Leukaemias

Leukemia

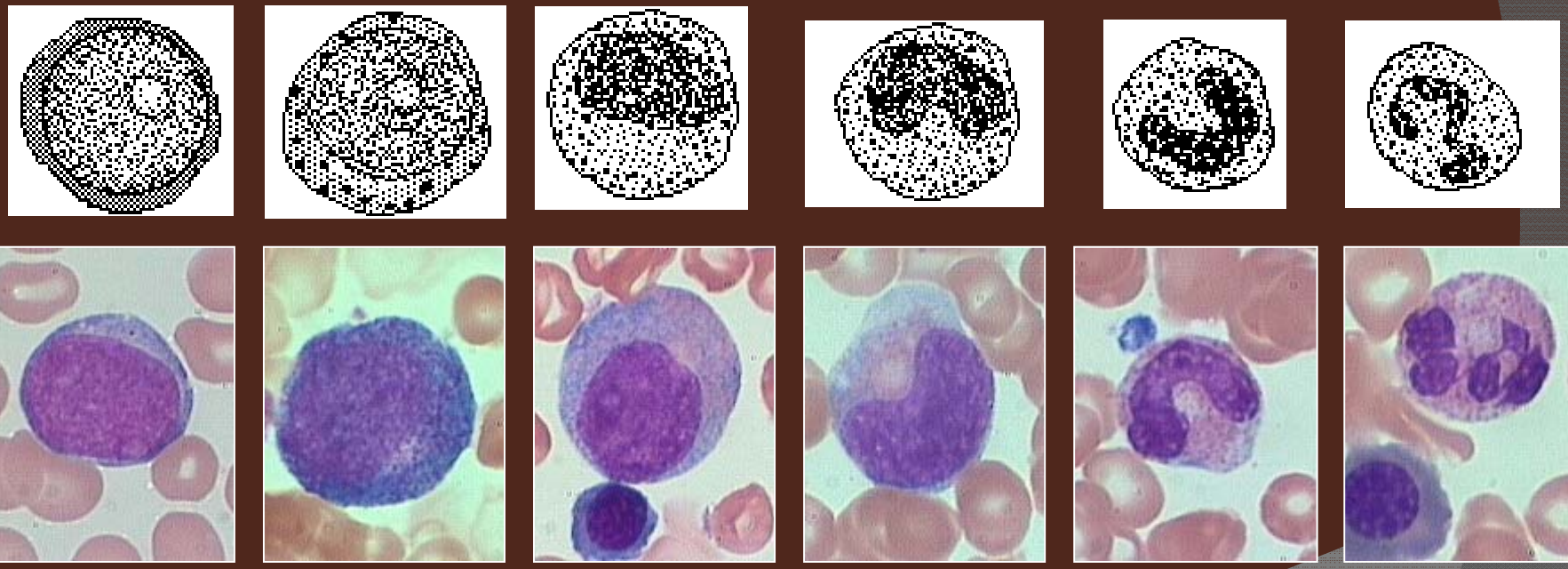
- Is a malignant hematologic disorder characterized by a proliferation of abnormal white cells that infiltrate the bone marrow, peripheral blood and organs

Classification of leukemias

	Acute	Chronic
Myeloid origin	Acute Myeloid Leukemia (AML)	Chronic Myeloid Leukemia (CML)
Lymphoid origin	Acute Lymphoblastic Leukemia (ALL)	Chronic Lymphocytic Leukemia (CLL)

Myeloid maturation

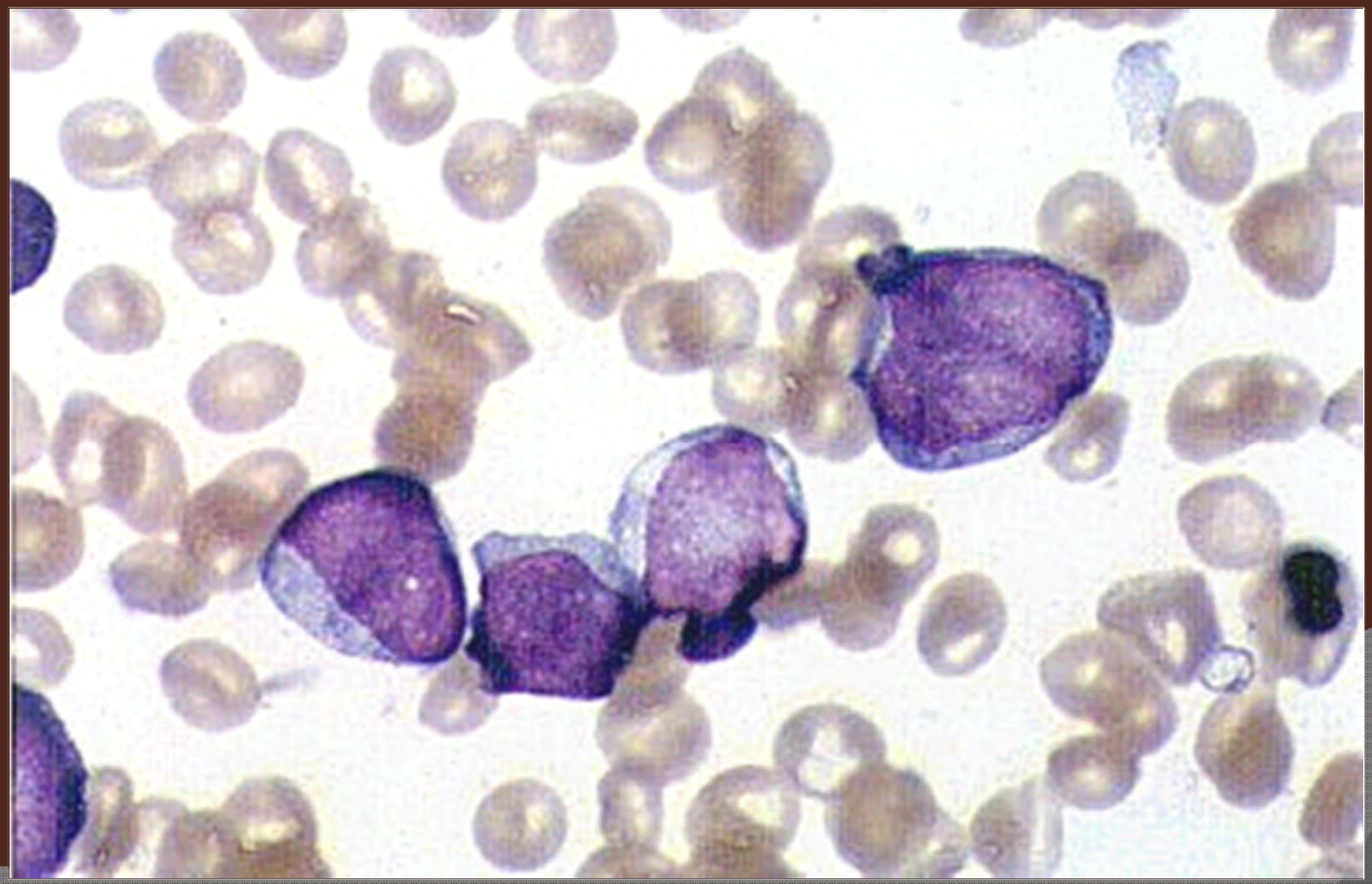
myeloblast promyelocyte myelocyte metamyelocyte band neutrophil



MATURATION

Acute Leukemia

- accumulation of blasts in the marrow



How to distinguish AML vs CML from looking at peripheral blood

Myeloid cell	CML	AML	normal
blasts	ɣ	ɣ	
promyelocytes	ɣ		
myelocytes	ɣ		
metamyelocytes	ɣ		
bands	ɣ		
neutrophils	ɣ	‘	ɣ

WHO classification (4th Edition- 2008) of myeloid neoplasms and acute leukemia

- Myeloproliferative neoplasms (MPN)
- Myeloid and lymphoid neoplasms associated with eosinophilia and abnormalities of *PDGFRA*, *PDGFRB*, or *FGFR1*
- Myelodysplastic/myeloproliferative neoplasms (MDS/MPN)
- Myelodysplastic syndrome (MDS)
- Acute myeloid leukemia and related neoplasms
- Acute leukemias of ambiguous lineage
- B lymphoblastic leukemia/lymphoma
- T lymphoblastic leukemia/lymphoma

WHO classification of myeloid neoplasms and acute leukemia

Myeloproliferative neoplasms (MPN)

- Chronic myelogenous leukemia, *BCR-ABL1*–positive
- Chronic neutrophilic leukemia
- Polycythemia vera
- Primary myelofibrosis
- Essential thrombocythemia
- Chronic eosinophilic leukemia, not otherwise specified
- Mastocytosis
- Myeloproliferative neoplasms, unclassifiable

Myeloid and lymphoid neoplasms associated with eosinophilia and abnormalities of *PDGFRA*, *PDGFRB*, or *FGFR1*

- Myeloid and lymphoid neoplasms associated with *PDGFRA* rearrangement
- Myeloid neoplasms associated with *PDGFRB* rearrangement
- Myeloid and lymphoid neoplasms associated with *FGFR1* abnormalities

WHO classification of myeloid neoplasms and acute leukemia

Myelodysplastic/myeloproliferative neoplasms (MDS/MPN)

- Chronic myelomonocytic leukemia
- Atypical chronic myeloid leukemia, *BCR-ABL1*–negative
- Juvenile myelomonocytic leukemia
- Myelodysplastic/myeloproliferative neoplasm, unclassifiable
  - *Provisional entity: refractory anemia with ring sideroblasts and thromb*

Myelodysplastic syndrome (MDS)

- Refractory cytopenia with unilineage dysplasia
  - Refractory anemia
  - Refractory neutropenia
  - Refractory thrombocytopenia
- Refractory anemia with ring sideroblasts
- Refractory cytopenia with multilineage dysplasia
- Refractory anemia with excess blasts
- Myelodysplastic syndrome with isolated del(5q)
- Myelodysplastic syndrome, unclassifiable
- Childhood myelodysplastic syndrome
  - *Provisional entity: refractory cytopenia of childhood*

WHO classification of myeloid neoplasms and acute leukemia

Acute myeloid leukemia and related neoplasms

- Acute myeloid leukemia 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*
  - APL with t(15;17)(q22;q12); *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*
  - *Provisional entity: AML with mutated NPM1*
  - *Provisional entity: AML with mutated CEBPA*
- Acute myeloid leukemia with myelodysplasia-related changes
- Therapy-related myeloid neoplasms

- Acute myeloid leukemia, not otherwise specified
  - AML with minimal differentiation
  - AML without maturation
  - AML with maturation
  - Acute myelomonocytic leukemia
  - Acute monoblastic/monocytic leukemia
  - Acute erythroid leukemia
    - Pure erythroid leukemia
    - Erythroleukemia, erythroid/myeloid
  - Acute megakaryoblastic leukemia
  - Acute basophilic leukemia
  - Acute panmyelosis with myelofibrosis
- Myeloid sarcoma
- Myeloid proliferations related to Down syndrome
  - Transient abnormal myelopoiesis
  - Myeloid leukemia associated with Down syndrome
- Blastic plasmacytoid dendritic cell neoplasm

WHO classification of myeloid neoplasms and acute leukemia

Acute leukemias of ambiguous lineage

- Acute undifferentiated leukemia
- Mixed phenotype acute leukemia with t(9;22)(q34;q11.2); *BCR-ABL1*
- Mixed phenotype acute leukemia with t(v;11q23); *MLL* rearranged
- Mixed phenotype acute leukemia, B-myeloid, NOS
- Mixed phenotype acute leukemia, T-myeloid, NOS
- *Provisional entity: natural killer (NK) cell lymphoblastic leukemia/lymphoma*

B lymphoblastic leukemia/lymphoma

- B lymphoblastic leukemia/lymphoma, NOS
- B lymphoblastic leukemia/lymphoma with recurrent genetic abnormalities
  - B lymphoblastic leukemia/lymphoma with t(9;22)(q34;q11.2);*BCR-ABL 1*
  - B lymphoblastic leukemia/lymphoma with t(v;11q23);*MLL* rearranged
  - B lymphoblastic leukemia/lymphoma with t(12;21)(p13;q22) *TEL-AML1* (*ETV6-RUNX1*)
  - B lymphoblastic leukemia/lymphoma with hyperdiploidy
  - B lymphoblastic leukemia/lymphoma with hypodiploidy
  - B lymphoblastic leukemia/lymphoma with t(5;14)(q31;q32) *IL3-IGH*
  - B lymphoblastic leukemia/lymphoma with t(1;19)(q23;p13.3);*TCF3-PBX1*

T lymphoblastic leukemia/lymphoma

FAB Classification ofAML

FAB	Morphology	MPO	SE	NSE	Comment
M0	Min. different ion	neg	neg	neg	primitive (bad?)
M1	w/out maturation	pos	neg	neg	primitive (bad?)
M2	With maturation	pos	pos	neg	t8;21
M3	APL	pos	pos	neg	t15;17 (APL)
M4	Myelomonocytic	pos	pos	pos	INV16/M4eo
M5	Monocytic	pos	neg	pos	11q23, FLT3
M6	Erythroleukemia	pos	neg	neg	BAD…
M7	Megakaryocytic	pos	neg	neg	BAD…

The FAB Classification

FAB Classification of Acute Myeloid Leukemia–I

FAB type	Blasts, percent		Erythroid progenitors	Morphology	Cytochemistry
	All cells	NEC			
M0	>30	>90	<50	Blasts resemble L2 variant of ALL; Cytoplasmic granules and auer rods are not seen.	<3% PXase+ or SBB+
M1	>30	>90	<50	>30% type 1 and type 2 blasts; <10% differentiated myeloid cells; Auer rods seen in about 50% of cases	>3% PXase+ or SBB+
M2	>30	>30-89	<50	>30% type 1 and type 2 blasts; >10% differentiated myeloid cells; Auer rods seen in about 70% of cases	PXase+ SBB+ NSE+<20% PAS-
M3	>30†	>30-89	<50	>20% abnormal hypergranular progranulocytes; blast count may be <30%; Auer rods and faggot cells seen in virtually all cases	PXase+ SBB+ PAS- NSE±
M3V	>30†	>30-89	<50	>20% abnormal hypogranular progranulocytes; blast count may be <30%; Auer rods and faggot cells seen in virtually all cases	PXase+ SBB+ PAS- NSE±
M4	>30	>30-79	<50	>20% promonocytes and monocytes; >20% granulocytic cells; peripheral monocytosis (>5 x 10^9) ± elevated serum or urine lysozyme; Auer rods seen in about 65% of cases	PXase+ >20% NSE+

†Abnormal progranulocytes and blasts
NEC = nonerythroid cells; PXase = peroxidase; SBB = Sudan black; NSA = nonspecific esterase; PAS = periodic acid-Schiff

FAB Classification of Acute Myeloid Leukemia–II

FAB type	Blasts, percent		Erythroid progenitors	Morphology	Cytochemistry
	All cells	NEC			
M4eo	>30	>30–79	<50	>5% eosinophils and cells with mixed basophilic and eosinophilic granules, plus M4 features	PXase+ >20%NSE+
M5a		>80#	<50	>80% of nonerythroid cells are monoblasts; Auer rods usually not seen	NSE+
M5b		>80#	<50	>80% of nonerythroid cells are monocytes, promonocytes, and monoblasts; Auer rods can be seen in a minor population of myeloblasts (30 percent of cases)	NSE+
M6		>30	**>50**	Erythroid predominance and dysplasia; >30% blasts among non-erythroid cells; Auer rods present in blasts in 60% of cases	PAS+ (erythroid cells); blasts are PXase+
M7	>30		<50	Blasts with cytoplasmic blebbing ± platelet shedding; marrow fibrosis; Auer rods are not seen	Platelet PXase+ on EM

†Abnormal progranulocytes and blasts

NEC = nonerythroid cells
PXase = peroxidase
SBB = Sudan black
NSA = nonspecific esterase
PAS = periodic acid-Schiff

Web Table 14.4: WHO Classification of Myeloid Neoplasms.

I. MYELOPROLIFERATIVE DISEASES
   1. Chronic myeloid leukaemia (CML), {Ph chromosome t(9;22)(q34;11), BCR/ABL-positive}
   2. Chronic neutrophilic leukaemia
   3. Chronic eosinophilic leukaemia/ hypereosinophilic syndrome
   4. Chronic idiopathic myelofibrosis
   5. Polycythaemia vera (PV)
   6. Essential thrombocythaemia (ET)
   7. Chronic myeloproliferative disease, unclassifiable

II. MYELODYSPLASTIC/MYELOPROLIFERATIVE DISEASES
   1. Chronic myelomonocytic leukaemia (CMML)

III. MYELODYSPLASTIC SYNDROME (MDS)
   1. Refractory anaemia (RA)
   2. Refractory anaemia with ring sideroblasts (RARS)
   3. Refractory cytopenia with multilineage dysplasia (RCMD)
   4. RCMD with ringed sideroblasts (RCMD-RS)
   5. Refractory anaemia with excess blasts (RAEB-1)
   6. RAEB-2
   7. Myelodysplastic syndrome unclassified (MDS-U)
   8. MDS with isolated del 5q

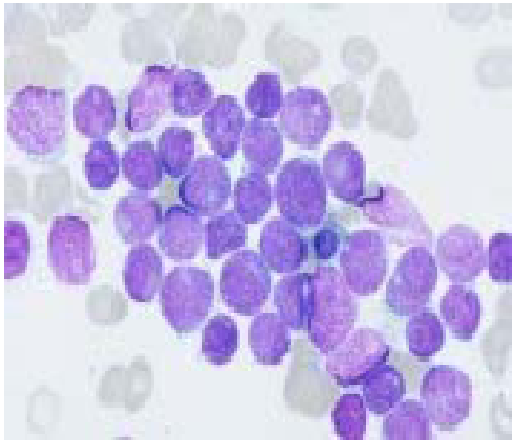
IV. ACUTE MYELOID LEUKAEMIA (AML)
   1. AML with recurrent cytogenetic abnormalities
      i) AML with t(8;21)(q22;q22)
      ii) AML with abnormal bone marrow eosinophils {inv(16)(p13q22)}
      iii) Acute promyelocytic leukaemia {t(15;17)(q22;q12)}
      iv) AML with 11q23 abnormalities (MLL)
   2. AML with multilineage dysplasia
      i) With prior MDS
      ii) Without prior MDS
   3. AML and MDS, therapy-related
      i) Alkylating agent-related
      ii) Topoisomerase type II inhibitor-related
      iii) Other types
   4. AML, not otherwise categorised
      i) AML, minimally differentiated
      ii) AML without maturation
      iii) AML with maturation
      iv) Acute myelomonocytic leukaemia (AMML)
      v) Acute monoblastic and monocytic leukaemia
      vi) Acute erythroid leukaemia
      vii) Acute megakaryocytic leukaemia
      viii) Acute basophilic leukaemia
      ix) Acute panmyelosis with myelofibrosis
      x) Myeloid sarcoma

V. ACUTE BIPHENOTYPIC LEUKAEMIA

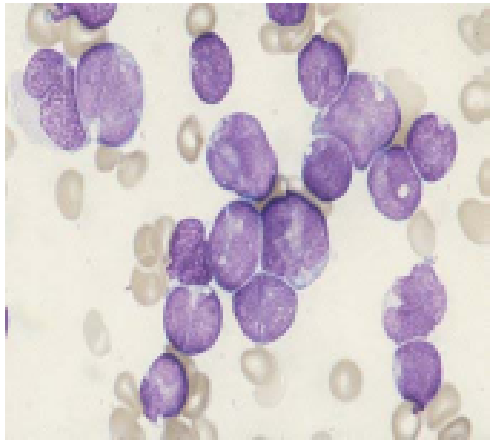
ALL SUBTYPES

- L1 ALL:
  - Blasts are small and relatively uniform
  - Round nucleus and regular cellular outline
  - Nucleoli are absent or inconspicuous and the nuclear-cytoplasmic ratio is high, chromatin pattern is fairly homogenous
- L2 ALL:
  - Lower nuclear cytoplasmic ratio typically with prominent nucleoli
  - Macroblasts sometimes two and a half times larger are identifiable
- L3 ALL:
  - Large, relatively round cells
  - Nuclei finely dispersed
  - Cytoplasm is strongly basophilic, contains prominent vacuoles

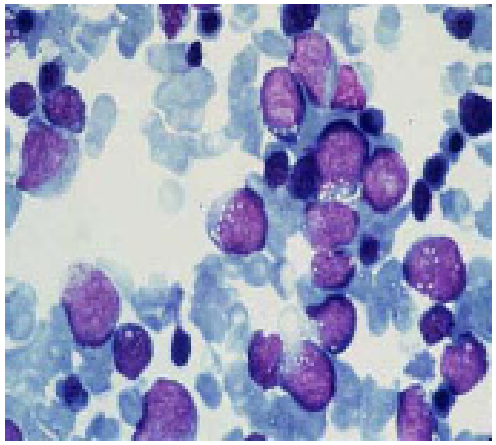
FAB Classification of ALL



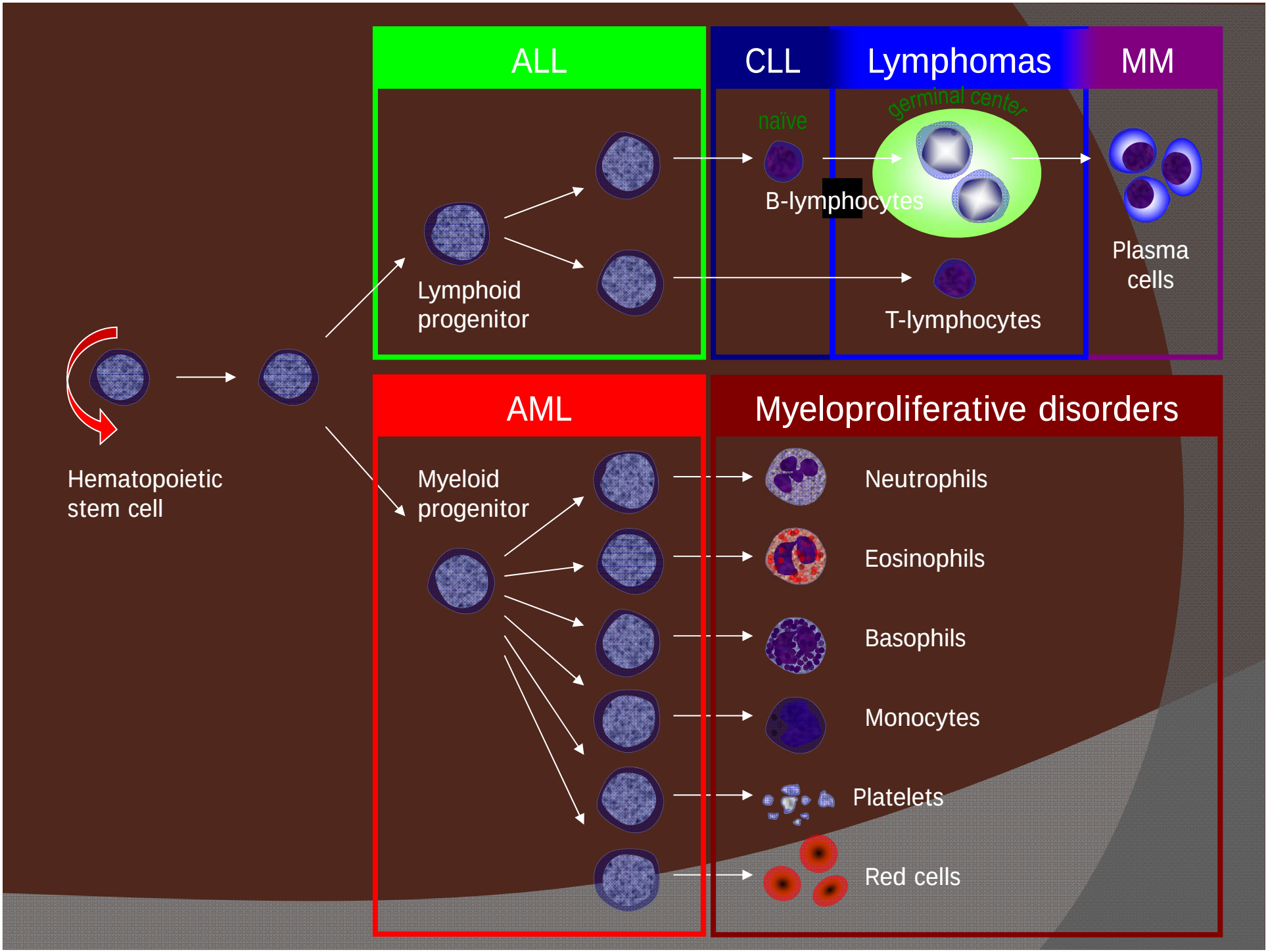
L1-ALL: Complete replacement by small/medium sized blasts with scanty cytoplasm and round nuclei with dense chromatin.



L2-ALL: Pleiomorphic blasts with variable amounts of cytoplasm, twisted irregular nuclei and multiple indistinct nucleoli.



ALL-L3: Large lymphoid blasts of with high nuclear to cytoplasmic ratio, dark-blue cytoplasm, and small lipid-containing vacuoles in the cytoplasm and over the nucleus. ALL-L3 are high grade B cell malignancies.


ALL
CLL
Lymphomas
MM
naïve
germinal center
B-lymphocytes
Plasma cells
Lymphoid progenitor
T-lymphocytes
Hematopoietic stem cell
AML
Myeloproliferative disorders
Myeloid progenitor
Neutrophils
Eosinophils
Basophils
Monocytes
Platelets
Red cells

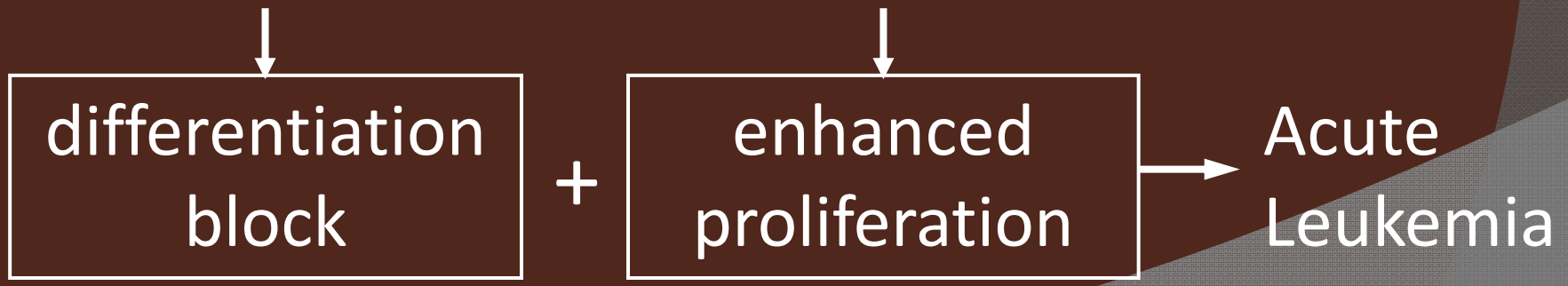
Two-hit model of leukemogenesis

Loss of function of transcription factors needed for differentiation

eg. AML1-ETO
PML-RARα

Gain of function mutations of tyrosine kinases

eg. FLT3, c-KIT mutations
N- and K-RAS mutations
BCR-ABL
TEL-PDGFβR



Causes of acute leukemias

- idiopathic (most)
- underlying hematologic disorders
- chemicals, drugs
- ionizing radiation
- viruses (HTLV I)
- hereditary/genetic conditions

Clincal manifestations

- symptoms due to:
  - marrow failure
  - tissue infiltration
  - leukostasis
  - constitutional symptoms
  - other (DIC)
- usually short duration of symptoms

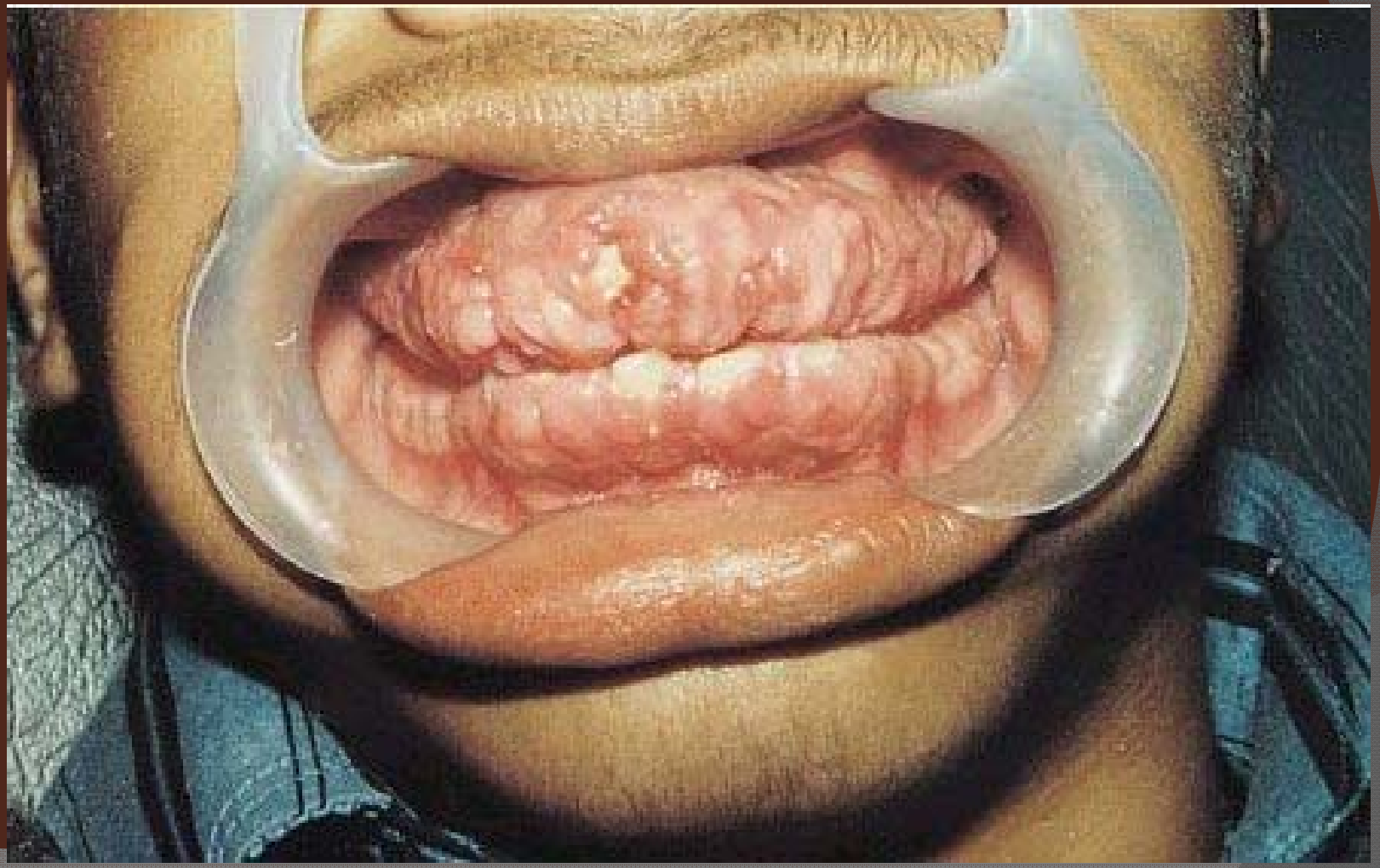
Marrow failure

- neutropenia: infections, sepsis
- anemia: fatigue, pallor
- thrombocytopenia: bleeding

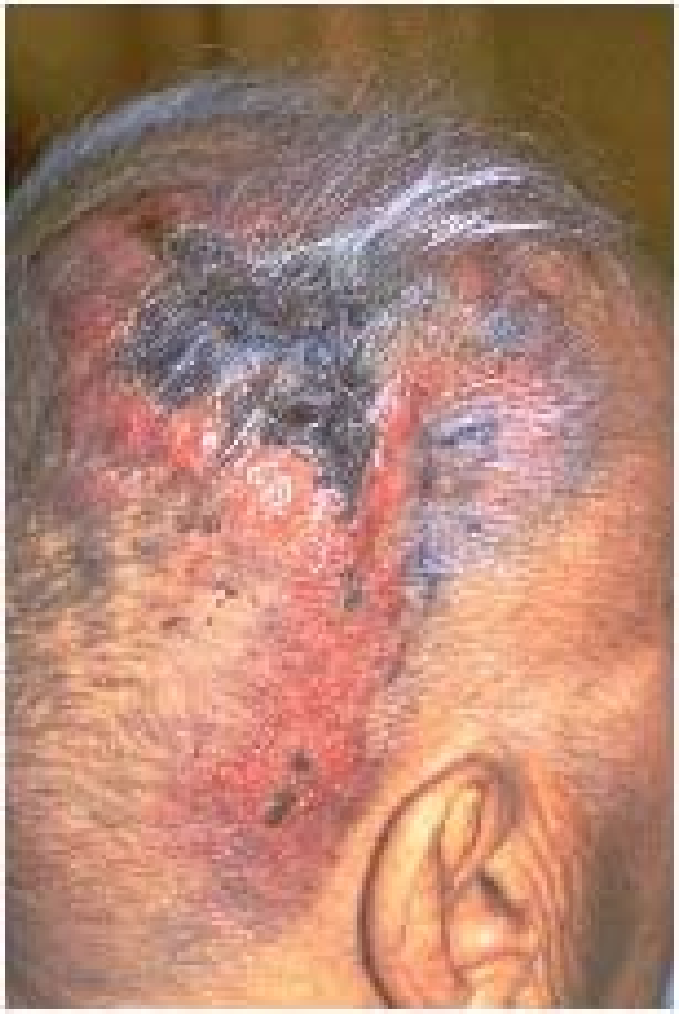
Infiltration of tissues/organs

- enlargement of liver, spleen, lymph nodes
- gum hypertrophy
- bone pain
- other organs: CNS, skin, testis, any organ

Gum hypertrophy



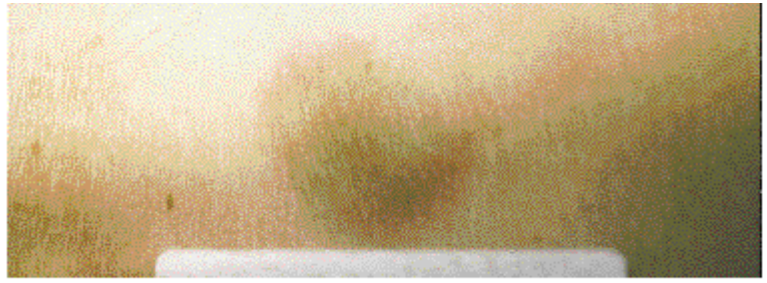
Chloromas



A

B

C



NEJM 1998

Leukostasis

- accumulation of blasts in microcirculation with impaired perfusion
- lungs: hypoxemia, pulmonary infiltrates
- CNS: stroke
- only seen with WBC >> 50 x 10^9/L

Constitutional symptoms

- fever and sweats common
- weight loss less common

Laboratory features

- WBC usually elevated, but can be normal or low
- blasts in peripheral blood
- normocytic anemia
- thrombocytopenia
- neutropenia
- DIC

Bone marrow in acute leukemia

- necessary for diagnosis
- useful for determining type
- useful for prognosis
- Acute leukemias are defined by the presence of > 20% blasts in bone marrow (% of nucleated marrow cells)
- > 30% blasts in bone marrow (% of nucleated marrow cells)FAB Classification

How do we apply WHO 2008?

Acute Leukemia

>20% blasts in peripheral blood or bone marrow

What are blasts?

Morphology

<u>Exceptions</u>

Small size

Granular blasts

Abnormal promyelocytes – in AMLM3

Promonocytes – in AMLM5

Cells which look like blasts

Hematogones, Erythroblasts, Regenerating cells, Lymphoma cells, etc

Common morphological problems

CLL vs ALL-L1

MCL vs ALL

Round cell tumor vs Burkitt's lymphoma

Evolution of leukemia classification

FAB Classification

Morphology, cytochemistry and immunophenotyping

WHO classification

It has classified myeloid and lymphoid malignancies according to cell of origin, based on morphologic, immunologic, genetic and clinical characteristics

FAB vs WHO

- Lack of immediate availability of genetic information is an obstacle to the utilization of the WHO classification
- Rapidly advancing technology of genetic analysis

Morphology + Cytochemistry

Stains(cytochemistry)

Giemsa

Myeloperoxidase (APML)

SBB

Non specific esterase(naphthyl –butyrate &naphthyl acetate)

Specific estrases

PAS

Blasts and cytochemistry

MPO+ (in AML)
M1, M2, M3 t (15;17), M4, M6

Only NSE+ (in AML)
M4, M5,

Both positive (only in AML)
M3, M4

Both negative
ALL,
AMLM0, M7
CMLBC, BPT

Acute leukemia - Diagnosis

- Investigations:
  - Complete blood counts
  - Peripheral Blood Smear (200 cell counts),
  - Bone Marrow Aspirate/ Imprint (500 cell counts)
  - BM Biopsy (paraffin block, CD34)

- **Cytochemical stains:**
  - MPO for myeloblasts, and
  - NSE for monoblasts

- Immunophenotyping by ***flow cytometry***

- Cytogenetics & Molecular Diagnostics

IMMUNOPHENOTYPING

FCM - MULTICOLOR IMMUNOPHENOTYPING

IMMUNOHISTOCHEMISTRY – MOSTLY SINGLE COLOR

CD markers - Acute Leukemia

All lymphoid cells	CD45+ (LCA)
B-cells	CD19, CD10, cCD22
T-cells	CD3, cCD3
Myeloid cells	CD13, CD33, CD117, anti MPO
Megakaryocytic	CD41, CD61
Blasts	CD34, Tdt, CD99
Others	HLA-DR

PANELS FOR ACUTE LEUKEMIA

A) PRIMARY PANEL:

B-CELLS - CD10, CD19

T-CELLS - CD3, CD7, *CD4, CD8*

MYELOID - CD13, CD33, CD117

NON-LINEAGE - HLADR, CD34

POSITIVE CONTROL: CD45 (LCA)

NEGATIVE CONTROL: ISOTYPE IGG1

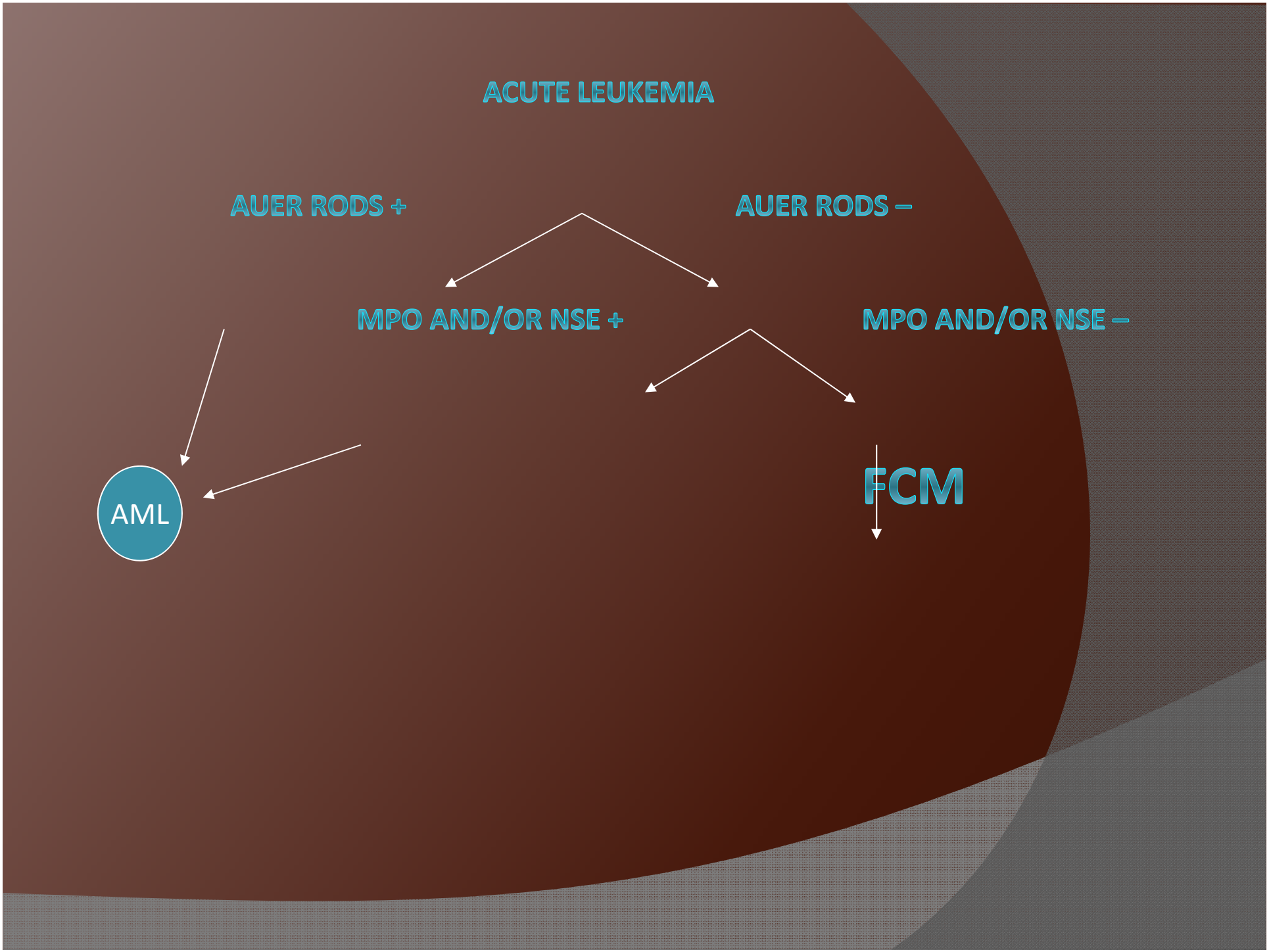
B) SECONDARY PANEL:

B-LINEAGE SPECIFIC - CYTOCD22 / CYTOCD79A

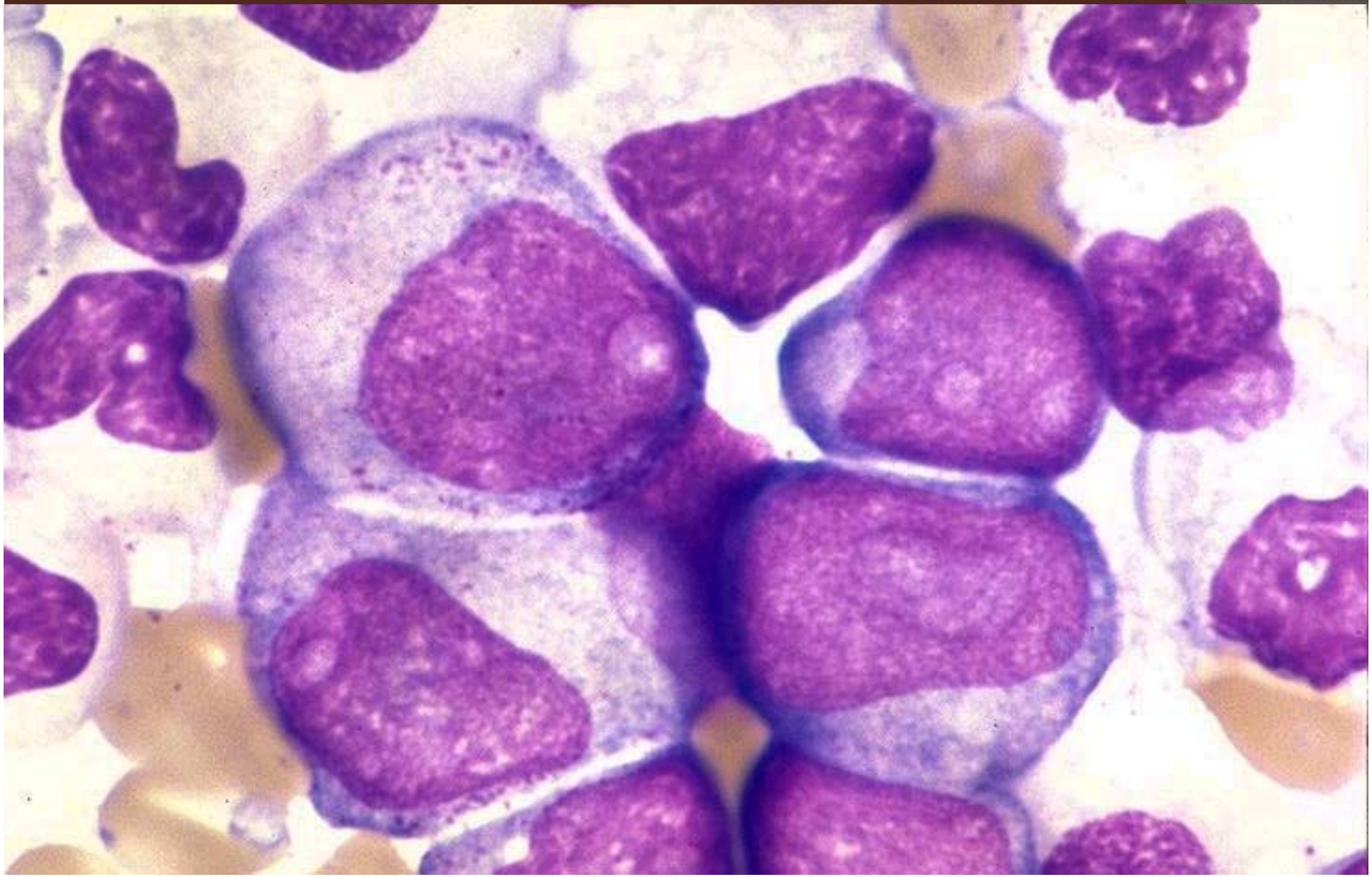
T-LINEAGE SPECIFIC - CYTOCD3

MYELOID LINEAGE SPECIFIC - ANTI-MPO

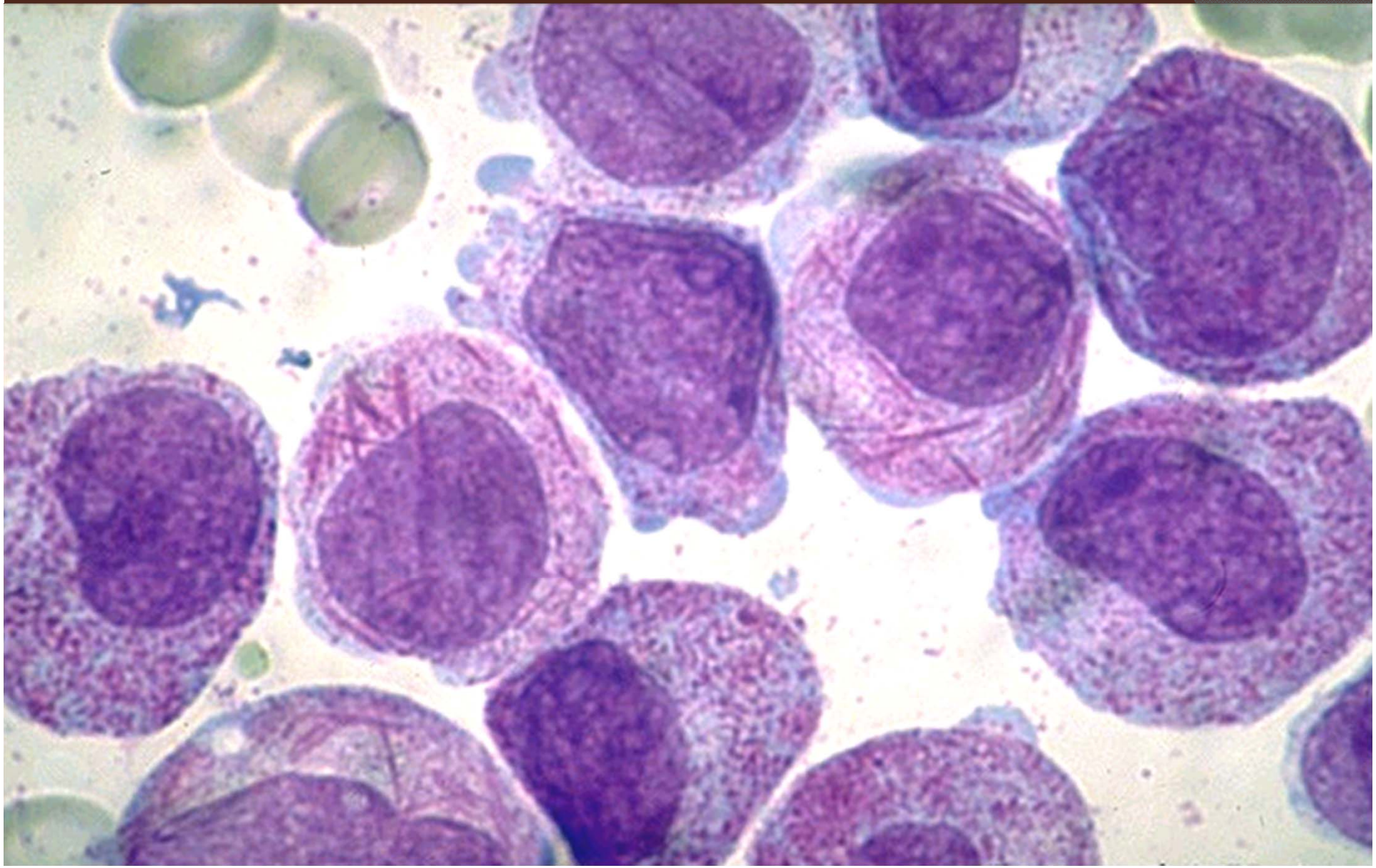
OTHER MARKERS – TDT, CD99, CD41, CD61, SMIG & CD56


ACUTE LEUKEMIA
AUER RODS +
AUER RODS –
MPO AND/OR NSE +
MPO AND/OR NSE –
AML
FCM

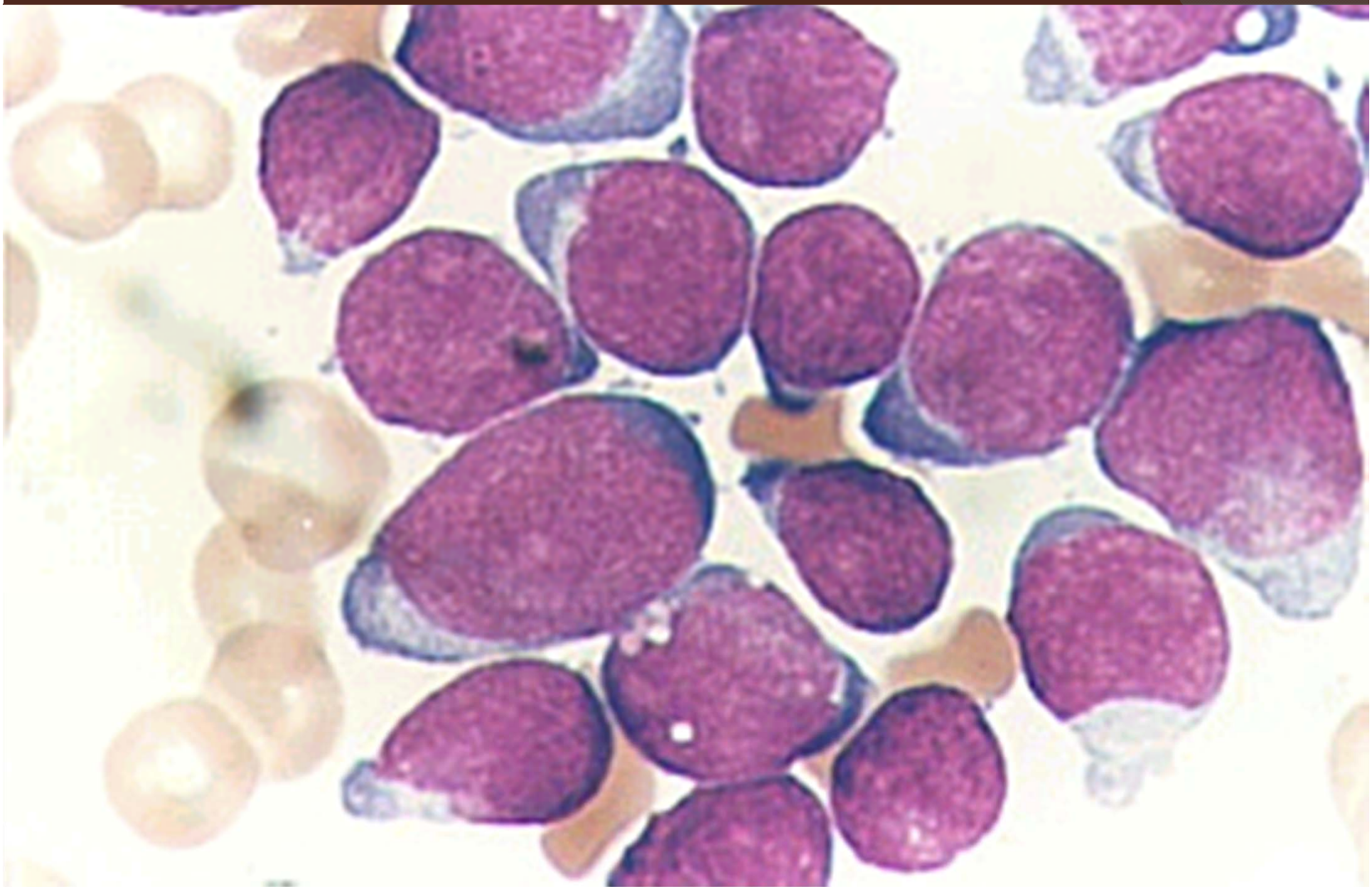
AML



Auer rods in AML



ALL



Treatment of acute leukemias

Choice of Rx is influenced by:

- type (AML vs ALL)
- age
- curative vs palliative intent

Principles of treatment

- combination chemotherapy
  - first goal is complete remission
  - further Rx to prevent relapse
- supportive medical care
  - transfusions, antibiotics, nutrition
- psychosocial support
  - patient and family

Chemotherapy for acute leukemias

- Phases of ALL treatment
  - induction
  - intensification
  - CNS prophylaxis
  - maintenance

  } post-remission therapy

- Phases of AML treatment
  - induction
  - consolidation (post-remission therapy)

Hematopoietic stem cell transplantation

- permits “rescue” from otherwise excessively toxic treatment
- additional advantage of graft-vs-leukemia effect in allogeneic transplants

Prognosis

Adult AML

Age	CR	DFS
< 60	75%	~ 30%
> 60	50%	5-15%

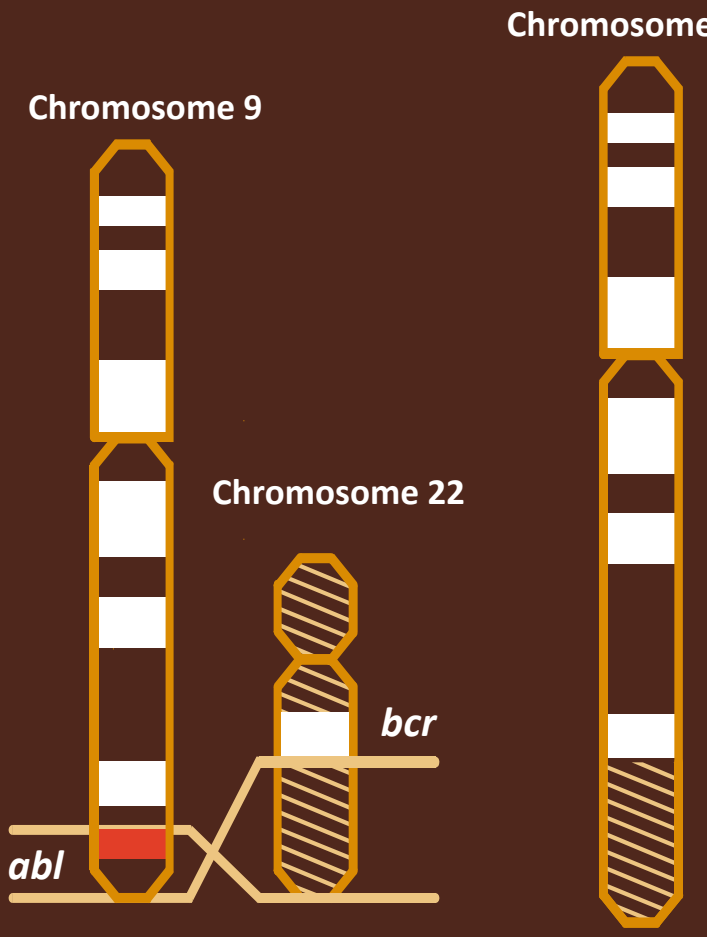
Adult ALL

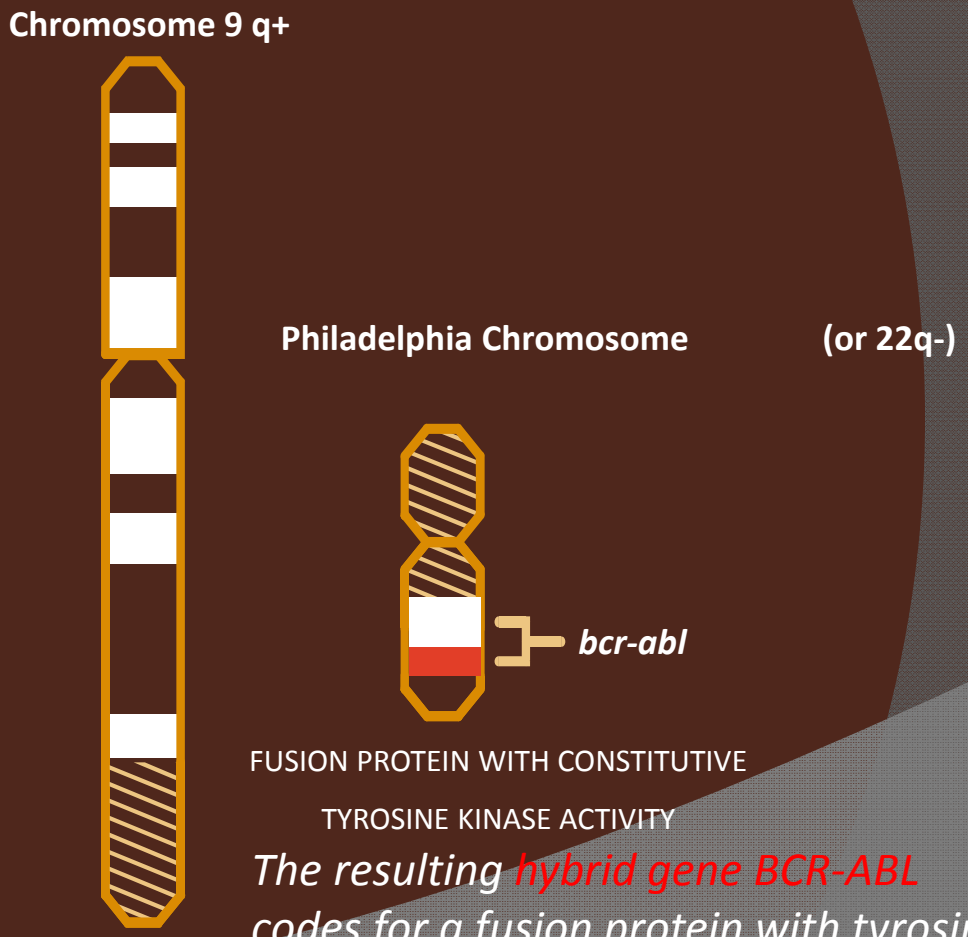
similar to or worse than AML

Chronic myelogenous leukemia (CML

- It is a type of myeloproliferative disease associated with a characteristic chromosomal translocation called the Philadelphia chromosome
- **CLONAL DISORDER**
- **Arises in a abnormal bone marrow stem cell**
- **A granulocyte precursor**
- increased and unregulated growth of predominantly myeloid cells in the bone marrow and the accumulation of these cells in the blood.

The Ph Chromosome and the *bcr-abl* Gene: The t(9;22) Translocation



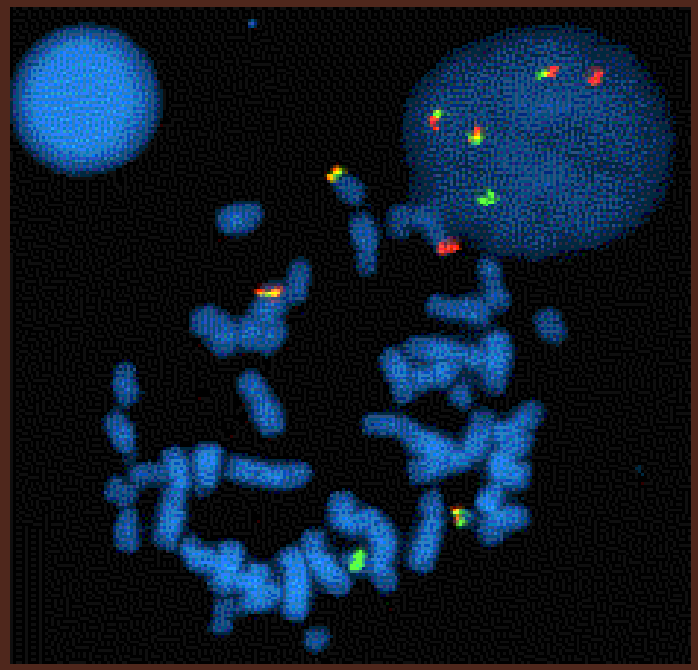


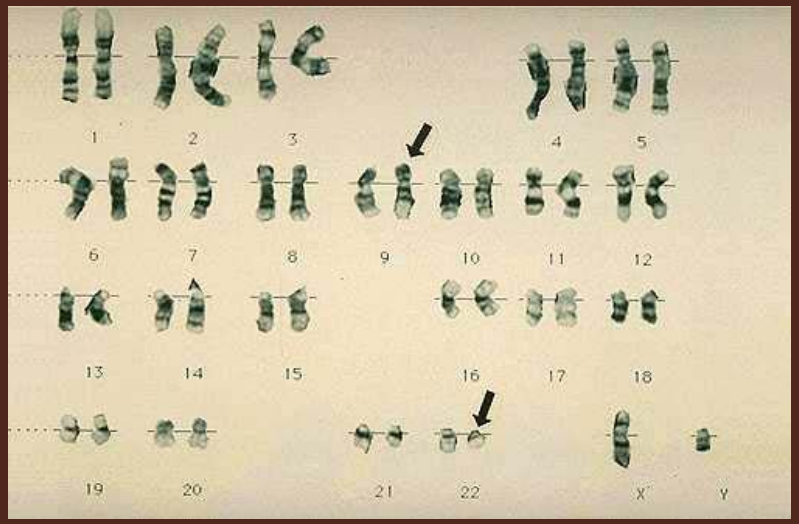
FUSION PROTEIN WITH CONSTITUTIVE TYROSINE KINASE ACTIVITY

The resulting hybrid gene BCR-ABL codes for a fusion protein with tyrosine kinase activity, which activates signal transduction pathways, leading to

CML

- Philadelphia chromosome
  - Translocation 9:22
    - BCR-ABL – tyrosine kinase
- In CML, the clone contains this translocation – proliferative advantage
- Myeloid series,endothelial cells,lymphoid cells
- Fusion gene product 210 KD protein
- ALL-180 KD

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Epidemiology

- CML occurs in all age groups, but most commonly in the middle-aged and elderly.
- Its annual incidence is 1–2 per 100,000 people
- slightly more men than women are affected.
- CML represents about 15–20% of all cases of adult leukemia in Western populations.

- The only well-described risk factor for CML is exposure to ionizing radiation; for example, increased rates of CML were seen in people exposed to the atomic bombings of Hiroshima and Nagasaki
- Known from 1981 or before that Benzene (a byproduct of the use of laser printers and copy machines) caused the mutation leading to CML in humans

Symptoms and Diagnosis

- CML can be discovered in a routine physical & LAB.examination
- 70% of those diagnosed with CML had symptoms including: fatigue, abdominal discomfort (**splenomegaly**), weight loss, and sweating ANAEMIA
- **Lymphadenopathy : uncommon**
- **Hepatomegally : variable**
- **Signs of leukemic infilterations**

Lab. findings

- anemia
- WBCs : Total count: may be very high: 200 – 400,000/µl
- Differential count: predominantely myelocytes, some mature granuulocytes, Myeloblastes (1- 5%) if more __ > acute exacerbation
- Basophils, Eosinophils are increased. platelets: very early may be rise, late decrease

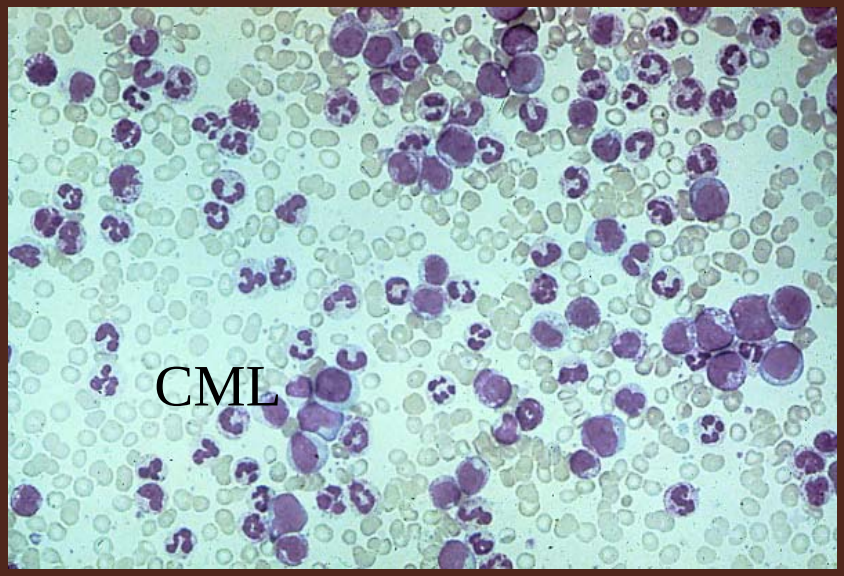
Leucocyte alkaline phosphatase: (LAP) score (20-140)

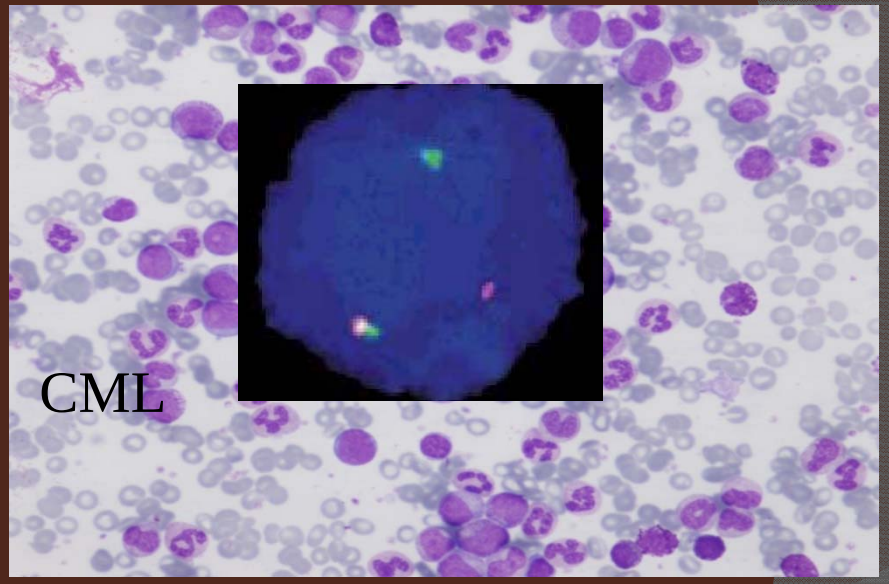
- Low/-nt-CML
- HIGH-?

BM Aspiration& trephine:

- complete replacement of fat-hyper cellular
- by cellular elements mostly granulocytes, but few blasts
- MEGAKARYOCYTIC hyperplasia including micromegakaryocytes
- Erythroid - Normal
- MARROW fibrosis

Ultimately, CML is diagnosed by detecting the Philadelphia chromosome. This characteristic chromosomal abnormality can be detected by routine cytogenetics, by fluorescent in situ hybridization, or by PCR for the bcr-abl fusion gene


CML


CML

Classification

- CML is often divided into three phases based on clinical characteristics and laboratory findings.
- CML typically begins in the *chronic* phase, and over the course of several years progresses to an *accelerated* phase and ultimately to a *blast crisis*.

Chronic phase

- Approximately 85% of patients with CML are in the chronic phase at the time of diagnosis.
- The duration of chronic phase is variable and depends on how early the disease was diagnosed as well as the therapies used.
- Ultimately, in the absence of curative treatment, the disease progresses to an accelerated phase.

- Blast crisis is the terminal phase of CML and clinically behaves like an acute leukemia.
- One of the drivers of the progression from chronic phase through acceleration and blast crisis **is the acquisition of new chromosomal abnormalities (in addition to the Philadelphia chromosome).**
- Some patients may already be in the accelerated phase or blast crisis by the time they are diagnosed.

Accelerated phase

- Criteria for diagnosing transition into the accelerated phase are somewhat variable; the most widely used criteria are those put forward by investigators at World Health Organization.
- *10–19% myeloblasts in the blood or bone marrow
- *>20% basophils in the blood or bone marrow
- *Platelet count <100,000, unrelated to therapy
- *Platelet count >1,000,000, unresponsive to therapy

Accelerated phase

- Persistent or increase in splenomegaly unresponsive to therapy.
- Clonal cytogenetic evolution with new abnormalities in addition to the Philadelphia chromosome

The patient is considered to be in the accelerated phase if any of the above are present. **The accelerated phase is significant because it signals that the disease is progressing and transformation to blast crisis is imminent**

Blast crisis

- Blast crisis is the final phase in the evolution of CML, and behaves like an acute leukemia, with rapid progression and short survival. Blast crisis is diagnosed if any of the following are present in a patient with CML:
- *>20% myeloblasts or lymphoblasts in the blood or bone marrow
- *Large clusters of blasts in the bone marrow on biopsy
- *Development of a chloroma (solid focus of leukemia outside the bone marrow)

Clinical Course: Phases of CML

Chronic phase	Advanced phases	
	Accelerated phase	Blast crisis
Median 5–6 years stabilization	Median duration 6–9 months	Median survival 3–6 months

Treatment

- Chronic phase CML is treated with inhibitors of tyrosine kinase, the first of which was imatinib mesylate (marketed as Gleevec or Glivec.
- In the past, antimetabolites (e.g. cytarabine, hydroxyurea), alkylating agents, interferon alfa 2b, and steroids were used, but these drugs have been replaced by imatinib.

- Imatinib was approved by the United States FDA in 2001 and specifically targets BCR/abl, the constitutively activated tyrosine kinase fusion protein caused by the Philadelphia chromosome translocation.

- Stem cell transplantation

- CLL

Chronic lymphocytic leukemia

Chronic lymphocytic leukemia (1)

- Is characterised by the accumulation of nonproliferating mature-appearing lymphocytes in the blood, marrow, lymph nodes, and spleen
- In most cases, the cells are monoclonal B lymphocytes that are CD5+
- T cell CLL can occur rarely

CLL-Chronic Lymphocytic Leukemia

- CLL most common leukemia
- it accounts for 30% of leukemias
- CLL is 2 X as common as CML
- incidence increases with age, 65 avg, rare in people under 35
- males more common- 2X compared to women
- equal blacks/whites

Etiology

- Heredity- 2-7x increased risk if 1st relative has CLL
  - Most notable familial clustering of all leukemias
- Immunodeficiency syndromes and viruses
- There is increased incidence in farmers, rubber manufacturing workers, asbestos workers, and tire repair workers
- no conclusive link with radiation exposure

Etiology

- Cytogenetics
  - clonal chromosomal abnormalities are detected in approximately 50% of CLL patients
  - the most common clonal abnormalities are:
    - trisomy 12
    - structural abnormalities of chromosomes 13, 14 and 11,17

  patients with abnormal karyotypes have a worse prognosis
- Oncogenes
  - Unmutated igvH BAD PROGNOSIS
  - MUTATED----------GOOD PROGNOSIS

Clinical findings

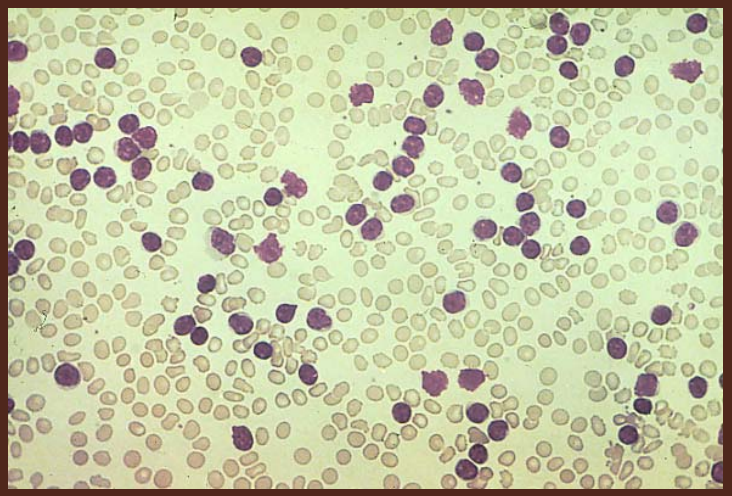
- Approximately 40% of CLL patients are asymptomatic at diagnosis

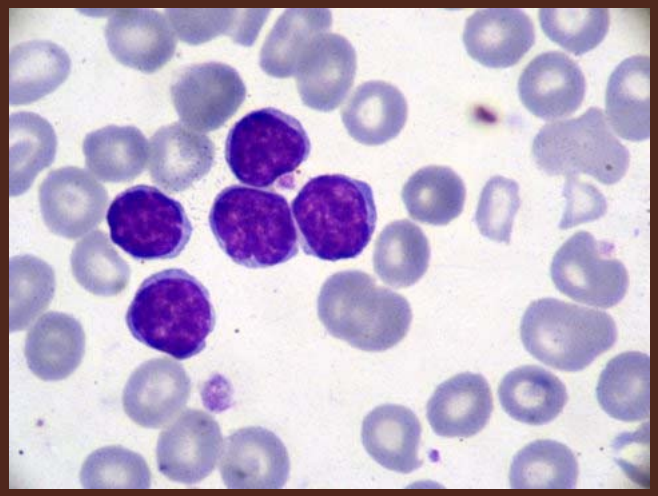
Clinical findings

- Most symptomatic patients have **enlarged lymph nodes** (more commonly cervical and supraclavicular) and **splenomegaly**
- The lymph nodes are usually discrete, freely movable, and nontender
- Hepatomegaly may occure
- Less common manifestation are infiltration of tonsils, mesenteric or retroperitoneal lymphadenopathy, and skin infiltration
- Patients rarely present with features of anemia, and bruising or bleeding

Laboratory findings

- Leucocytosis
- Absolute lymphocyte count above 5,0 00/µl
- In most patients the leukemic cells have the morphologic appearance of normal small lymphocytes
- In the blood smears are commonly seen ruptured lymphocytes (“basket” or “smudge” cells)
- Careful examination of the blood smear can usually differentiate CLL, and the diagnosis can be confirmed by **immunophenotyping**





Laboratory findings

- Clonal expansion of B (99%) or T(1%) lymphocyte
  - In B-cell CLL clonality is confirmed by
    - the expression of either κ or λ light chains on the cell surface membrane
    - the presence of unique idiotypic specificities on the immunoglobulins produced by CLL cells
    - by immunoglobulin gene rearrangements
    - typical B-cell CLL are unique in being CD19+ and CD5+
- Hypogammaglobulinemia or agammaglobulinemia are often observed

- 10 - 25% of patients with CLL develop autoimmune hemolytic anemia, with a positive direct Coombs' test-**Evan syndrome**
- The marrow aspirates shows greater than 30% of the nucleated cells as being lymphoid

The diagnostic criteria for CLL

1) A peripheral blood lymphocyte count of greater than 5,0 00/µl
2) The cell should have the presence of Bcell-specific differentiation antigens (CD19, CD20, and CD23) and be CD5(+),dim surface expression of IgM/D CD10 NEGATIVE
3) A bone marrow aspirates showing greater than 30% lymphocytes

Differential diagnosis

- Infectious causes
  - bacterial (tuberculosis)
  - viral (mononucleosis)
- Malignant causes
  - B-cell
  - T-cell
    - leukemic phase of non-Hodgkin lymphomas
    - Hairy-cell leukemia
    - Waldenstrom macroglobulinemia
    - large granular lymphocytic leukemia

- If available immunophenotyping should be carried out to confirm the diagnosis
- Bone marrow biopsy and cytogenetic analysis is not routinely performed in CLL

Staging

- Rai Classification for CLL
  - 0 - lymphocytosis (>5 ooo/µl)
  - I - lymphocytosis + lymphadenopathy
  - II - lymphocytosis + splenomegaly +/-lymphadenopathy
  - III - lymphocytosis + anemia (Hb <11g%) +/-lymphadenopathy or splenomegaly
  - IV - lymphocytosis + thrombocytophenia (Plt <1 lac/µl) +/- anemia +/-lymphadenopathy +/- splenomegaly

Staging (2)

- Binet Classification for CLL
  - A. < 3 involved areas, Hb > 10g%, Plt >1lac/µl
  - B. > 3 involved areas, Hb > 10g%, Plt > 1lac/µl
  - C. - any number of involved areas, Hb < 10g%, Plt < 1 lac/µl

Prognosis

- Rai classification

stage	median survival (years)
0	>10
I	> 8
II	6
III	2
IV	< 2

- Binet classification

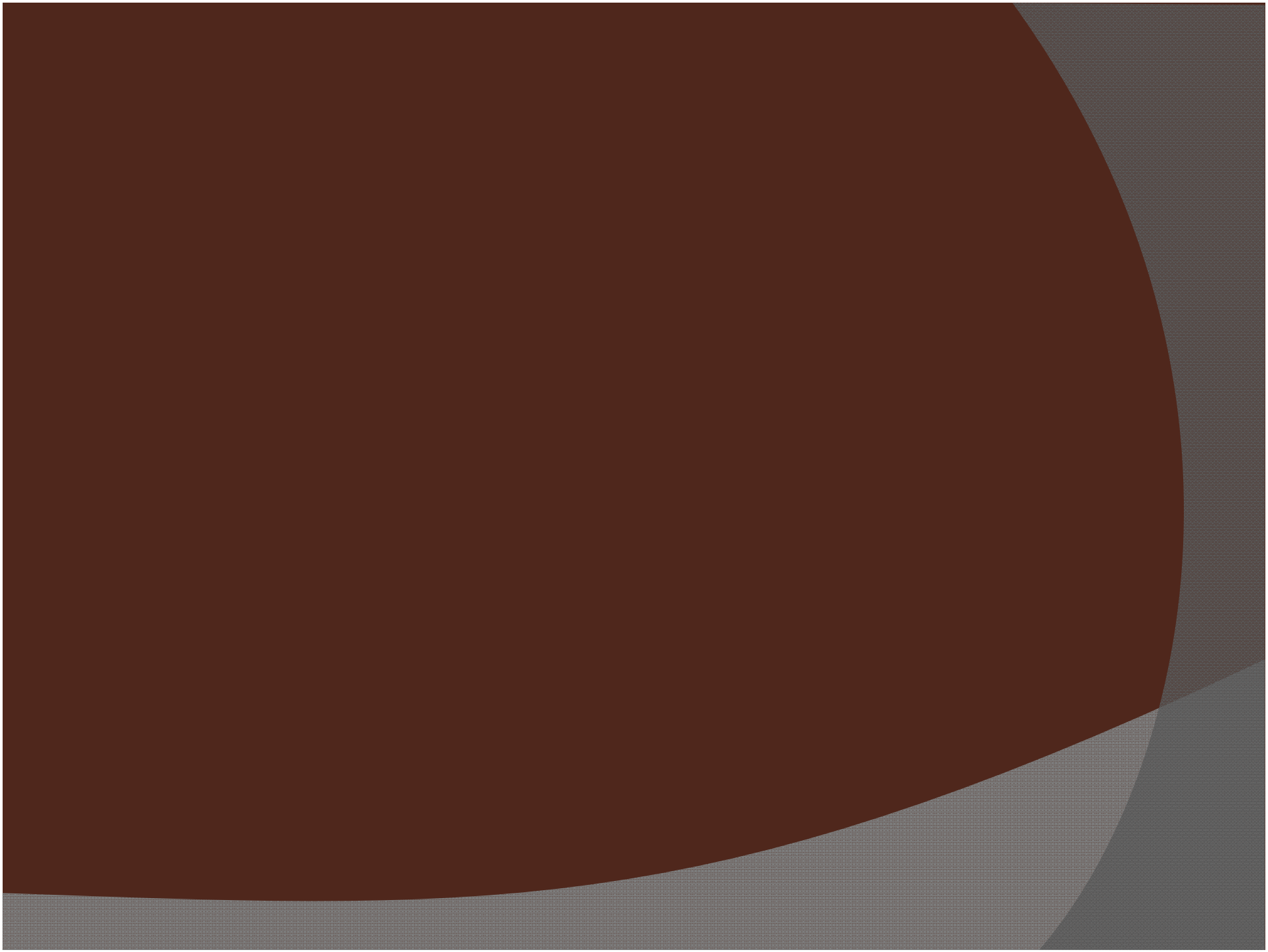
stage	median survival (years)
A	> 10
B	7
C	2

Markers of poor prognosis in CLL

- Advanced Rai or Binet stage
- Peripheral lymphocyte doubling time <12 months
- Diffuse marrow histology
- Increased number of prolymphocytes or cleaved cells
- Poor response to chemotherapy
- High β2- microglobulin level
- Abnormal karyotyping
- ZAP-70 mutation

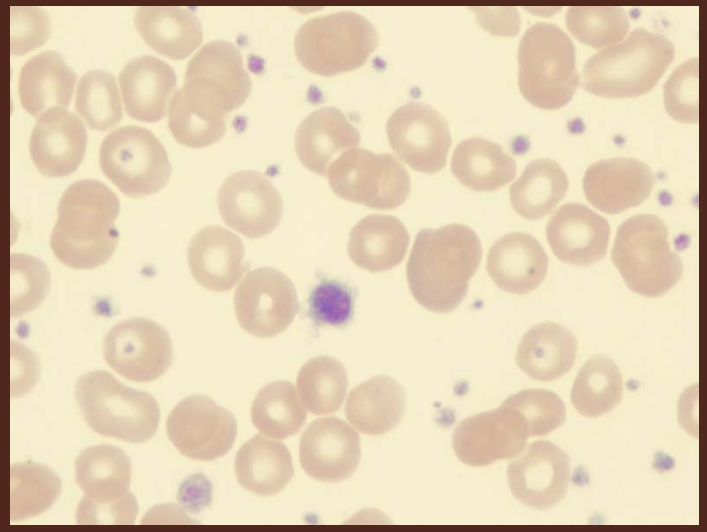
Treatment

- Treatment is reserved for patients **with low- or intermediate risk disease who are symptomatic or have progressive disease (increasing organomegaly or lymphocyte doubling time of less than 12 months) and patients with high -risk disease**
  - Alkylating agents (chlorambucil, cyclophosphamide)
  - Nucleoside analogs (cladribine, fludarabine)
  - Biological response modifiers
  - Monoclonal antibodies
  - Bone marrow transplantation
  - And systemic complications requiring therapy
    - antibiotics
    - immunoglobulin
    - steroids
    - blood products



ET

- MPD



- Plts >4.5 lac
- JAK2 mutation in 40-45% of cases

***Must r/o reactive thrombocytosis (fe deficiency, cancer, chronic inflammatory disorders, acute bleeding)

S/S

…

Neurologic:

- …Headache
- …TIA

…Thrombosis:

- …Coronary
- …Renal
- …Portal
- …DVT, Pul

…Bleeding:

- …GI
- …Skin
- …Eye
- …Brain
- …Urinary tract

Splenomegaly-uncommon

REACTIVE THROMBOCYTOSIS

… Infection

… Malignancy

… Autoimmune diseases

… Postsplenectomy

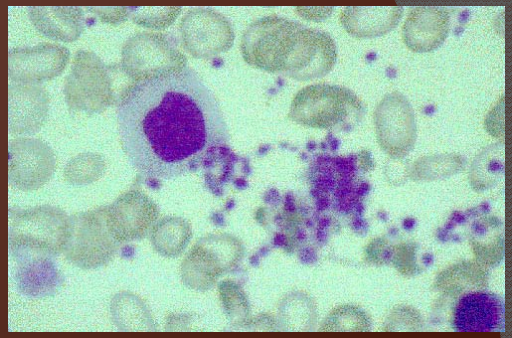
… Trauma

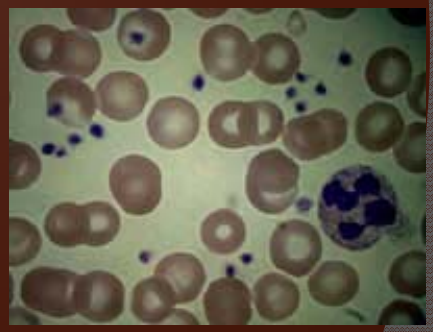
… Rebound thrombocytosis

… Anemia

… Hemorrhage

Drug(Vincristine,steroid), etc





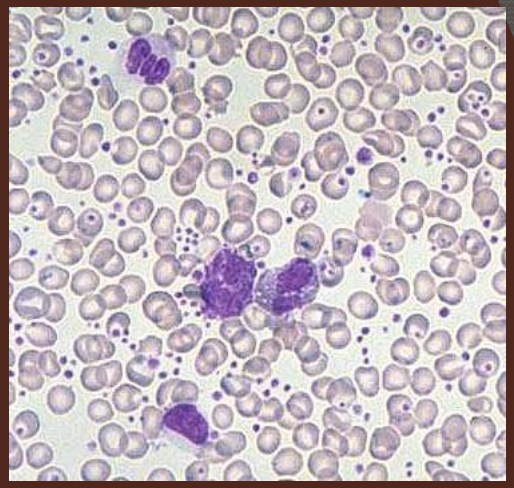
Diagnosis…Essential thrombocythemia

… Blood Smear

…Myelocyte

…Metamyelocyte

… increased Thrombocytes

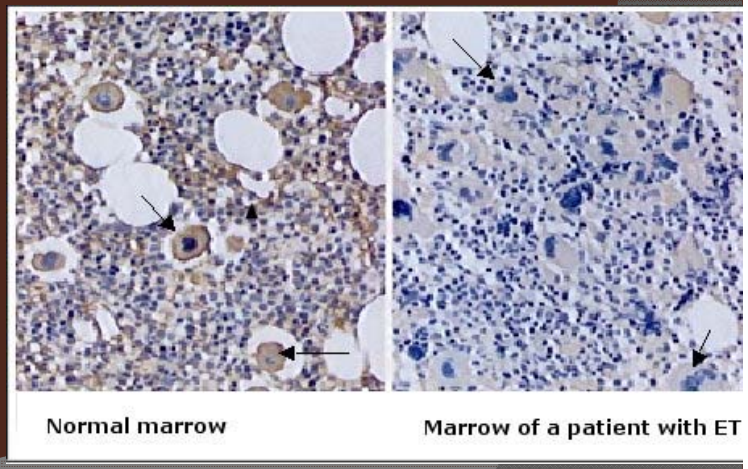


… Bone Marrow aspirate/biopsy

…hyperlobated megakaryocyte

… Hypercellularity

… Hyperplasia



Primary Myelofibrosis (PMF)

- Chronic Idiopathic Myelofibrosis
- Idiopathic Myelofibrosis
- Agnogenic Myeloid Metaplasia
- Myelosclerosis with Myeloid Metaplasia

- Myelofibrosis is a chronic myeloproliferative disease with clonal hematopoesis and secondary(non-clonal) hyperproliferation of fibroblasts
- (stimulated by PDGF, EGF, TGF-β released from myeloid cells, mainly from neoplastic megakaryocytes)
- increased collagen synthesis. It produces bone marrow fibrosis
- extramedullary hematopoesis in the spleen or in multiple organs.

MYELOFIBROSIS

- The incidence of Myelofibrosis is about 0.5/10,000.
- The median age at diagnosis was approximately 65 years.
- **Common complaints**:

  fatigue, weight loss, night sweats, bone pain, abdominal pain, fever
- **Physical findings:**

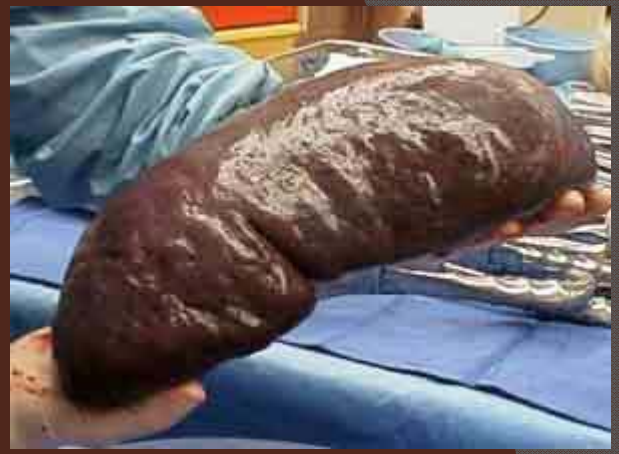
  splenomegaly (often giant), hepatomegaly(in about 50% of patients), symptoms of anaemia and thrombocytopenia

PMF

Splenomegaly

- Hallmark of PMF
  - Often marked, in two case series, 1/3 patients had splenomegaly to >10+ cm below costal margin and 1/4 had 16+ cm

Extramedullary Hematopoeisis results often in marked hepatosplenomegaly . EMH may occur in any organ pleural, pericardial, abdominal effusions, GI/GU tracts, may involve CNS – increased ICP, cord compression



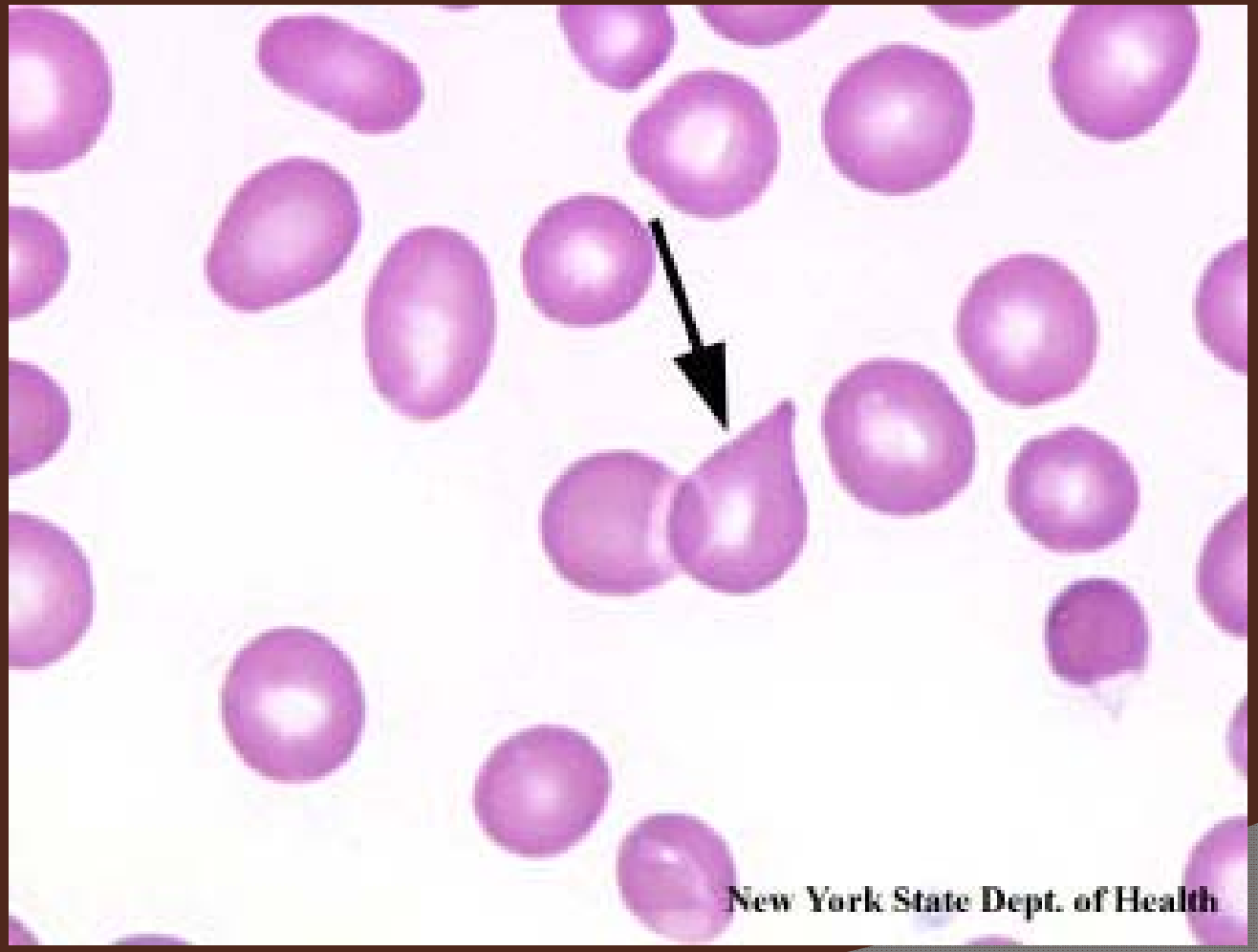
MYELOFIBROSIS
- laboratory findings

- Anemia - Hb<10g/dL in 60% of patients
- Leukocytosis with counts generally below 50000/µl(in about 50%), leukopenia (in about 25% at the time of diagnosis)
- thrombocytosis in 50% at the time of diagnosis, with disease progression thrombocytopenia becomes common
- eosinophilia and basophilia may be present
- reticulocytosis
- LAP score is usually elevated
- Increased level of lactate dehydrogenase
- uric acid level is increased in most patients

MYELOFIBROSIS
- laboratory findings(2)

- **Peripheral blood smear:**anisocytosis and poikilocytosis with the presence of teardrop-shaped and nucleated red cells, immature neutrophils but myeloblasts not always
- **Aspiration of bone marrow** is usually ansuccessful (dry tap).

  Smears from successful aspirates usually show neutrophilic and megakaryocytic hyperplasia
- **Trephine biopsy** often shows a hypercellular marrow with increased reticulin fibers and variable collagen deposition . Increased numbers of dysplastic megakaryocytes are frequently seen.

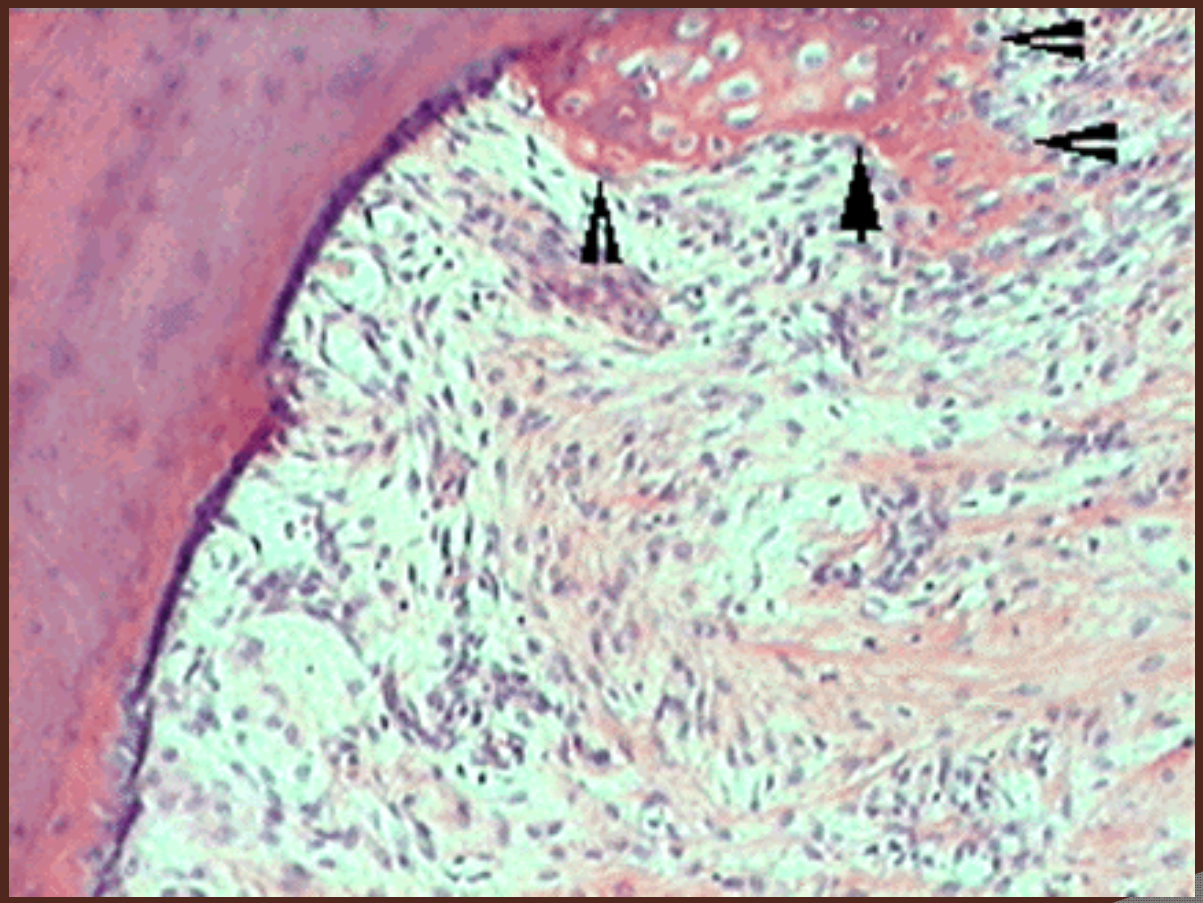

New York State Dept. of Health

Cytogenetics

- JAK2 mutation in 40%

PMF – Bone Marrow

- Up to 50% of bone marrow biopsy attempts will yield “dry tap” fibrosis
- Bone marrow biopsy is essential
  - initially hypercellular with large, dysplastic, clustered megakaryocytes and excess granulocytes; increased reticulin is present around clusters of megakaryocytes;
  - intermediate phase has alternating areas of hematopoiesis and fibrosis;
  - terminal phase is hypocellular and diffusely fibrotic with atypical megakaryocytes;
  - Ultimately marrow may be converted to bone (osteosclerosis)



Diagnosis:

- **Proposed revised WHO criteria for primary myelofibrosis**

- **Major criteria**
- Presence of megakaryocyte proliferation and atypia, usually accompanied by either reticulin and/or collagen fibrosis, or, in the absence of significant reticulin fibrosis, the megakaryocyte changes must be accompanied by an increased bone marrow cellularity characterized by granulocytic proliferation and often decreased erythropoiesis (ie, prefibrotic cellular-phase disease)
- Not meeting WHO criteria for PV , CML , MDS , or other myeloid neoplasm
- Demonstration of JAK2 617V>F or other clonal marker (eg, MPL 515W>L/K), or in the absence of a clonal marker, no evidence of bone marrow fibrosis due to underlying inflammatory or other neoplastic diseases
- **Minor criteria**
- Leukoerythroblastosis
- Increase in serum lactate dehydrogenase level
- Anemia
- Palpable splenomegaly

Diagnosis requires meeting all 3 major criteria and 2 minor criteria.

PMF vs Other

- PMF must be distinguished from the other MPD as well as the MDS
  - presence of t(9:22) usu results in dx of CML
  - dyserythropoiesis (dysplastic bone marrow, associated with variable degrees of peripheral blood cytopenia with or without monocytosis) suggests MDS, especially in absence of splenomegaly
  - the Ph- MPD are PV and ET. PV identified with increased red cell mass and PMF vs ET dep't largely on degree of fibrosis and splenomegaly.

MYELOFIBROSIS - therapy

1. **Androgens**(oxymetholone in anemia from decreased red cell production -overall response is about 40%
2. **Corticosteroids**(prednisone in anemia with shortened red cell life-span-response in 25-50% of patients
3. **Hydroxsyurea** for the control of leukocytosis, thrombocytosis, or organomegaly
4. **Allopurinol-**to prevent hyperuricaemia
5. **Transfusions of packed red cells** for anemia or **platelets** for thrombocytopenia with bleeding

MYELOFIBROSIS - therapy

6. **Splenectomy should be considered for:** portal hypertension, painful splenomegaly, refractory anemia and thrombocytopenia, or excessive transfusion requirement. However,the procedere is hazardous (an operative mortality is up to 38%).
7. **Splenic irradiation:** when there is a contrindication to splenectomy
8. **Allogeneic stem-cell transplantation:** for young patients who have a poor prognosis and have a suitable donor identified.
9. **Experimental therapies:** Interferon-α, antifibrotic and antiangiogenic drugs (anagrelide, suramin, pirfenidone, thalidomide,)

MYELOFIBROSIS

- prognosis

- a median survival of 3.5 to 5.5 years
- the principal causes of death are infections, thrombohemorrhagic events, heart failure, and leukemic transformation
- leukemic transformation occurs in approximately 20% of patients during first 10 years

- Myelodysplastic Syndromes (MDS)

Myelodysplastic Syndromes (MDS)

- MDS – characterized by:
  - a dysplastic marrow (hyper or occasionally hypoplastic)
  - variable degrees of peripheral cytopenias
  - may demonstrate monocytosis

- *somewhat of a paradox – usu hyperplastic marrow but with peripheral cytopenias – thought increased intramedullary apoptosis

MDS

- Arise from clonal disorders of hematopoietic stem cells that occur predominantly in patients >50 and are characterized by ineffective hematopoiesis and peripheral cytopenias
- Patients are usually initially evaluated _ transfusion dependent anemia and frequent infections

Myelodysplastic syndromes

FAB classification system

- **Refractory anemia (RA): cytopenia of one PB lineage; normo- or hypercellular marrow with dysplasias; < 1% PB blasts and <5% BM blasts**
- **Refractory anemia with ringed sideroblasts (RARS): cytopenia, dysplasia and the same % blasts involvement in BM and PB as RA. Ringed sideroblasts account for > 15% of nucleated cells in marrow.**
- **Refractory anemia with excess of blasts (REAEB): Cytopenia or two or more PB lineages; dysplasia involving all 3 lineages; < 5% PB blasts and 5-19% BM blasts**

- **Refractory anemia with excess blasts in transformation: (REAEB-t): hematologic features identical to RAEB. >5% blasts in PB or 20-29% blasts in BM, or the presence of Auer rods in the blasts**
- **Chronic myelomonocytic leukemia (CMML):monocytosis in PB>10^9/L; < 5% blast in PB and < 20% BM blasts**

WHO of MDS

- RA – refractory anemia
- RARS – refractory anemia with ringed sideroblasts
- RCMD – refractory cytopenia with multilineage dysplasia
- RCMD-RS – RCMD with ringed sideroblasts
- RAEB 1/2 - refractory anemia with excess blasts
- MDS Unclassified
- MDS assoc with del (5q)

RA – refractory anemia	Anaemia	Erythroid dysplasia only
RARS – refractory anemia with ringed sideroblasts	Anaemia	Erythroid dysplasia only >15% ring siderblasts
RCMD – refractory cytopenia with multilineage dysplasia	Bi/pancytopenia	Dysplasia in>10% cells of2 > cell lineages
RCMD-RS – RCMD with ringed sideroblasts	Bi/pancytopenia	Dysplasia in>10% cells of2 > cell lineages >15% ring siderblasts

RAEB 1/2 - refractory anemia with excess blasts	Bi/pancytopenia <5% blasts 5-19 blasts	Uni/multilineage dysplasia 5-9%blasts uni/multilineage dysplasia 10-19%biasts
MDS Unclassifie	Bi/pancytopenia	Myeloid/Megakaro. dysplasia
MDS assoc with del (5q	Anaemia,increased platelets	Megakaro. Increased,hypolobated nuclei

Myelodysplastic features in MDS

MDS	Bone marrow and/or peripheral blood findings
Dyserythropoiesis	Bone marrow: multinuclearity, nuclear fragments, megaloblastoid changes, cytoplasmic abnormalities, ringed sideroblasts Peripheral blood: Poikilocytosis, anisocytosis, nucleated red blood cells

Myelodysplastic features in MDS

MDS	Bone marrow and/or peripheral blood findings
Dysgranulopoiesis	Nuclear abnormalities including: hypolobulation, ring-shaped nuclei, hypogranulation
Dysmegakariopoiesis	Micromegakariocytes Large mononuclear forms Multiple small nuclei

Bone marrow biopsy

- Blood examination and bone marrow aspirate are sufficient for a diagnosis of MDS
- It is obviously important in cases of difficult diagnosis , and it could brink additional prognostic information in some cases
- normal or increased cellularity is seen in 85-90% od cases
- abnormal localization of immature precusors (ALIP)
- Fibrosis (significant in 15-20% of cases)

Diagnosis of MDS

- Aplastic anaemia and some disease accompanied by marrow dysplasia, including wit. B_{12} and/or folate deficiency, exposure to haevy metals, recent cytotoxic therapy and ongoing inflamation (including HIV and chronic liver disease/alcohol use) should be ruled out

cytogenetics

- **Good prognosis**

(–Y, $5q^-$, $20q^-$)

- **Intermediate prognosis**

(+8, miscellaneous singleabnormality, double abnormalities)

- **Poor prognosis**

(abnor. 7, complex- >3 abnor.)

MDS

- Median survival ranges from months to >5 years.
  - Those with few blasts (in bone marrow) and only anemia have longest median survival
  - May progress to acute leukemia (minority of patients)
  - Patients with >20% blasts are treated like acute leukemia although outcomes are worse
- Treatment is often supportive, ie transfusions, treatment of infections and epo/gcsf to decrease transfusion requirements

Diagnostic Criteria for Chronic Myelomonocytic Leukemia According to the World Health Organization
Persistent peripheral blood monocytosis is >1 × 10^9/L.
Absence of Philadelphia chromosome or BCR/ABL rearrangement.
Blasts[a] are <20% in peripheral blood or bone marrow.
Dysplasia in one or more myeloid lineages, or, in the absence of dysplasia, CMML can be diagnosed, if all other criteria are met, together with either of the following: Presence of a clonal cytogenetic abnormality. Monocytosis has been persistent for at least 3 months and all other causes of monocytosis have been excluded.
Subgroups CMML-1: Blasts <5% in peripheral blood and <10% in bone marrow. CMML-2: Blasts 5–19% in peripheral blood, 10–19% in bone marrow, or Auer rods are present and blasts are <20% in peripheral blood or bone marrow. CMML-1 or CMML-2 with eosinophilia: Criteria for CMML-1 and CMML-2 are present, and the eosinophil count in peripheral blood is >1.5 × 10^9/L.
CMML, chronic myelomonocytic leukemia. [a]Blasts include myeloblasts, monoblasts, and promonocytes