Acute Leukemias

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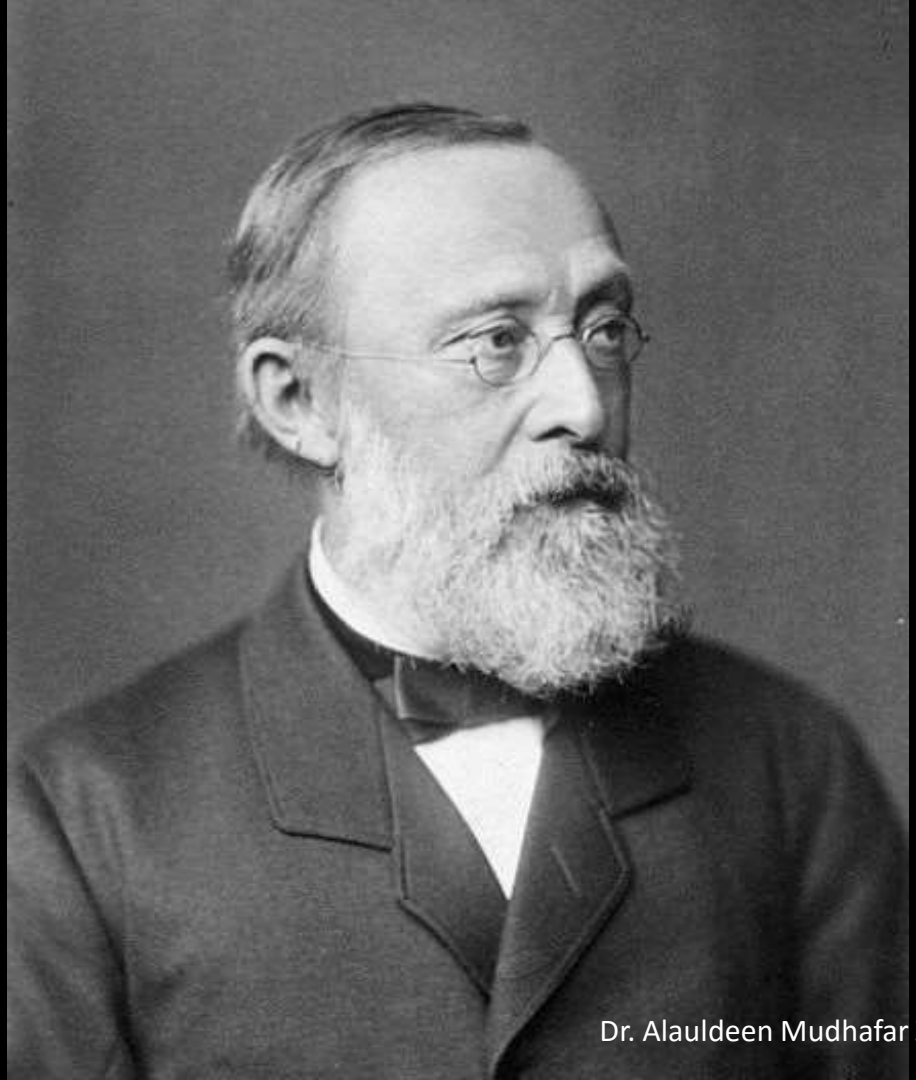
Leukemia is a disease resulting from the neoplastic proliferation of hemopoietic or lymphoid cells. It results from a mutation in a single stem cell, the progeny of which form a clone of leukemic cells. The cell in which the leukemic transformation occurs may be a lymphoid precursor, a myeloid precursor or a pluripotent stem cell capable of differentiating into both myeloid and lymphoid cells.

History

In 1845, Virchow and John Bennett independently observed abnormal increase in white blood cells in patients. Virchow correctly identified the condition as blood disease, and named it *leukämie* in 1847



Rudolf Carl Virchow
1821 – 1902

Leukemias are divided into:

1. Acute leukemia which if untreated leads to death in weeks or months.
2. Chronic leukemia which if untreated leads to death in months or years.

In acute leukemias, cells of the leukemic clone continue to proliferate without maturing to end cells and dying, so immature cells predominate. Chronic leukemias are characterized by an expanded pool of proliferating cells that retain their capacity to differentiate to end cells.

Predisposing factors:

1) Ionizing radiation: X-rays and other ionizing rays were the first identifiable agents associated with the induction of leukemia. This became apparent in Hiroshima and Nagasaki. AML and CML were the predominant types. Patients treated by irradiation for other malignancies develop myelodysplasia and AML often.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CORBIS

2) Chemicals: **Benzene** and other petroleum derivatives. There was a high incidence of leukemia and aplastic anemia in occupationally exposed people.

3) Alkylating agents: (Cyclophosphamide, chlorambucil, busalphan and melphalan).These drugs used to treat malignancies and they are associated with a high incidence of myelodysplasia and AML. Acute leukemias following irradiation and/or chemotherapy are called secondary acute leukemias.

4) Genetic disorders:

- Down's syndrome: trisomy 21
- Bloom's syndrome: AR, short stature, predisposition to cancer.
- Fanconi's anemia: AR, aplastic anemia
- Ataxia telangiectasia: AR

Acute Leukemias

- Acute leukemia is defined by the presence of 20% or more blast cells in the peripheral blood and/or bone marrow or finding a specific cytogenetic abnormality like t(15;17).
- Blasts cells are primitive looking immature cells. They proliferate in the bone marrow and spill into blood and infiltrate other organs.

Incidence:

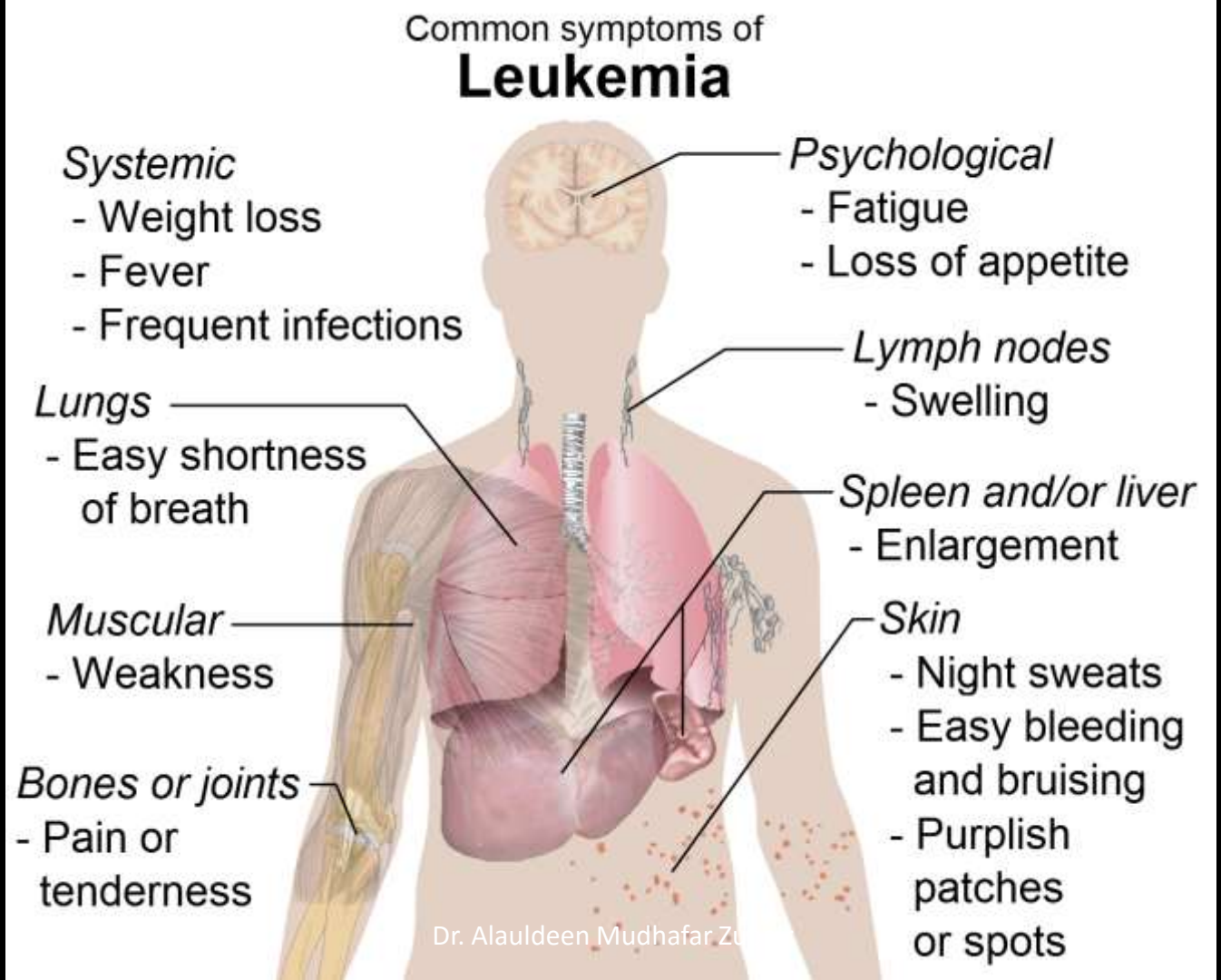
ALL constitute over 80% of childhood cases, while AML comprises around 80% of adult cases. Among adults the incidence of AML rises with age, from approximately 1/100000/year in the fourth decade, to approximately 10/100000/year in these over 70 years. AML is common in males. The incidence of ALL in children up to the age of 15 years is 2.5 – 3.5/100000/year. It is commoner in males.

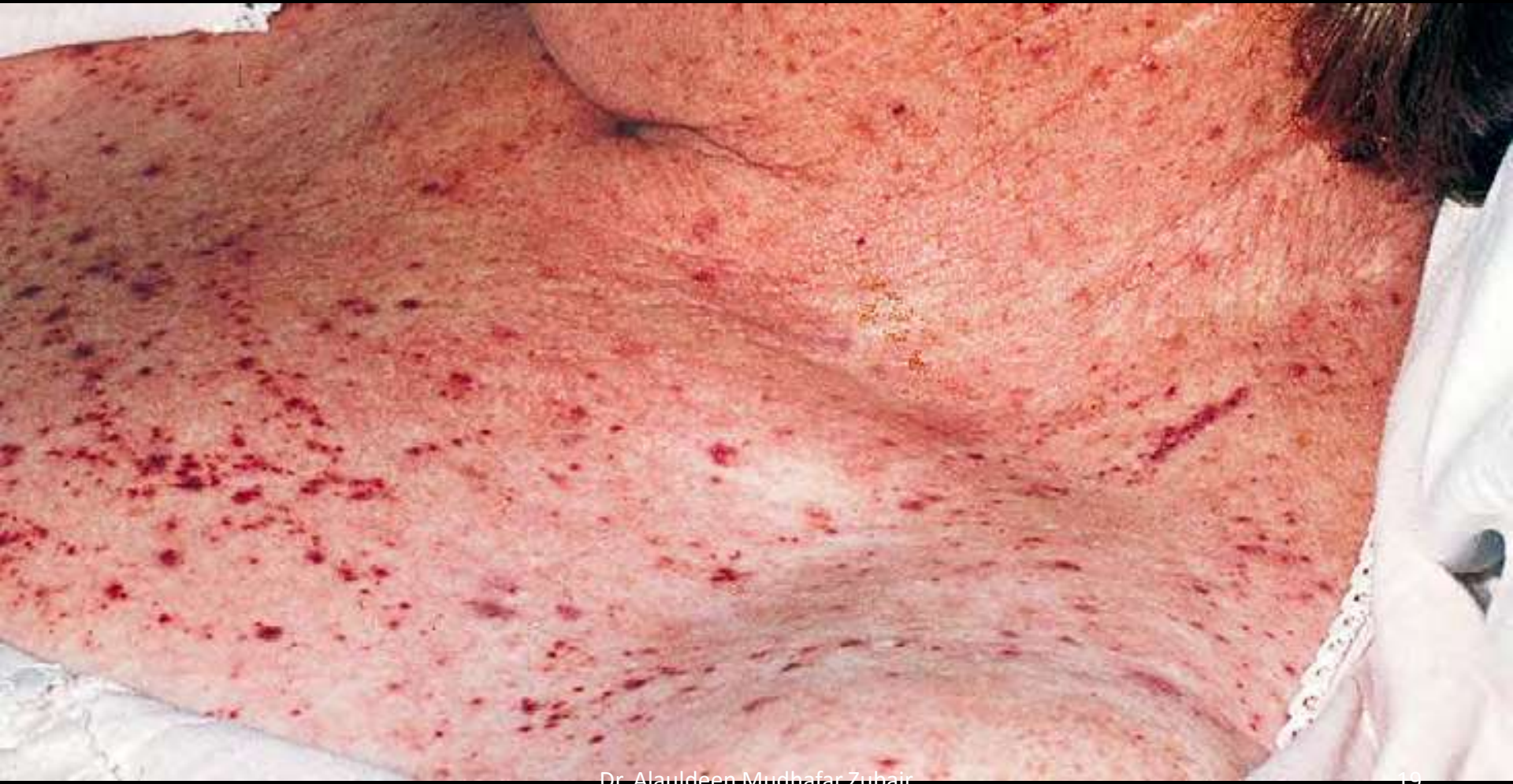
Clinical features:

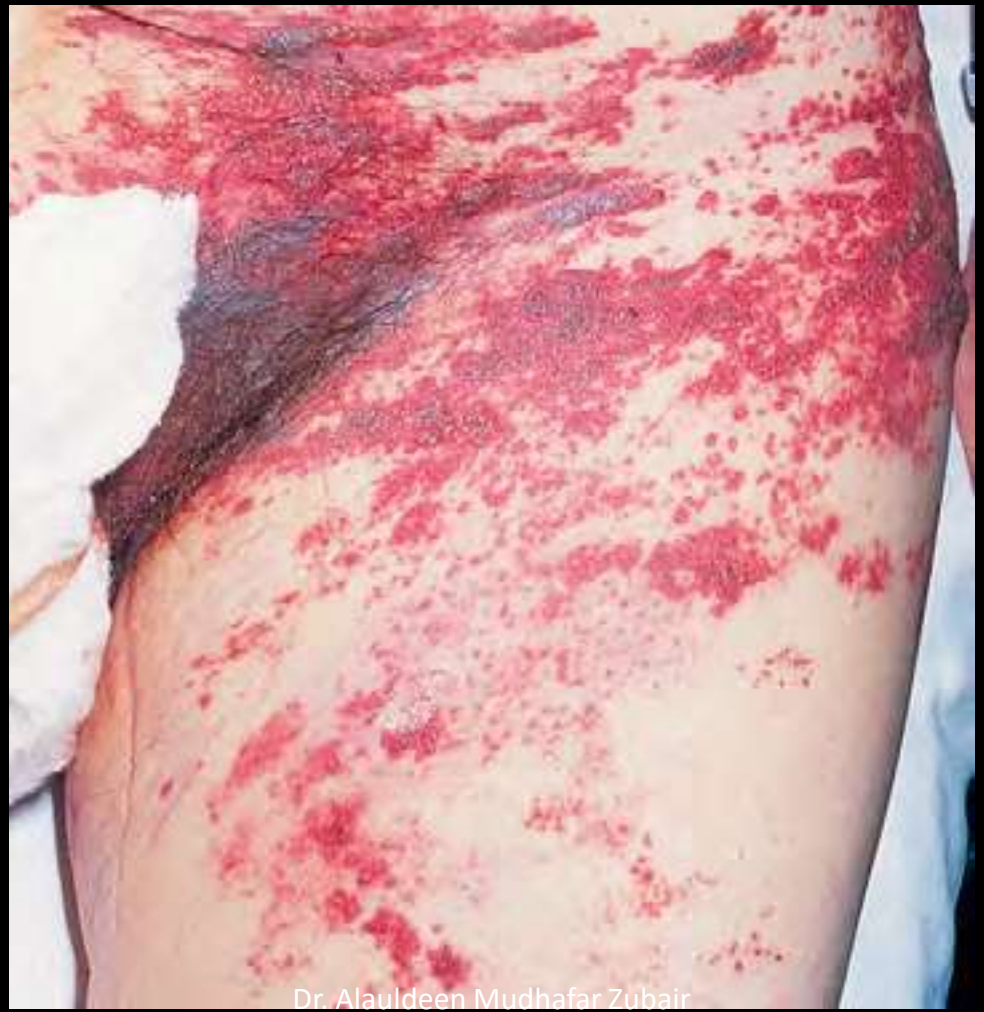
A.Resulting from bone marrow failure

1) Anemia: pallor, dyspnea, and easy fatigability.

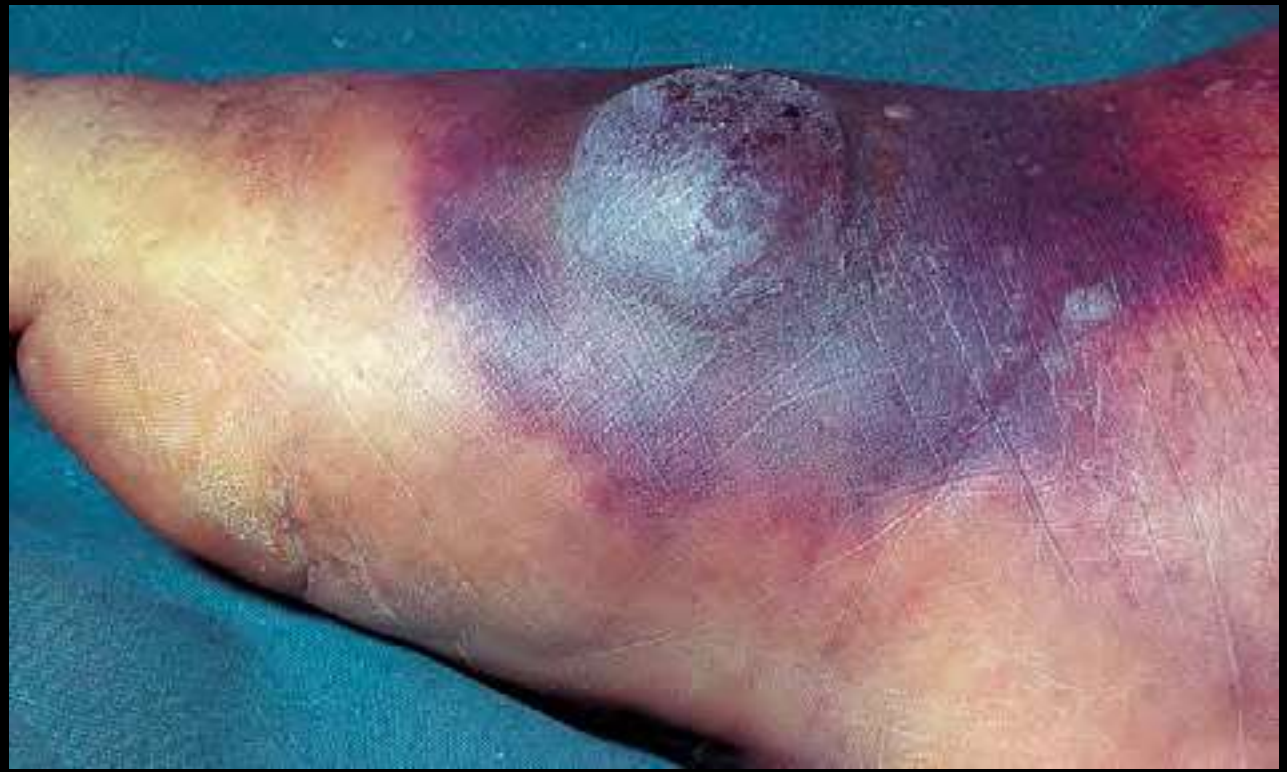
2) Thrompocytopenia: Leads to bleeding tendency; spontaneous bruises, purpura, bleeding gums, menorrhagia and bleeding from venepuncture sites. A bleeding tendency due to DIC is characteristic of AML/M3.

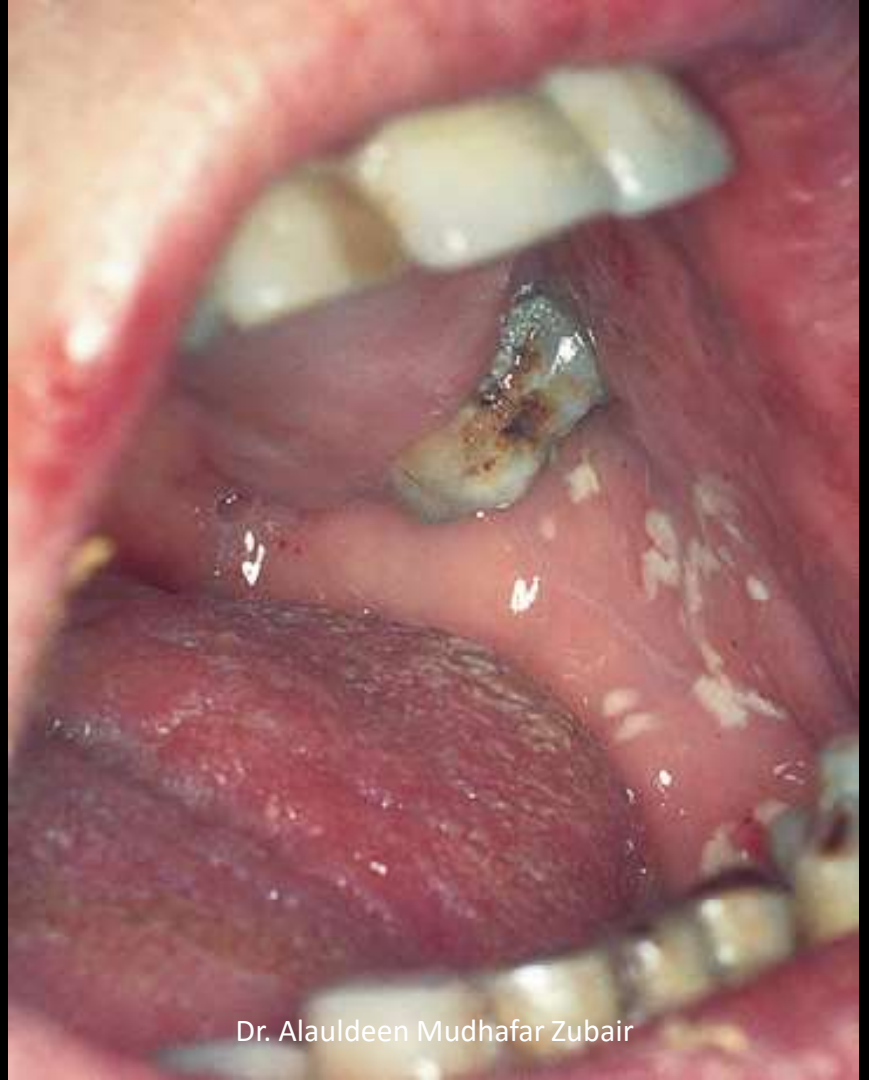

Common symptoms of
Leukemia
Systemic
- Weight loss
- Fever
- Frequent infections
Psychological
- Fatigue
- Loss of appetite
Lymph nodes
- Swelling
Lungs
- Easy shortness of breath
Spleen and/or liver
- Enlargement
Muscular
- Weakness
Skin
- Night sweats
- Easy bleeding and bruising
- Purplish patches or spots
Bones or joints
- Pain or tenderness





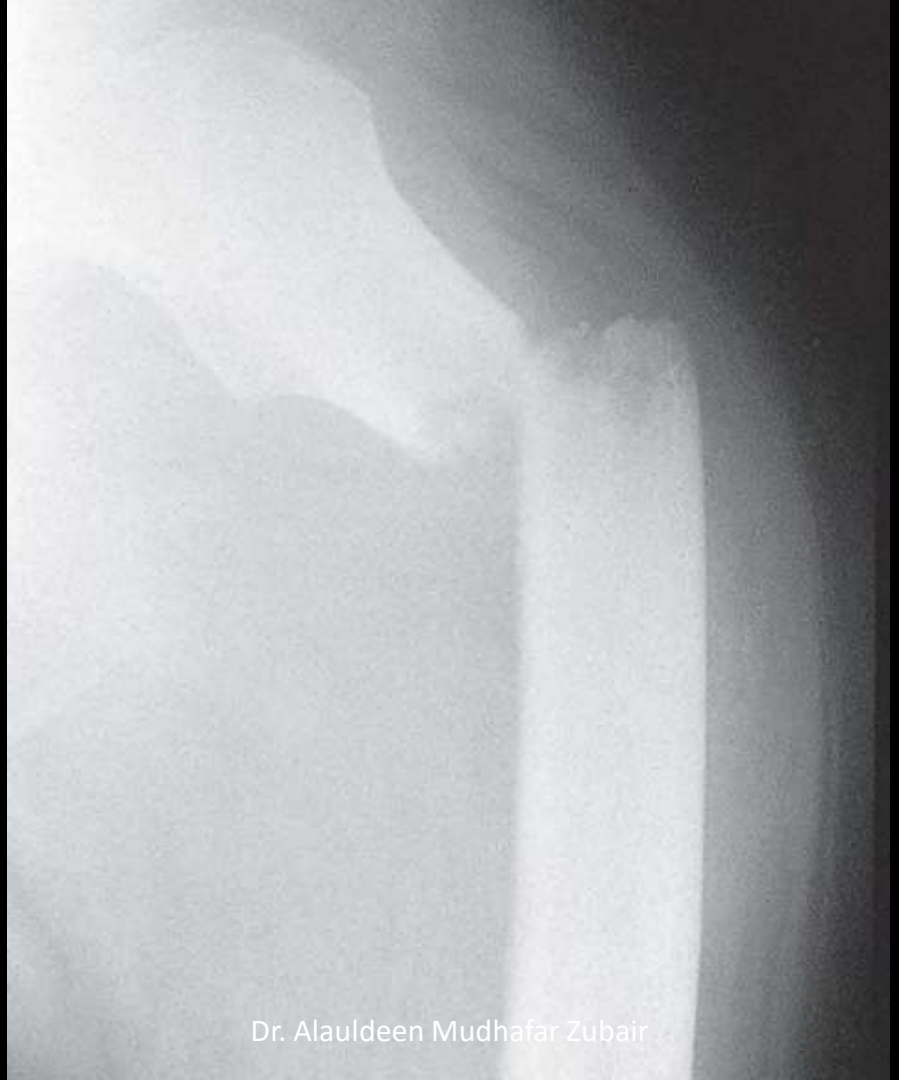
3) Neutropenia: fever, malaise, infections of the followings (mouth, throat, skin, respiratory tract and even septicemia). The infections are predominantly bacterial (gram positive and gram negative), but also viral (e.g.; herpes simplex and zoster), fungal (e.g.; candida) and protozoal (e.g.; Toxoplasma gondii) infection occur with increased frequency.

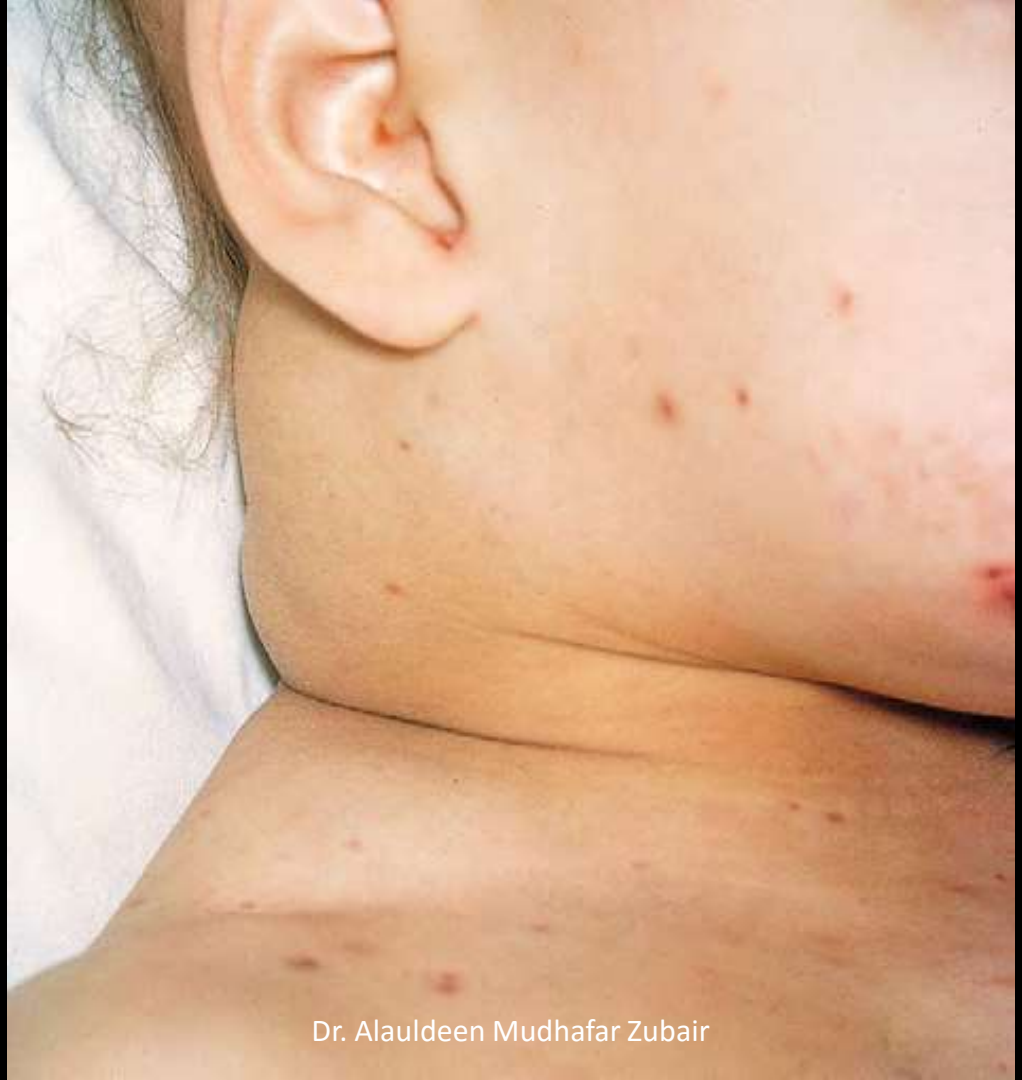


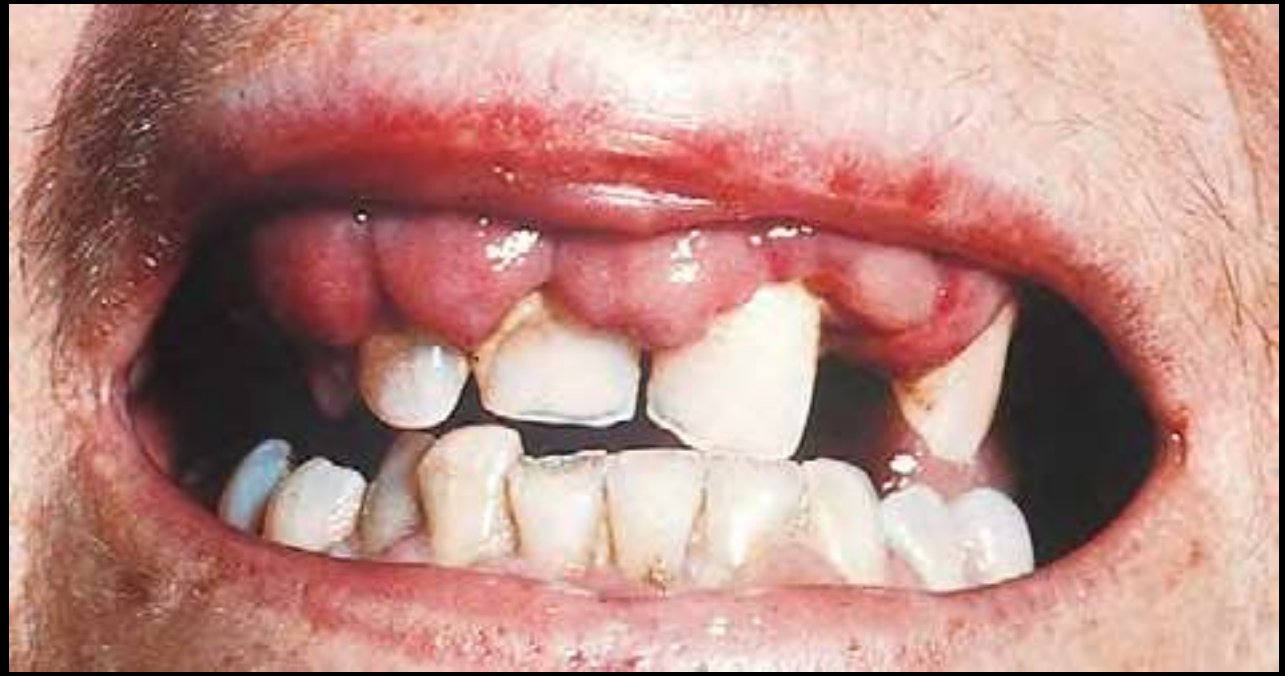


B. Due to organ infiltration:

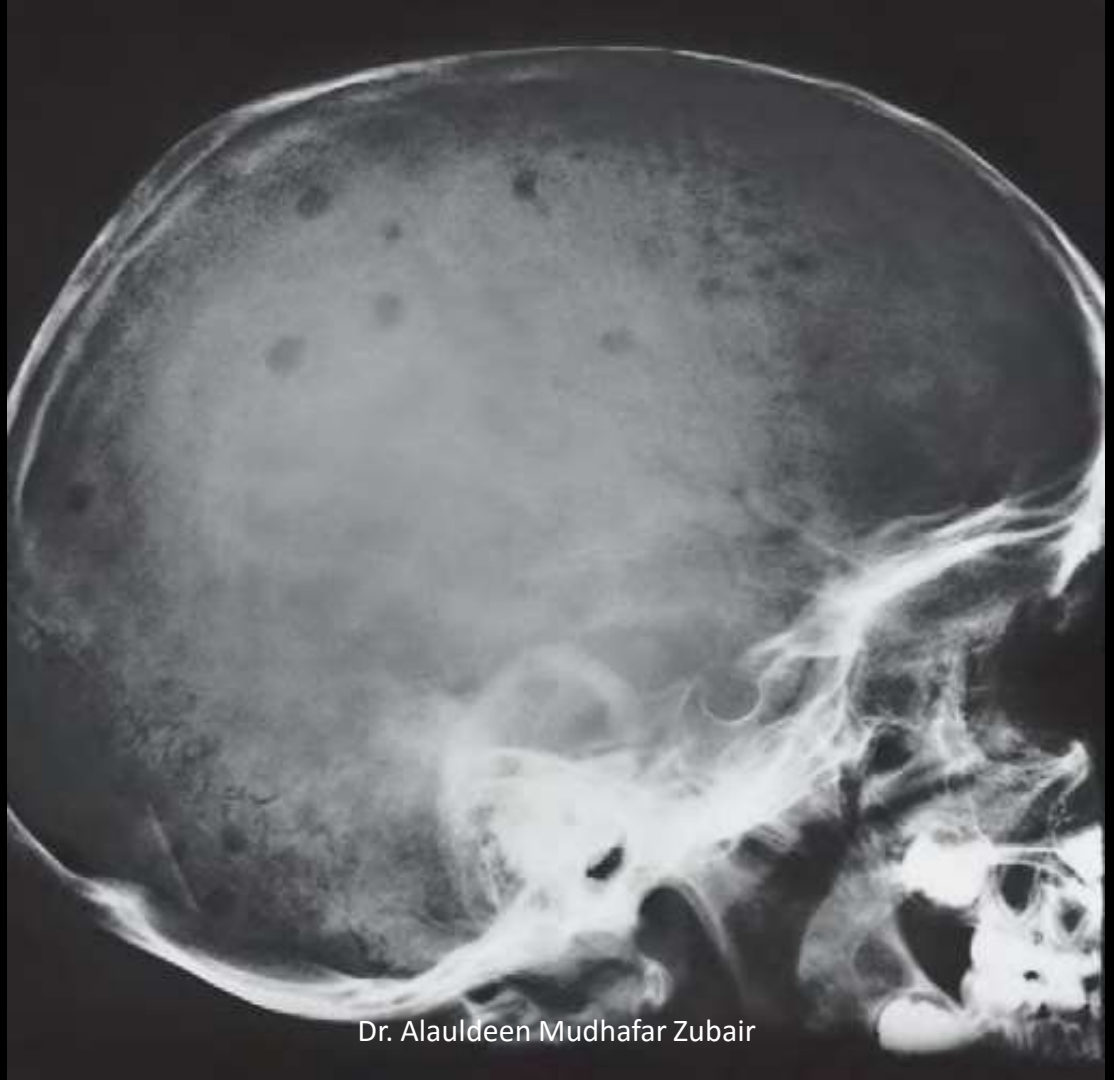
4) Tender bones, especially in children.
5) Superficial lymphadenopathy in ALL.
6) Moderate splenomegaly, hepatomegaly in ALL.
7) Gum hypertrophy and infiltration, skin involvement especially in M4 & M5 subtypes. Lysozyme released by the blast cells may cause renal damage with potassium leakage and hypokalemia in M5.





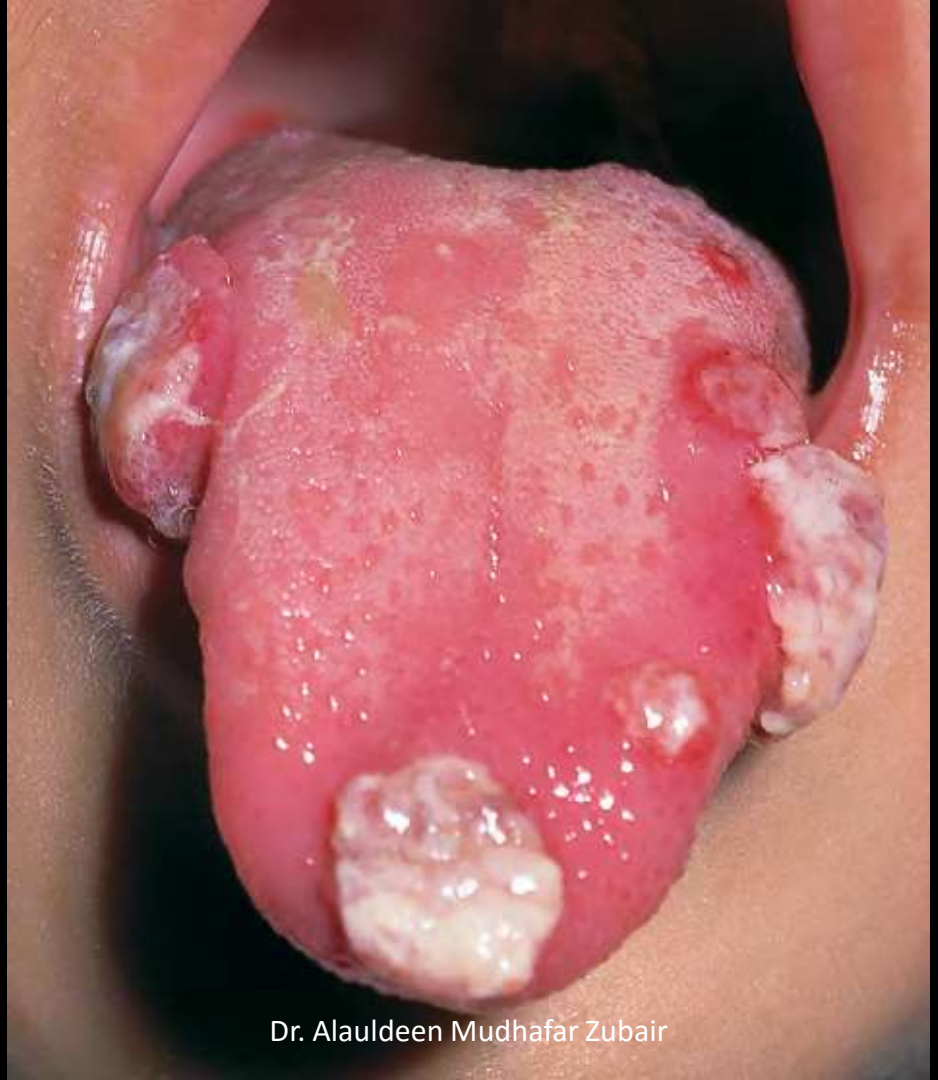


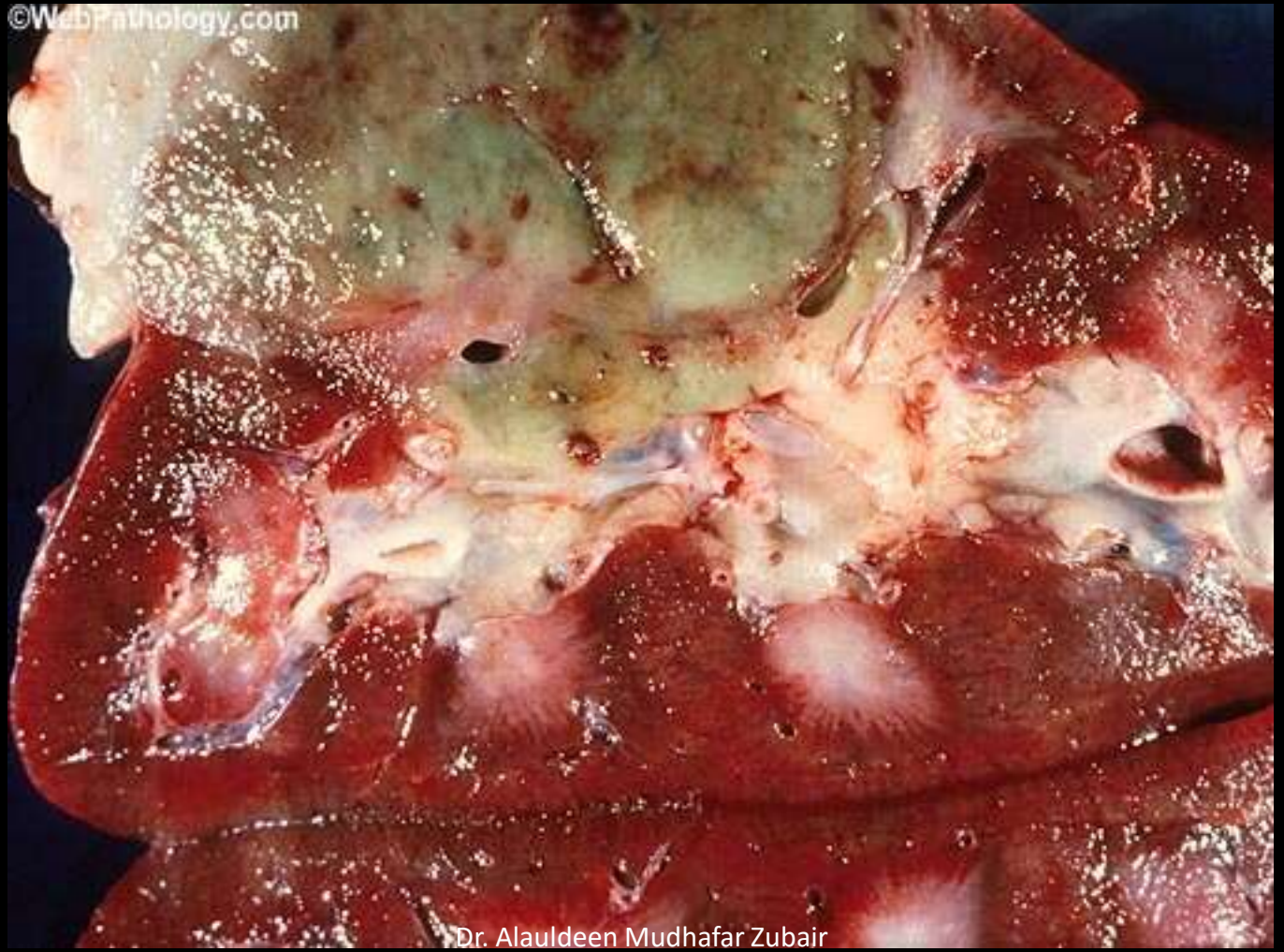
8) Meningeal syndrome, particularly in ALL and M4, M5. It manifests as headache, nausea and vomiting, blurred vision and diplopia. Fundal examination may reveal papilloedema and hemorrhage. This syndrome results from leukemic infiltration to the CNS.



9) Other occasional organ infiltration includes testicular swelling in ALL and mediastinal lymphadenopathy and compression in T-ALL.

10) AML may be presented as an extramedullary tumor called chloroma or granulocytic sarcoma.

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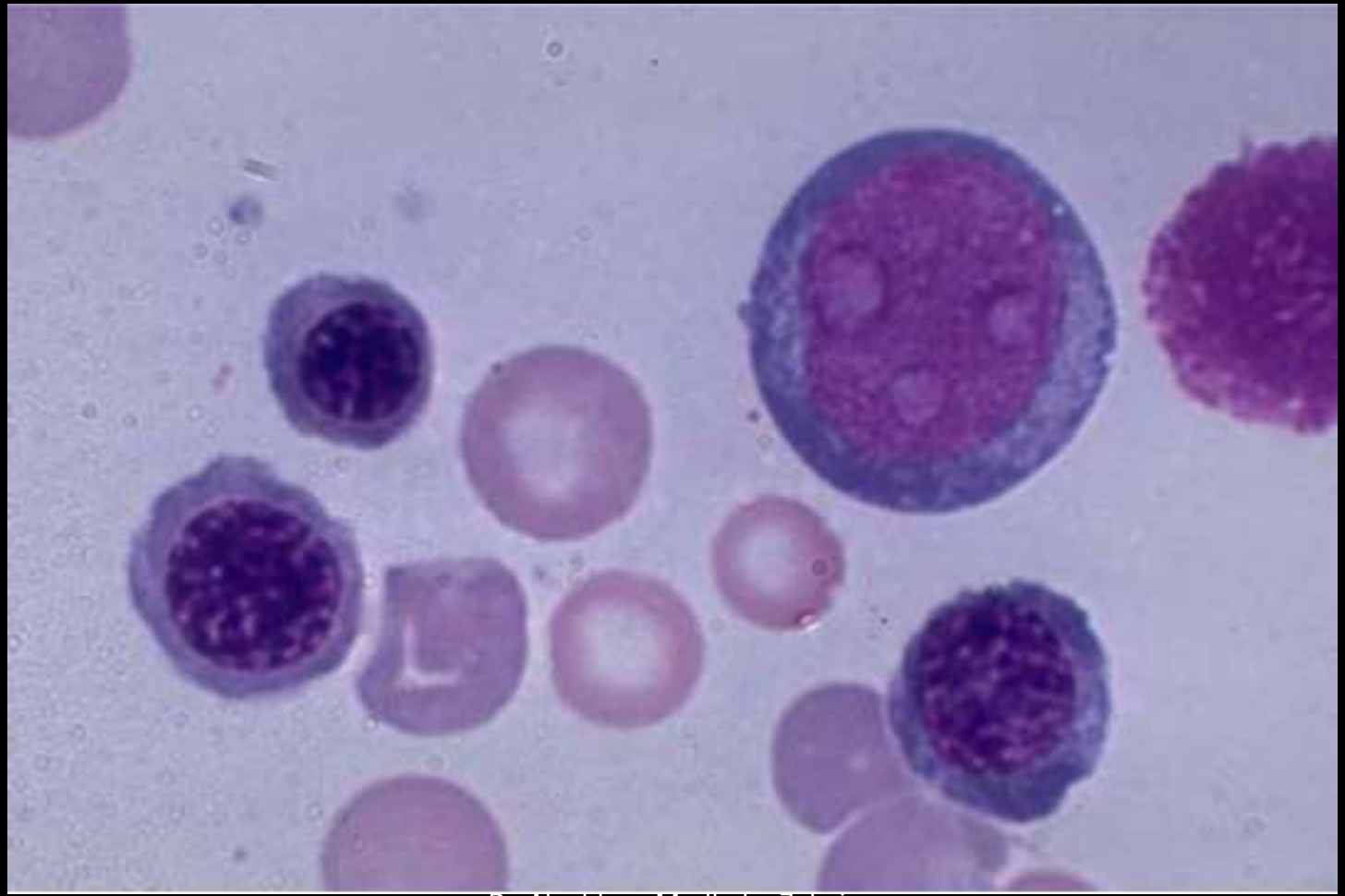
Laboratory findings:

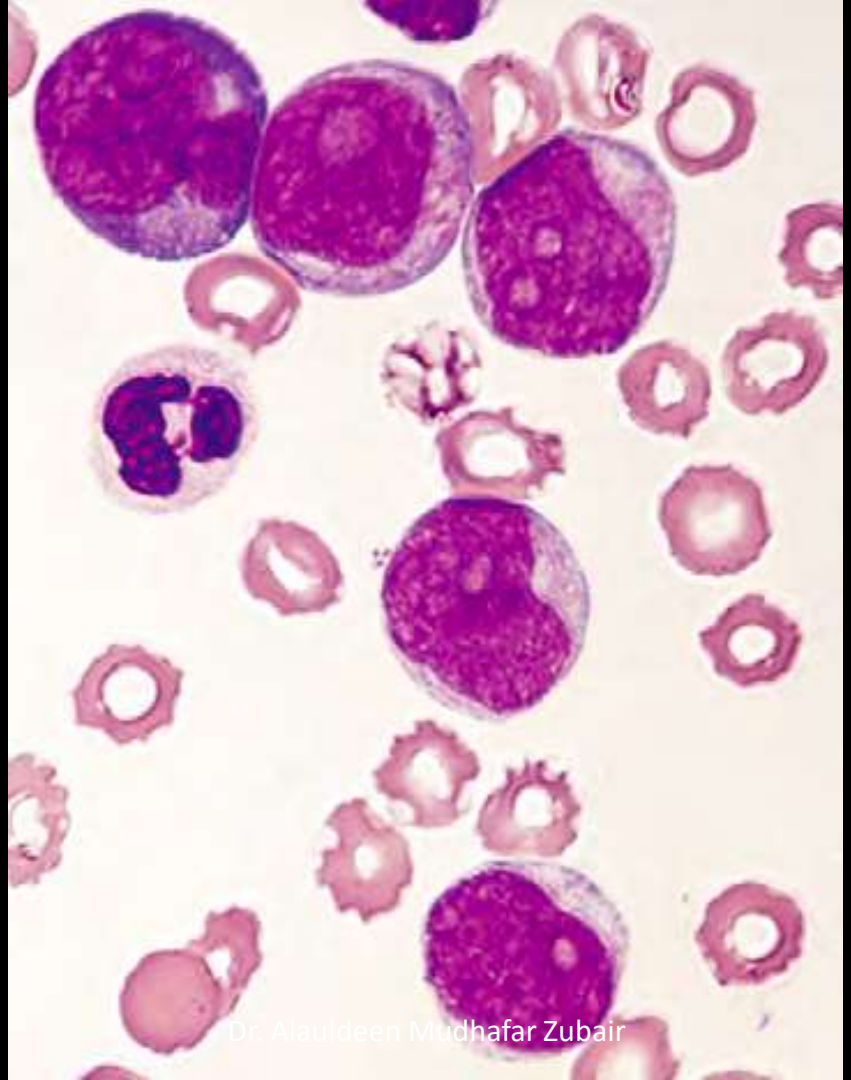
i. Anemia: commonly normochromic normocytic due to marrow infiltration.

ii. The total white cell count may be decreased, normal or increased.

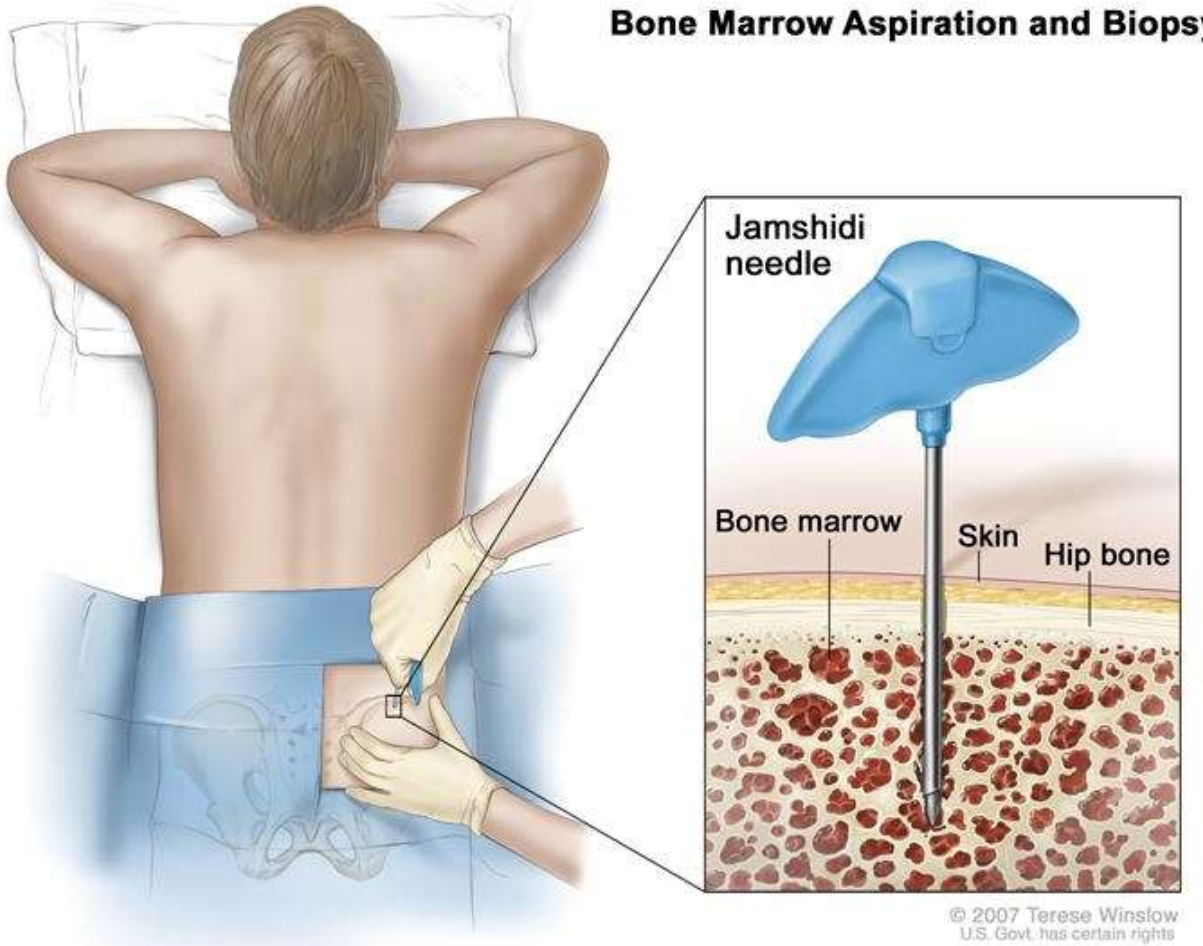
iii. Thrombocytopenia in most cases.

iv. Blood film examination shows variable number of blast cells. Other abnormal cells may be present according to the subtype. In AML, the blasts may contain Auer rods.





V.Bone marrow aspirate is typically hypercellular with suppression of all the normal hemopoeitic elements due to infiltration by leukemic blast cells. Diagnosis of acute leukemia requires that at least 20% of total nucleated cells in the bone marrow are blast cells. Sometimes the bone marrow may be normocellular or hypocellular.


Bone Marrow Aspiration and Biopsy
Jamshidi needle
Bone marrow
Skin
Hip bone
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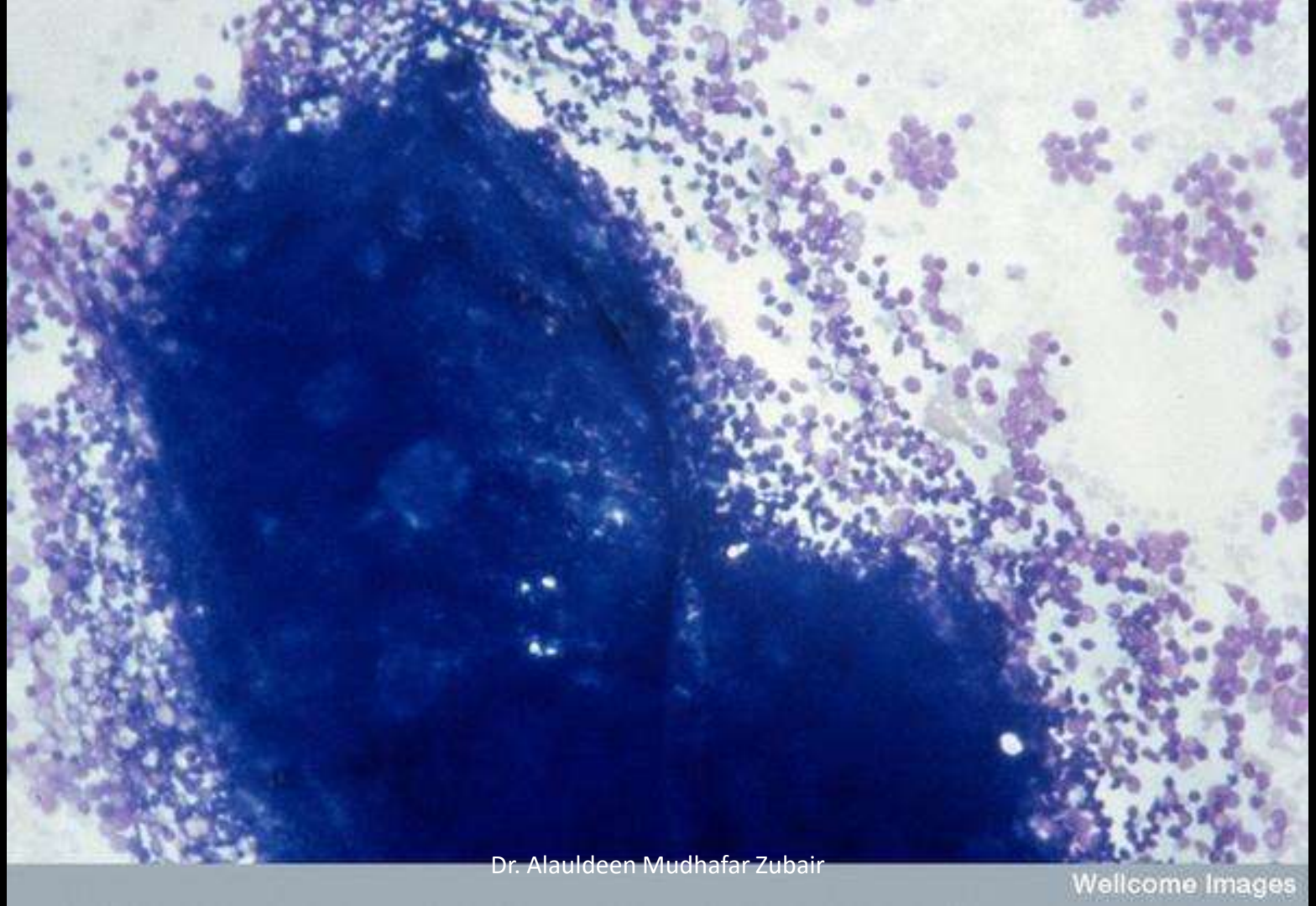
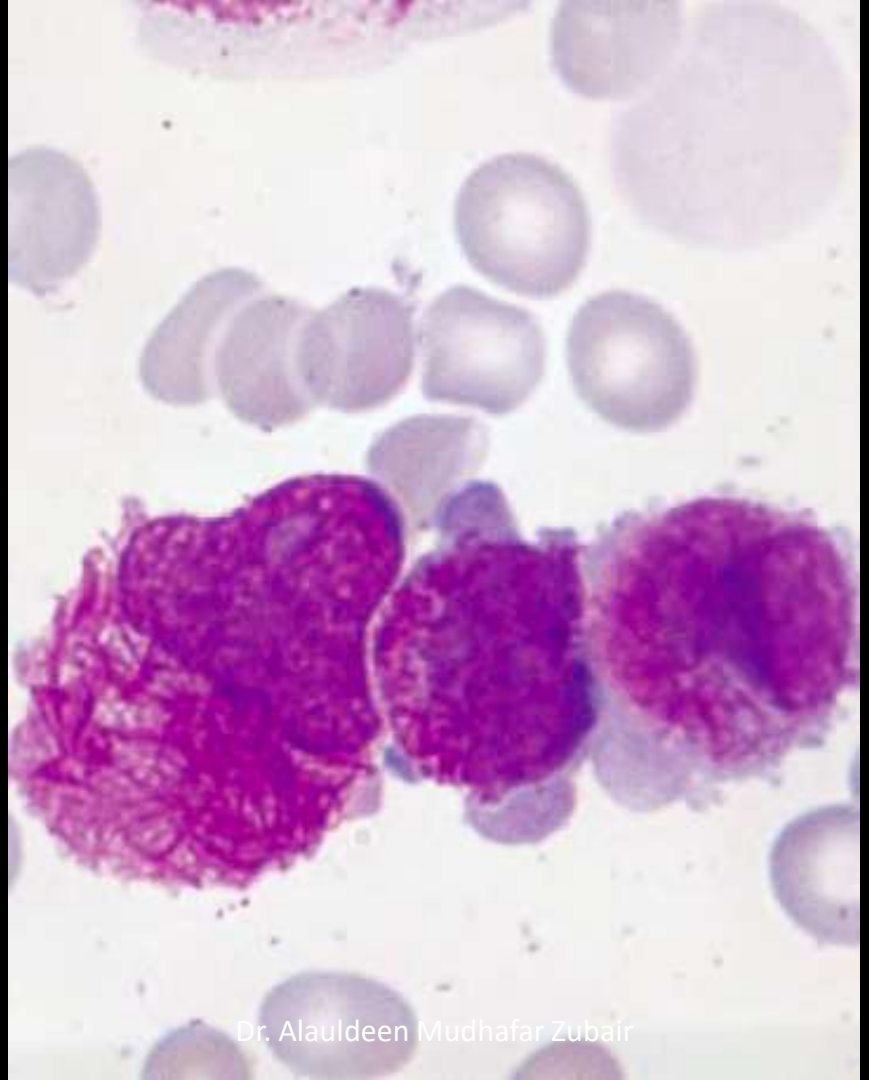
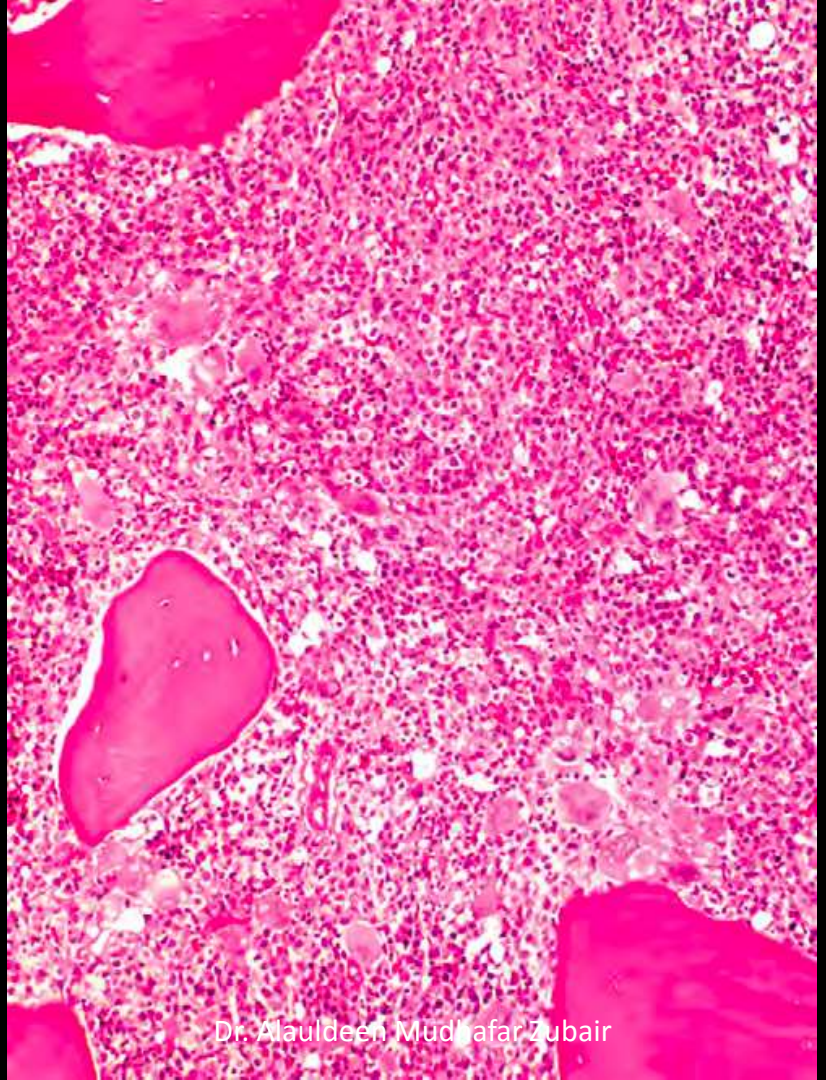

Wellcome Images



VI. Bone marrow biopsy: usually not needed unless the marrow is difficult to aspirate because of fibrosis or extreme hypercellularity.



Classification of acute leukemias

- FAB classification(1975): old system based on morphology and cytochemical stains. Easy to implement in underdeveloped countries.
- WHO classification of tumors of hematopoietic and lymphoid tissues(2008): recent, based mainly on cytogenetics and immunophenotyping in addition to morphology.

ACUTE MYELOID LEUKEMIA LEUKEMIA (AML) AND RELATED PRECURSOR NEOPLASMS

AML with recurrent genetic abnormalities

- AML with t(8;21)(q22;q22); RUNX1-RUNX1T1
- AML with inv(16)(p13.1q22) or t(16;16)(p13.1;q22); CBFB-MYH11
- Acute promyelocytic leukemia with t(15;17)(q22;q11-12); PML-RARA

- AML with t(9;11)(p22;q23); MLLT3-MLL
- AML with t(6;9)(p23;q34); DEK-NUP214
- AML with inv(3)(q21q26.2) or t(3;3)(q21;q26.2); RPN1-EVI1
- AML (megakaryoblastic) with t(1;22)(p13;q13); RBM15-MKL1
- AML with mutated NPM1
- AML with mutated CEBPA

- AML with myelodysplasia-related changes Therapy-related myeloid neoplasms
- Acute myeloid leukemia, NOS
- Myeloid sarcoma
- Myeloid proliferations related to Down syndrome

1. Transient abnormal myelopoiesis
2. Myeloid leukemia associated with Down syndrome

TABLE 2: WHO 2008 classification of acute lymphoblastic leukemia (ALL)

Precursor lymphoid neoplasms
B-cell lymphoblastic leukemia/lymphoma, not otherwise specified
B-cell lymphoblastic leukemia/lymphoma with recurrent genetic abnormalities
B-cell lymphoblastic leukemia/lymphoma with t(9;22)(q34;q11.2); *BCR-ABL1*
B-cell lymphoblastic leukemia/lymphoma with t(v;11q23); *MLL* rearranged
B-cell lymphoblastic leukemia/lymphoma with t(12;21)(p13;q22); *TEL-AML1 (ETV6-RUNX1)*
B-cell lymphoblastic leukemia/lymphoma with hyperploidy
B-cell lymphoblastic leukemia/lymphoma with hypoploidy (hypodiploid ALL)
B-cell lymphoblastic leukemia/lymphoma with t(5;14)(q31;q32); *IL3-IGH*
B-cell lymphoblastic leukemia/lymphoma with t(1;19)(q23;p13.3); *E2A-PBX1 (TCF3-PBX1)*
T-cell lymphoblastic leukemia/lymphoma

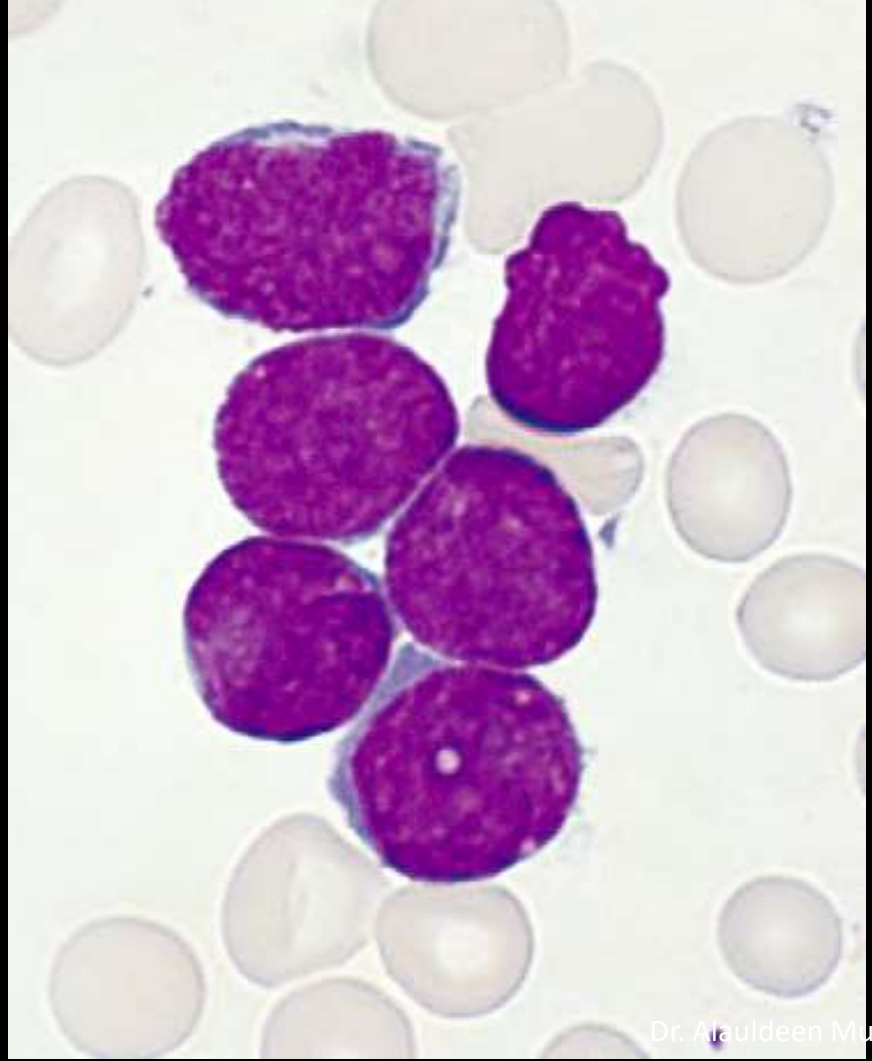
WHO = World Health Organization

Swerdlow SH, Campo E, Harris NL, et al (eds): WHO classification of tumours of haematopoietic and lymphoid tissues. Lyon, France: IARC Press; 109–138, 2009.

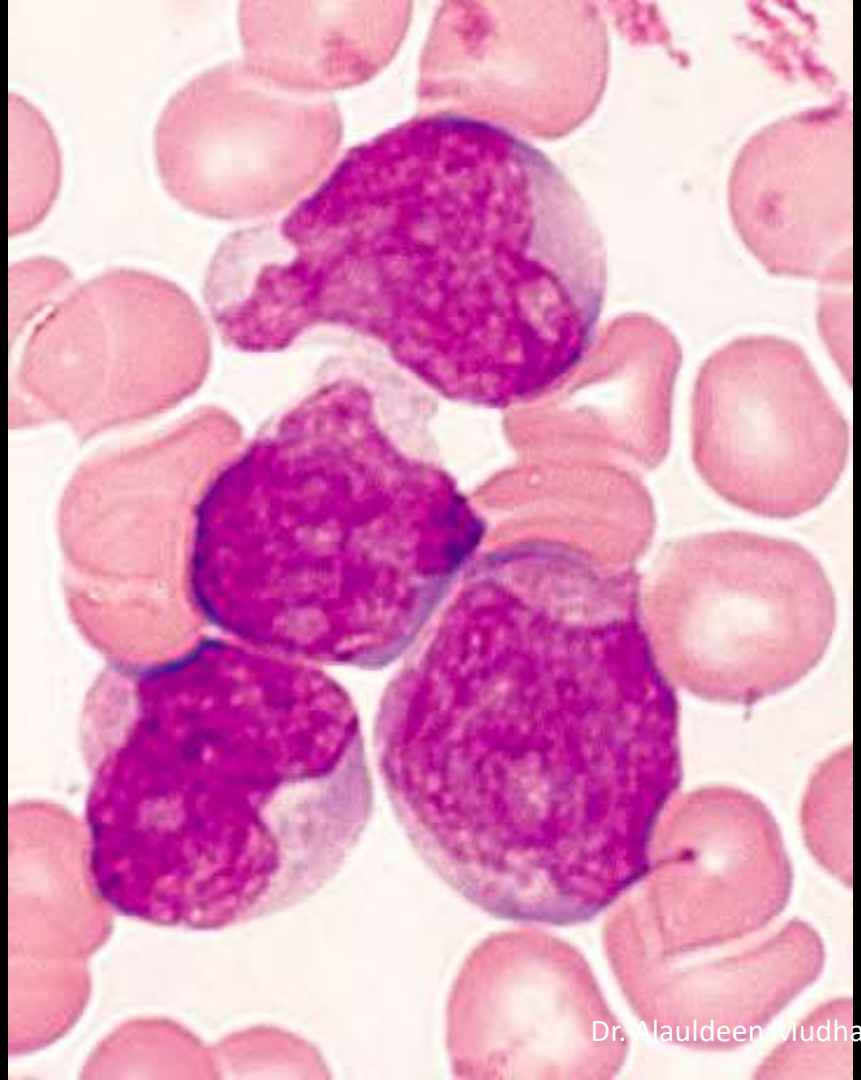
FAB (French American British) group Classification of acute leukemia

Acute Lymphoblastic Leukemia (ALL):

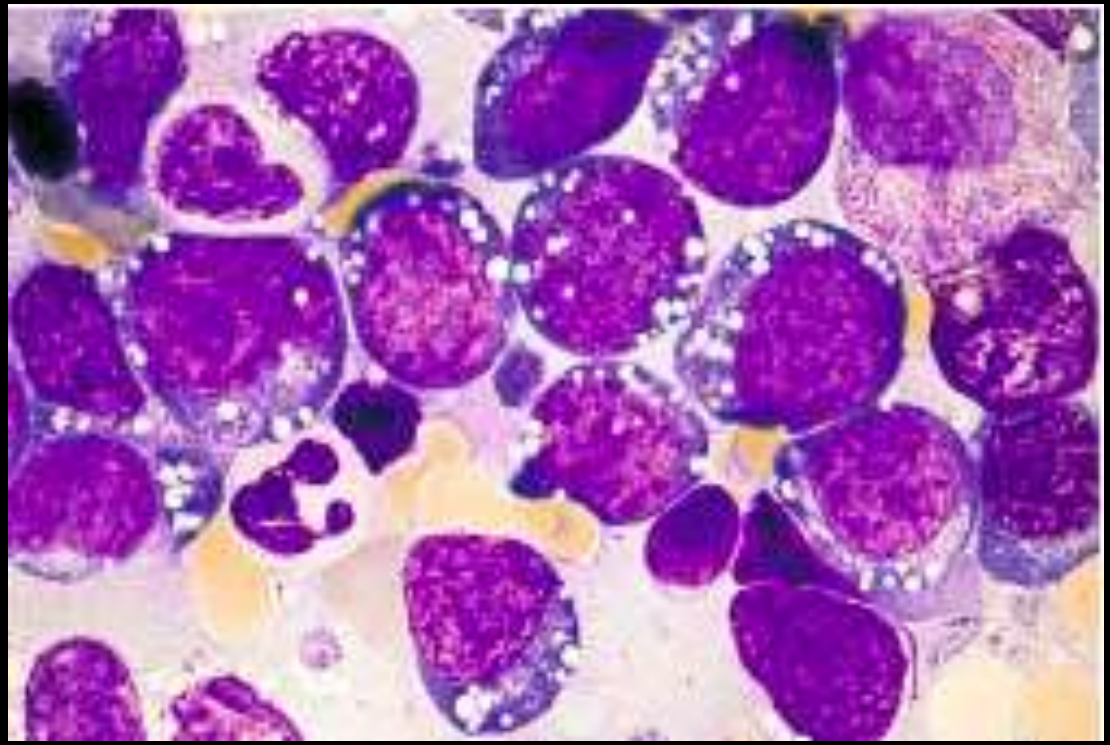
L1	Lymphoblasts are small, monomorphic with high N:C ratio
L2	Lymphoblasts are large, heterogenous, nucleolated with a low N:C ratio
L3	Burkitt-cell type, basophilic, vacuolated cytoplasm



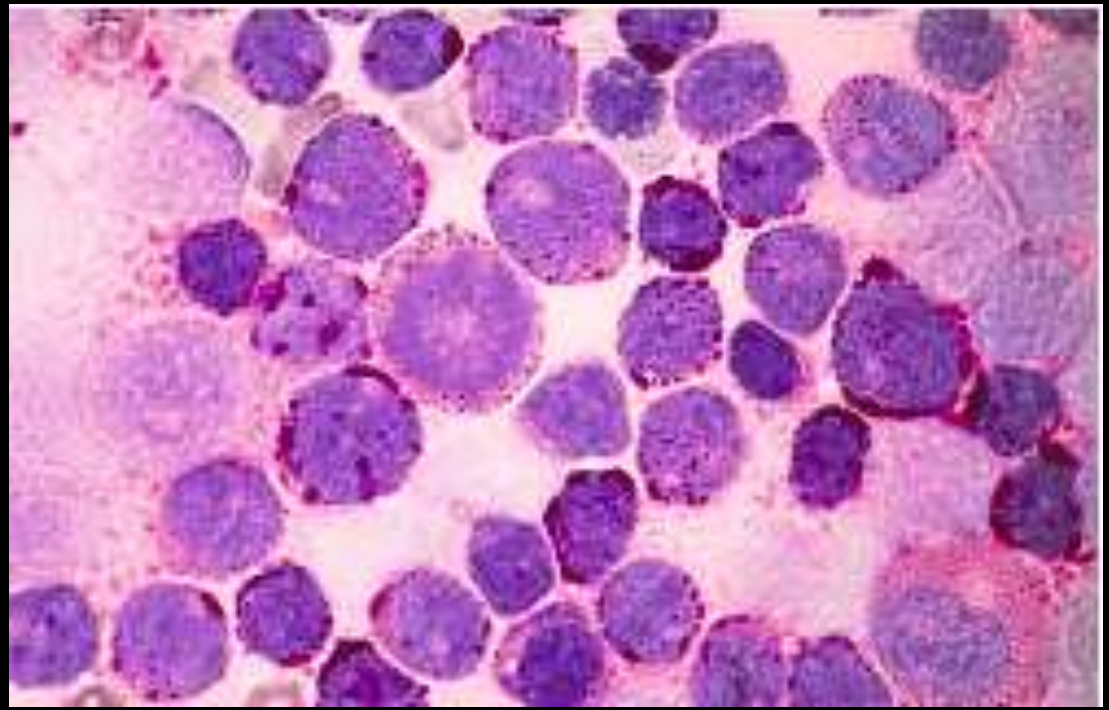
ALL: L1



ALL: L2



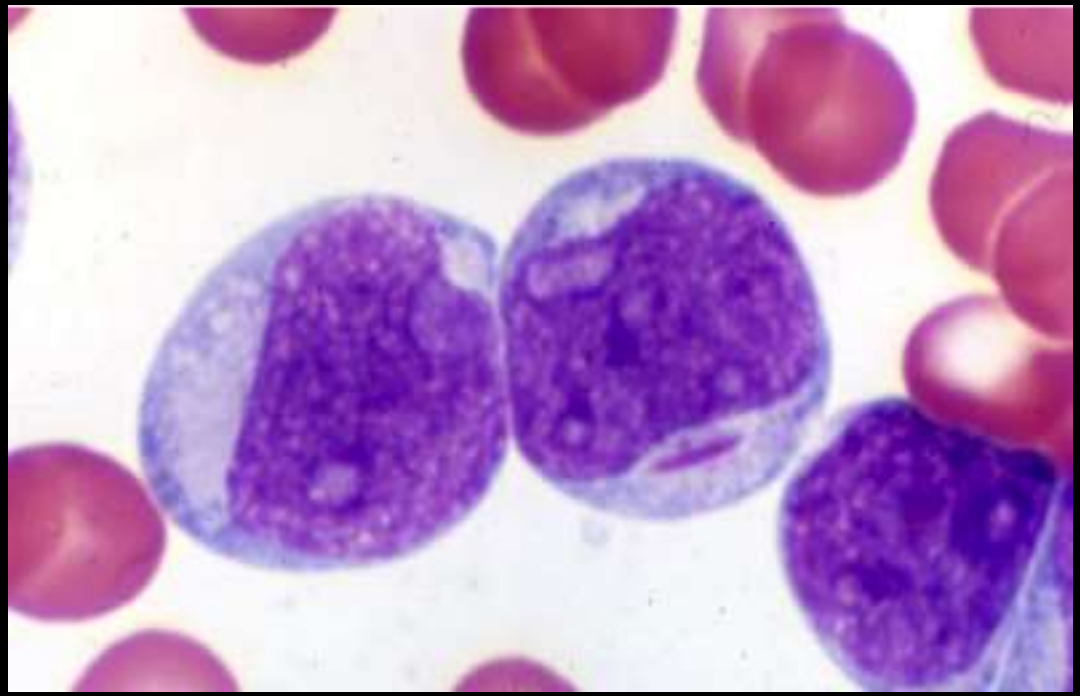
L3

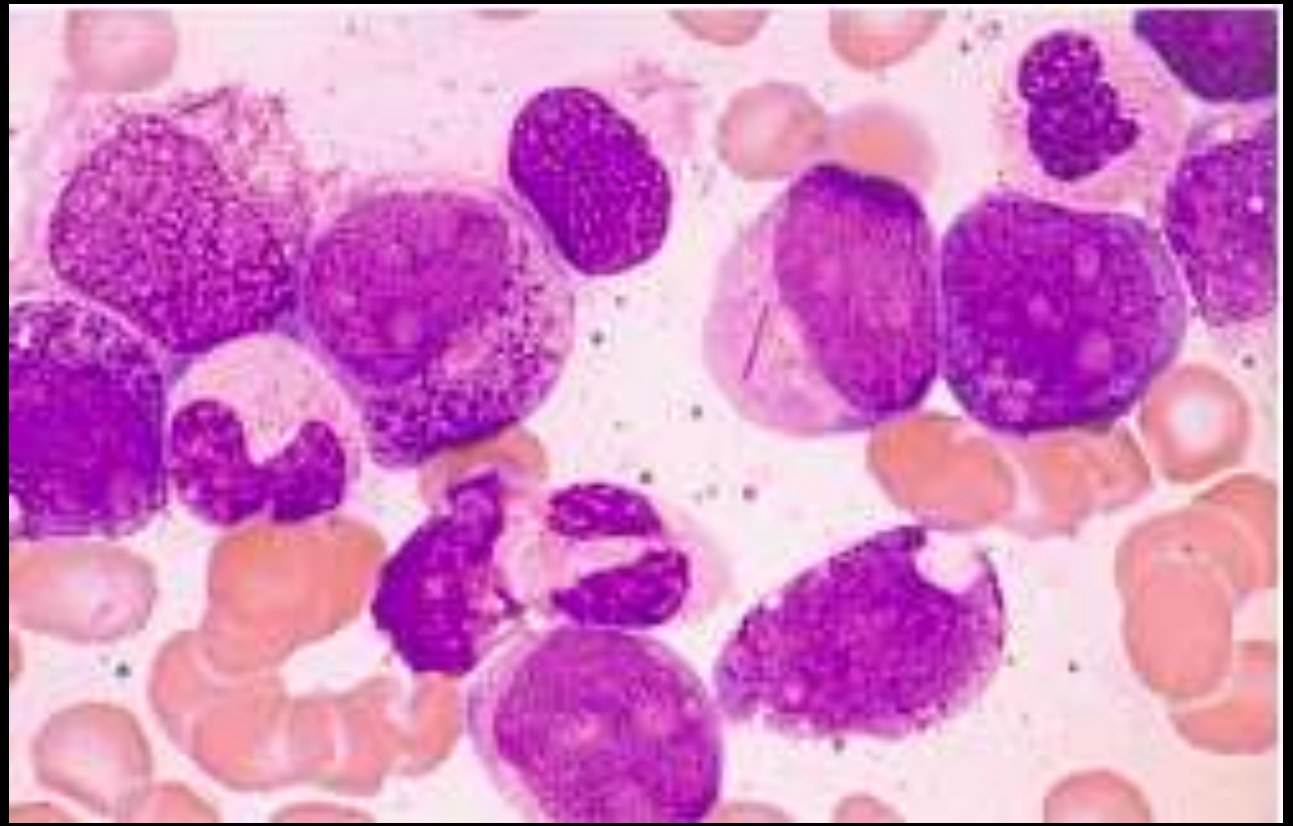


PAS

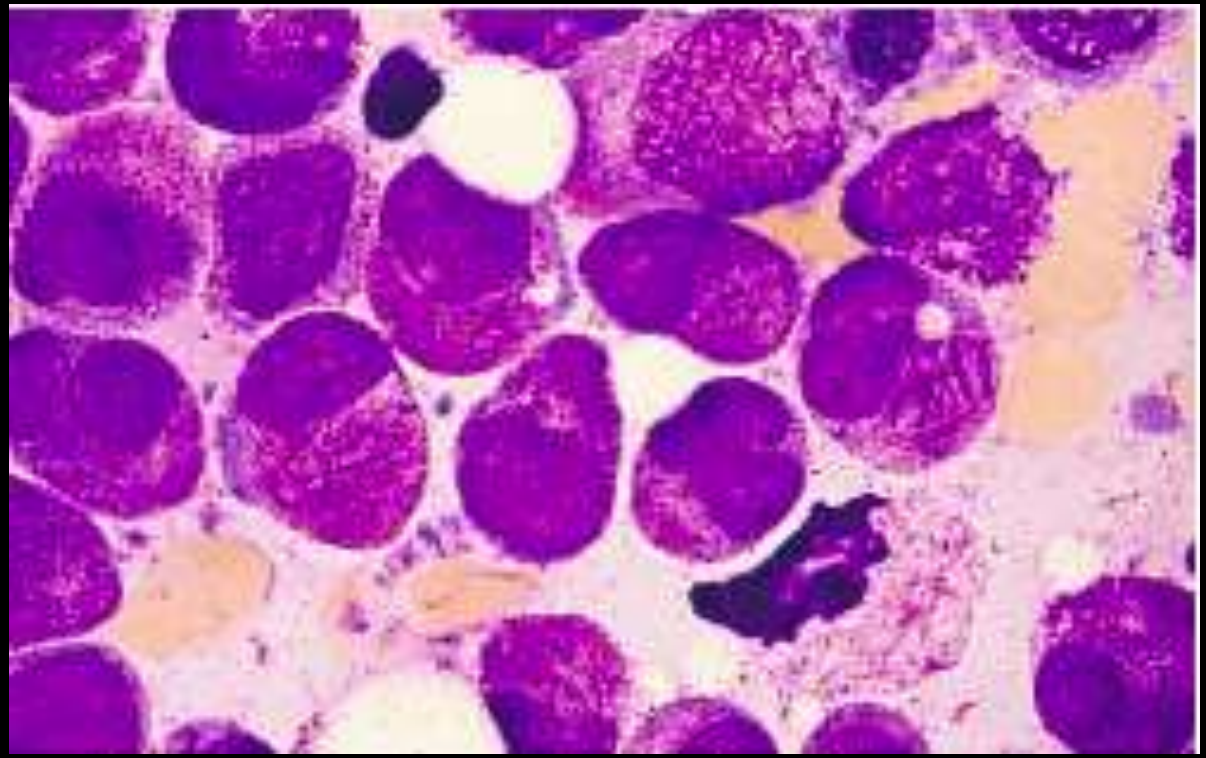
FAB	Common Name	Predominant Cell Type
M-0	Undifferentiated	Myeloblasts - no differentiation
M-1	Minimally differentiated	Myeloblasts - minimal differentiation
M-2	Myelocytic (-blastic)	Myeloblasts -with differentiation
M-3	Promyelocytic	Hypergranular promyelocytes
M-4	Myelomonocytic	Myelomonoblasts
M-5	Monocytic (-blastic)	Monoblasts
M-6	Erythroleukemia	Erythroblasts and Myeloblasts
M-7	Megakaryocytic	Megakaryoblasts

M2: Auer rod

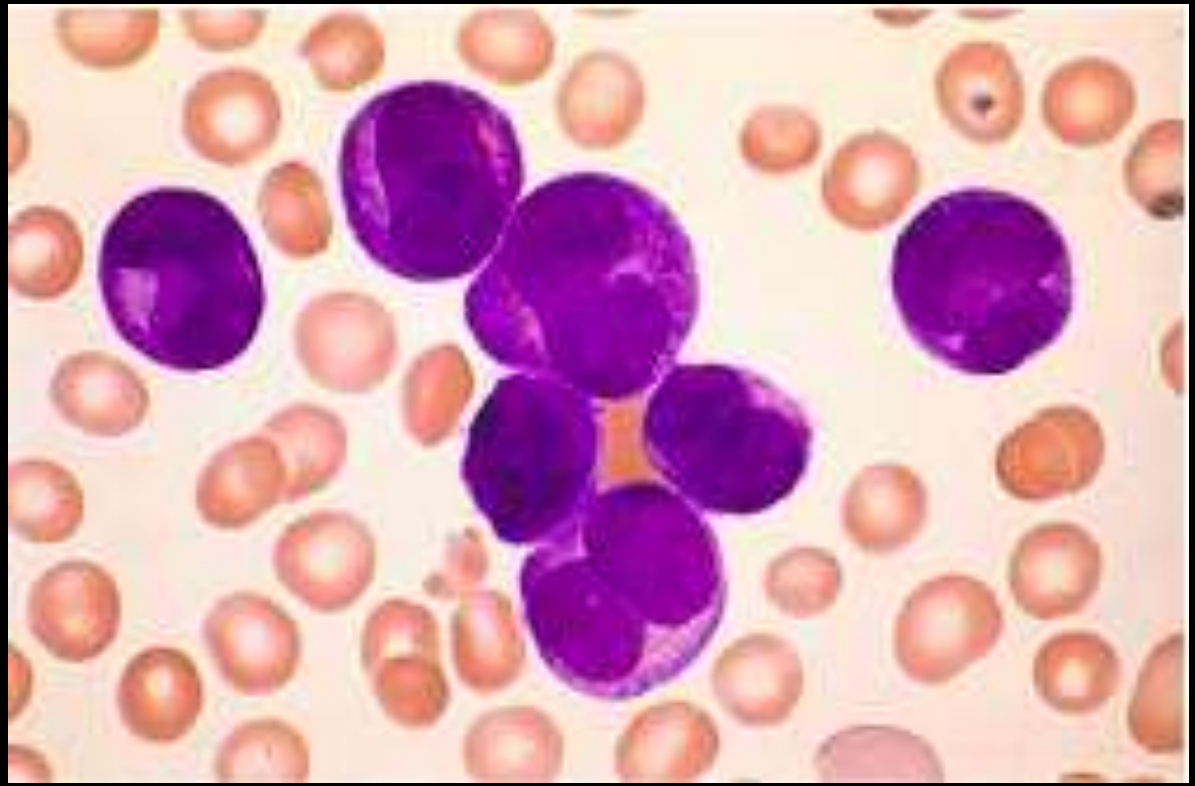




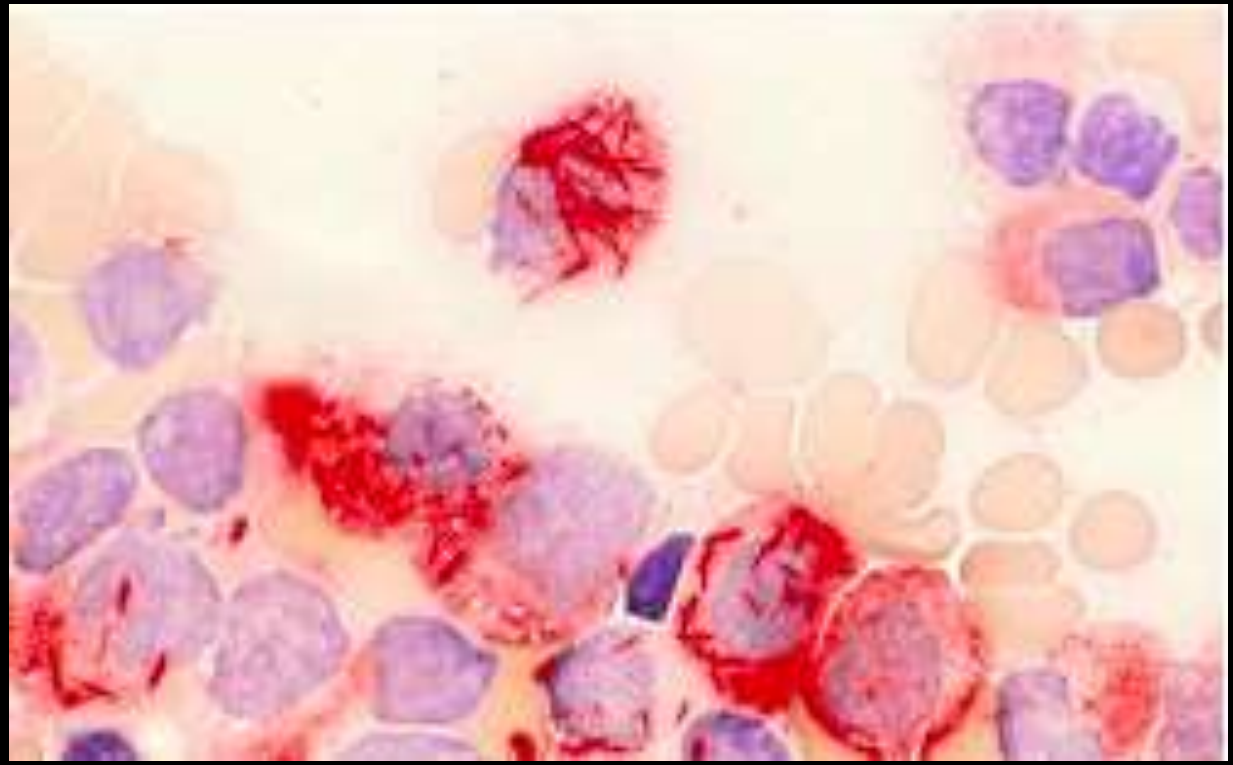
Auer rods



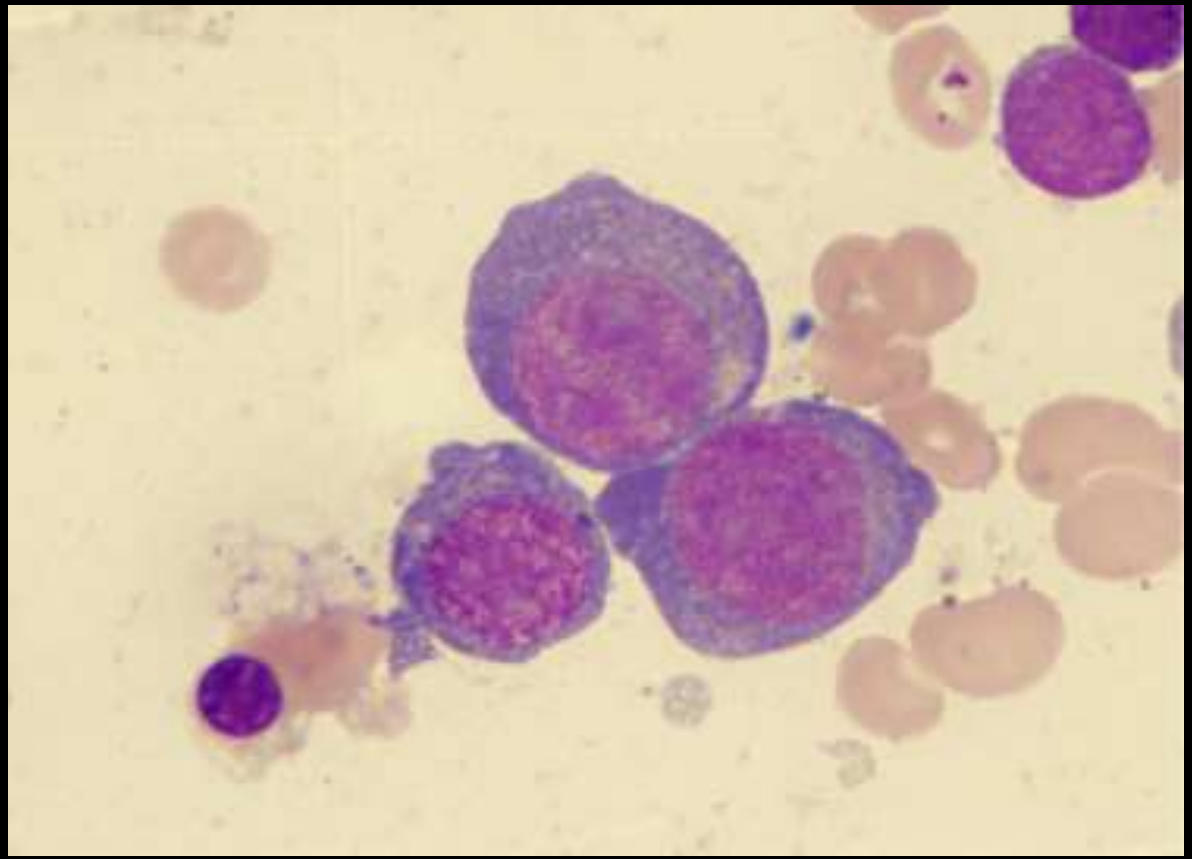
Acute promyelocytic leukemia:M3



M3 variant



Chloroacetate esterase



Erythroleukemia: M6

Special investigations in acute leukemia:

- Special test are carried out to:

① Confirm the diagnosis.

② Differentiate AML & ALL.

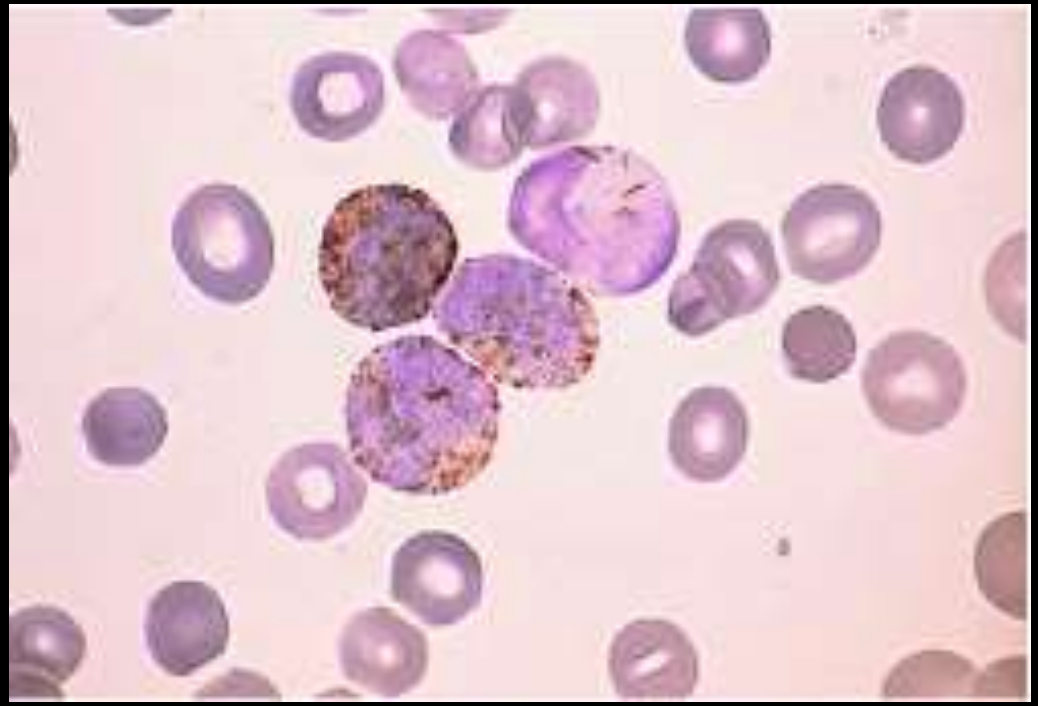
③ Diagnose the subtypes of AML & ALL.

④ Provide prognostic data.

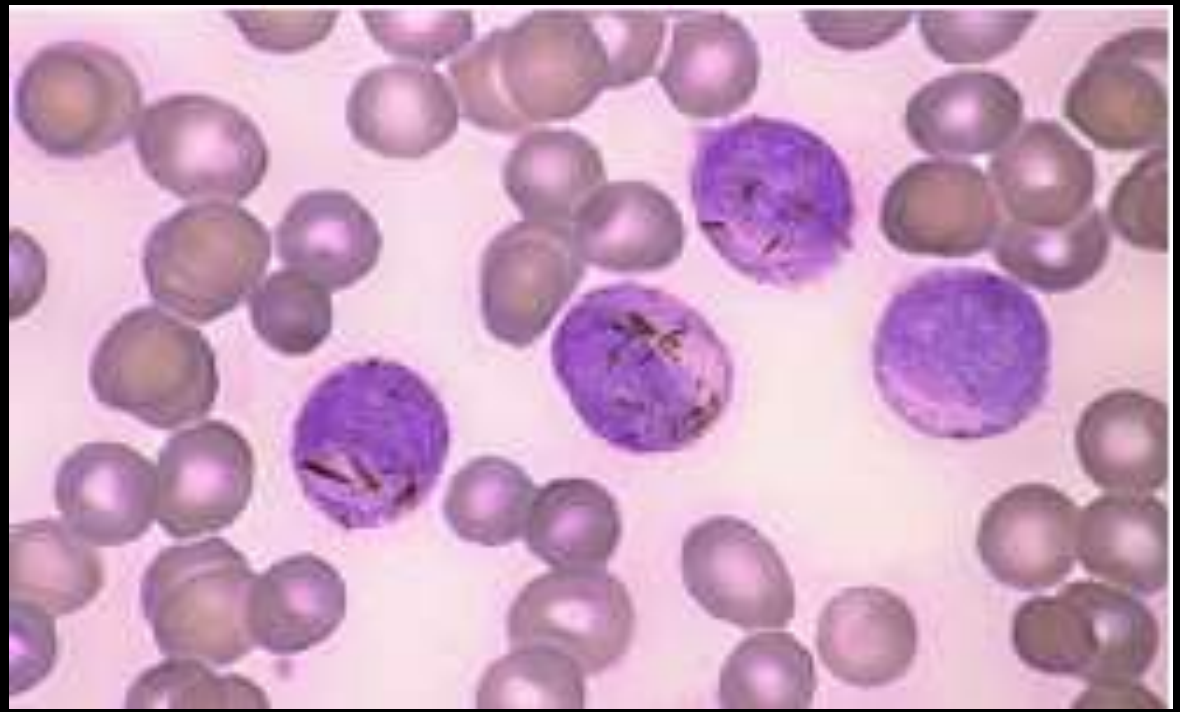
⑤ Provide therapeutic information.

Cytochemical Stains:

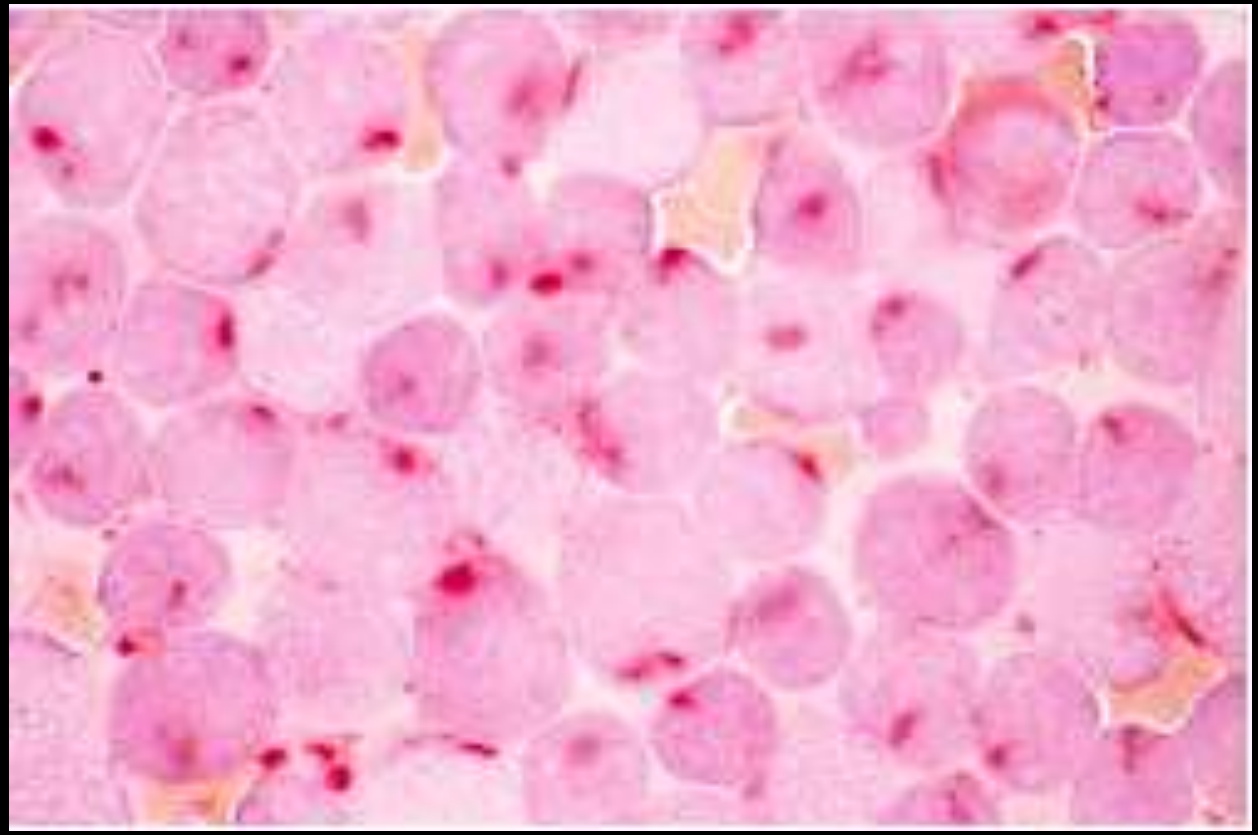
- Myeloperoxidase: positive in AML.
- Sudan black B: positive in AML.
- Non-specific esterases: positive in M4 & M5.
- Acid phosphatase: positive in T-lineage ALL.
- Periodic acid Schiff (PAS): positive in B-lineage ALL.



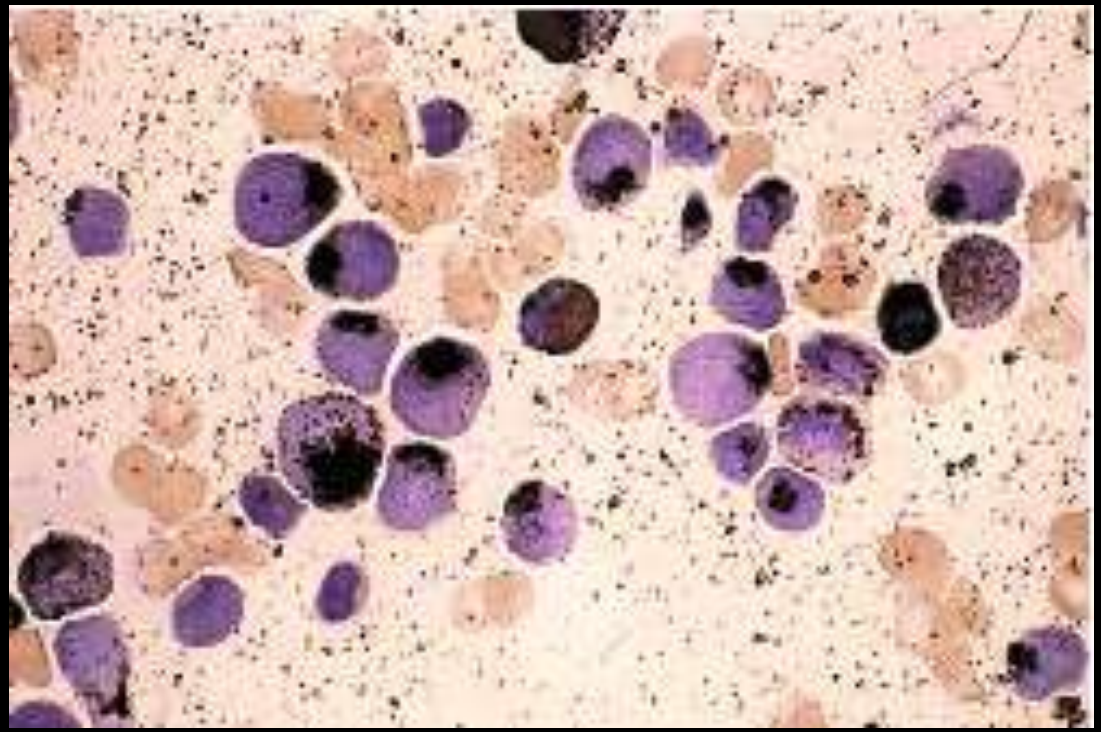
Myeloperoxidase



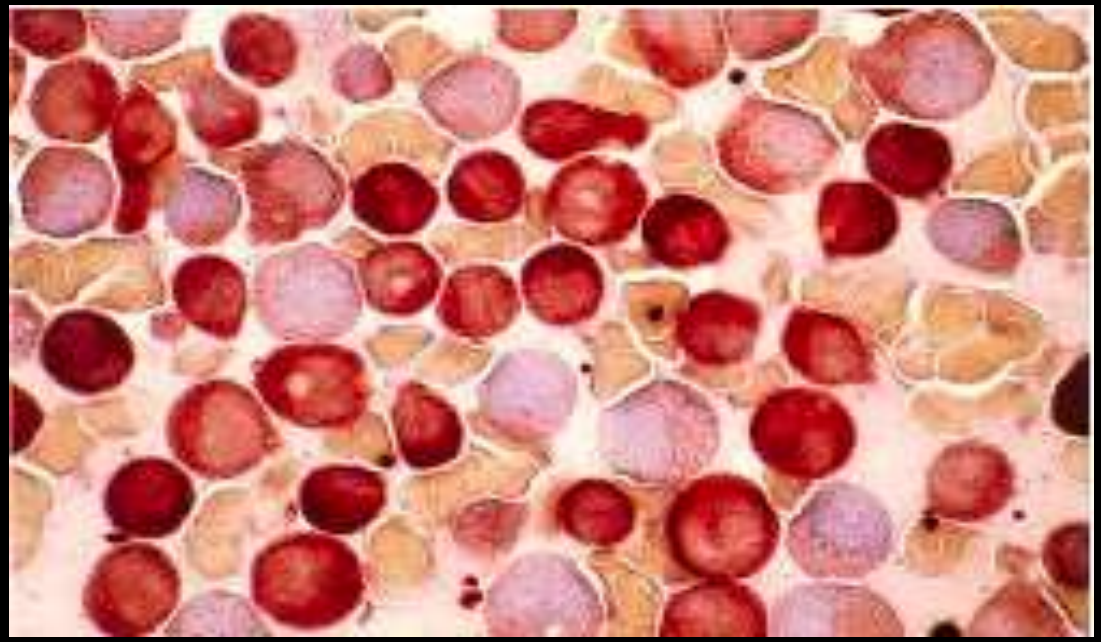
Peroxidase: Auer rods



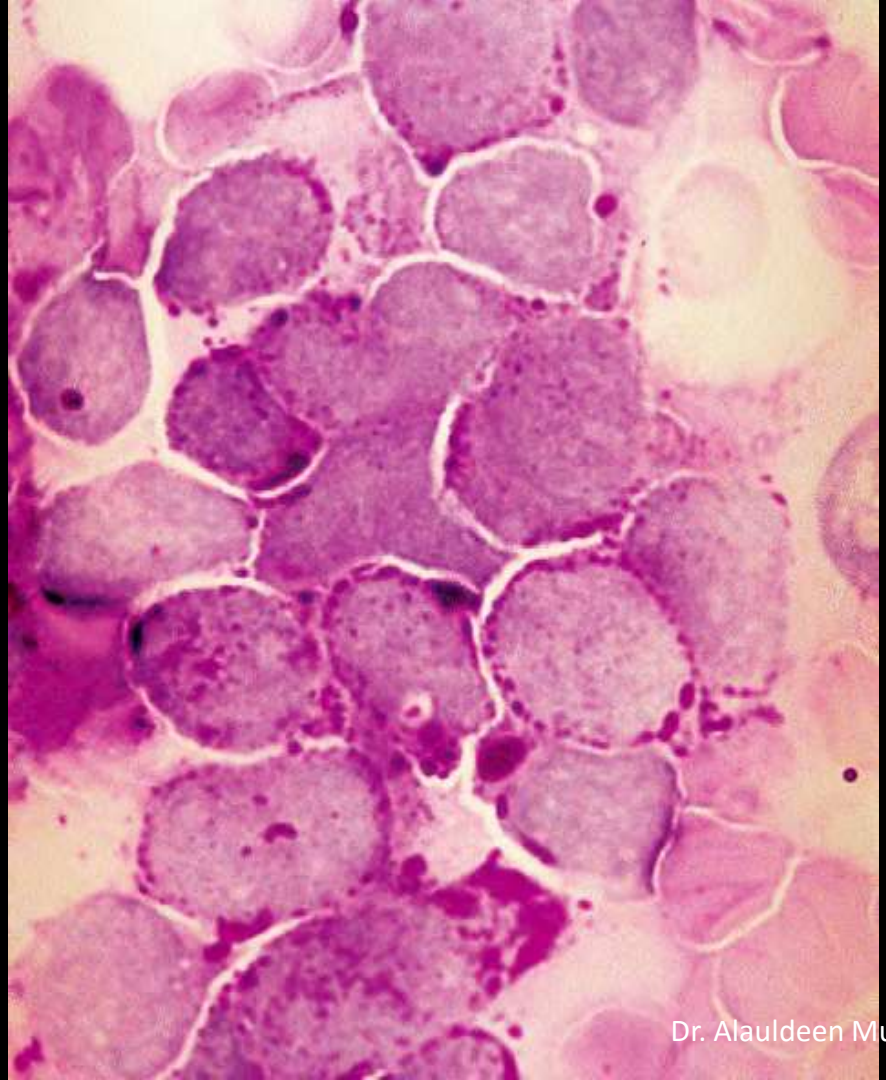
Acid phosphatase



Sudan Black



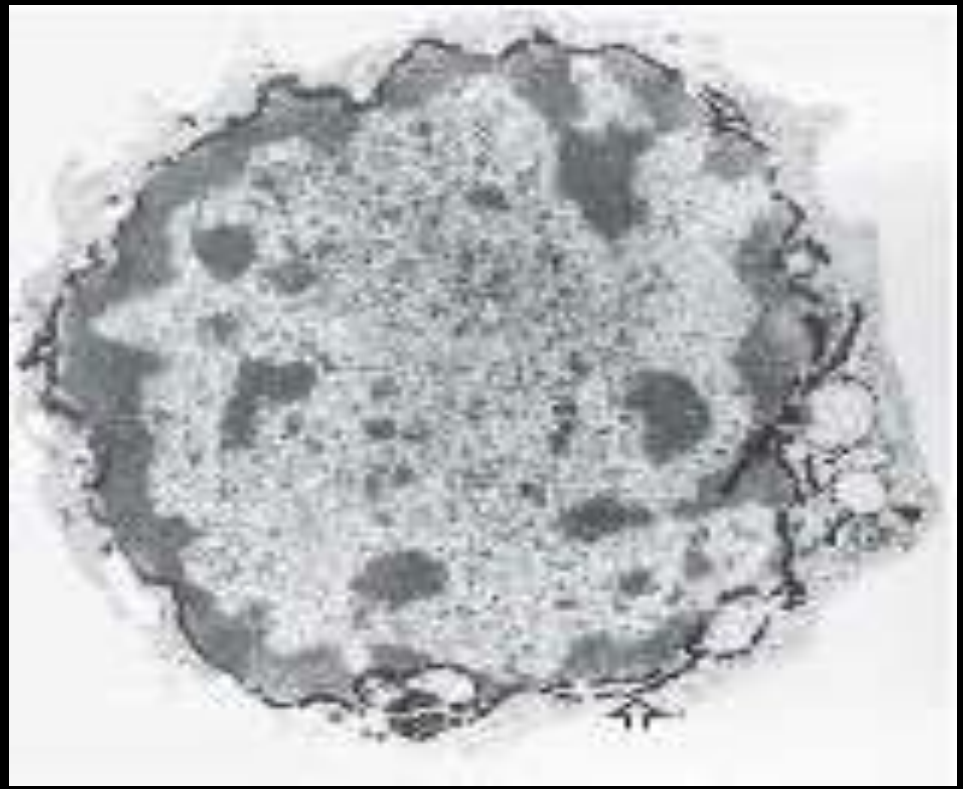
Non-specific esterase



Acute lymphoblastic leukemia, precursor B: PAS positive

Electron Microscopy:

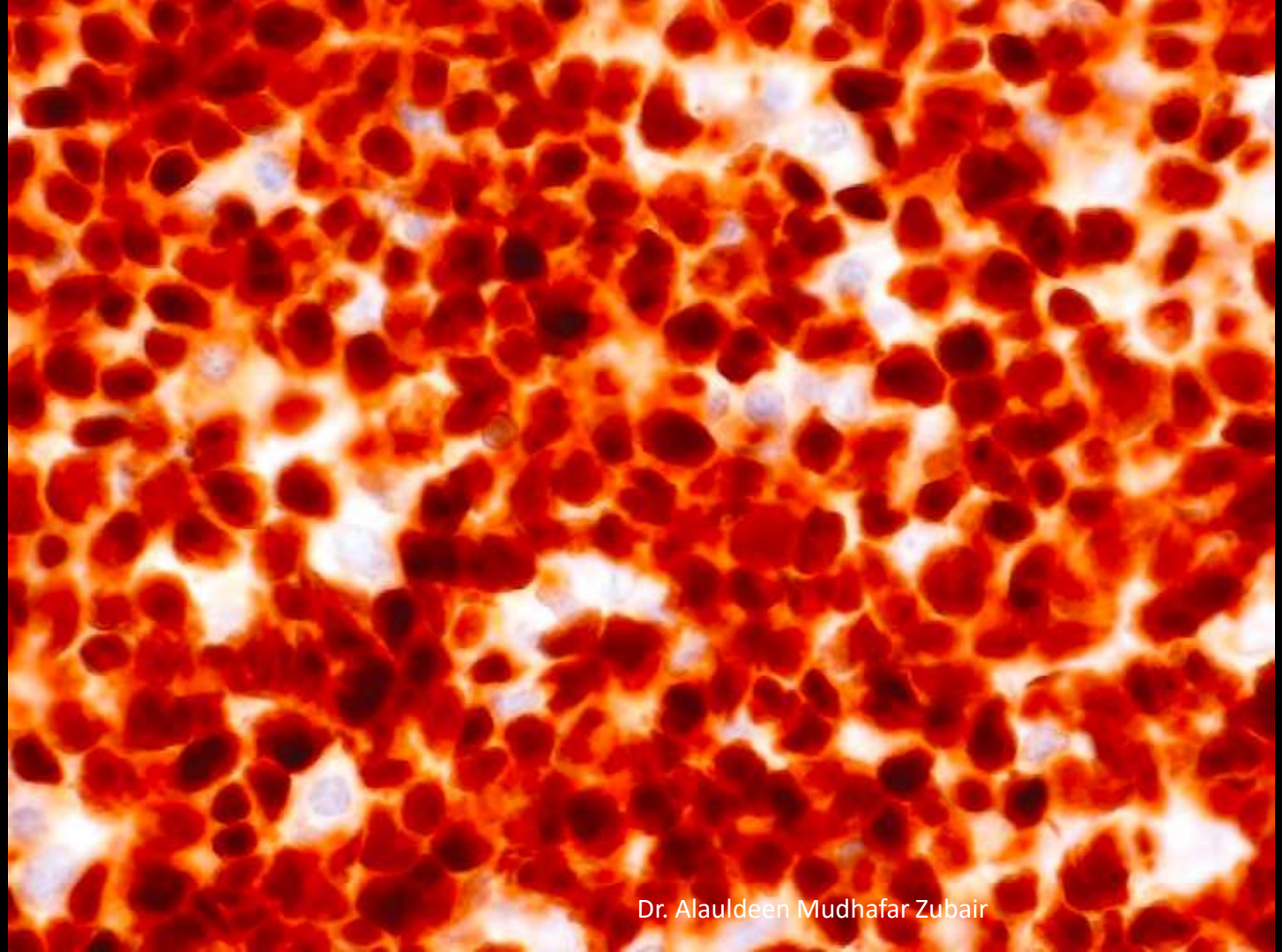
This permits more detailed examination of blast cells and the recognition of some structures which may not be apparent by light microscopy. This may help in the classification of a particular cell type, but it can not be used to establish a diagnosis of leukemia.



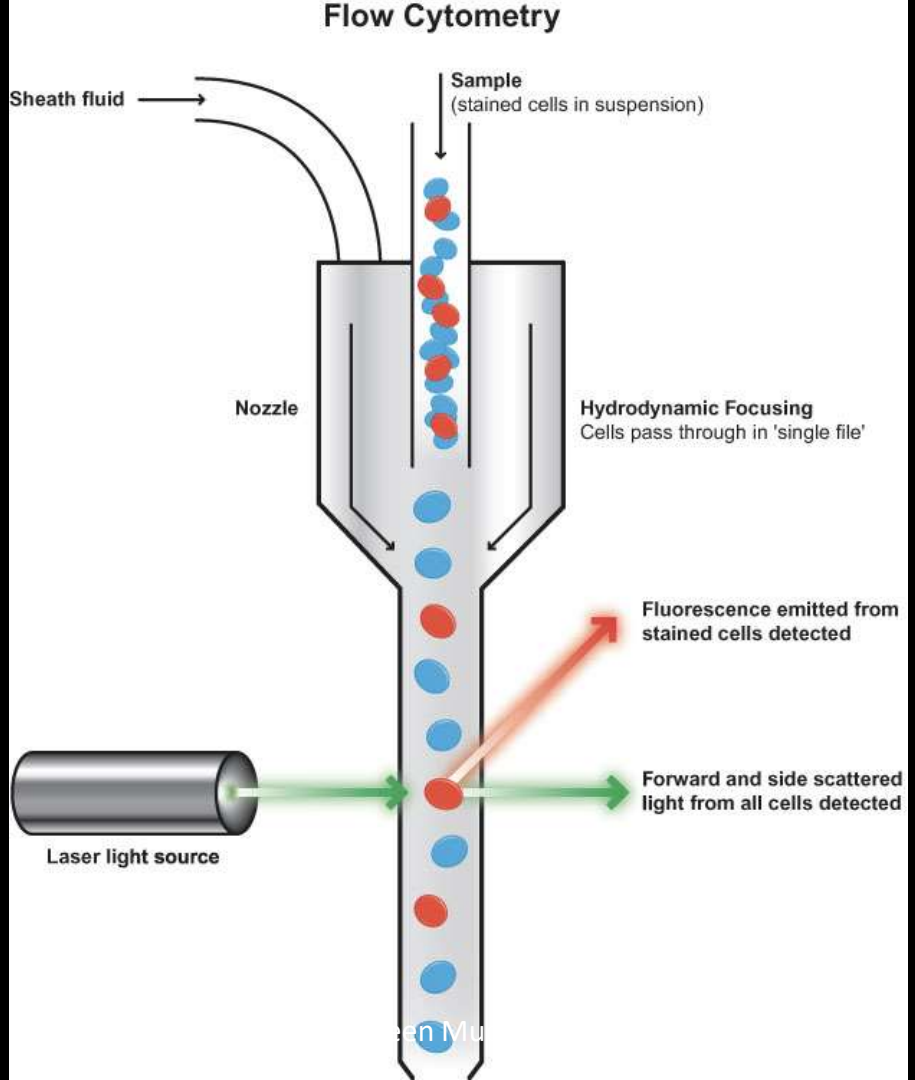
EM: platelet peroxidase in megakaryoblast

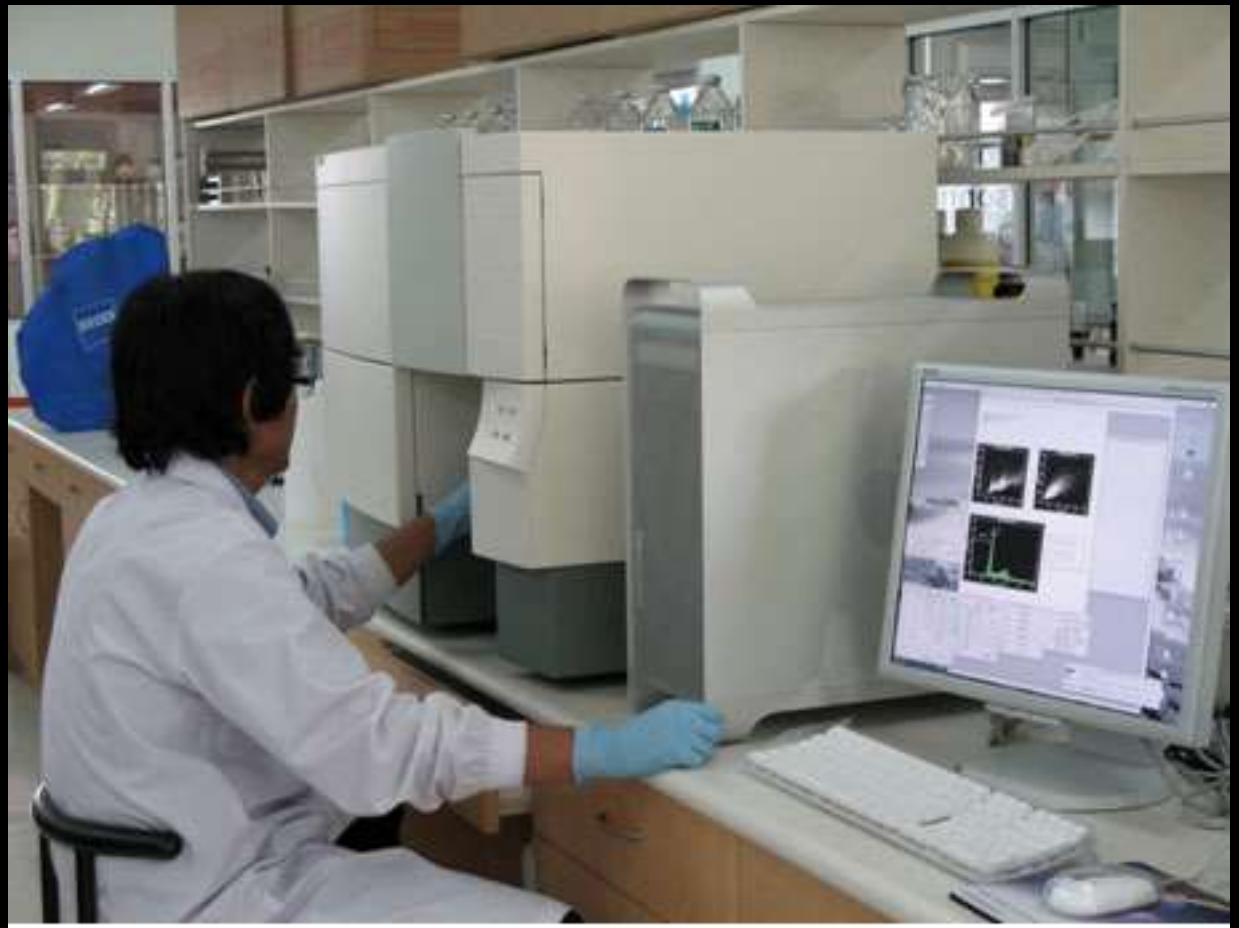
Immunophenotyping (Cell markers):

Characterization of the immunophenotype is referred to as immunophenotyping and is achieved by means of labeled antibodies that recognize specific epitopes of cellular antigens. In general the most useful antibodies are monoclonal antibodies produced by hybridoma technology, but for some antigens polyclonal antibodies are better. The techniques employed for immunophenotyping include immunoenzymatic technique, immunofluorescence technique and flow cytometry.

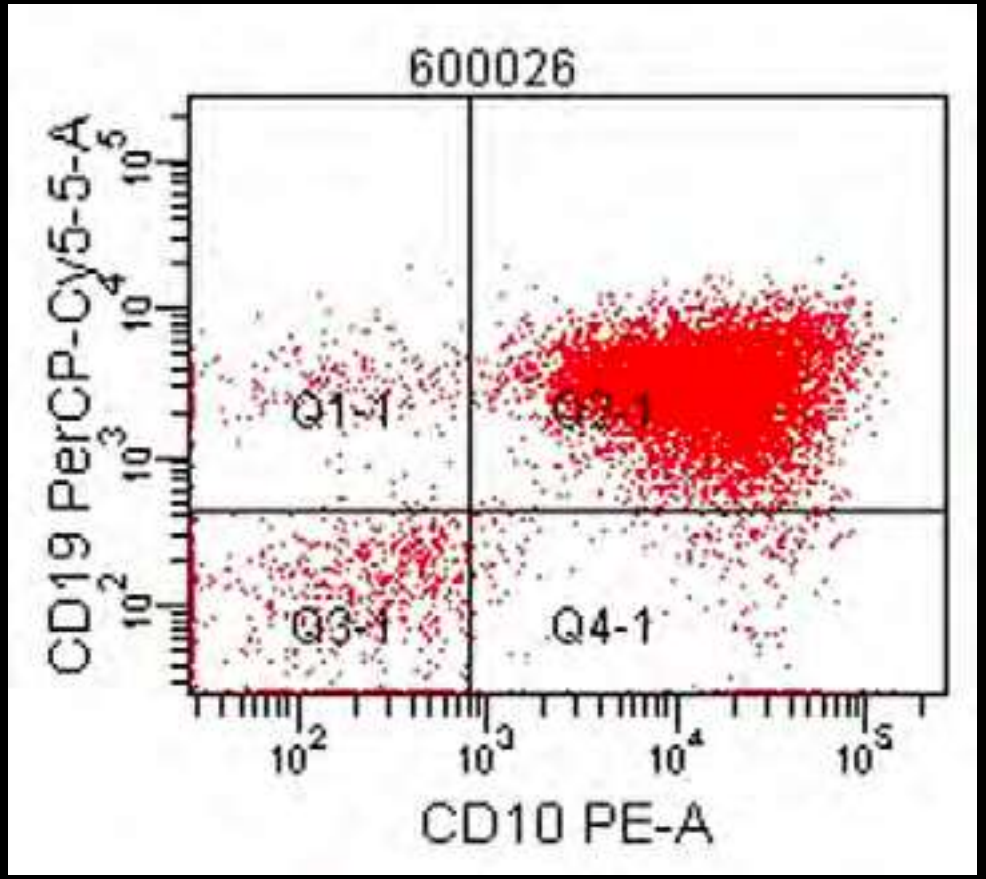


ALL: TdT positive.


Flow Cytometry
Sheath fluid
Sample
(stained cells in suspension)
Nozzle
Hydrodynamic Focusing
Cells pass through in 'single file'
Fluorescence emitted from
stained cells detected
Forward and side scattered
light from all cells detected
Laser light source



BD FACSCalibur Flow Cytometer



Acute lymphoblastic leukemia: B-lineage flow cytometry

Immunophenotypic classification of ALL

	*Adults (%)**	*Surface marker*	*Cytogenetics†*	*Molecular genetics†*
B-lineage		HLA-DR$^+$, TdT$^+$, CD19$^+$ and/or CD79a$^+$ and/or CD22$^+$		
Pro B-ALL	11	No further differentiation markers	t(4;11)	*ALL1(MLL)–AF4*
Common-ALL	50	CD10$^+$	t(9;22)	*BCR–ABL*
Pre B-ALL	12	CD10$^{+/-}$, cyIgM$^+$	t(9;22), t(1;19)	*BCR–ABL, E2A–PBX1*
B-ALL	5	CD10$^{+/-}$, sIgM$^+$	t(8;14)	*MYC–IGH*
T-lineage		TdT$^+$, cyCD3$^+$ or sCD3$^+$		
Early T-ALL	6	cyCD3$^+$, CD7$^+$, CD5$^{+/-}$, CD2$^{+/-}$	t(11;14)	*LMO1*–TCRα/δ
Cortical T-ALL (Thy ALL)	10	cyCD3$^+$, CD7$^+$, CD1a$^+$, sCD3$^{+/-}$	t(10;14)	*HOX11*–TCRα/δ
Mature T-ALL	6	sCD3$^+$, CD1a$^-$		

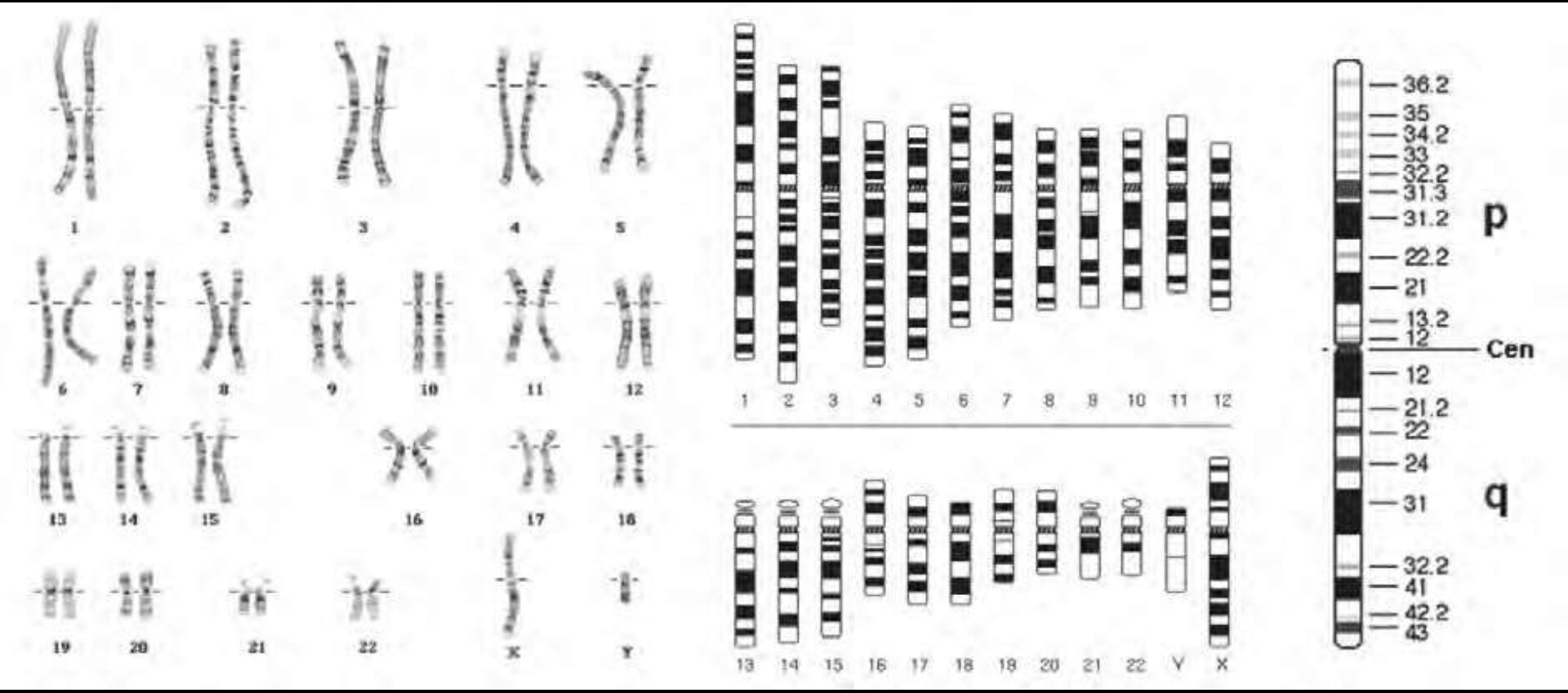
Cytogenetic (chromosomal) analysis and molecular genetic analysis

- These are used to:

1. Establish evidence of clonality.
2. Aid the diagnosis: certain subtypes are associated with characteristic anomalies e.g.: t (15; 17) with M3, t (8; 21) with M2.
3. Provide prognostic information: some anomalies are known to confer good prognosis while other anomalies predict a poor prognosis.
4. Monitor treatment and predict relapse.

Cytogenetics in AML

- The following cytogenetics confer a good response to treatment and good prognosis
- **M2 t(8;21) (q22;q22)**
- **M3 t(15;17) (q22;q21)**
- **M4 inv(16) (p13;q22) or del(16)**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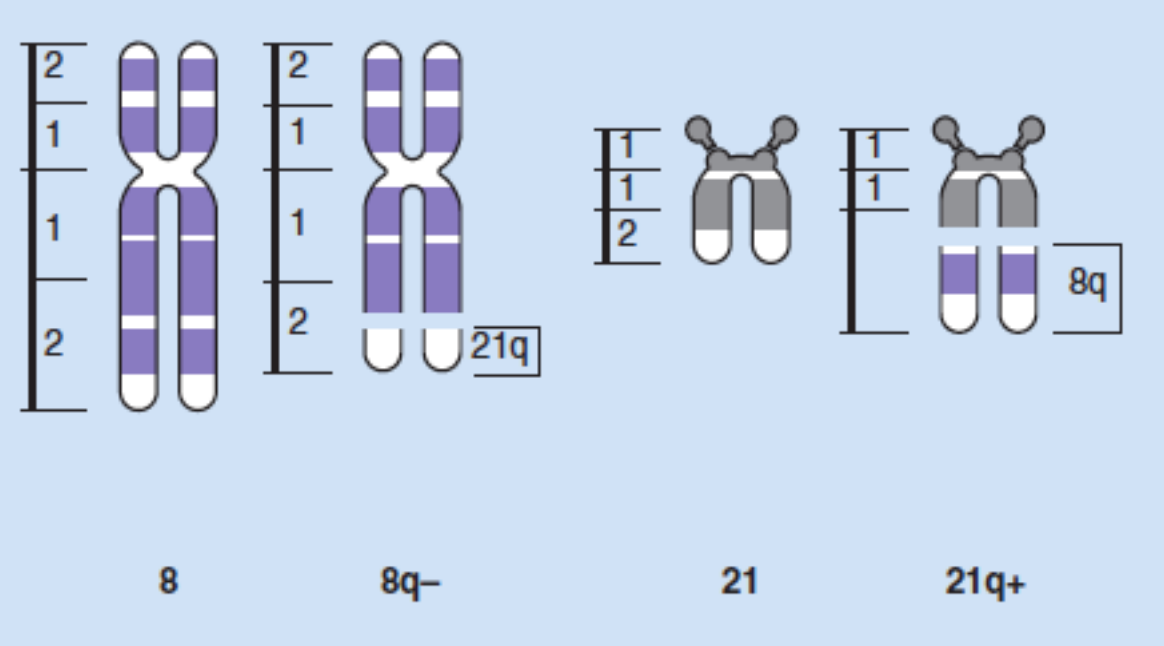
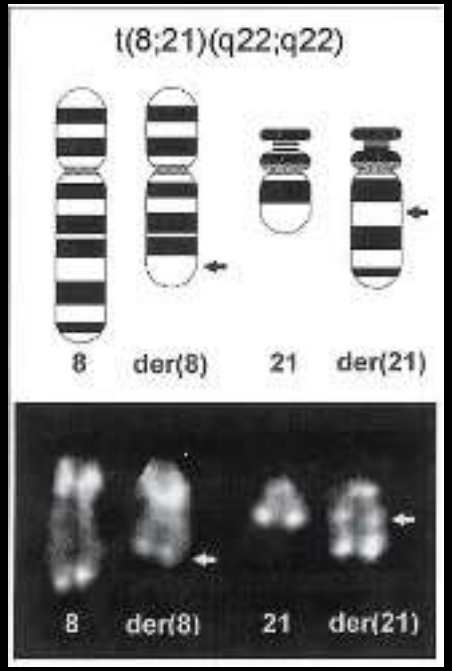
21
22
X
Y
1 2 3 4 5 6 7 8 9 10 11 12
13 14 15 16 17 18 19 20 21 22 V X
36.2
35
34.2
33
32.2
31.3
31.2
22.2
21
13.2
12
Cen
12
21.2
22
24
31
32.2
41
42.2
43
p
q

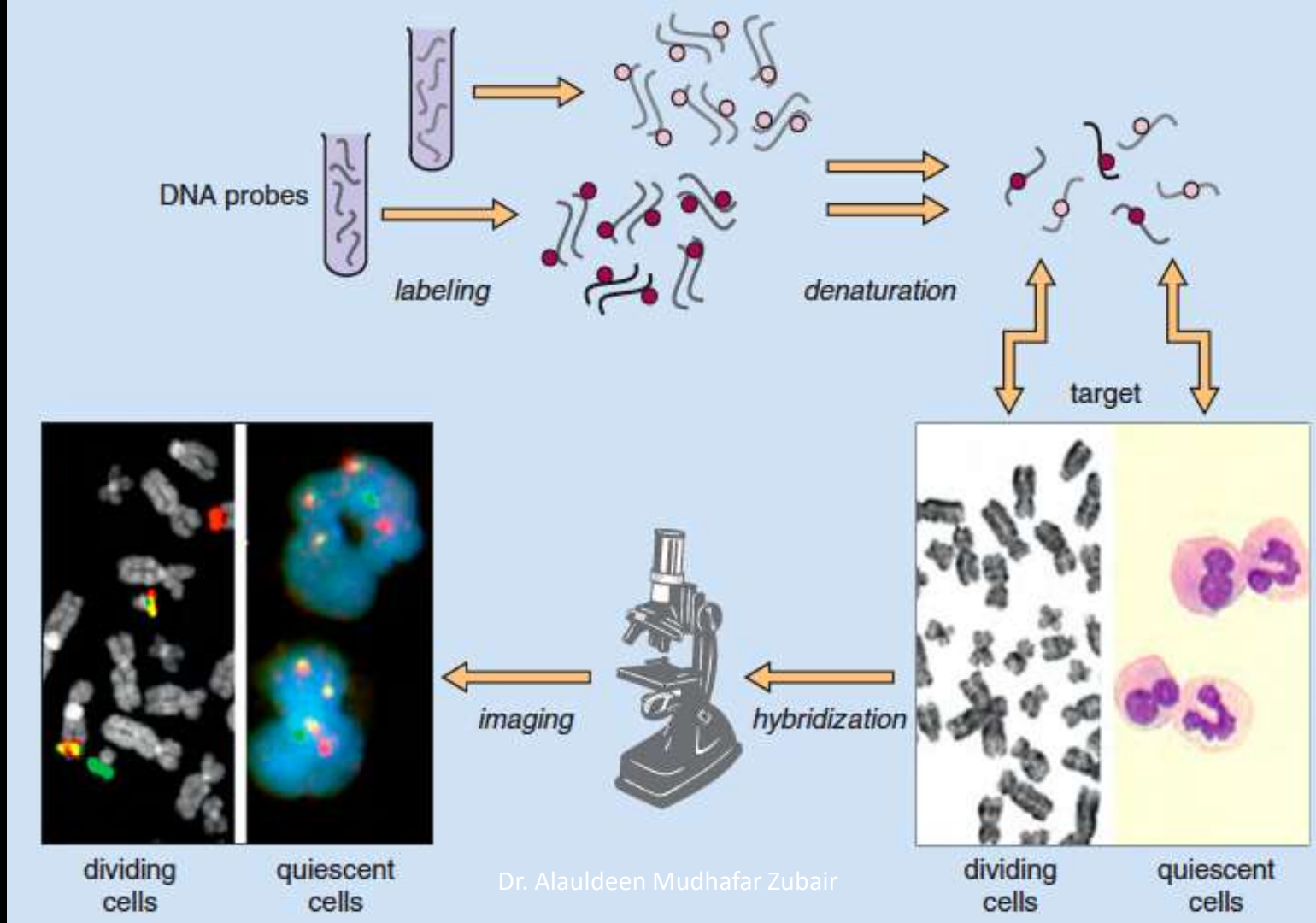


t(8;21)


t(8;21)(q22;q22)
8
der(8)
21
der(21)
8
der(8)
21
der(21)

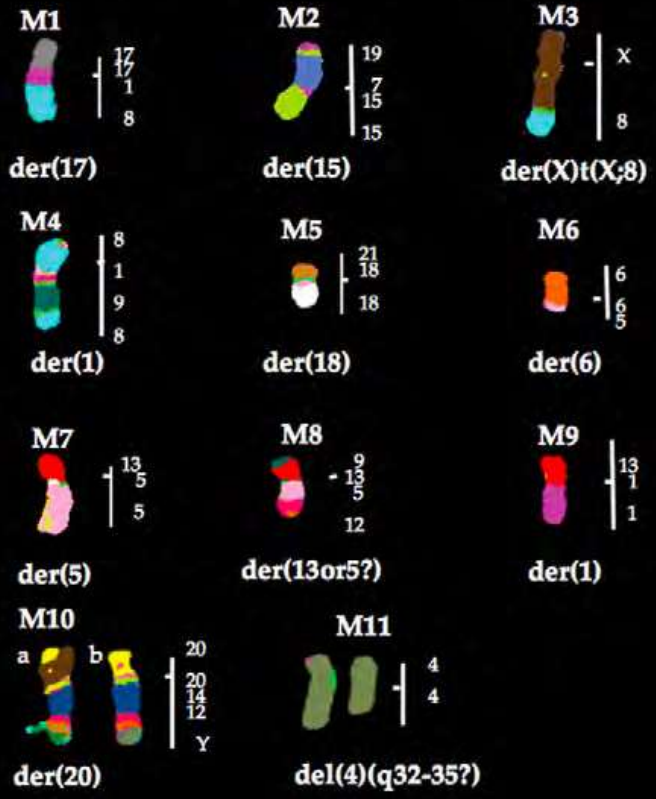
FLUORESCENT IN SITU HYBRIDIZATION

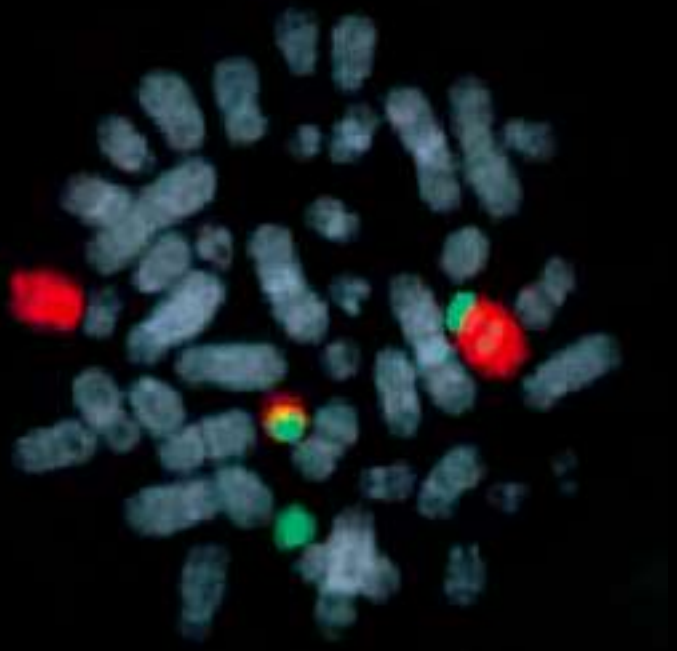
Fluorescent in situ hybridization (FISH) can be used to examine interphase as well as dividing cells. It is able to detect gain or loss of all or part of a chromosome or a translocation, in some cases where the translocated or lost material is not visible by light microscopy


DNA probes
labeling
denaturation
target
imaging
hybridization
dividing cells
quiescent cells
dividing cells
quiescent cells

M-FISH resolves multiple complex rearrangements at a resolution of single chromosome band (~10 Mbp)

AML (M1)





Metaphase from a childhood patient with ALL showing the translocation t(12;21) by chromosome “painting.” Chromosomes 12 are painted red and chromosomes 21 green.