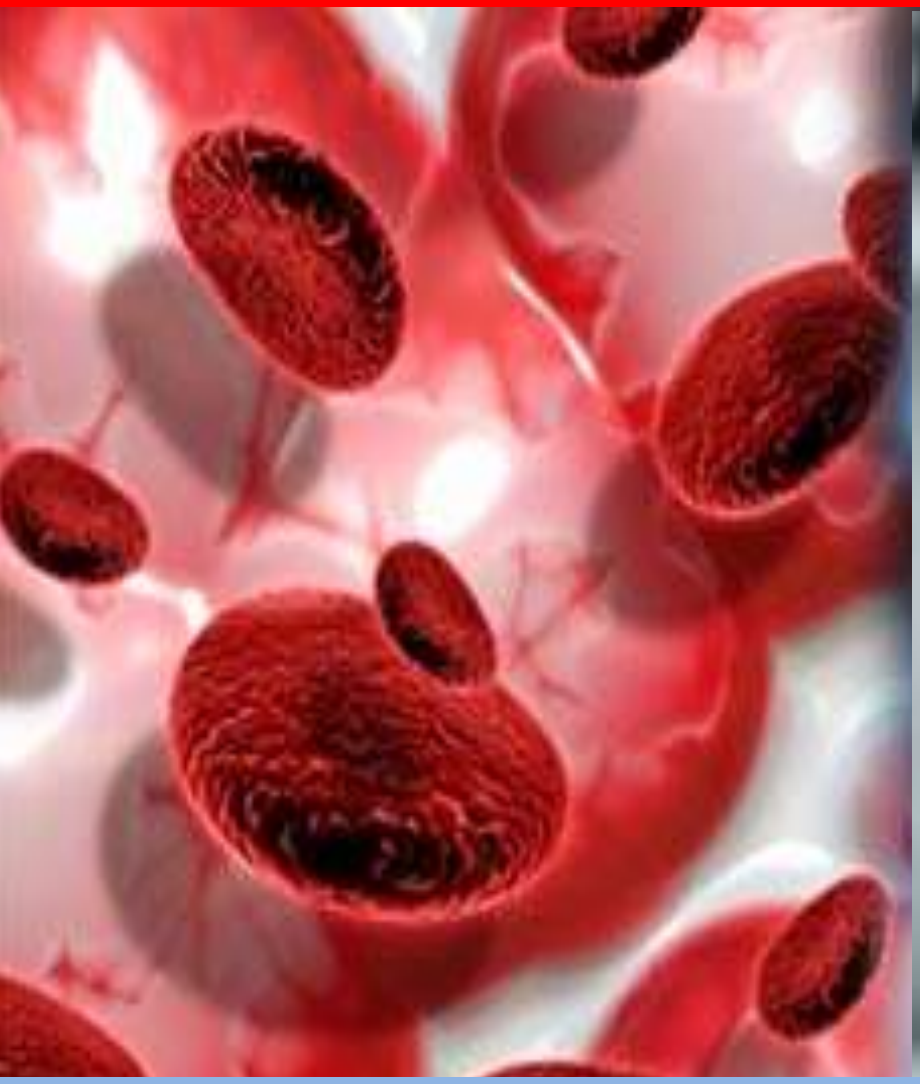
LEUKEMIA of LABORATORY



Dr. dr. Sulistyo Mulyo Agustini.,Sp.PK

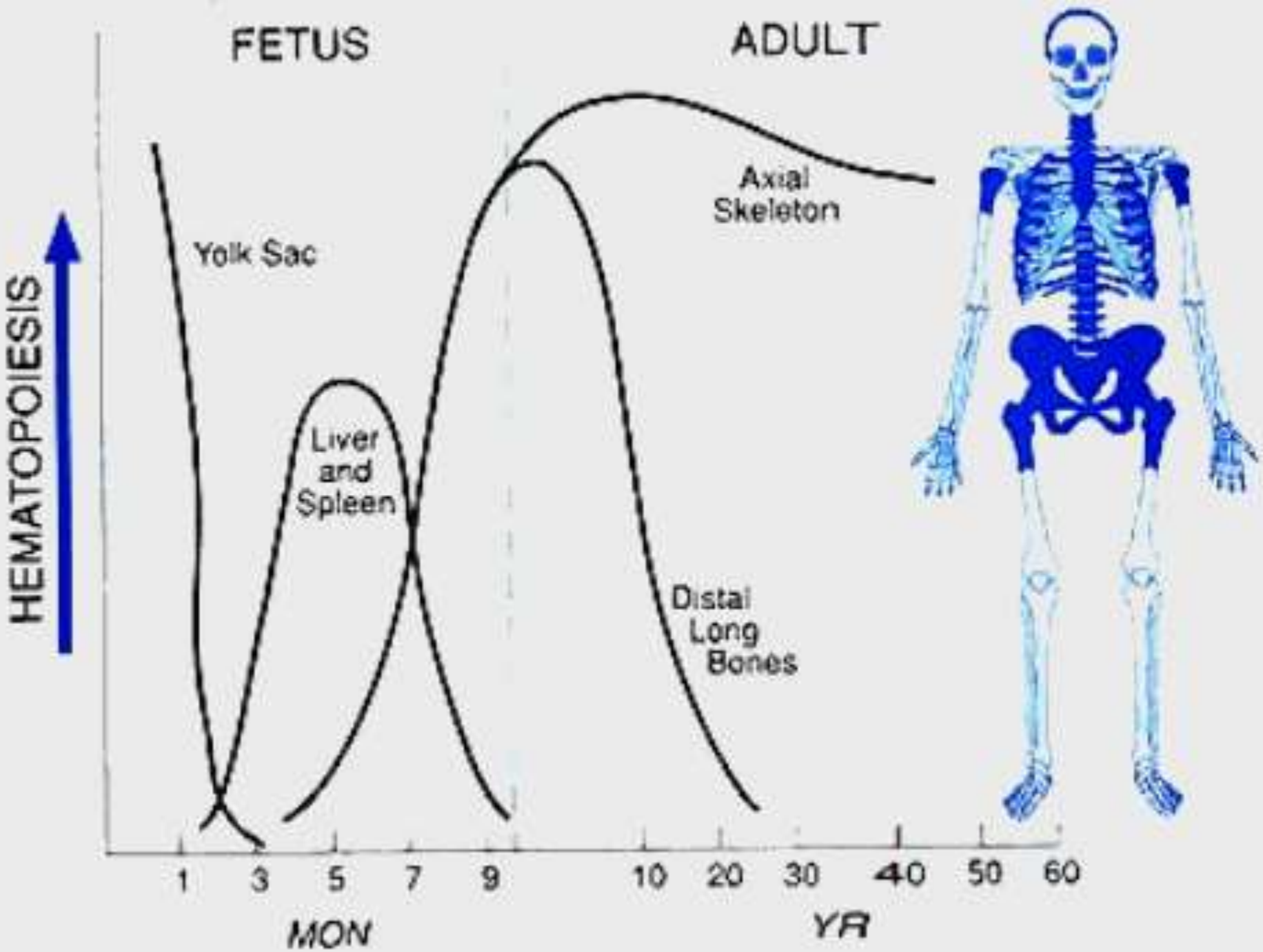
FK UMM 2023

REFERENCE

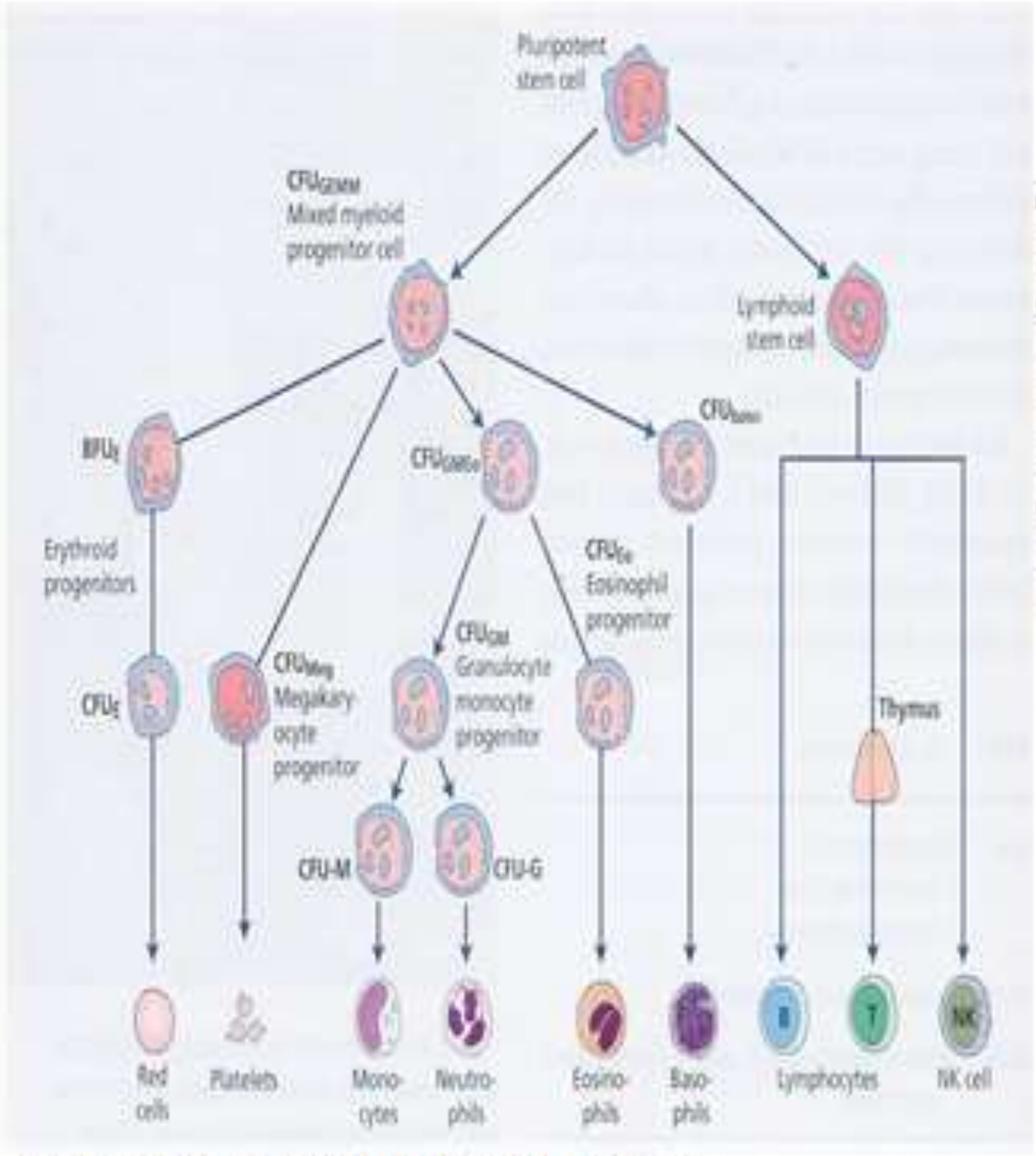
- **Agustini S M**. 2016. Model Support System of Prediction Diagnosis and Classification Leukemia Based on Information Technology, photonjournal@yahoo.com
- **Agustini SM**., Utomo D. 2019. An in silico approach toward wheatgrass extract-induced apoptosis of human acute myeloid leukemia cells,. Drug Invention Today Journal
- **Agustini SM**, Widjajanto E 2, Rifa'I M3, Mubarika S H4, Nurdiana5, Lyrawati D5, Sukorini U6, Lestari ND. 2023. Anti-leukemic activity of Cyperus rotundus L.on human acute myeloid leukemia HL-60 cells in vitro. Journal of Pharmacy & Pharmacognosy Research, 11 (1), 191-197, 2023. ISSN 0719-4250. DOI: https://doi.org/10.56499/jppres22.1502_11.1.191
- M Setiawan [1], **S M Agustini** [2], Patmawati [3], N D Lestari.2024. Anti-leukemic activity of Cyperus rotundus L.on human acute myeloid leukemia HL-60 cells in vitro. Brazilian Journal of Biology, 2024, vol. 84, e278323 https://doi.org/10.1590/1519-6984.2783. 24 May 10:84:e278323..doi: 10.1590/1519-6984.278323. eCollection
- McCance Huether, PATHOPHYSIOLOGY "The Biologic Basic for Disease in Adults and Children, Fifth edition, Mosby-Elsevier, 2006
- Hoffbrand AV, Pettit JE and Moss PAH, Essential HAEMATOLOGY, fourth edition, 2003
- Howard MR, Hamilton PJ, HAEMATOLOGY An Illustrated Colour Text, tjird edition, 2008
- Kumar Hagler Schneider, Robin and Cotran PATHOLOGIC BASIC of DISEASE, 7 th edition, 2005
- Abeloff D et al, CLINICAL ONCOLOGY, 3 rd edition, 2004
- Abeloff D. M, Armitage O. J, Niederhuber E J, Kastan B M, McKenna W.G, Childhood Leukemia in Clinical Oncology, 3rd edition, USA, Elsevier Inc, 2004; 2731 – 2754
- Craig FE and Foon K A, Fow cytometric immunophenotyping for hematologic neoplasms, Blood, 2008: 3941-3967
- Haferlach T, et al. 2005. Global approach the diagnosis of leukemia using gene expression profiling, The American Society of Hematology; 1189-1198
- Hoffbrand AV, Pettit JE, Moss PA. H. 2001. Acute leukaemia. In Essential Haematology. 4th ed. Victoria: Blackwell science ; p.1 – 11, 146, 162- 79
- Kumar, Hagler, Schneider. 2005. Tissue Renewal and Repair, Robbin and Cotran In Pathologic Basis of Disease, 7th edition, Elsevier Sauders Phildelphia; 87- 98 and 145 – 149.
- Vardiman JW, et al, The 2008 revision of the World Health Organization (WHO) classification of myeloid neoplasms and acute leukemia: rationale and important changes, Blood, 2009;114:937-951)

Introduction

- Leukemia is a heterogeneous group of hematologic malignancies that arise from the dysfunctional proliferation of developing leukocytes
- Leukemia is the 10th-most common cancer diagnosed in the United States
- The disease is characterized by the uncontrolled growth of blood cells, usually white blood cells in the bone marrow
- The exact cause of leukemia isn't known→mutations in the DNA (genetic or environmental factors)
- Some risk factors for leukemias→Long-term exposure to radiation or other carcinogenic chemicals, like benzene; Smoking; A family history of leukemia; Certain blood disorders, like polycythemia vera; Certain congenital syndromes, like Down syndrome and Fanconi anemia (Progressive bone marrow failure with pancytopenia typically presents in the first decade, often initially with thrombocytopenia or leukopenia) &Viruses: Human T-lymphocyte leukemia virus (HTLV1), EBV.


FETUS
ADULT
HEMATOPOIESIS
Yolk Sac
Liver and Spleen
Axial Skeleton
Distal Long Bones
1
3
5
7
9
10
20
30
40
50
60
MON
YR

Haemopoiesis



Hoffbrand AV, Essential Haematology, 2003

CFU: Colony-forming unit
BFU: Burst-forming unit
GEMM: (Granulocyte, Erytroid Monocyte, Megakaryocyte)
E : Erytroid:
Eo: Eosinophils
GM: Granulocyte, Monocyte
Meg: Megakaryocyte
NK: Natural Keiler

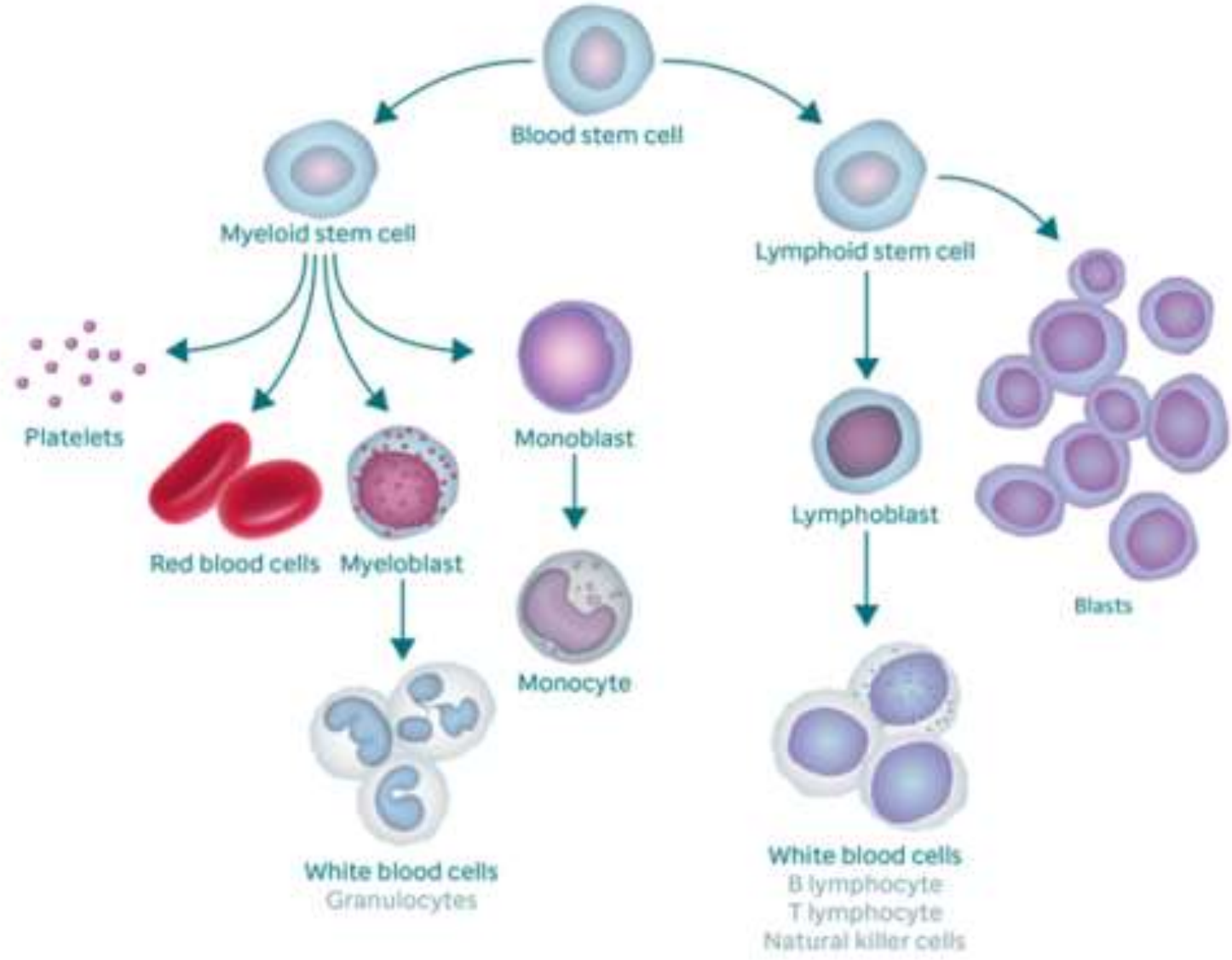
Patogenesis

- *Stem cells disease:*
- *dishematopoisis, uneffective hematopoisis*
maturation arrest
- *uncontrolled proliferation*
- *delay apoptosis*
- *clonal expansion*
- supresi hematopoisis lainnya

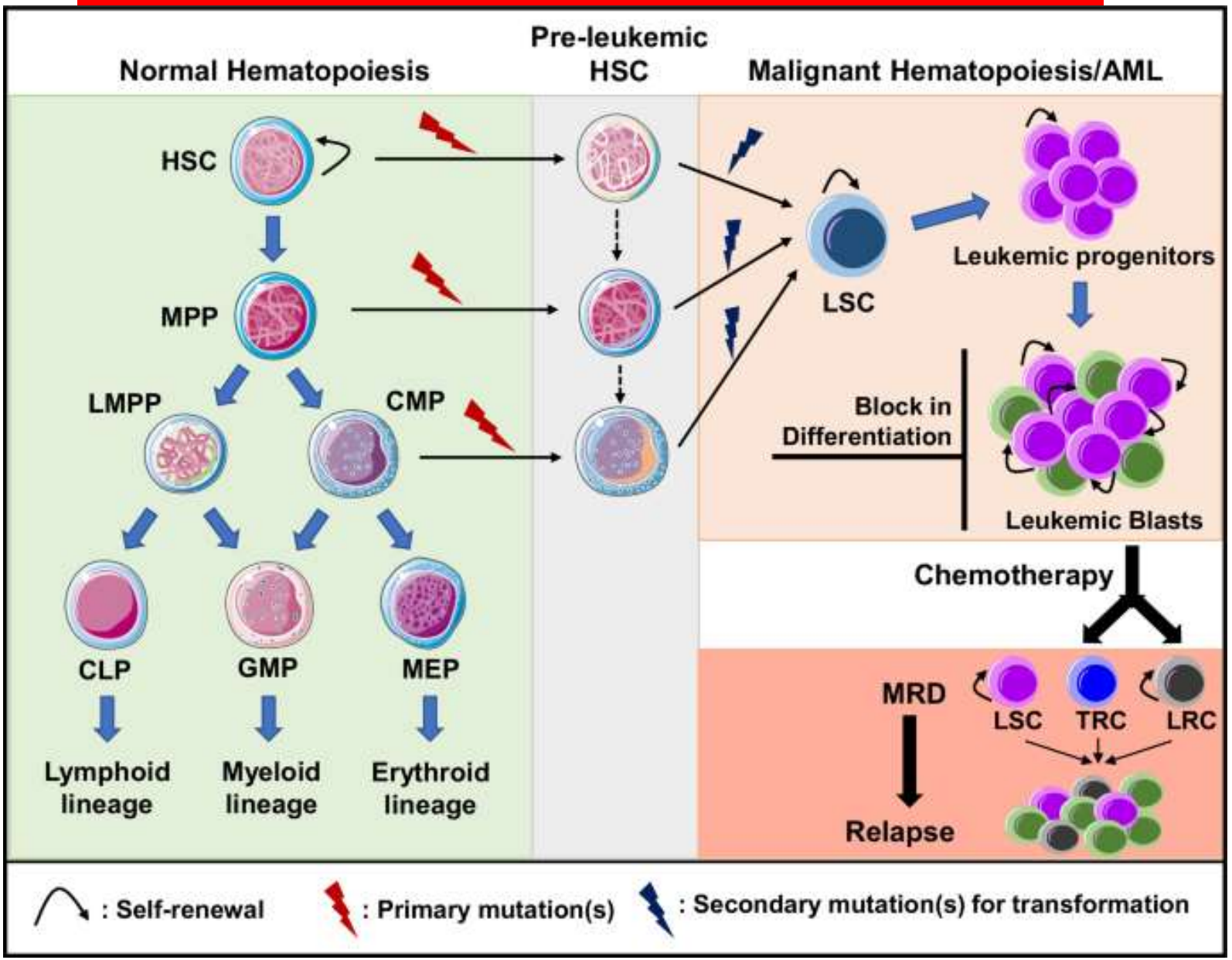
- Leukemic cells produce symptoms because of:

Bone marrow failure (i.e. anemia, neutropenia, thrombocytopenia)

- Infiltration of body organs (e.g. liver, spleen, lymph nodes, meninges, brain, skin or testes)


Blood stem cell
Myeloid stem cell
Lymphoid stem cell
Platelets
Red blood cells
Myeloblast
Monoblast
Monocyte
Lymphoblast
Blasts
White blood cells
Granulocytes
White blood cells
B lymphocyte
T lymphocyte
Natural killer cells

PATHOPHYSIOLOGY OF LEUKEMIA



Diagnostic tests for leukemia

- **Complete blood count (CBC) test**→throughout treatment to closely monitor a patient's blood counts

- Immunophenotype analysis or Flow cytometry
- Genetic tests→ cytogenetics, cytogenetic testing, molecular genetics, molecular testing, karyotyping or FISH tests
- **DNA test**

Basic Blood Tests

Complete Blood Count (CBC)

1. White blood cell count (WBC):

   Differential white blood cell count (Diff)

2. Red blood cell count (RBC)

   Hemoglobin (Hb): component RBC

   Hematocrit (Hct): mengukur masa RBC

   Red blood cell index→ (MCV; MCHC;MCH)

   Red blood cell distribution width (RDW)

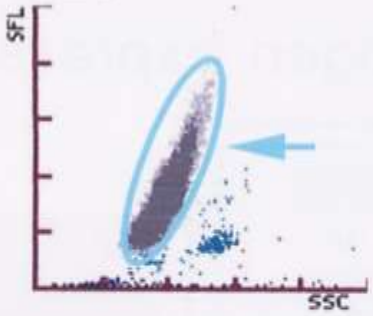
→Stained red cell examination (blood smear)

3. Platelet count (PLT)

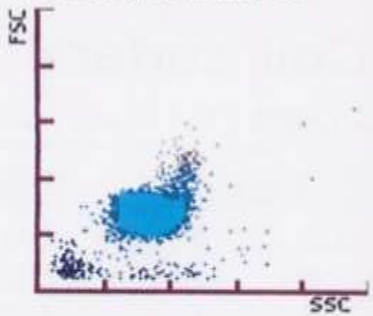
Autoanalizer Hemotologi Complete Blood Count

WBC	52.39 *	[10^9/L]		
RBC	1.44 -	[10^{12}/L]		
HGB	47 -	[g/L]		
HCT	14.4 -	[%]		
MCV	100.0	[fL]		
MCH	32.6	[pg]		
MCHC	326	[g/L]		
PLT	55 *	[10^9/L]		
RDW-SD	51.5	[fL]		
RDW-CV	15.2	[%]		
PDW	9.6 *	[fL]		
MPV	9.0 *	[fL]		
P-LCR	17.3 *	[%]		
PCT	0.05 *	[%]		
NEUT	----	[10^9/L]	----	[%]
LYMPH	----	[10^9/L]	----	[%]
MONO	----	[10^9/L]	----	[%]
EO	0.01 *	[10^9/L]	0.0 *	[%]
BASO	0.08 *	[10^9/L]	0.2 *	[%]
RET	0.14	[%]	2.0	[10^9/L]
IRF	17.7	[%]		
LFR	82.3	[%]		
MFR	11.8	[%]		
HFR	5.9	[%]		

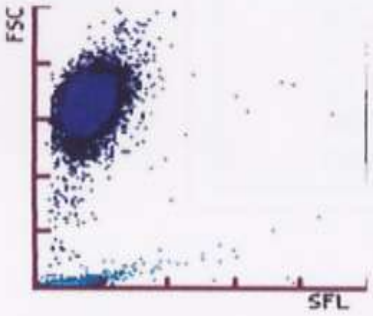
DIFF



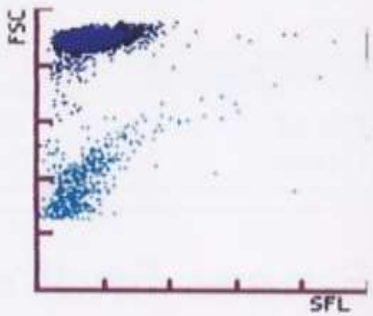
WBC/BASO



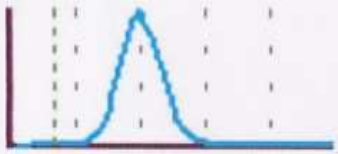
RET



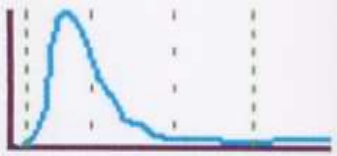
PLT-O



RBC



PLT



WBC IP Messages

WBC Abn Scattergram
Leukocytosis

Blasts?

RBC/RET IP Messages

Anemia

PLT IP Messages

Thrombocytopenia

PLT Clumps?

Visual count data

Stab	0 [%]
Seg	2 [%]
Lymph	4 [%]
Mono	0 [%]
Eo	0 [%]

Most Common Types of Leukemia

knowmedge

Acute Myelogenous Leukemia (AML)

Occurs in both children and adults

Acute Lymphocytic Leukemia (ALL)

Most common type of Leukemia in children. Also affects adults.

Chronic Myelogenous Leukemia (CML)

Mainly affects adults

Chronic Lymphocytic Leukemia (CLL)

Most often in people over age 55

* Classification of leukemia:

1. Acute Leukemia:

- Acute lymphoblastic leukemia (ALL): L1 – L3.
- Acute myeloblastic leukemia: (AML): M0 – M7.

2. Chronic Leukemia:

- Chronic lymphocytic leukemia (CLL).
- Chronic myelocytic leukemia (CML).

Acute Leukemia

- Chromosomal abnormality: Imbalance between oncogene and anti-oncogene
- Blast cells: Uncontrolled proliferation of this clone results in:

  1- Arrest of cellular differentiation.

  2- Reduced apoptosis.

  3- Inhibition of normal marrow elements
- Tissue infiltration: Hepatomegaly More common with ALL than AML.Splenomegaly

Acute Lymphoblastic Leukemia (ALL)

- It is the result of clonal proliferation of lymphoid progenitor cells originating in the marrow
- Clinical features
- The disease affects children, Incidence: Under 10 – 15 years (Childhood onset)→B-cell ALL or T-cell
- Bone marrow failure: RBCs → Anemia: pallor, lethargy, malaise and dyspnea.Leukocytes → neutropenia: fever, malaise, features of mouth, skin, respiratory infections. Platelets → throbombocytopenia: spontaneous bruises, pupura, bleeding gums, respiratory infections due to neutropenia. Spontaneous bruises, pupura, bleeding gums due to thrombocytopenia.
- Generalized lymphadenopathy.Hepatomegaly.Splenomegaly.Organ infiltration:Bony infiltration.Testicular infiltration.C.N.S. infiltration.Fever.

Laboratory Investigations of ALL

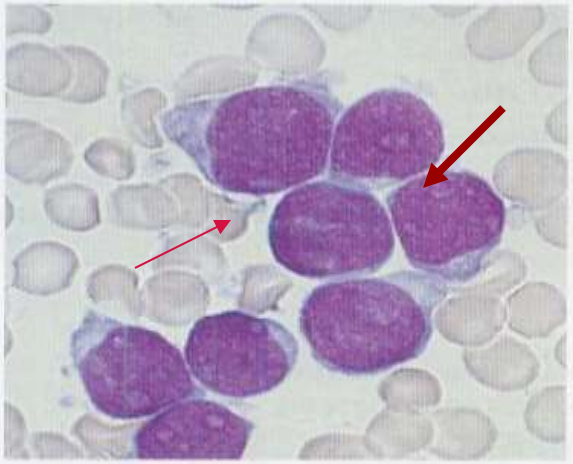
- CBC:↑↑ WBCs with lymphoblasts in the peripheral blood; RBCs: ↓ (anaemia).Platelets: ↓
- Bone marrow Aspirate: All normal marrow elements are depressed and replaced by abnormal blast cells
- Morphological classification of ALL L1 L2 L3

L1 subtype-blasts show scanty cytoplasm without granules.

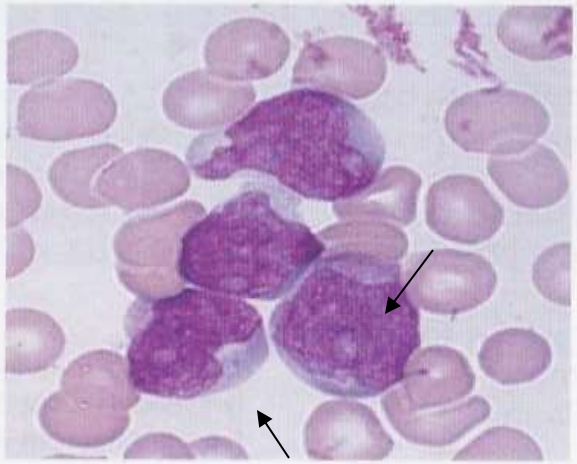
L2 subtype-blasts are larger and heterogeneous with more abundant cytoplasm.

L3 subtype-blasts are deeply basophilic with cytoplasmic vacuolation

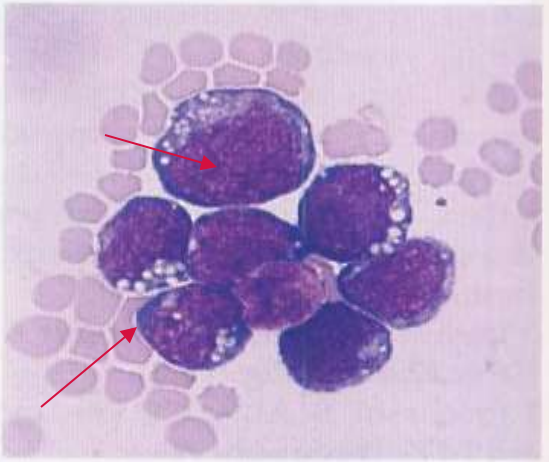
Subtype & morphology ALL



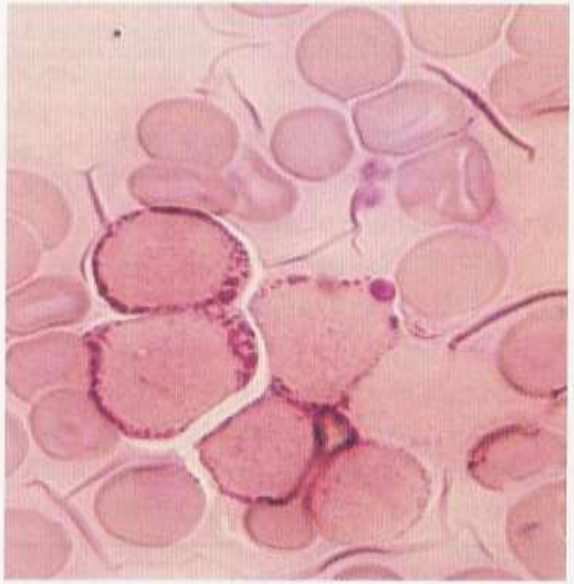
(a) L1. subtype (granulasi-)



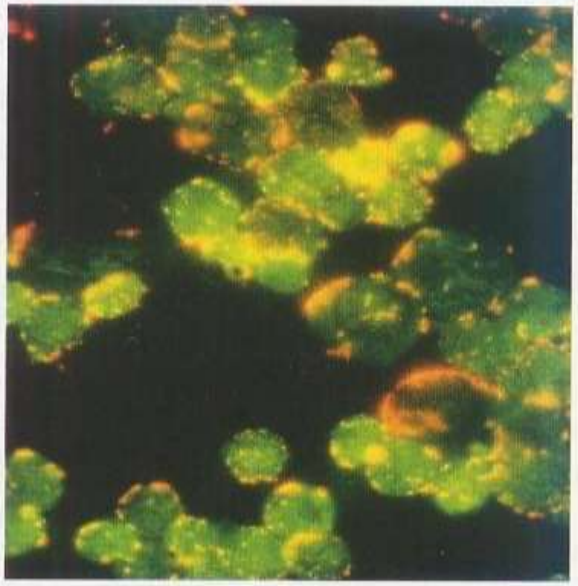
(b) L2. blst langer cytoplasma penuh



(c) L3. (basophilic dg cytoplasma vacuolation)



(d) Staining PAS



(e) Indirect immunofluorescene

Fig. 12.1 Acute lymphoblastic leukaemia. (a) L_1 subtype—blasts show scanty cytoplasm without granules. (b) L_2 subtype—blasts are larger and heterogeneous with more abundant cytoplasm. (c) L_3 subtype—blasts are deeply basophilic with cytoplasmic vacuolation. (d) Periodic acid–Schiff (PAS) staining reveals coarse granules. (e) Indirect immunofluorescence reveals nuclear terminal deoxynucleotidyl transferase (TdT) (green) and membrane CD10 (orange). (Courtesy of Professor G. Janossy.)

Sumber : Hoffbrand AV, 2001

The French-American-British (FAB) Classification for Acute Lymphoblastic Leukemia (ALL)

	L1	L2	L3
Size of blasts	Small,uniform	Large,variable	Medium-large,uniform
Amount of cyplasm	Scanty	variable	moderate
Cytoplasmic basophilia	moderate	variable	intense
Cytoplasmic basophilia	moderate	variable	prominent
Nucleus	Regular,occasional clefting, homogenous chromatin	Irregular, clefting common, heterogeneous chromatin	Regular, nonclefted, homogeneous,finely stipped chromatin
Nucleolus	0-1 inconspicuous	≥ 1,prominent	2-5, prominent
N/C ratio	high	low	low

T **Sun Tsieh, 2008 case 14, pag.137**

Acute Myeloid Leukemia (AML)

- Clonal proliferation of *myeloid precursor cells* with reduced capacity to differentiate into more mature cellular elements
- Results in accumulation of leukemic forms in bone marrow, peripheral blood, and other tissues.Leads to reduction in RBCs, platelets, PMNs
- Several morphologic variants; each one has a characteristic clinical & lab. features (M0 – M7)
- Incidence: 80% of AML is in adults.20% of AML is in children
- Clinical Picture: Anemia→weakness and easy fatigue; Neutropenia → infections; Thrombocytopenia →gingival bleeding, ecchymoses, epistaxis, menorrhagia, Anorexia, weight loss, fever & organomegaly

	Acute leukemias **(proliferations of immature cells, >20% blasts)**	**Chronic leukemias** **(proliferations of mature cells)**
Myeloid	Acute Myeloid Leukemia (AML)	Chronic Myeloid Leukemia (CML, now considered an MPN)
Lymphoid	Acute Lymphoblastic Leukemia (ALL)	Chronic Lymphocytic Leukemia (CLL, now considered lymphoma)

- treatments and prognosis are VERY different for
  - acute vs. chronic leukemias
  - myeloid vs. lymphoid leukemias

Laboratory diagnosis of AML

- **CBC**:↑↑ WBCs with myeloid cells in the peripheral blood; RBCs: ↓ (anaemia).Platelets: ↓
- Bone Marrow Exam: Aspiration (BMP) Morphologic classification using cytochemistry and immunophenotyping
- Cytochemistry:
  - Peroxidase: +
  - Sudan black: +
  - Specific esterase: +
- Immunophenotyping: Pan myeloid markers: + CD13, CD33

TABLE 1. WHO classifications for AML subtypes

Type	Name
M0	Minimally differentiated acute myeloblastic leukemia
M1	Acute myeloblastic leukemia (t(8;21)(q22,q22))
M2	Acute myeloblastic leukemia (t(6;9))
M3	Acute promyelocytic leukemia (APL)
M4	Acute myelomonocytic leukemia
M4eo	Myelomonocytic leukemia with bone marrow eosinophilia
M5	• Acute monoblastic leukemia (M5a) • Acute monocytic leukemia (M5b)
M6	Acute erythroid leukemias, including —Erythroleukemia (M6a) —Very rare pure erythroid leukemia (M6b)
M7	Acute megakaryoblastic leukemia
M8	Acute basophilic leukemia

Key: AML, acute myeloid leukemia; t, translocation; WHO, World Health Organization.

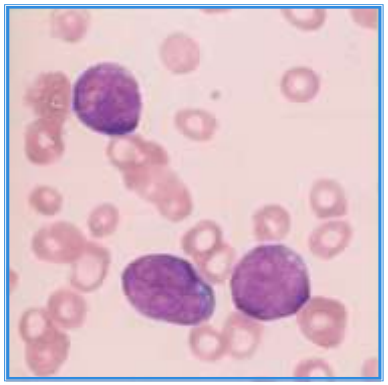
Source: Acute myeloid leukemia classification. News-Medical.net Web site. http://www.news-medical.net/health/Acute-Myeloid-Leukemia-Classification.aspx. Accessed March 9, 2012.

Morfologi & Sitokimia

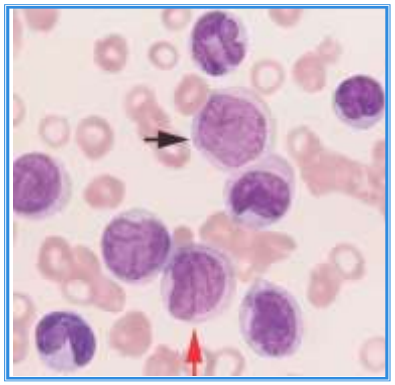
Romanowsky (wright-Giemsa)

- Tidak spesifik

Hapusan Darah Pada ALL

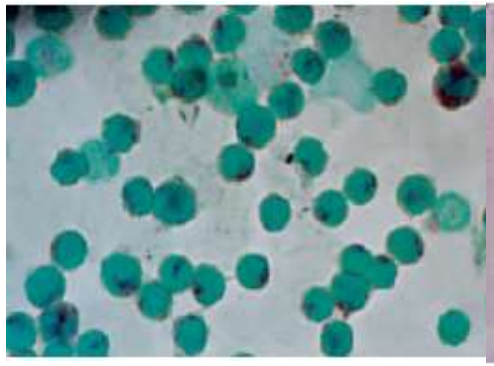


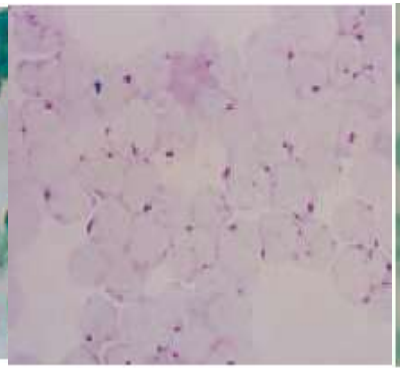
Hapusan Darah Pada AML

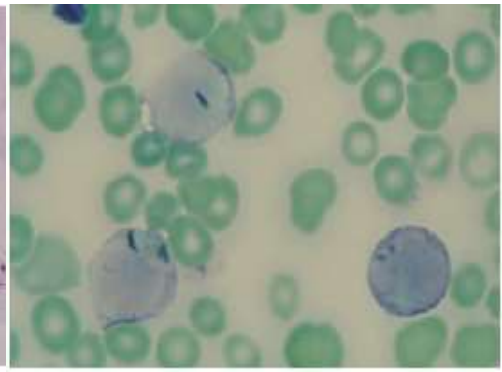


Sitokimia (Pewarnaan spesifik)

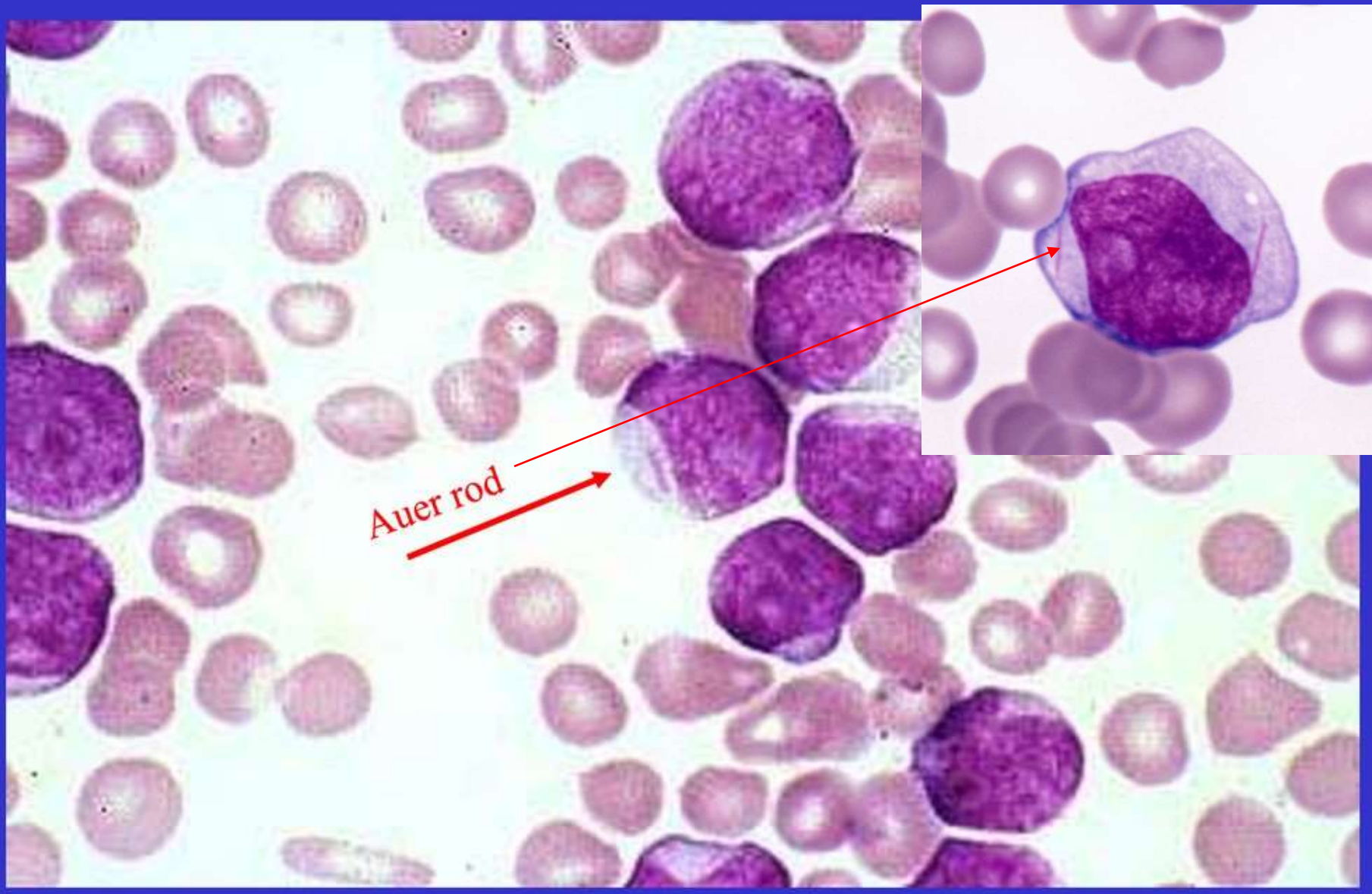
- Non enzim : Periodic Acid Schiff (PAS) →Lymphosit / ALL (+)
- Sudan Black B→AML (+)
- Enzim : Myeloperoksidase (MPO)→ AML (+) terlihat multiple Auer rods







Myeloblasts - AML



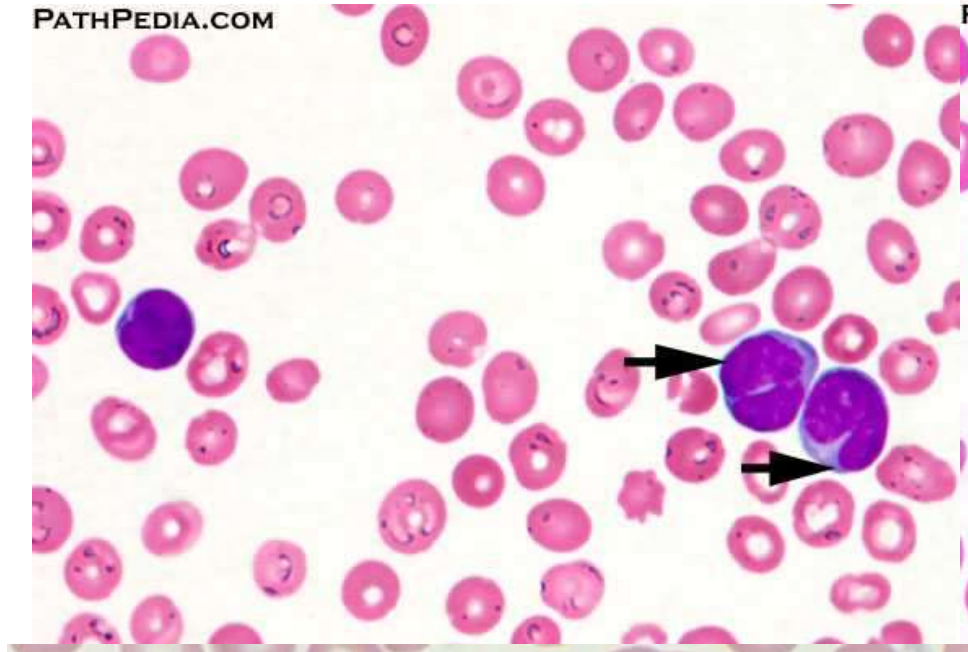
Chronic leukemia

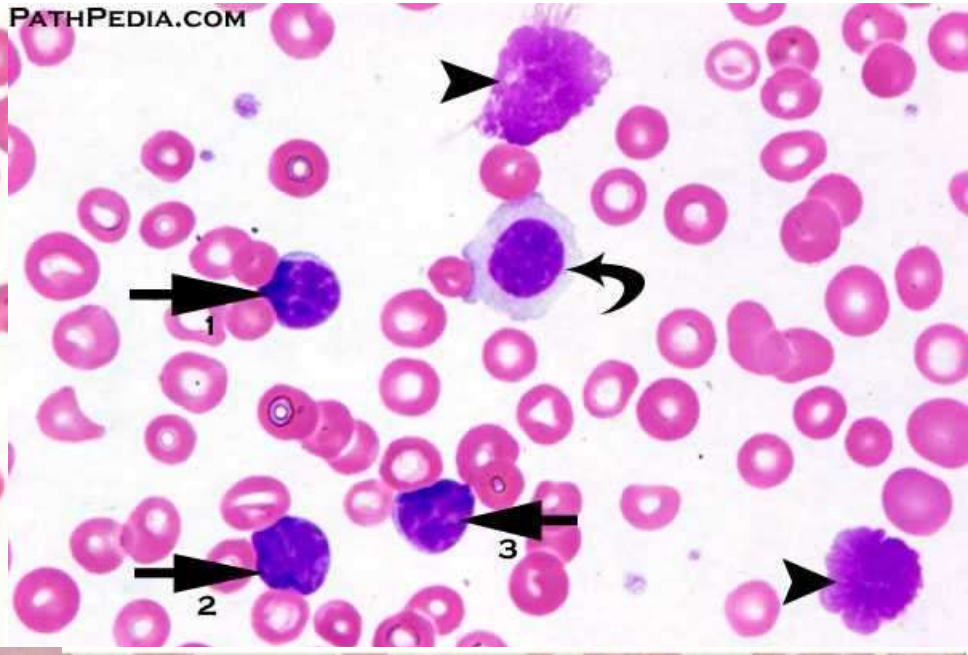
- Chronic leukemia progresses more slowly and results in the accumulation of relatively mature, but still abnormal, white blood cells, more difficult to treat
- Diagnosis is typically based on blood tests finding high numbers of mature lymphocytes and smudge cells

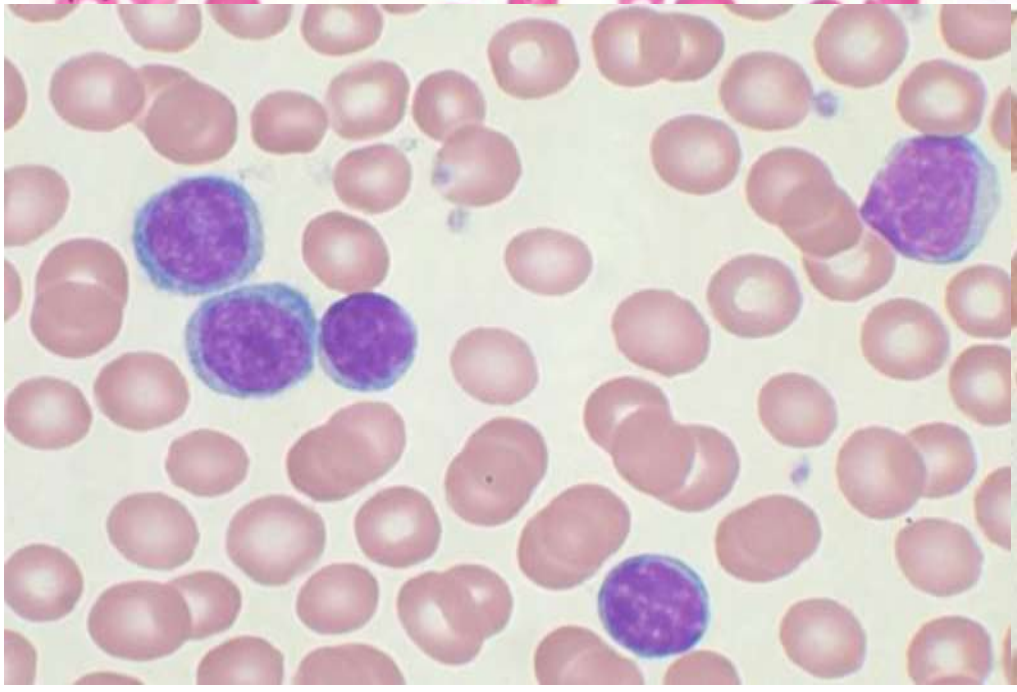
Chronic Lymphocytic Leukemia

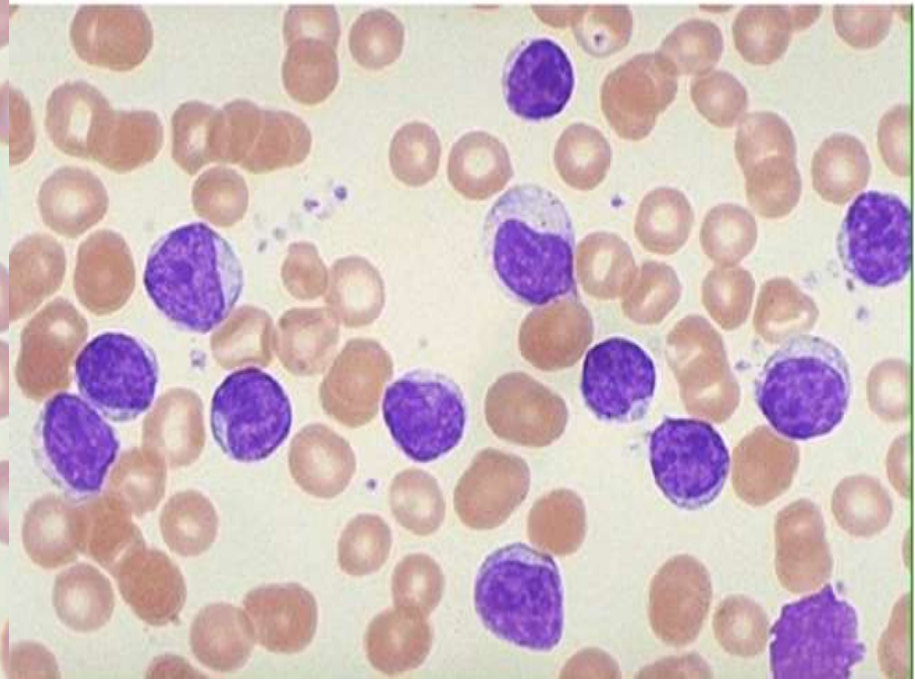
- characterized by the clonal proliferation and accumulation of neoplastic B lymphocytes in the blood, bone marrow, lymph nodes, and spleen
- The median age of patients at diagnosis is 65 years, with only 10 to 15 percent under 50 years of age
- more men than women are affected

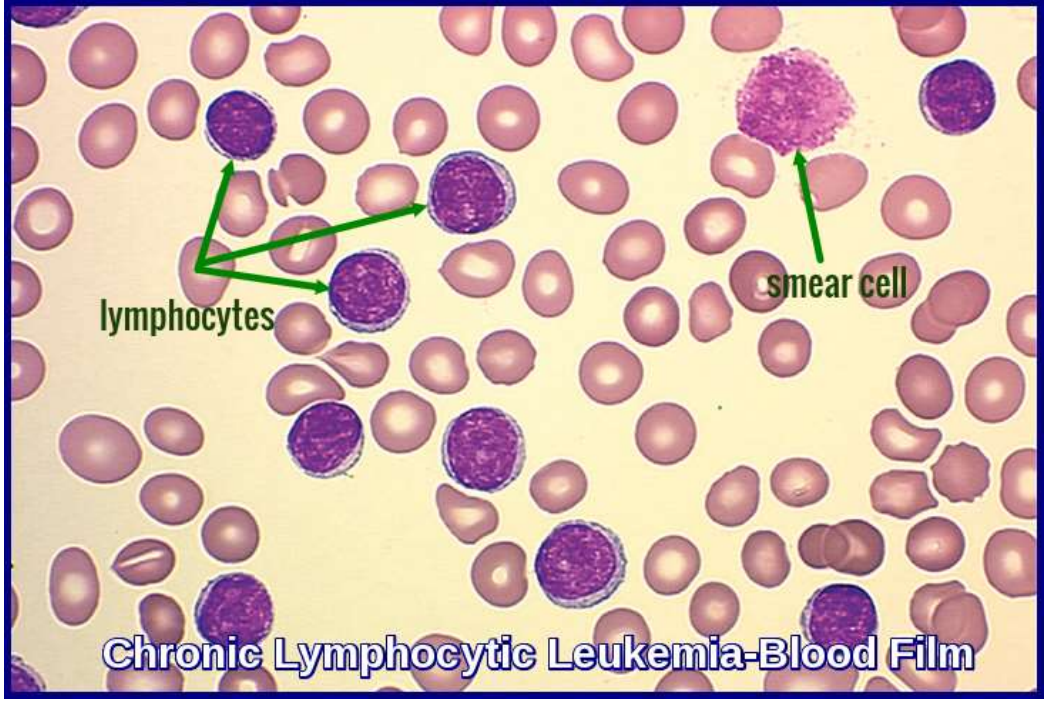
Chronic Lymphocytic Leukemia

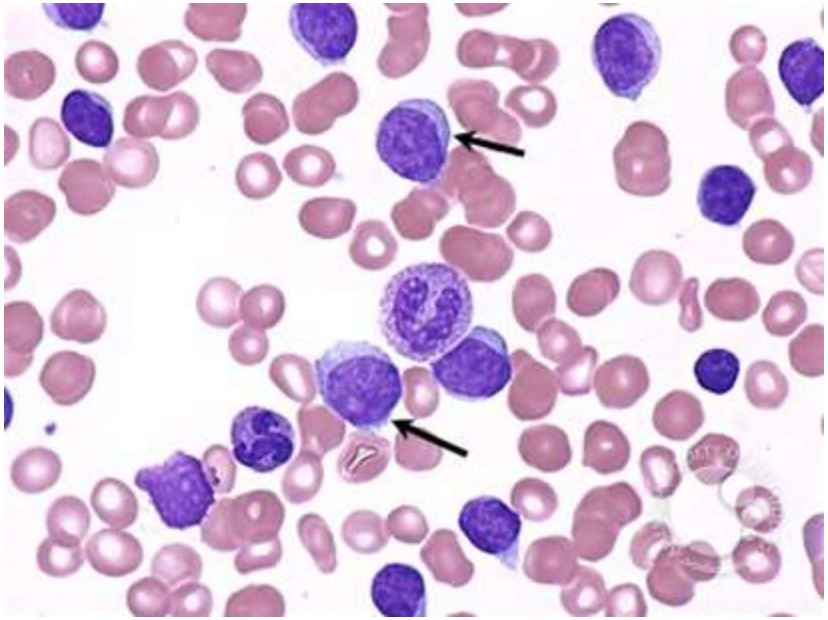








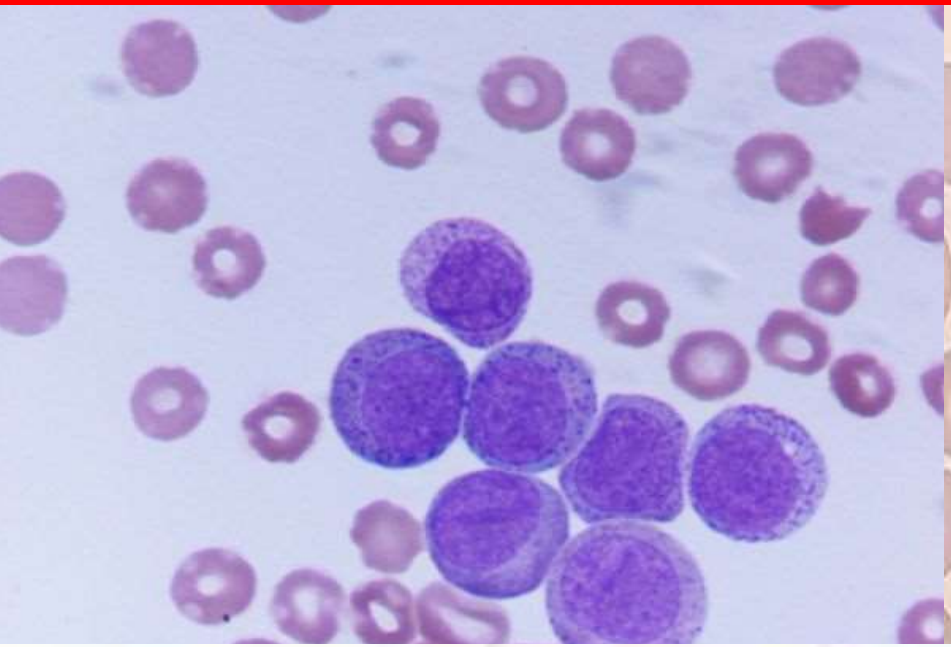

lymphocytes
smear cell
Chronic Lymphocytic Leukemia-Blood Film

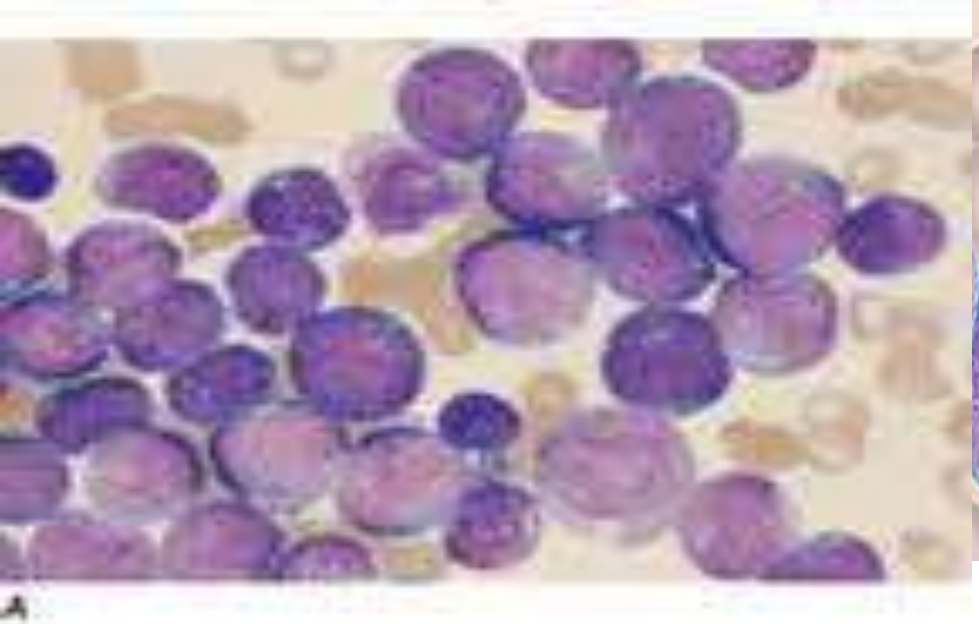


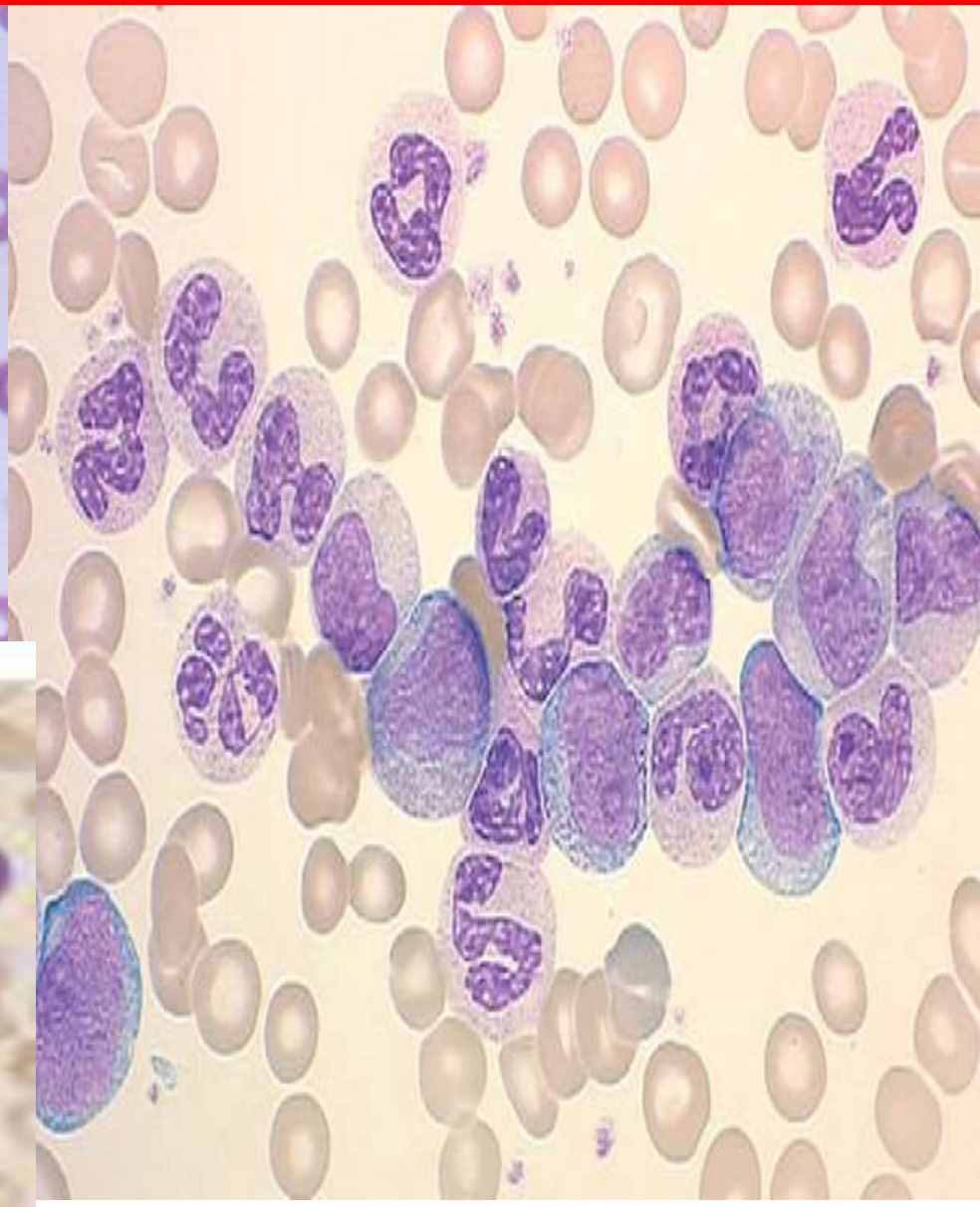
Chronic myeloid leukemia (CML)

- also known as chronic myelogenous leukemia, begins in the blood-forming cells of the bone marrow and then, over time, spreads to the blood→ Eventually, the disease spreads to other areas of the body
- slow-growing, but once it starts causing symptoms, these may include fatigue, fever, weight loss and an enlarged spleen
- Around half of CML cases are diagnosed by a blood test before symptoms have begun. About 15 percent of leukemias are CML

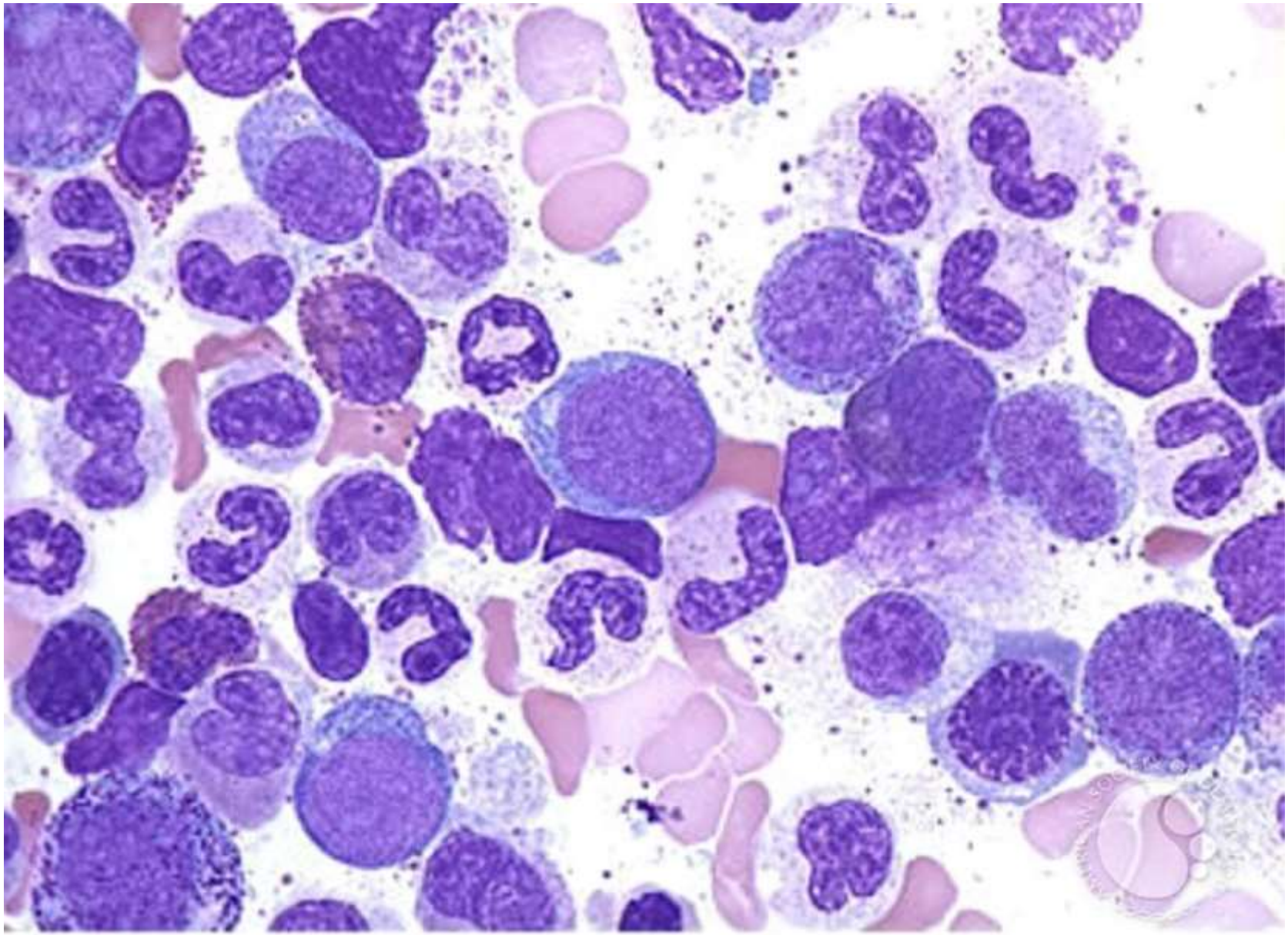
Chronic Myelocytic Leukemia (CML)

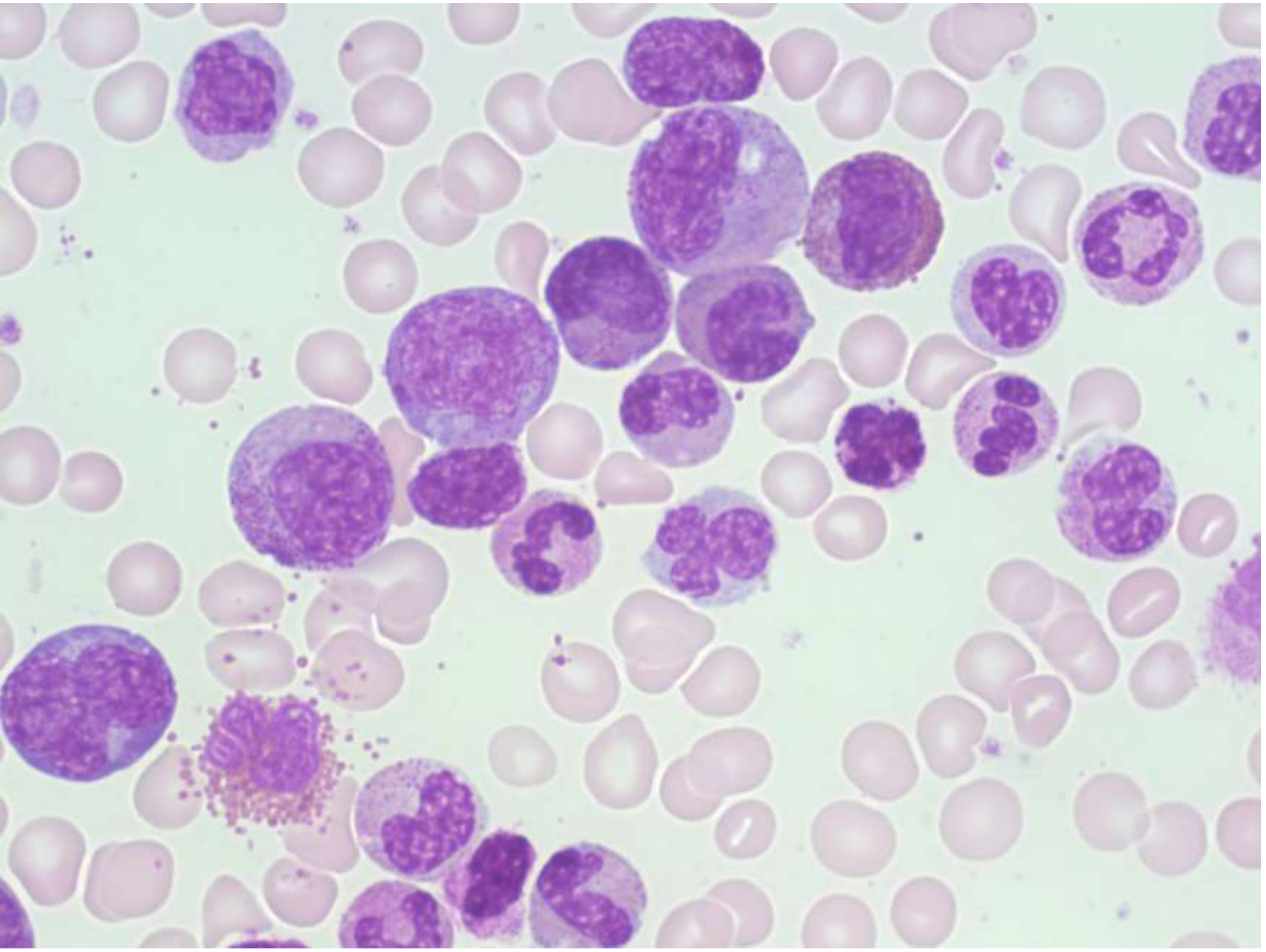






Chronic myeloid leukemia (CML)

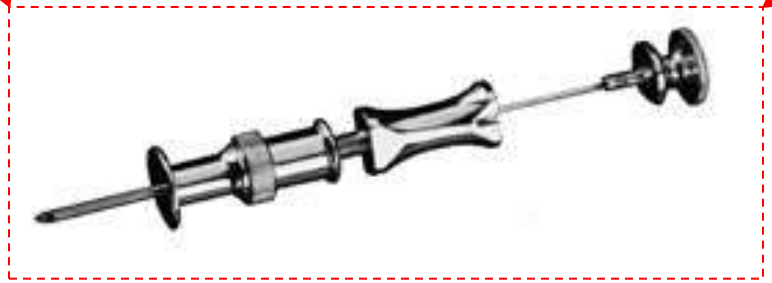




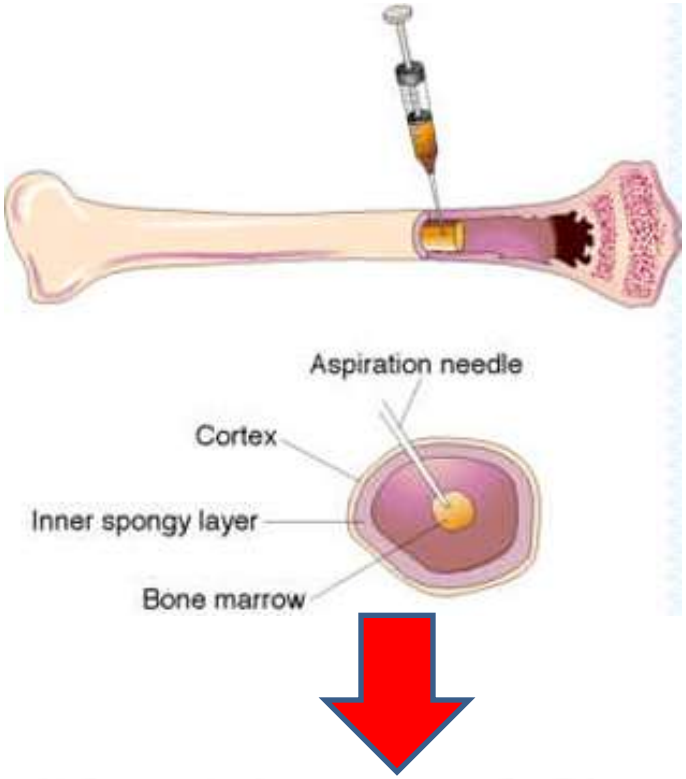
Role of BMP

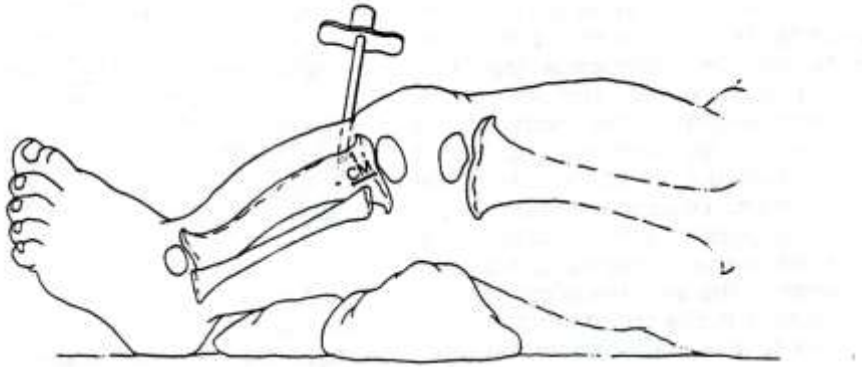
- Differentiate : non neoplastic and neoplastic lesion
- Differentiate : benign and malignant tumor
- Differentiate Malignant tumors : primary, metastatic, or recurrence.
- Rapid diagnosis for emergency case :spinal lesion with cord compression.
- Determine staging of tumor.

BONE MARROW ASPIRATION PROCEDURE



BONE MARROW ASPIRATION PROCEDURE





Bone marrow

- ⇨ **soft material in the center of bones**
- ⇨ **contain : termed cellular marrow**

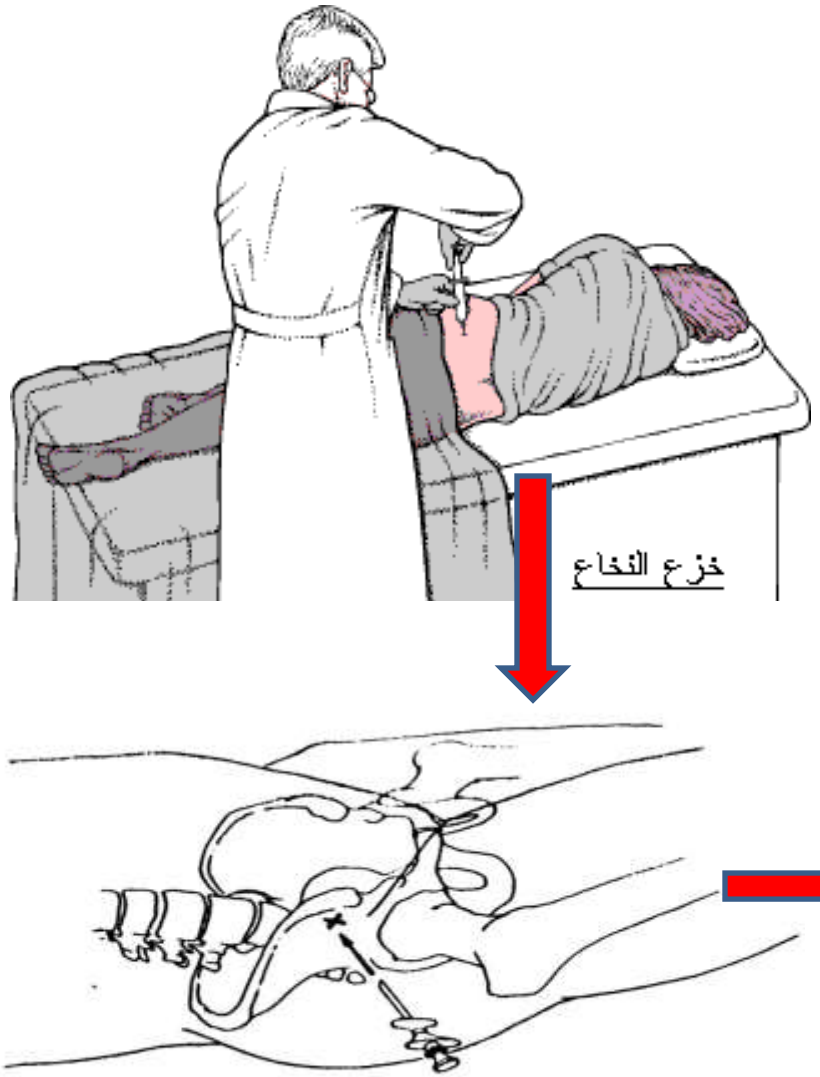
  different types of cells

  - ⇨ **red cells**

    white cells

    platelets

BONE MARROW ASPIRATION PROCEDURE



- **The skin is cleansed with an antiseptic**
- **Local anesthetic is injected at the procedure site to numb the skin and tissue down to the surface of the bone**
- **A small nick is made in the skin**

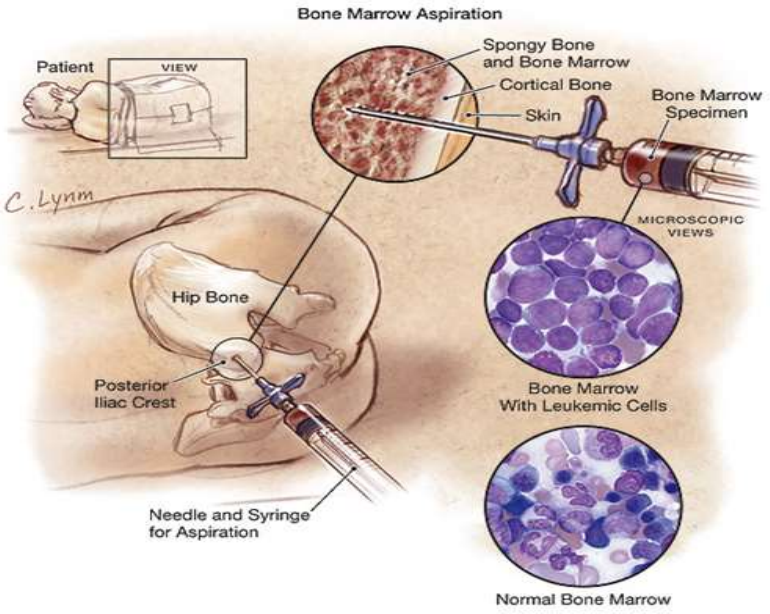
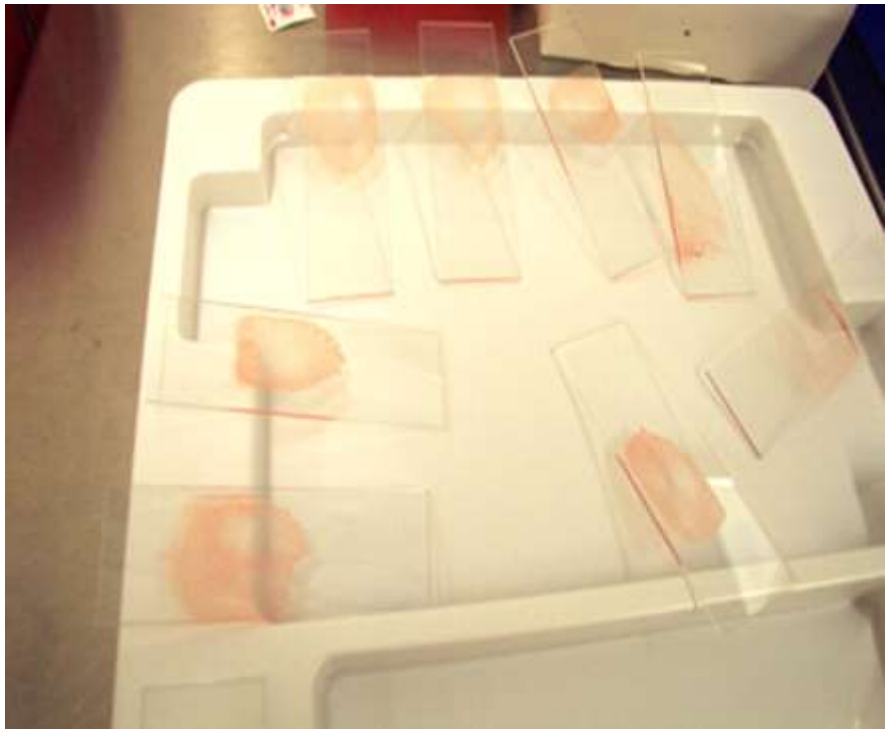
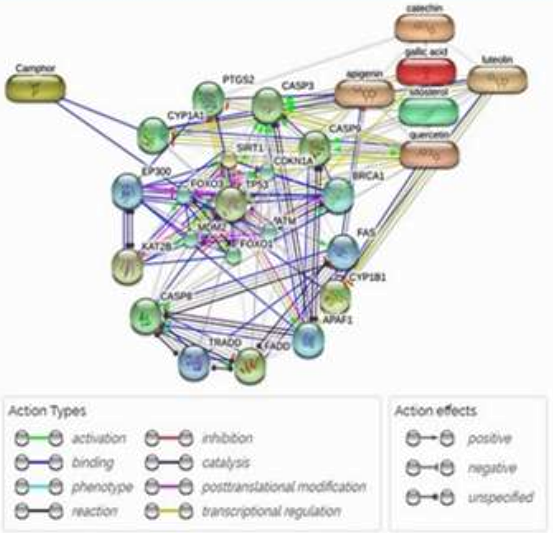


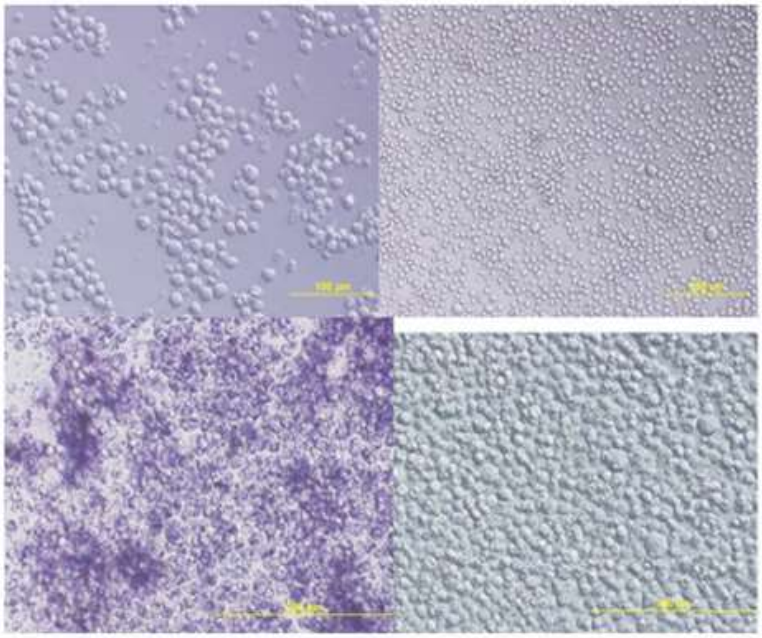
Photo credit: Mihaela Onciu, MD/St Jude Children's Research Hospital

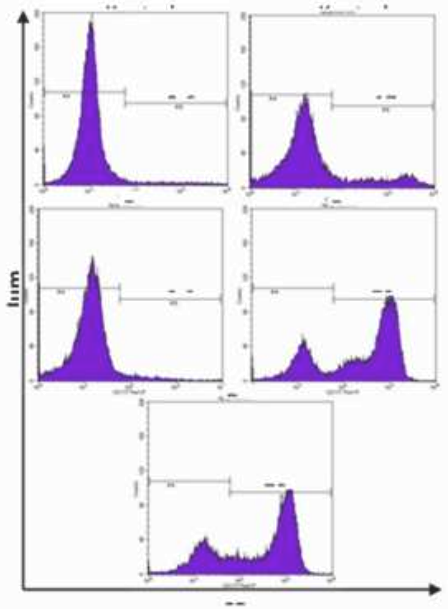
BONE MARROW ASPIRATION PROCEDURE



- **Aspirate of the bone marrow is spread on glass slides by selecting the marrow particles and avoiding dilution with blood**
- **The routine Wright or Giemsa staining were performed**
- **The slides examined under the microscope**


catechin
gallic acid
luteolin
Camphor
PTGS2
CASP3
apigenin
isoscutellarein
CYP1A1
CASP9
quercetin
SIRT1
CDKN1A
EP300
FOXO3
TP53
BRCA1
ATM
MDM2
FAS
KAT2B
FOXO1
CYP1B1
CASP8
APAF1
TRADD
FADD
Action Types
activation
inhibition
binding
catalysis
phenotype
posttranslational modification
reaction
transcriptional regulation
Action effects
positive
negative
unspecified




Num

Road Map Penelitian Ekstrak (Cyperus rotundus)

2018-2022

- determinasi umbi rumput teki (Cyperus rotundus) by Meteria Medica
- uji senyawa aktif CRT(GCMS/LCMS)
- Studi awal Bioinformatika: *in silico & Docking*
- Uji MTT, IC.50 (Konsentrasl 367 - 384 µg/mL)
- *In vitro* dg HL-60 cell line AML (APL)
- Terdaftar PATEN No.P00202205683 (22 Mei 2022)

-

2023-2024

- Uji efektifitas CRT Adjuvant kemoterapi pd mencit yg diinjeksi 4T1 yg diberi kemoterapi

Uji toxisitas akut & sub kronis (in vivo) tikus (Rattus norvegicus) sbb:

-tes faal ginjal

-tes fs liver

- toksisitas thd limfosit
- Efikasi profil molekul proinflamasi
- Efikasi ekstract CRT thd Makropatologi & Histopatologi

2024-2026

- Adjuvant kemoterapi berbahan umbi rumput teki (*Cyperus rotundus*) sebagai imunomodulator dalam menjaga sistem imunitas pada mencit model kanker injeksi 4T1
- Prototipe senyawa ekstrak CRT dg air

-Analisa senyawa kimia CRT (FTIR), HPLC, DPPH

- UJI antioksidan DPPH
- Uji sinergisitas CRT dg Rosella


Selamat belajar & Sukses