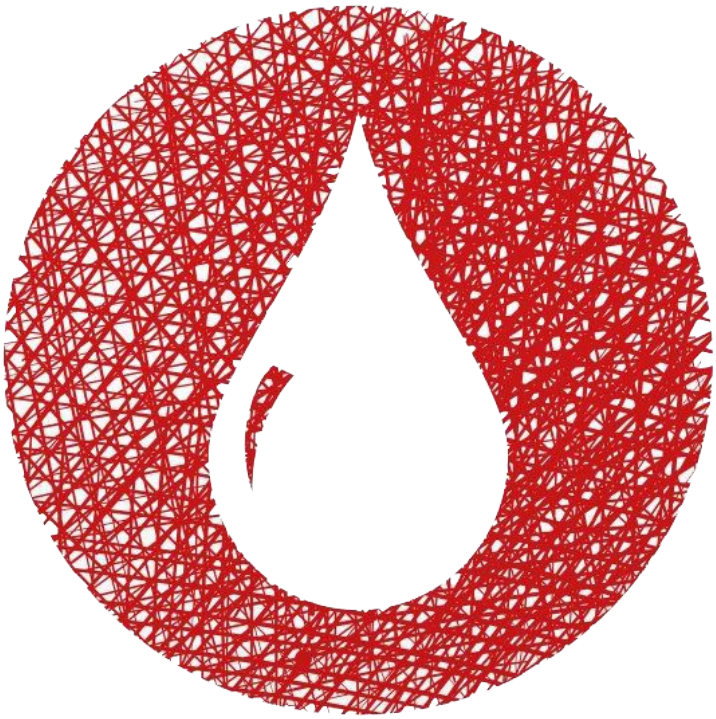


Acute leukemia

Team leaders : Abdulrahman Alageel, Ebtesam Almutairi.
Done by : Maan Shukr

Impotent
Notes
Doctor's slides

Acute leukemia

Aggressive malignant hematopoietic disorders which characterized by **accumulation of abnormal blasts** (**Immature** precursors of WBC) in **bone marrow** and blood leading to:

1. Bone marrow failure (anemia ,neutropenia & thrombocytopenia).
2. Organ infiltration (hepatosplenomegaly ,lymphadenopathy).Organ infiltration of blasts interferes with their functions

Pathogenesis of acute leukemia

Genetic predisposition	Environmental factors	Infection	Previous therapy	Other hematological disorders

Most common environmental factor is smoking

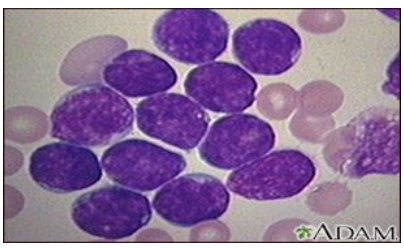
Worst type of predisposition

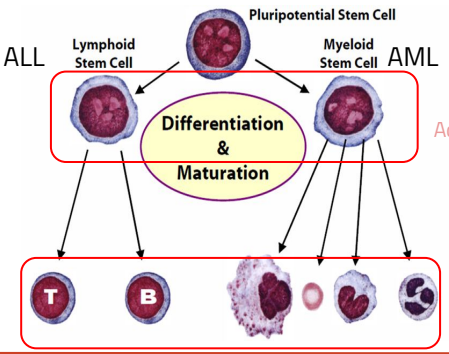
+Down syndromes (megaloblastic Anemia)

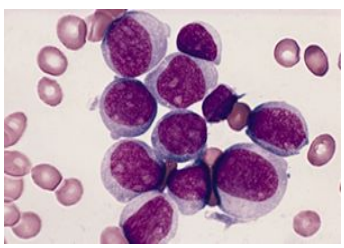
Unknown mechanism

Genetic alteration in the immature precursors

Block of differentiation ,Enhanced proliferation & Decreased apoptosis







Acute (immature)

Chronic (mature)

Epidemiology

- AL represent about 8% of neoplastic disease & cause about 4% of malignancy related deaths !
- AML (in adults) has an incidence of 2 – 3 per 100 000 per year in children, rising to 15 per 100 000 in adults.
- ALL (in children) has an incidence of 30 per million & represent about 76% of childhood leukemia.

General Classification

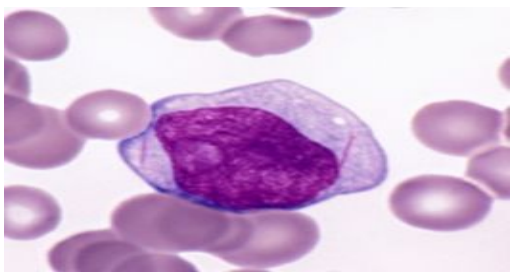
- Acute Myeloid Leukemia "AML".
- Acute Lymphoid Leukemia "ALL"
- Acute Leukemia of Ambiguous Lineage (very rare)

Basis of Classification

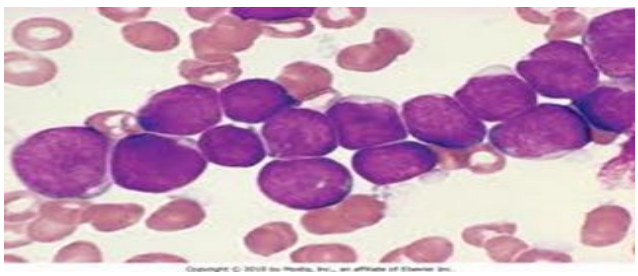
1. Clinical history (**Previous therapy**)
2. Morphology .
3. Flow cytometry.
4. Chromosomal Karyotyping.
5. Molecular study .

Light microscopy

- Blast count : it should be >20% out of the total cells Below 20% count in Light microscopy doesn't exclude AML
- Blast morphology :



Myeloblast
Size: **medium-Large**
Nucleus: round, oval or irregular
Nucleolus: **prominent**
Cytoplasm: abundant, granular
Auer rods is characteristic

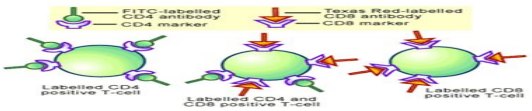


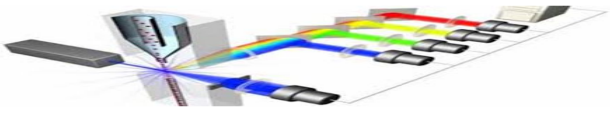
Lymphoblast
Size: **small- medium**
Nucleus: round
Nucleolus: not prominent
Cytoplasm: scanty ,agranular
may be **vacuolated**

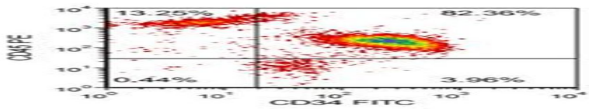
Flow cytometry:

CD34 result in flow cytometry always indicates Stem cells while CD45 is the leukocyte common origin

Laser based technology allows for cells counting & detection of their surface & cytoplasmic markers by suspending them in a stream of fluid followed by analysis through electronic system.







Stem Cell Markers: (CD34& TDT)

If these positive —> blast

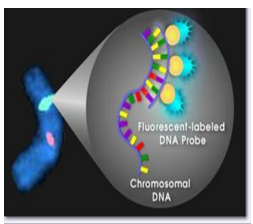
Myeloid	B-Lymphoid	T-Lymphoid
MPO CD13 CD33 CD14 CD64 CD41 CD235a	CD10 CD19 CD22 CD79a	**CD3** CD4 CD5 CD7 CD8

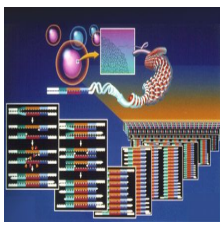
Chromosomal Karyotype

Set of the chromosomes from one cell during metaphase to **study the numerical (deletion trisomy) and structural (translation & inversion) abnormality.**

Molecular studies:

Several techniques used to detect and localize the presence or absence of **specific DNA sequences on chromosomes.**





Recurrent genetic abnormalities

AML	
Karyotype	Molecular
t (8;21)	**AML1-ETO**
t (16;16) or inv(16)	**CBFB-MYH11**
t (15;17)	**PML-RARA**
t (9;11)	MLLT1-MLL

ALL	
Karyotype	Molecular
t (9;22)	**BCR-ABL1**
t (4;11)	AF4-MLL
t (12;21)	ETV6-RUNX1
t (5;14)	IL3-IGH

Absence of MPO does not exclude Myeloid origin of leukemia, however, we have to have 2 markers in order for it to be proven. -CD13 and 33 are myeloid while CD14 and 64 are monocytes
-CD19 is the strongest B-lymphoid marker
-Without CD3 it can not be T-Lymphoid

Acute Myeloid Leukemia

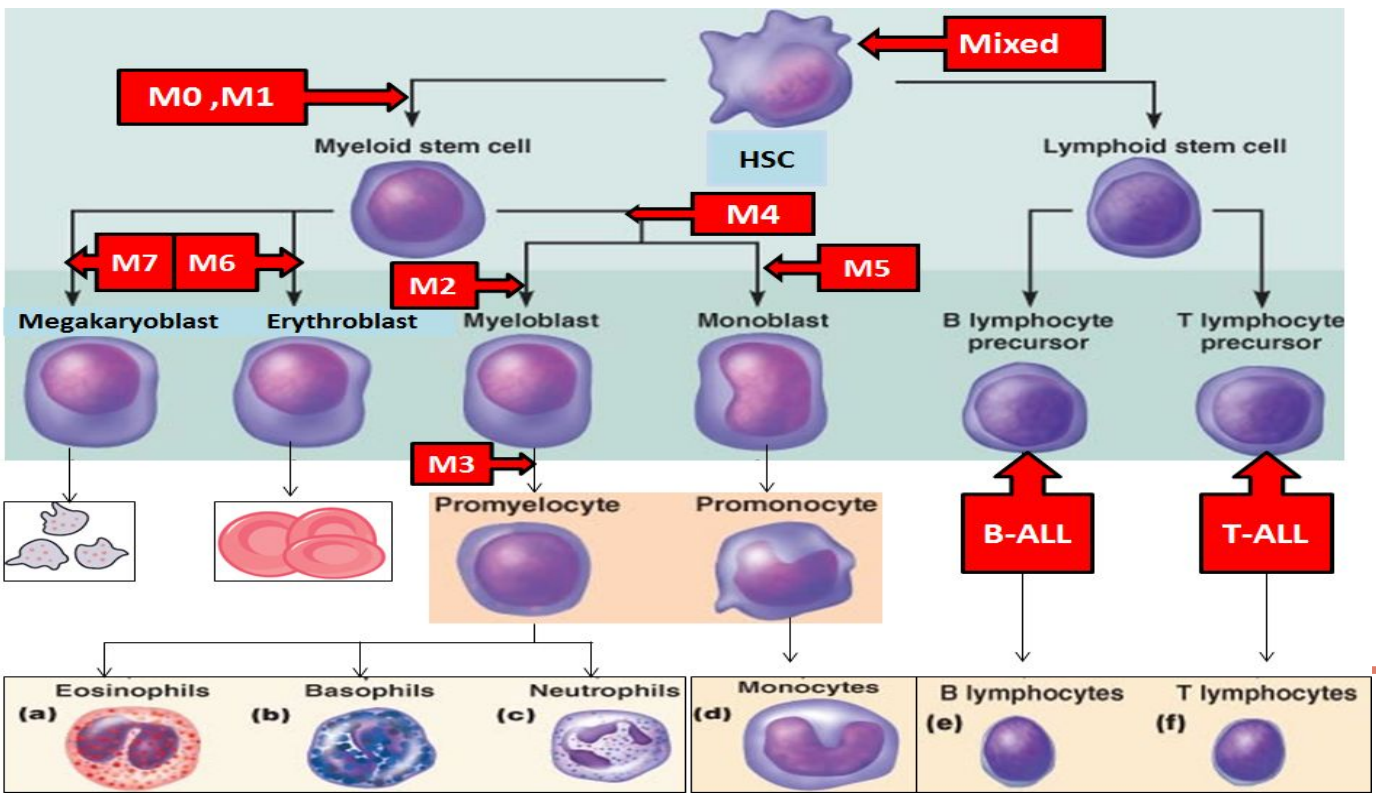
- Group of hematopoietic neoplasms caused by proliferation of malignant myeloid blasts in bone marrow and blood.
- The blast ≥20% or t(8;21) t (16;16) or t(15;17).
- More in Adults (do occur in infants)
- Worse than ALL

FAB Classification

Promyelocytic Leukemia is the most dangerous type of AML and it involves consumption of platelets

Based on **morphology & flow cytometry.**

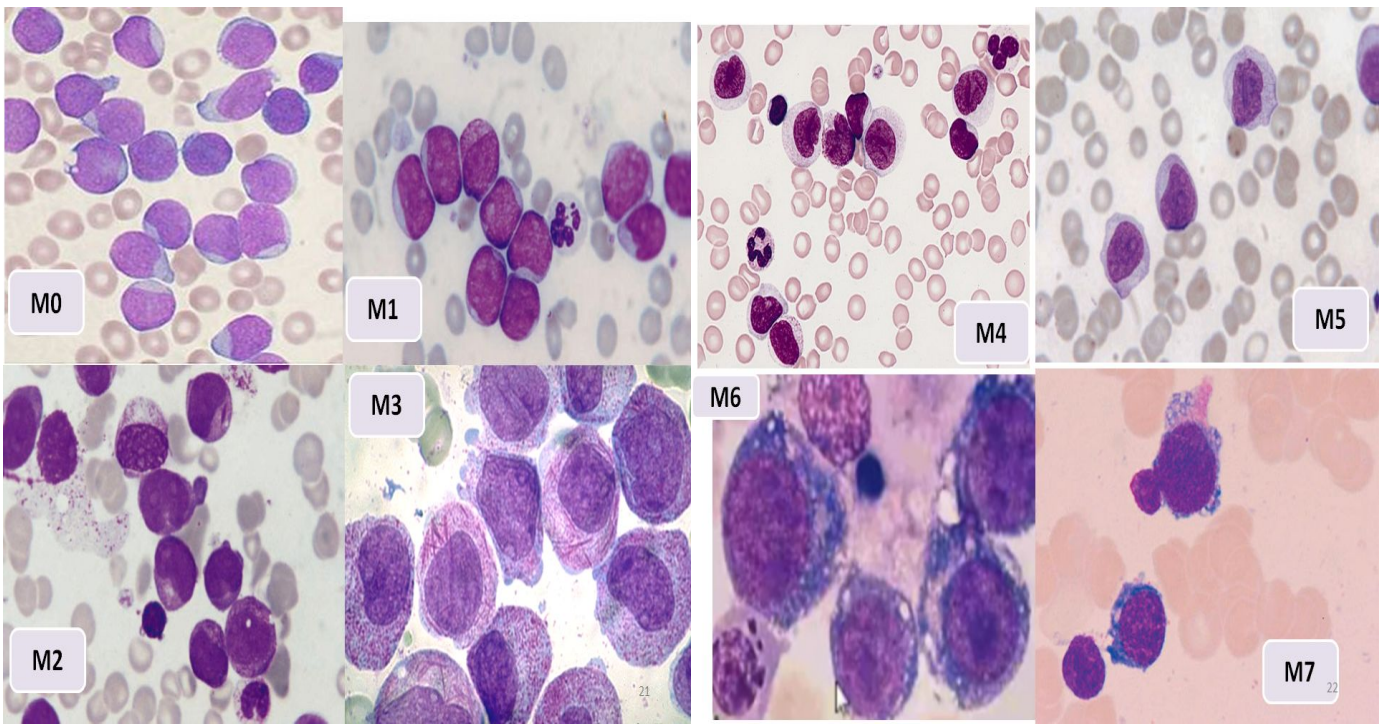
Subtype	Features	Genetics in WHO	Notes
Mo	Minimal differentiation		
M1	Without maturation		
M2	With maturation	t(8;21)	
M3	Promyelocytic	t(15;17)	DIC
M4	Granulocytic and monocytic	t or inv(16;16)	Gum hypertrophy
M5	Monoblastic (M5a) Monocytic (M5b)	t(9;11)	
M6	Erythroid		CD235a
M7	Megakaryocytic		CD41
M8	Basophilic		



AML Classification (WHO) :

New classification based on genetic.

AML with recurrent genetic abnormalities	Myelodysplasia related AML	Therapy related AML	AML, not otherwise specified (FAB)
1- t(8;21) 2- t(16;16) 3- t(15;17) **Prognosis:** Good	•Blasts≥ 20% •Significant dysplasia **Prognosis:** poor	•Blasts≥ 20% •Previous chemotherapy **Prognosis:** poor	•Blasts≥ 20% •Genetic: N •No dysplasia **Prognosis:** Standard

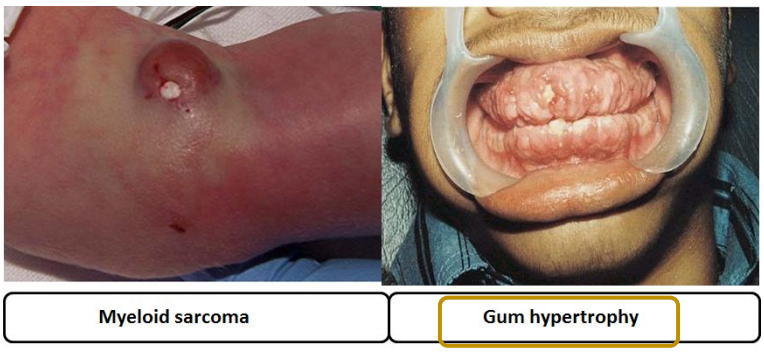


- During M0 we can not differentiate lymphoblast
- During M1 there is minimal differentiation
- During M2 there are many neutrophils + promyelocytes but we will see Aur rods
- During M3 we will see many Aur rods which raises Tissue factor and stimulates DIC , t(15, 17)
- During M6 we ee erythroblasts
- During M7 we see megakaryoblasts

Clinical Features of AML:

1. Pancytopenia: Acute Onset
   - ↓ WBC → infection (fever ,septic shock).
   - ↓ Hb → anemia (fatigue , headache , pallor ,SOB....)
   - ↓ Platelets → bleeding (bruises , epistaxis ,menorrhagia...)

2. Organ infiltration:
   - Hepatosplenomegaly.
   - Lymphadenopathy (rare).
   - Myeloid sarcoma.
   - Gum hypertrophy .
   - CNS disease.



3. Leukostasis (increased blood viscosity).
4. Disseminated Intravascular Coagulation (DIC):
   Widespread activation of coagulation system leading to intravascular fibrin deposition & consumption of platelet and coagulation factors which can be manifested as bleeding (85%) or thrombosis (15%) More with Acute Promyelocytic leukemia (M3)

Prognosis of AML:

Better prognosis if:

- Genetics: t(8;21), inv(16;16) or t(15;17) are invovlved.
- Age: < 60 years.
- Primary better than secondary.

Treatment of AML:

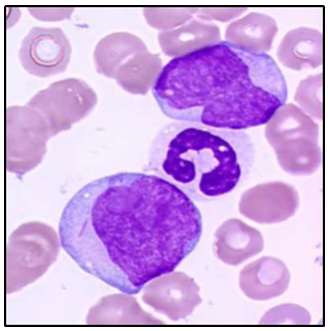
- Chemotherapy:

1. AML: M0-M8 but **not M3** (same protocol)
2. **AML: M3 (ATRA** (works on t(15;17) **or arsenic)**

- Stem cell transplantation

Case Study

- 65 years old male presented to ER with fatigue ,fever and nose bleeding for 2 weeks.
- O/E : moderate hepatosplenomegaly & multiple bruises.
- CBC :
  WBC :40 x109/L.
  HB: 7g/dL.
  PLT: 51 x109/L.



- Blood smear & bone marrow:

- Flow cytometry :
  The blast are positive for CD34 ,CD13,CD33,CD117 and MPO They are negative for CD3,CD10,CD19&CD79a.

  Karyotype:

What is the final diagnosis?

AML with t(8;21)

ACUTE LYMPHOBLASTIC LEUKEMIA

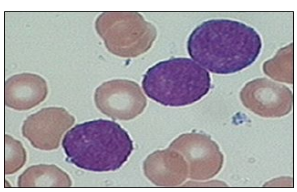
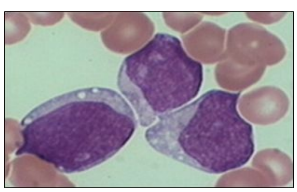
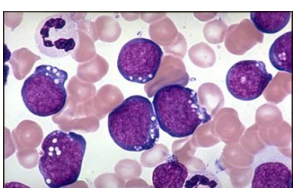
- Acute leukemia characterized by proliferation of malignant lymphoid blasts in bone marrow and blood.
- **B and T cells.**
- More common in Children.
- Better than AML.

Clinical Features of ALL:

1. Pancytopenia:
   - ↓WBC → infection (fever ,septic shock).
   - ↓Hb → anemia (fatigue , headache , pallor ,SOB….)
   - ↓Platelets → **bleeding** (bruises , **epistaxis** ,menorrhagia…)
2. Organ infiltration:

- **Lymphadenopathy (very common)** due to maturation of cells in them
- Hepatosplenomegaly.
- **testicles involvement**
- CNS disease
- **Mediastinal mass** which is characteristic of T-cell type because they mature in thymus

Morphological subtypes (FAB) of ALL: Important

	L1	L2	L3 Burkitt's
Morphology	Homogenous	Heterogeneous	Homogenous
Size	Small	Variable	Small
Cytoplasm	Little	More	Vaculated
Nucleoli	Not prominent	Prominent	Prominent
Genetics	Variable	Variable	t(8;14) cmyc
			

Immunophenotypic Subtypes (WHO): Important

	B cell	T cell
Markers	CD19,CD10 CD79a	CD3
Percentage	80%	20%
Age	Younger	Older
Clinical	Lymphadenopathies are more common in B cell type	Mediastinal mass CNS relapse
WBC count	Less	Higher
Prognosis	Better	Worse
Genetics	t(9;22),t(4;11),t(12;21)	-------

Notice that L3 (Burkitt's) represents mature lymphoid neoplasm so it is a type of lymphoma not Acute lymphoblastic leukaemia.it is negative for TDT or CD34 in flow cytometry

Prognosis:

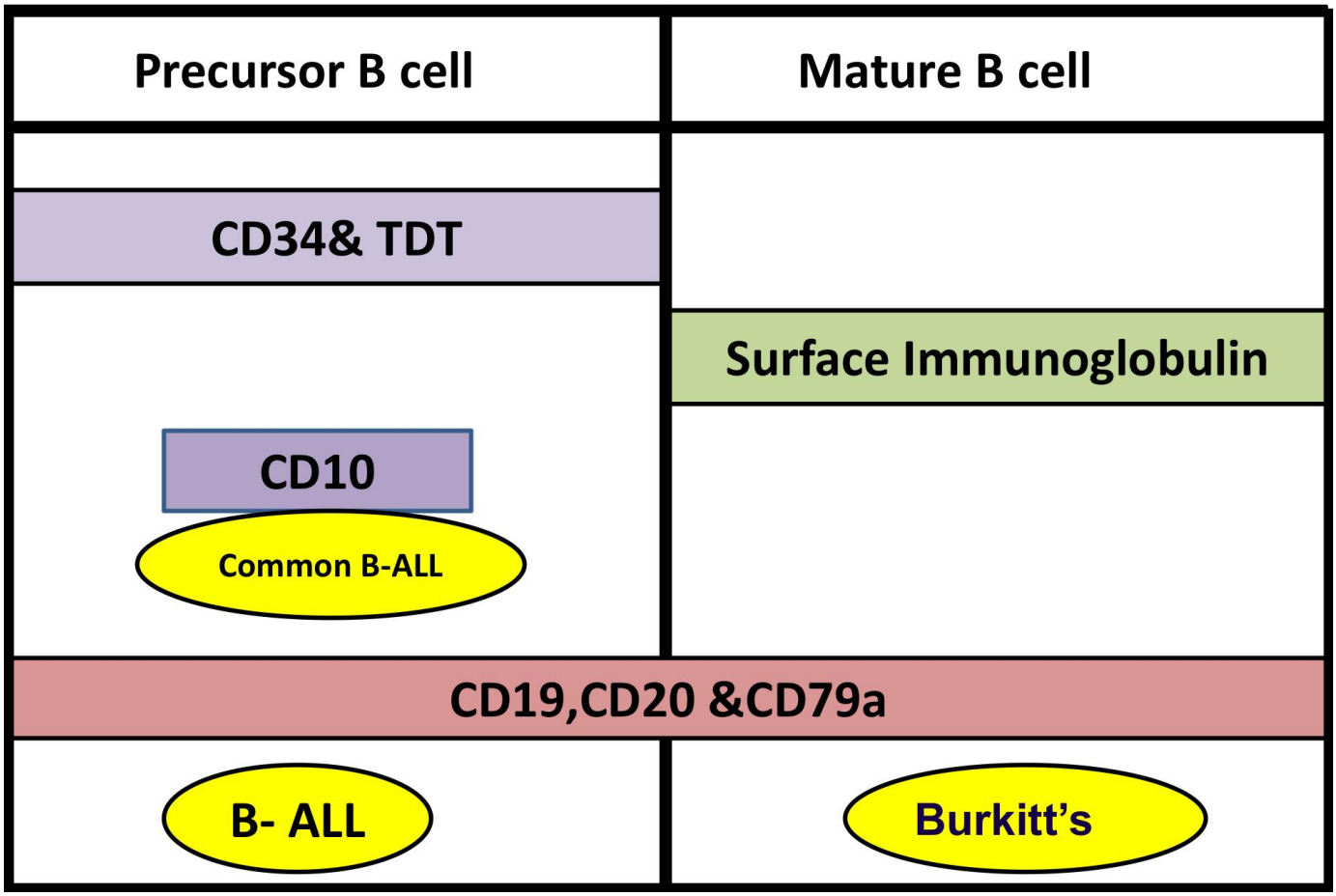
	Better	Worse
Age	2 - 10 yrs	<2 - >10 yrs
Gender	F	M
WBC count	Low	High
Cell type	B cell	T cell
B-ALL phenotype	Common	Others
B-ALL genetics	Hyperdiploidy t(12;21)	Hypodiploidy t(9;22)
CNS involvement	No	Yes

- Hyperdiploidy is the presence of more than 46 chromosomes
- Hypodiploidy is the presence of less than 46 chromosomes and it is harder to treat because it is easier to limit a new function the compensate for a lost one.
- t(9;22) os harder to target well than rest of translocation because it is triphasic (it is seen in AML, CML, and ALL)

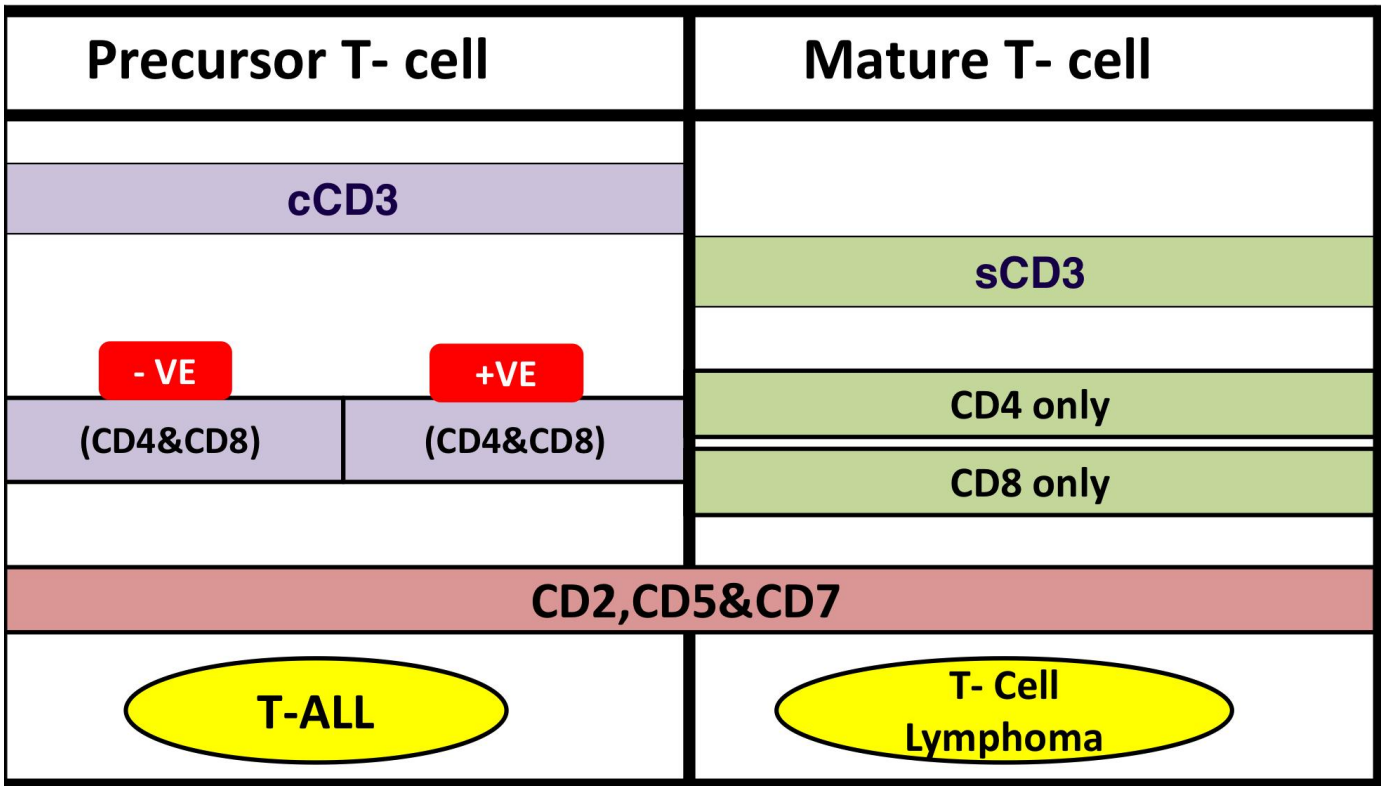
Treatment:

- Chemotherapy (high cure rate) .
- Stem cell transplantation.

B-cell


Precursor B cell
Mature B cell
CD34& TDT
Surface Immunoglobulin
CD10
Common B-ALL
CD19,CD20 &CD79a
B- ALL
Burkitt's

T-cell


Precursor T- cell
Mature T- cell
cCD3
sCD3
- VE
+VE
(CD4&CD8)
(CD4&CD8)
CD4 only
CD8 only
CD2,CD5&CD7
T-ALL
T- Cell Lymphoma

Remember ! Important

- Acute leukaemia is a fatal neoplastic condition.
- 20% or more blasts = Acute leukaemia.
- Diagnosis requires special investigations.
- Auer rods = AML.
- AML M3 = DIC &target therapy.
- Gum hypertrophy = mostly M4 or M5.
- Mediastinal = T-ALL.
- Subtypes of AML (M0-M8) + cytogenetic abnormalities.
- Subtypes of ALL (T or B cell).
- Main lineages markers are MPO, CD19 and CD3.
- Stem cell markers are CD34,TDT.
- FAB classification based mainly on morphology.
- WHO classification focused more on genetics.

Doctors notes:

- Organ infiltration of blasts interferes with their functions
- Examples of genetic predispositions are trisomy 21 (down syndrome), and RGB gene mutation
- Most common environmental factor is smoking
- Examples of infectious predispositions to leukemia are malaria and EBV
- Worst type of predisposition is Previous chemotherapy
- There are two types of Acute leukemia of ambiguous lineage: 1) type expressing myeloid and lymphoid blast at the same time 2) type expressing stem cells markers without lymphoid or myeloid
- Patients who have previous chemotherapy should be treated with stem cell therapy immediately.
- Below 20% count in Light microscopy doesn't exclude AML
- Auer rods are made out of myeloid peroxidase??
- Flow cytometry reads the ID of cells
- CD34 result in flow cytometry always indicates Stem cells while CD45 is the leukocyte common origin (hematopoietic cells whether they are mature or not.
- Absence of MPO does not exclude Myeloid origin of leukemia, however, we have to have 2 markers in order for it to be proven.
- CD13 and 33 are myeloid while CD14 and 64 are monocytes
- CD19 is the strongest B-lymphoid marker
- Without CD3 it can not be T-Lymphoid
- Best example of translocation is 15,17
- From clinical information we need to obtain: 1) history 2)physical examination. From lab we obtain morphology. From karyotyping we know about number of chromosomes. And from molecular studies we need to know about genes
- Myeloid stem cells to megakaryocyte, erythrocyte, monocytes, myeloblast is controlled by growth factor
- Promyelocytic Leukemia is the most dangerous type of AML and it involves consumption of platelets
- During M0 we can not differentiate lymphoblast
- During M1 there is minimal differentiation
- During M2 there are many neutrophils + promyelocytes but we will see Aur rods
- During M3 we will see many Aur rods which raises Tissue factor and stimulates DIC
- During M6 we ee erythroblasts
- During M7 we see megakaryoblasts
- leukostasis is caused by increased number of WBCs
- ATRA works on fixing t(15;17)

Doctors notes:

- B cell ALL is the most common and best prognosis type
- Clinical features of ALL are similar to AML except for lymphadenopathies commonality (due to maturation of cells in them) , testicle involvement, and mediastinal mass (which is characteristic of T-cell type because they mature in thymus)
- Myeloid cells mature in spleen and bone marrow
- Even though FAB classification classifies Burkitt's as leukemia it is a lymphoma
- Lymphadenopathies are more common in B cell type
- t(12;21) has the best type of prognosis
- L3(Burkitt's) is negative for TDT or CD34 in flow cytometry
- We must tell apart burkitt's from leukemia because treatment is massively different
- Hyperdiploidy is the presence of more than 46 chromosomes
- Hypodiploidy is the presence of less than 46 chromosomes and it is harder to treat because it is easier to limit a new function the compensate for a lost one.
- t(9;22) is the most common translocation
- t(9;22) os harder to target well than rest of translocation because it is triphasic (it is seen in AML, CML, and ALL)