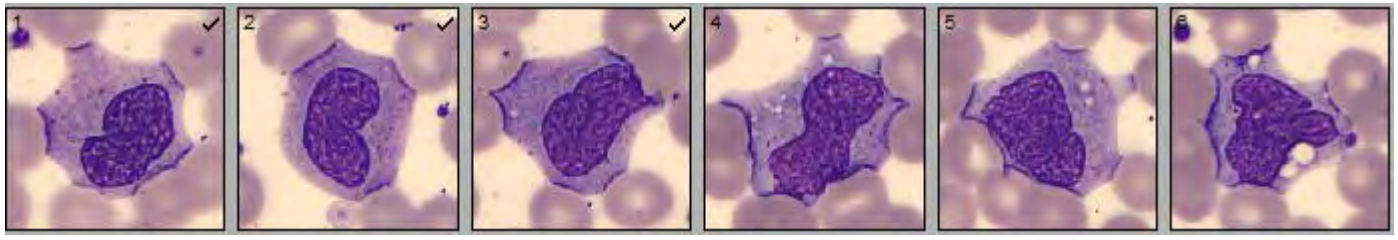


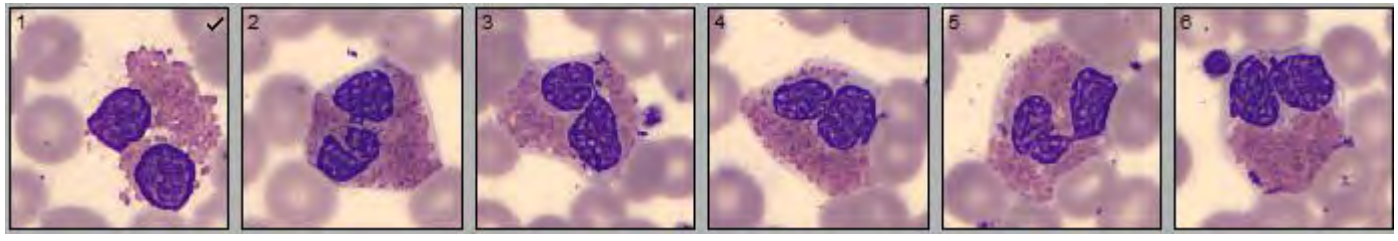
WBC	HGB	MCV	PLTo	PLTi	Ne	Ly	Mo	Eo	Ba	NRBC	VLYM
6.6	14.4	84.00	216	220	43	18.5	3.9	4.4	1		29.3



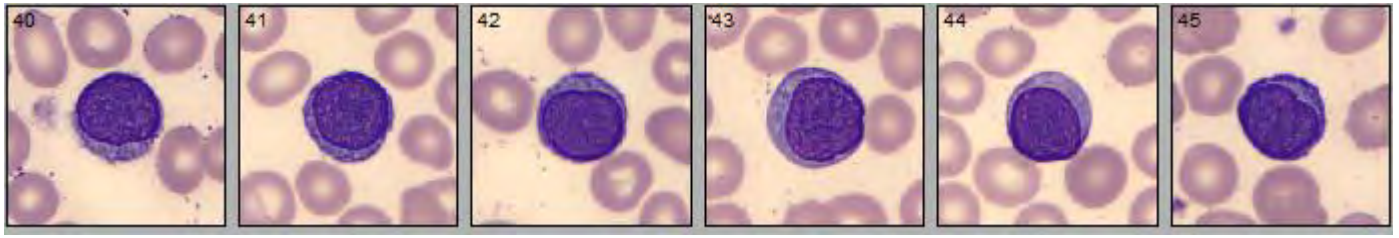


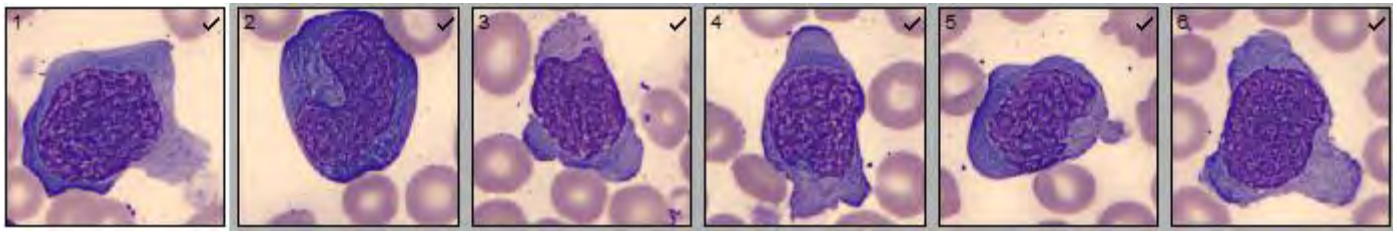




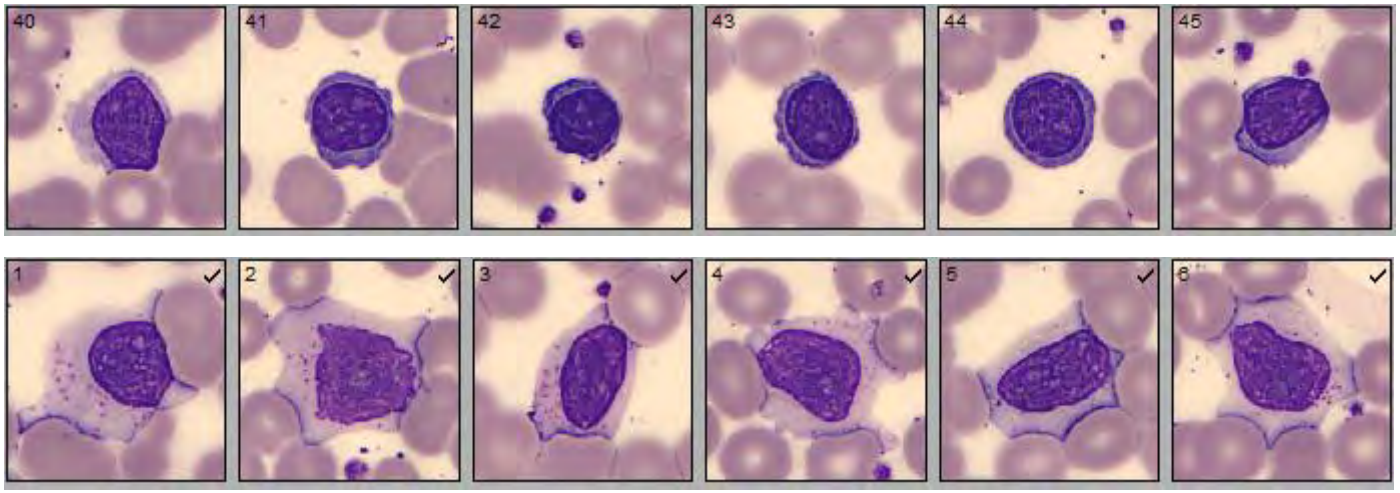


WBC	HGB	MCV	PLTo	PLTi	Ne	Ly	Mo	Eo	Ba	NRBC	VLYM	Meta	Myelo	Pro	Blast	AbLy
13.6	8.9	69.50	377	417	17.8	79.7	2	0.5								37.7

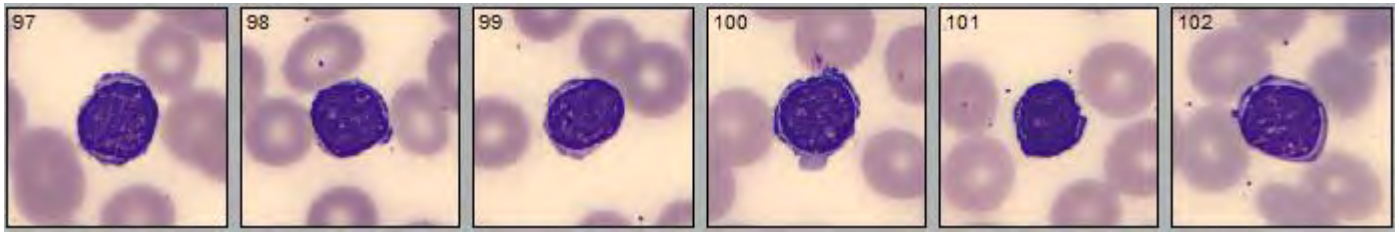


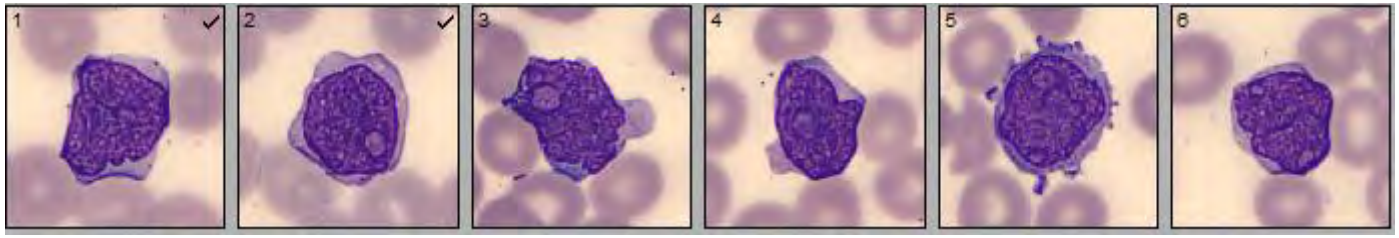


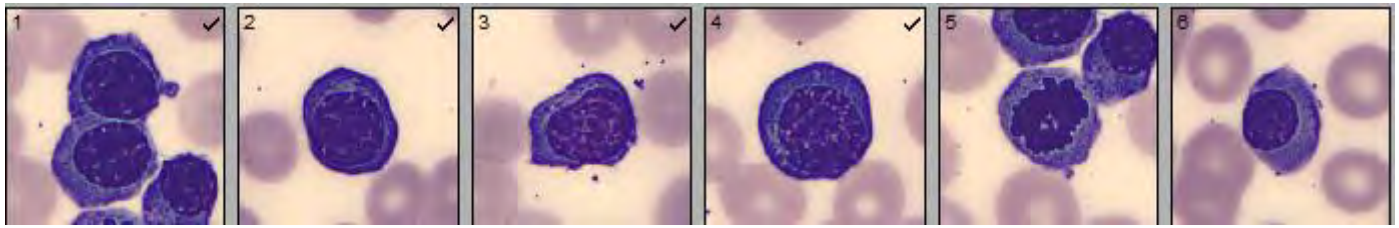
WBC	HGB	MCV	PLTo	PLTi	Ne	Ly	Mo	Eo	Ba	NRBC	VLYM
8.1	14.7	93.50	346	342	44.8	28.4	8.5	2			16.4

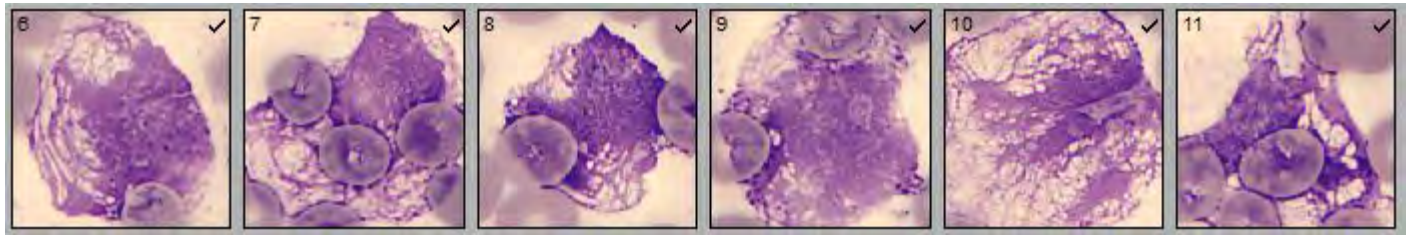


WBC	HGB	MCV	PLTo	PLTi	Ne	Ly	Mo	Eo	Ba	NRBC	Comments
5.9	9.0	86.30	4	11	4.2	95.3	0.5			9.8	Large number of the lymphocytes are abnormal, many with blast like fefatures; 43.5% smudge cells

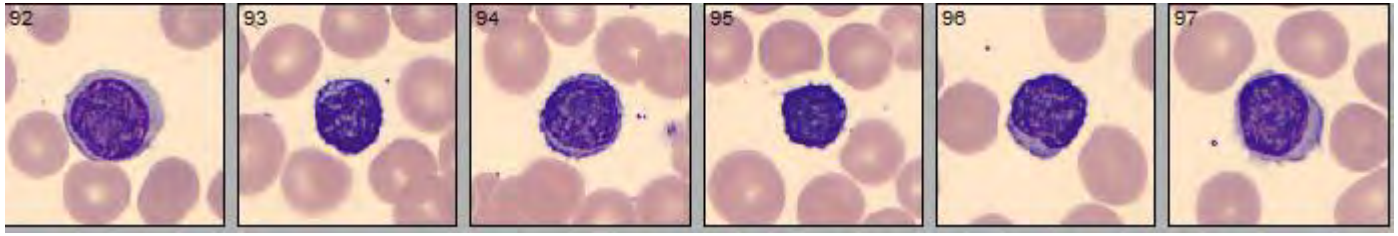


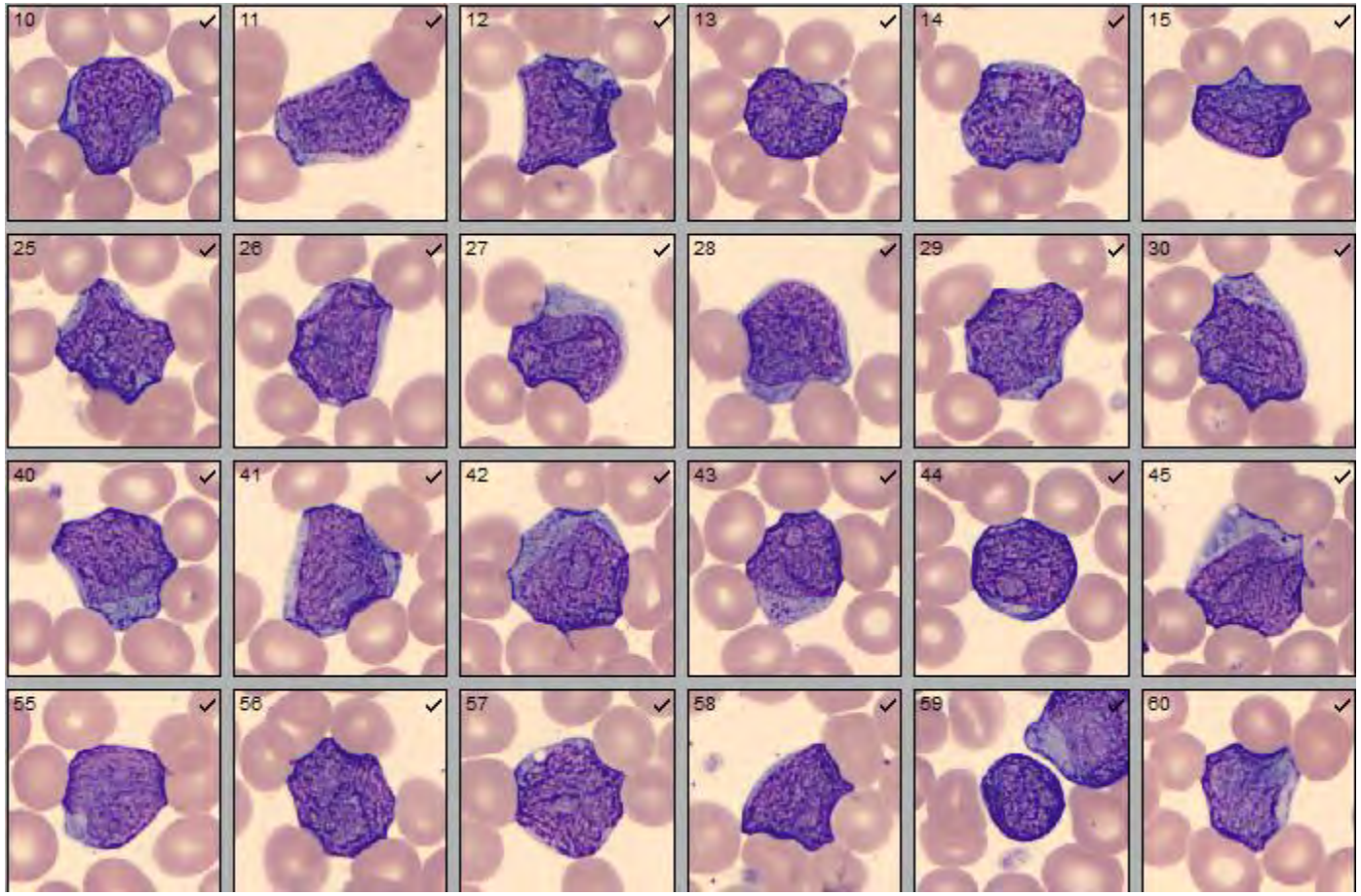




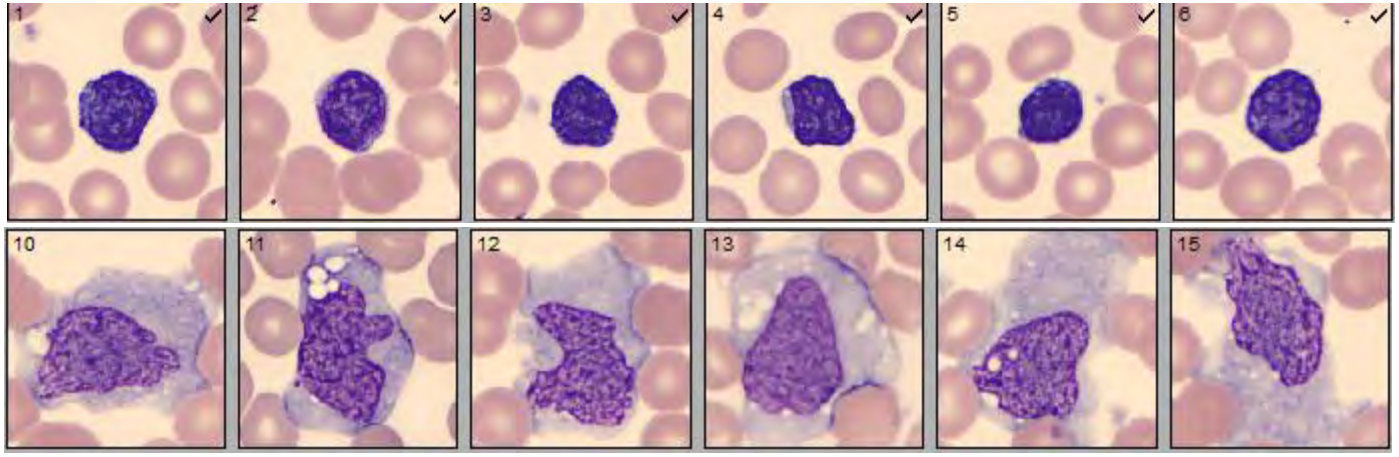


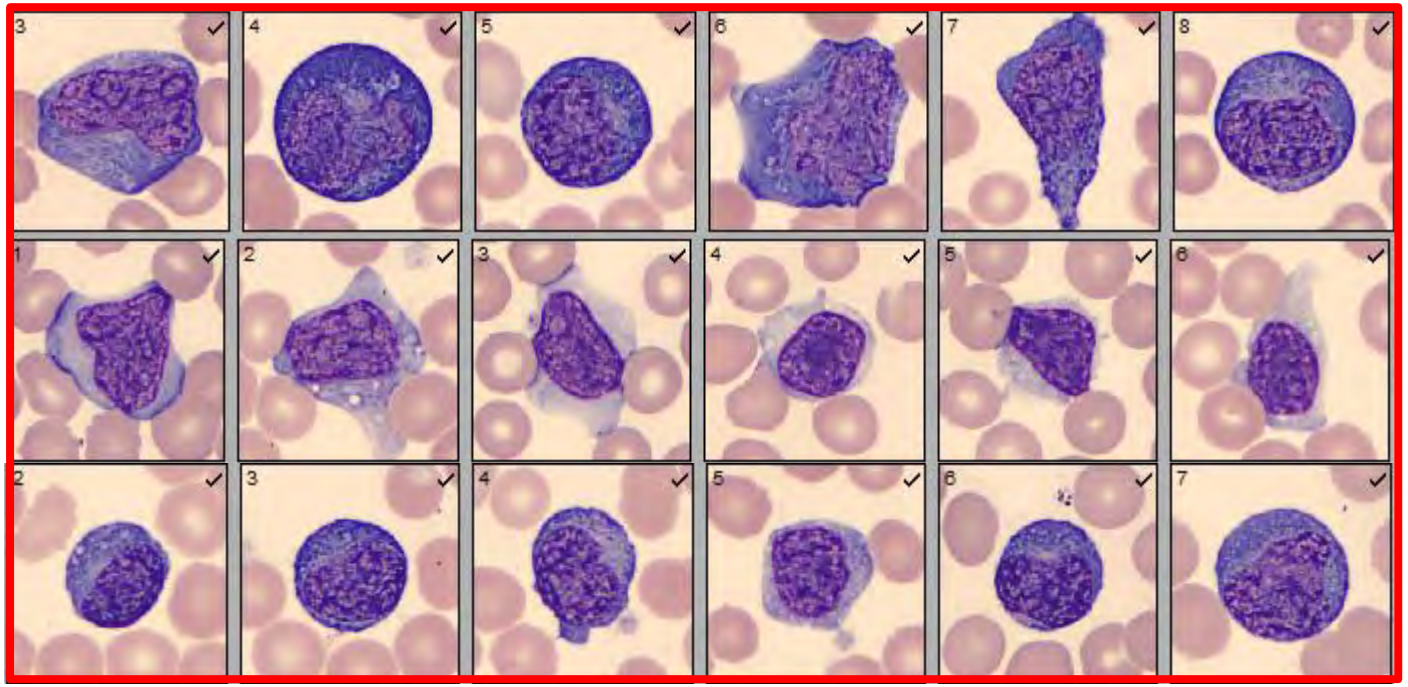
WBC	HGB	MCV	PLTo	PLTi	Ne	Ly	Mo	Eo	Ba	Blast
3.6	10.3	91.10	85	95	4.5	46.6	0.5	0.9	0.5	47.1



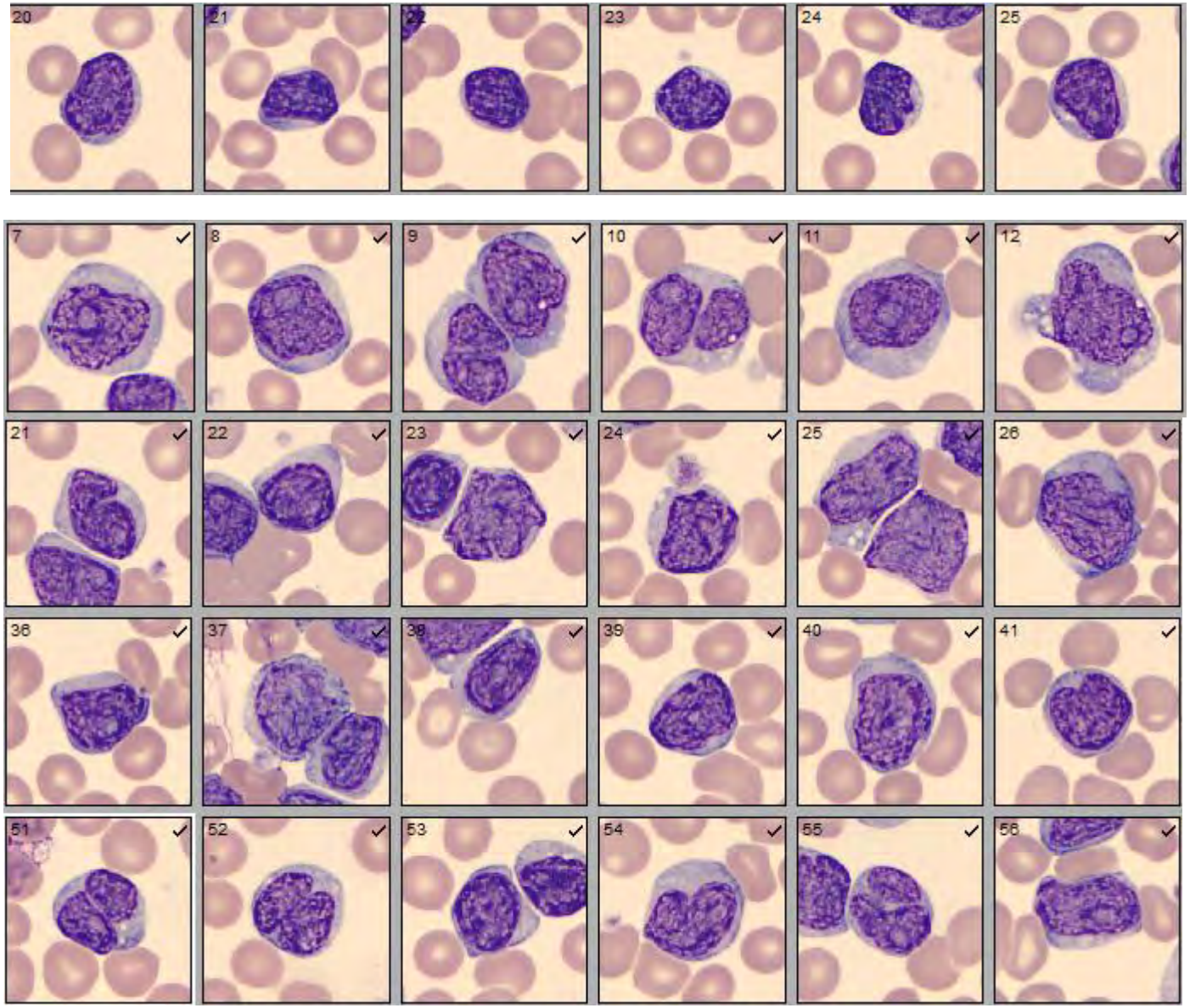


WBC	HGB	MCV	PLTo	PLTi	Ne	Ly	Mo	Eo	Ba	NRBC	VLYM
5.3	11.9	79.20	207	218	35.2	37.9	10		0.5		16.3

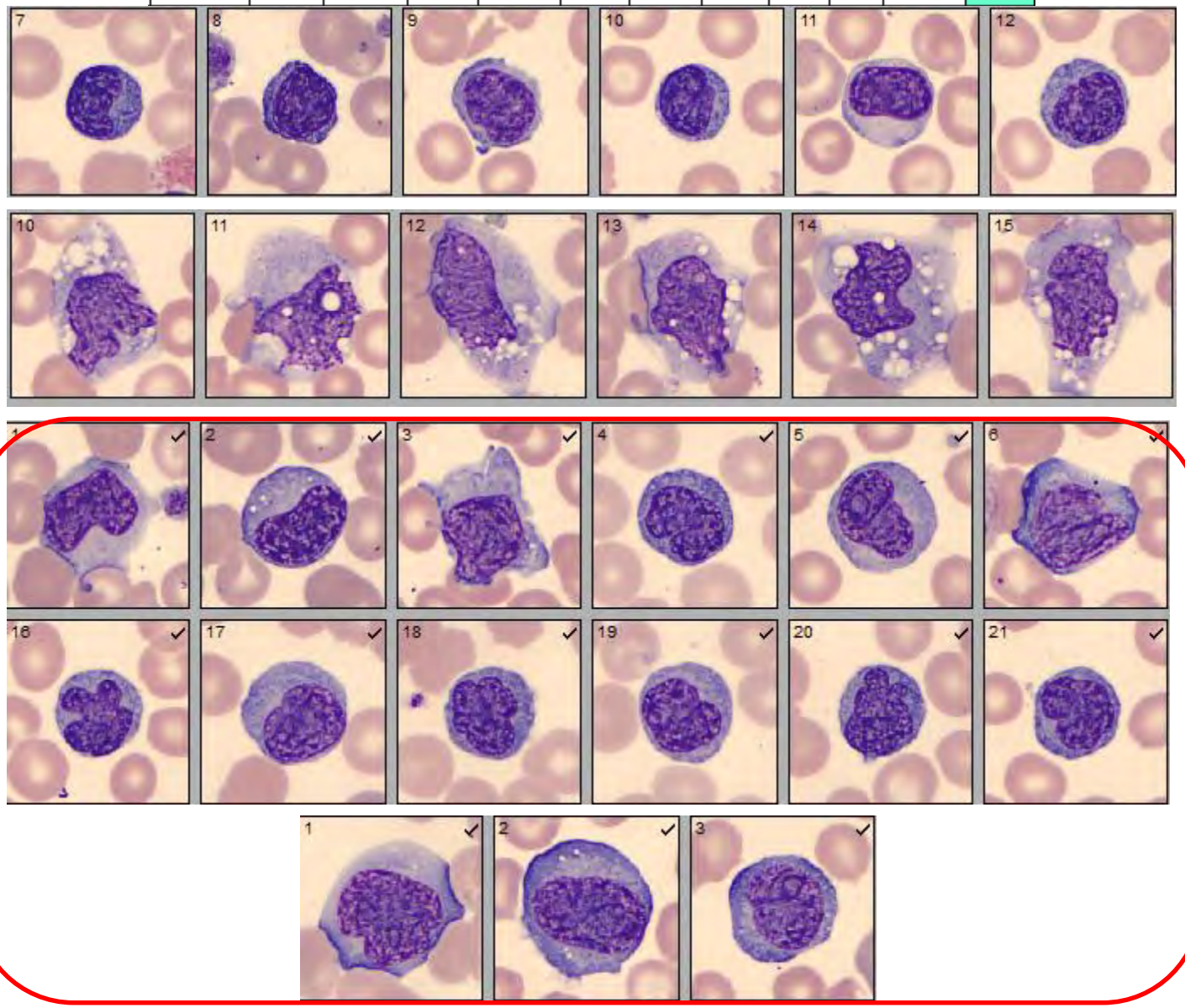


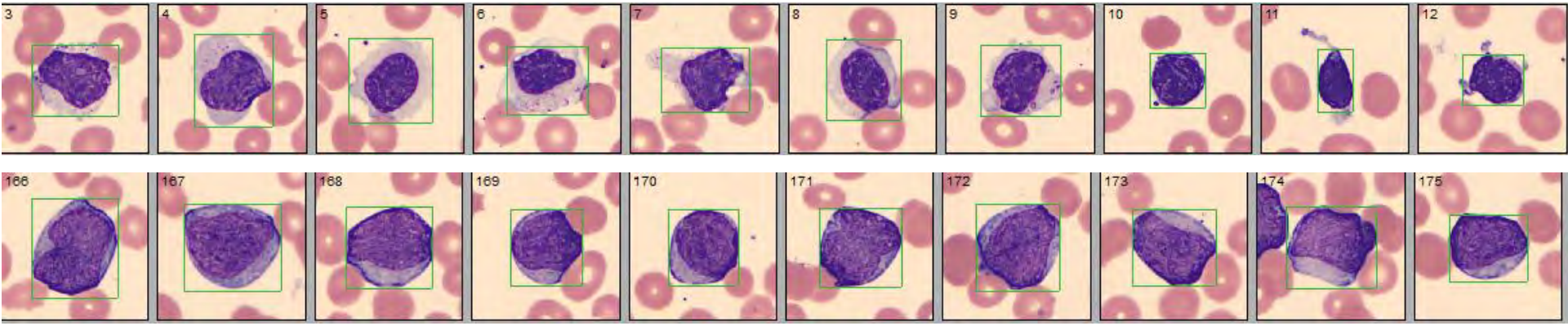


WBC	HGB	MCV	PLTo	PLTi	Ne	Ly	Mo	Eo	Ba	OTHR
230.0	13.6	88.90	166	174	6.1	1.7	0.3			91.9

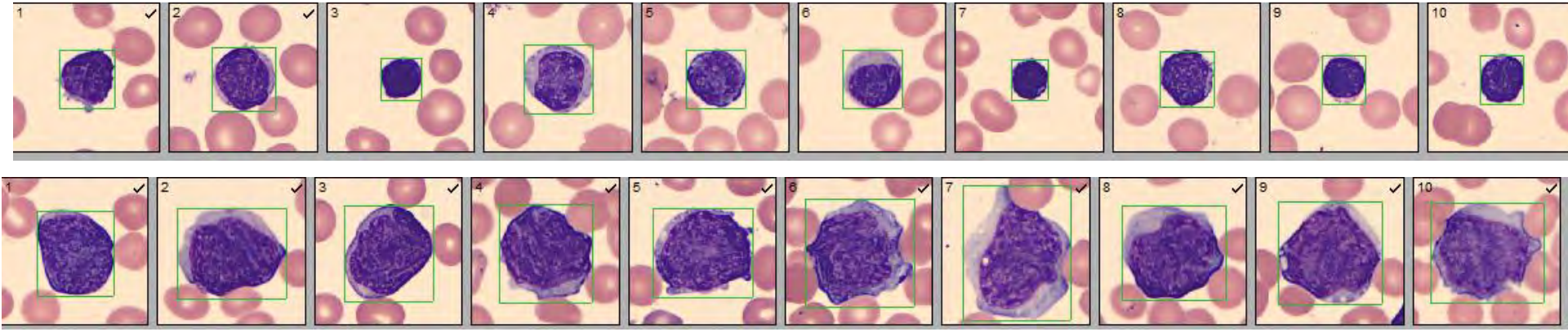


WBC	HGB	MCV	PLTo	PLTi	Ne	Ly	Mo	Eo	Ba	NRBC	VLYM
19.7	12.0	91.80	352	384	25.3	39.6	6.4	6.9	0.5	1	21.3



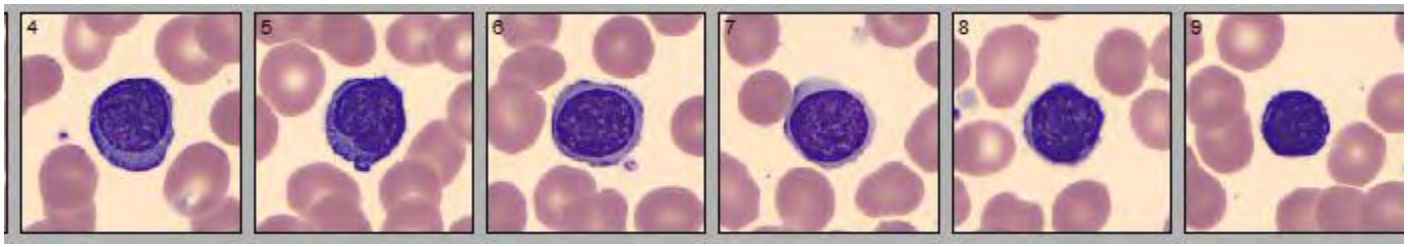


Blasts



Mature lymphoid neoplasm

Normal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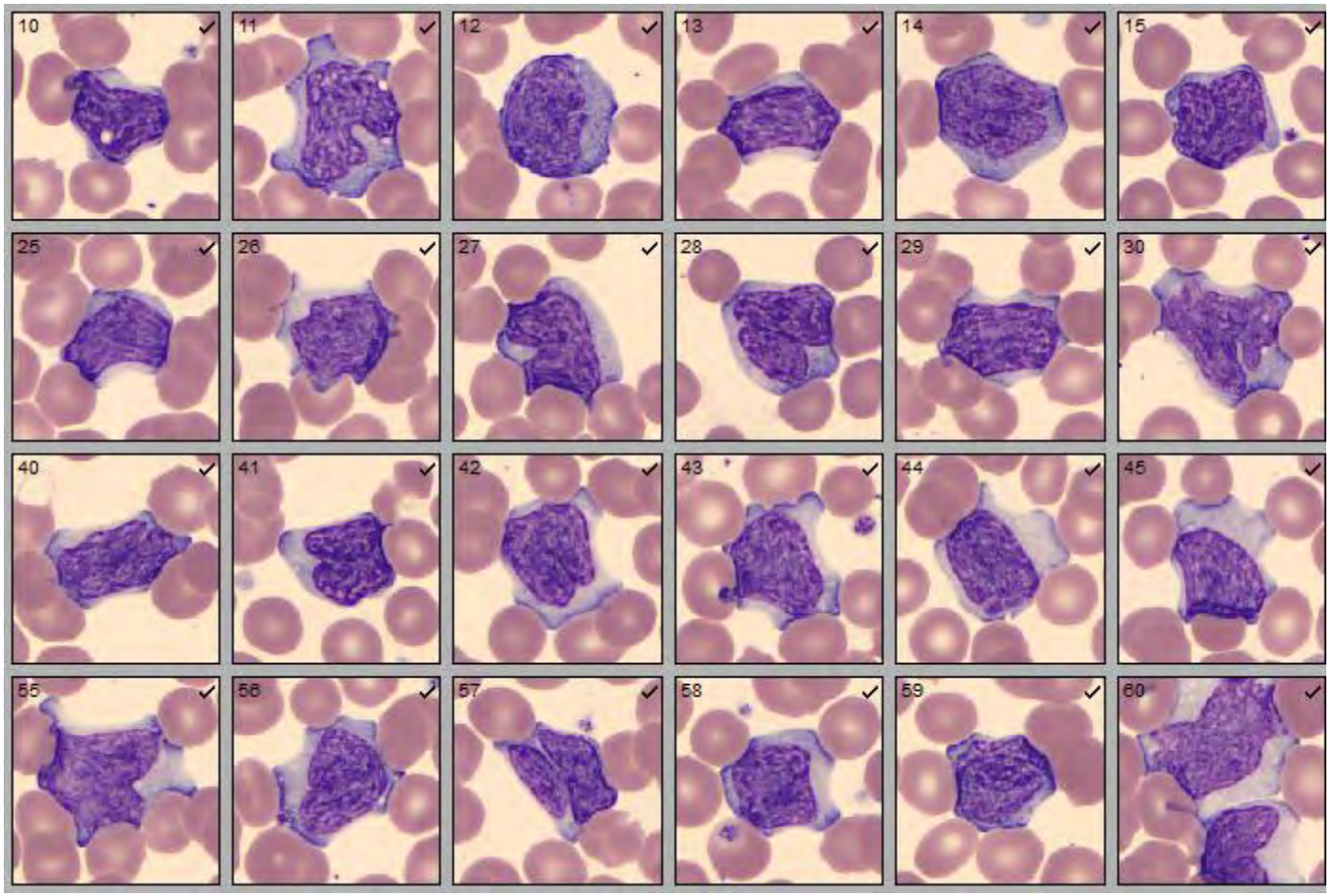
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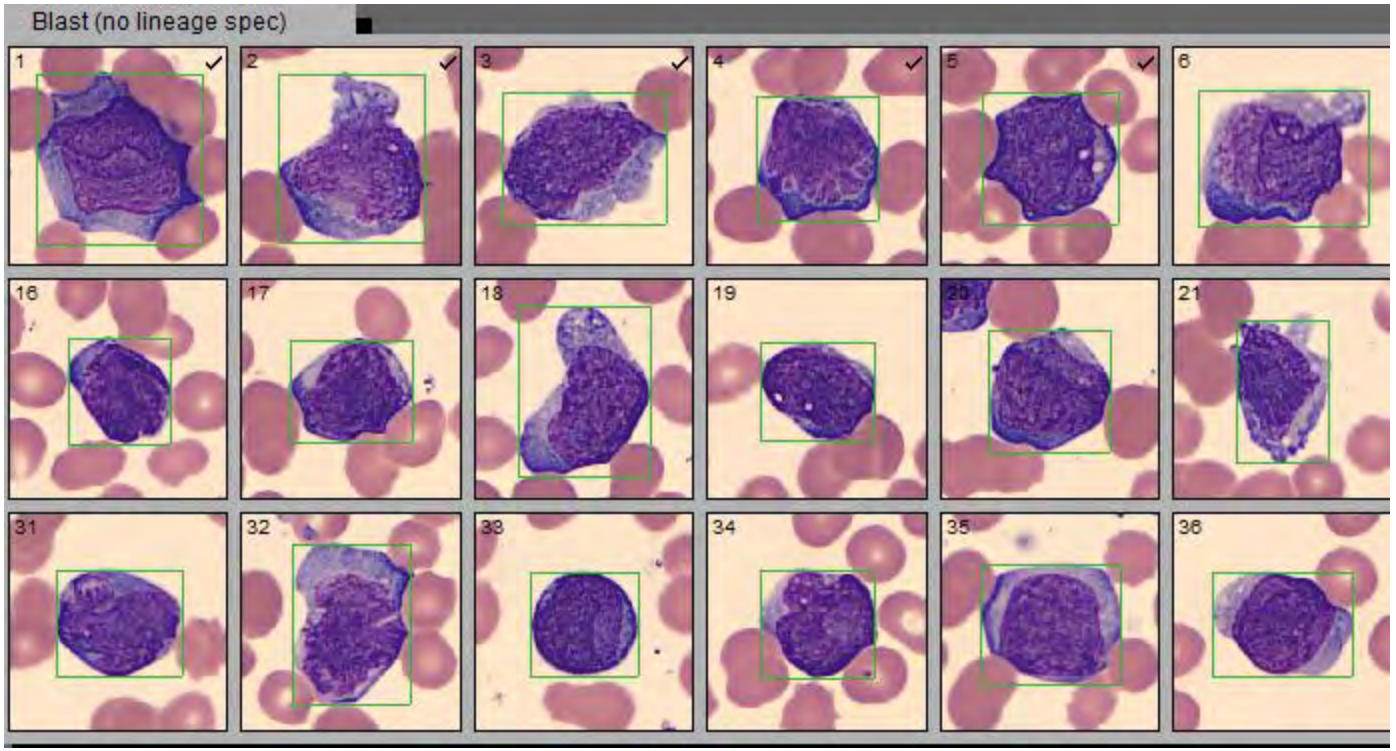

Lymphoma or blasts?

Thank you

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