# WHITE BLOOD CELLS (LEUKOCYTES)

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**Ph.D in Hematology & Blood Banking**

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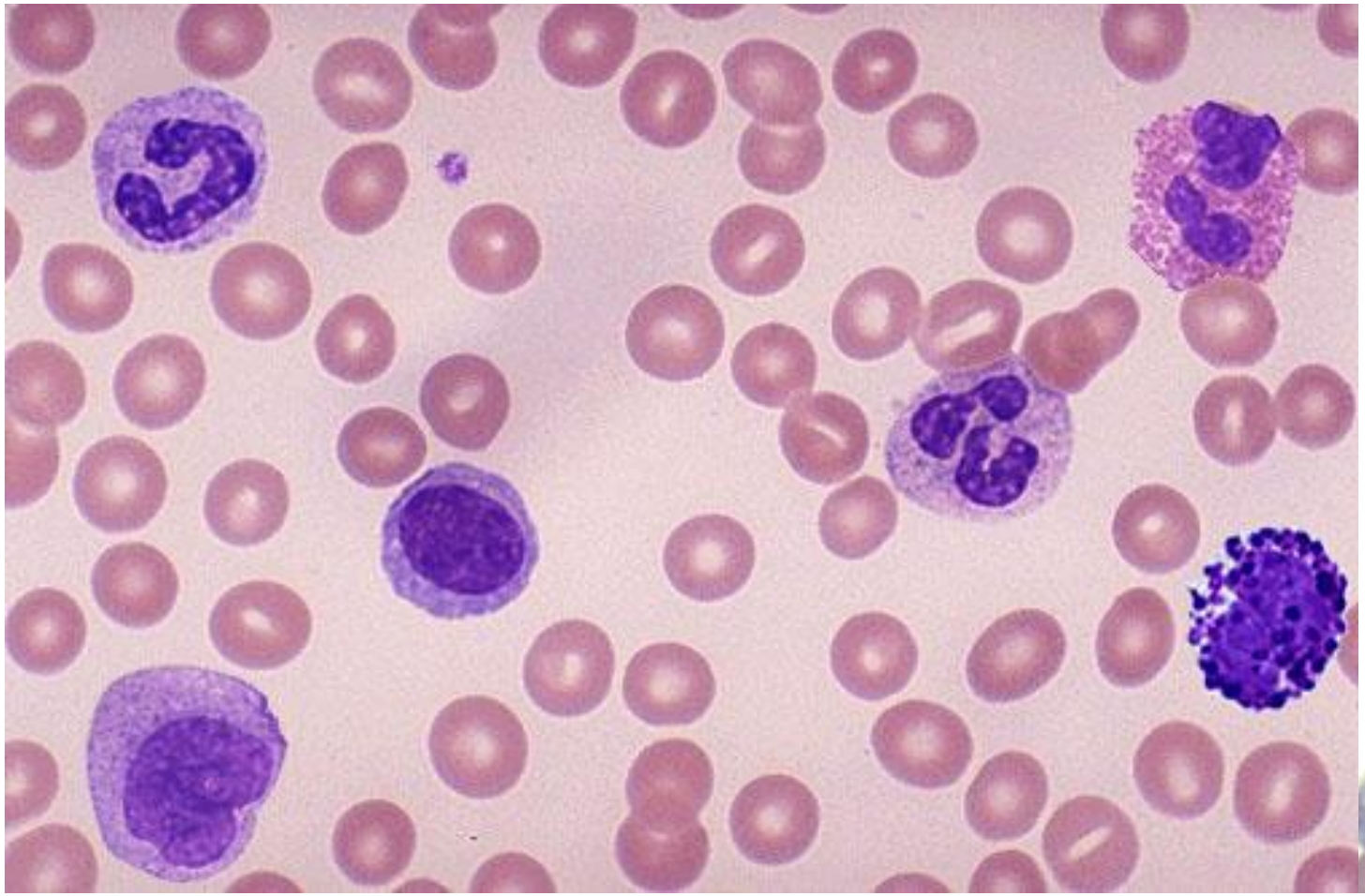
# OBJECTIVES

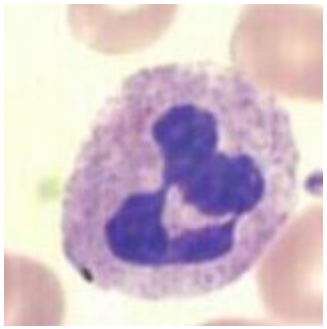
- Introduction & Review of WBC Morphology
- Diff; Relative % vs. absolute number
- Review: mature & immature WBC
- nonneoplastic WBC alterations
- Acute leukemias
- Myeloprolifrative Neoplasm
- Lymphoprolifrative Neoplasm
- Lymphoma

# IN NORMAL PERIPHERAL BLOOD, THERE ARE THREE BASIC TYPES OF LEUKOCYTES

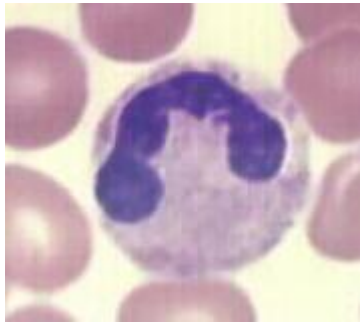


# REFERENCE RANGE (RELATIVE %)

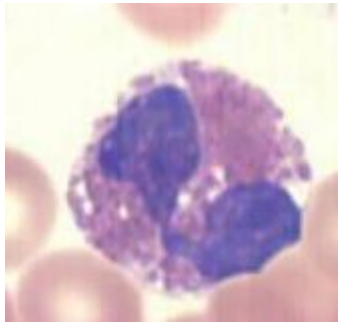
- Reference ranges (values considered to be normal) for differential WBC counts may vary among laboratories, but are usually about:



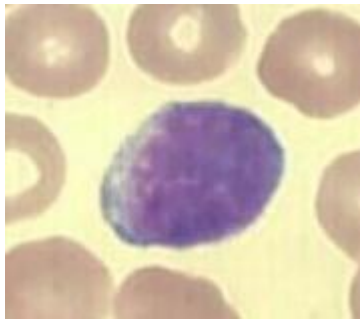
**Segmented Neutrophils 50-70%**



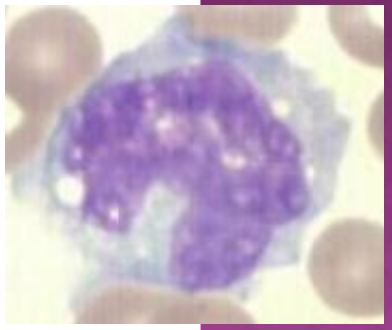
**Band neutrophils 0- 5%**



**Eosinophils 1-5%**



**Lymphocytes 20-40%**



**Monocytes 1-6%**

# The Absolute Number Of Each Cell Type/µl

- For example: given a patient with a total WBC count of 8,000/mL and the differential WBC count shown below (i.e., the number observed for each cell type in the 100 white cell count):
- Segmented neutrophils 60%
- Band neutrophils 5%
- Lymphocytes 30%
- Monocytes 2%
- Eosinophils 2%
- Basophils 1%

# REFERENCE RANGES

| CELL TYPE | RELATIVE % | ABSOLUTE NO./µl |
|---|---|---|
| Segmented neutrophils | 42 - 72 | 1800 - 8000 |
| Neutrophilic bands | 0 - 5 | 0 - 550 |
| Eosinophils | 1 - 6 | 45 - 550 |
| Basophils | 0 – 0.2 | 0 - 200 |
| Lymphocytes | 24 - 45 | 1100 - 5000 |
| Monocytes | 0.4 - 10 | 200 - 1100 |

# The Absolute Number Of Each Cell Type/µl

| | | | | |
|---|---|---|---|---|
| Segmented neutrophils: | 60% | x | 8,000 = | 4,800 |
| Band neutrophils: | 5% | x | 8,000 = | 400 |
| Lymphocytes: | 30% | x | 8,000 = | 2,400 |
| Monocytes: | 2% | x | 8,000 = | 160 |
| Eosinophils: | 2% | x | 8,000 = | 160 |
| Basophils: | 1% | x | 8,000 = | 80 |

Total = 100% = 8,000

# DO THE RELATIVE VALUES ALWAYS INDICATE WHICH CELL LINE IS ↓ OR ↑ ?

- If the total WBC count is “normal” (i.e., within the established reference range), the relative values are a good reflection of the number of  each cell type present, including increases or decreases.
- However , if the total WBC count is abnormal (i.e., increased or decreased), the relative percentage must be converted to an absolute number of each cell type present in order to determine which cell line is involved.

# The Absolute Number Of Each Cell Type/µl

- For example: given a patient with a total WBC count of 15,000/mL and the differential WBC count shown below (i.e., the number observed for each cell type in the 100 white cell count):
- Segmented neutrophils 65%
- Band neutrophils 5%
- Eosinophils 2%
- Lymphocytes 25%
- Monocytes 3%

# The Absolute Number Of Each Cell Type/µl

| | | | | | |
|---|---|---|---|---|---|
| Segmented neutrophils: | 65% | X 15000 = | 9750 | H | (1800 -8000) |
| Band neutrophils: | 5% | X 15000 = | 750 | H | (0 - 550) |
| Eosinophils: | 2% | X 15000 = | 300 | | (0 - 600) |
| Lymphocytes: | 25% | X 15000 = | 3750 | | (1100 - 5000) |
| Monocytes: | 3% | X 15000 = | 450 | | (200 - 1100) |

# The Absolute Number Of Each Cell Type/µl

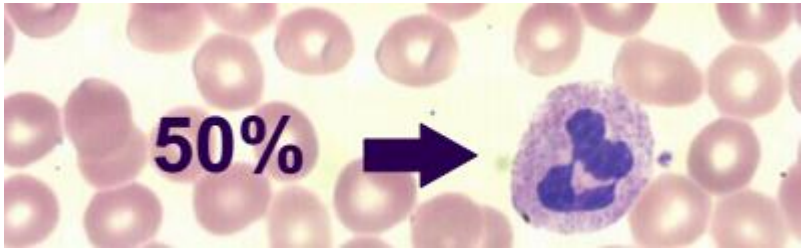
- For example: given a patient with a total WBC count of 15,000/mL and the differential WBC count shown below (i.e., the number observed for each cell type in the 100 white cell count):
- Segmented neutrophils 75% High
- Band neutrophils 5%
- Eosinophils 2%
- Lymphocytes 15%  Low
- Monocytes 3%

# The Absolute Number Of Each Cell Type/µl

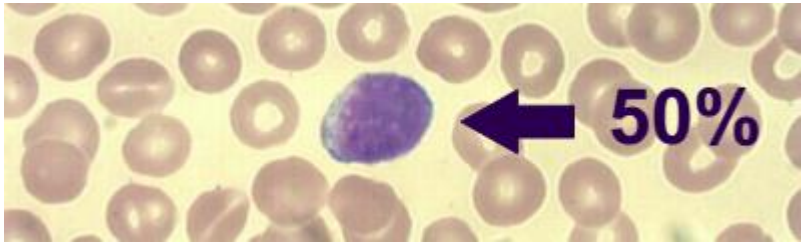
| | | | | | |
|---|---|---|---|---|---|
| Segmented neutrophils: | 75% | X 15000 = | 11,250 | H | (1800 -8000) |
| Band neutrophils: | 5% | X 15000 = | 750 | H | (0 - 550) |
| Eosinophils: | 2% | X 15000 = | 300 | | (0 - 600) |
| Lymphocytes: | 15% | X 15000 = | 2250 | | (1100 - 5000) |
| Monocytes: | 3% | X 15000 = | 450 | | (200 - 1100) |

# WHAT IF THE PATIENT'S TOTAL WBC IS DECREASED? FOR EXAMPLE…

If a patient's total WBC count is 2,500/mL and the following relative distribution is found on the differential WBC count, what interpretation can be made regarding increased or decreased cell lines?



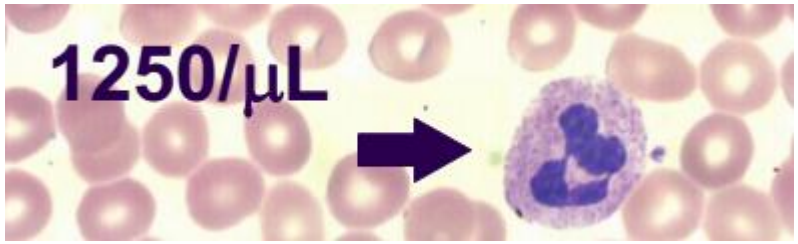

Neutrophils (Normal)



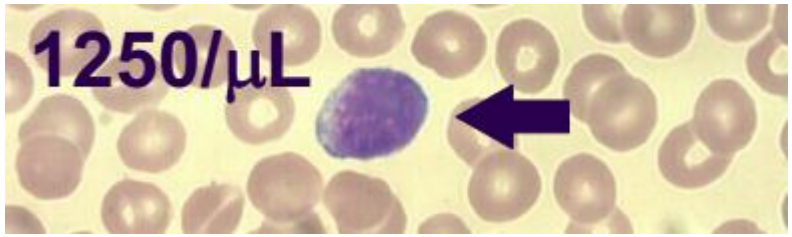

Lymphocytes (Increased)

**Does this patient have *lymphocytosis?***

**the absolute numbers are:**



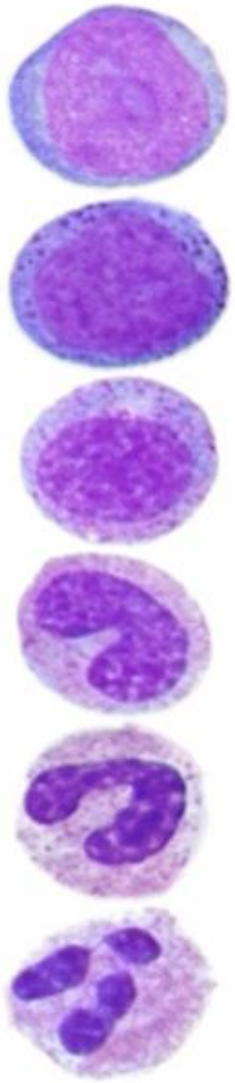

**Neutropenia(<1800/µL)**




**Normal(1100 – 5000/µL)**

# Total Recall: ID Criteria

1. Cell size
2. Nucleus size to cytoplasm volume. [N:C]
3. Cytoplasm
   a. Color
   b. Presence/absence of granules
   c. Size and *color* of granules
4. Nucleus
   a. Shape
   b. Color
   c. CHROMATIN PATTERN
   d. Presence or absence of nucleoli



# MATURATION SEQUENCES: MYELOID SERIES

Myeloblast

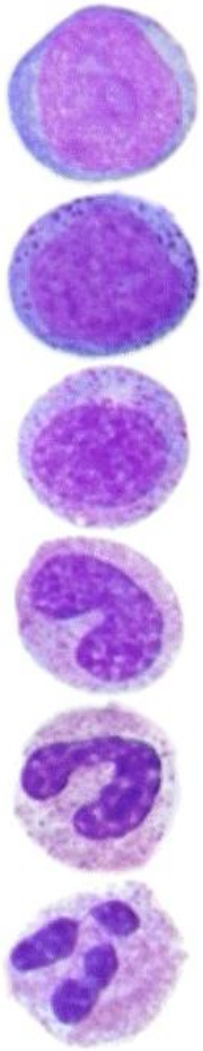
Promyelocyte

Myelocyte

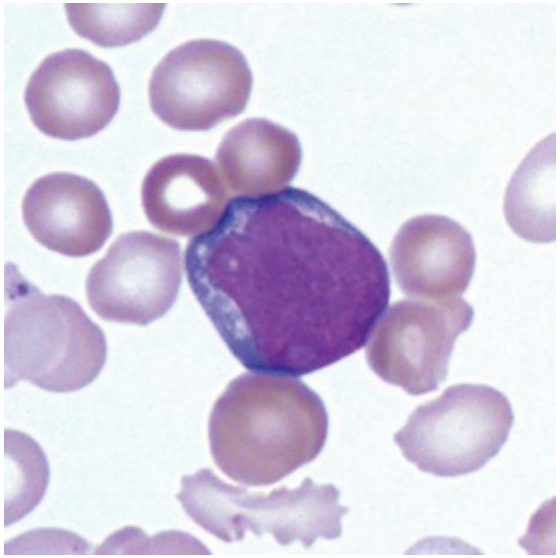
Metamyelocyte

Band

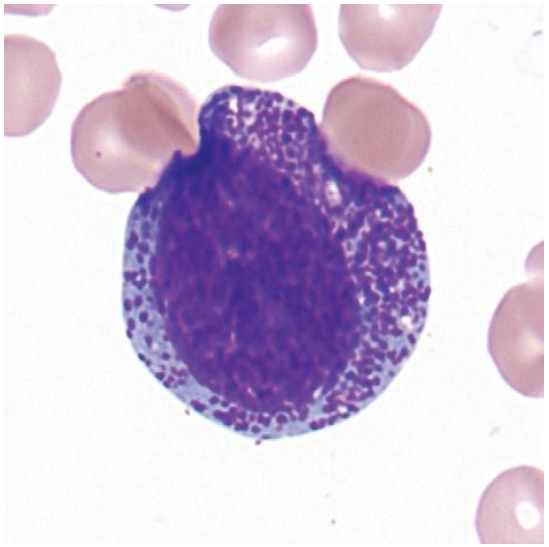
Neutrophil



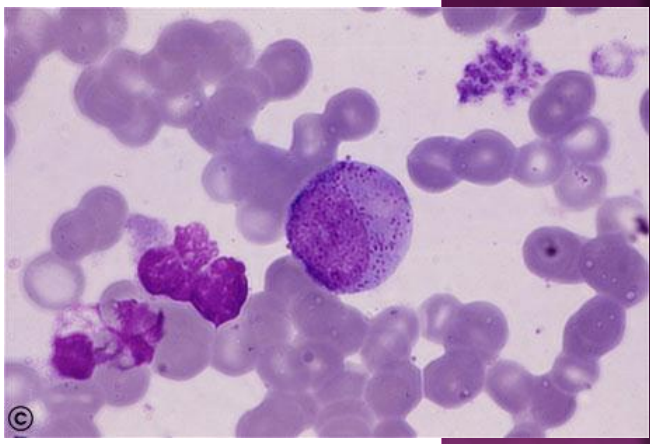
- ✓Cell size
- ✓Cytoplasm color, volume, granulation
- ✓Nucleus size, color, chromatin pattern
- ✓Nucleoli presence



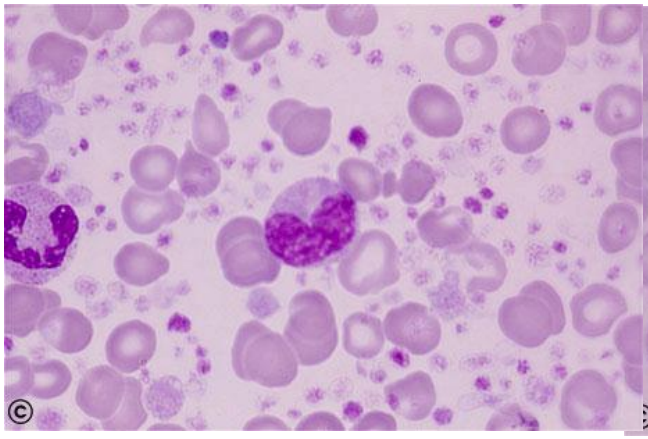
MYELOBLAST



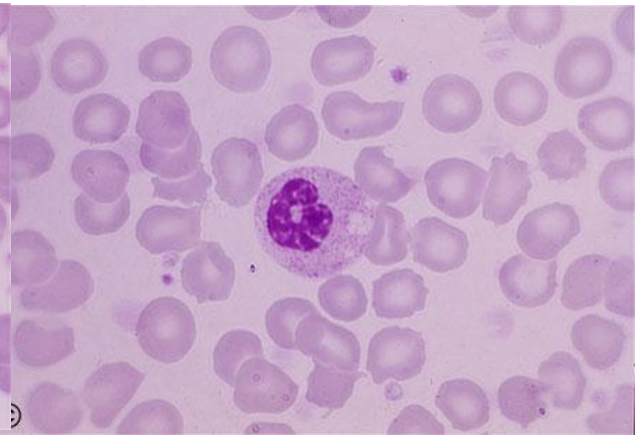
PROMYELOCYTE



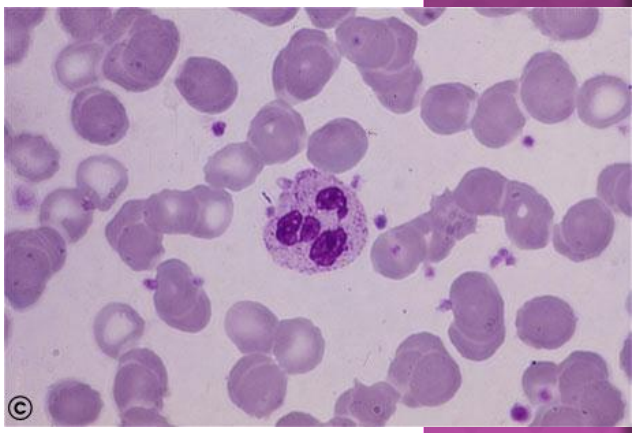
MYELOCYTE



METAMYELOCYTE

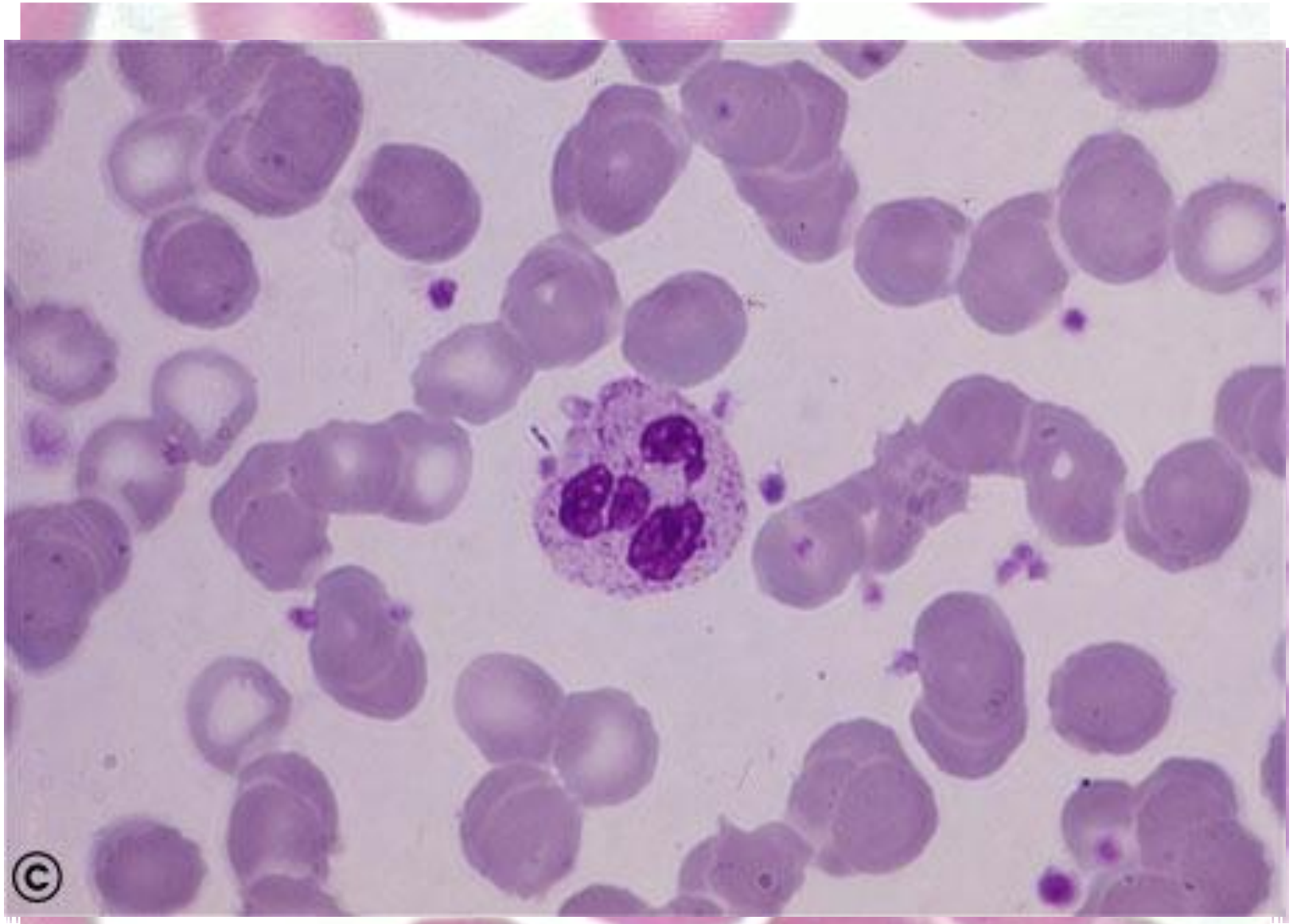


BAND

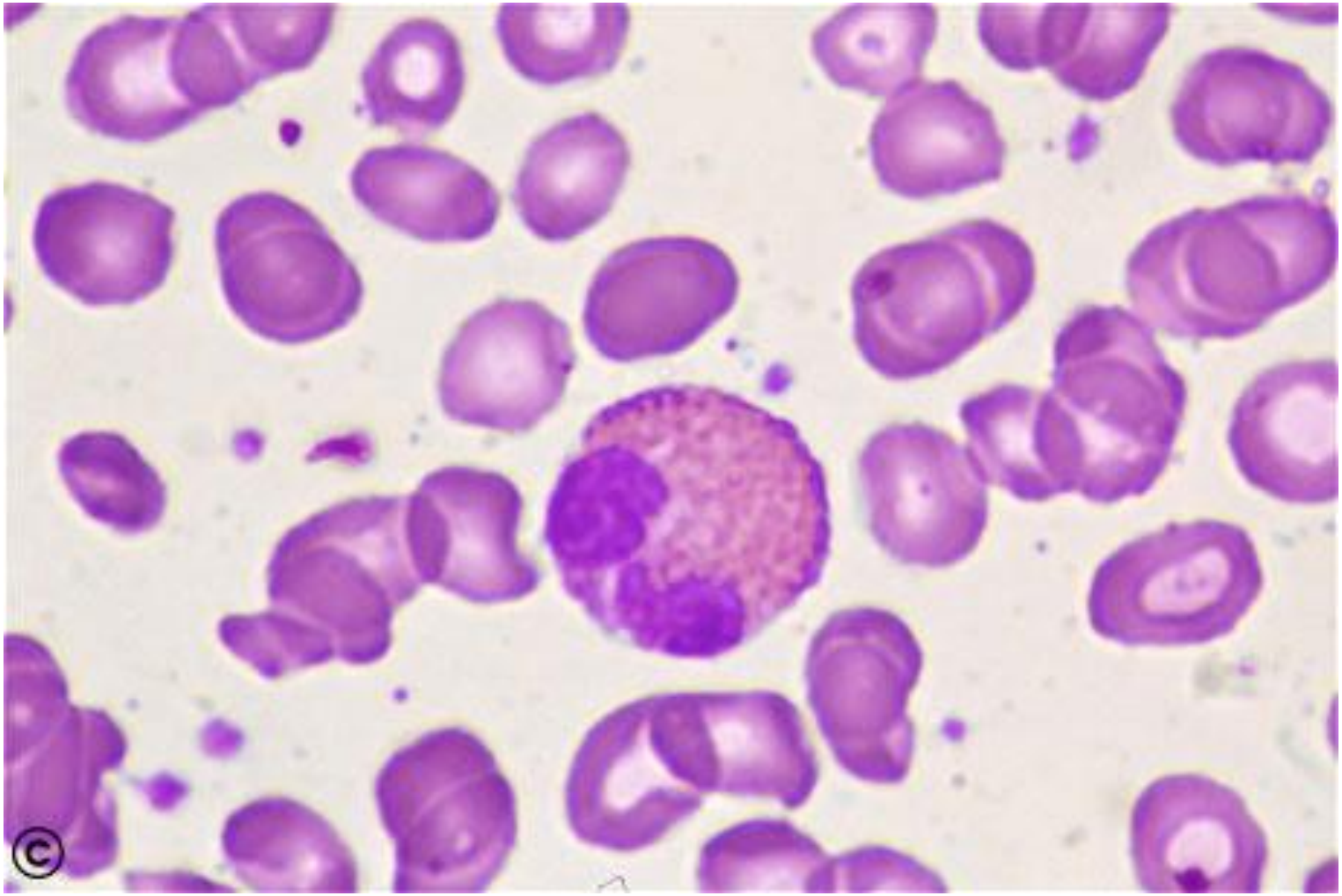


NEUTROPHIL

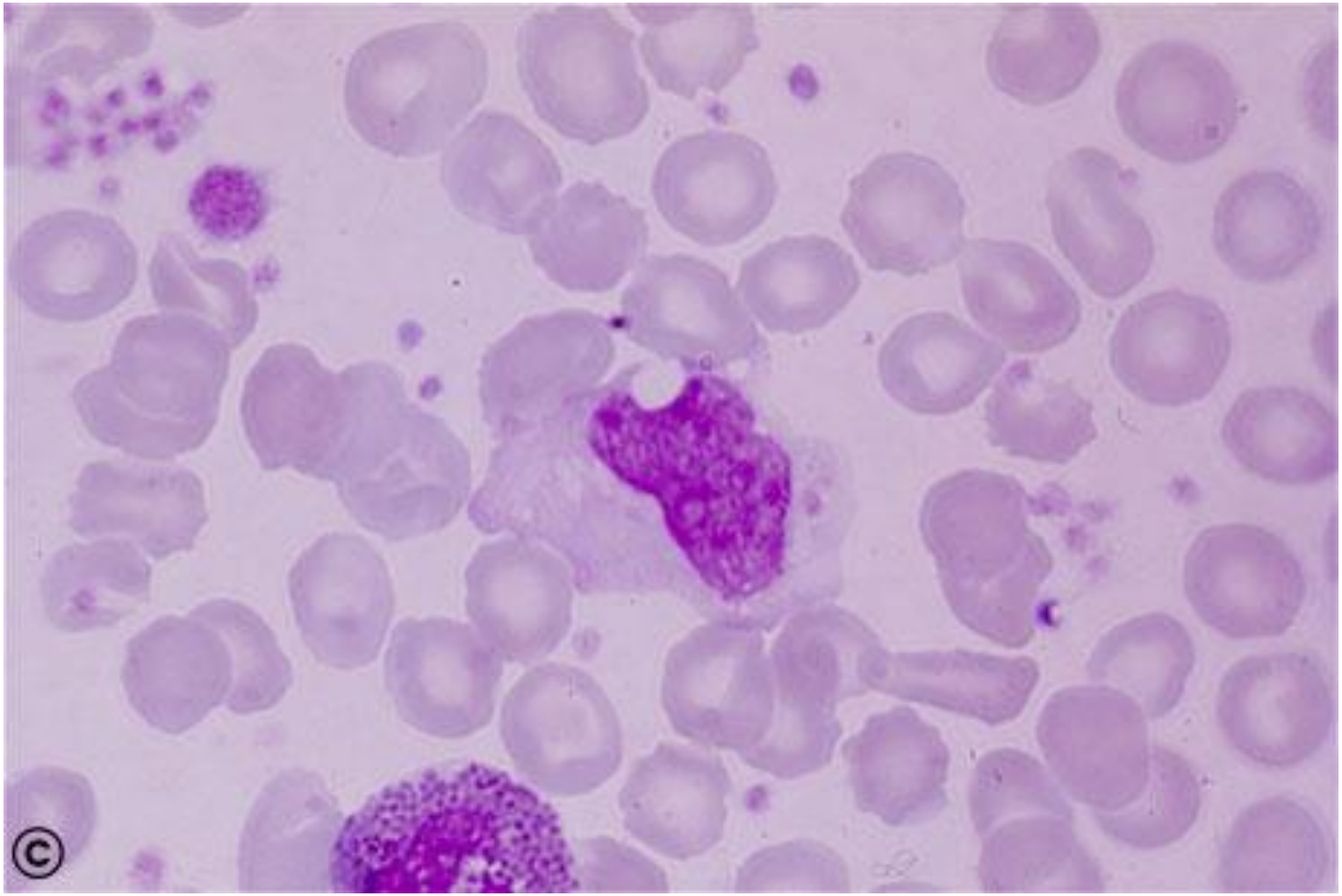
# NEUTROPHIL MATURATION



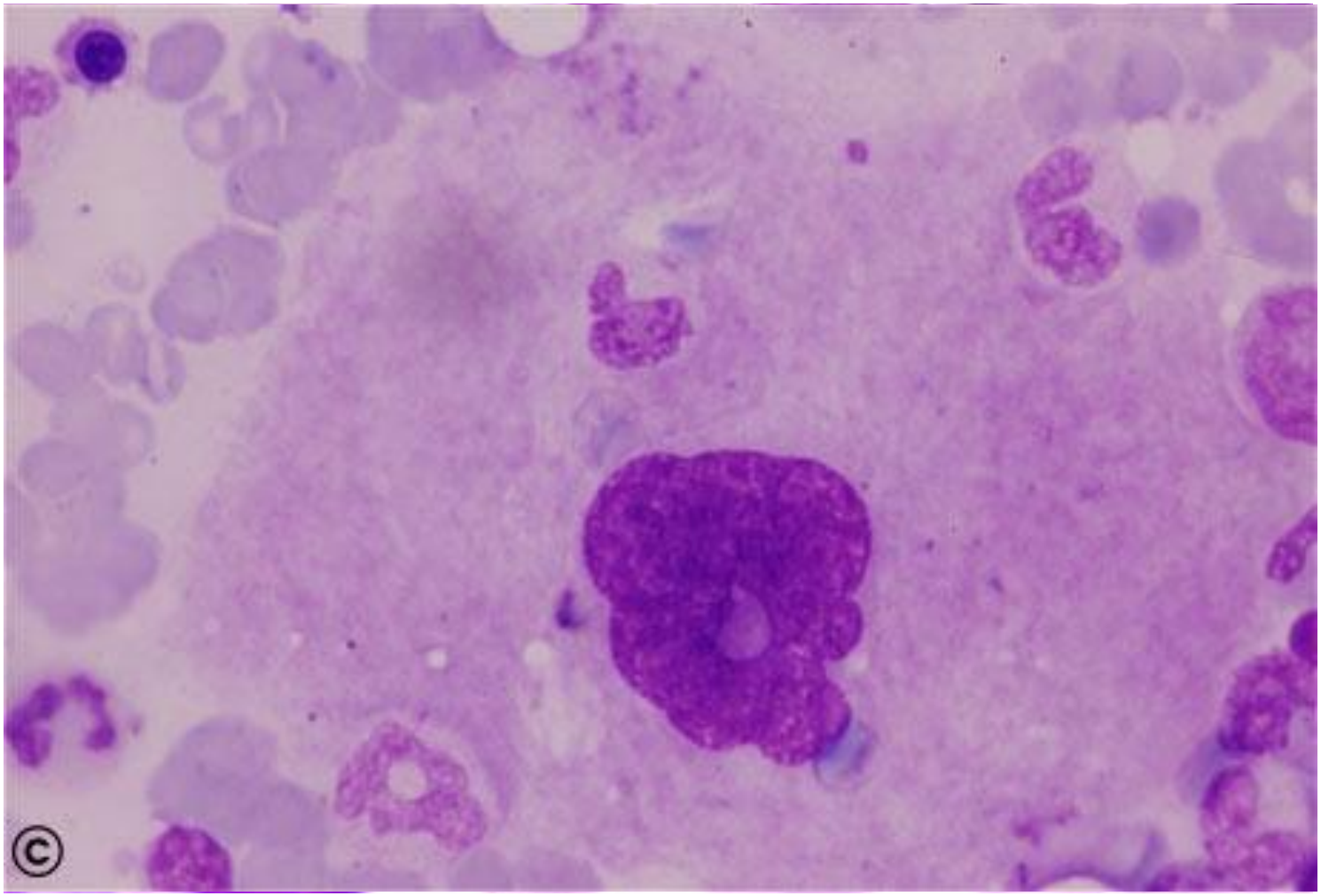
# EOSINOPHIL MATURATION



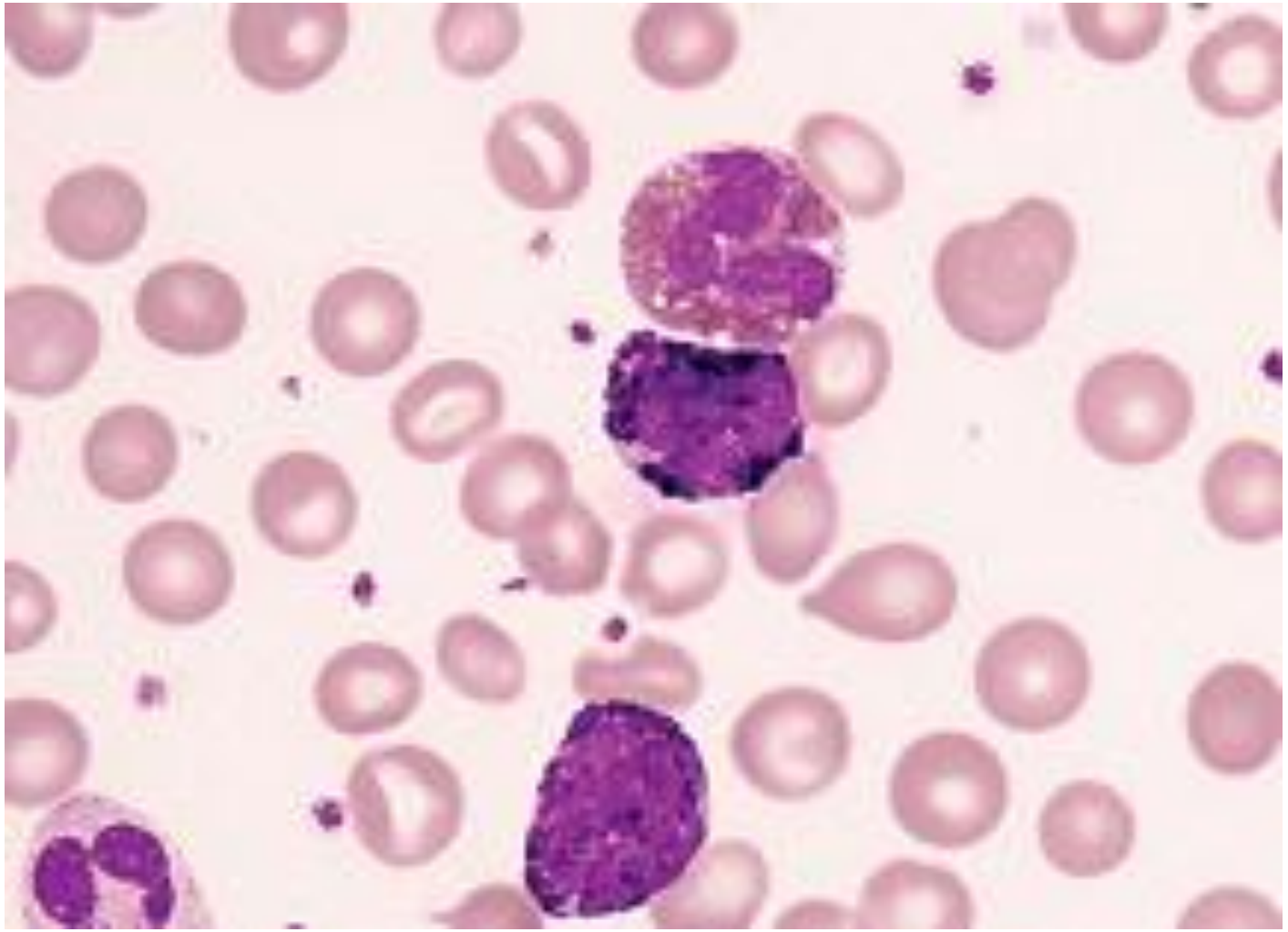
# MONOCYTE MATURATION



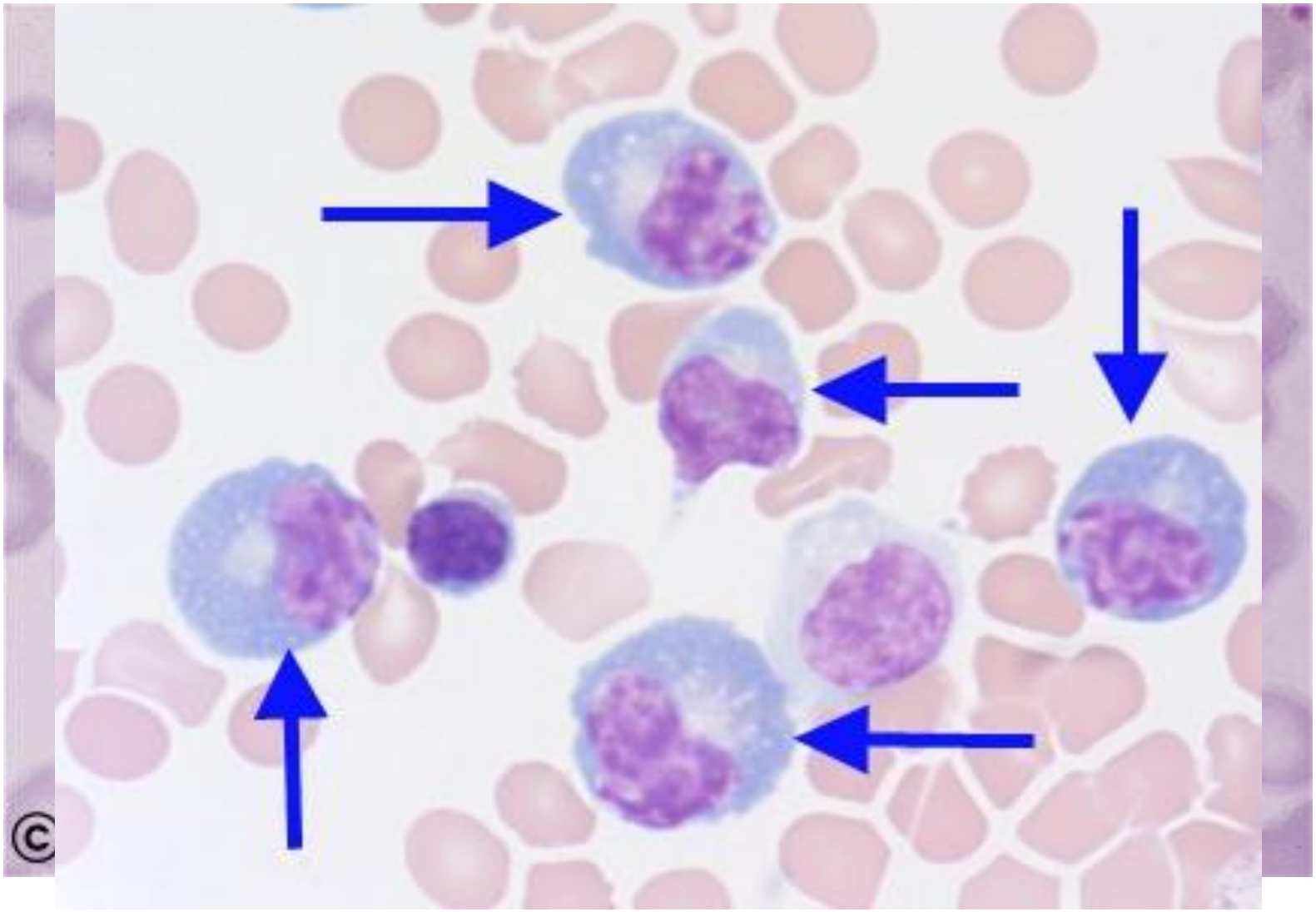
# PLATELET MATURATION



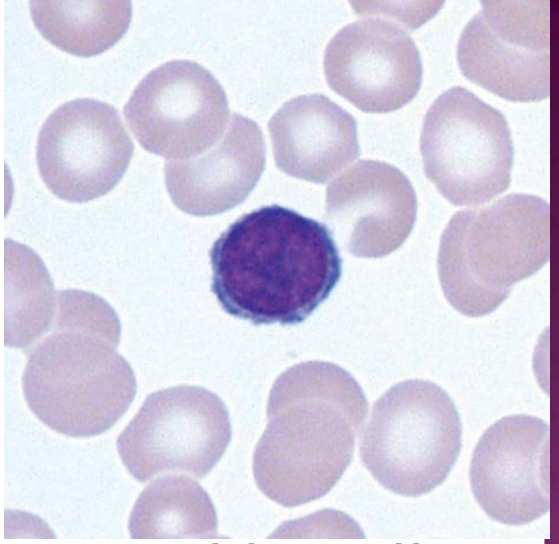
# BASOPHIL



# LYMPHOCYTE MATURATION



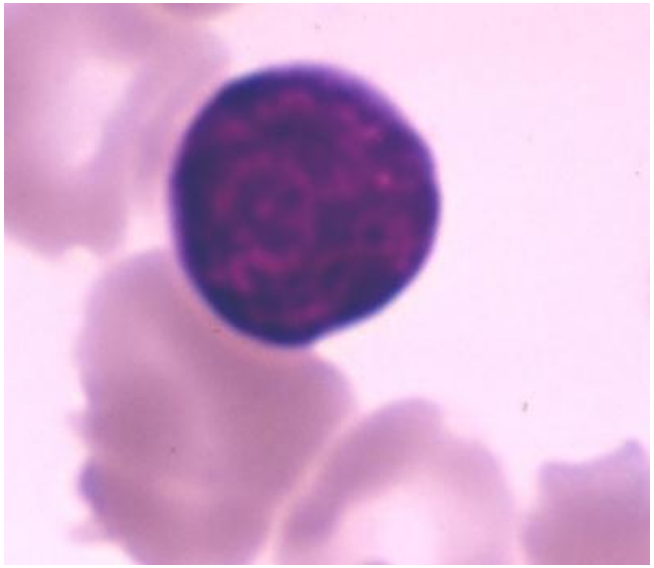
# LYMPHOCYTES:

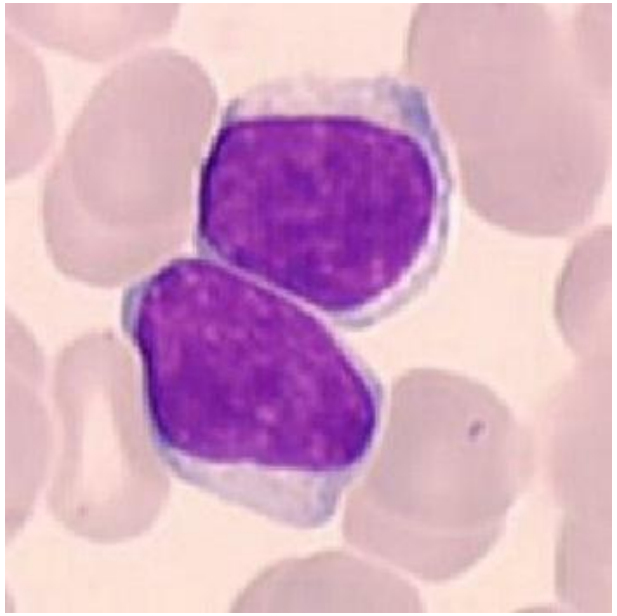


- 2 types of lymphocytes
  - Morphologically: small & large
  - Functionally: T & B lymphocytes
- Small lymphocytes: 7-10 µm

Nucleus rounded, cytoplasm: just rim is seen. Older cells.

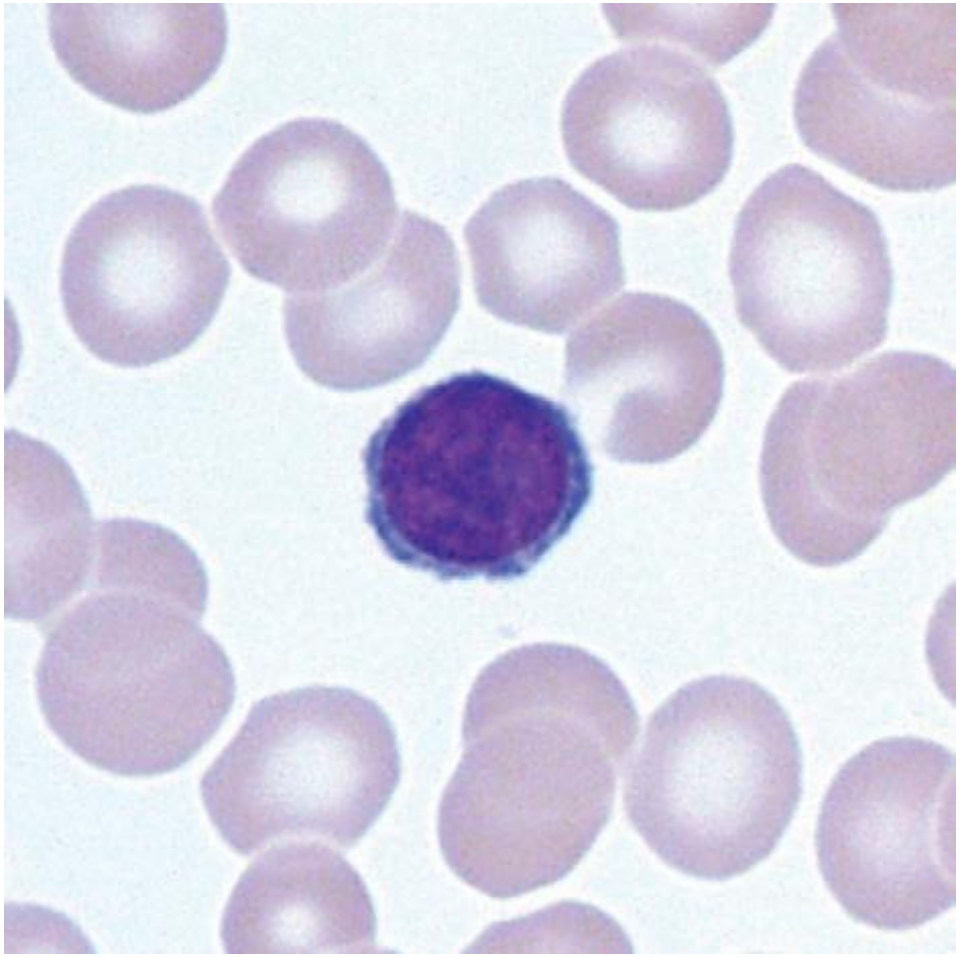
- Large lymphocytes: 10-14 µm Nucleus is big with indentation, definite cytoplasm is seen. Precursor of small lymphocyte.



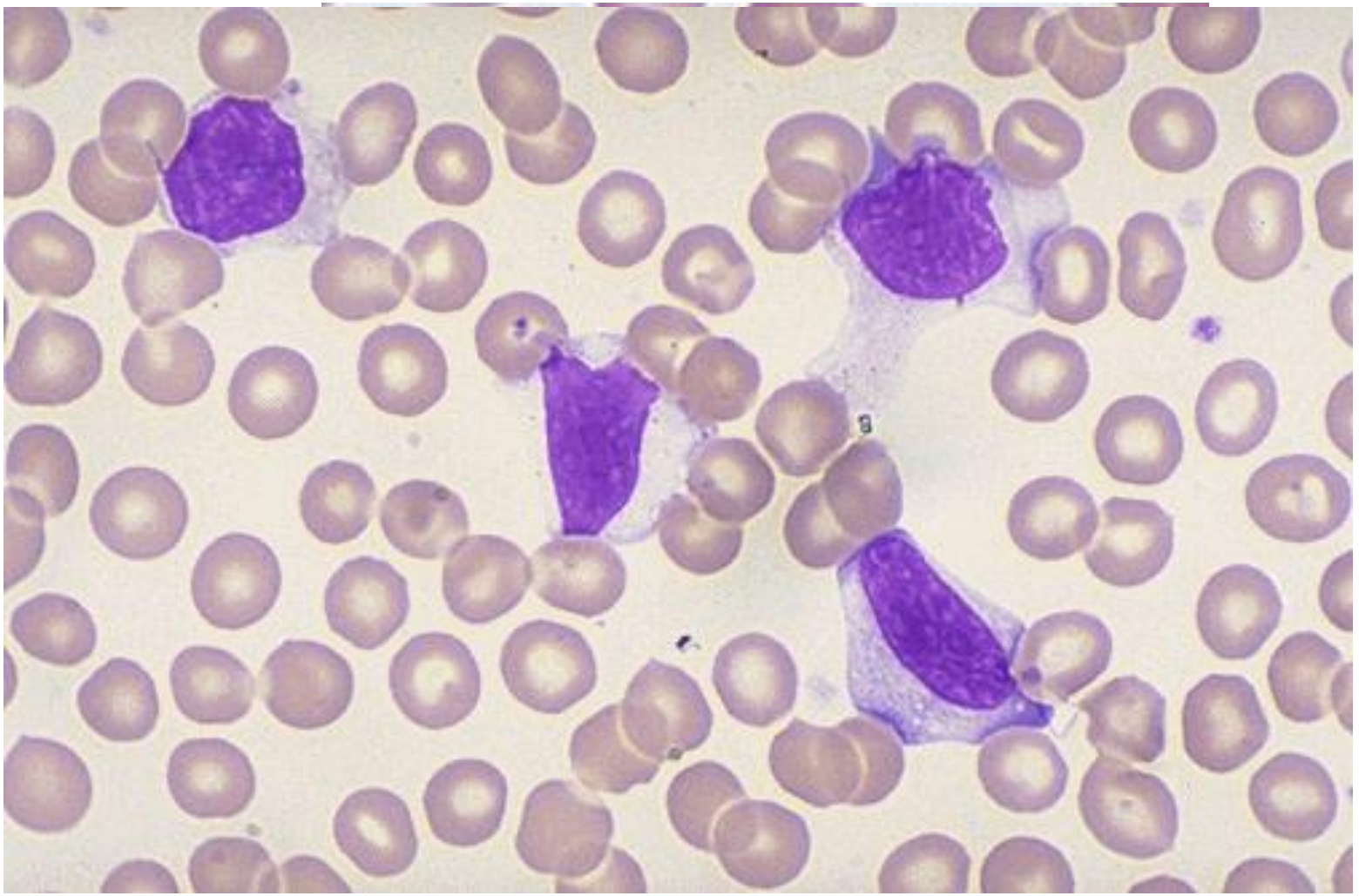


# LYMPHOCYTES MORPHOLOGICALLY : SMALL & LARGE

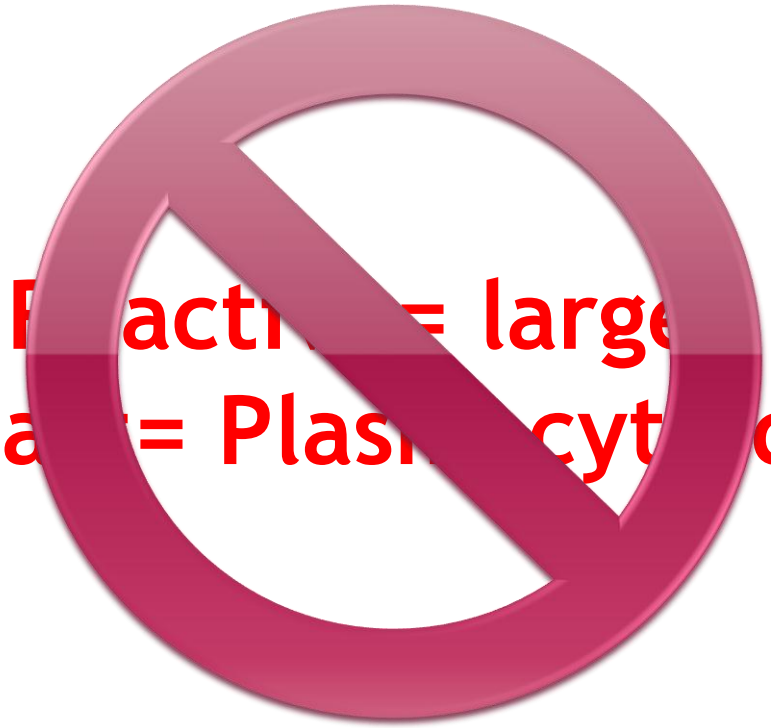
- Small lymphocyte= Normal lymphocyte



# ATYPICAL= REACTIVE= LARGE= VIROCYTE= IMMUNOBLAST= PLASMACYTOID

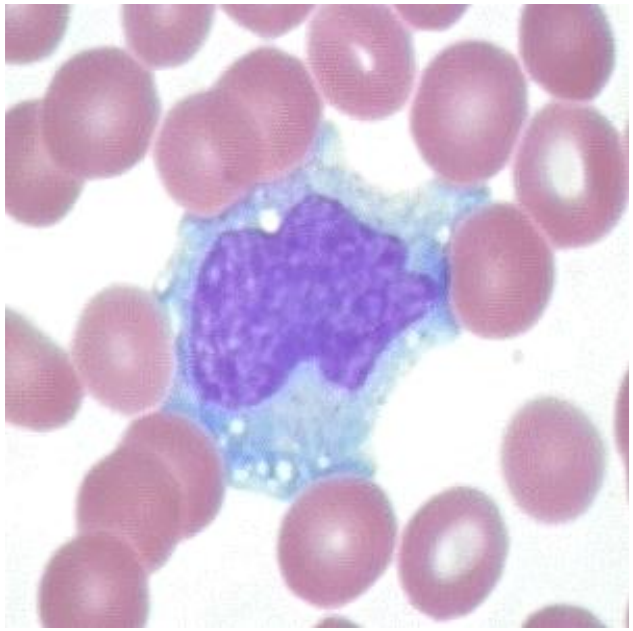


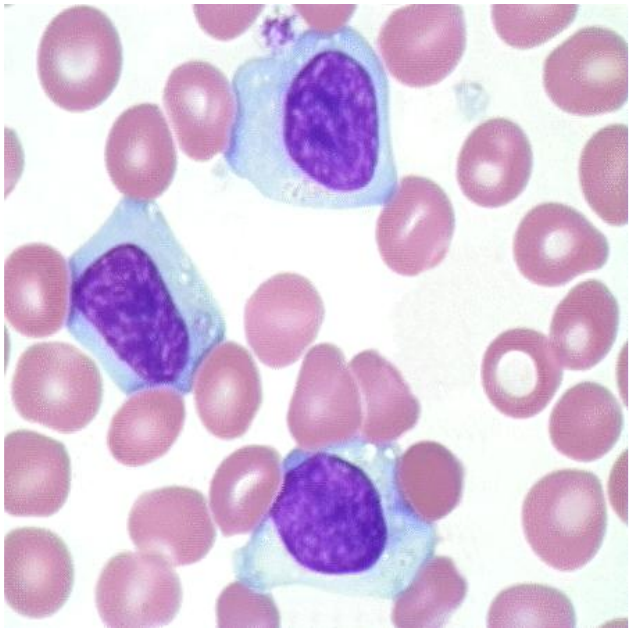
Atypical= R[illegible]activ[illegible]= large[illegible] Virocyte= Immunobla[illegible]= Plasm[illegible]cyt[illegible]d=Downey cell …



***Lymphocyte variant form***

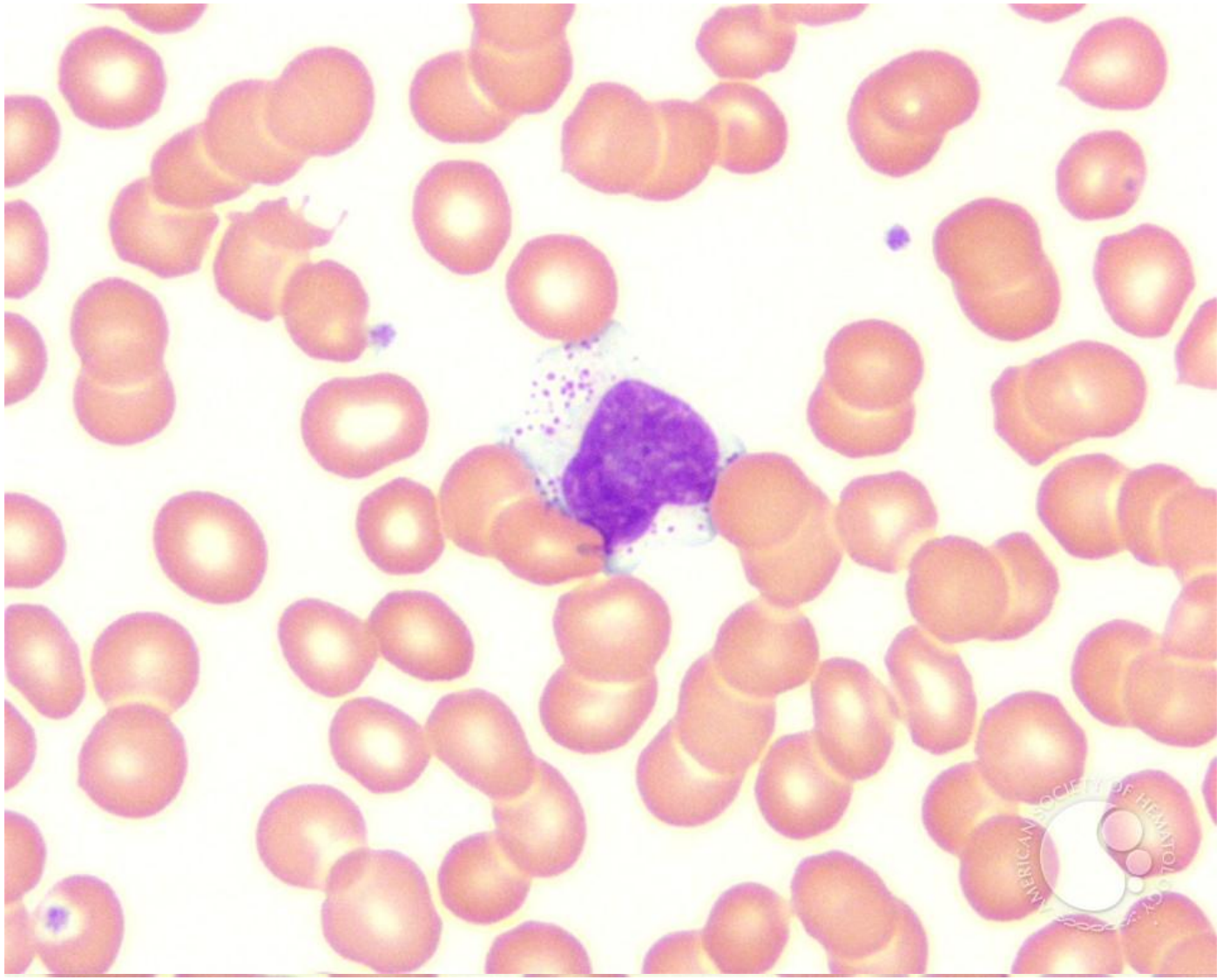
# MONOCYTES VS LYMPHOCYTES



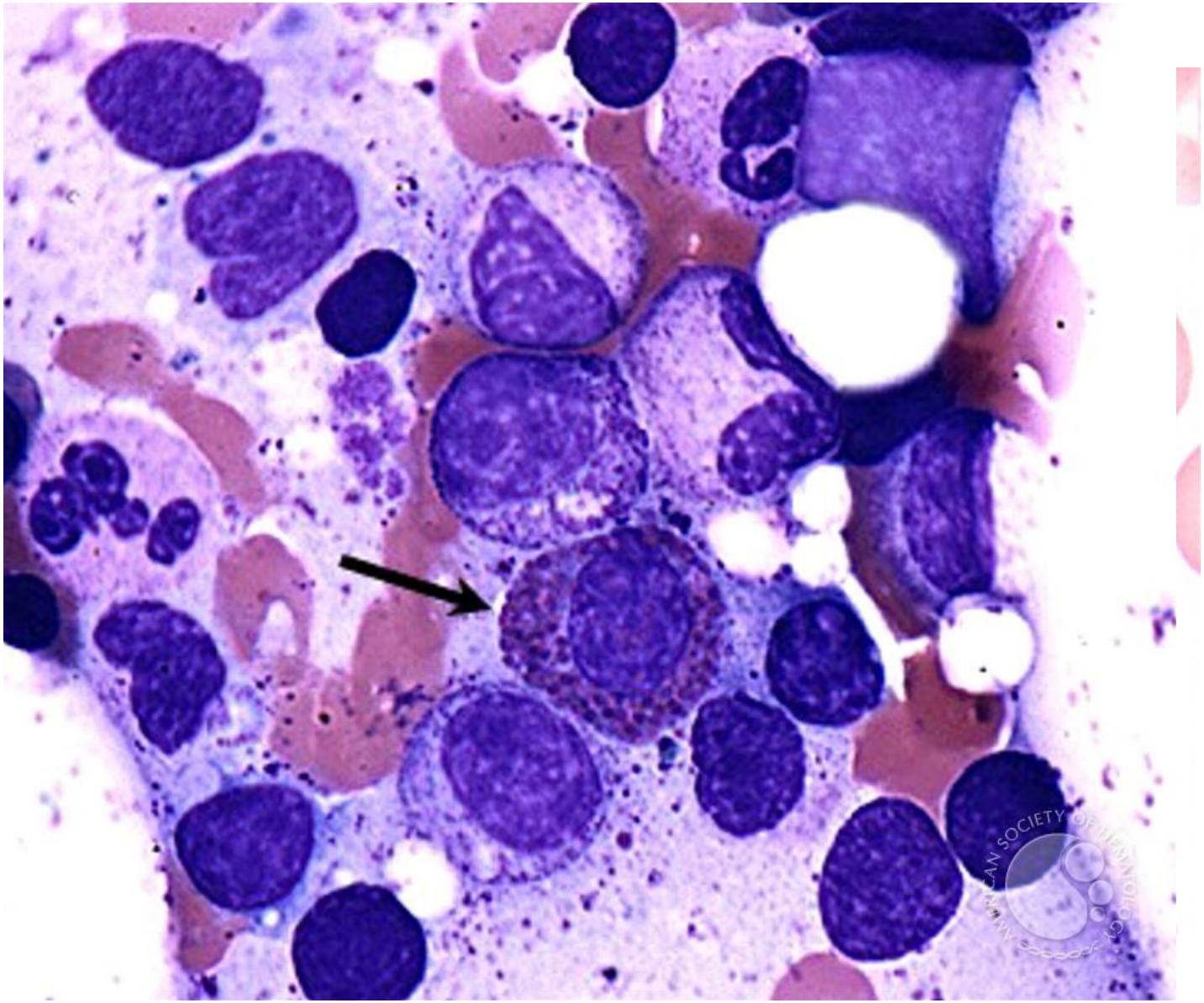


| | |
|---|---|
| •Cell size | monocyte generally larger |
| •Nuclear shape | amorphous, indented vs round-oval |
| •Nuclear color | light-staining vs dark-staining |
| •Chromatin | “clumped” vs fine lacy |
| •Cytoplasm color | light clear sky-blue vs gray-blue |

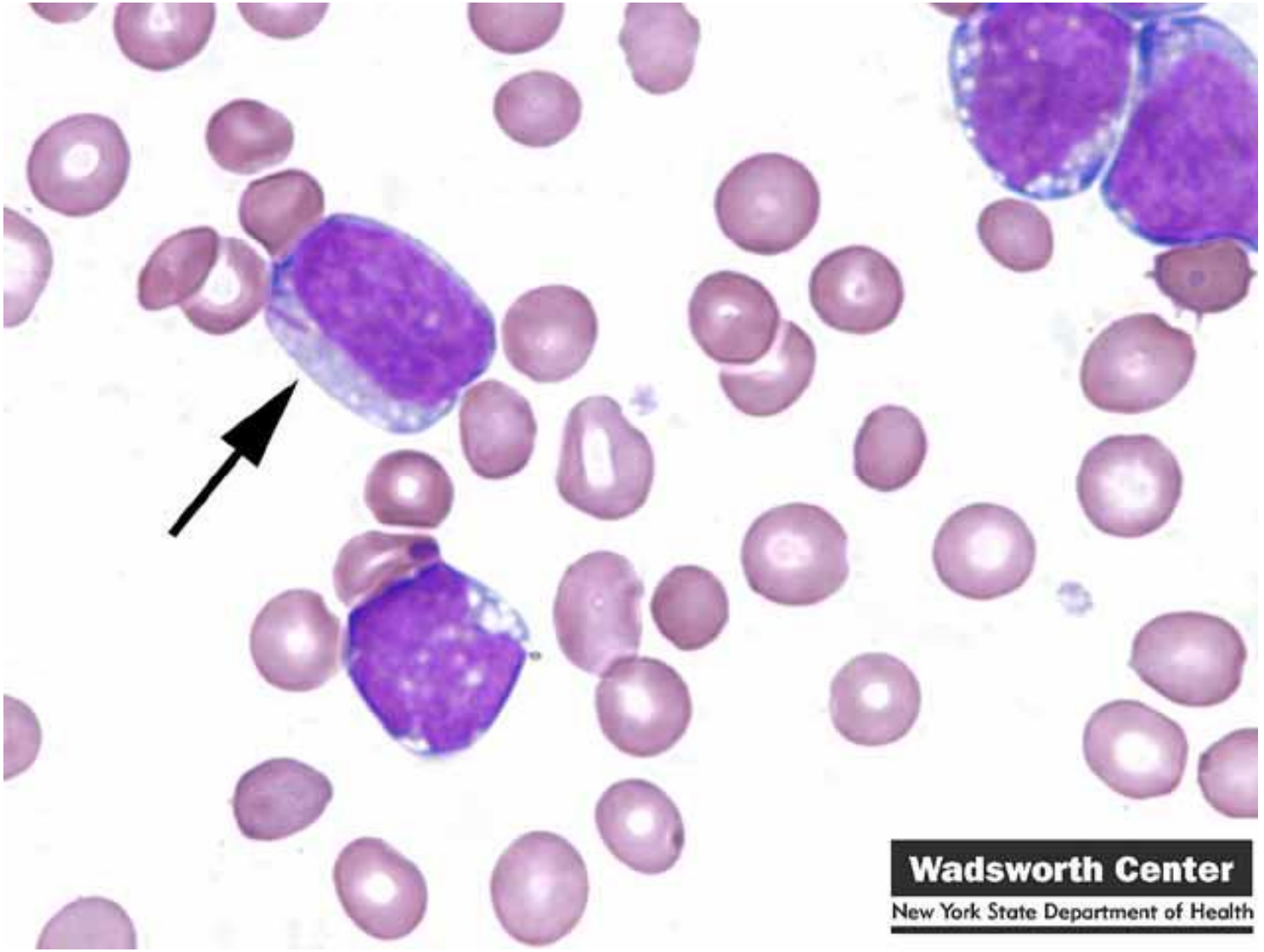
# LARGE GRANULAR LYMPHOCYTE



# WHAT IS YOUR OPINION?

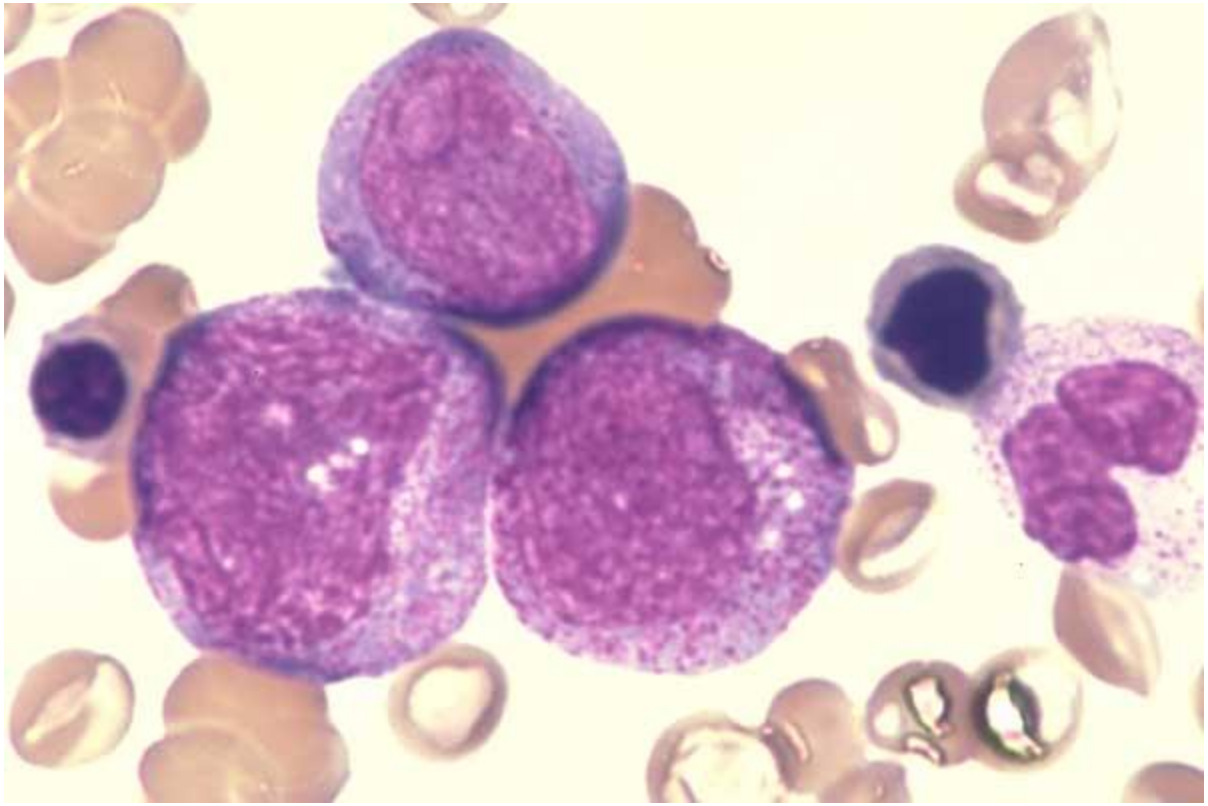


# WHAT IS THIS CELLS?




Graphic accessed http://www.wadsworth.org/chemheme/heme/glass/cytopix/slide036blastsauer.jpg, 1998.

# WHAT ARE THESE CELLS?



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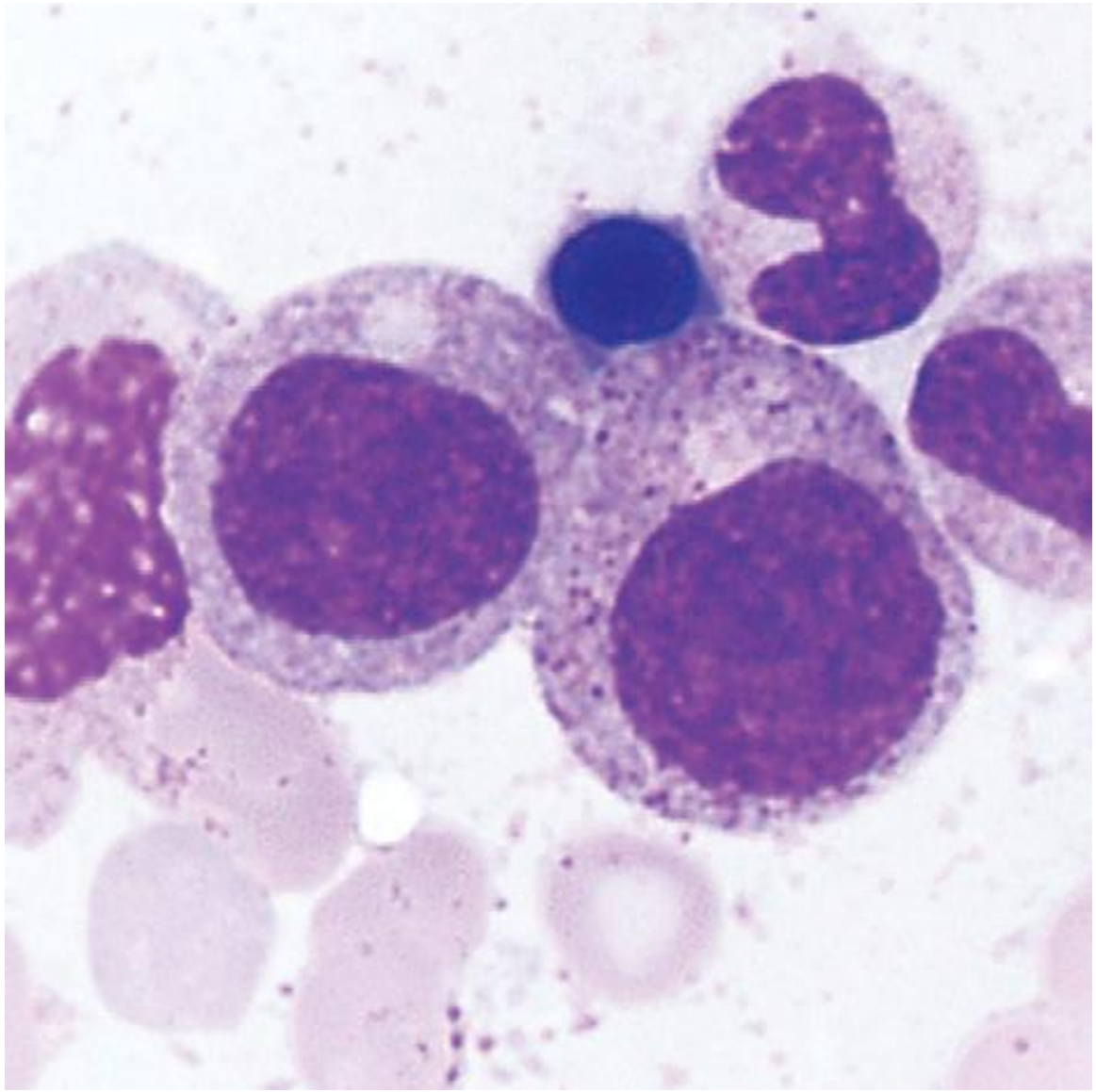

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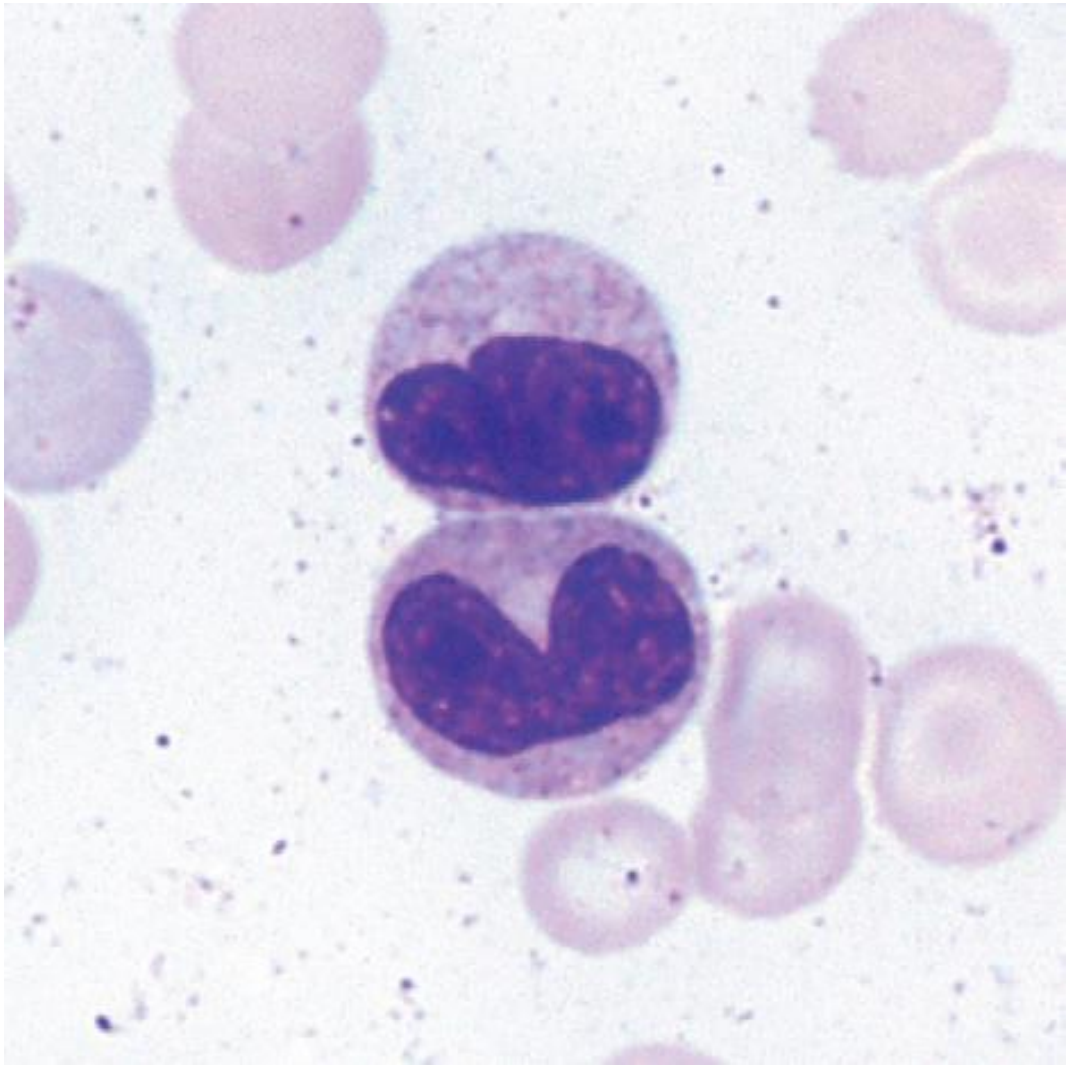

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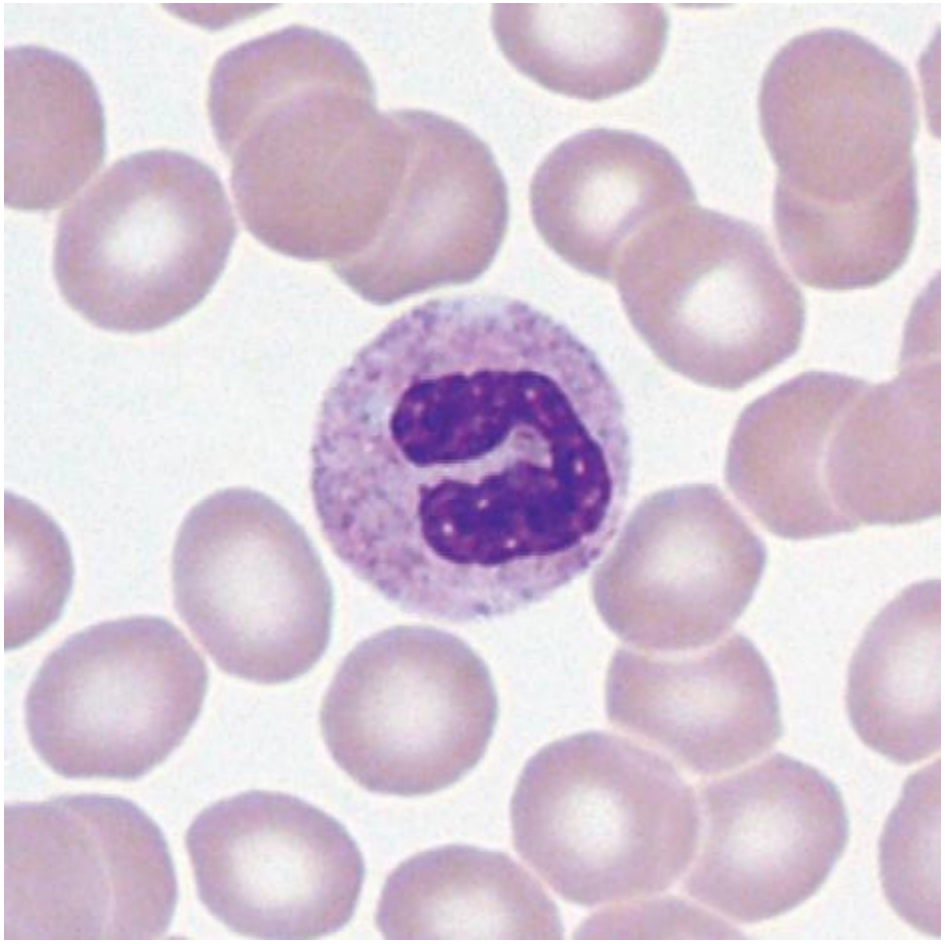

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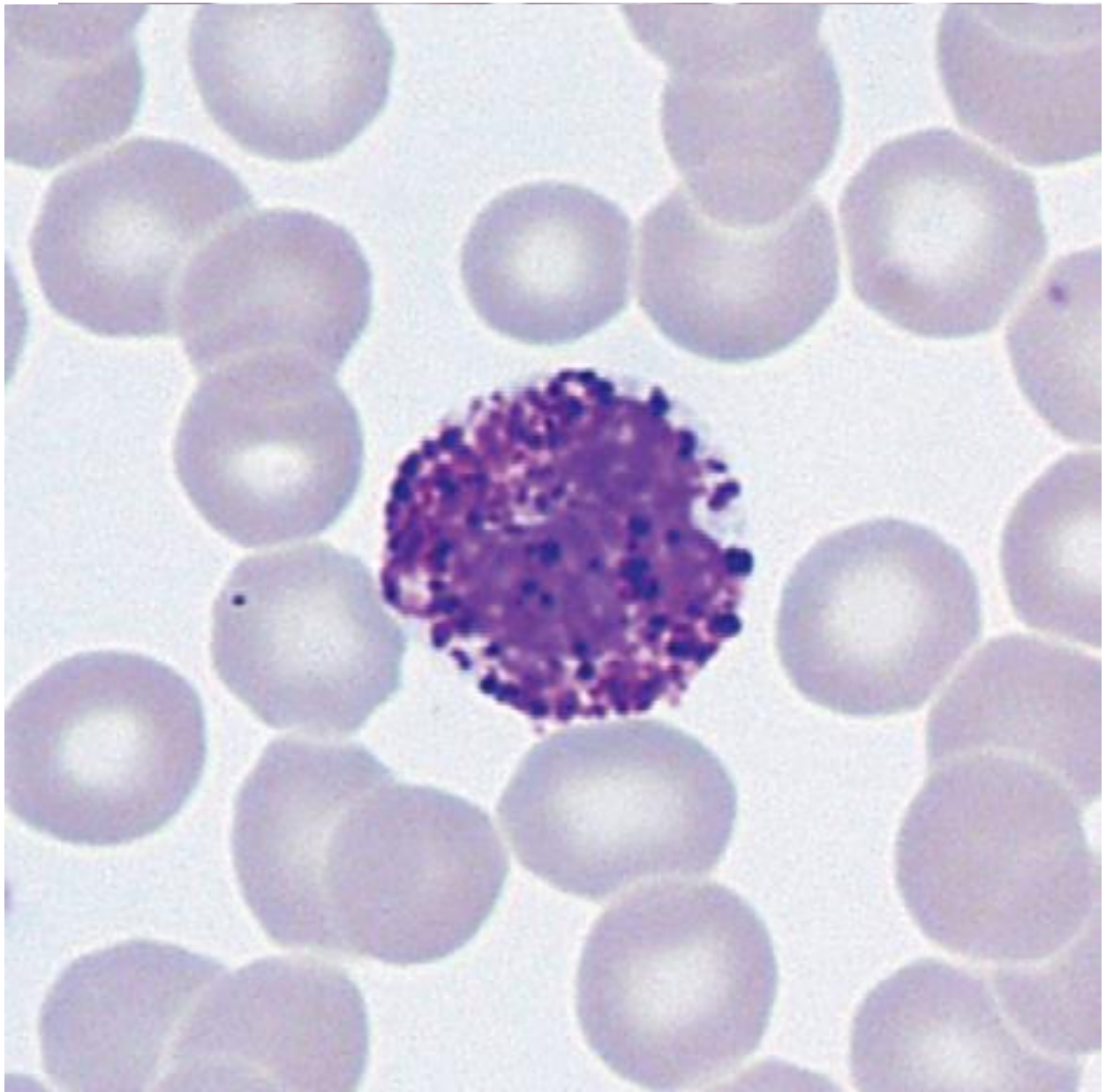
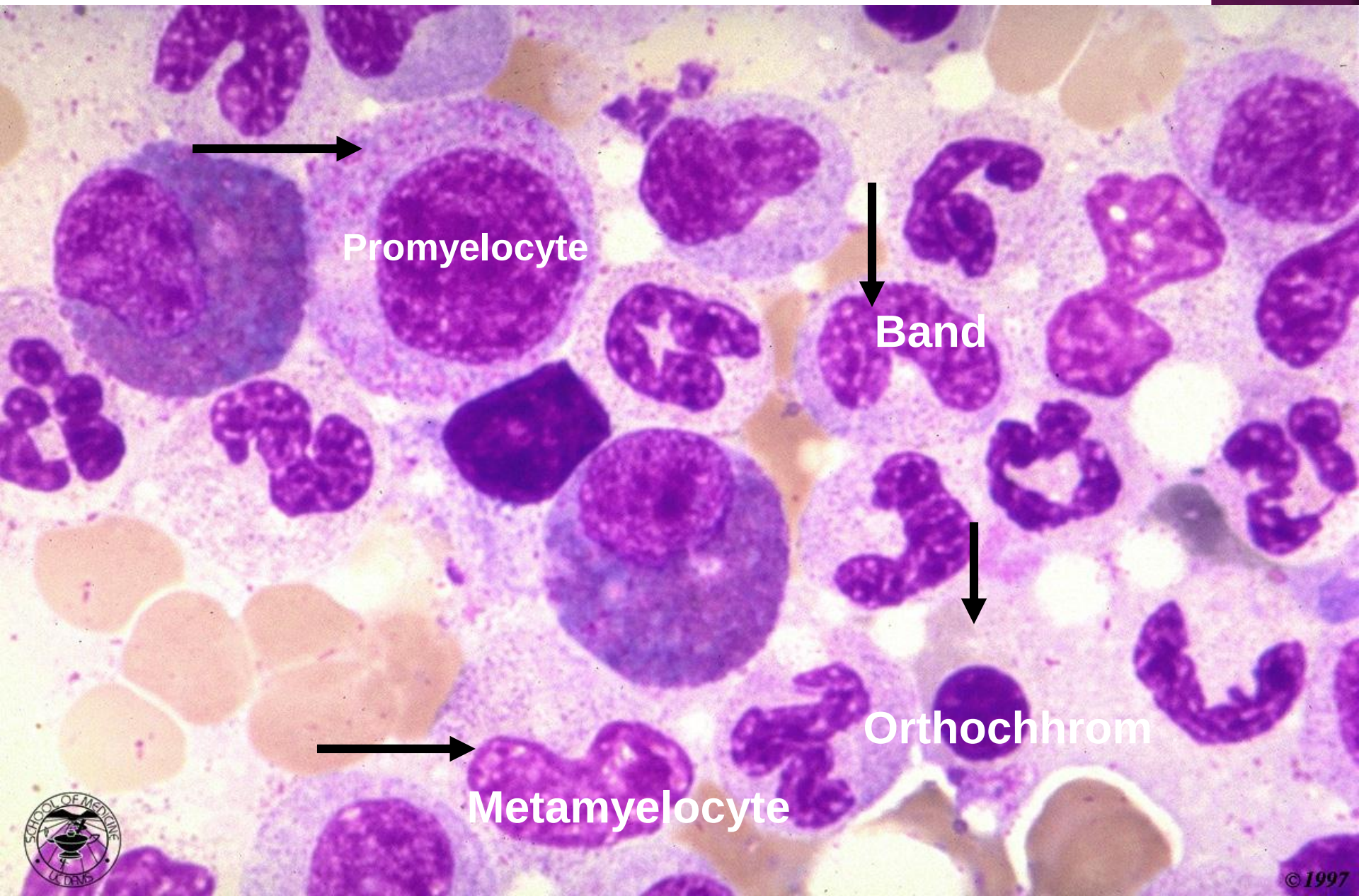


# NAME THE CELLS



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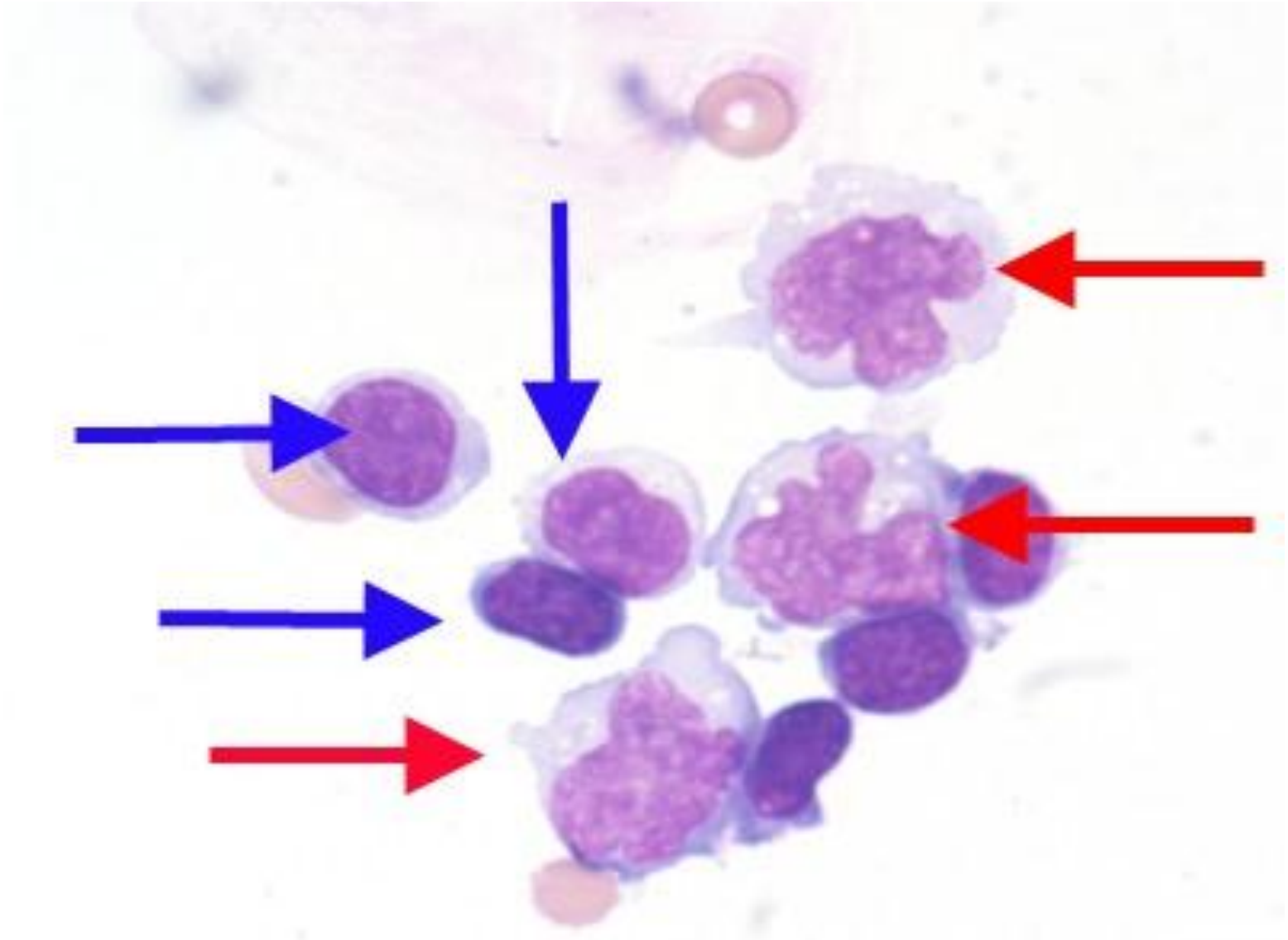
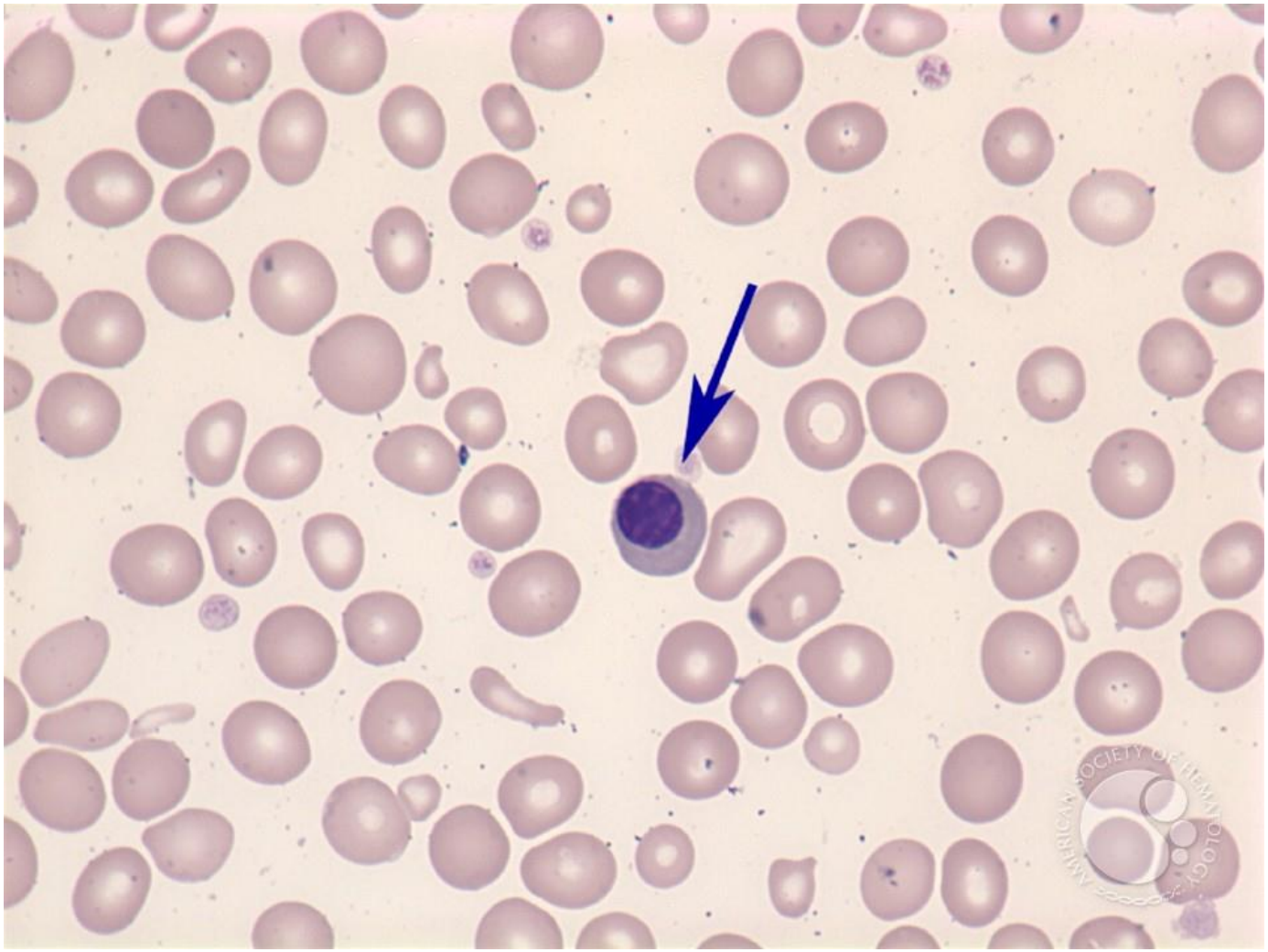


# WHAT IS YOUR OPINION?

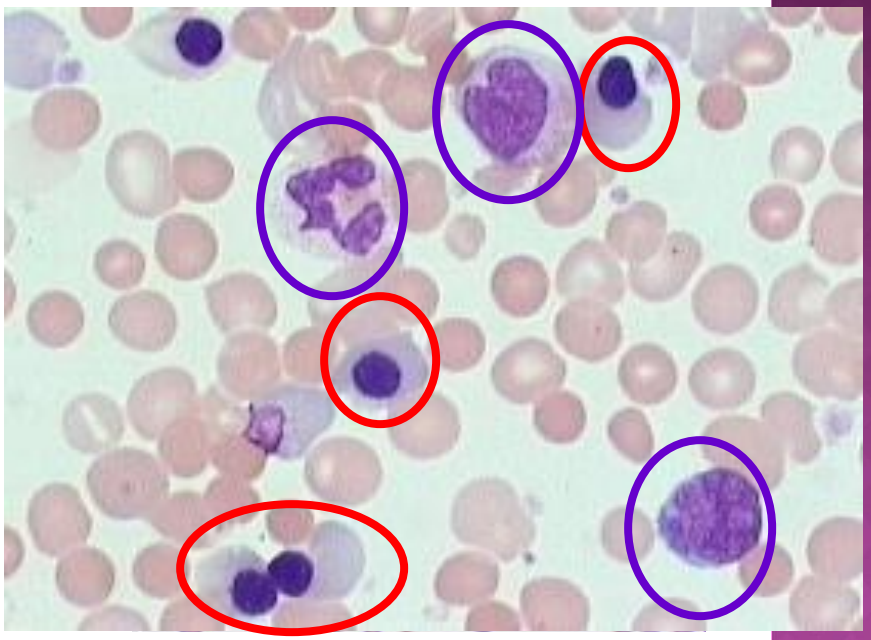


# LEUKOCYTE DISORDERS
## NONNEOPLASTIC DISORDERS

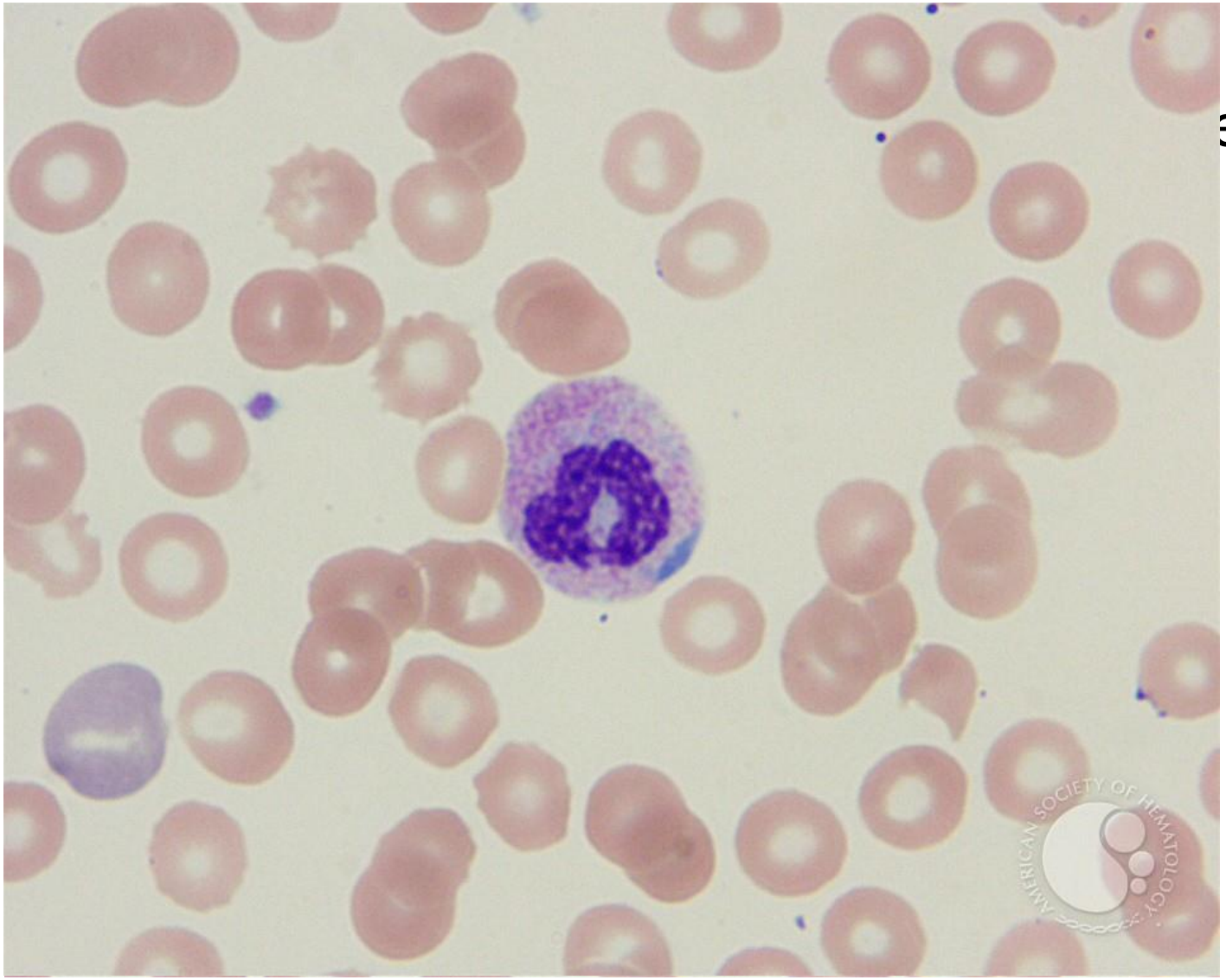
- WBC with acquired non-neoplastic alterations
- WBC with inherited non-neoplastic alterations

# DEFINITIONS WHITE CELL NUMBERS

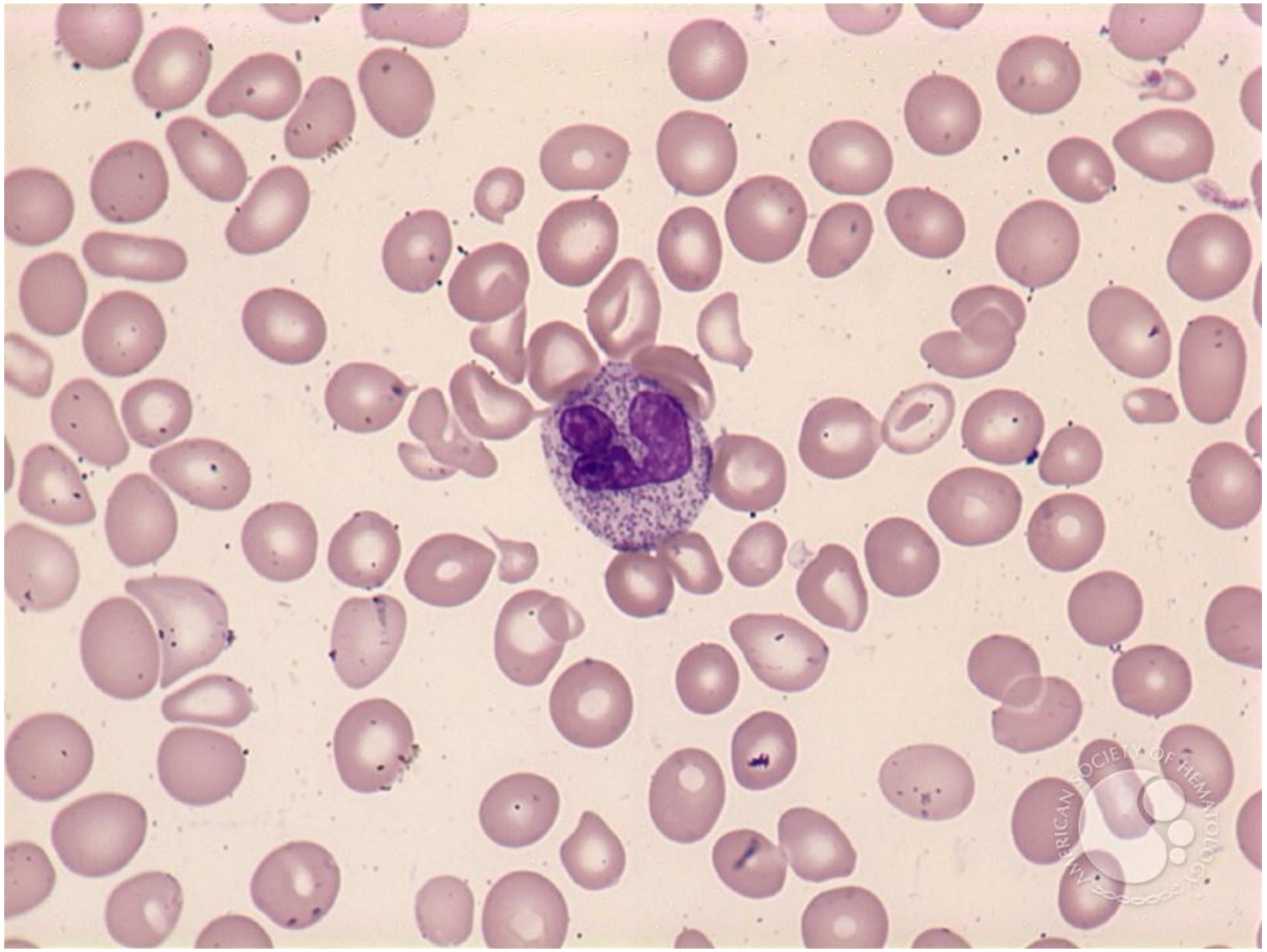
- ***Leukocytosis:*** increase in the numbers of circulating white cells
  - >12,000/uL
- ***Leukopenia:*** decrease in the numbers of circulating white cells
  - < 4,000/uL
- ***Left Shift*** – increased circulating numbers of immature neutrophils
- ***Leukoerythroblastic Reaction*** – leukocytosis with a left shift accompanied by nucleated red cells: seen in malignancy.
- ***Leukemoid Reaction*** – benign excessive leukocytosis accompanied by an exaggerated neutrophilia and a left shift in response to an infection; the WBC > 50 x $10^9$/L



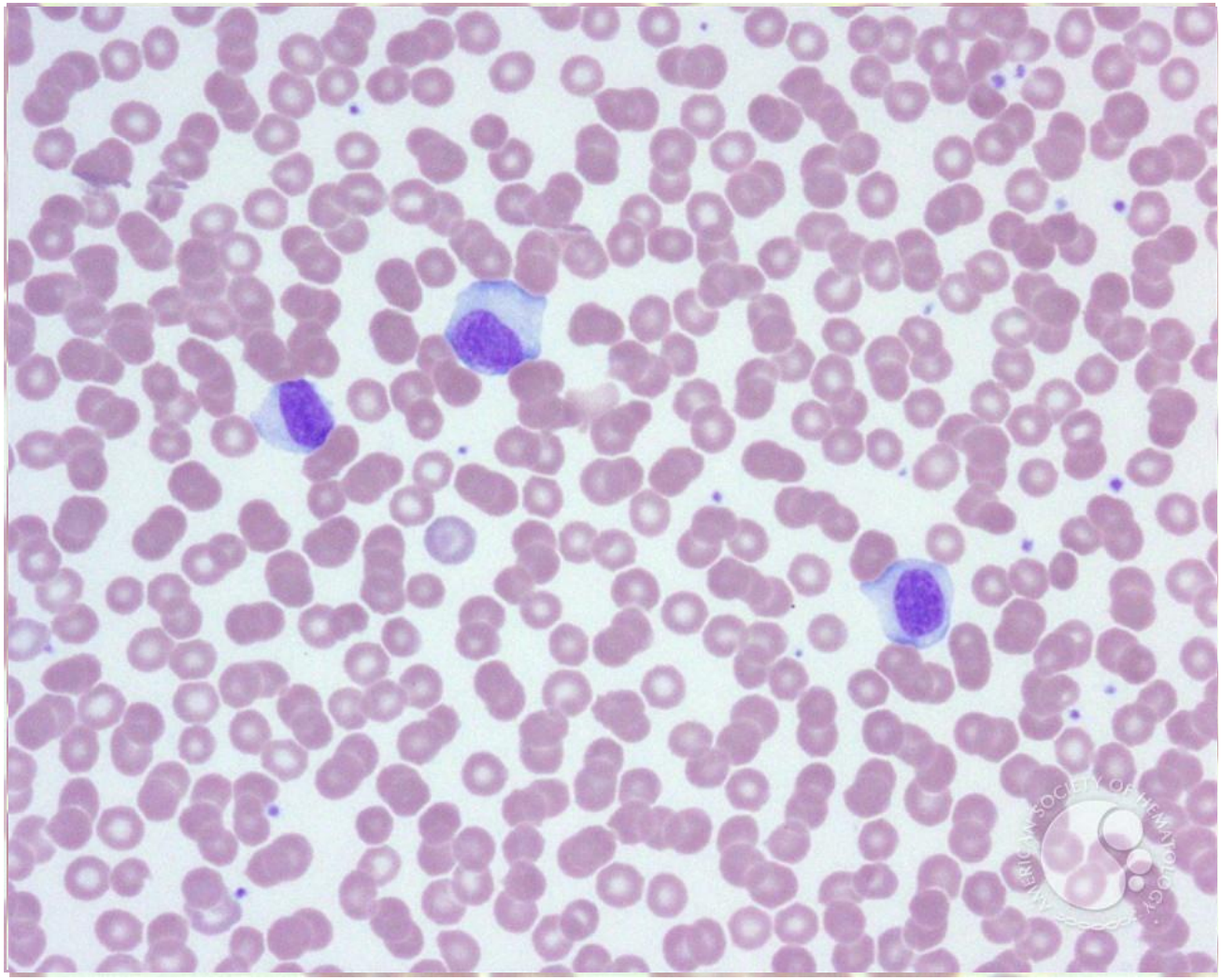
# DOHLE INCLUSION BODIES

- 

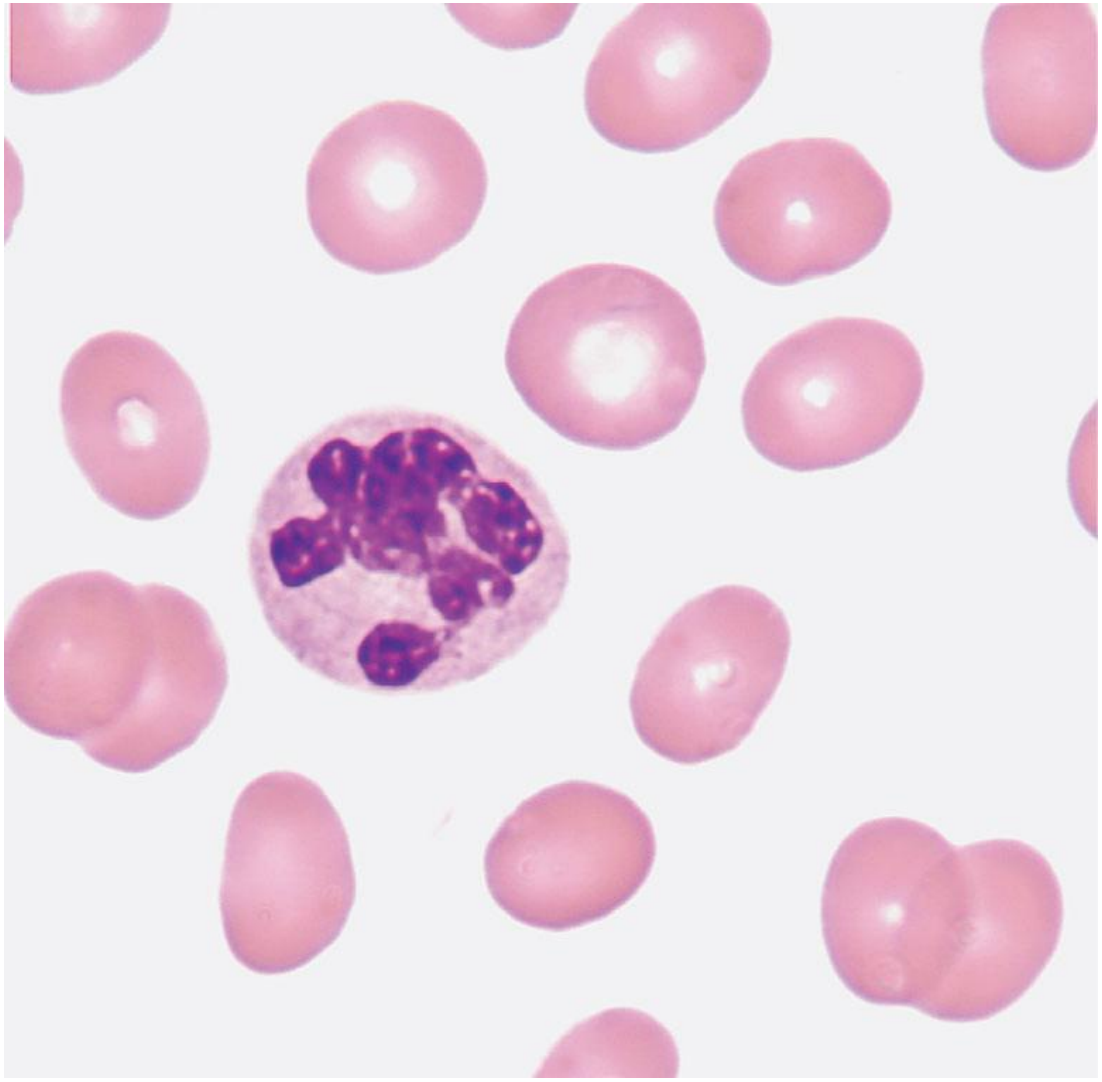
# TOXIC GRANULATION



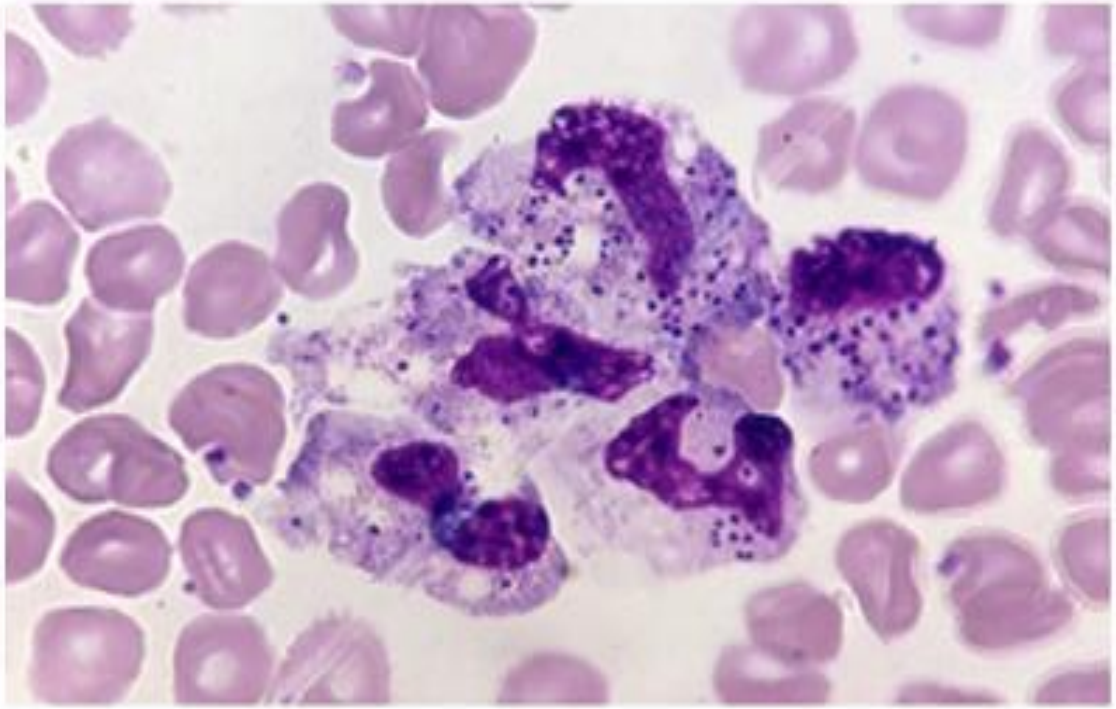
# LYMPHOCYTE VARIANT FORM IN INFECTIOUS MONONUCLEOSIS OR CMV



# HYPERSEGMENTATION

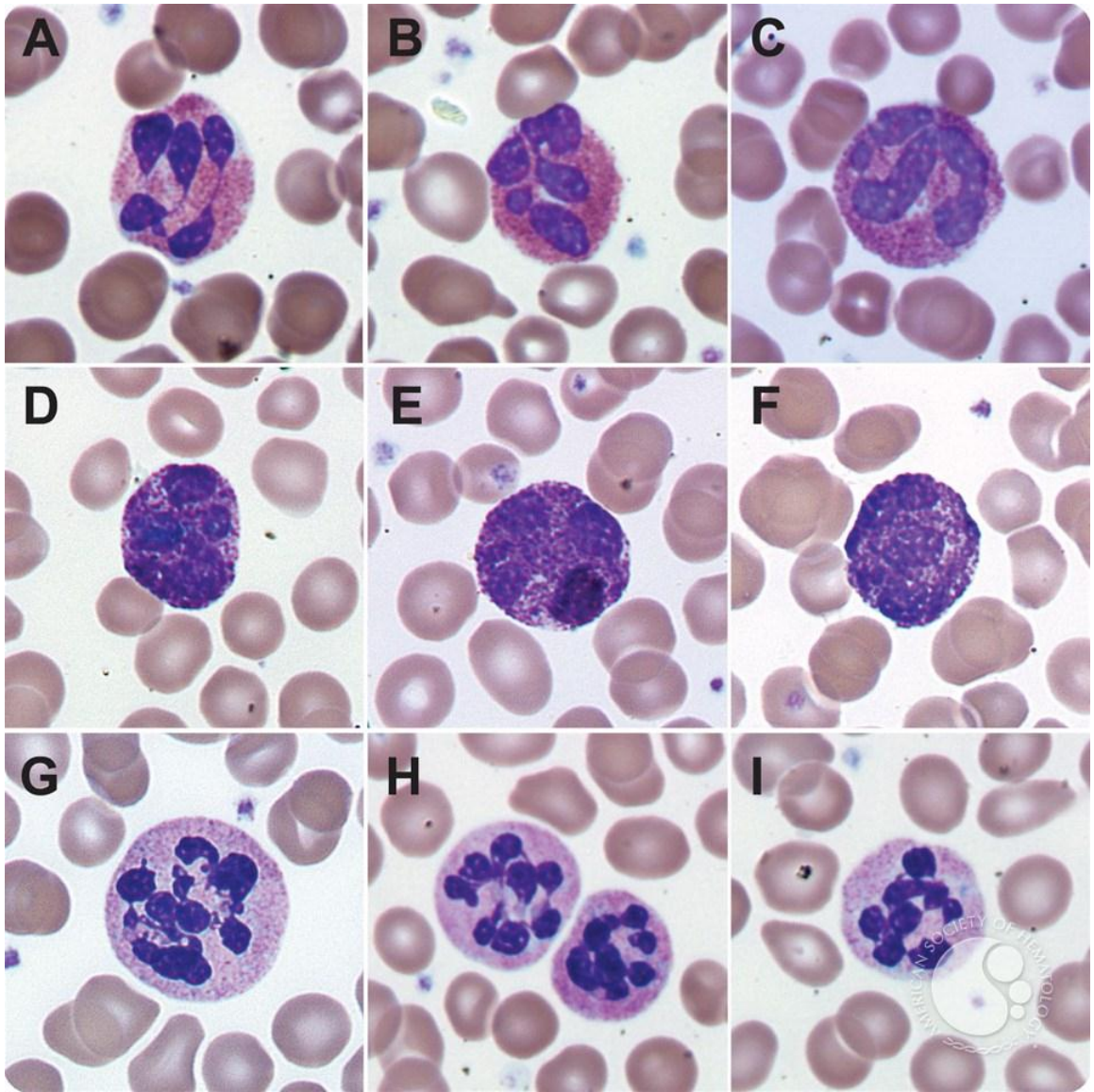


. NEUTROPHILS WITH INGESTED BACTERIA

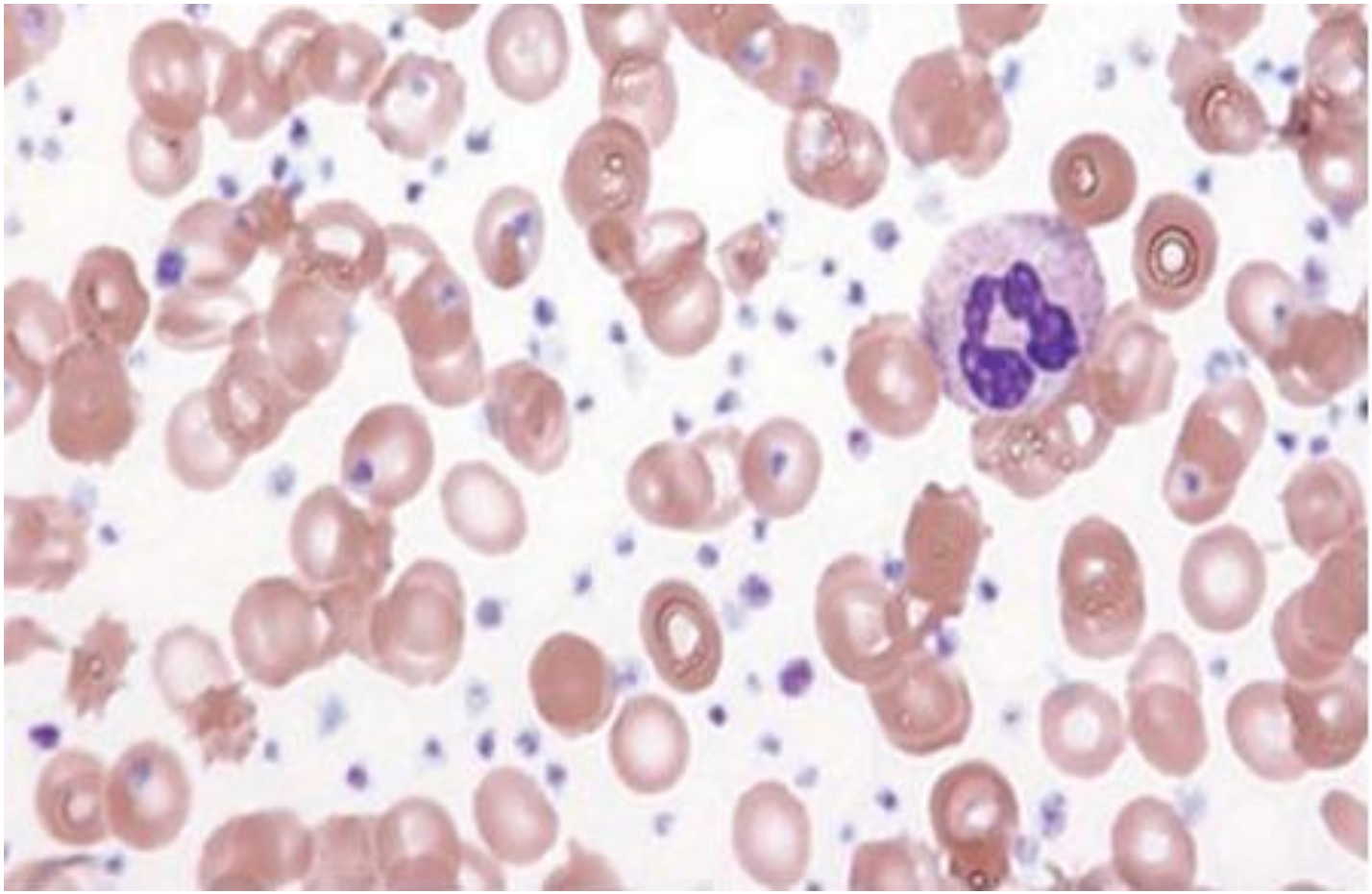


باکتری های گرام مثبت یا منفی در رنگ آمیزی رایت یا گیمسا به رنگ آبی بنفش در می آید.

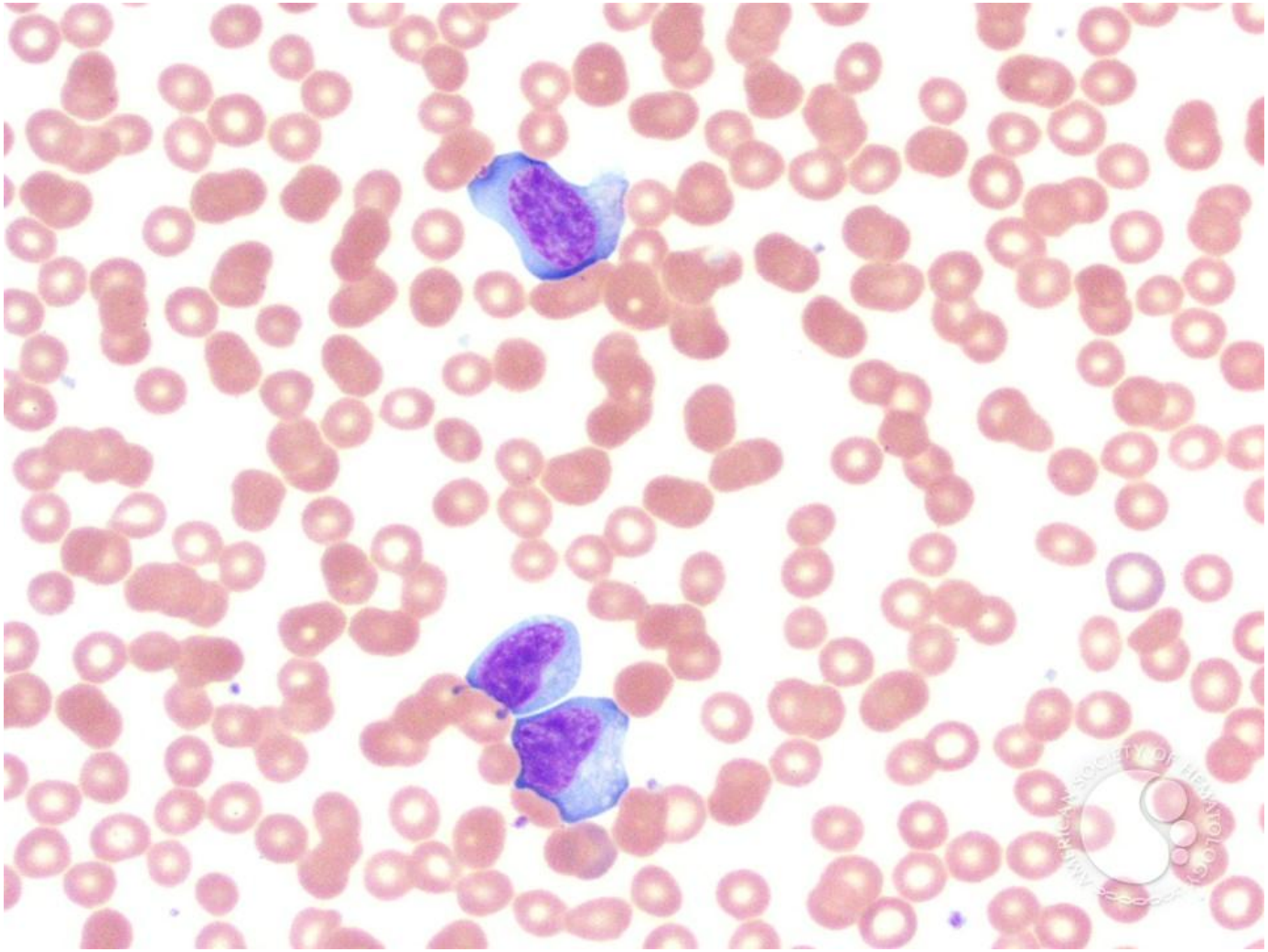
# NUCLEAR HYPERSEGMENTATION OF NEUTROPHILS, EOSINOPHILS, AND BASOPHILS DUE TO HYDROXYCARBAMIDE (HYDROXYUREA)



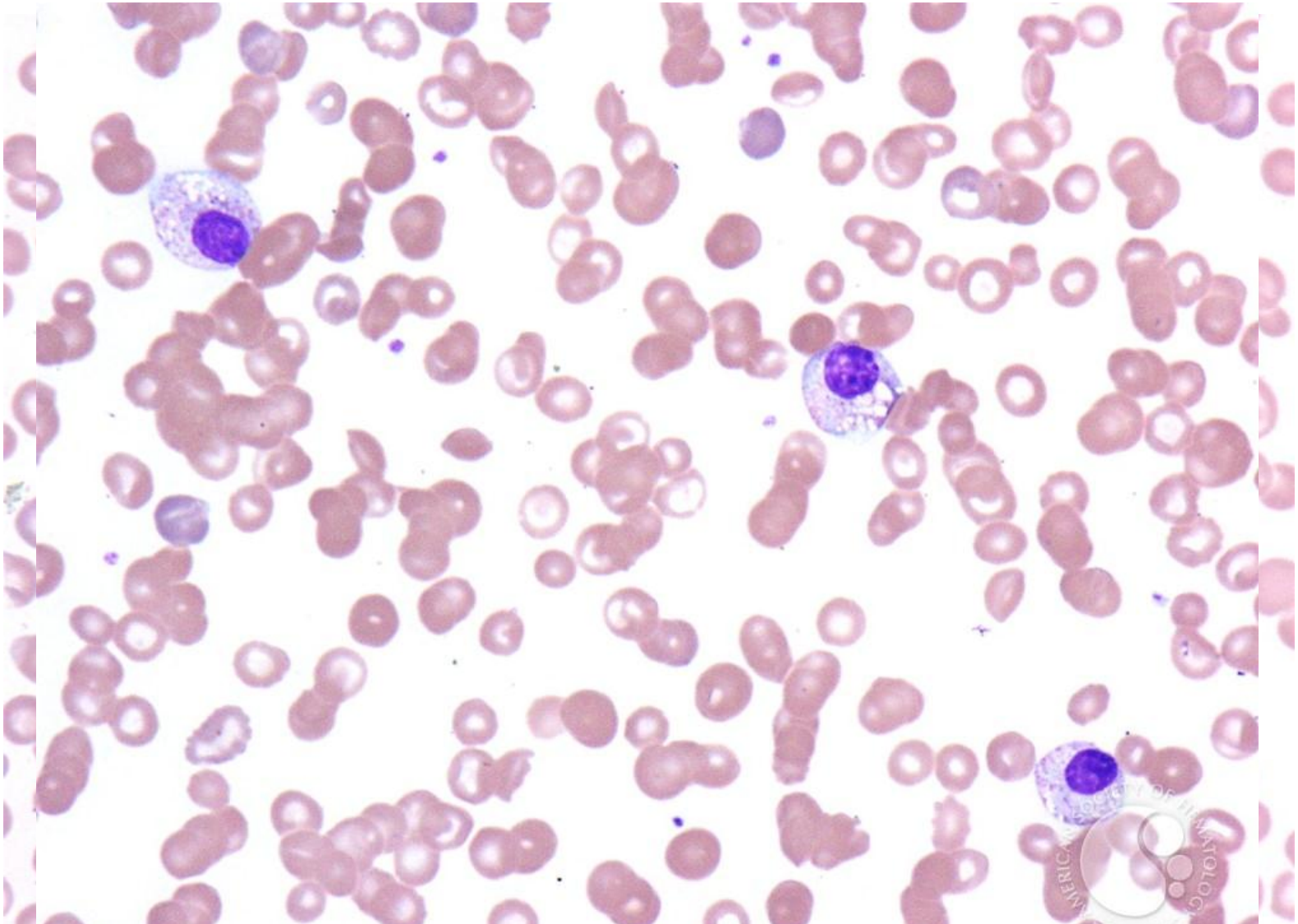
# PLATELATE IN INFECTION



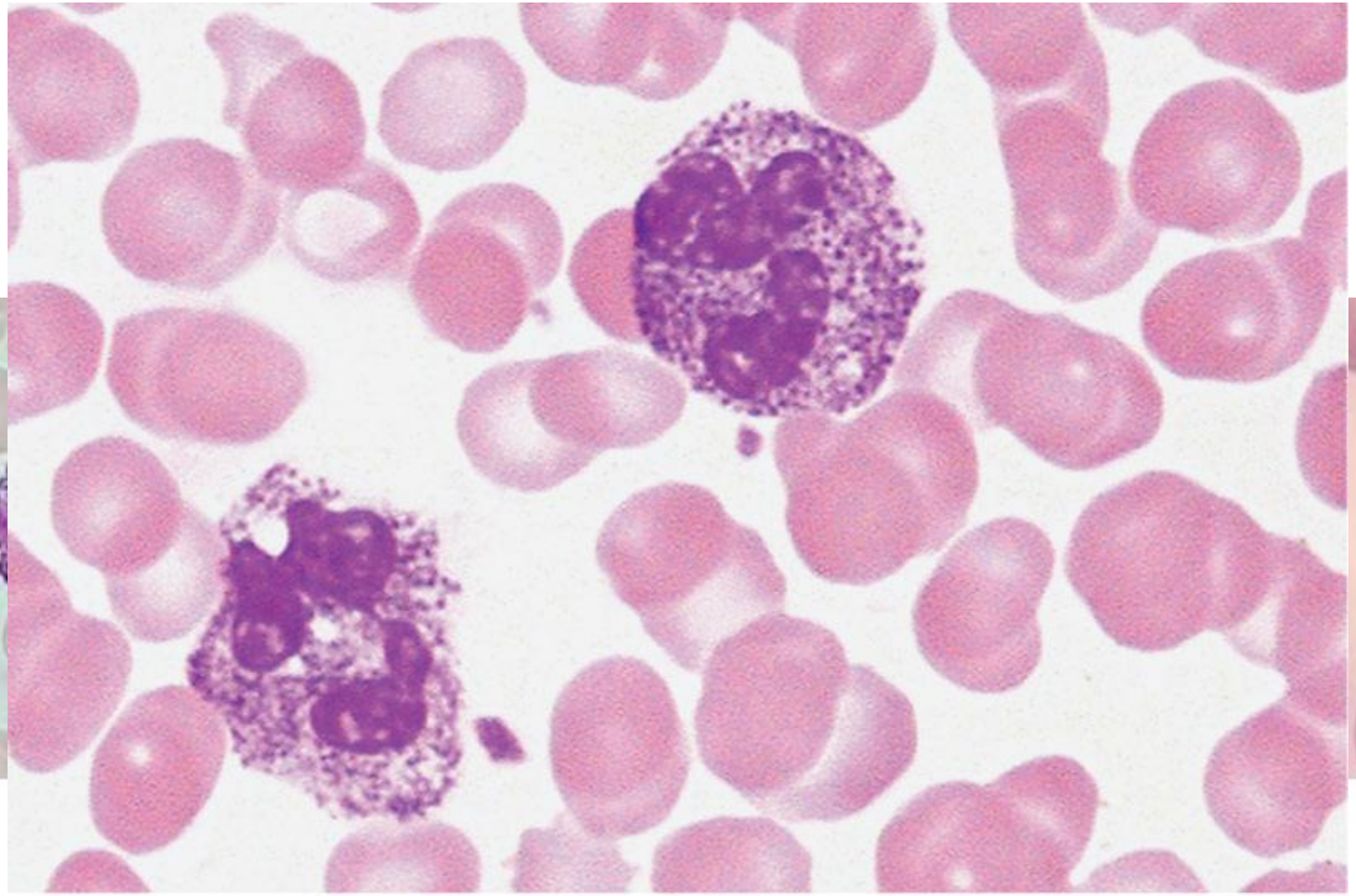
# WHAT IS YOUR INTERPRETATION?



# PELGER-HUET ANOMOLY



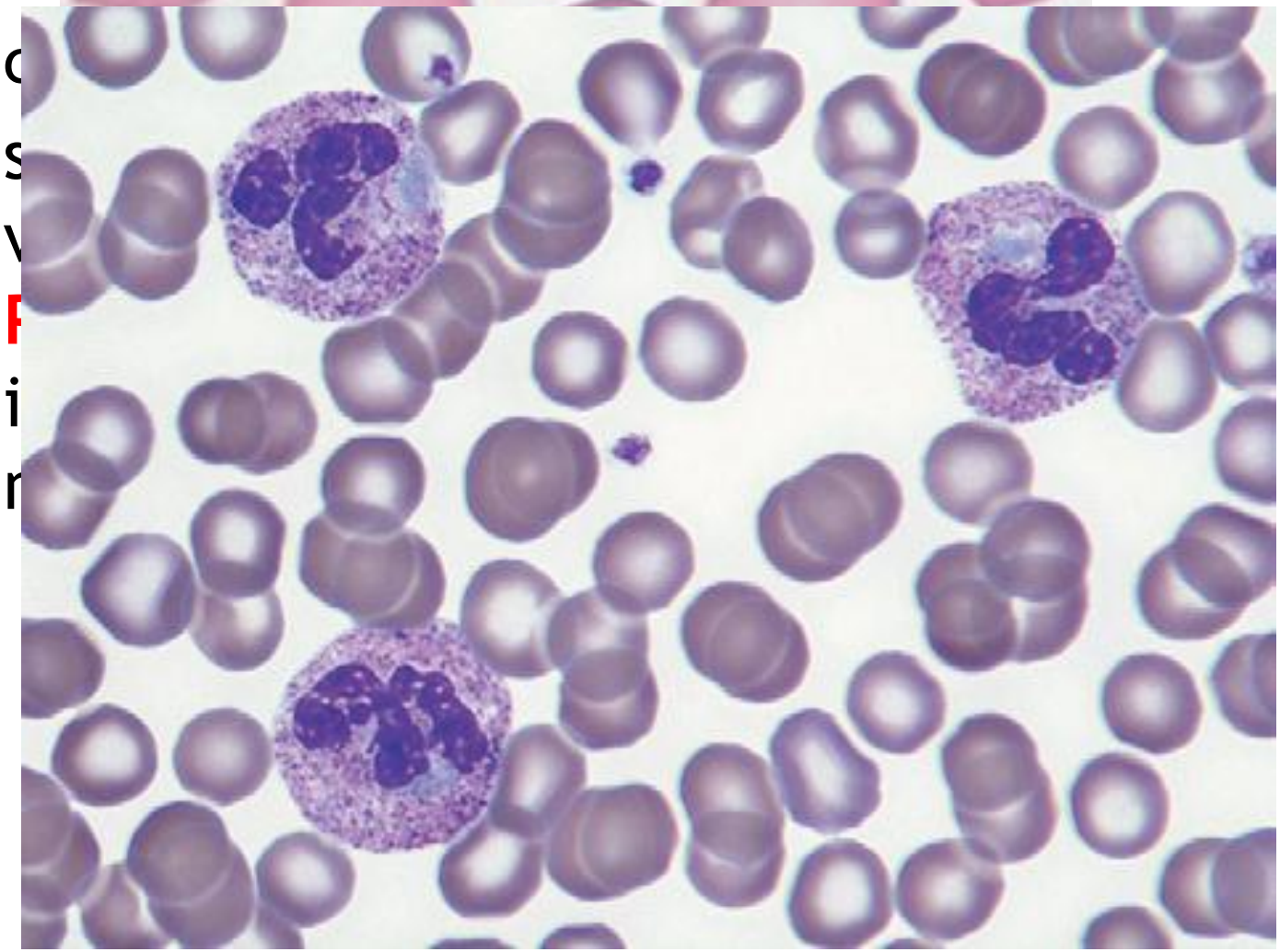
# ALDER-REILLY ANOMALY



(Courtesy of Dennis P. O'Malley, M.D., US Labs; Irvine, Calif.)

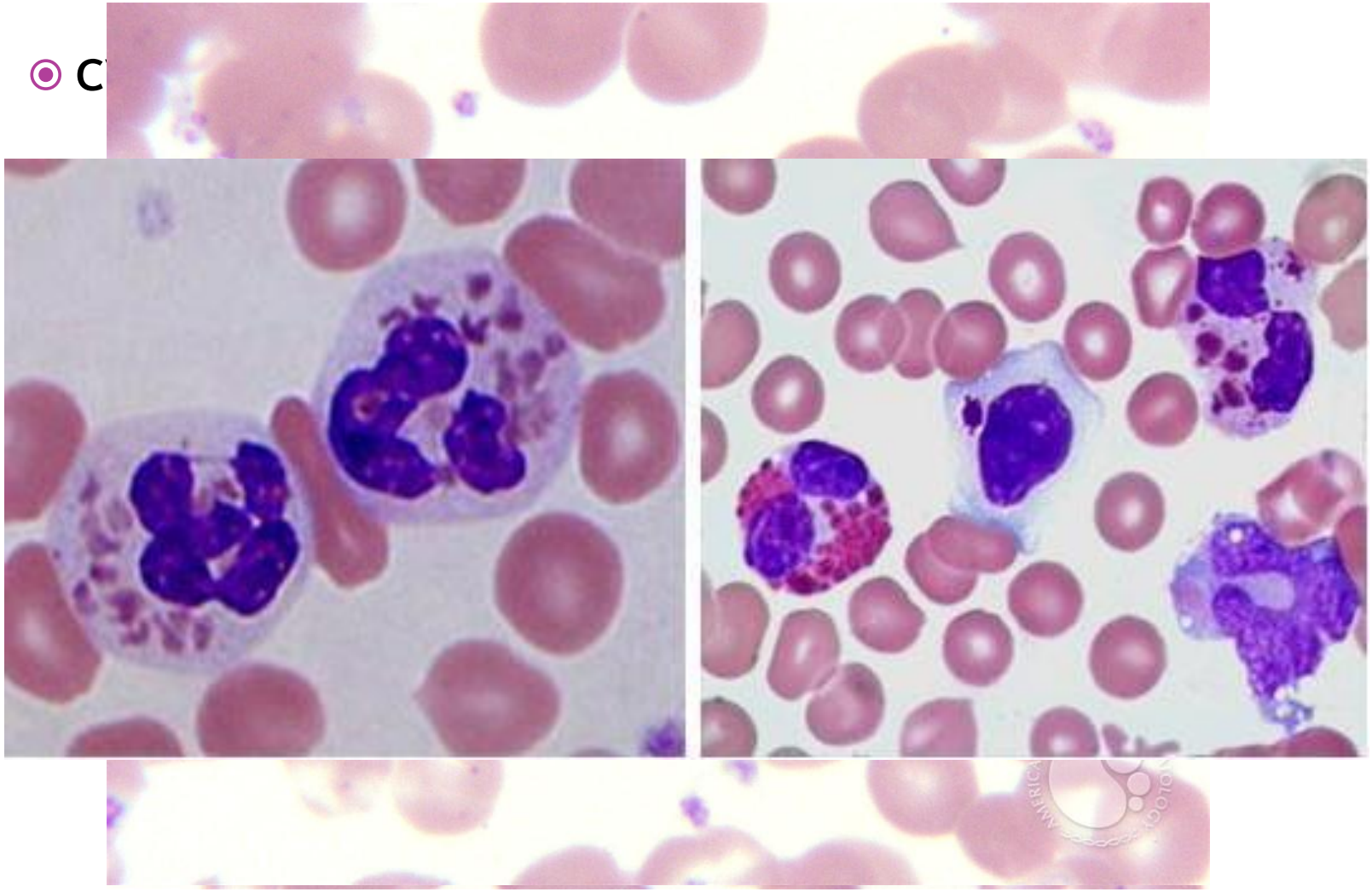
# MAY-HEGGLIN ANOMALY

- C
- S
  V
  P
  i
  r



# CHEDIAK-HIGASHI SYNDROME

- C



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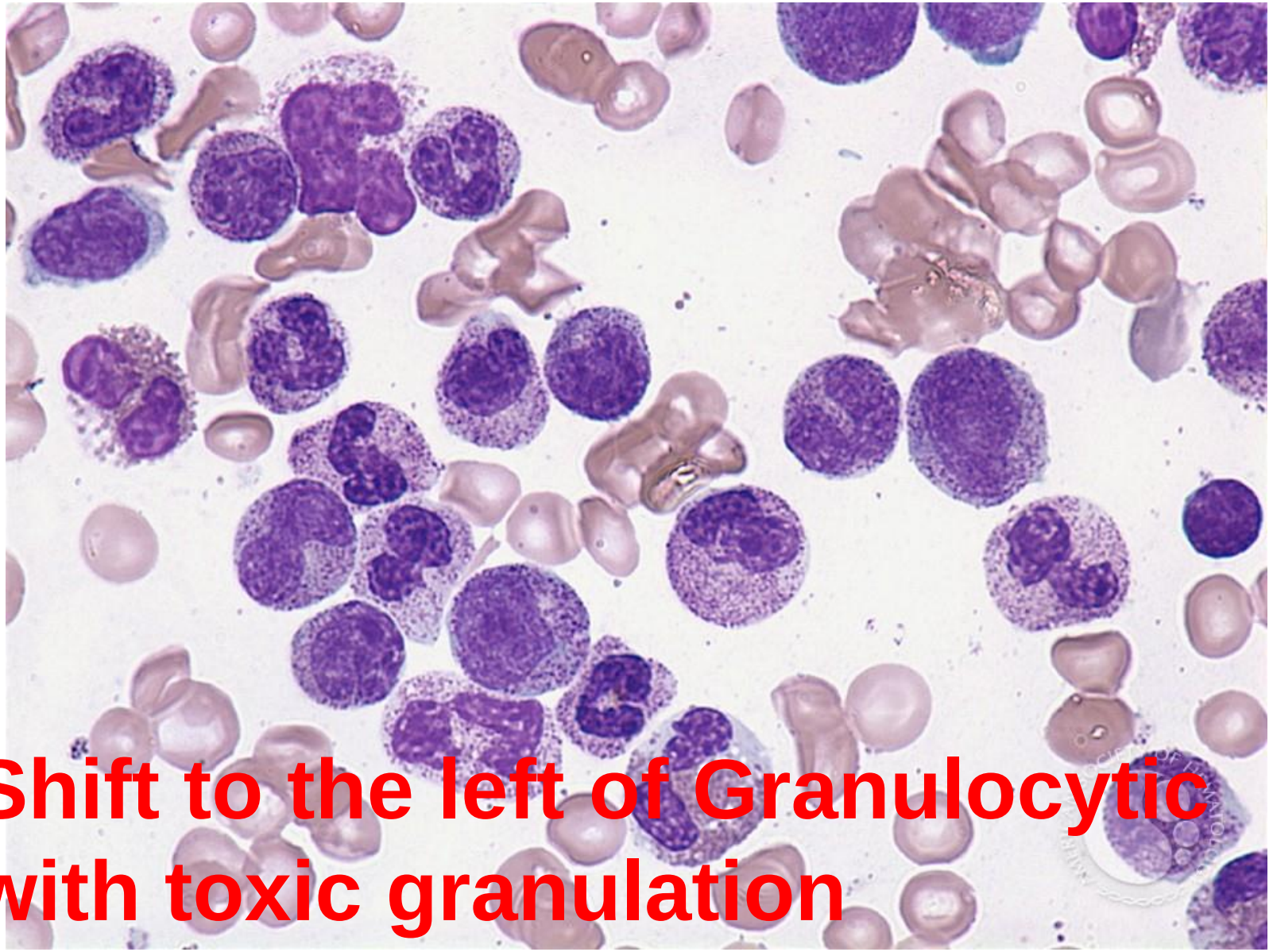
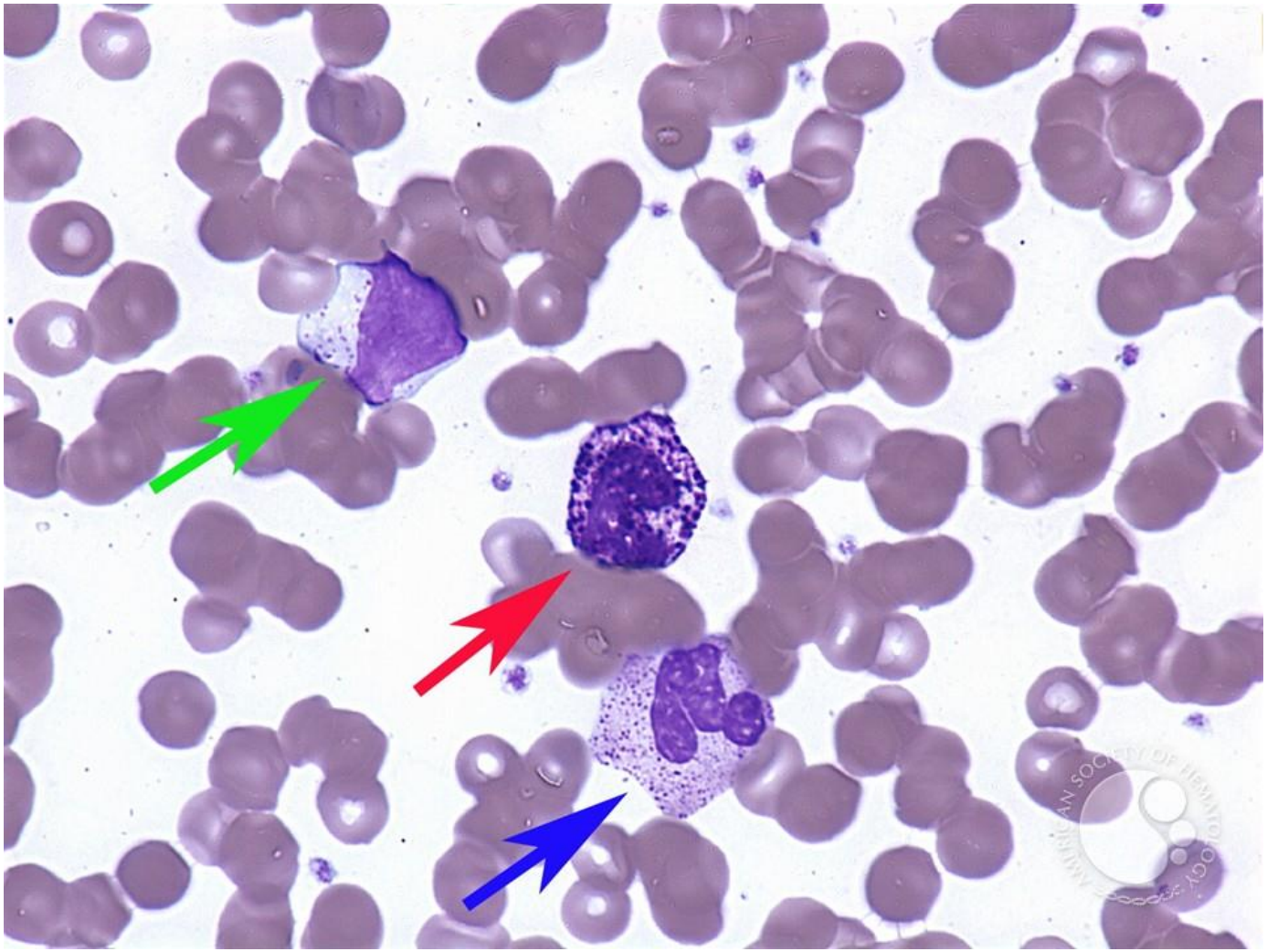
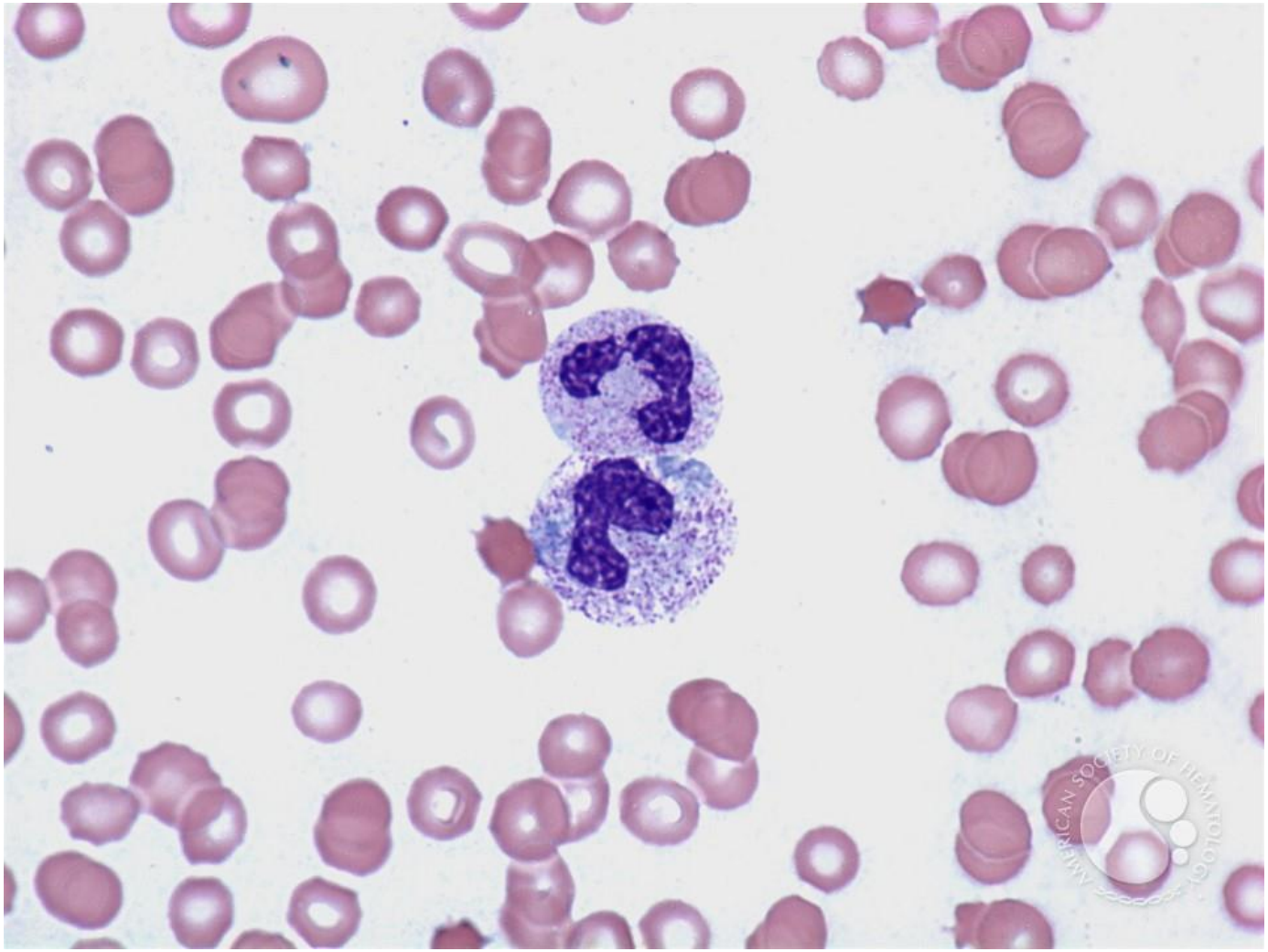


**Shift to the left of Granulocytic with toxic granulation**

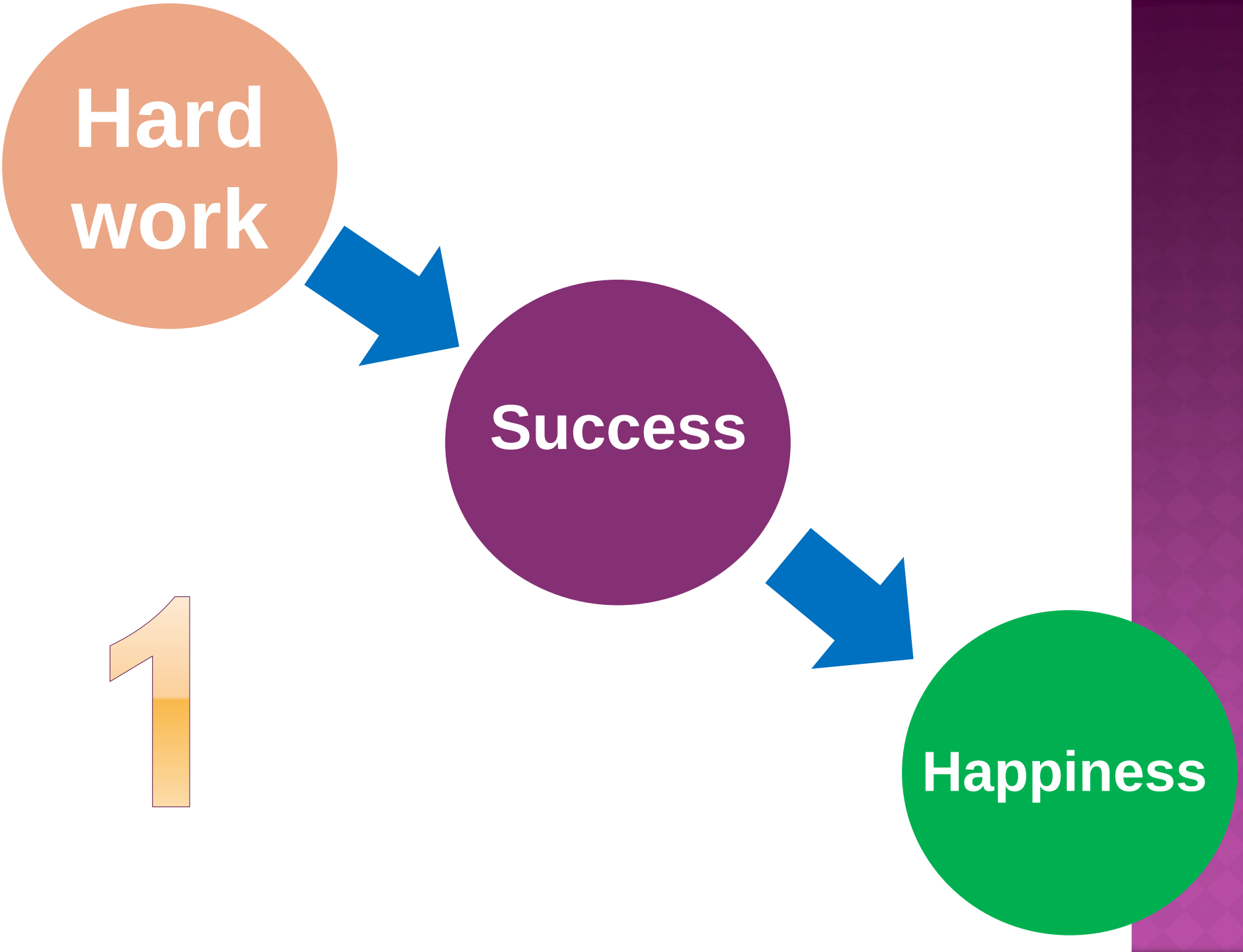
# NAME THESE CELLS?

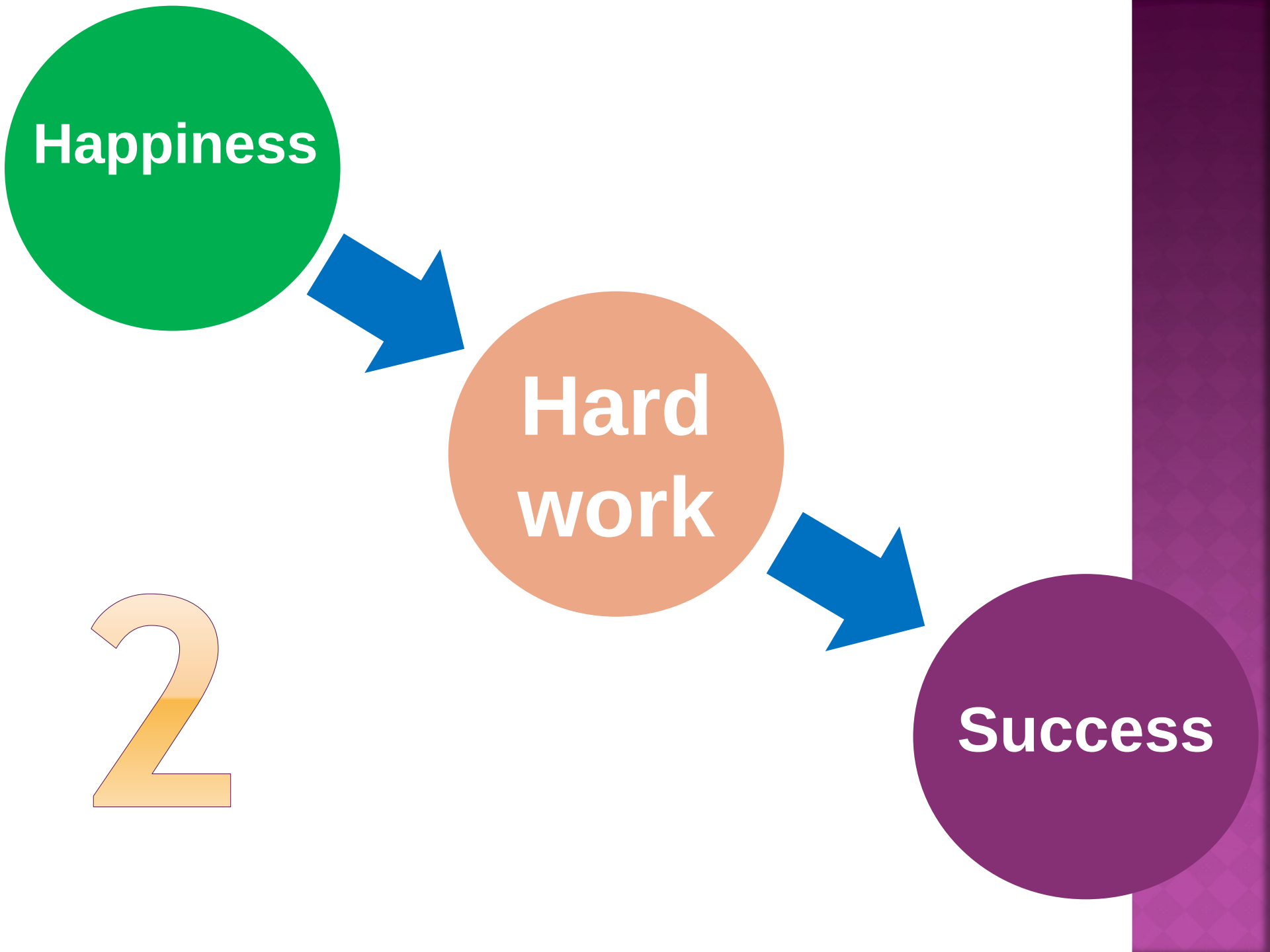


# WHAT IS YOUR INTERPRETATION?



# WHAT IS YOUR INTERPRETATION?

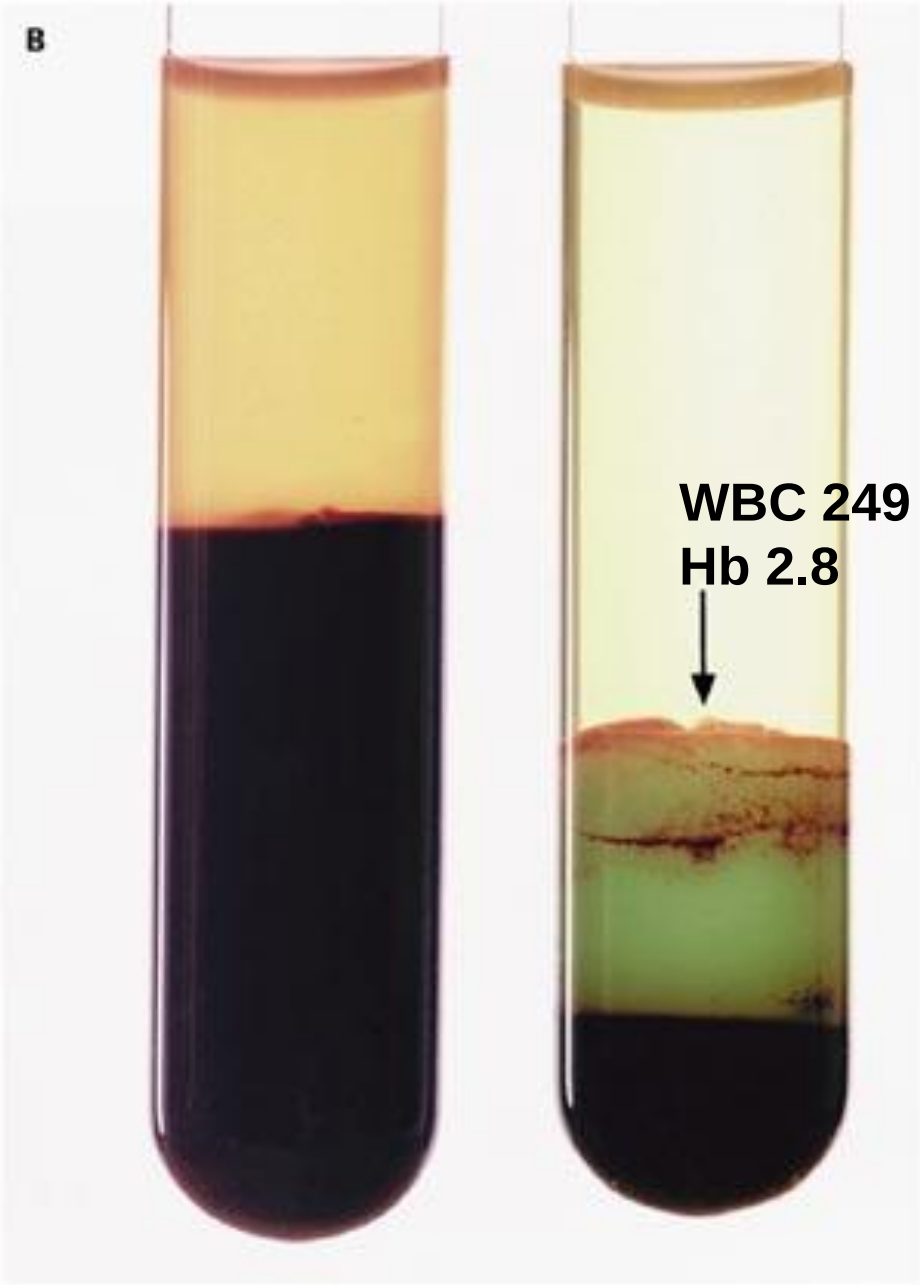

Hard work
Success
Happiness
1


Happiness
Hard work
Success
2

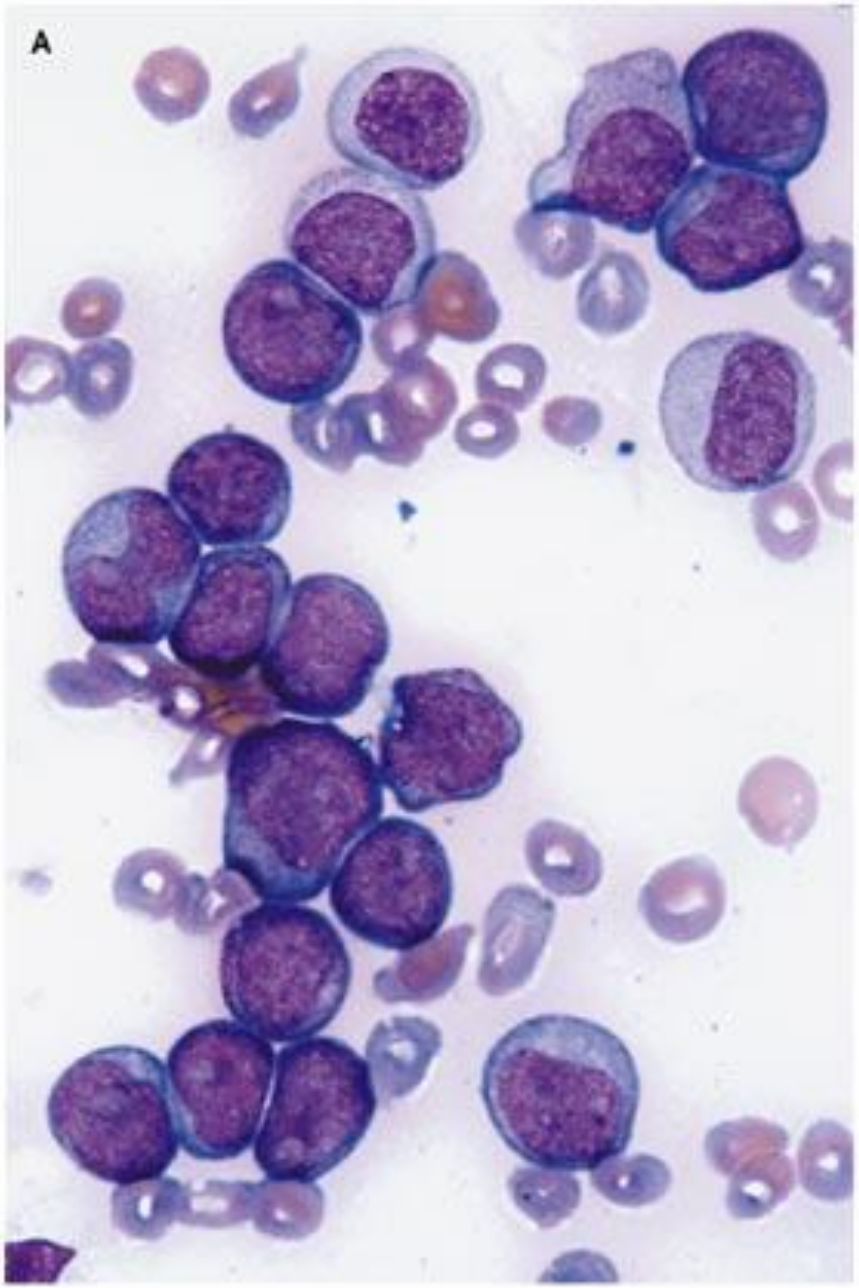
# ACUTE MYELOID LEUKEMIA (AML)
# ACUTE LYMPHOBLASTIC LEUKEMIA (ALL)

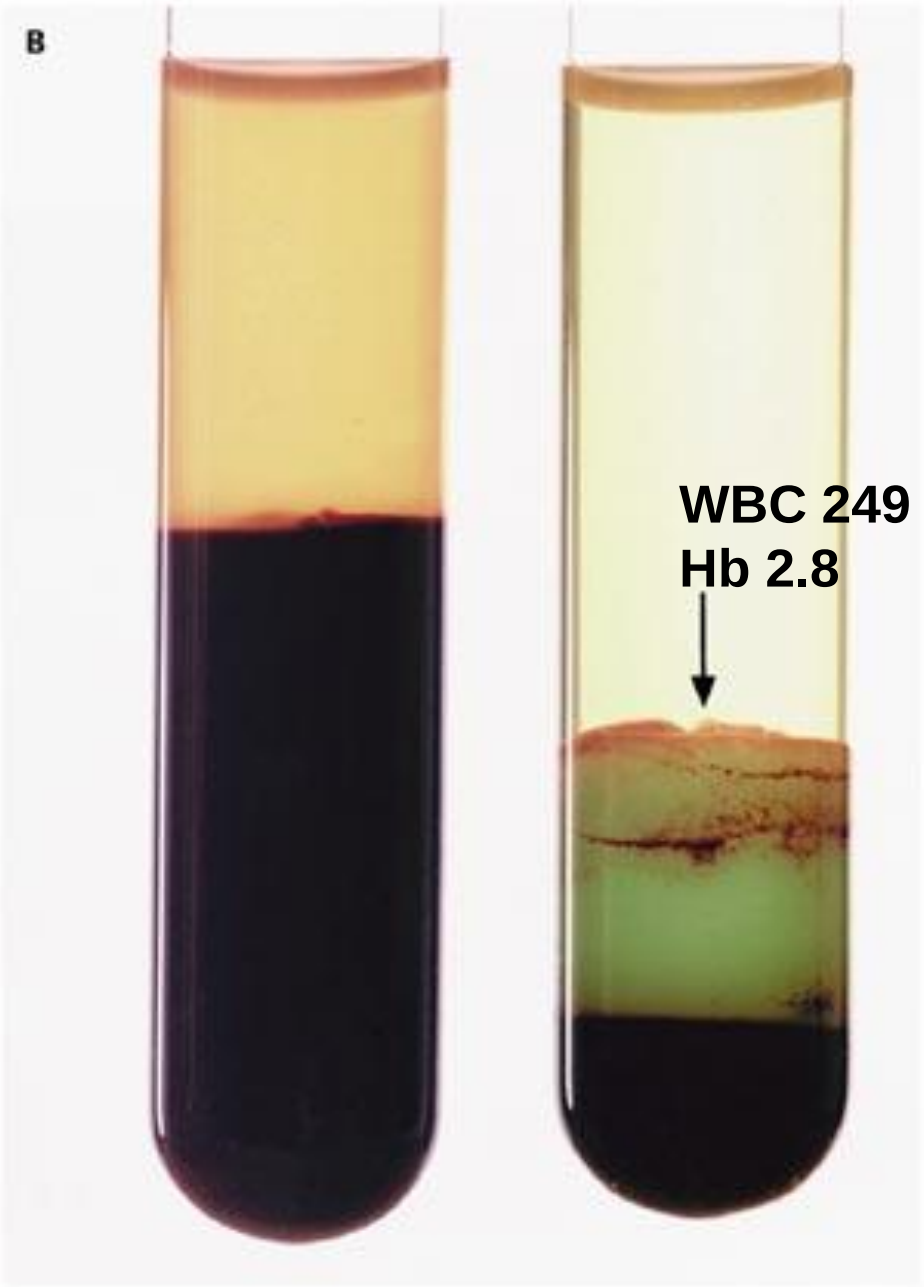
**leukemia**

**Greek words "leukos" meaning "white" and "haima" meaning "blood,**




N Engl J Med 2003; 349:767, Aug 21, 2003. **Images in Clinical Medicine**

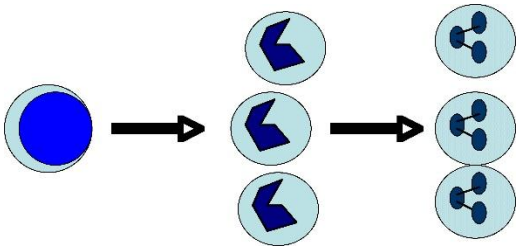


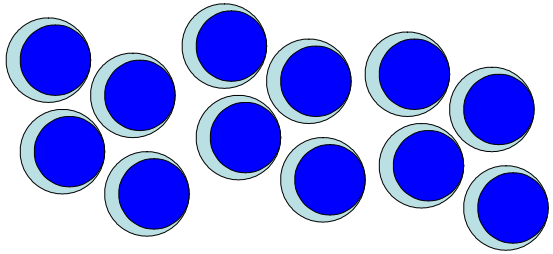

N Engl J Med 2003; 349:767, Aug 21, 2003. **Images in Clinical Medicine**

# DEFINITION

- Acute myeloid leukemia is a clonal myeloid stem cell disorder
- Expansion of immature cells (Blasts)
- Maturation block



Normal hematopoiesis



Acute leukemia

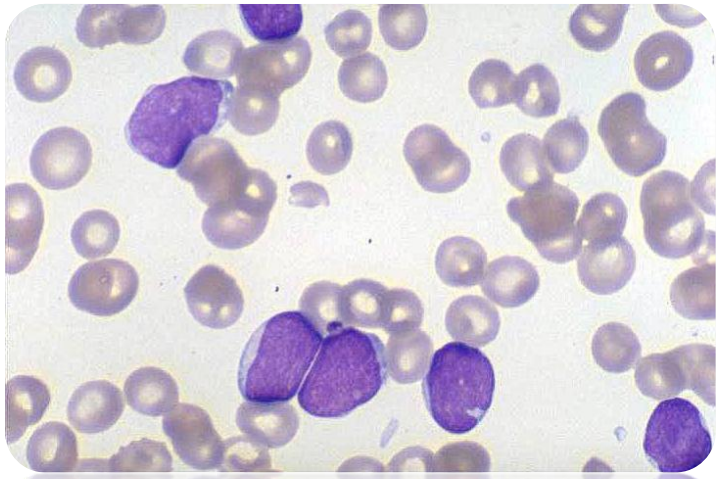
# DIAGNOSIS OF ACUTE LEUKEMIA

- **Demonstrate an increase in blasts**
  - >20% blasts in the peripheral blood or bone marrow
- **Lineage**
  - Myeloid--Acute myeloid leukemia (AML)
  - Lymphoid—Acute lymphoid leukemia (ALL)
- **Classification**
  - FAB
  - WHO (2001, 2008)

- **Tools for diagnosis**
  - Morphology
  - Cytochemistry
  - Immunophenotype/Flow cytometry
  - Genetics

# MORPHOLOGY
# >20% BLASTS

## AML



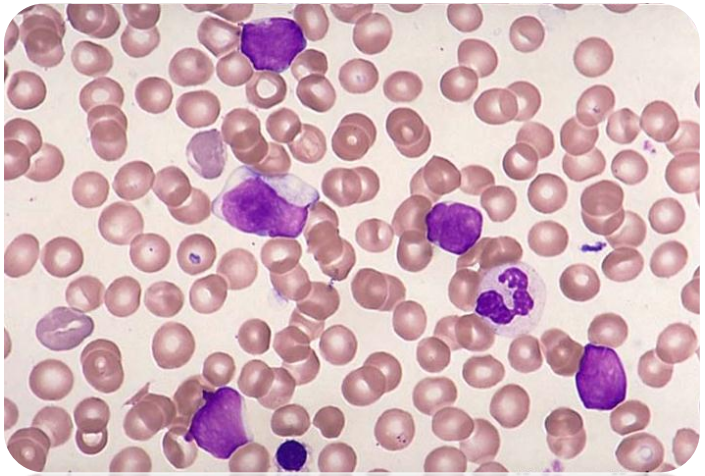
Larger

Slightly more cytoplasm, may be granular

**AUER ROD**

Larger more open nuclei with prominent nucleoli

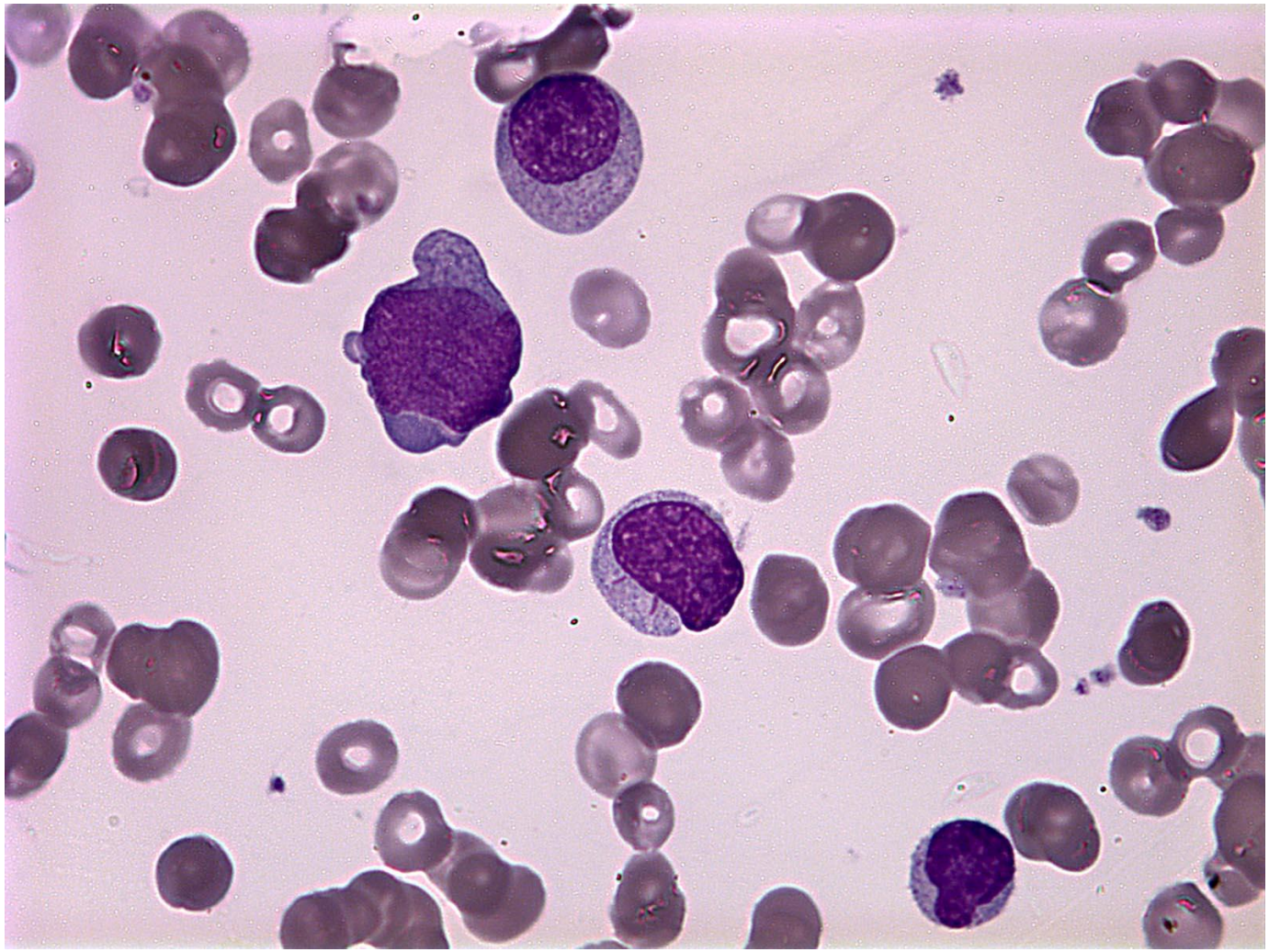
## ALL



Smaller

High NC ratio, usually cytoplasm lacks granules

Smaller nuclei, less open chromatin with indistinct nucleoli

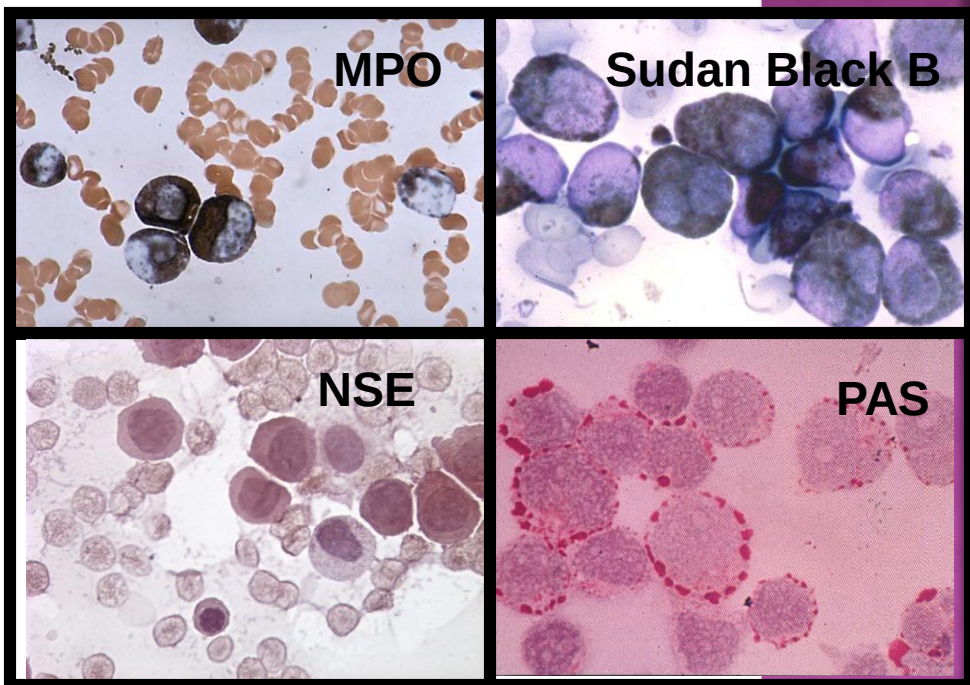


# Cytochemistry

AML ⟷ ALL

- **Myeloid**
  - MPO
    - Myeloperoxidase activity
    - Enzyme deteriorates as slides age
  - Sudan Black B
    - Lipid in myeloid primary granules
    - Slightly less specific than MPO
- **Monocytic**
  - Non Specific Esterase
    - Alpha napthyl acetate
      - Stains Megakaryocyes
        - Punctate pattern
        - Fluoride resistant
    - Alpha napthyl butyrate

- **PAS**
  - Stains Glycogen
  - Lymphoblasts—block positivity
  - Myeloid cells diffusely faintly positive




pathy.med.nagoya-u.ac.jp/atlas/img/t8/img73.jpg

# FLOW CYTOMETRY

## AML

**CD34**

**CD13, CD33, CD117**

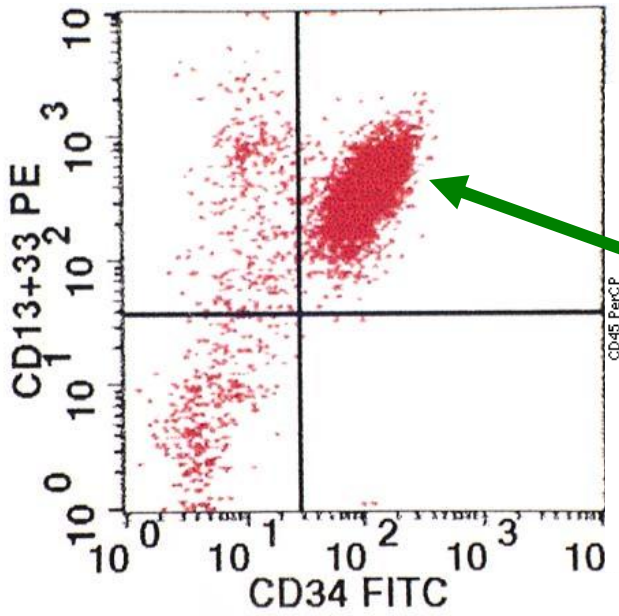
**CD64, glycophorin, CD61**

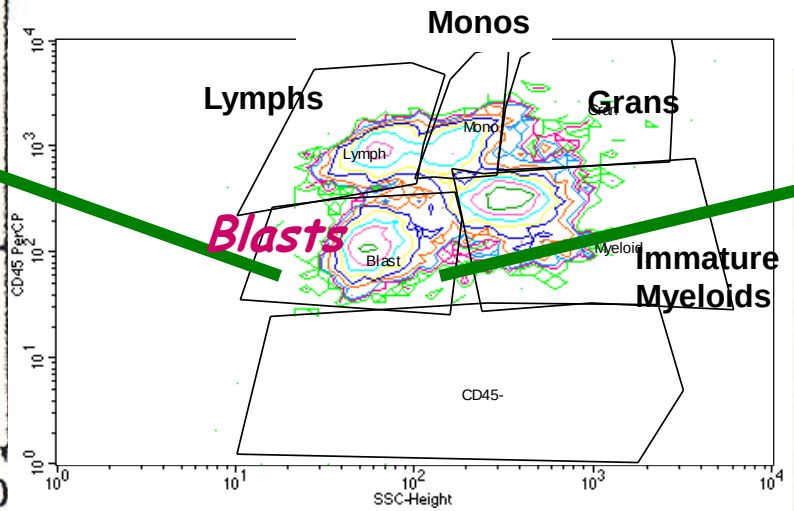
## ALL

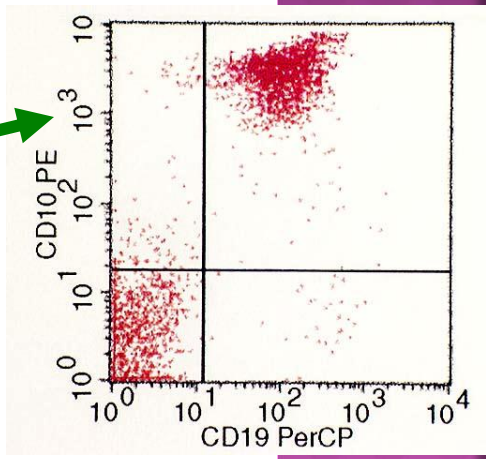
**TdT, CD34, CD10, HLA-DR**

**B-Cell-CD19, CD22, CD20**

**T-Cell-CD2, CD5, CD7,cCD3**

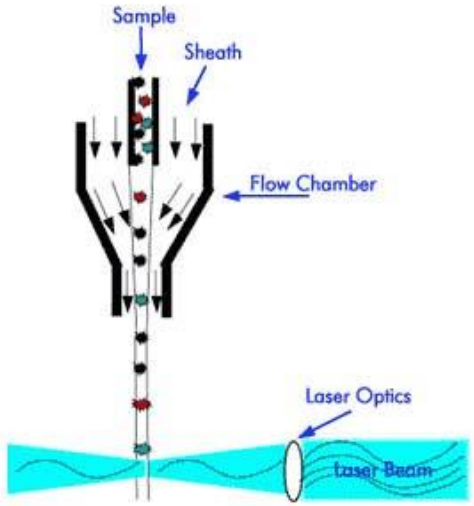


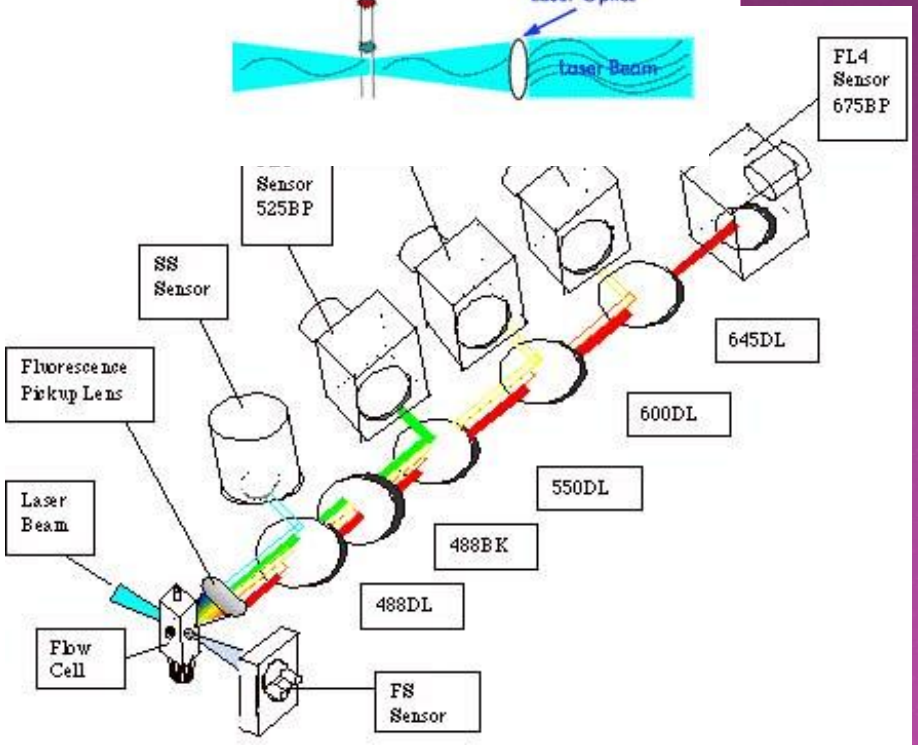



# IMMUNOPHENOTYPE

- Flow cytometry
  - Fast and accurate way to identify, quantify and determine lineage
  - Physical properties
    - Forward scatter--cell size
    - Side scatter--cytoplasmic granularity
  - Cells can be stained with fluorescently labeled antibodies that recognize cell markers
    - CD34
      - Stem cell marker
    - CD117, CD33, CD13, MPO
      - Myeloid markers
    - CD14, CD64
      - Monocytic marker
    - Glycophorin A
      - Erythroid marker
    - CD41, CD61
      - Megakaryocytic markers



# Genetics

**AML** **ALL**

## Recurrent cytogenetic abnormalities

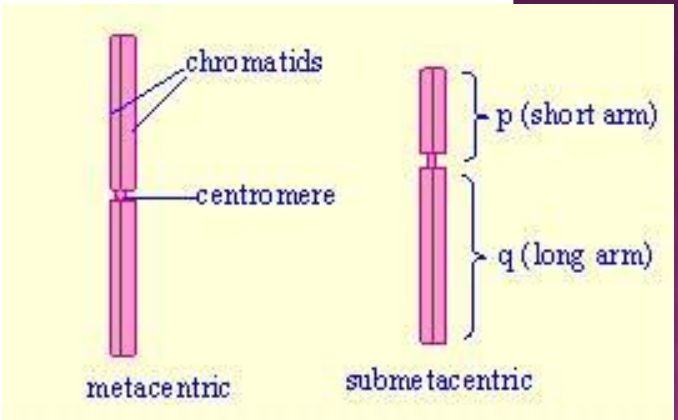
t(15;17)(q22,q12)
t(8;21)(q22,q22)
inv(16)(p13,q12)
11q23

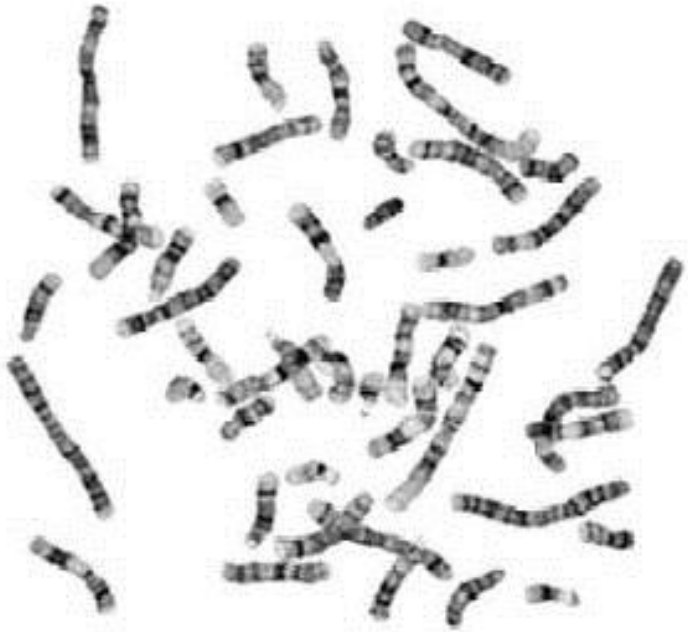
**Hyperdiploidy >50 (+4,+10,+17)**
**t(12;21)(p13;q22)**
**t(9;22)(q34,q11.2)**
**t(4;11)(q21,q23)**
**t(1;19)(q23;p13.3)**

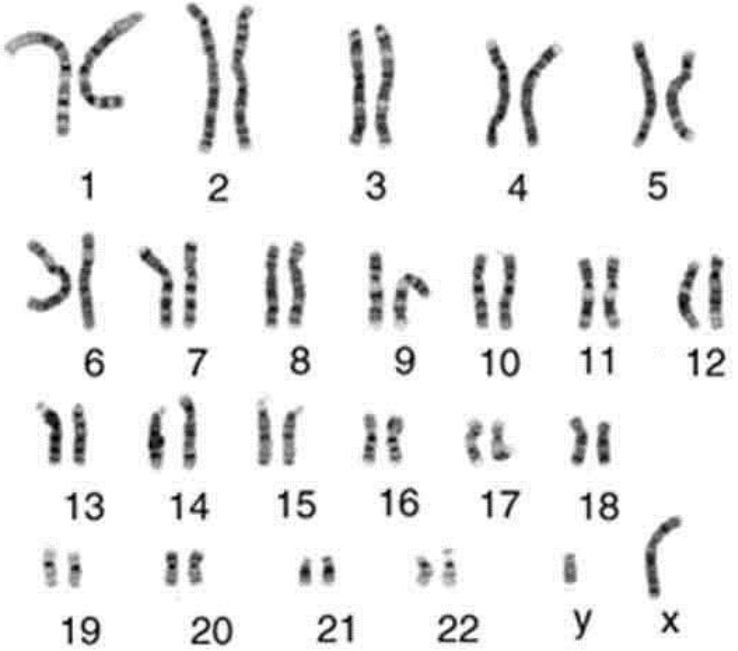
- **Cytogenetics** correlate with survival and response to therapy in both AML and ALL
- WHO classification defines subtypes of AML by recurrent cytogenetic changes
- Some translocations seen in AML have morphologic correlates (more to follow)

# CONVENTIONAL CYTOGENETICS

- **Conventional cytogenetics**
  - Requires dividing cells (culture 24-48h for marrow)
  - Examine metaphase spread
    - Colcemid→expose cells to hypotonic solution (swell)→fix→place on cover slip→rupture membranes→stain
  - Allows identification of numeric and structural abnormalities





# GENETICS

- **Conventional cytogenetics**
  - Requires dividing cells (culture 24-48h for marrow)
  - Examine metaphase spread
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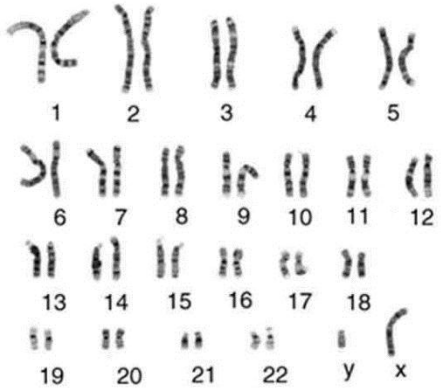
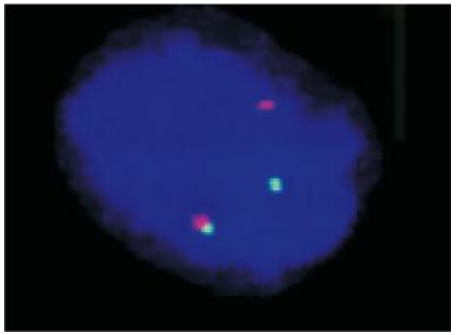
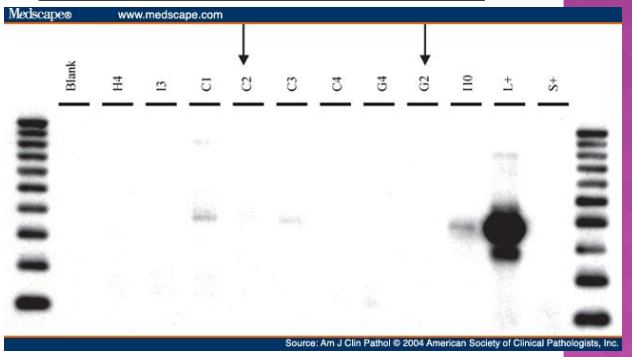
  - Allows identification of numeric and structural abnormalities




- **FISH—fluorescent in situ hybridization**
  - Probe for the abnormality (structural or numeric) of interest using specific probes
  - Faster than conventional cytogenetics
  - Must know what to look for



- **Molecular studies**
  - Fast
  - Must know what to look for



# GENE MUTATIONS IN AML

In patients with AML with normal karyotype:

- FLT3 ITD ~30%
- NPM ~50%
- CEBPA 15-18%

- Good prognosis with:
  - NPM mutated and FLT3 wild type

  Or

  CEBPA mutated FLT3 wild type

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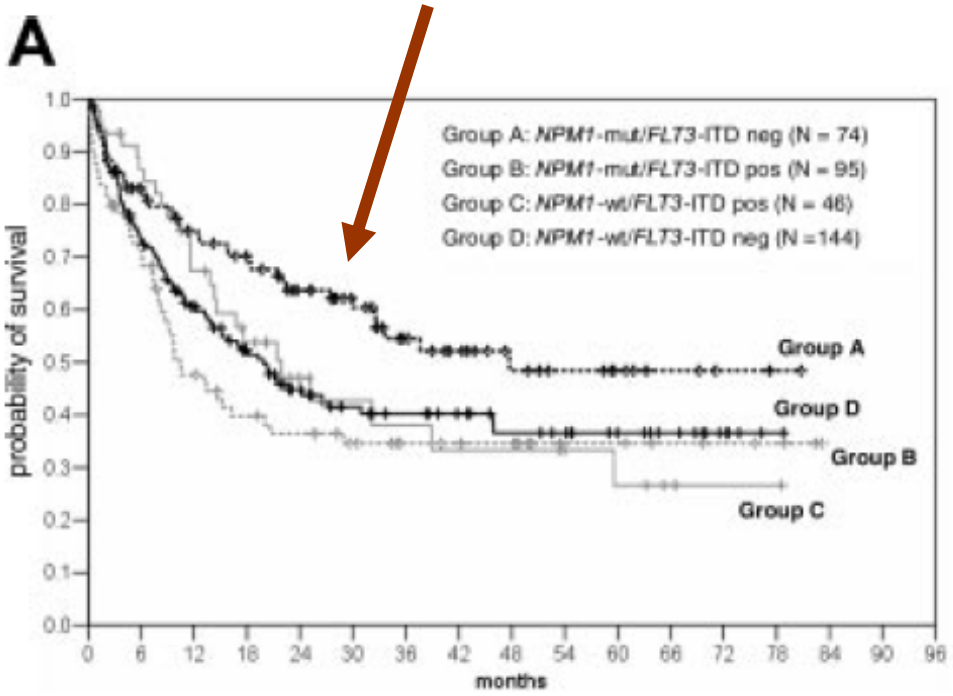
Thiede et al. p 4011



# ACUTE MYELOID LEUKEMIA

## Classification

- FAB-1985
- WHO-2001, 2008

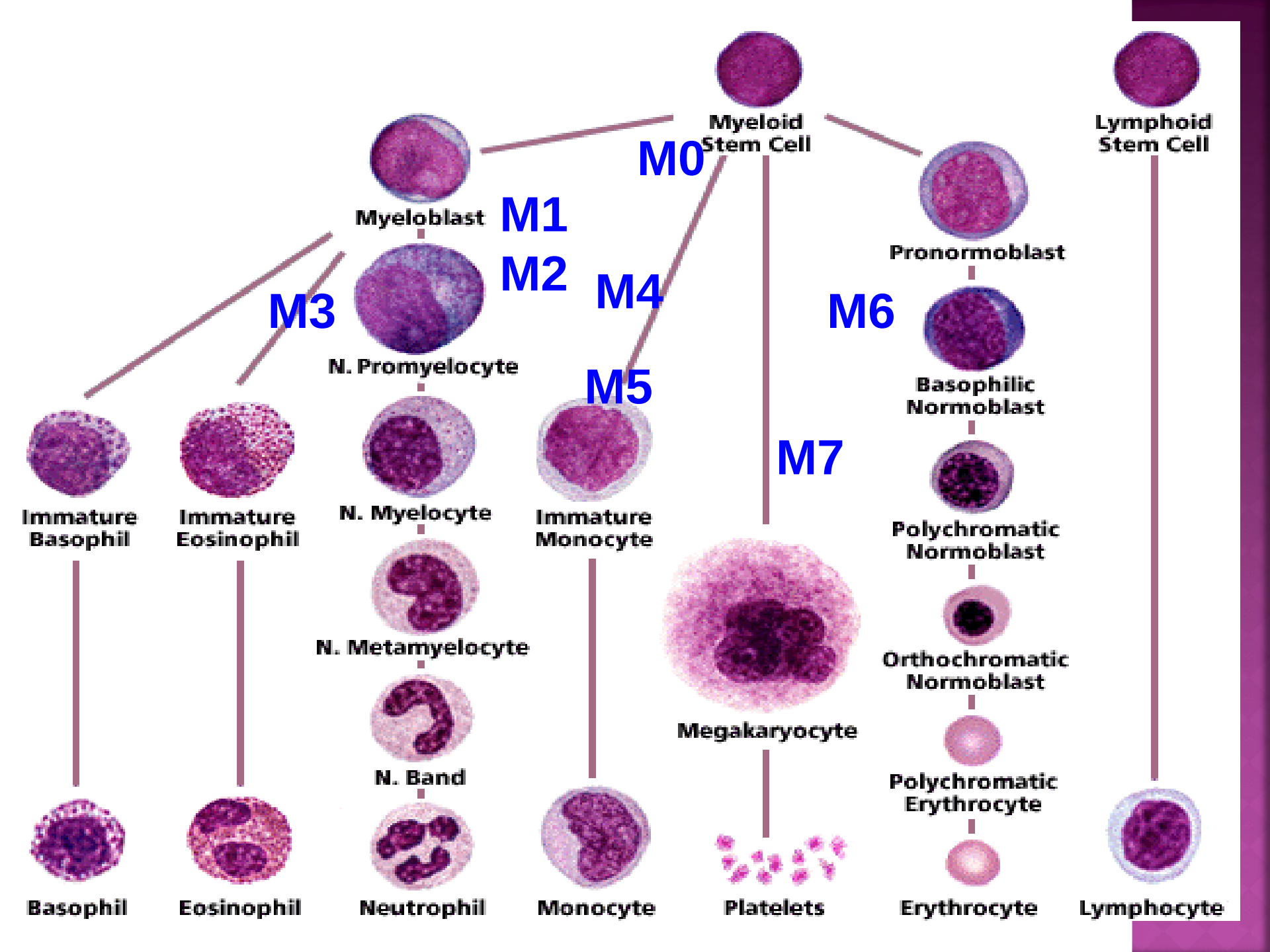
# CLASSIFICATION OF AML

- FAB-French American British
  - Introduced in 1976 (revised in 1985)
  - Based primarily on morphology and cytochemistry
  - Leukemia is defined by the presence of >30% blasts in the peripheral blood and/or marrow
  - Provides and organized way to think about AML
  - Limited value in predicting prognostically significant groups

**WHO >20% blasts = AML**

# FAB CLASSIFICATION OF AML

| | |
|---|---|
| M0—Minimally differentiated | >30% blasts, <3% blasts MPO+, SBB+ |
| M1—Without maturation | >30% blasts, >3% blasts MPO+, SBB+<br><10% maturing myeloids (promyelocyte and beyond) |
| M2—With maturation<br>t(8;21) | >30% blasts<br>>10% maturing myeloids (promyelocyte and beyond) |
| M3—Promyelocytic<br>t(15;17) | >30% blast equivalents<br>(neoplastic promyelocytes and myeloblasts) |
| M4—Myelomonocytic<br>M4 eos Acute myelomonocytic leukemia with eosinophilia<br>inv (16) | >30% blast equivalents<br>(myeloblasts, monoblasts and promonocytes)<br>>20% monocytic elements (NSE+)<br>>20% myeloid elements (MPO+, SBB+) |
| M5<br>M5a—Monoblastic(>80% monoblasts)<br>M5b—Monocytic (<80% monoblasts) | >30% blast equivalents<br>(myeloblasts, monoblasts and promonocytes)<br>>80% NSE+ monocytic elements<br><20% MPO+, SBB+ myeloid elements |
| M6—Erythroid<br>M6a—Erythroleukemia<br>M6b—Pure erythroid leukemia | >50% erythroid elements<br>>30% of nonerythroid elements are myeloid blasts<br>>80% immature erythroid elements |
| M7—Megakaryocytic | >30% blasts of megakaryocytic lineage |

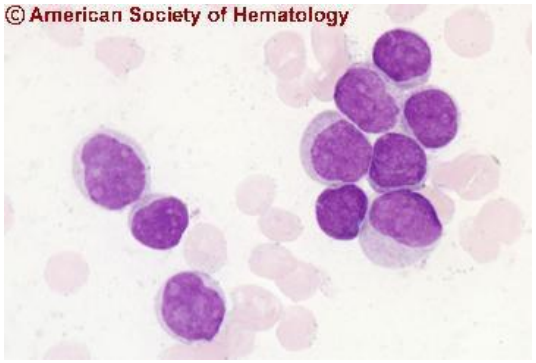

Myeloid Stem Cell
Lymphoid Stem Cell
M0
Myeloblast
M1
M2
M3
M4
M5
M6
M7
N. Promyelocyte
Pronormoblast
Basophilic Normoblast
Immature Basophil
Immature Eosinophil
N. Myelocyte
Immature Monocyte
Polychromatic Normoblast
N. Metamyelocyte
Orthochromatic Normoblast
Megakaryocyte
N. Band
Polychromatic Erythrocyte
Basophil
Eosinophil
Neutrophil
Monocyte
Platelets
Erythrocyte
Lymphocyte

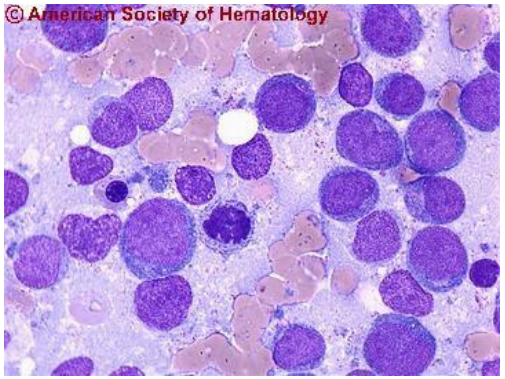
# FAB CLASSIFICATION OF AML

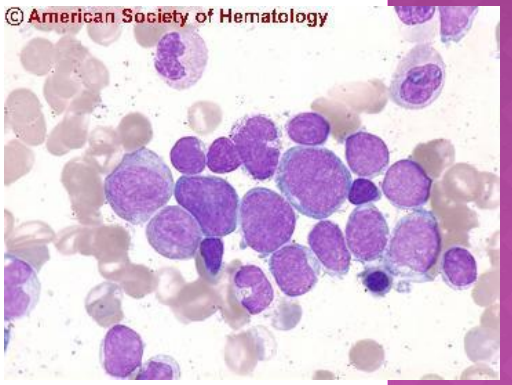
- **AMLM0 5%**
  - AML with minimal differentiation
    - Myeloid blasts >30%
    - <3% blasts are SBB and/or MPO
- Somewhat poorer overall prognosis
  - Poor cytogenetic features are overrepresented in this group

- **AMLM1 30-45%**
  - AML without maturation
    - Myeloid blasts >30%
    - **>3% blasts are SBB and/or MPO+**
    - <10% maturation to the promyelocyte stage and beyond

- **AMLM2 10%**
  - AML with maturation
    - Myeloid blasts >30%
    - >3% blasts are SBB and/or MPO+
    - **>10% maturation**
  - Frequent association with t(8;21)

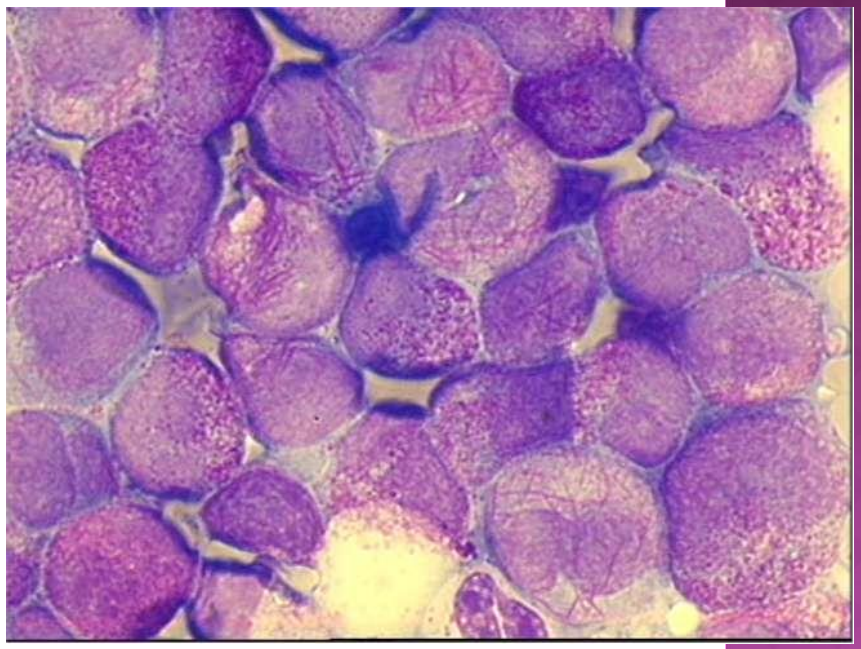


# FAB CLASSIFICATION OF AML

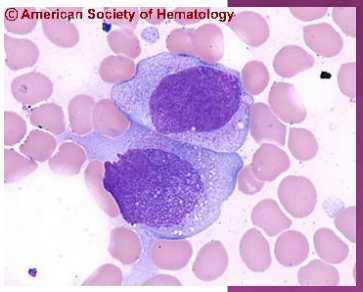
- AML-M3 (5-8%)
  - Acute promyelocytic leukemia
  - Maturation arrest at the stage of promyelocytes
    - >30% promyelocytes and blasts
  - Clinical--DIC
  - Genetics t(15;17)
    - PML-RARα
  - Treatment--ATRA



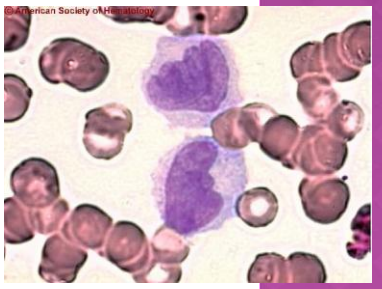
# FAB CLASSIFICATION OF AML

- **AML-M4 15-25%**
  - Acute myleomonocytic leukemia
    - >20% myeloid elements (MPO, SBB)
    - >20% monocytoid elements (NSE)
    - >30% blasts (myeloblasts and monoblasts) and promonocytes
  - AML M4eo
    - Acute myelomoncytic leukemia with abnormal eosinophils
    - Inversion (16)

- **AML-M5**
  - > 80% monocytic elements
  - > 30% monoblasts and promonocytes
  - **M5a 5-8%**
    - Acute monoblastic leukemia
    - >80% monoblasts



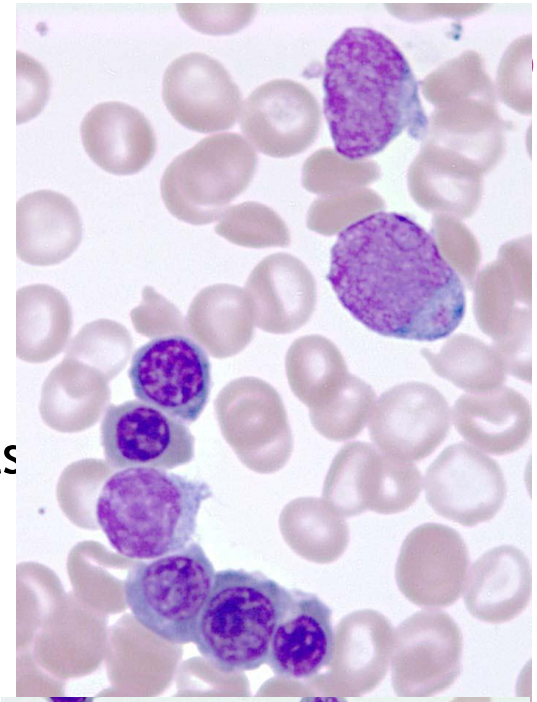

  - **M5b 3-6%**
    - Acute monocytic leukemia
    - <80% monoblasts, more promonocytes

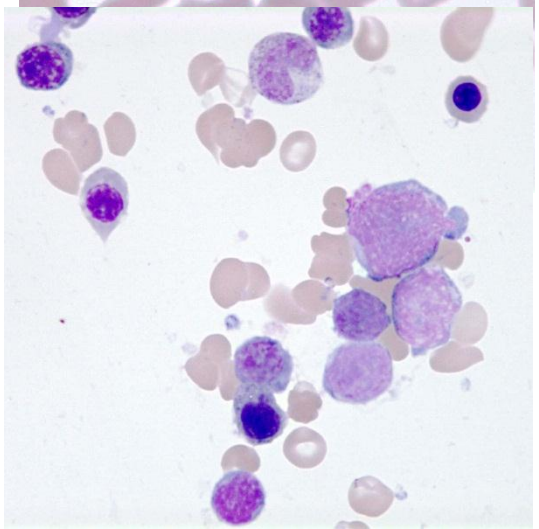


http://www.medinfo.ufl.edu/year2/path/lectures/11

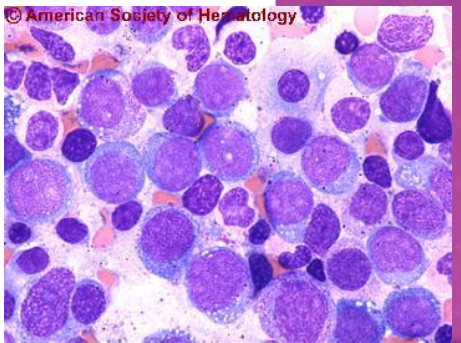
# FAB CLASSIFICATION OF AML

- AML M6a--5-6%
  - Erythroleukemia
    - >50% erythroid elements
    - >30% of non erythroid elements are myeloblasts



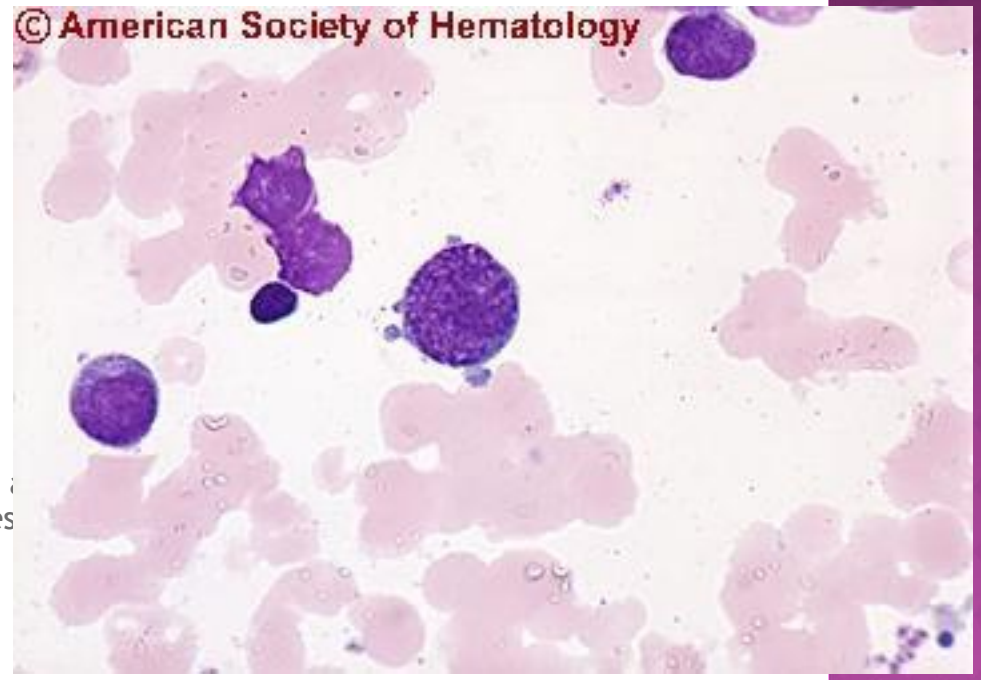


- AML M6b--rare
  - Pure erythroid leukemia
    - >80% immature erythroid elements
    - Erythroblasts have vacuolated cytoplasm and may have lobular positivity by PAS



# FAB CLASSIFICATION OF AML

- AML-M7 3-5%
  - Acute megakaryoblastic leukemia
  - Acute leukemia (>30% blasts)
  - >50% of the blasts are of megakaryocytic lineage
    - Basophilic cytoplasm
    - Blebbing
      - "shedding platelets"
    - ANA
    - Platelet peroxidase
    - CD61, CD41
    - **Marrow fibrosis**
      - May have a "dry tap"
      - Megakaryocytes are thought to secrete a platelet mitogenic factor that promotes fibroblast growth.
  - Genetic correlates
    - Down's syndrome +21
    - t(1;22)(p13;q13)--children, organomegally



# WHO CLASSIFICATION OF AML

- **WHO Classification of AML (1995→2001, 2008)**
  - **> 50 Pathologists**
  - **> 40 Hematologist/Oncologists**
- Takes into account additional diagnostic tools and prognostic data
  - Blast threshold (20%)
  - Cytogenetics
  - Prior chemotherapy
  - Dysplasia

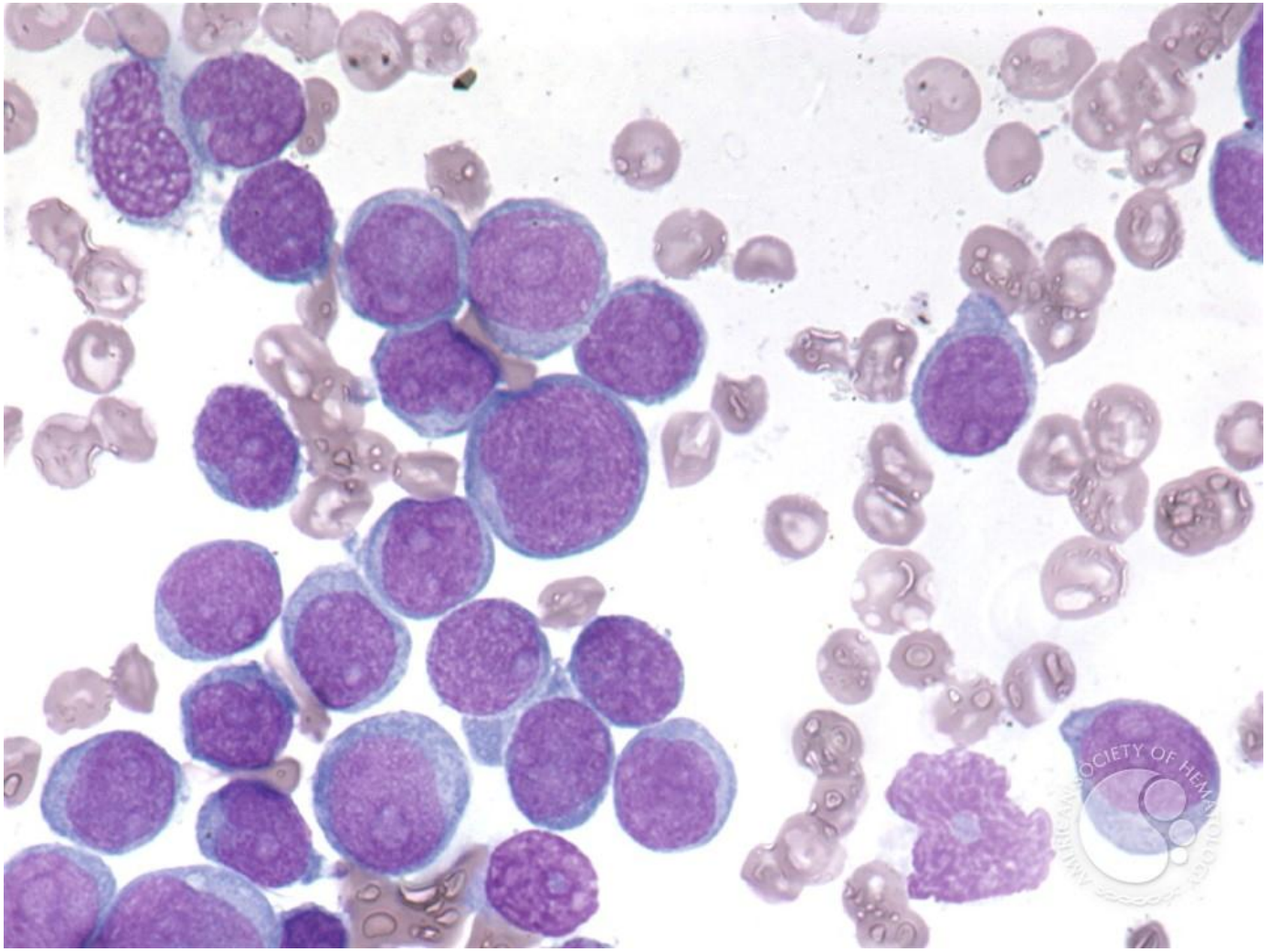
# WHO CLASSIFICATION OF AML

- **WHO** Classification is based on:
  - Morphology
    - Blasts 30%→20%
    - Dysplasia
  - Cytochemistry
    - Less emphasis than in the FAB classification
  - Flow cytometry
  - Cytogenetics
  - Previous Therapy
    - Alkylating agents
    - Topoisomerase inhibitors

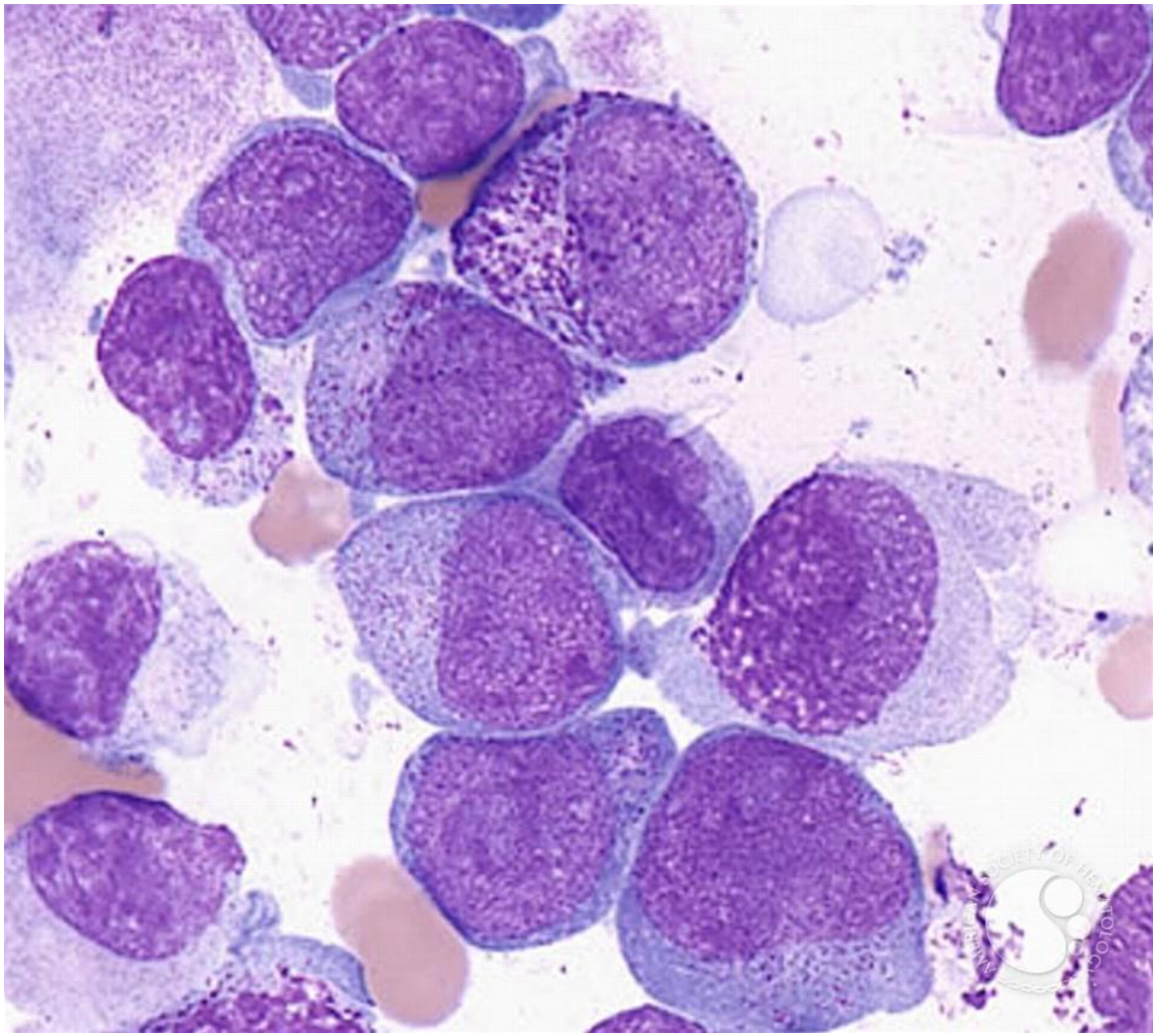
# 2008 WHO CLASSIFICATION OF AML

- **AML with recurrent cytogenetic abnormalities**
  - AML with t(8;21)(q22;q22), (*AML1/ETO*) — 5%
  - AML with inv(16)(p13q22) or t(16;16)(p13;q22), (*CBFbeta/MYH11*) — *5-8%*
  - AML with t(15;17)(q22;12), (*PML/RARalpha*) and variants — 5-8%
  - AML with t(9;11)(p22q23) (*MLL*) — 2% adults
  - AML with t(6;9)(p23q34) (DEK-NUP214) — 1-2%
  - AML with inv(3)(q21q26.2) or t(3;3)(q21;q26.2) (RPN1-EVI1) — 1-2%
  - AML with t(1;22)(p13q13) (RBC15-MKL1) — <1%
  - Provisional: AML with gene mutations (FLT3ITD, NPM1, CEBPA)
- **AML with myelodysplasia related changes** — 24-35%
- **Therapy related myeloid neoplasms** — 10-20%
- **AML not otherwise categorized**
  - AML minimally differentiated — <5%
  - AML without maturation — 5-10%
  - AML with maturation — 10%
  - Acute myelomonocytic leukemia — 5-10%
  - Acute monoblastic and monocytic leukemia — <5%, <5%
  - Acute erythroid leukemia — <5%, rare
  - Acute megakaryoblastic leukemia — <5%, [+21, t(1;22)]
  - Acute basophilic leukemia — <1%
  - Acute panmyelosis with myelofibrosis — rare
  - Myeloid sarcoma

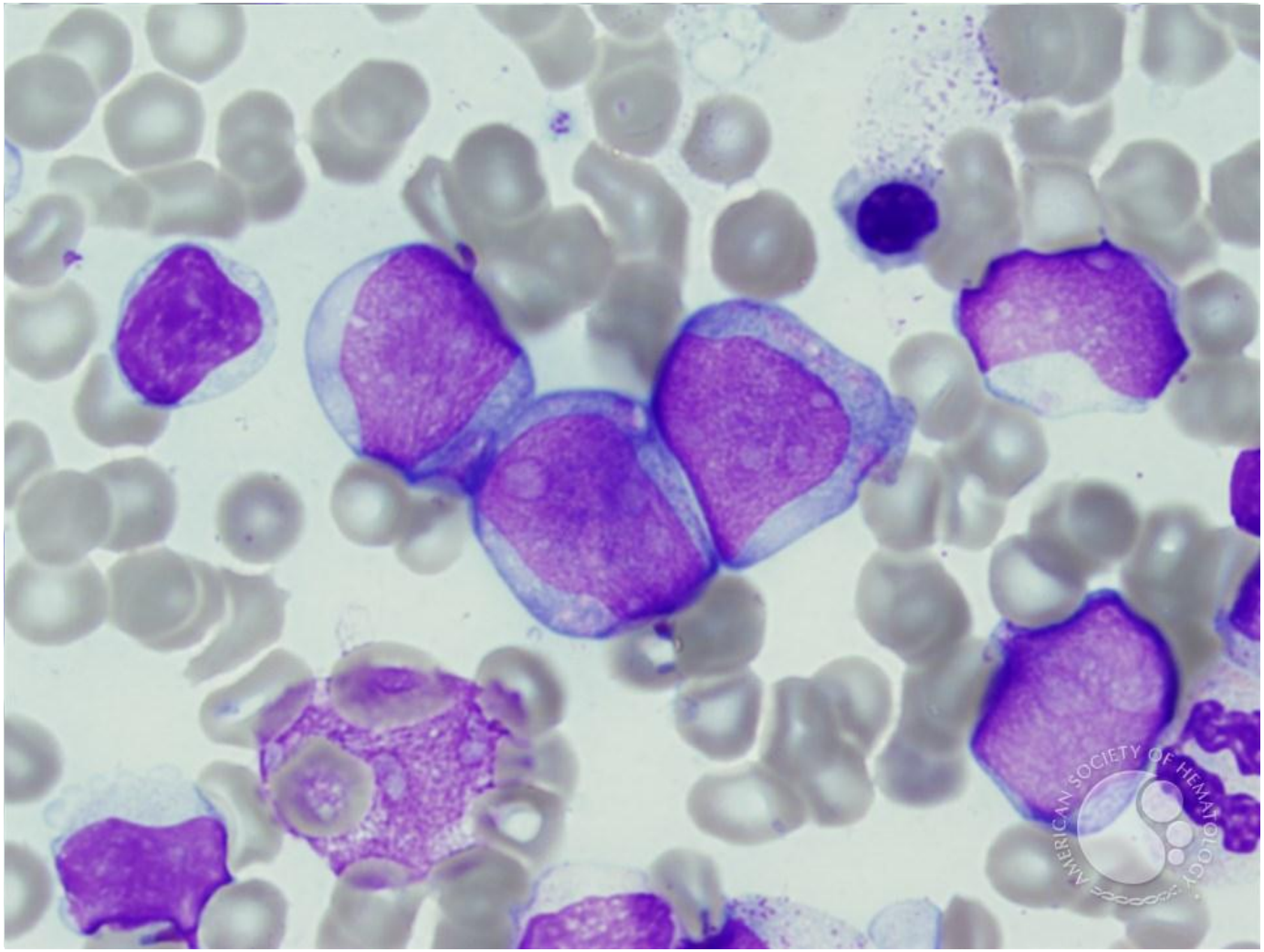
# AML WITHOUT MATURATION - 2.
# THESE BLASTS LACK AZUROPHILIC GRANULES OR AUER RODS.



# Acute myeloid leukemia with maturation blasts (types I, II, and III) seen with promyelocyte.

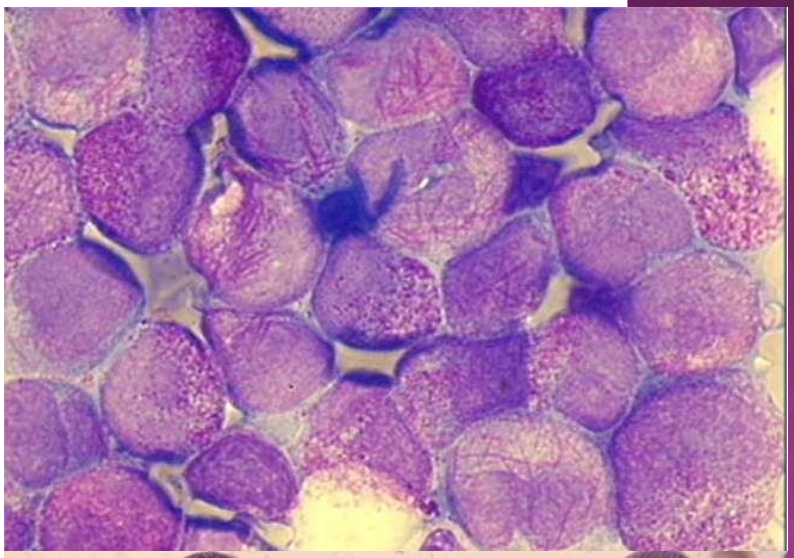


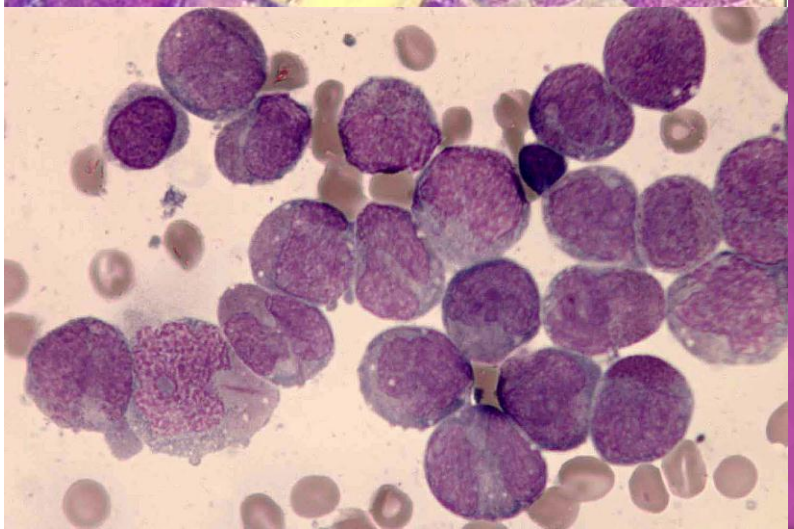
# AML WITH MUTATED CEBPA
# HIGHER POWER OF BONE MARROW ASPIRATE SHOWING BLASTS INCLUDING ONE WITH MULTIPLE AUER RODS



# AML WITH RECURRENT CYTOGENETIC ABNORMALITIES: T(15;17)

- Morphologic correlate
- FAB AML-M3
  - Acute promyelocytic leukemia (APL)
- Closest link between morphology and genetics
- Clinically critical to identify
  - **Clinical presentation**
    - DIC
      - If left untreated DIC can cause pulmonary or cerebrovascular hemorrhage in up to 40 percent of patients with APL
      - Some studies report a 10 to 20 percent incidence of early hemorrhagic deaths
  - **Therapy**
    - ATRA



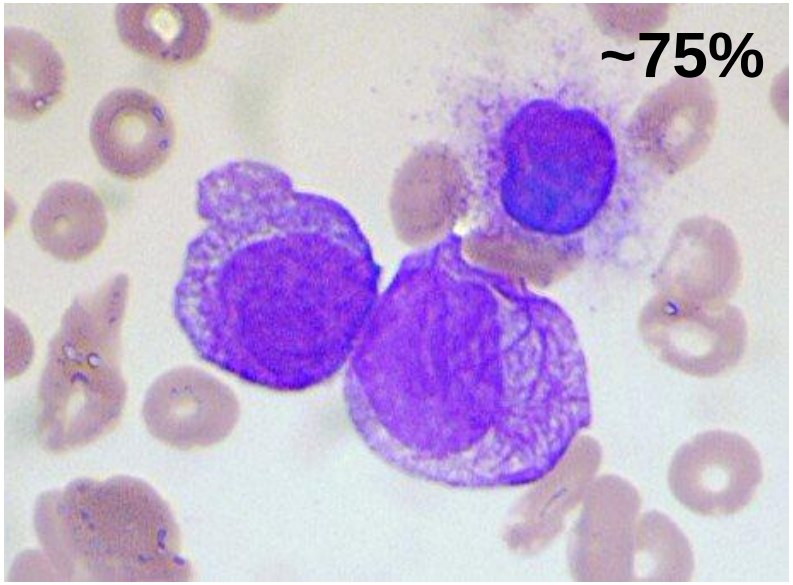


# DIAGNOSIS OF APL

- Morphology
- Immunophenotype
- PML immunocytochemistry
  - Nuclear granular pattern with nucleolar exclusion (normal promyelocyte or blast from other forms of AML—speckled nuclear pattern)
- **Genetics—Gold standard**
  - Cytogenetics
  - FISH
  - Molecular studies

# MORPHOLOGY

## Hypergranular type

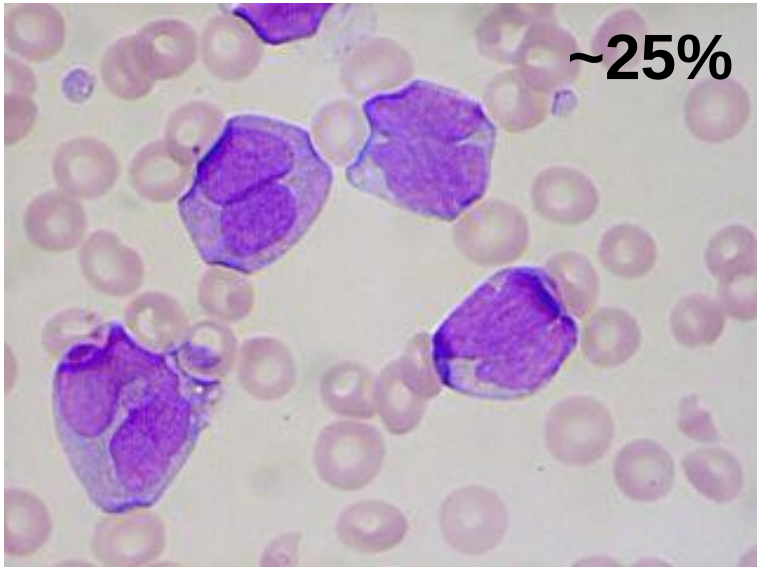



- Cytoplasm is packed with densely packed, sometimes coalescent large granules
- Auer rods (often large, sometimes in bundles)

Low WBC

Strongly MPO+

## Microgranular type

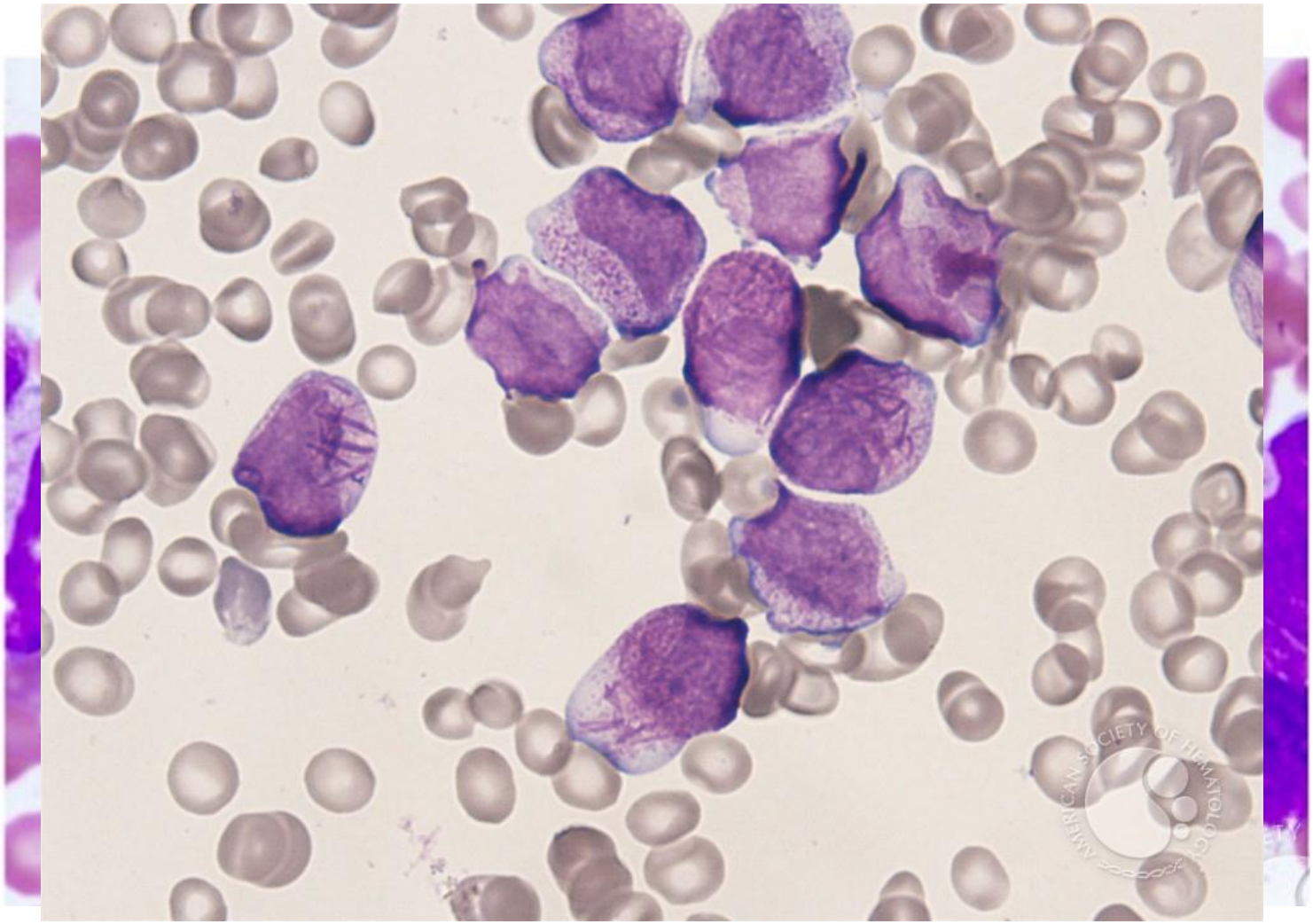



- Bilobed nuclei, apparent paucity of granules (submicroscopic granules)
- May be mistaken for monoblasts
- Auer rods may be present

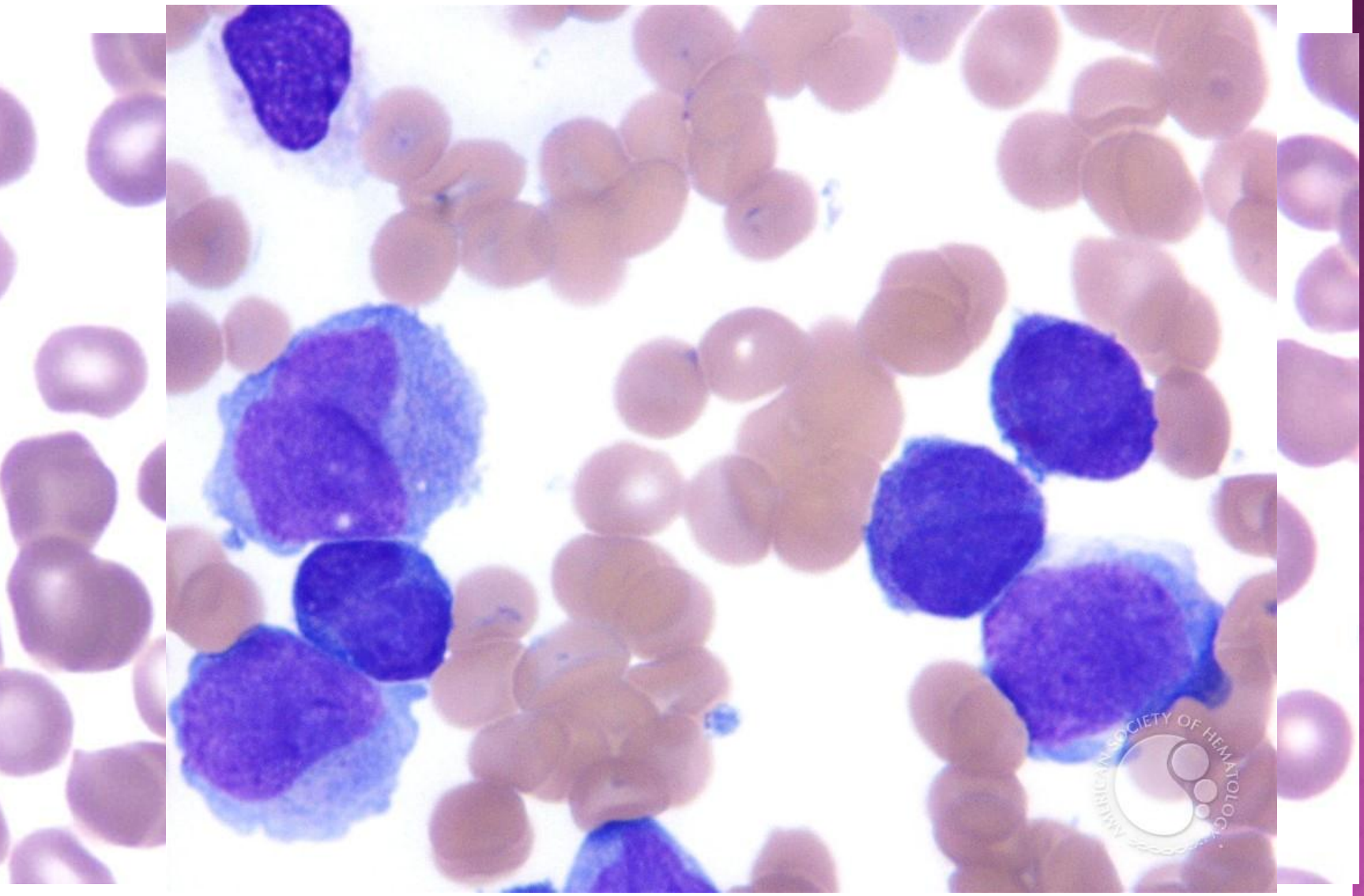
High WBC 50,000 to 200,000/µL

Strongly MPO+

# HYPERGRANULAR ACUTE PROMYELOCYTIC LEUKEMIA



# ACUTE PROMYELOCYTIC LEUKEMIA, MICROGRANULAR VARIANT,



# IMMUNOPHENOTYPE

- Strong side scatter
- Express myeloid markers:
  - MPO, CD13, CD33, CD117
- **Typically lack:**
  - **HLADR (4% vs 96%)**
  - **CD34 (2.5% vs 90.5%)**
  - Integrin expression low
    - CD11a (1.0% vs 93.4%)
    - CD18 (13% vs 95%)
- Microgranular variant
  - CD2
  - CD34
  - CD45

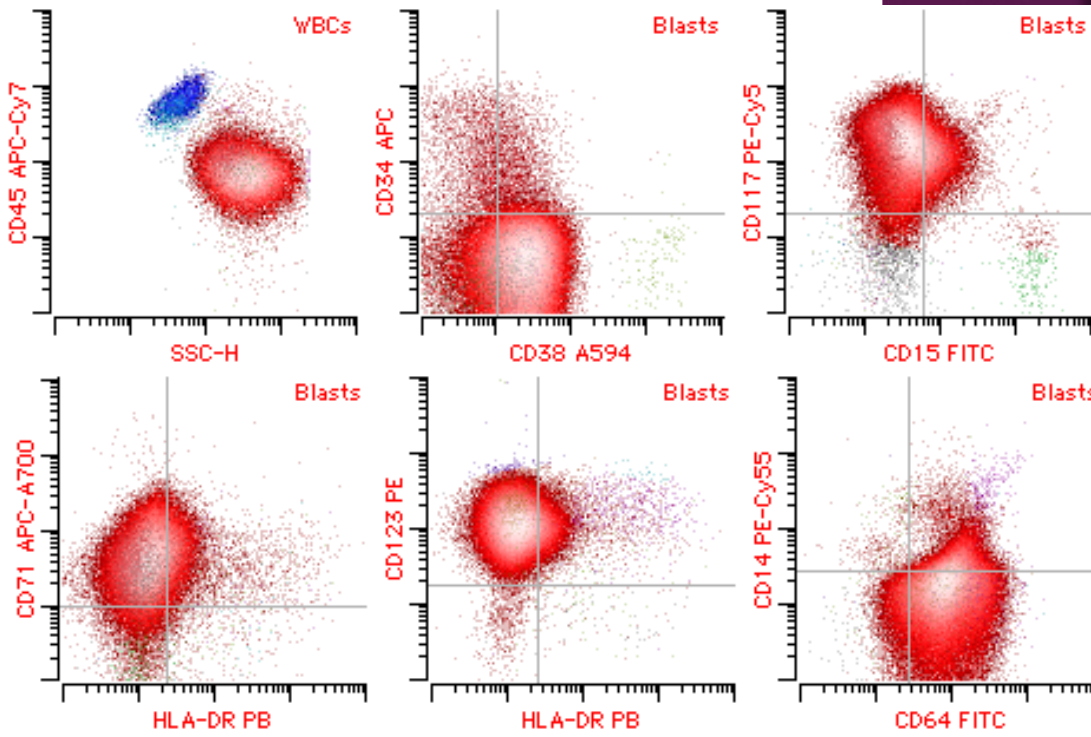



Table 2A
*Descriptive Statistics of Significantly Different Antigenic Features in APL with M3 Versus APL With M3v Morphology*

| Antigenic features[a] | APL FAB M3 (N = 102) | APL FAB M3v (N = 30) |
|---|---|---|
| % CD2 | 3.0 (2,12.5) | 44.0 (7.5,56.5) |
| % CD34 | 3.0 (1,11) | 32.5 (3.5,45) |
| CD45 MFC# | 25.5 (16,34.5) | 66.0 (41,113) |

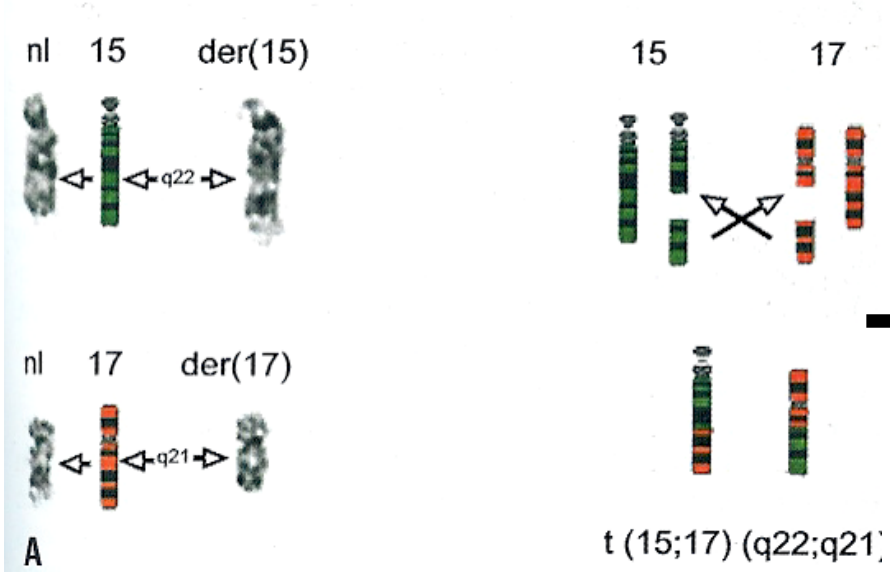
[a]Data are presented as medians, with the lower and upper quartiles in parentheses. MFC, mean fluorescence channel.

Paietta et al. Cytometry B Clin Cytom. 2004 May;59(1):1-9.

# GENETICS AND PATHOGENESIS

**PML**

**RARα**



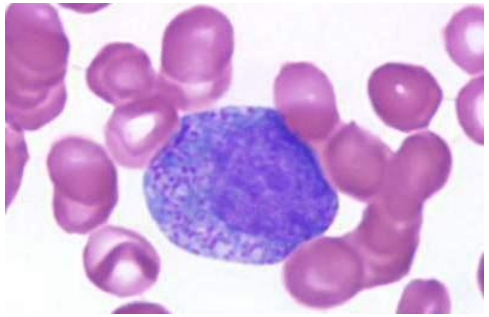

Acute promyelocytic leukemia (100%)

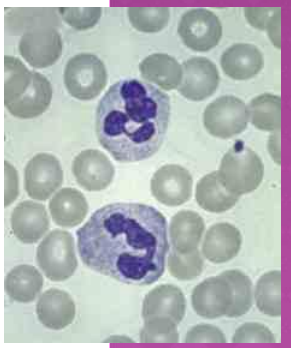
**PML-RARα**

**Blocks differentiation beyond the promyleocyte stage**

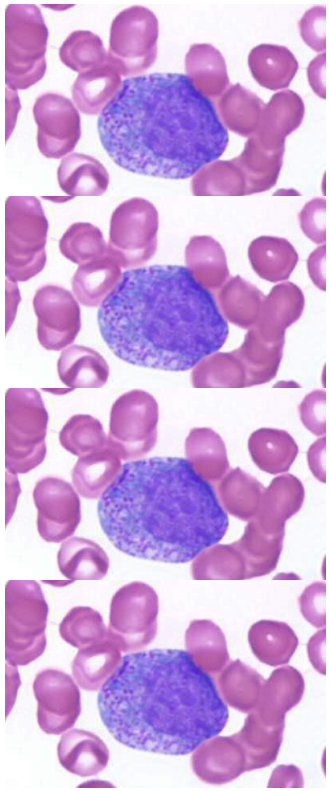
**Rare cases have variant translocations involve RARa and other partners:**

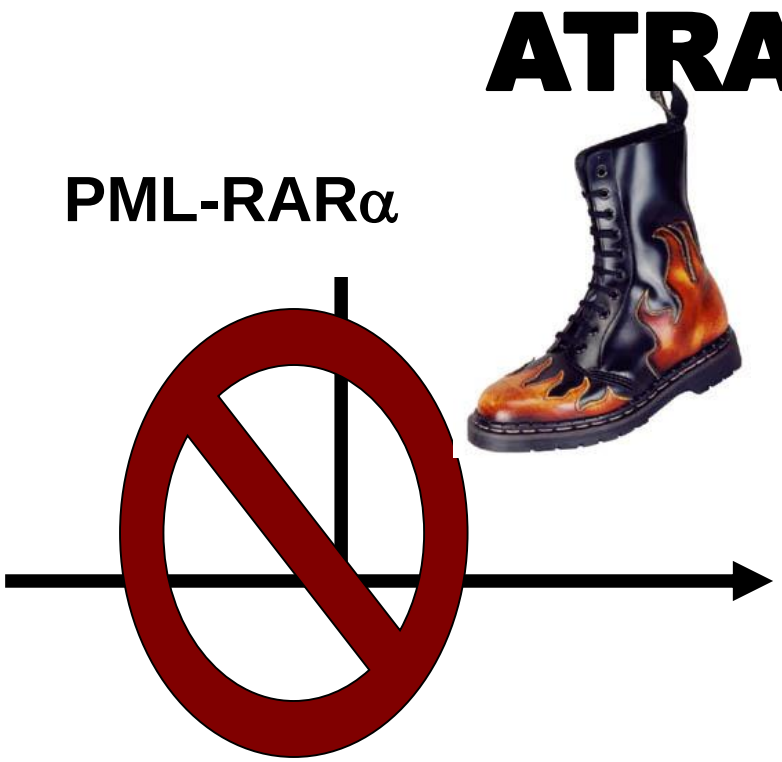
**t(11;17)PLZF-RARA**
**t(11;17) NuMA1-RARA**
**t(5;17) MPM1-RARA**
**t(17;17)STATB5-RARA**

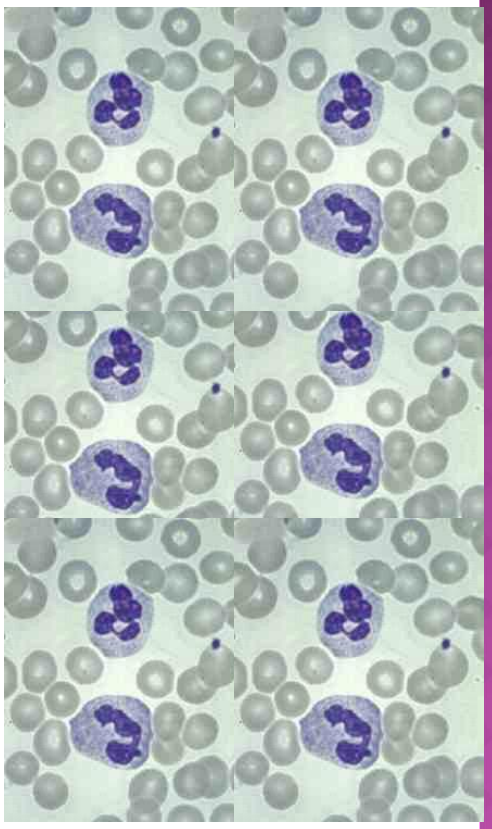




# ALL TRANS RETINOIC ACID (ATRA)

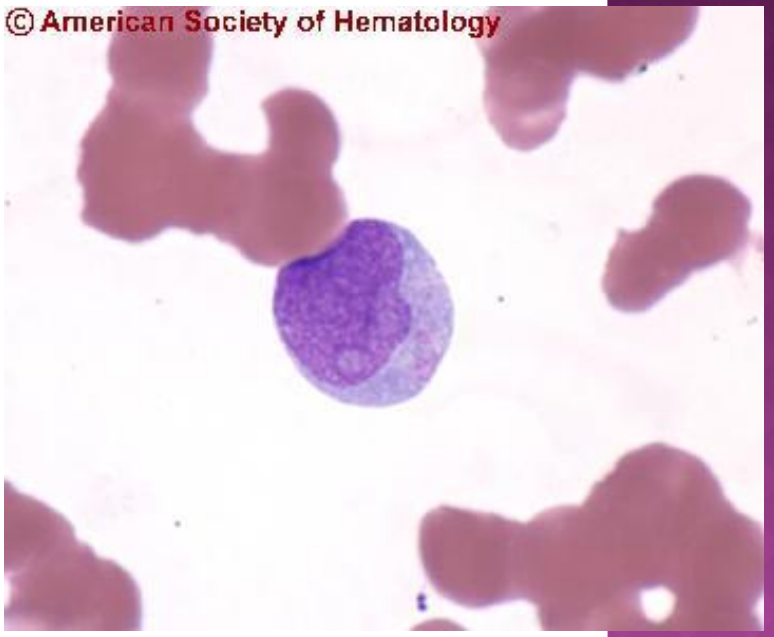




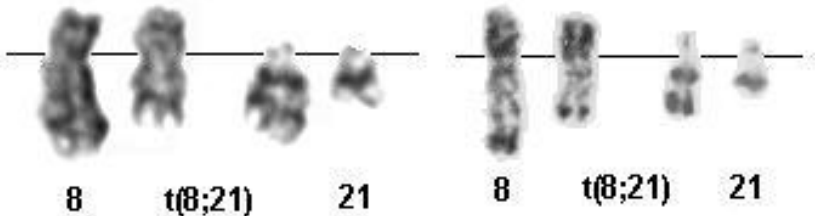
# AML WITH RECURRENT CYTOGENETIC ABNORMALITIES: T(8;21)

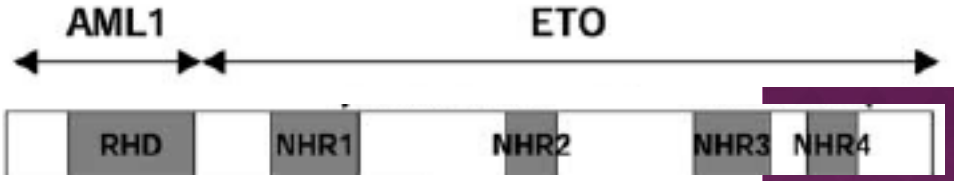
- t(8;21)(q22;q22) AML-1/ETO
- Morphology
  - Salmon pink granules
  - Present in 1/3 of karyotypically abnormal AML-M2—AML with maturation
  - AML-M2 is the most common morphologic type but cases without maturation and with monocytic differentiation have been described
  - Rare cases with <20% blasts have been described but the presence of the translocation defines this as AML
  - **Better prognosis**




- Immunophenotype
  - Myeloid markers
    - CD13, CD33, MPO
  - CD34
  - Frequent co-expression of CD56 and/or CD19 on a subset of blasts

# GENETICS

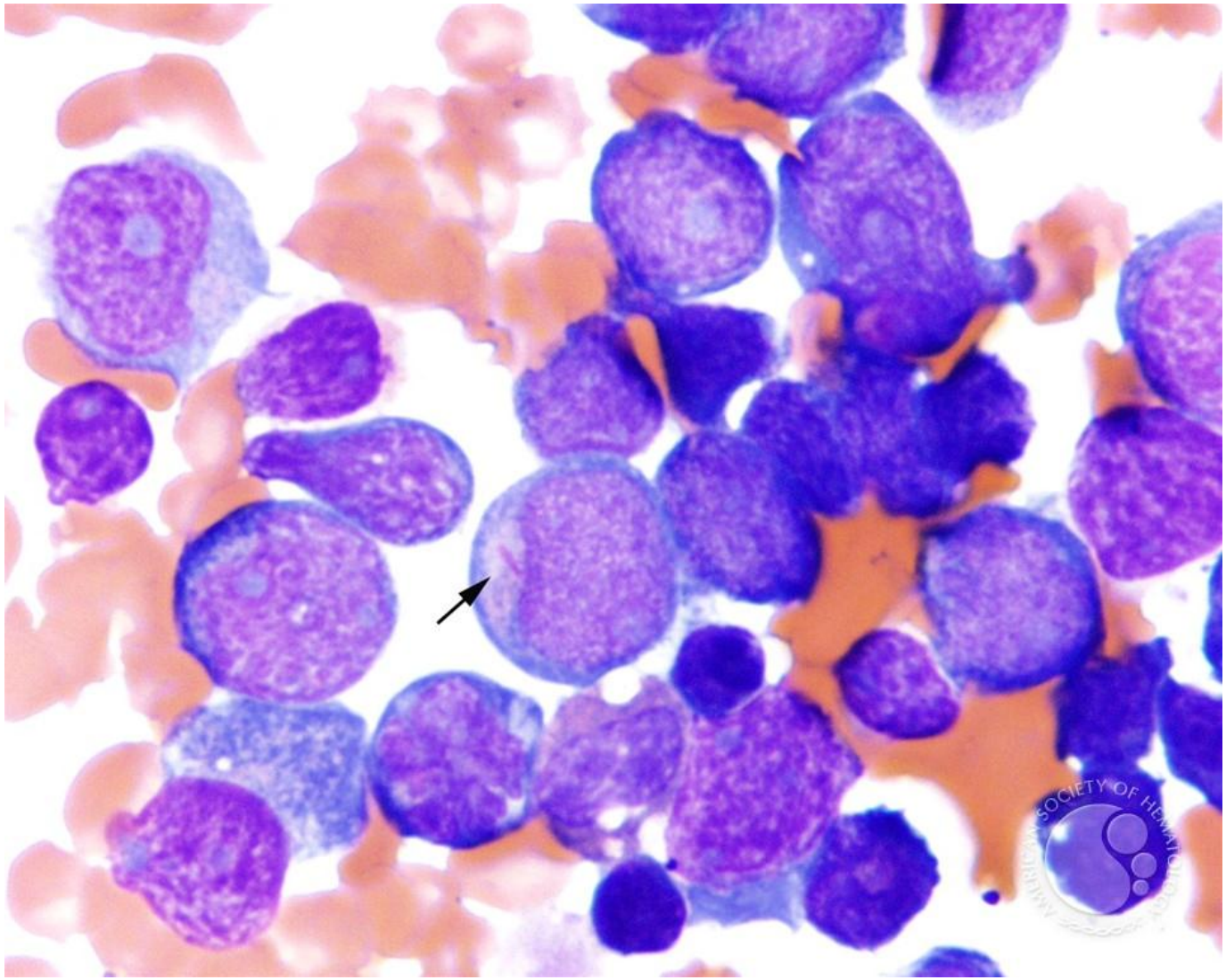



Oncogene (2004) 23, 4255–4262

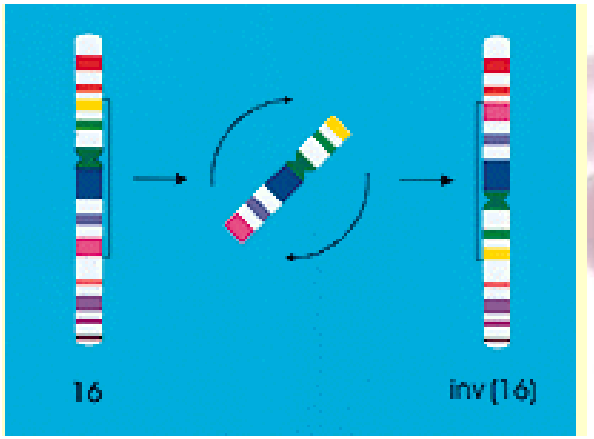
- 8q22 ETO
- 21q22 AML1=RUNX1=CBF α
  - **Core Binding Factors (CBF α and β)** are transcription factors necessary for normal hematopoiesis (myeloid and lymphoid)
- AML1-ETO fusion protein negatively regulates AML1 target genes (IL3, GMCSF receptor, TCRβ) thereby disrupting normal cell proliferation, differentiation and survival
- Alterations of these transcription factors have been described in both AML and ALL
  - CBF AML inv(16); t(8;21)--> 15-20% of AML in adults <60
  - B-ALL t(12;21)→ ~25% of childhood precursor B-ALL

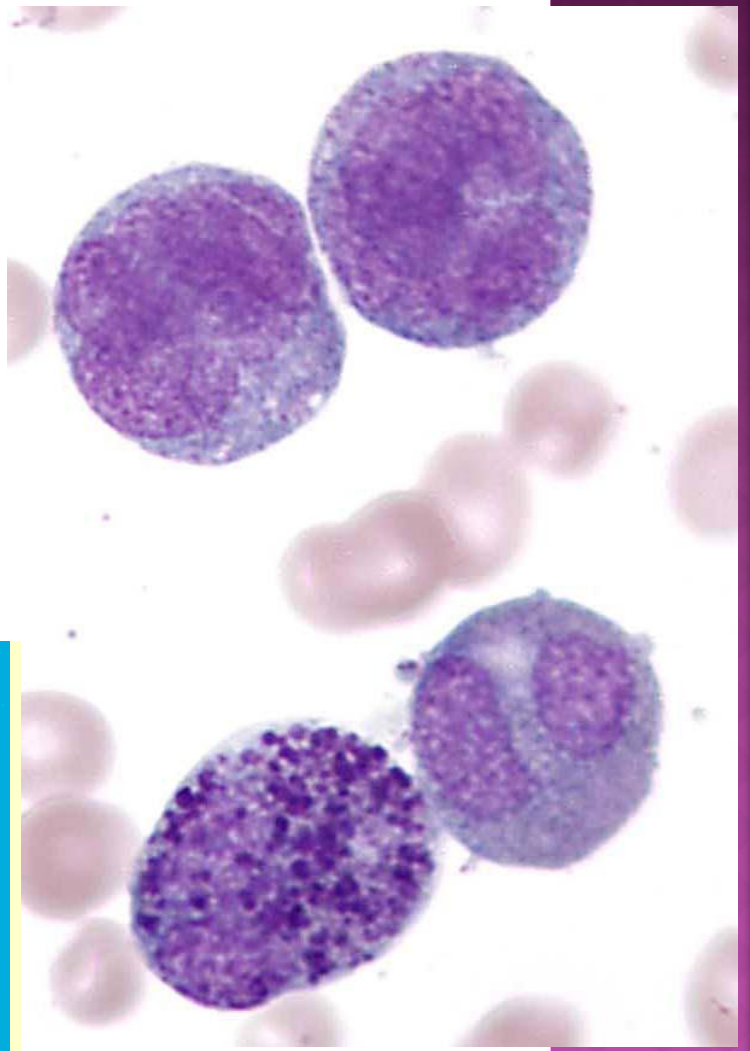
# AML WITH T(8;21)



# AML WITH RECURRENT CYTOGENETIC ABNORMALITIES: INV(16)

- Inv(16)(p13;q22) or t(16;16)(p13;q22)
- Morphologic equivalent AML-M4eo
  - Acute myelomonocytic leukemia with abnormal eosinophils
    - >20% blasts
    - >20% myeloid and monocytic elements
    - Abnormal eosinophils
      - Immature forms contain basophilic granules “eo-basos”
- Genetics
  - another “core binding factor AML”
- Better Prognosis

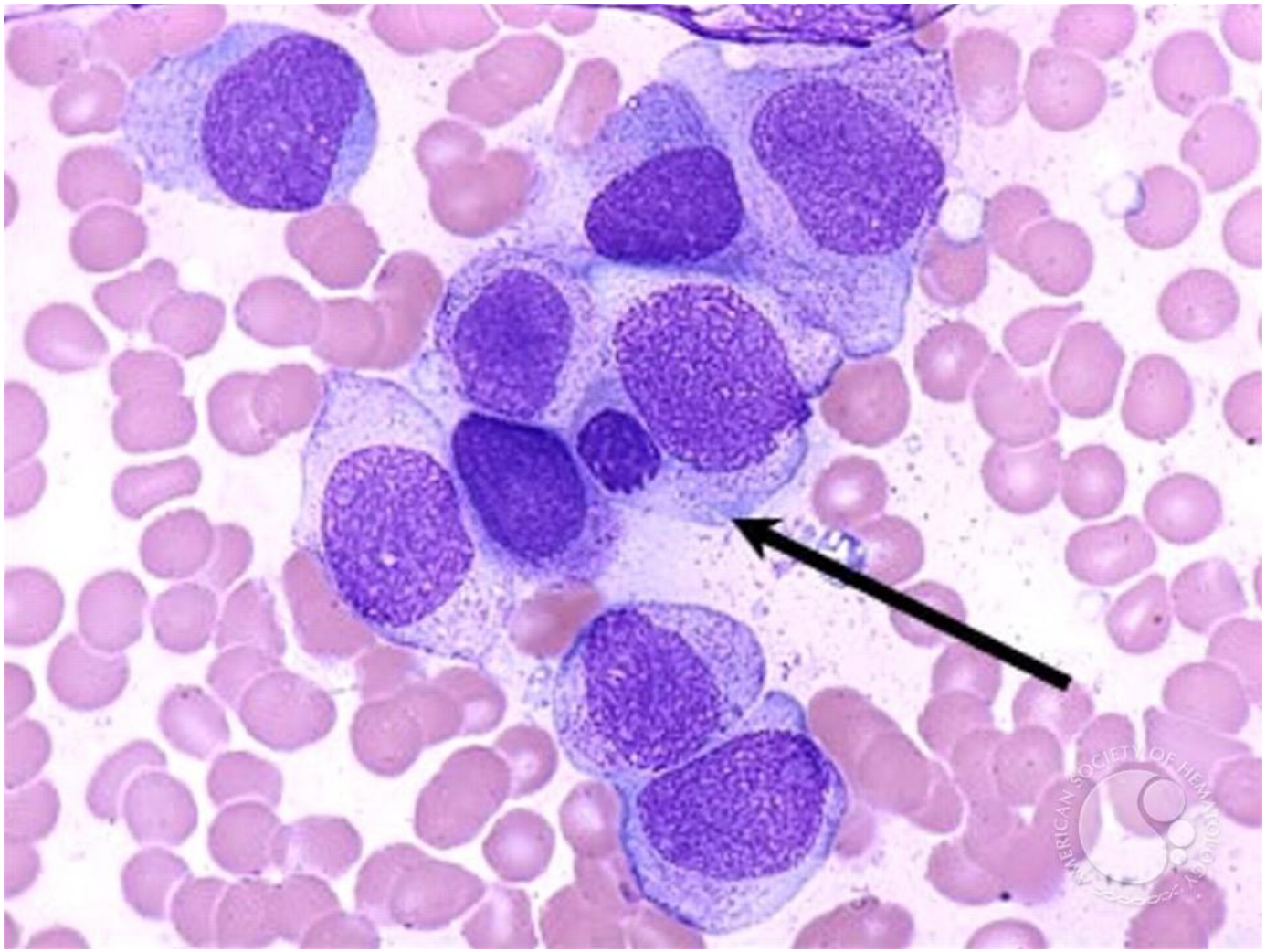


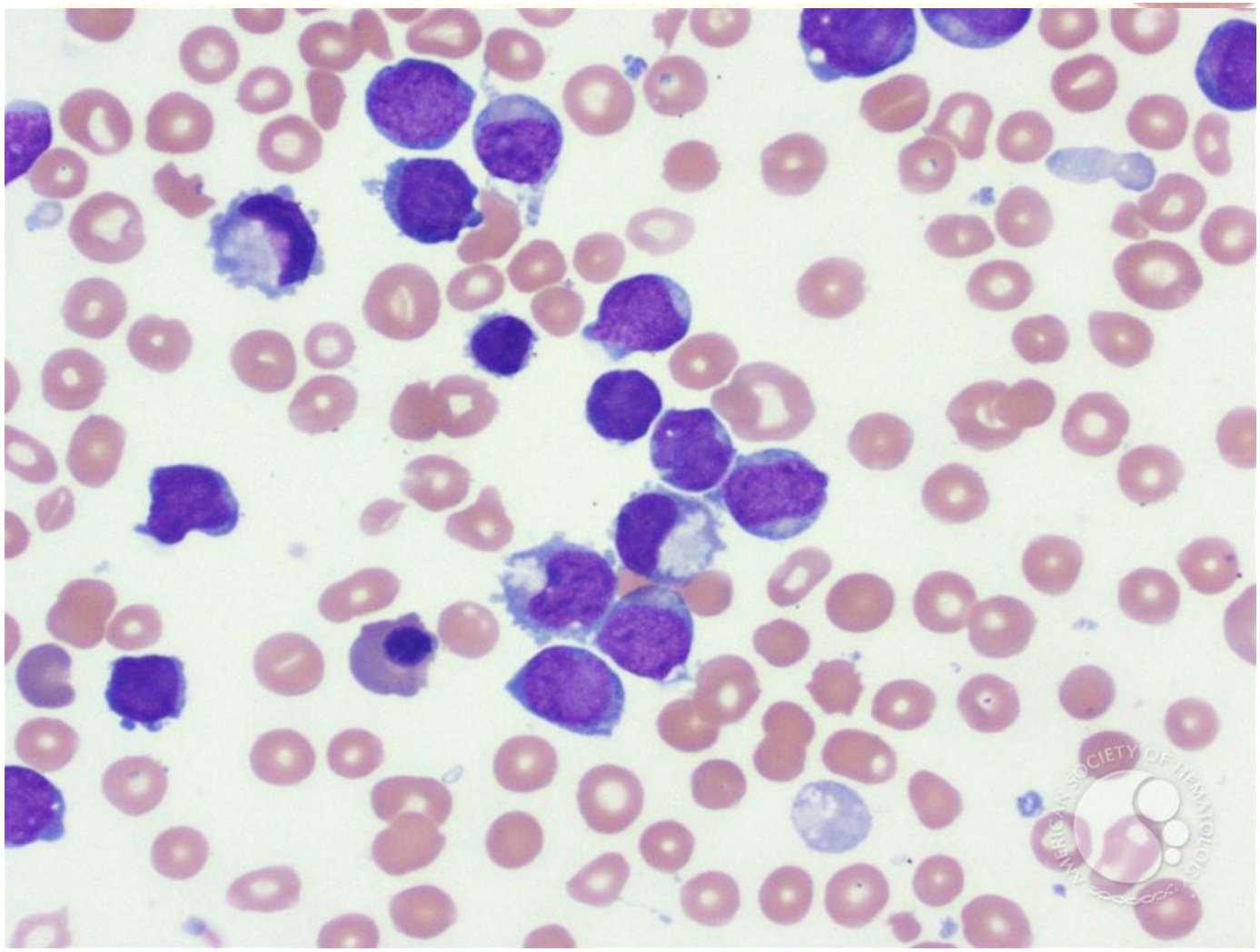
www.meds.com/leukemia/facts/facts_m4.html

# ACUTE MONOCYTIC LEUKEMIA

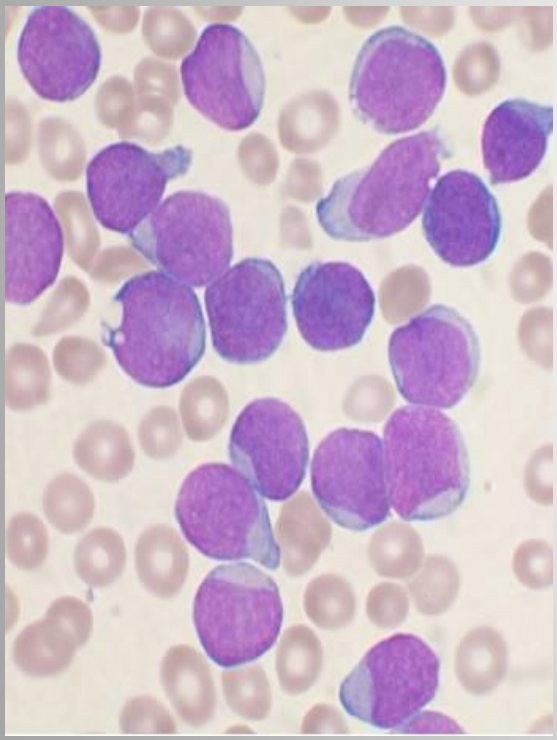
## IN ACUTE MONOBLASTIC LEUKEMIA, MONOBLASTS CONSTITUTE GREATER THAN 80% OF THE MONOCYTIC COMPONENT OF THE BONE MARROW.

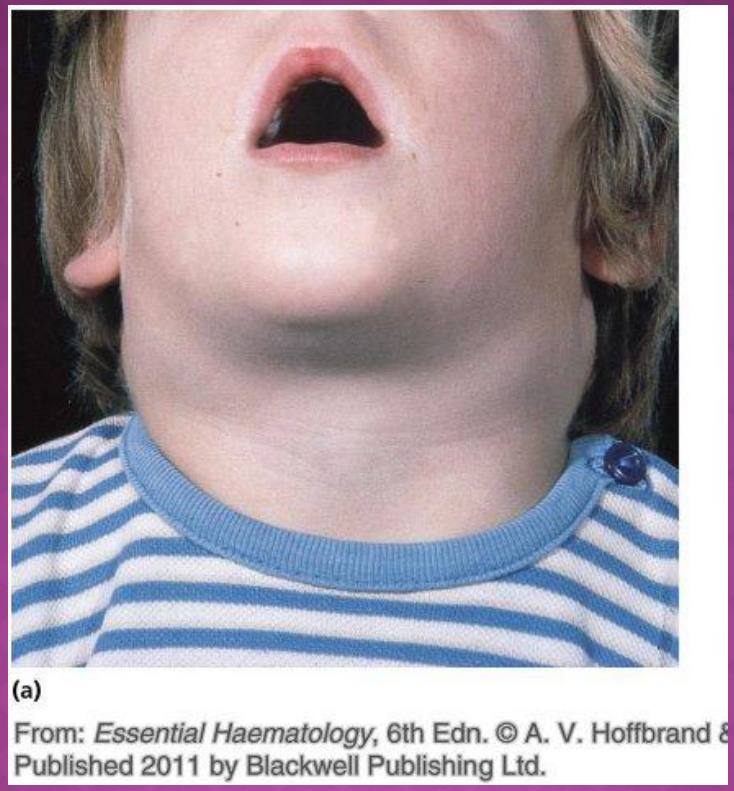


# ACUTE MEGAKARYOCYTIC LEUKEMIA MEGAKARYOBLASTS



# ACUTE LYMPHOBLASTIC LEUKEMIA (ALL)





(a)

# ALL - CLASSIFICATION

- **FAB** ( French-American-British) classification
  - Based largely on morphology
  - Little prognostic or therapeutic information to help guide treatment decisions.

- **WHO** ( World Health Organization) classification(WHO) classification
  - Revised in 2008
  - Discarded the FAB terms since morphological classification has no clinical or prognostic relevance.
  - Changed the classification to reflect increased understanding of the biology and molecular pathogenesis of ALL.

# ALL – CLASSIFICATION FAB

| Subtype | Morphology | Occurrence (%) |
|---|---|---|
| L1 | Small round blasts clumped chromatin | 75 |
| L2 | Pleomorphic larger blasts clefted nuclei, fine chromatin | 20 |
| L3 | Large blasts, nucleoli, vacuolated cytoplasm | 5 |

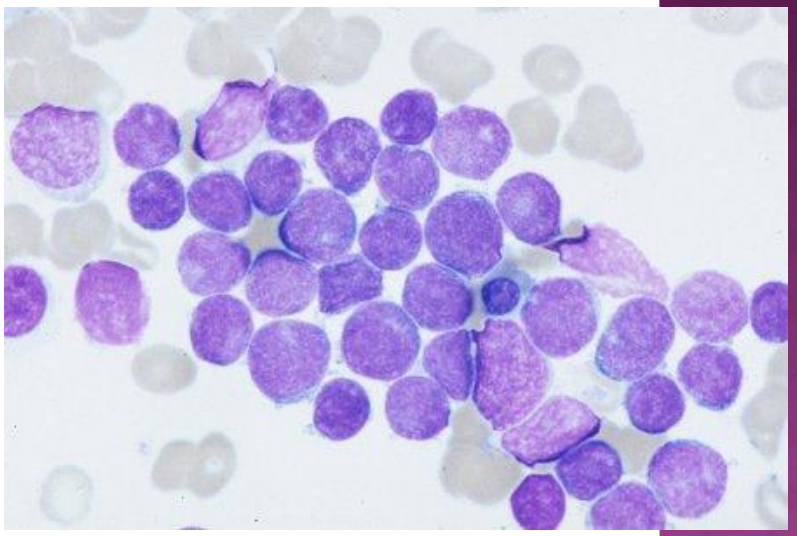
# FAB ACUTE LYMPHOBLASTIC LEUKEMIA

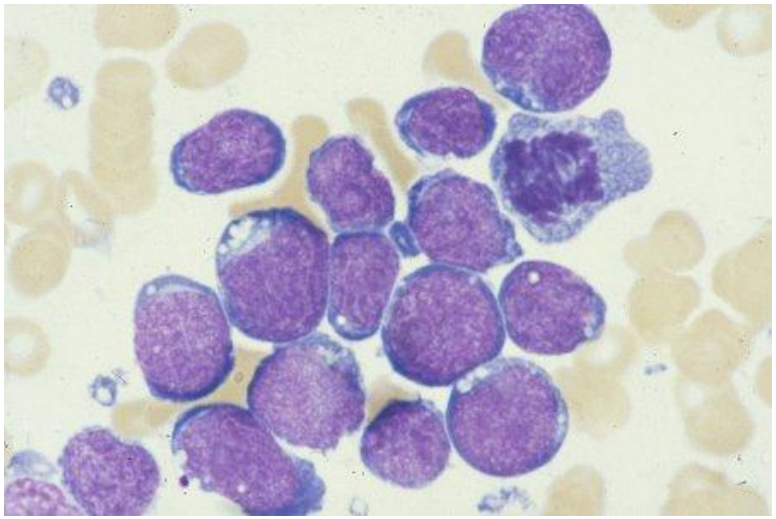
Acute lymphoblastic leukemia (ALL)*

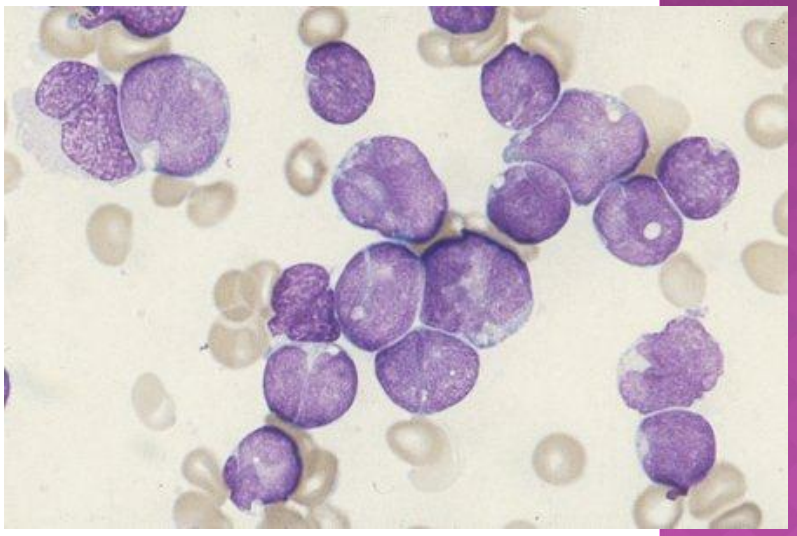
L-1 85%

L-2 14%

L-3 (Burkitt's) 1% childhood







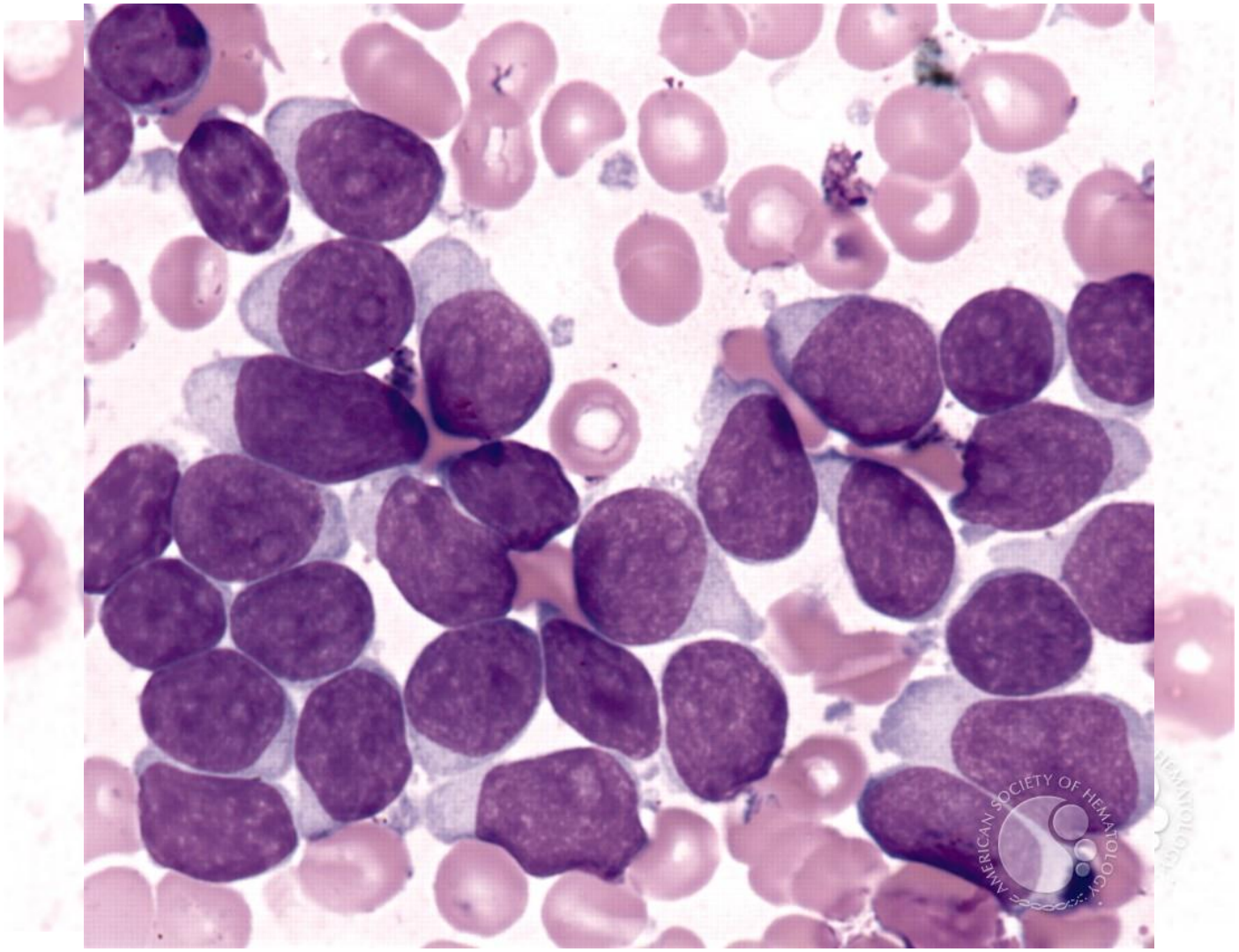
# ALL - CLASSIFICATION WHO

- Uses immunophenotypic classification :
  - Acute lymphoblastic leukemia/lymphoma (Former Fab L1/L2)
    - **Precursor B** acute lymphoblastic leukemia/lymphoma.
      - Cytogenetic subtypes:
        - t(12;21)(p12,q22) TEL/AML-1
        - t(1;19)(q23;p13) PBX/E2A
        - t(9;22)(q34;q11) ABL/BCR
        - T(V,11)(V;q23) V/MLL
    - **Precursor T** acute lymphoblastic leukemia/lymphoma
  - Burkitt's leukemia/lymphoma (Former FAB L3) ( mature B cell ALL)
  - Biphenotypic acute leukemia ( 2 to 5%)

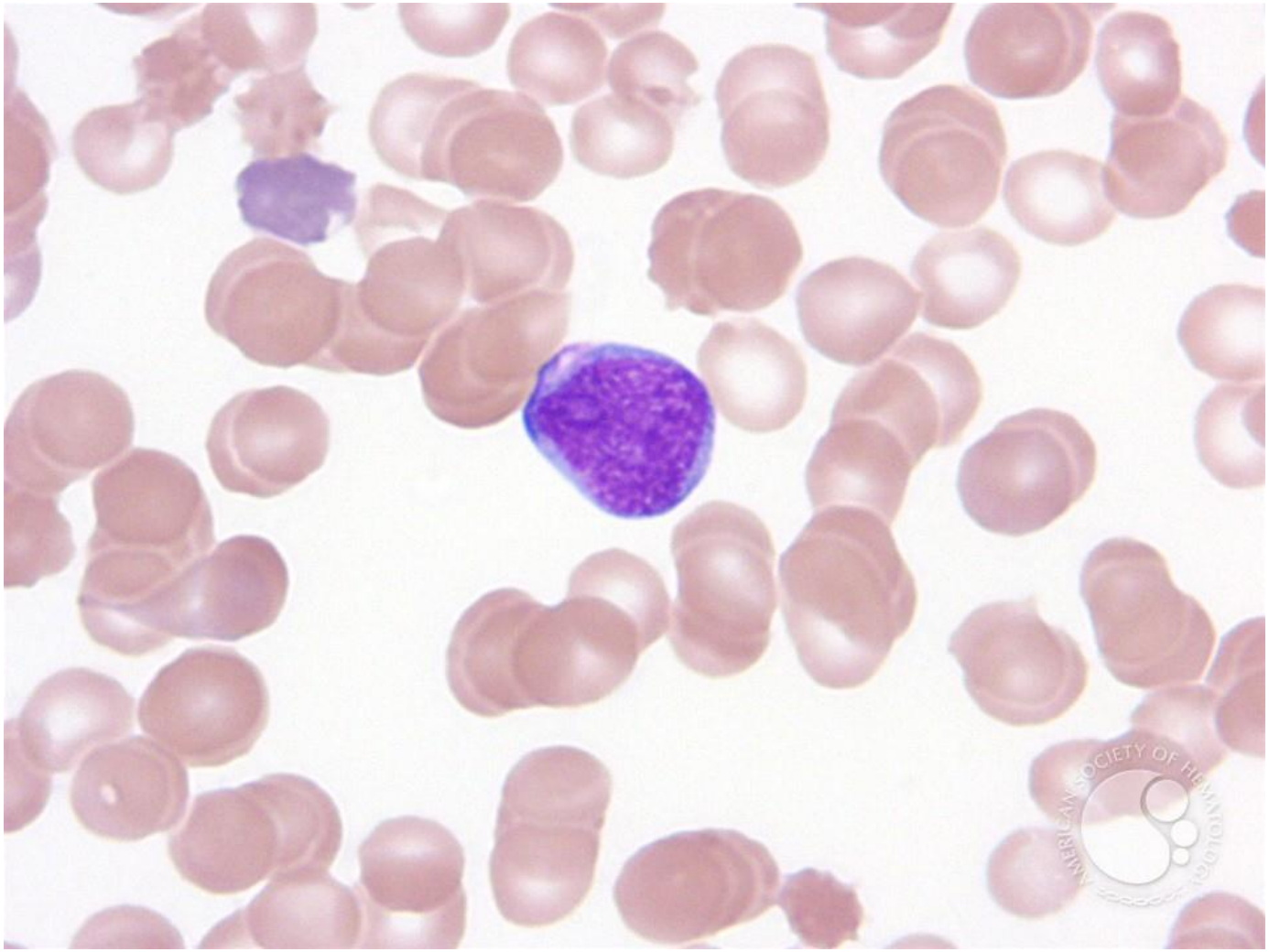
# ALL: WHO CLASSIFICATION

- Precursor B-cell
  - TdT+, CD34+, surface Ig negative
  - CD19, CD22, CD79a positive, CD 20 variable
- Precursor T-cell
  - TdT+
  - CD3+, often CD2, CD4, CD8 positive
- Mature B-cell (Burkitt)
  - CD19, CD22+
  - Surface Ig positive

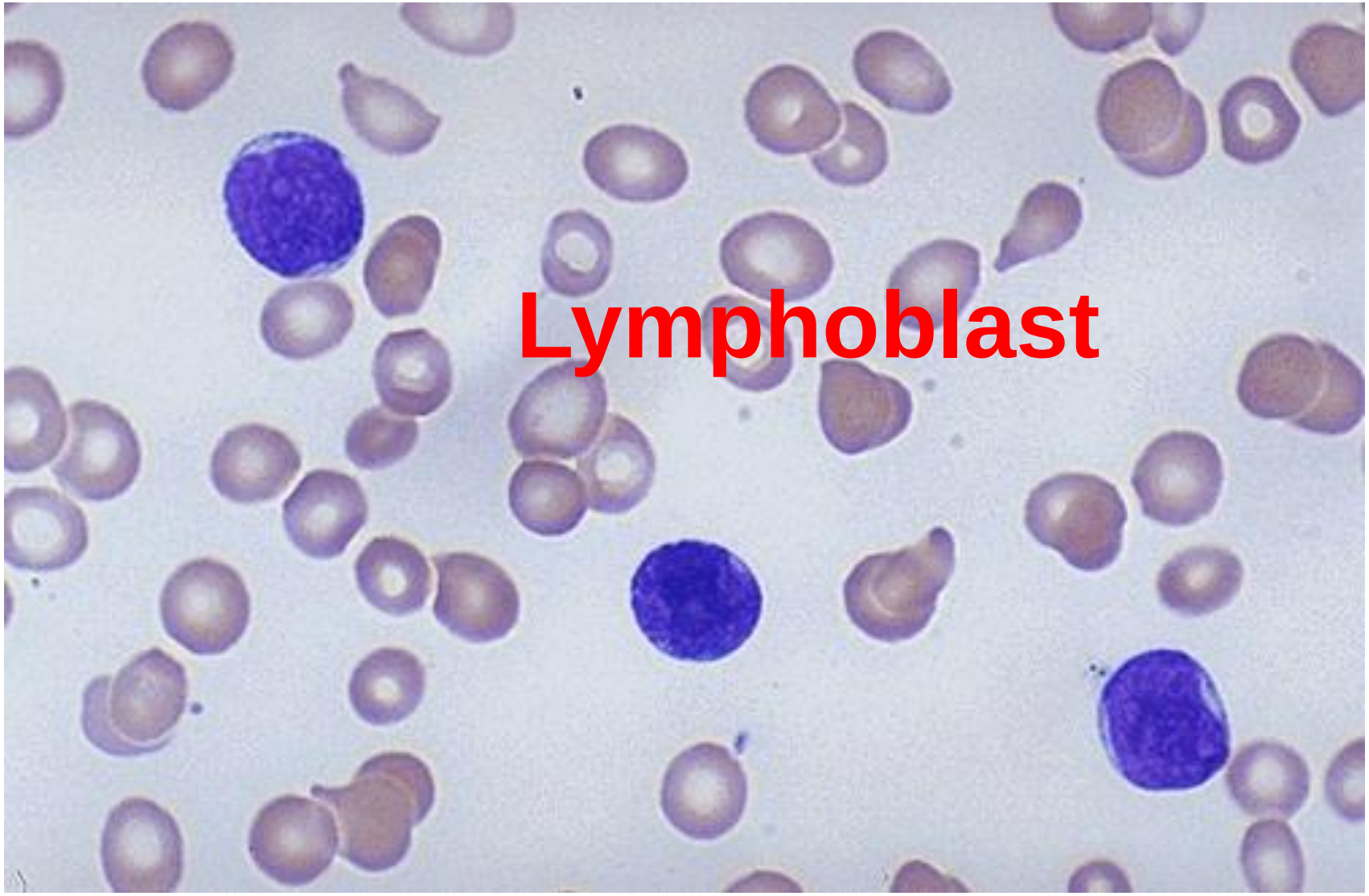
# PRECURSOR B-LYMPHOBLASTIC LEUKEMIA



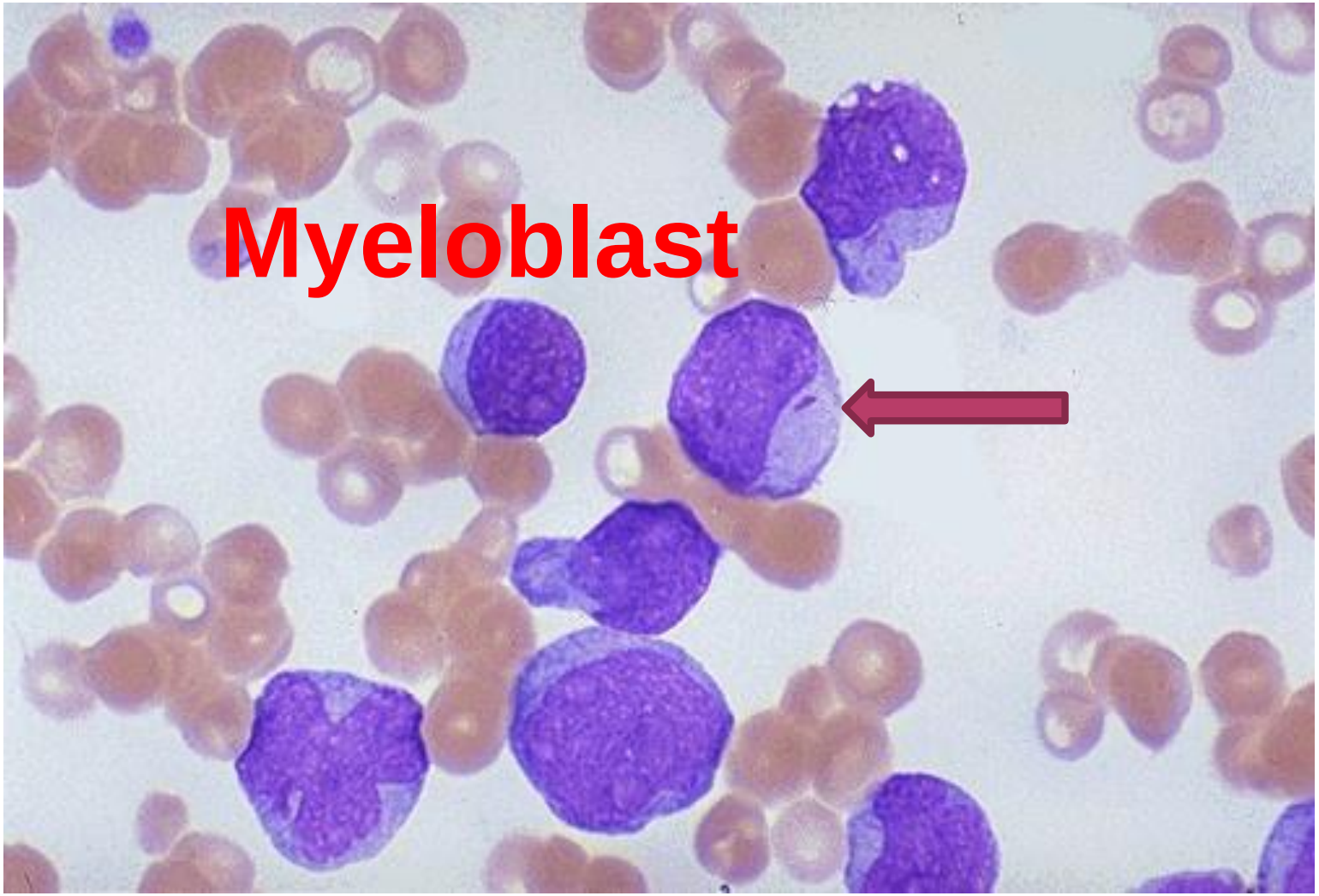
# WHAT IS THIS CELL?



# WHAT ARE THESE CELLS?



# WHAT ARE THESE CELLS?

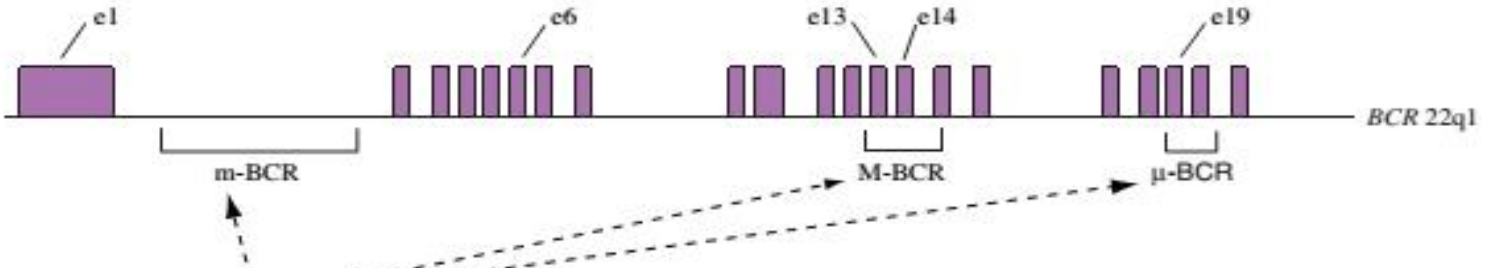


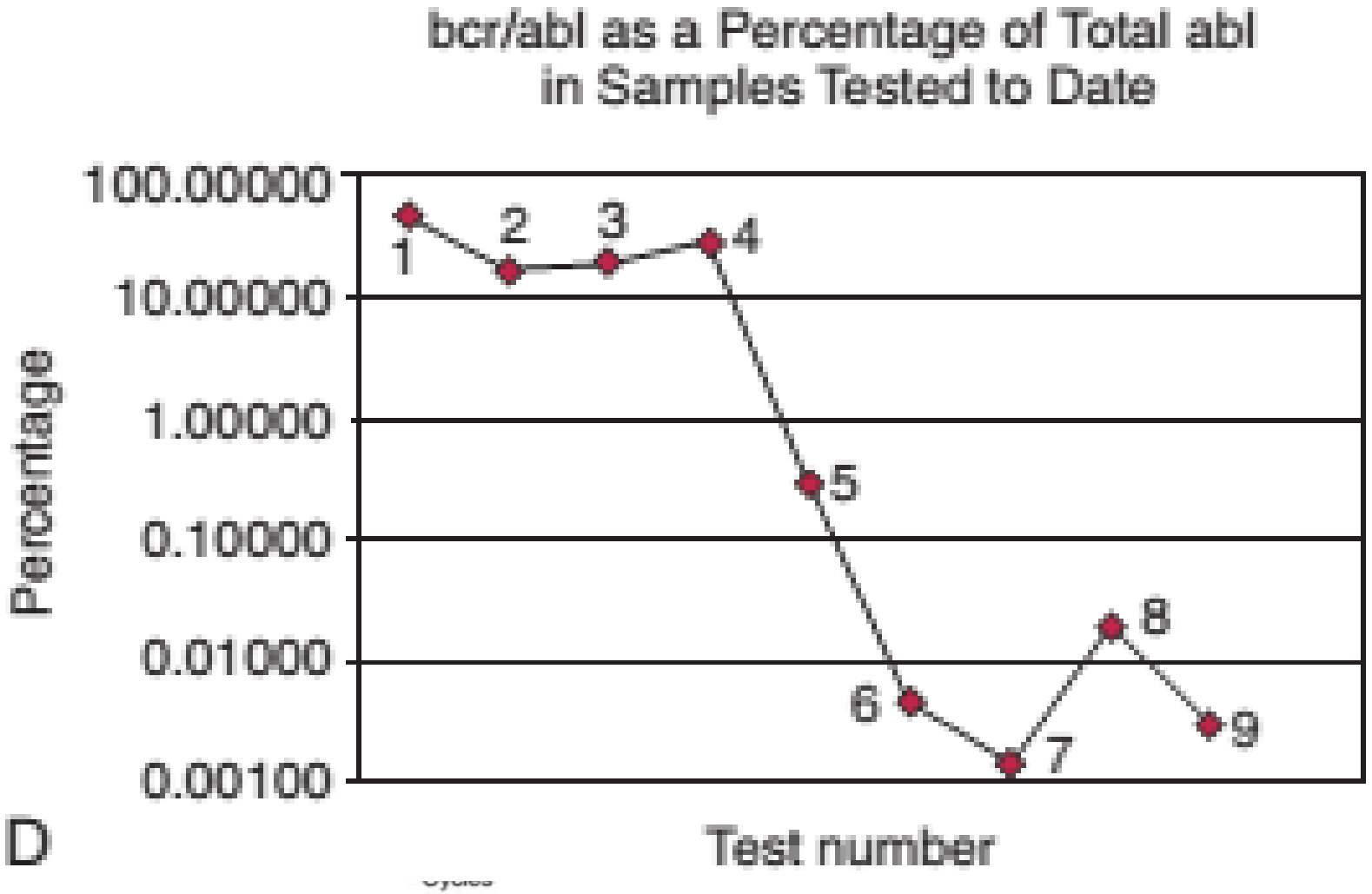
# MYELOPROLIFERATIE NEOPLASMS

# MYELOPROLIFERATIVE NEOPLASMS

- Chronic Myelogenous Leukemia, BCR-ABL1 Positive
- Ph-Negative Myeloproliferative Neoplasms:

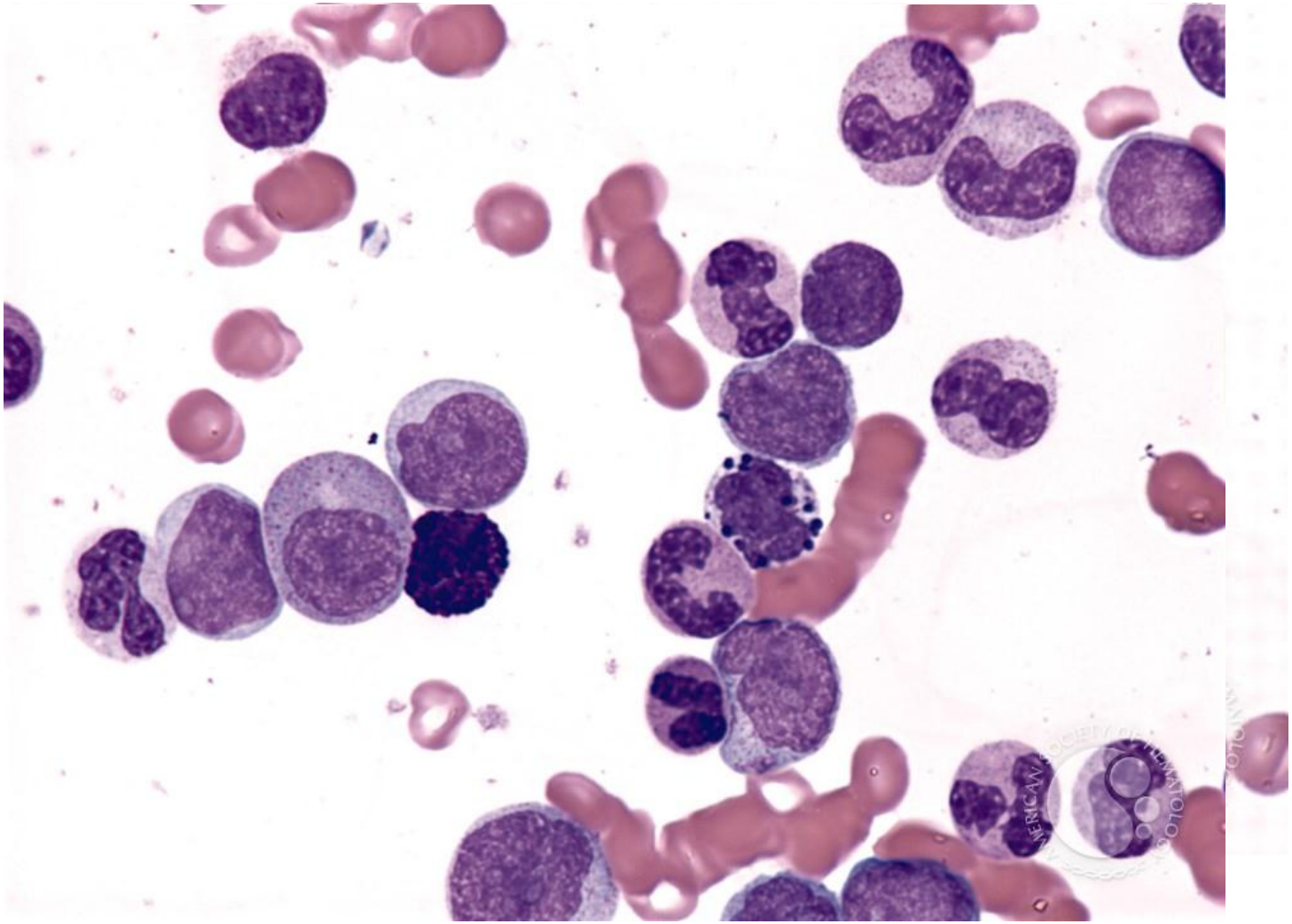
1. Polycythemia Vera,
2. Essential Thrombocythemia
3. Primary Myelofibrosis
4. juvenile myelomonocytic leukemia
5. Hypereosinophilic syndrome (HES)
6. Chronic Neutrophilic leukemia
7. Mastocytosis and the KIT D816V Gene Mutation
8. Neoplastic Disorders Associated with Eosinophilia


e1
e6
e13
e14
e19
BCR 22q1
m-BCR
M-BCR
µ-BCR

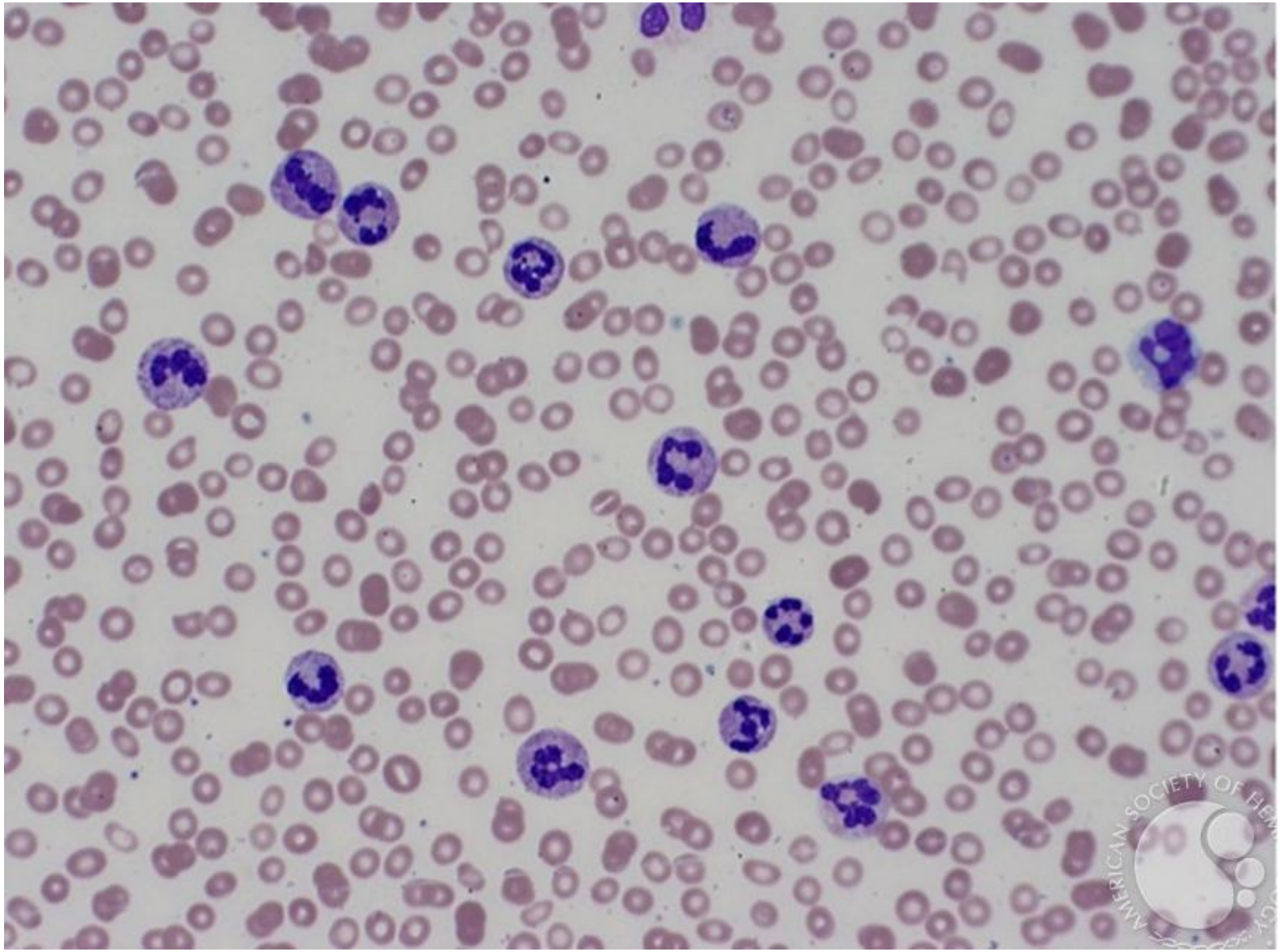

bcr/abl as a Percentage of Total abl
in Samples Tested to Date
100.00000
10.00000
1.00000
0.10000
0.01000
0.00100
Percentage
1
2
3
4
5
6
7
8
9
Test number

D

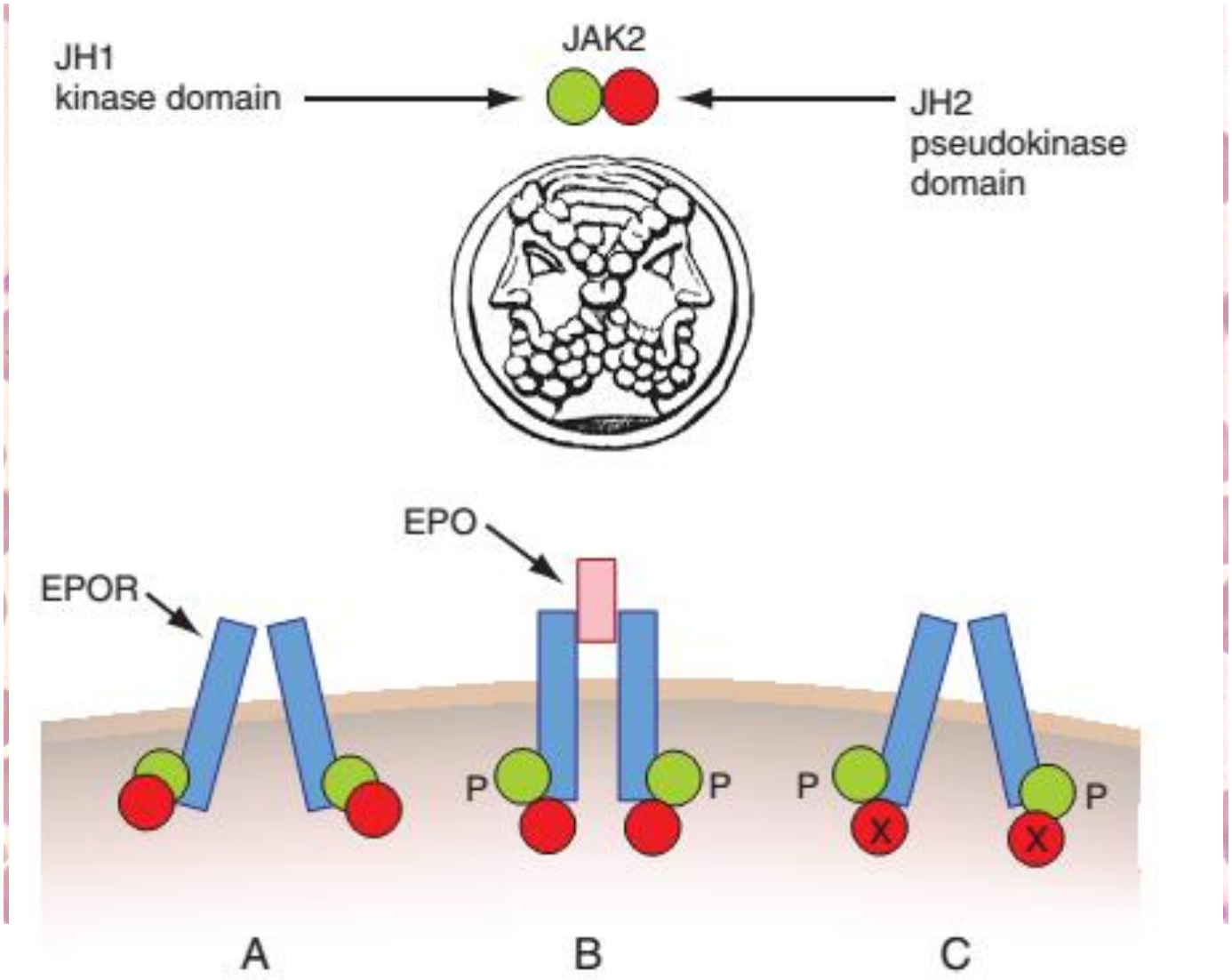
# ACCELERATED PHASE OF CML



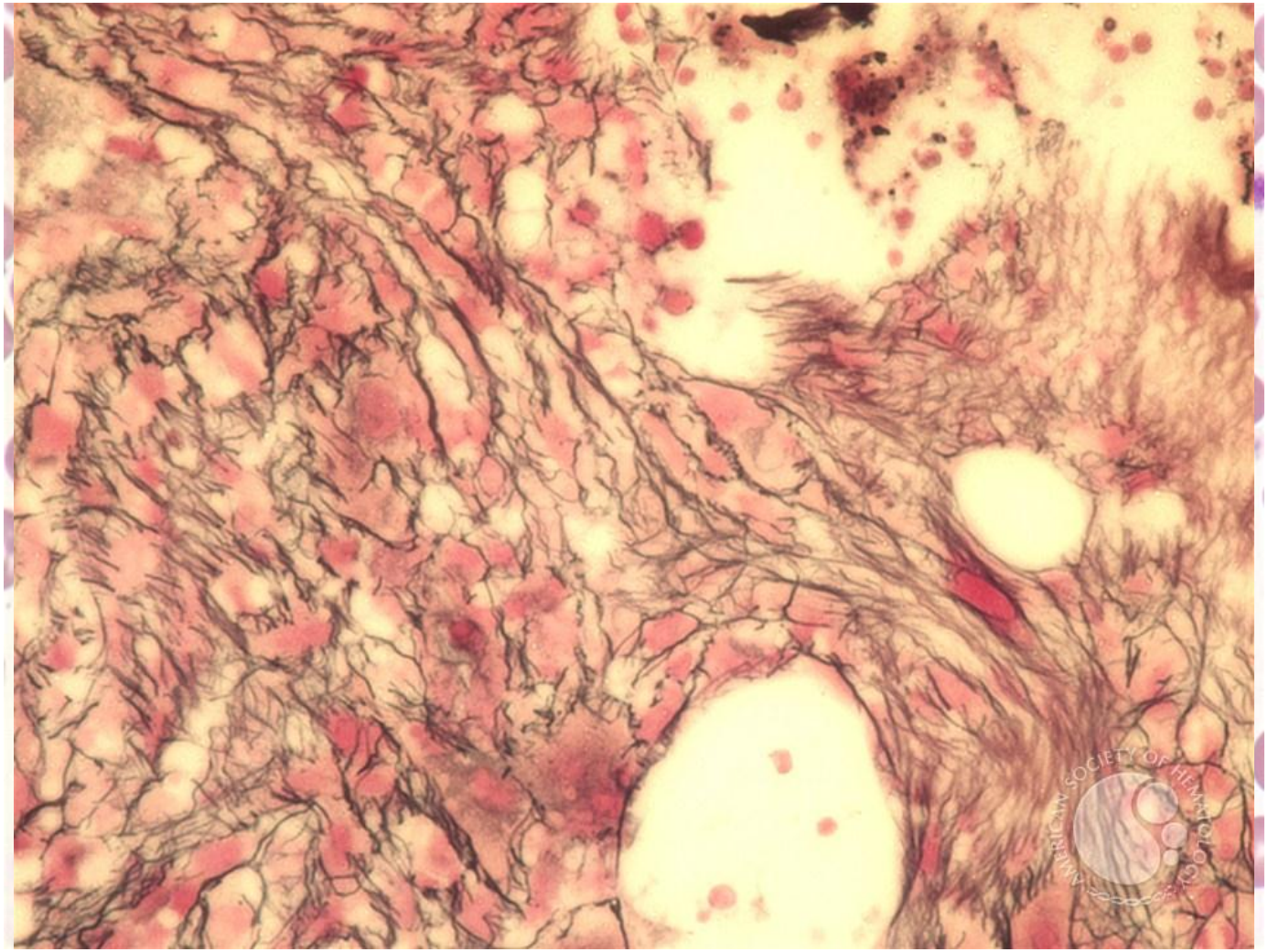
# CHRONIC NEUTROPHILIC LEUKEMIA



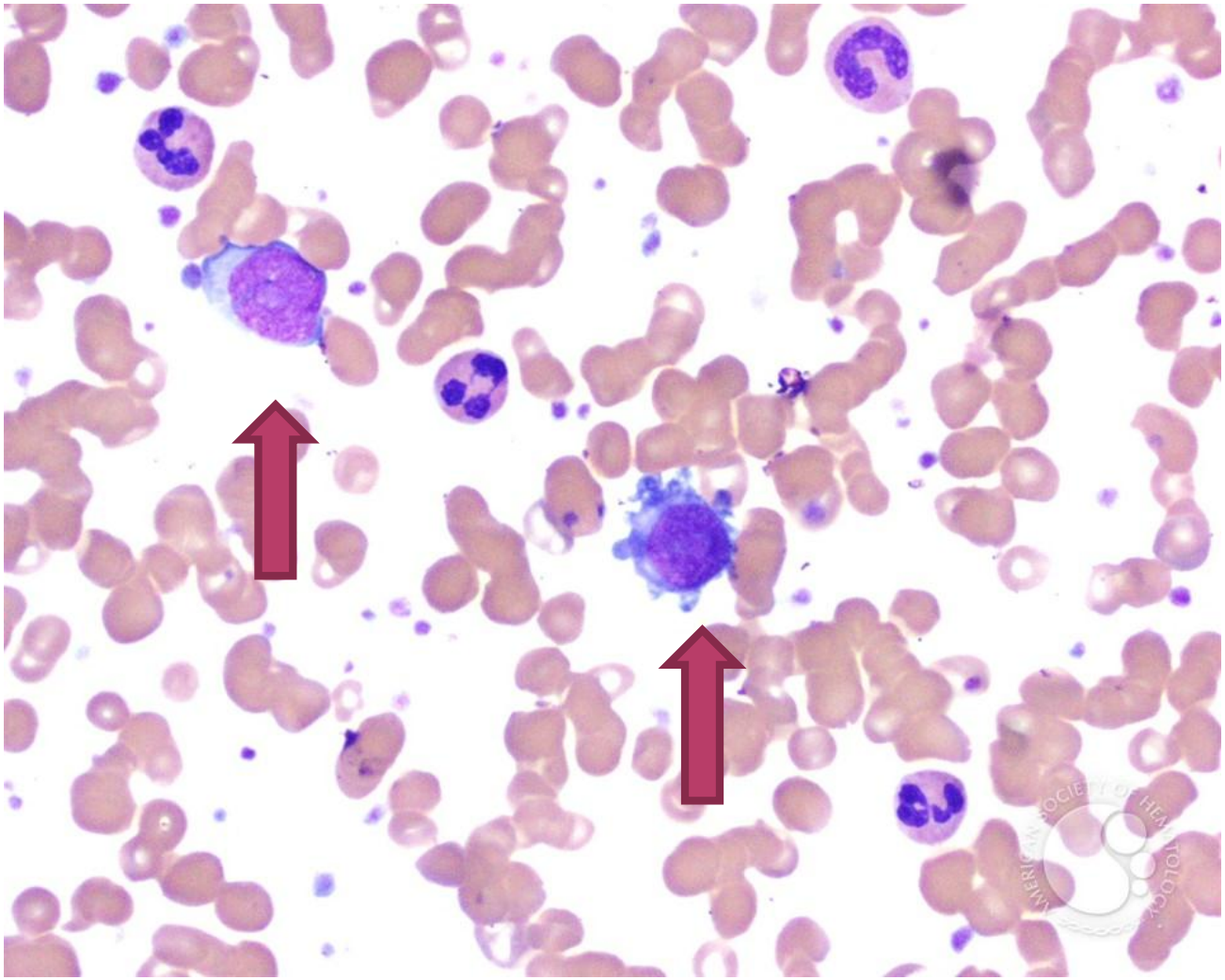
# PH-NEGATIVE MYELOPROLIFERATIVE NEOPLASMS: POLYCYTHEMIA VERA, ESSENTIAL THROMBOCYTHEMIA, AND PRIMARY MYELOFIBROSIS-JAK2 AND MPL GENE MUTATIONS



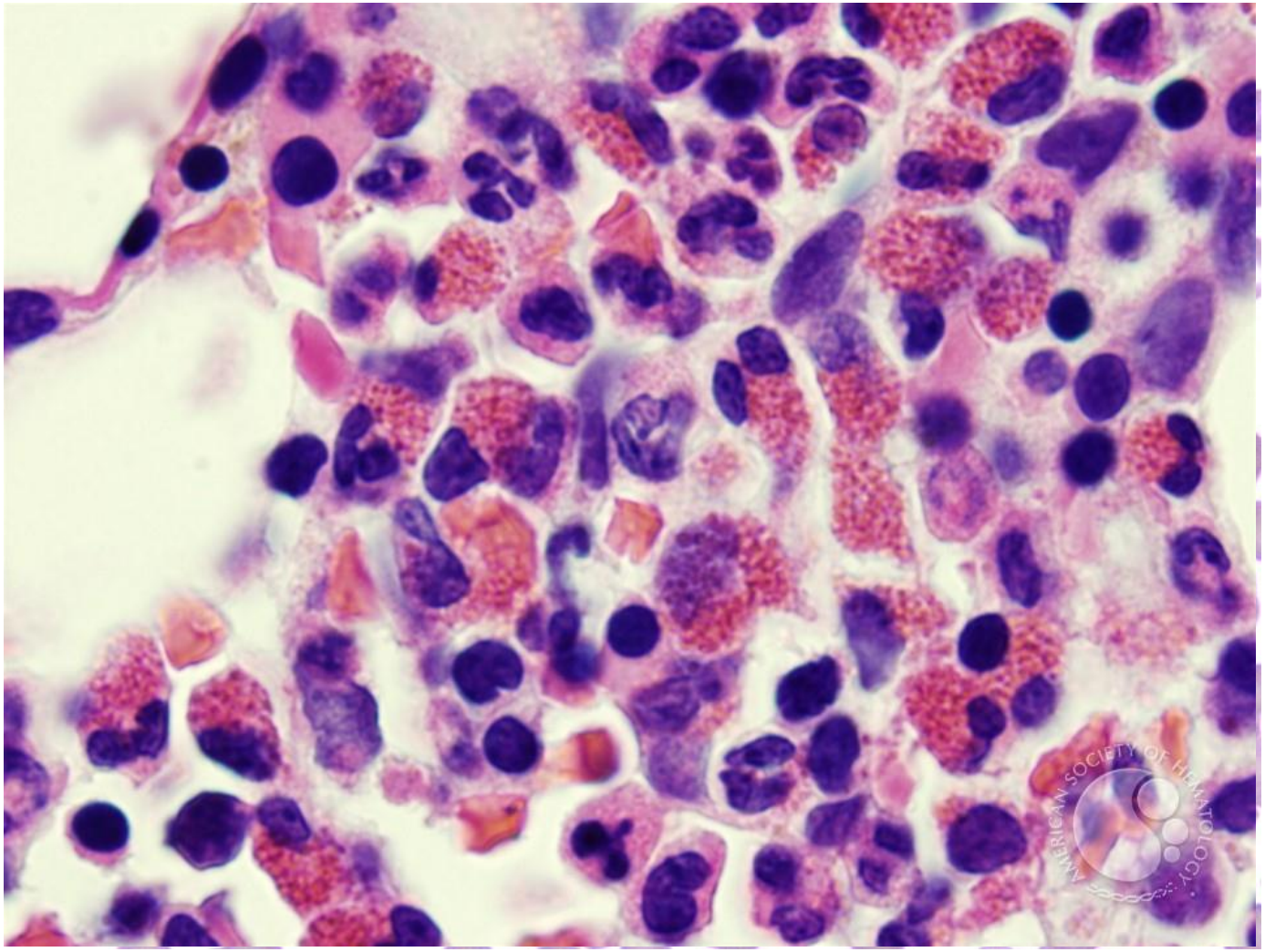
# MYELOFIBROSIS: PERIPHERAL BLOOD
# A MEGAKARYOCYTIC NUCLEUS ASSOCIATED WITH LARGE PLATELET FORMS IS PICTURED WITH AN EOSINOPHIL.



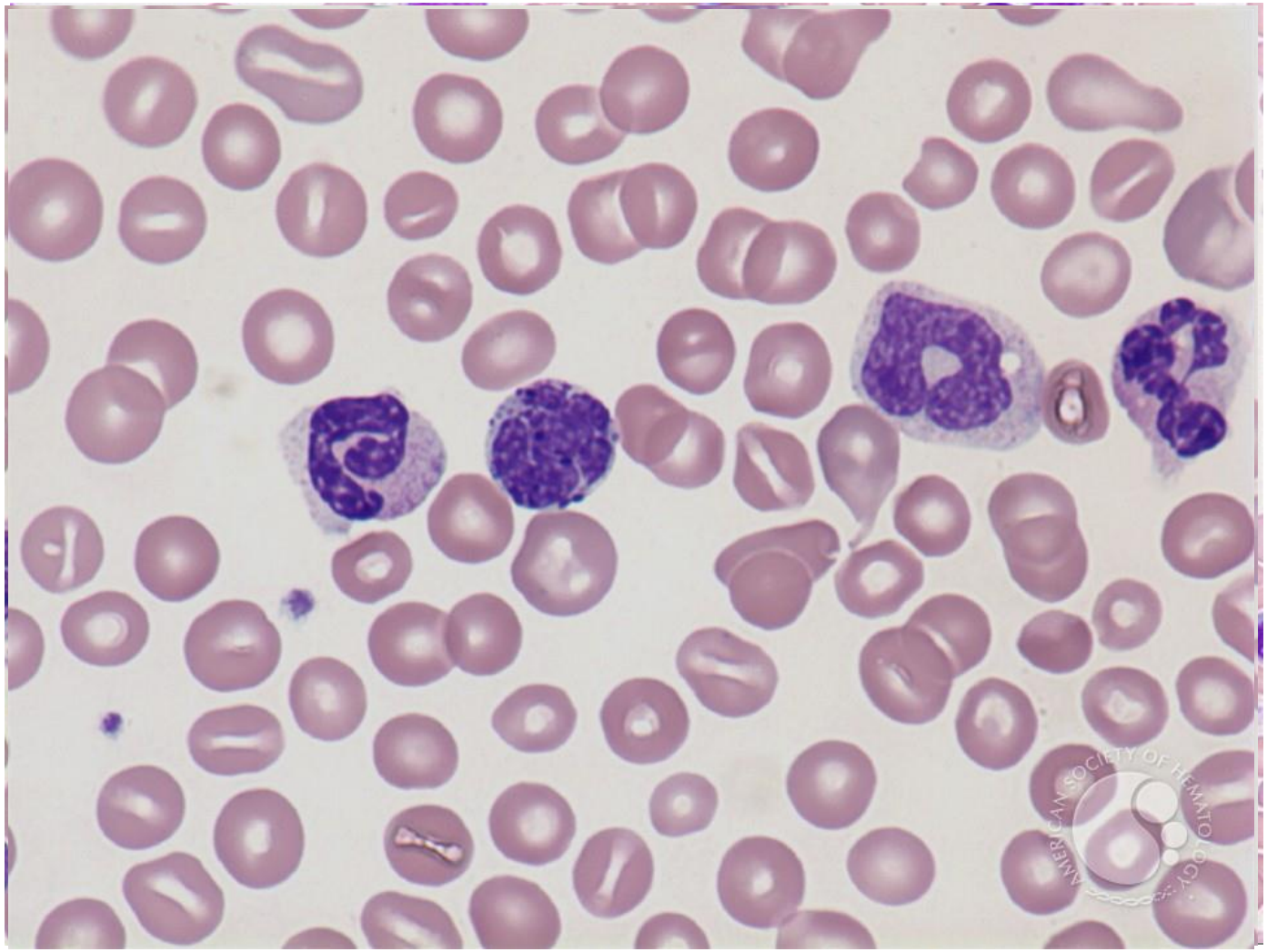
# MYELOFIBROSIS



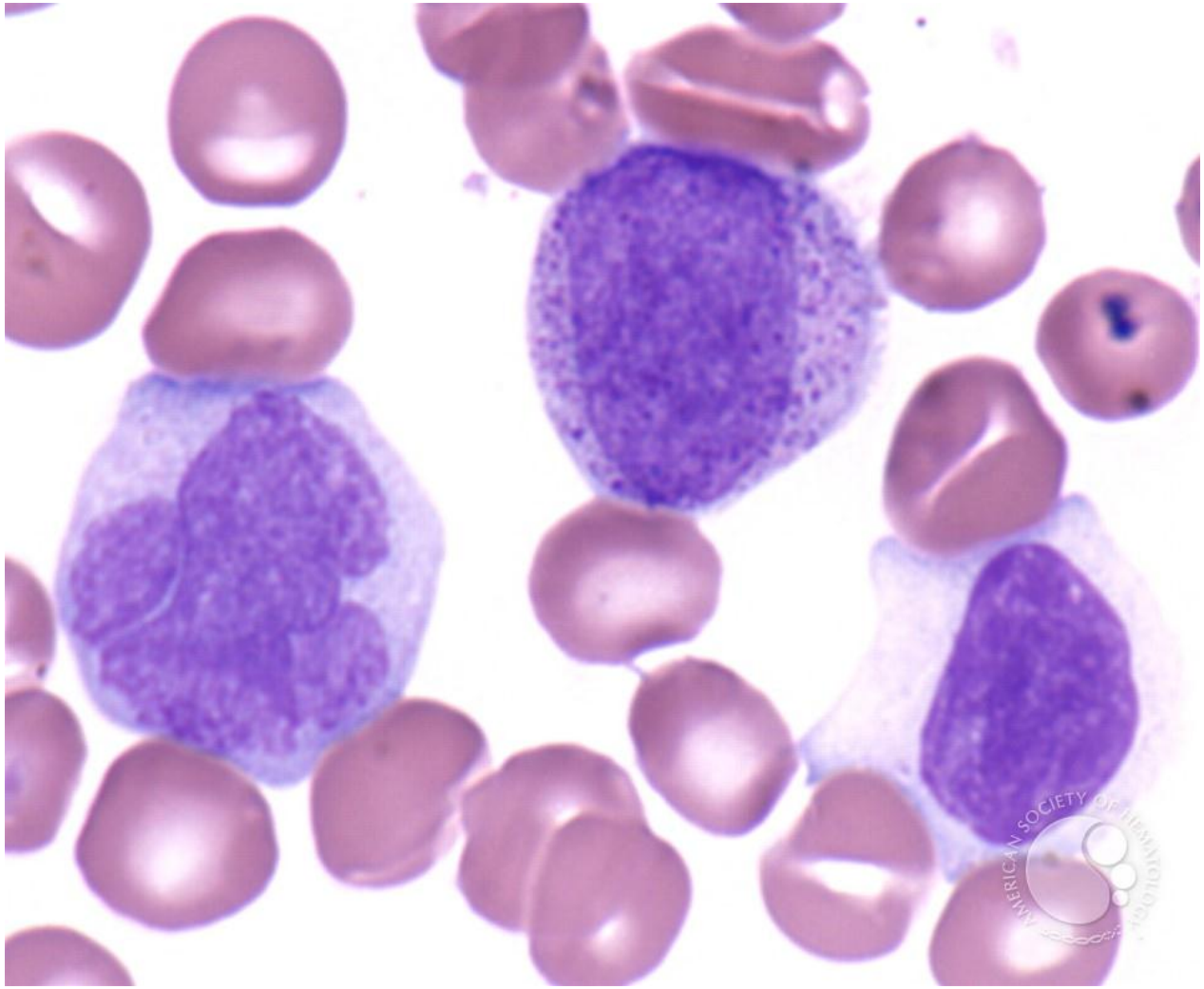
# HYPEREOSINOPHILIA



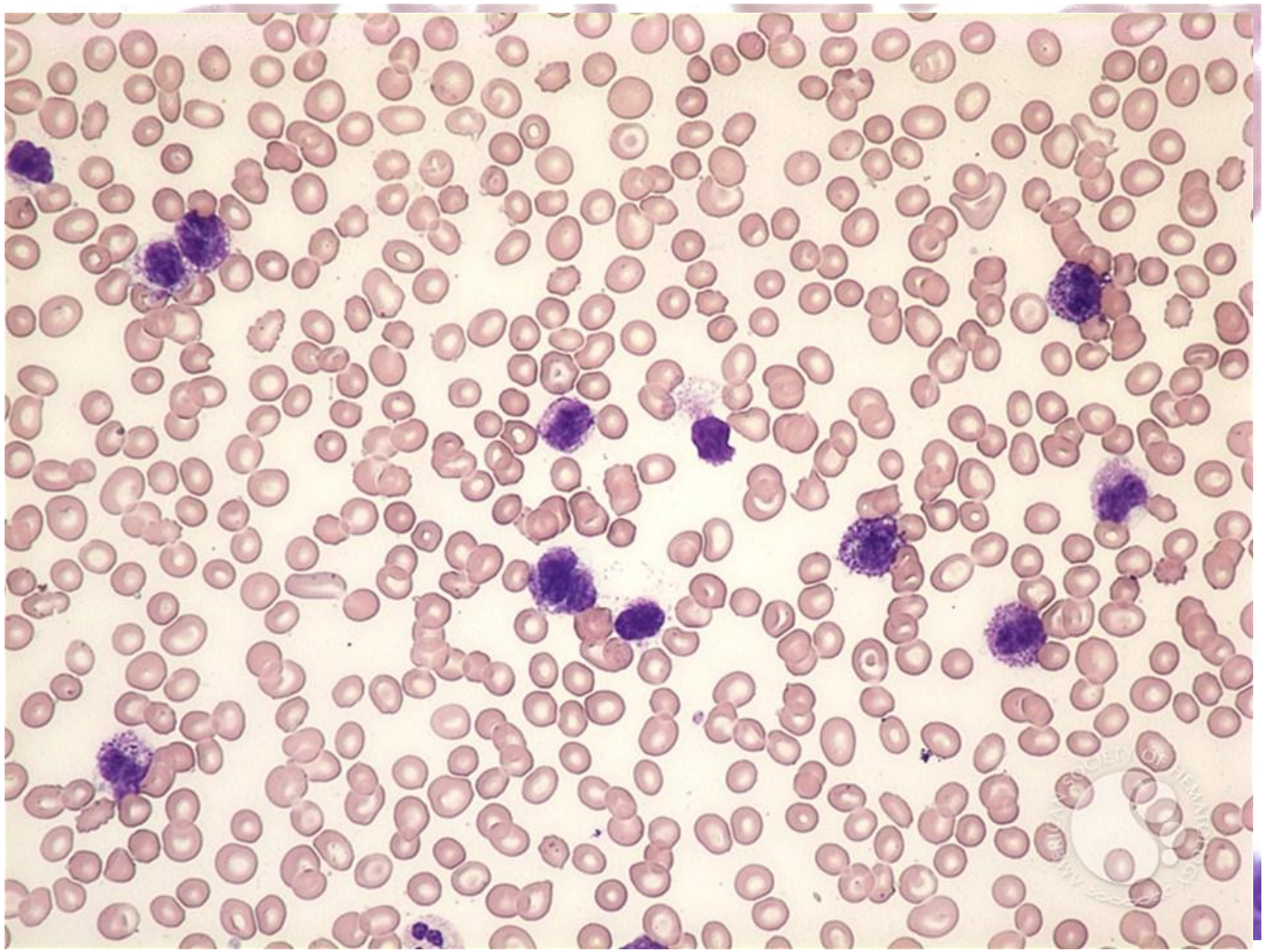
# JUVENILE MYELOMONOCYTIC LEUKEMIA
# BASOPHILS MAY BE FOUND IN THE PERIPHERAL BLOOD



# CMMOL



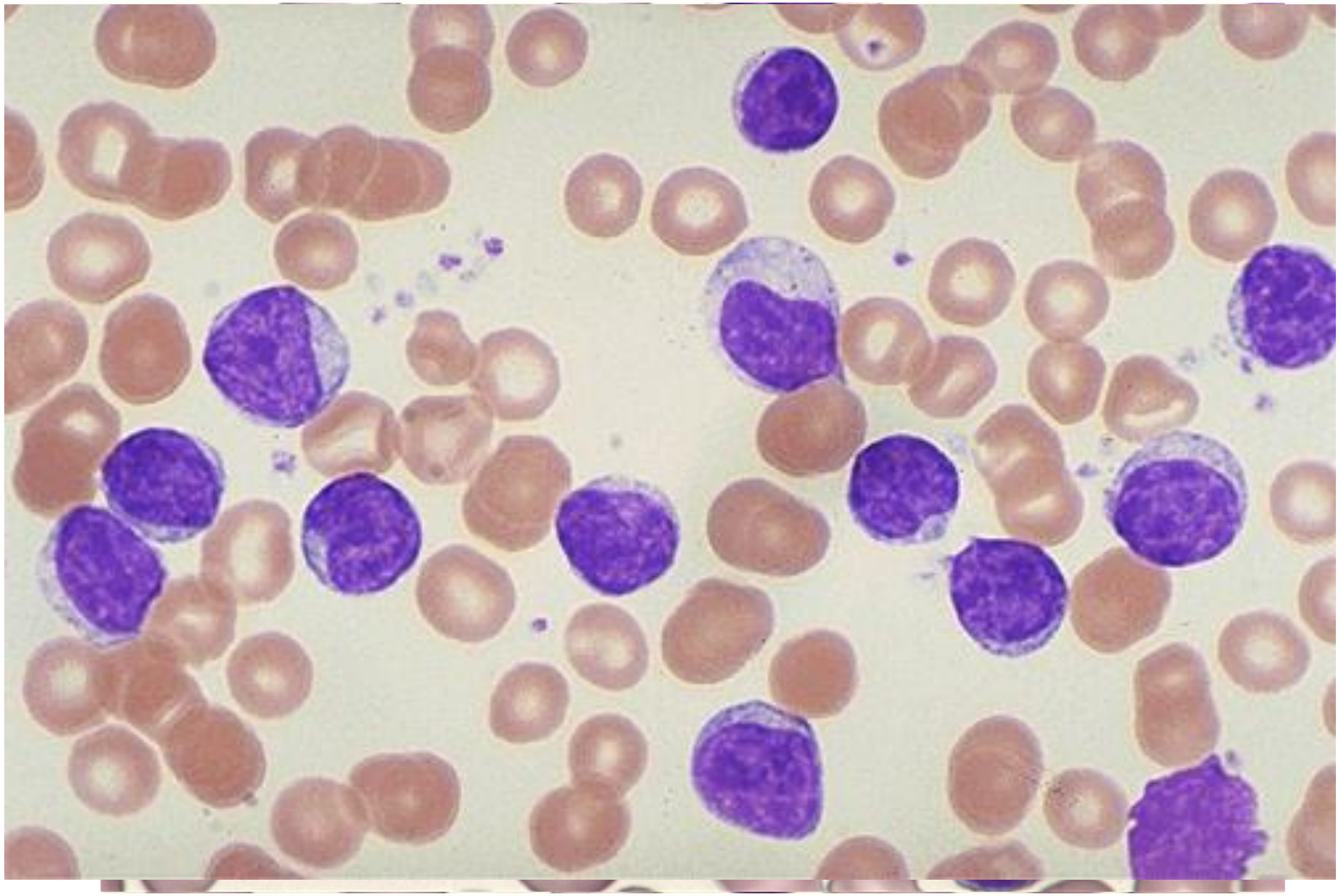
# MAST CELL LEUKEMIA
# SOME OF THE CIRCULATING MAST CELLS SPONTANEOUSLY DEGRANULATE.



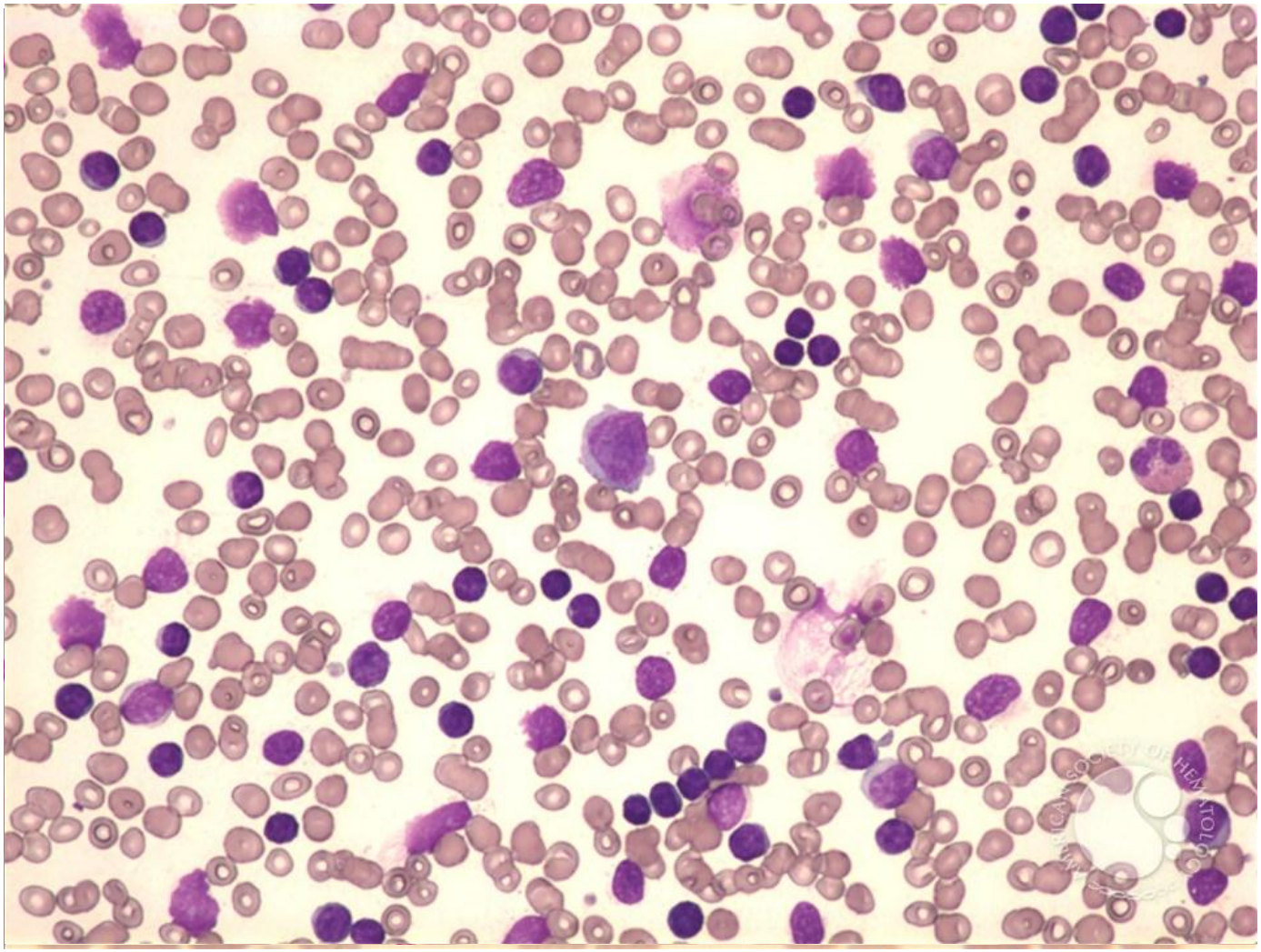
# LYMPHOPROLIFRATIVE NEOPLASM

# CHRONIC LYMPHOCYTIC LEUKEMIA

## FEW PROLYMPHOCYTES (UP TO 10%) MAY BE SEEN IN MIXED CELL TYPE CLL.

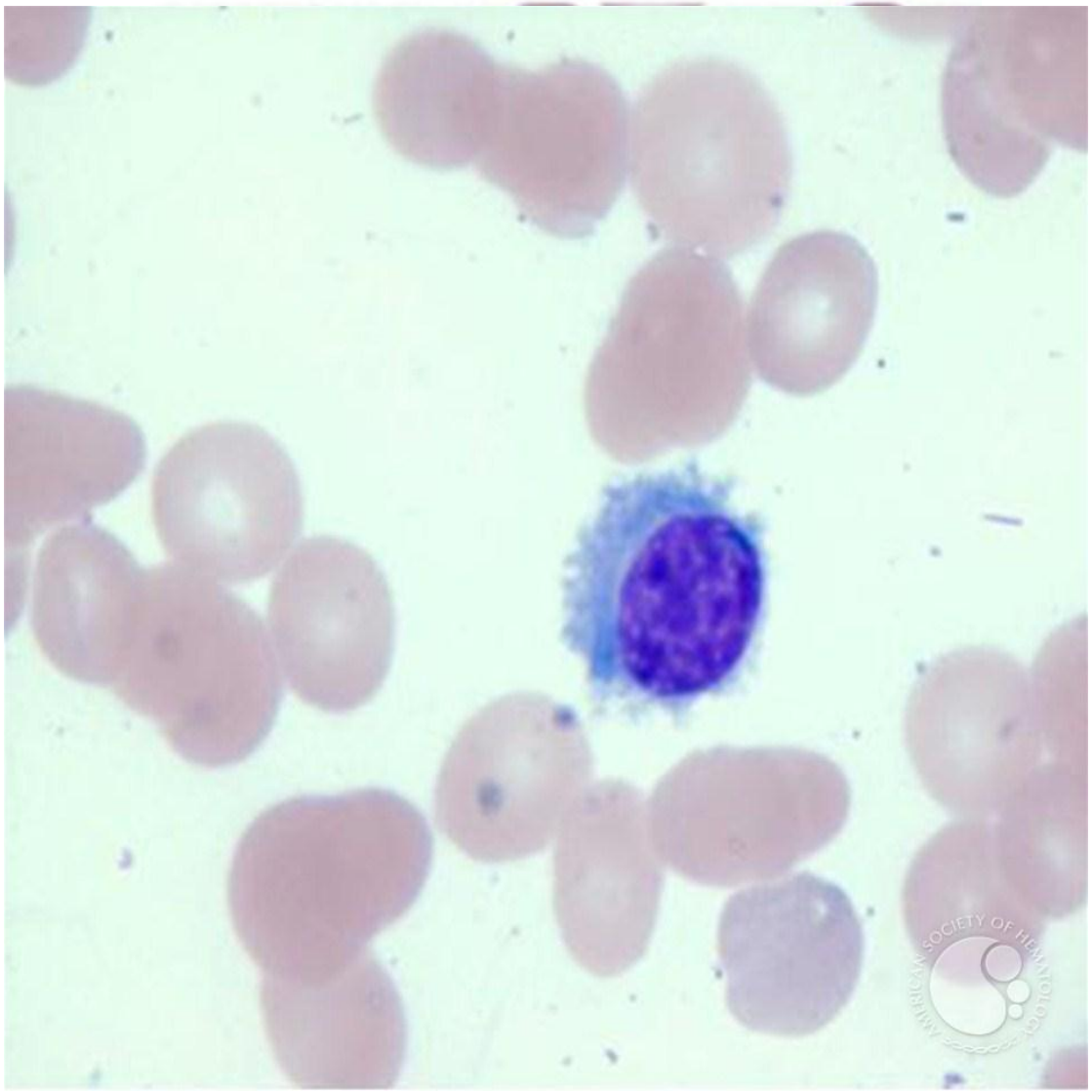


# PROLYMPHOCYTES IN PLL

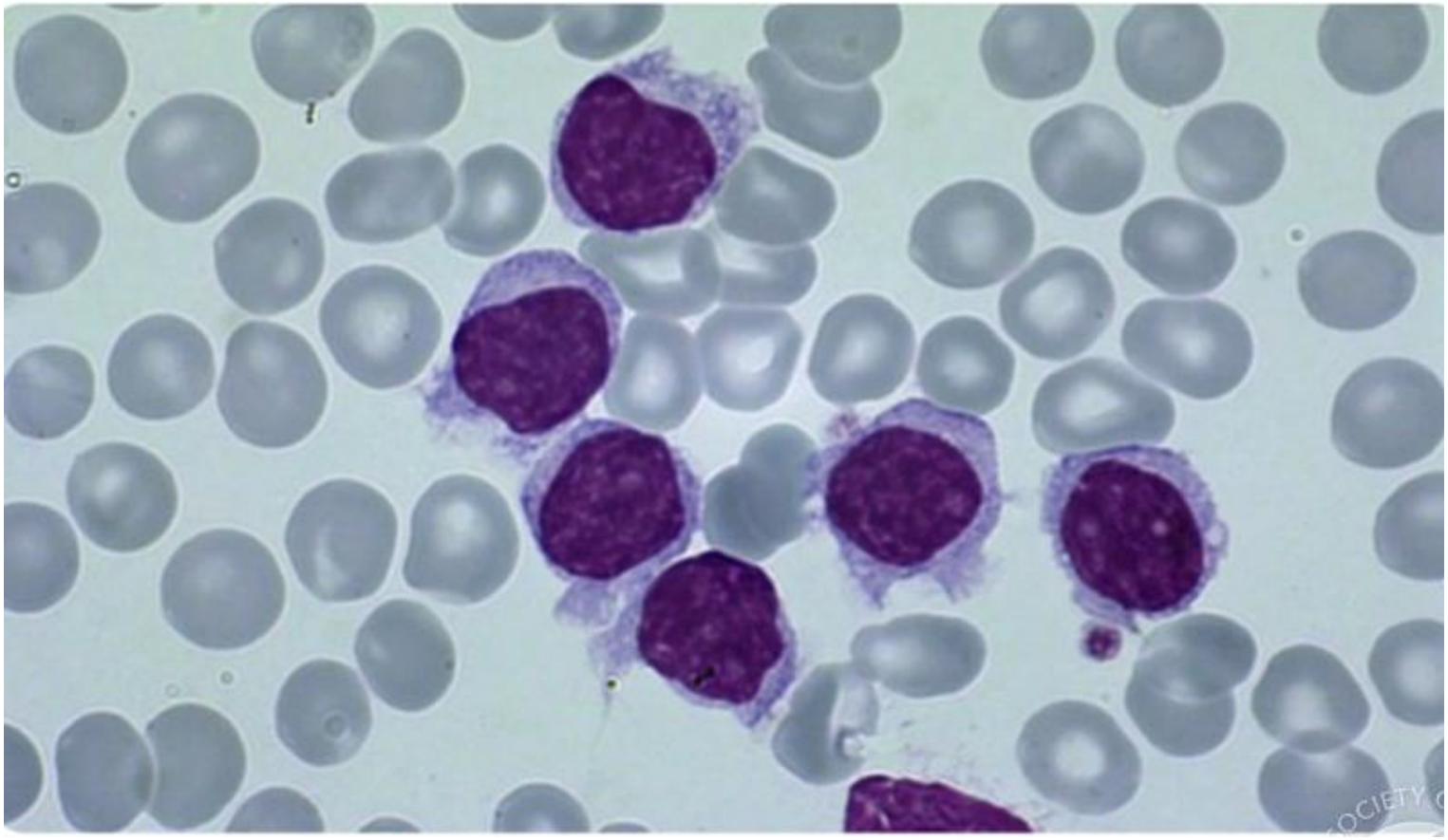


# HAIRY CELL LEUKEMIA

## (HAIRY CELL)

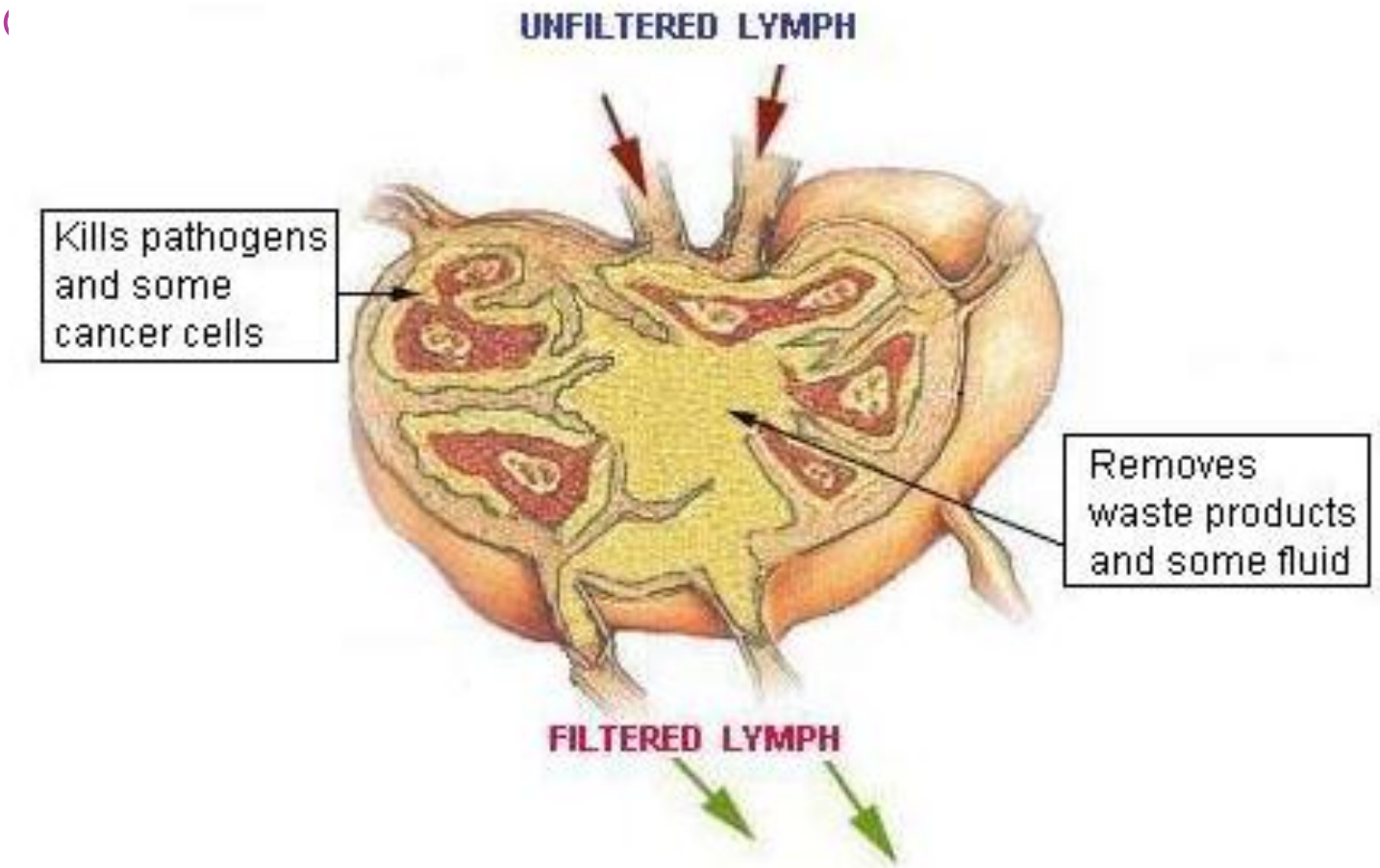


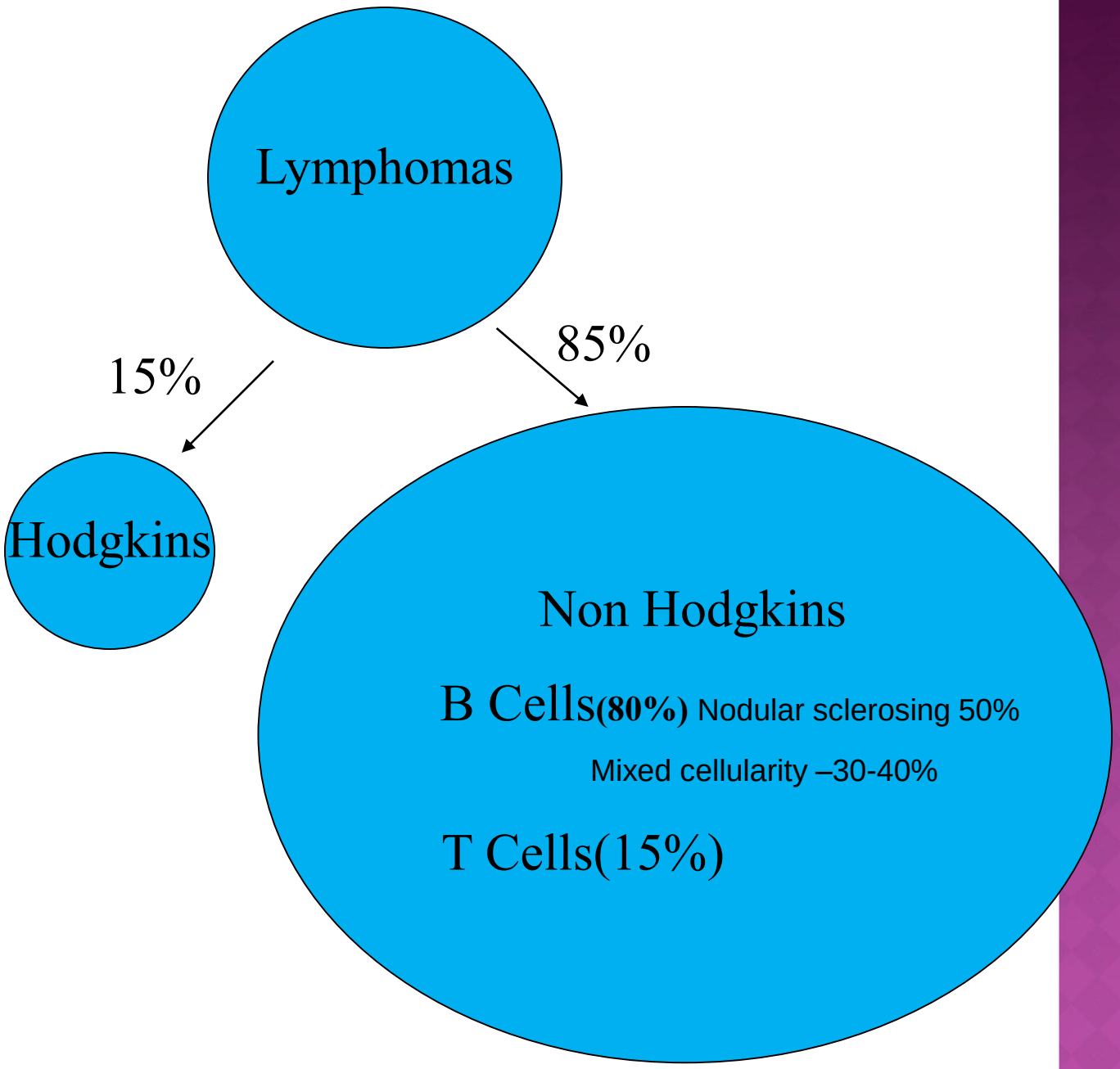
# WHAT IS YOUR INTERPRETATION?



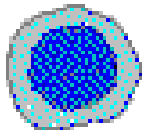
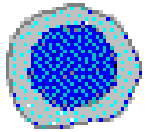
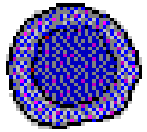
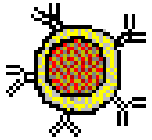
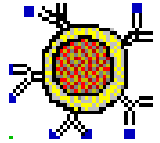
# LYMPHOMA

# LYMPHOMA'S
## WHERE THEY BEGIN

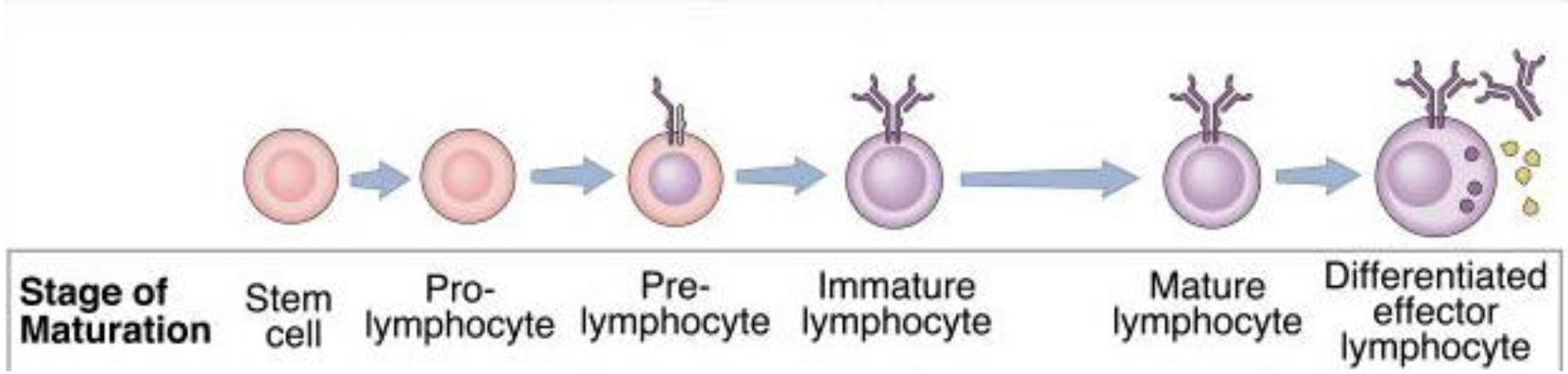



Lymphomas
15%
85%
Hodgkins
Non Hodgkins
B Cells(80%) Nodular sclerosing 50%
Mixed cellularity –30-40%
T Cells(15%)

# B-CELL CANCERS BY CELL DEVELOPMENT

| Cell Stage | | Malignancy |
|---|---|---|
| Stem Cell |  | acute lymphocytic leukemia (ALL)<br>lymphoblastic lymphoma/leukemia |
| Precursor B-Cell |  | Precursor B-lymphoblastic<br>lymphoma / leukemia |
| Immature B-Cell |  | Small Non-Cleaved Cell<br>Lymphoma (Burkitt's/Non-Burkitt's)<br>(Possibly) |
| B-Cell Before<br>Antigen Exposure |  | Chronic Lymphcytic Leukemia (CLL)<br>Small Lymphocytic Lymphoma |
| B-Cell After<br>Antigen Exposure |  | Follicular Lymphomas<br>Large Cell Lymphoma<br>Immunoblastic Lymphoma |
| Antibody Producing<br>B-Cell |  | |

# DISEASE STATES CORRELATE WITH STAGES IN NORMAL B-CELL DEVELOPMENT




**Diseases:**

AML

Pro-B-ALL

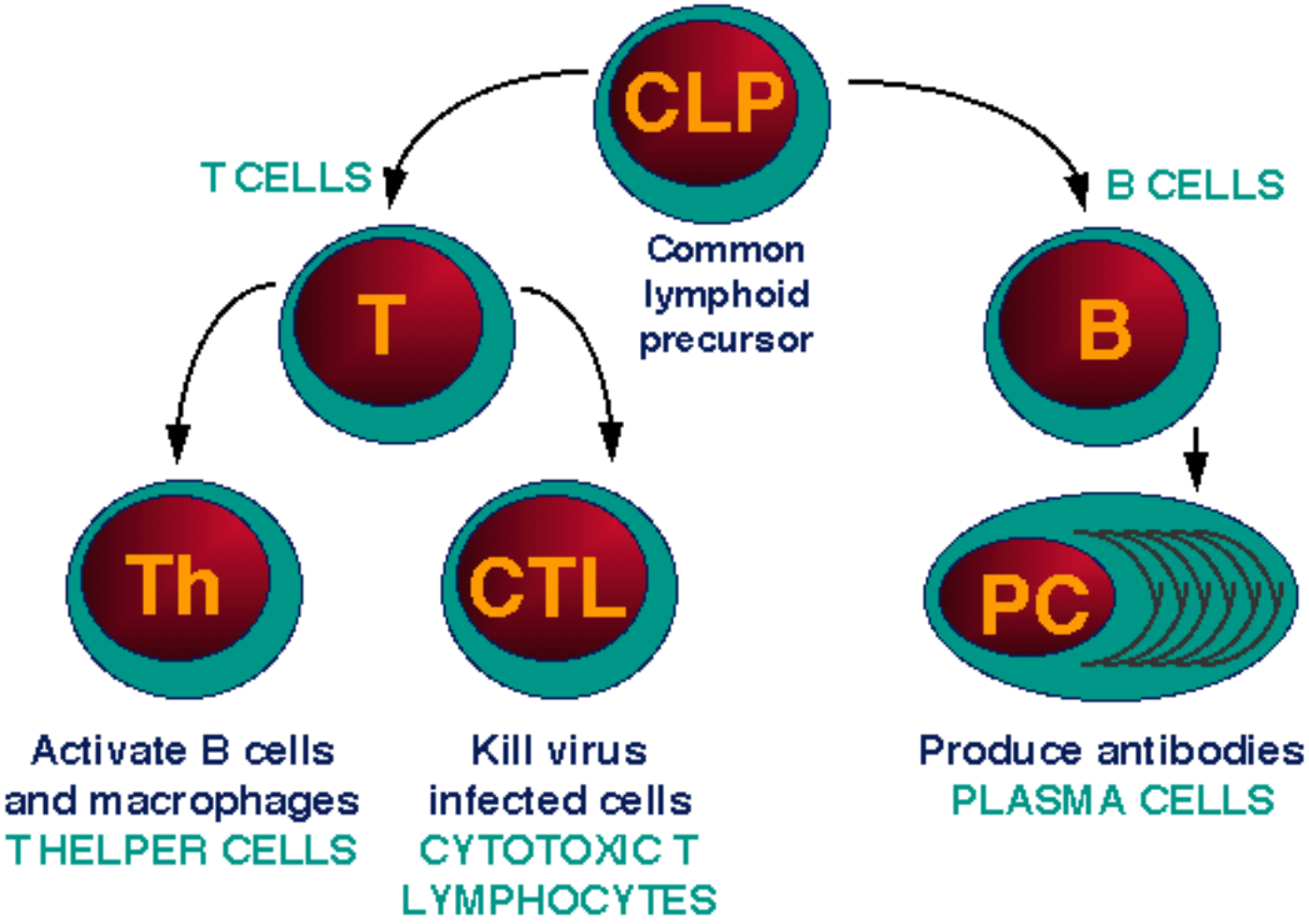
Pre-B-ALL

B-ALL

-B-CLL
-DLBCL
-FLL
-BL
-Mantle Cell lymphoma
-Marginal Zone lymphoma
-MALT
-GALT
-Hodgkins (?)

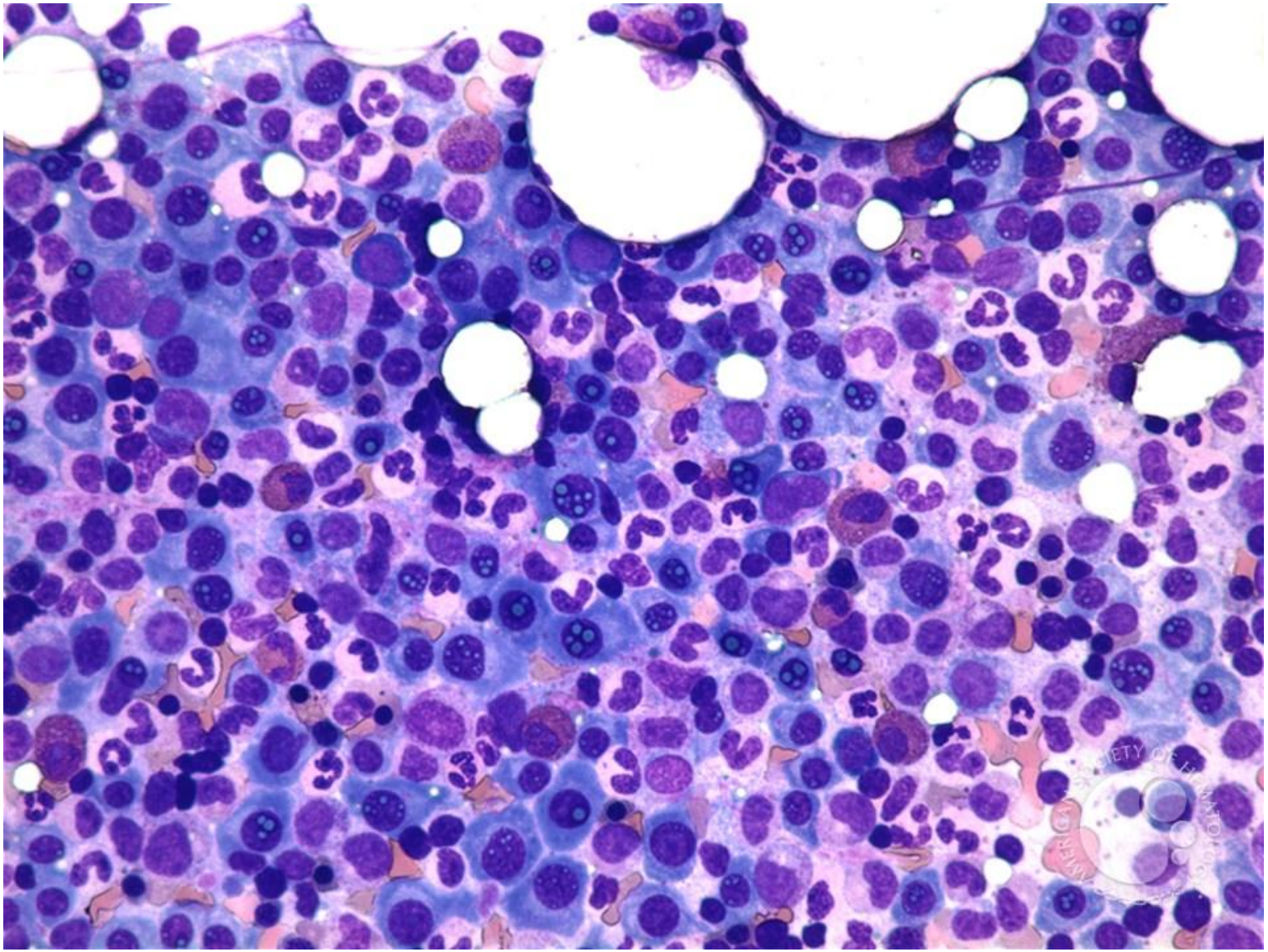
-MGUS
-Multiple Myeloma
-Plasmacytoma

# Lymphocyte subsets



# NON-HODGKIN'S LYMPHOMA

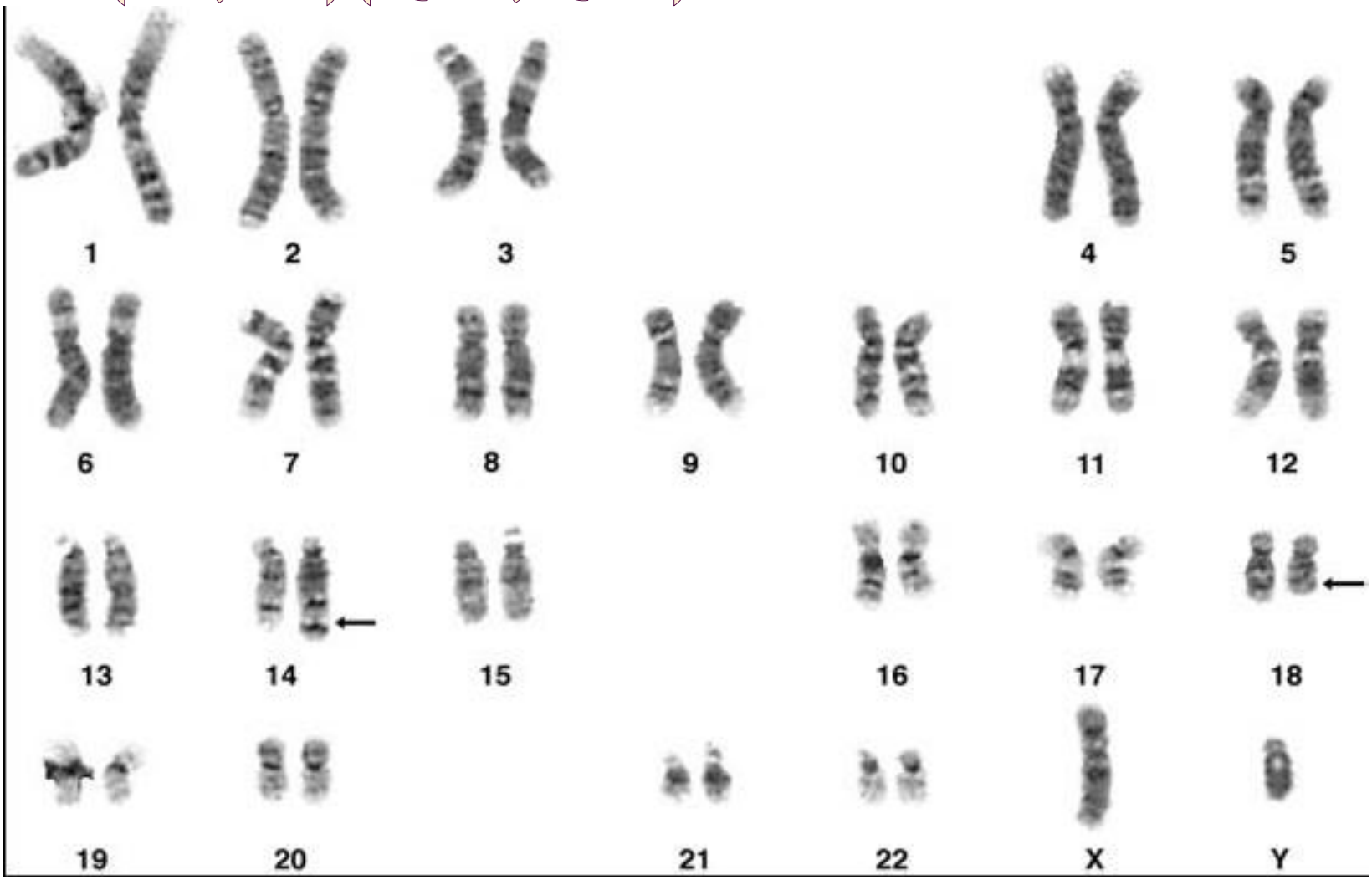
# MULTIPLE MYELOMA



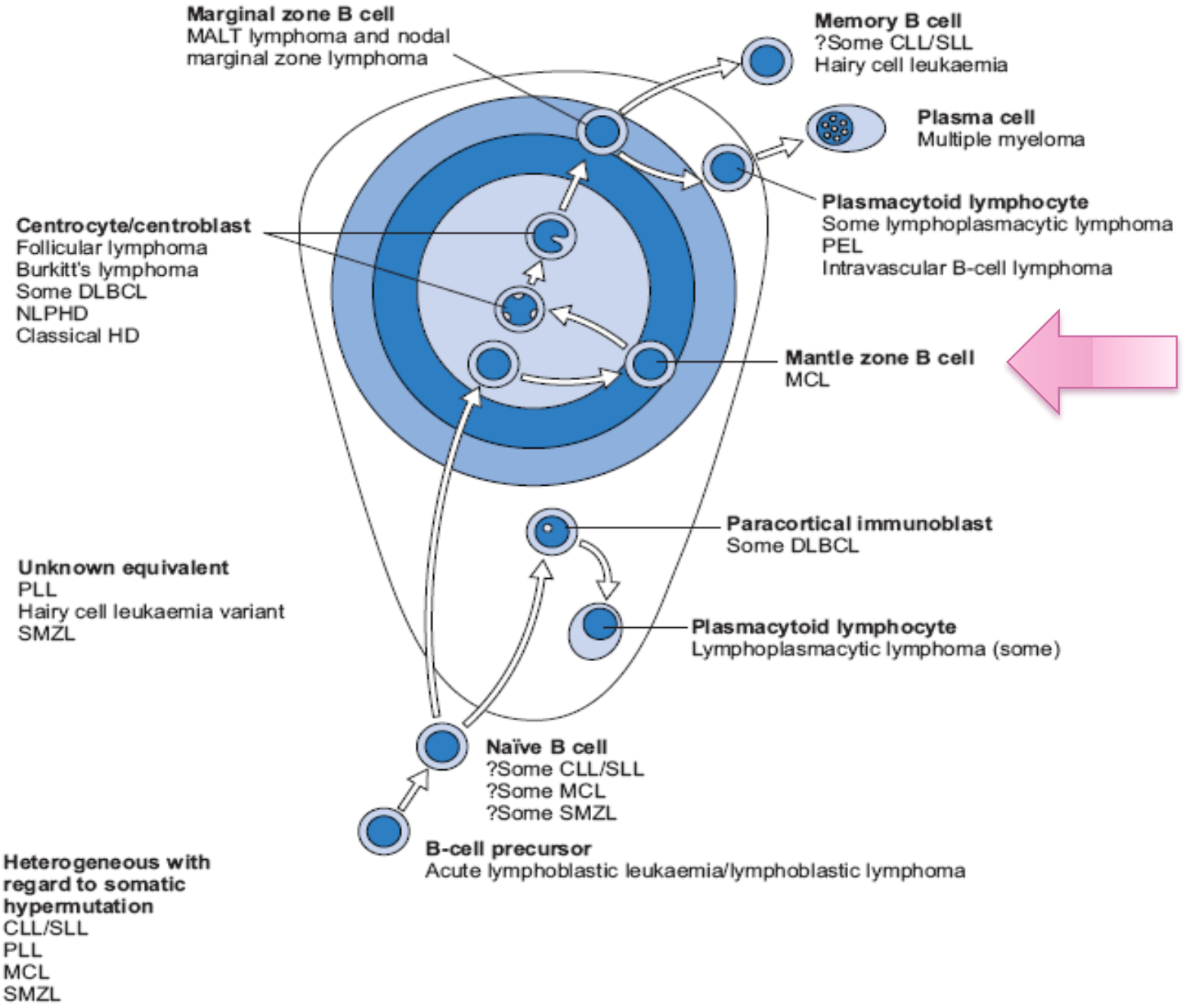
# FOLLICULAR LYMPHOMA

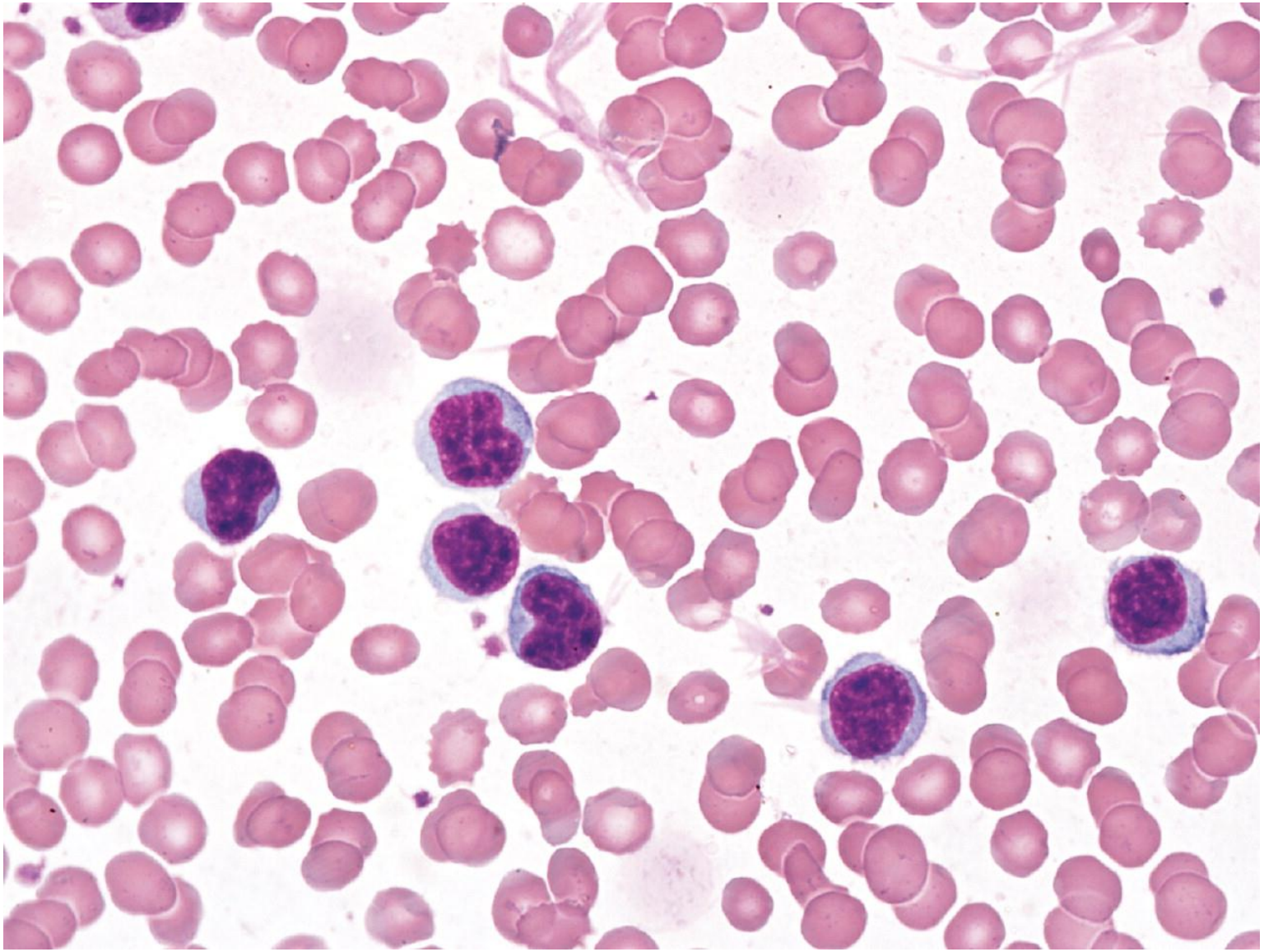
- F > M
- median age 59 years
- Peripheral lymphadenopathy dominates, with central adenopathy, BM and splenic involvement frequent
- Positive
  - CD19, 20, 22, 23, 79a, 10, bcl-2, bcl-6
- Negetive
  - CD5, 43
- Cytogenetics
  - t(14;18)(q32;q21)

# A KARYOGRAM SHOWING T(14;18)(Q32;Q21)

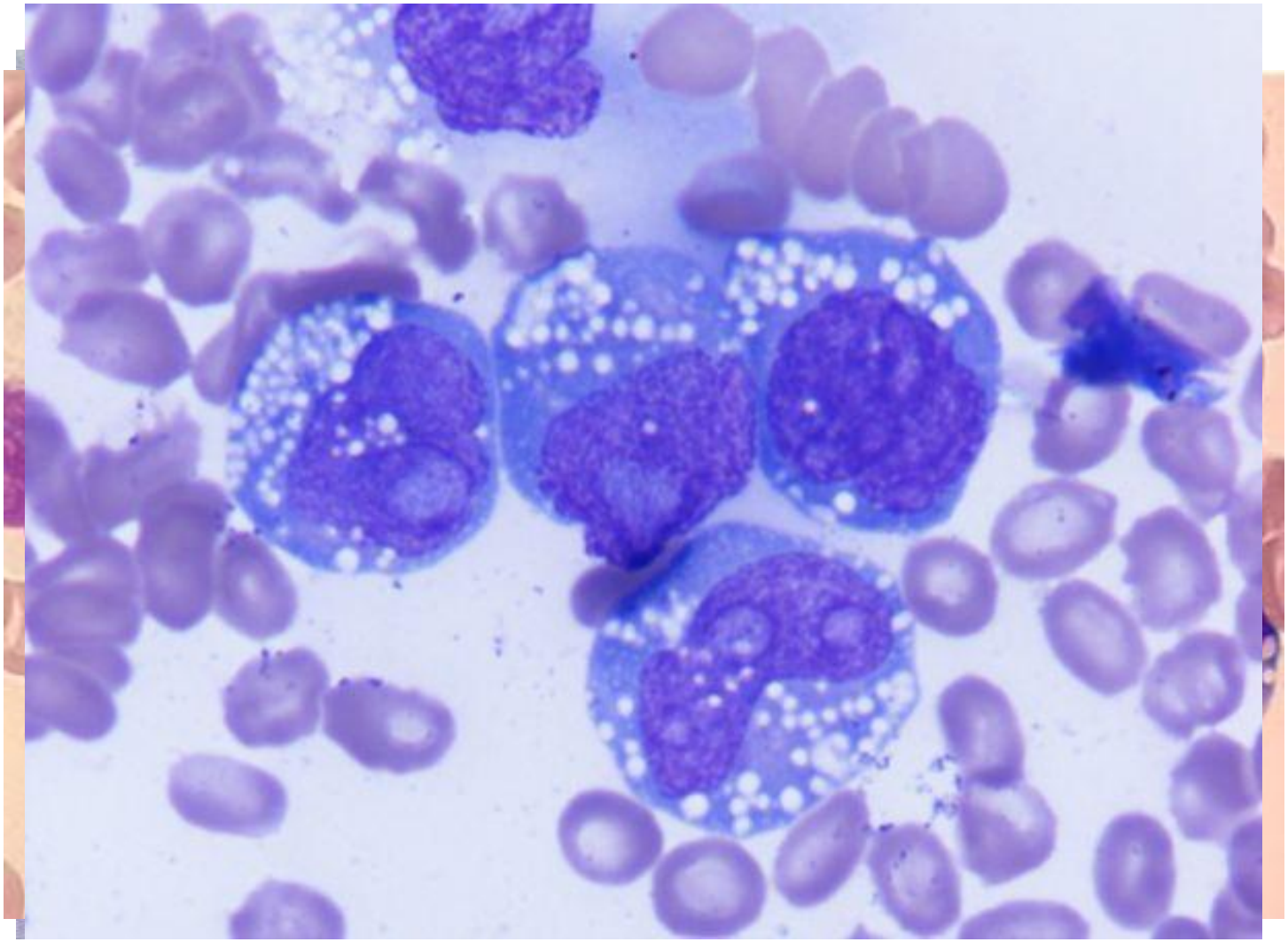


# MANTLE CELL LYMPHOMA


Marginal zone B cell
MALT lymphoma and nodal marginal zone lymphoma
Memory B cell
?Some CLL/SLL
Hairy cell leukaemia
Plasma cell
Multiple myeloma
Plasmacytoid lymphocyte
Some lymphoplasmacytic lymphoma
PEL
Intravascular B-cell lymphoma
Centrocyte/centroblast
Follicular lymphoma
Burkitt's lymphoma
Some DLBCL
NLPHD
Classical HD
Mantle zone B cell
MCL
Paracortical immunoblast
Some DLBCL
Unknown equivalent
PLL
Hairy cell leukaemia variant
SMZL
Plasmacytoid lymphocyte
Lymphoplasmacytic lymphoma (some)
Naïve B cell
?Some CLL/SLL
?Some MCL
?Some SMZL
B-cell precursor
Acute lymphoblastic leukaemia/lymphoblastic lymphoma
Heterogeneous with regard to somatic hypermutation
CLL/SLL
PLL
MCL
SMZL



# BURKITT LYMPHOMA



# MALT LYMPHOMA

## MUCOSA ASSOCIATED LYMPHOID TISSUE

# SPLENIC MARGINAL ZONE LYMPHOMA (SMZL)

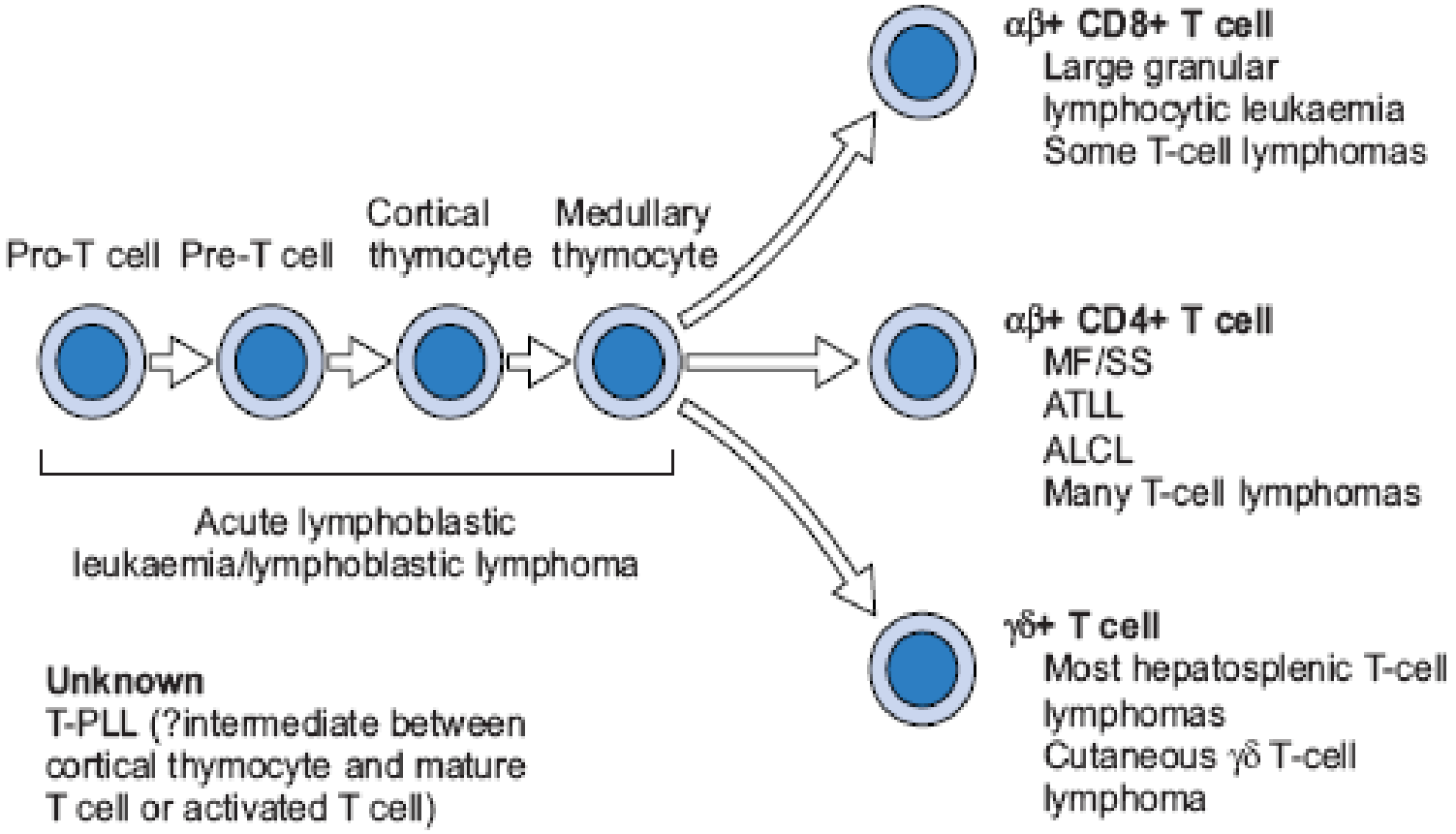
- F > M
- median age 61
- Indolent course involving stomach, other GI sites
- Associated with antecedent autoimmune disease (**Sjögren's syndrome**, Hashimoto's) or *H. pylori* infection

- **Positive**
- CD19, 20, 22, 79a, sIg, bcl-2

- **Negetive**
- CD5, 10, 23, bcl-6

- **Cytogenetics**
- +3, t(11;18)(q21;q21)

# LEUKAEMIAS OF MATURE T AND NK CELLS

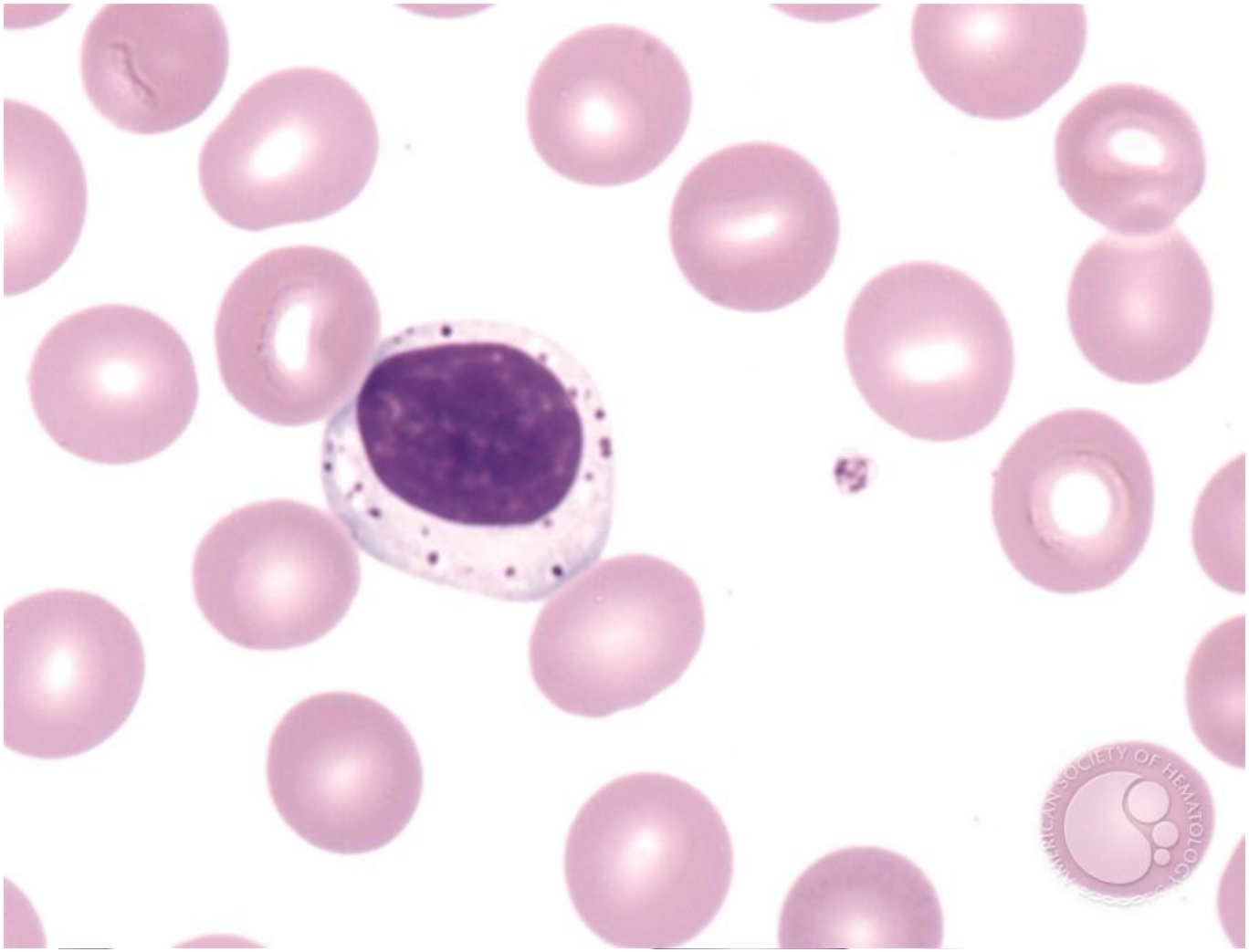



**Putative relationship between normal T-cell differentiation and T-lineage neoplasms. Abbreviations: ALCL, anaplastic large cell lymphoma; ATLL, adult T-cell leukaemia/lymphoma; MF, mycosis fungoides; SS, Sezary syndrome; T-PLL, T-cell prolymphocytic leukaemia.**

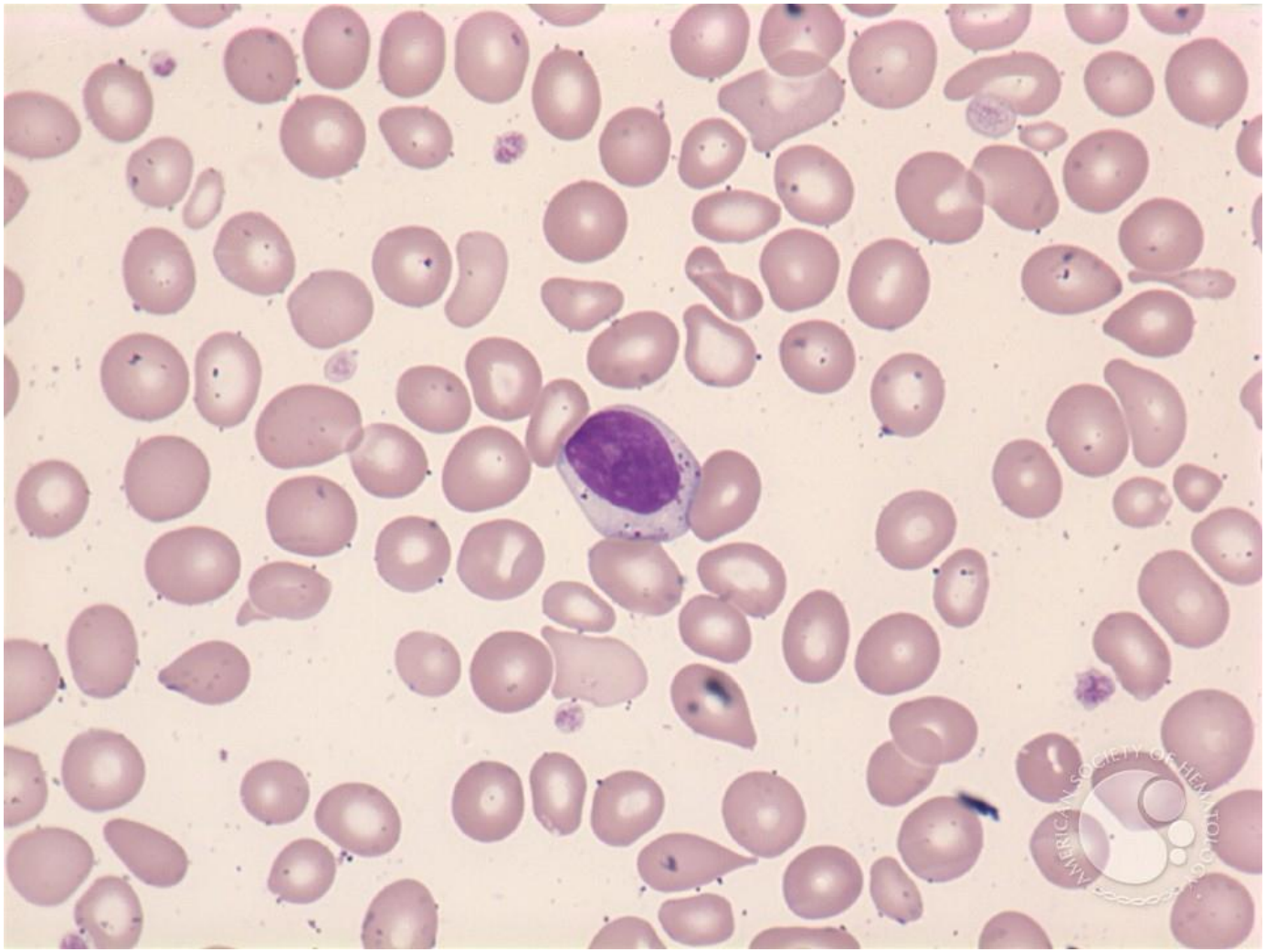
# T-CELL LARGE GRANULAR LYMPHOCYTE LEUKAEMIA (LGL LEUKEMIA)

- Median age 63
- Neutropenia causing infections
- Anemia
- mild lymphocytosis
- LGL > $2 \times 10^3$
- Morphology
  - Moderately sized cell with condensed chromatin
  - abundant pale blue cytoplasm
  - azurophilic granules
- Cell Surface Markers
  - CD3, 8, 57, TCRα-β
- Gene Rearrangements
- TCRγ, β

# LARGE GRANULAR LYMPHOCYTE



# WHAT IS THIS CELL?

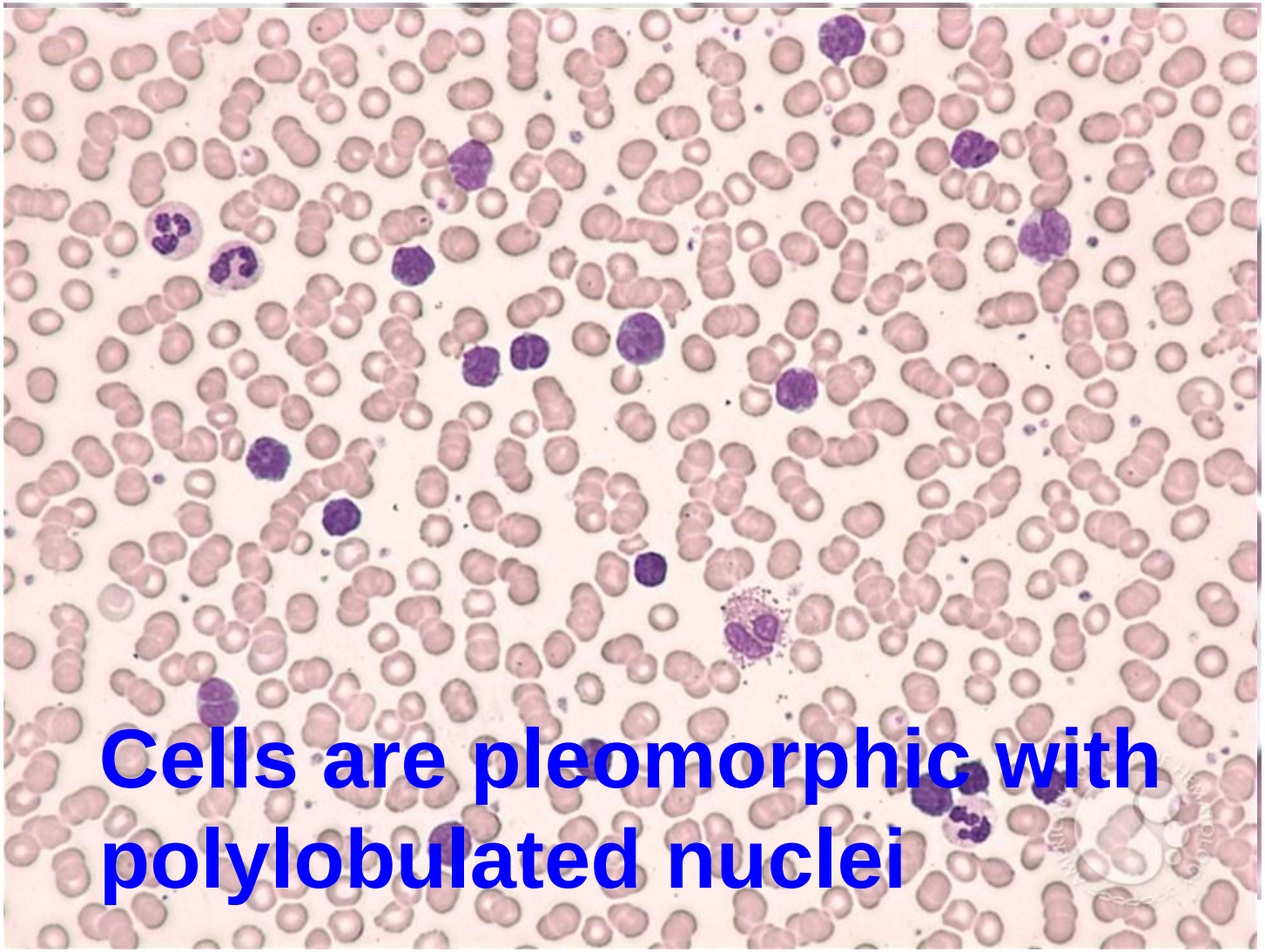


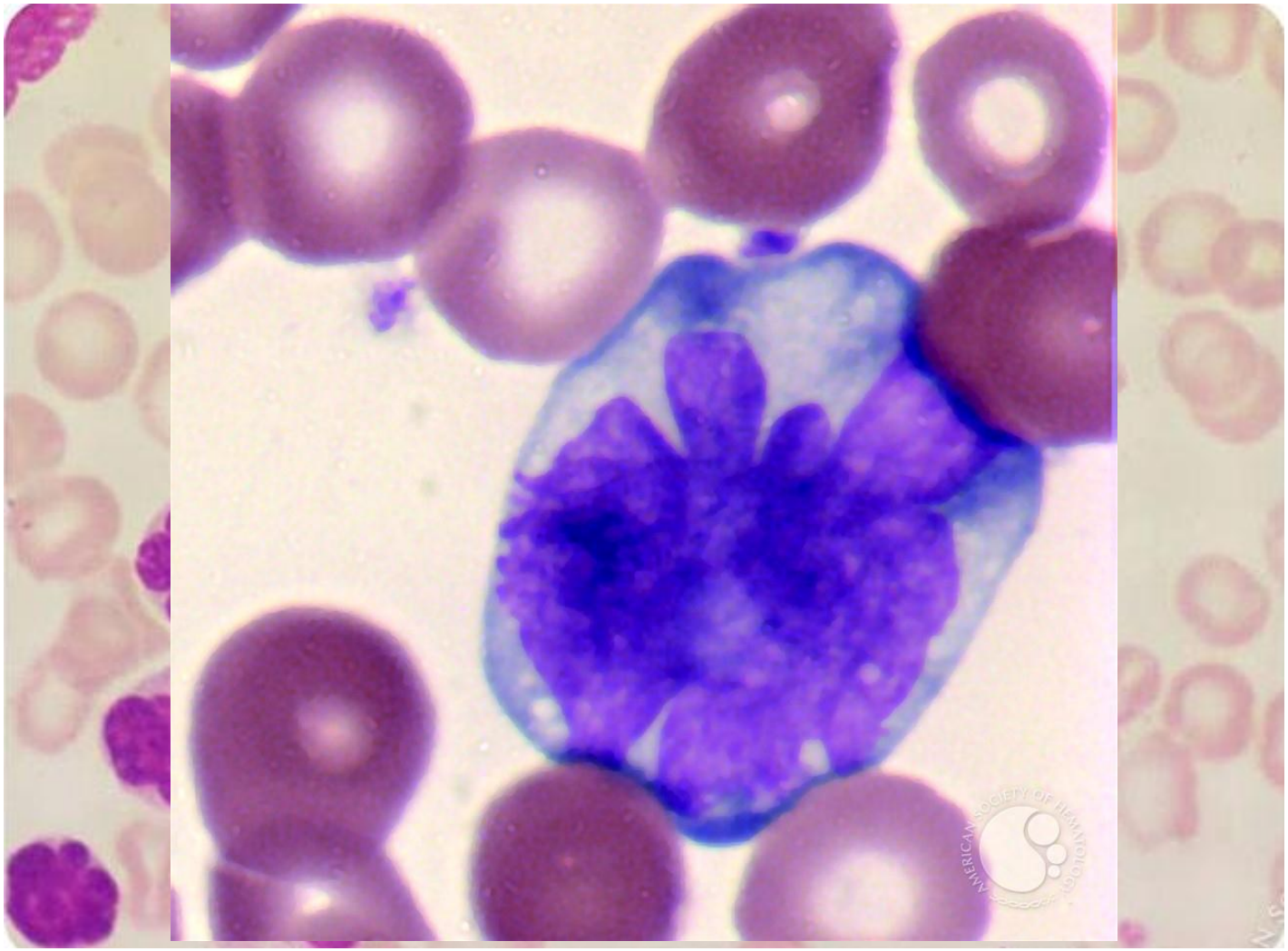
# ADULT T-CELL LEUKAEMIA/LYMPHOMA

- Associated with HTLV-1
- frequent in Japan, Caribbean, central Africa
- Acute variant with skin, lymph node involvement
- Hypercalcemia
- Morphology
- Moderately large, blastic cells with convoluted nuclei (floret cells),
- agranular, basophilic cytoplasm
- Cell Surface Markers
- CD 2, 3, 5, 25, often CD30
- Gene Rearrangements
- TCR Genes

# ADULT T-CELL LEUKEMIA / LYMPHOMA

## A HIGH POWER VIEW REVEALS ABERRANT LOBULATION OF THE NUCLEUS.





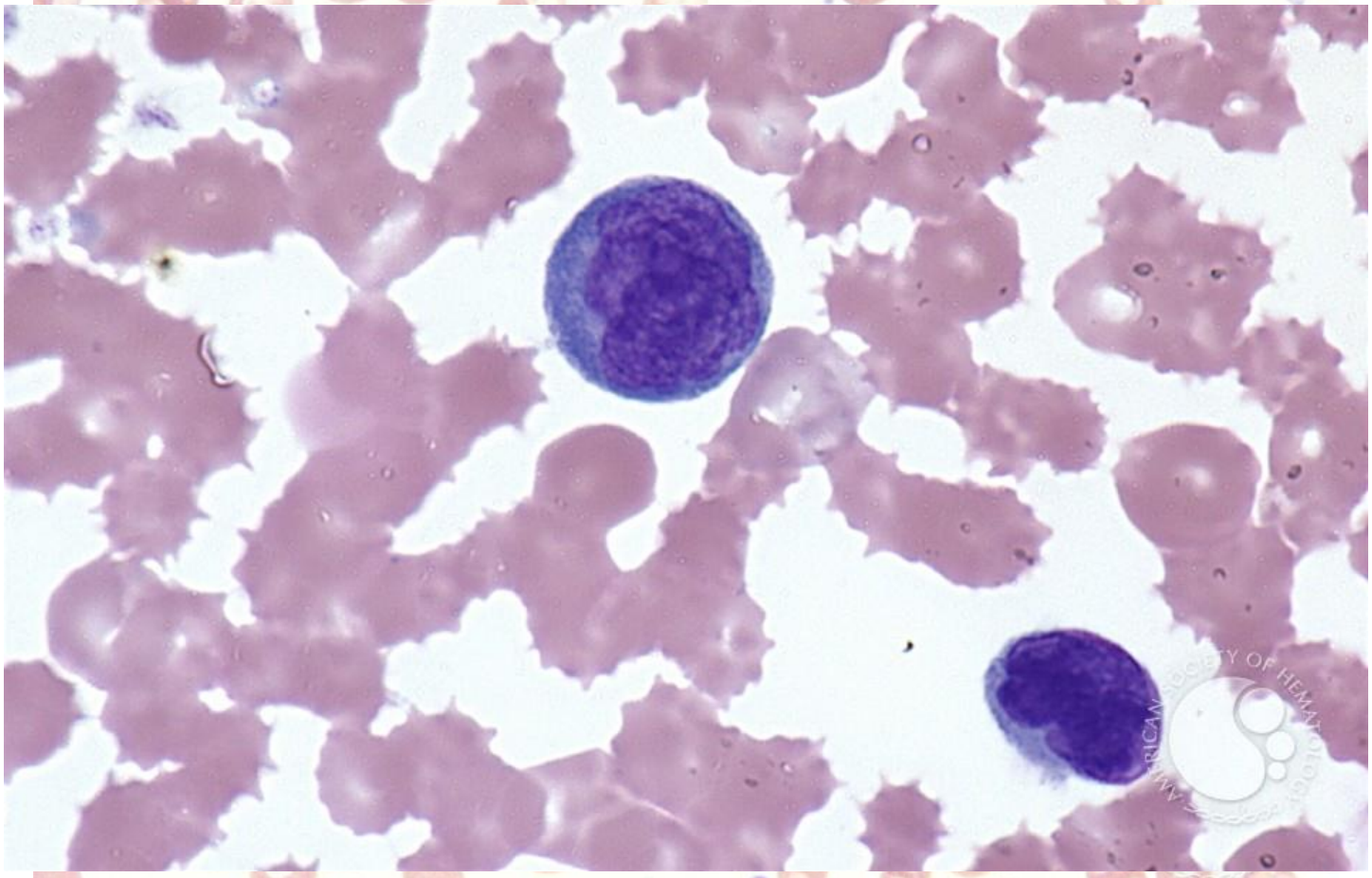
Peripheral blood film showing two lymphoma cells, one of which is a '**flower cell' (convoluted nuclei).**

# MYCOSIS FUNGOIDES, SÉZARY SYNDROME (SS)

- ✓ **M > F**
- ✓ **middle-age–older**
- ✓ **Dermatitis progressing to ulcerated lesions**
- ✓ **PB blood involvement in SS**
- ◉ **Morphology**
- ❑ **Dermal band-like infiltrates of lymphocytes with cerebriform nuclei**
- ❑ **Microabscesses**
- ◉ **Cell Surface Markers**
- ◉ **CD2, 3, 4**
- ◉ **Gene Rearrangements**
- ◉ **TCR genes**

# SEZARY SYNDROME

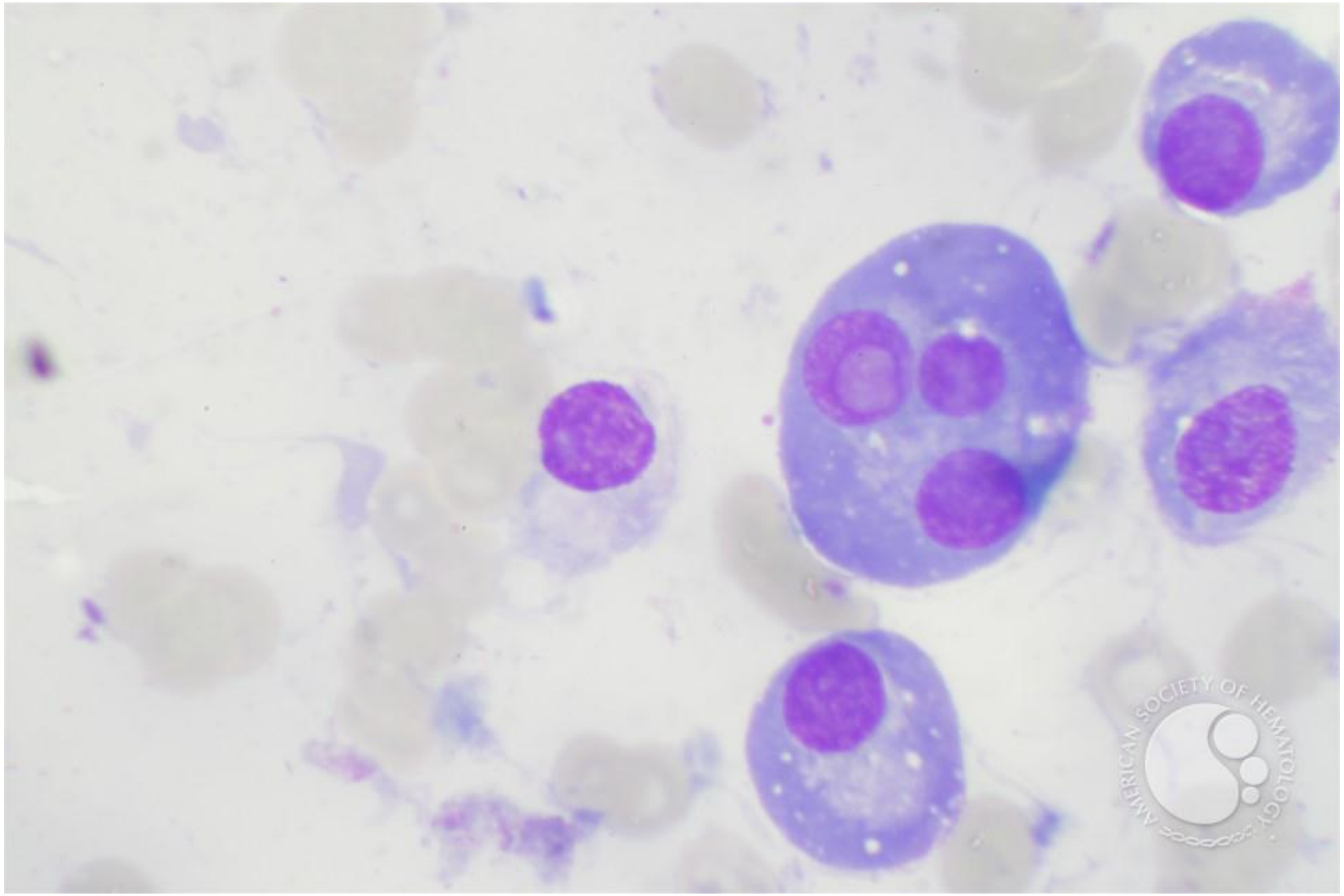
THREE OF THE FOUR LYMPHCYTES IN THIS VIEW HAVE AN IRREGULAR NUCLEAR BORDER AND A NUCLEAR PATTERN WITH FOLDER NUCLEI.



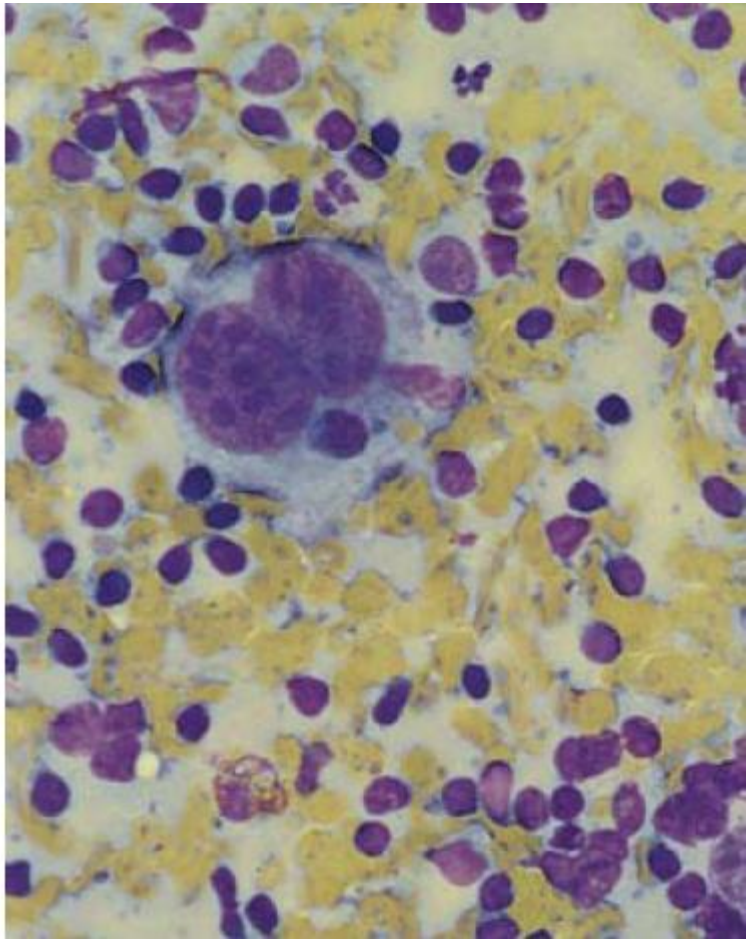
# ANAPLASTIC LARGE CELL LYMPHOMA

- M≈ F
- teens, young adults
- Peripheral, abdominal adenopathy
- extranodal and BM involvement
- Morphology
  - Pleomorphic large cells
  - wreath-like nuclei
  - multiple nucleoli
  - abundant cytoplasm
- Cell Surface Markers
  - CD30 (cytoplasmic and Golgi)
  - CD2, 4
- Gene Rearrangements
  - t(2;5)(p23;q35)
  - Other variants involve 2p23

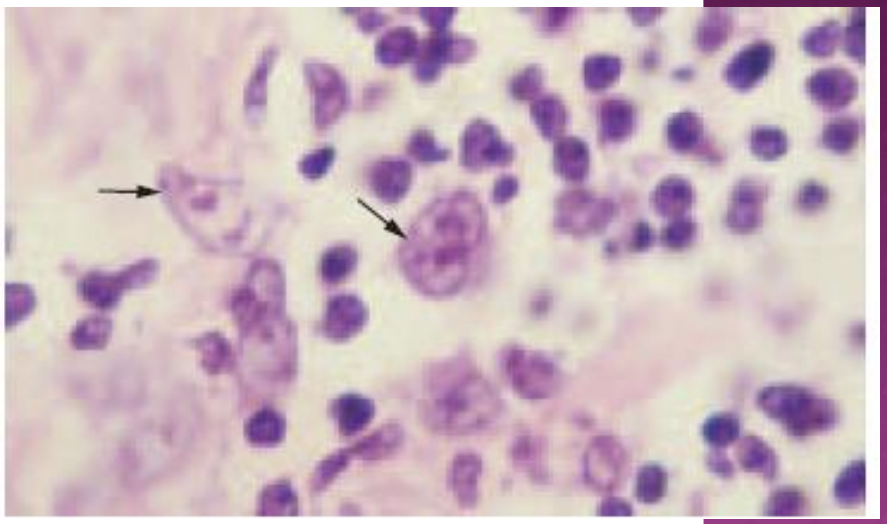
# WHAT ARE THESE CELLS?



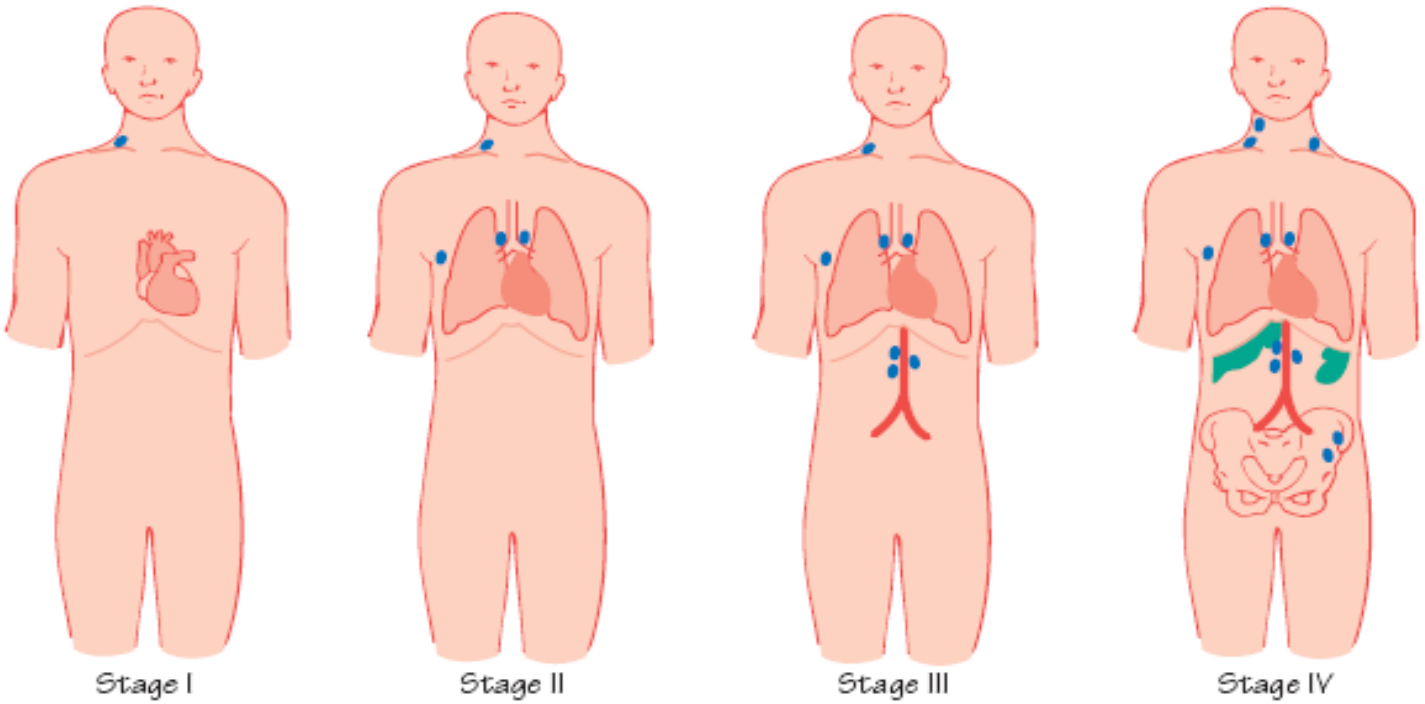
# HODGKIN'S DISEASE



Hodgkin disease: giant binuclear cell (Reed–Sternberg giant cell).



Hodgkin lymphoma: lymph node biopsy showing a Reed–Sternberg cell (multinucleate cell) (arrows).




**Hodgkin lymphoma: clinical features and staging.** Stage I: involvement of a single lymph node region or structure; stage II: involvement of two or more lymph node regions on the same side of the diaphragm; stage III: involvement of lymph node regions or structures on both sides of the diaphragm; stage IV: involvement of other organs, e.g. liver, bone marrow, CNS. A: no symptoms; B: fever, night sweats, weight loss >10% in preceding 6 months; X: bulky disease; >1/3 widening of mediastinum; 10cm max dimension of nodal mass; E: extralymphoid disease (e.g. in lung, skin).

دنیا آنقدر وسیع هست که برای همه مخلوقات جایی باشد، پس به جای آنکه جای کسی را بگیریم تلاش کنیم جای واقعی خود را بیابیم.