Acute myeloid Leukemia

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Definition

Acute myeloid leukemia (AML) *develops as the consequence of a series of genetic changes in a hematopoietic precursor cell. These changes alter normal hematopoietic growth and differentiation,* Clonal cells proliferation, resulting in an accumulation of large numbers of abnormal, immature myeloid cells in the bone marrow and peripheral blood. These cells are capable of dividing and proliferating, but cannot differentiate into mature hematopoietic cells (ie, neutrophils).

Pathophysiology

maturational arrest of bone marrow cells in the earliest stages of development.

Congenital disorders

-Bloom syndrome, Down syndrome, congenital neutropenia, Fanconi anemia, and neurofibromatosis.

Usually, these patients develop AML during childhood

-Polymorphisms of NAD(P)H:quinone oxidoreductase (NQO1), an enzyme that metabolizes benzene derivatives, are associated with an increased risk of AML.

Increased risk exists for AML that occurs after chemotherapy for another disease or for de novo AML with an abnormality of chromosomes 5, 7, or both.

Polymorphisms in glutathione *S*-transferase are associated with secondary AML after chemotherapy for other malignancies.

FAMILIAL SYNDROMES

-Germline mutations in the gene *AML1* (*RUNX1, CBFA2*) .

- Mutation of *CEBPA*

Environmental exposures

-Radiation exposure, patients receiving therapeutic irradiation for ankylosing spondylitis were at increased risk of leukemia.

-Survivors of the atomic bomb explosions in Japan.

-Exposure to benzene is associated with aplastic anemia and pancytopenia. These patients often develop AML. Many of these patients have the erythroleukemia subtype of AML (AML-M6).

Previous exposure to chemotherapeutic agents

- Patients with previous exposure to chemotherapeutic agents can be divided into 2 groups:
- (1) those with previous exposure to alkylating agents
- (2) those with exposure to topoisomerase-II inhibitors.
- Patients with a previous exposure to alkylating agents, with or without radiation, often have a myelodysplastic phase before the development of AML.
- Cytogenetics testing frequently reveals -5 and/or -7 (5q- or monosomy 7).
- Patients with a previous exposure to topoisomerase-II inhibitors do not have a myelodysplastic phase. Cytogenetics testing reveals a translocation that involves band 11q23. Less commonly, these patients develop leukemia with other balanced translocations, such as inversion 16 or t(15;17).

Diagnosis

- **Clinical: Signs and Symptoms**
- AML often manifests with clinical sequelae attributable to pancytopenia.
- -Patient complaints of weakness, fatigue, or dyspnea on exertion. Pallor is a common finding on physical examination.
- -Infection can result from insufficient numbers of white cells or impaired white blood cell function.
- -Collections of leukemic cells, seen in leukemia cutis, granulocytic sarcomas, or chloromas, can also occur. These collections represent extramedullary sites of disease and often involve cutaneous and visceral tissues.
- -Low numbers of platelets can lead to petechiae, gingival bleeding, ecchymosis, epistaxis, or menorrhagia.
- -Palpable lymphadenopathy and hepatosplenomegaly are rare findings in AML.

Diagnosis

- The diagnosis of AML requires the identification of 20% or more leukemic blasts in the bone marrow.
- Further analysis then must separate AML from acute lymphoblastic leukemia by showing evidence for commitment to the myeloid lineage.
- Immunohistochemical staining for myeloperoxidase is the best method for determining which cells are committed to the myeloid lineage.
- The leukemic clone giving rise to AML can occur at any point in the differentiation of the myeloid cell, creating heterogeneity among patients. Flow cytometry and cytogenetics are then used to differentiate the various AML subtypes.

FAB subtype	Name
M0	Undifferentiated acute myeloblastic leukemia
M1	Acute myeloblastic leukemia with minimal maturation
M2	Acute myeloblastic leukemia with maturation
M3	Acute promyelocytic leukemia (APL)
M4	Acute myelomonocytic leukemia
M4	Acute myelomonocytic leukemia with eosinophilia
M5	Acute monocytic leukemia
M6	Acute erythroid leukemia
M7	Acute megakaryoblastic leukemia

FAB Classification of AML

- M0 undifferentiated acute myeloblastic leukemia (5%)
- M1 AML with minimal maturation (20%)
- M2 AML with maturation (30%)
  - t(8;21)
- M3 Acute promyelocytic leukemia (5%)
  - t(15;17)
- M4 Acute myelomonocytic leukemia (20%)
- M4 eos Acute myelomonocytic leukemia with eosinophilia (5%)
  - inv (16)
- M5 Acute monocytic leukemia (10%)
  - t(9;11)
- M6 Acute erythroid leukemia (3%)
- M7 Acute megakaryoblastic leukemia (3%)

TABLE 1: 2008 WHO classification of acute myelogenous leukemia (AML)

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*
AML 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*
AML with myelodysplasia-related changes
Therapy-related myeloid neoplasms
AML, not otherwise specified
AML with minimal differentiation
AML without maturation
AML with maturation
Acute myelomonocytic leukemia
Acute monoblastic/monocytic leukemia
Acute erythroid leukemias
Pure erythroid leukemia
Erythroleukemia, erythroid/myeloid
Acute megakaryoblastic leukemia
Acute basophilic leukemia
Acute panmyelosis with myelofibrosis
Myeloid sarcoma

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.

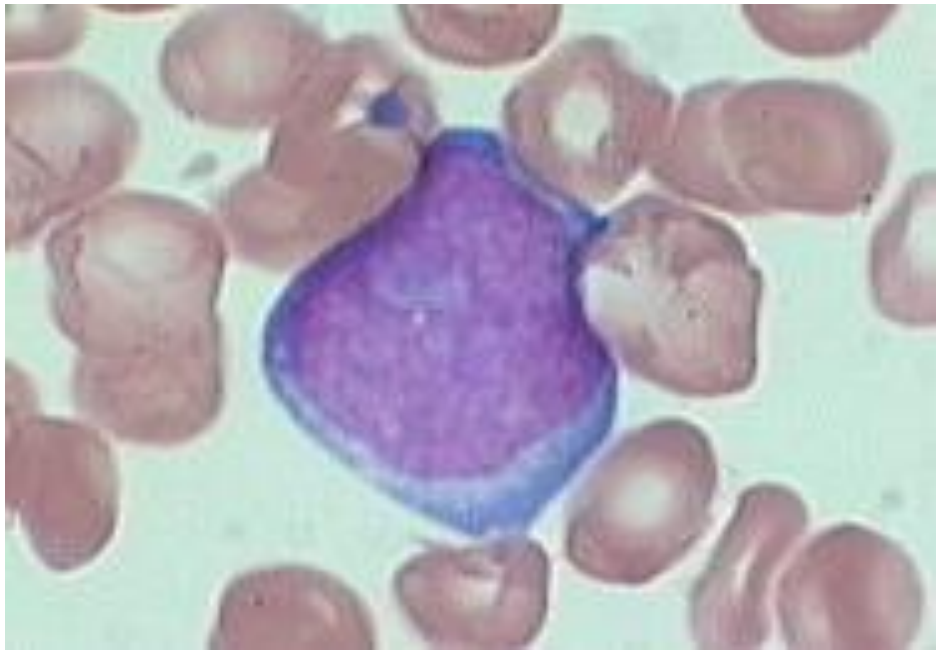
Vardiman JW, Thiele J, Arber DA, et al: The 2008 revision of the World Health Organization classification of myeloid neoplasms and acute leukemia: Rationale and important changes. Blood 114:937–951, 2009

New Acute Myeloid Leukemia subtypes 2016

- AML with *RUNX1* mutation (provisional)
  - Elderly male, poor prognosis
- AML with *BCR-ABL 1* (provisional)
  - Antigen receptor deletion (IGH)
- AML with biallelic *CEBPA* mutations ($CEBPA^{dm}$)
- Familial AML/MDS (multiple types)
- Promoted to full entity (No longer provisional)
  - Acute Myeloid Leukemia with NPM1 mutation
  - Acute Myeloid Leukemia with $CEBPA^{dm}$

M0

- 5% of cases of AML. Most cases are seen in adults.
- The blasts are of medium size with dispersed nuclear chromatin, round or indented nuclei with one or more nucleoli. The cytoplasm is agranular with varying degrees of basophila.



- Iph: CD45 dim

M0 blasts are negative for B and T lymphoid antigens, platelet glycoproteins GP Ib and GP IIb/IIIa, and erythroid glycophorin A. Myeloid antigens such as CD13, CD33 and CD11b are variably positive. CD34 and HLA-DR are generally positive. MPO is negative for these blasts.

- *These blasts are CD45 dim and express the primitive hematopoetic markers CD34, CD38 and HLA Dr. TdT may be expressed in 1/3 of cases. One or more of the following pan myeloid markers are expressed; CD13, CD33 and/or CD117. B and T restricted antibodies are not expressed (cCD3, cCD79a, cCD22). MPO is negative on these cases, though very few blasts may express MPO. Occassionally CD2 and/or CD7 (not lymphoid specific) are expressed on these cells.*

- *Cytochemistry*:
- M0 blasts are nondescript (no Auer rods) and are MPO, PAS and NSE negative (<3%).
- Electron microscopic ultracytochemical studies for platelet peroxidase are negative.

Genetics: There is no particular association with any chromosome abnormalities.

M1

- AML-M1 comprises approximately 10% of AMLs. It may occur at any age but the majority of patients are adults. The median age is 46 years of age.
- **Morphology**

In some cases the immature cells have abundant, frequently basophilic cytoplasm, with variable numbers of often indistinct, sometimes coalescent granules. If such immature cells are < 10% the diagnosis is M1

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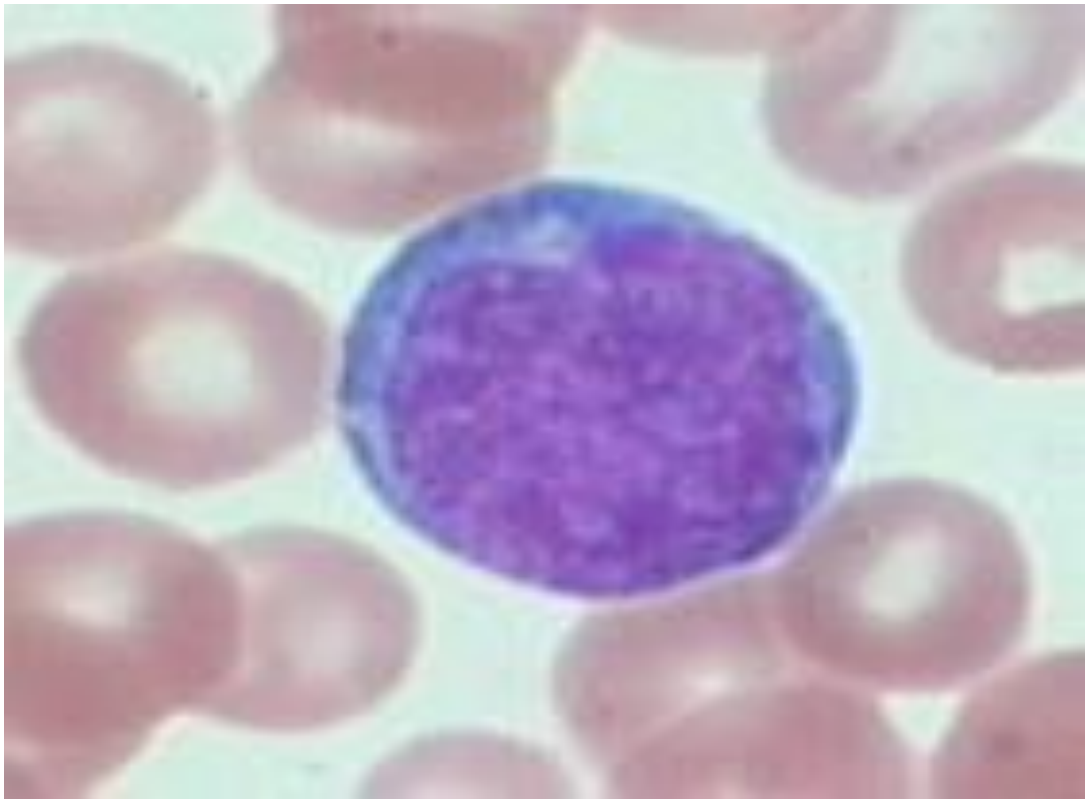

- ***Cytochemistry*:** Relatively few blasts (5-10%) are MPO (myeloperoxidase) positive. A minimum of 3% MPO positive blasts are required for diagnosis. NSE and PAS are generally negative.
- *Genetics*: Chromosome Abnormalities: t(9;22) Philadelphia chromosome, 8+, -5, and -7.
- **Flow Diagnosis**

AML-M1 and AML-M2 are initially stratified by morphology (see above). M1 blasts must express at least two of the following myeloid antigens: **CD13, CD33, CD117, MPO** and/or **HLA-DR**. **MPO** must be expressed on ≥ 3% on blasts.

M2

- Epidemiology

AML-M2 comprises approximately 30-45% of cases of AML. It occurs in all age groups; 20% of patients are less than 25 years of age and 40% are ≥60 years of age.

- **Morphology**

In some cases the immature cells have abundant, frequently basophilic cytoplasm, with variable numbers of often indistinct, sometimes coalescent granules. If such immature cells are < 10% the diagnosis is M1, but if > 10% the diagnosis becomes AML-M2. AML-M2 shows significant maturation:

Type II blasts are common and Auer rods are frequent (promyelocytes-myelocytes).

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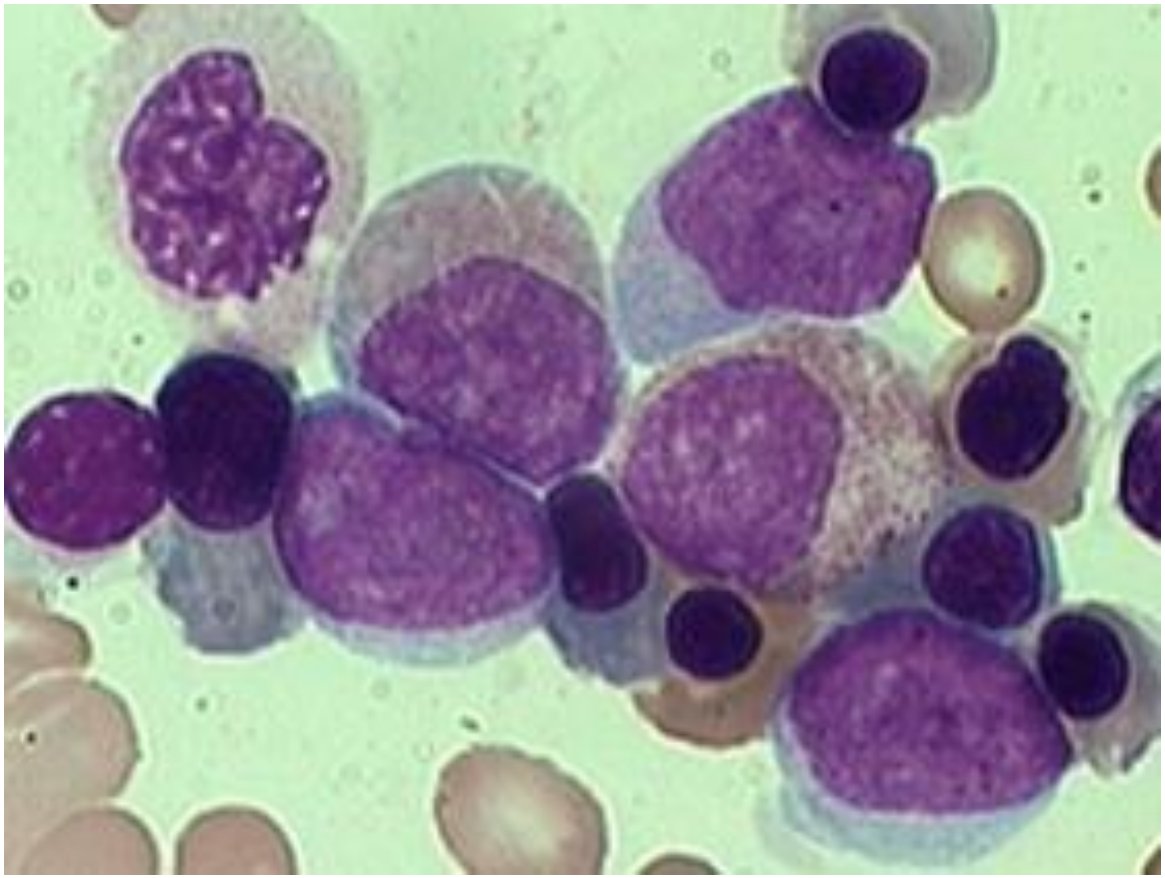
M2



- **Cytochemistry:** The blasts are largely MPO positive. NSE and PAS are generally negative.
- **Genetics**: Cases with increase basophils may have recurrent abnormalities including translocations and deletions at 12p (11-13) and t(6;9)(p23;q34). AML with the t(8;21)(q22;q22) is usually an AML-M2 and has a more favorable prognosis.
- **Flow Diagnosis**
- M2 blasts must express at least two of the following myeloid antigens: CD13, CD33, CD15, CD117, MPO and/or HLA-DR.
- MPO must be $\geq$ 3% on blasts.

IPH

- AML-M2 must express one or more of the myeloid associated antigens,
- CD13(mod), CD33(bright), and CD15.
- They may express CD117, CD34 and HLA Dr. CD4 may be present, but dim.
- These blasts may also express CD7 and/or CD56.
- These blasts are generally negative for the monocytic markers CD14 and CD11b.

Acute Promyelocytic Leukemia - AML with t(15;17)(q22;q12)

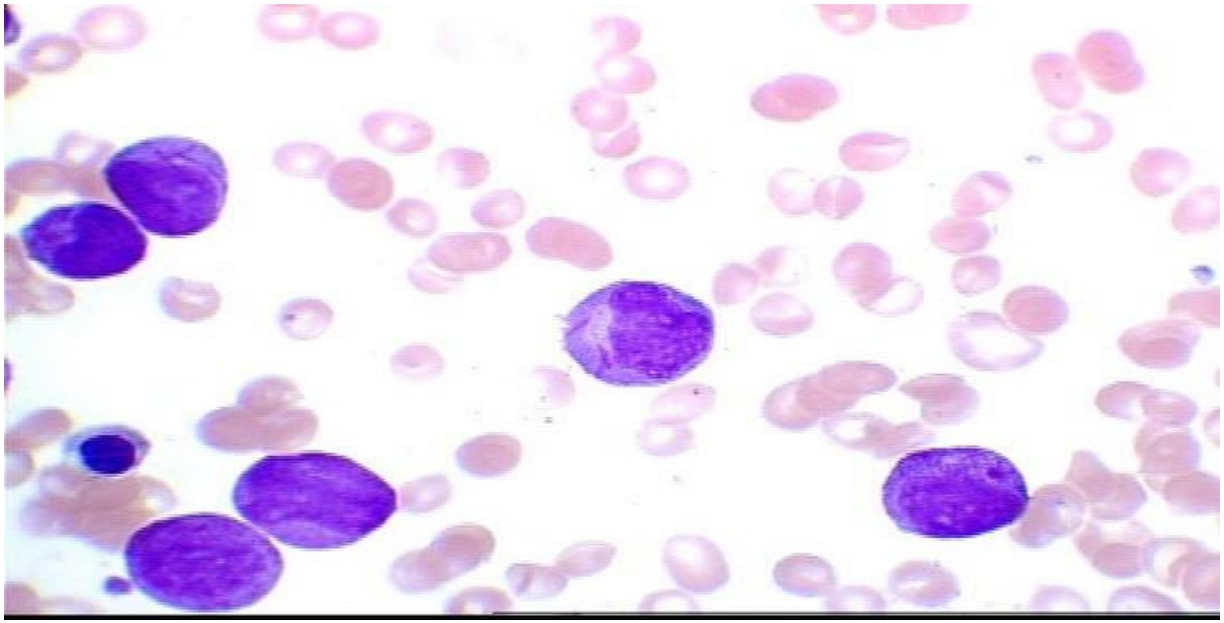
- **Definition**
- It is also known as acute progranulocytic leukemia; APL; AML with t(15;17)(q22;q12), PML-RARA and variants; FAB subtype M3 and M3 variant.
- In APL, there is an abnormal accumulation of immature granulocytes called promyelocytes. The disease is characterized by a chromosomal translocation involving the retinoic acid receptor alpha (*RARa* or *RARA*) gene and is unique from other forms of AML in its responsiveness to all *trans* retinoic acid (ATRA) therapy.

- **Epidemiology**

Acute promyelocytic leukemia (APL) represents 5-8% of AML in adults. The median age is approximately 40 years, which is considerably younger than the other subtypes of AML (70 years). The incidence is increased in patients originated in Latin American countries.

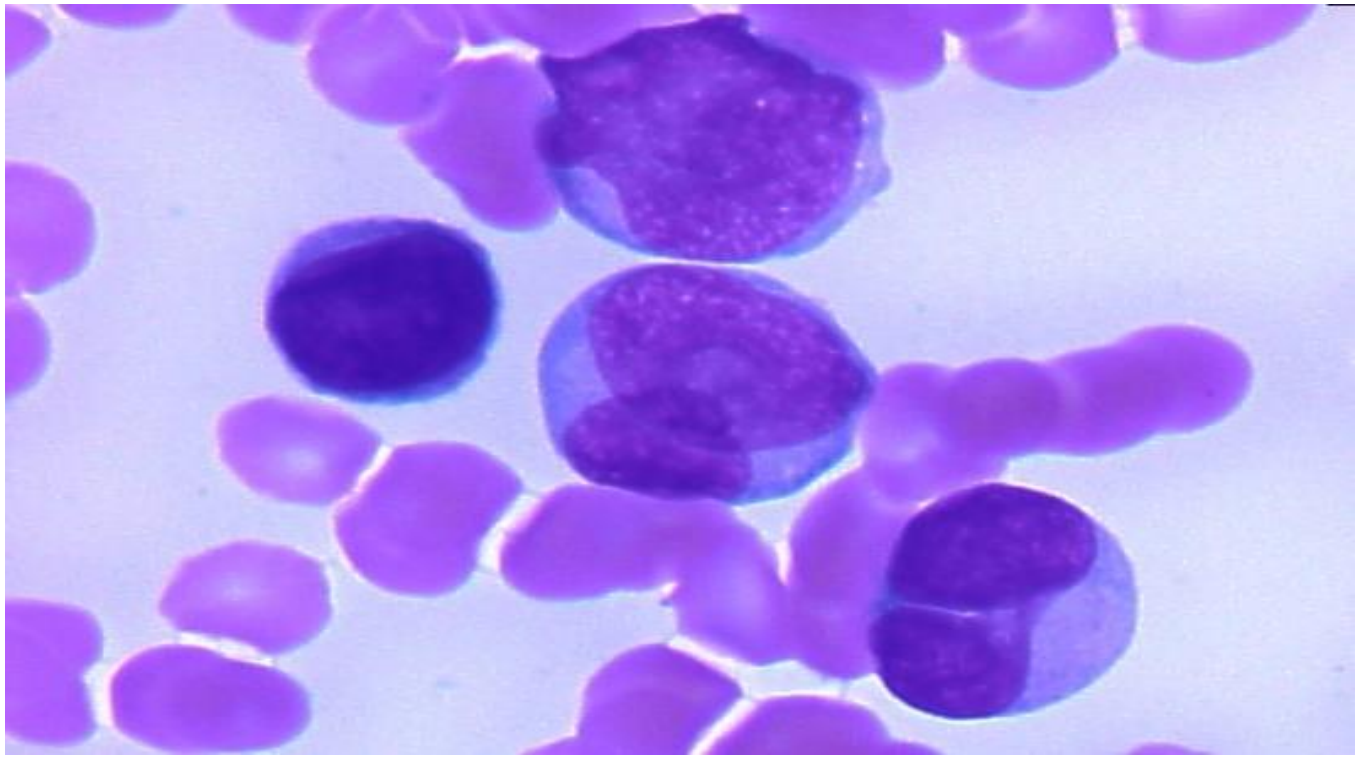
Morphology

- There are two subtypes of APL based on morphologic features;
- Hypergranular APL blasts vary greatly in size. The cytoplasm is densely packed with large granuoles occassionally obscurring the nucleus. Characteristic Auer rods or bundles of Auer rods are frequently present.



M3

- Microgranular/Hypogranular APL blasts have distinct morphologic features such as bilobed nucleus and paucity or absence of granules. many times these cells are confused with monoblasts.



CYROCHEMISTRY: STRONG PEROXIDASE +VE

- **Genetics:** In 95% of cases of APL, retinoic acid receptor-alpha (*RARα*) gene on chromosome 17 is involved in a reciprocal translocation with the promyelocytic leukemia gene (*PML*) on chromosome 15, a translocation denoted as t(15;17)(q22;q12).
- Four other gene rearrangements have been described in APL fusing *RARα* to promyelocytic leukemia zinc finger (*PLZF*), nucleophosmin (*NPM*), nuclear matrix associated (*NUMA*), or signal transducer and activator of transcription 5b (*STAT5B*) genes.

IPH:

- The myeloid markers **CD33** and **CD117** are positive.
- **CD13** and **CD34** are generally positive in the hypogranular variant.
- **HLA Dr** is characteristically negative

AMML (Acute Myelomonocytic Leukemia) (M4)

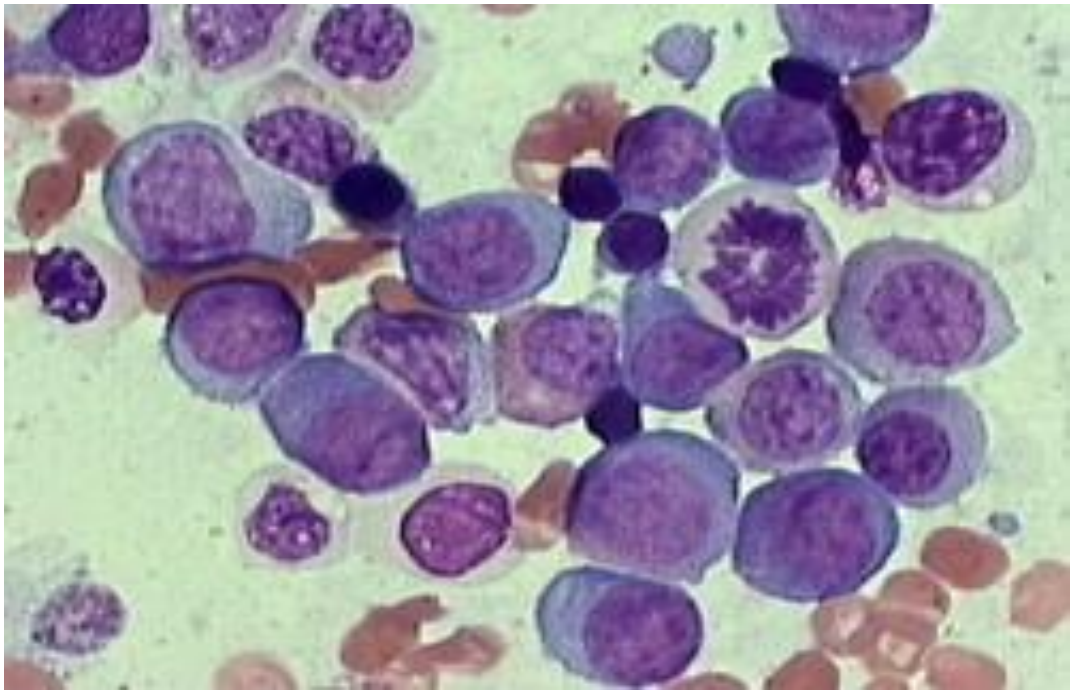
- Definition: acute leukemia with differentiation along both myeloid and monocytic lines. Monocytes and promonocytes represent > 20%, but < 80% of the marrow differential. Both myeloblasts and monoblasts are present. A high number of circulating monocytes may be present.

- **Epidemiology**

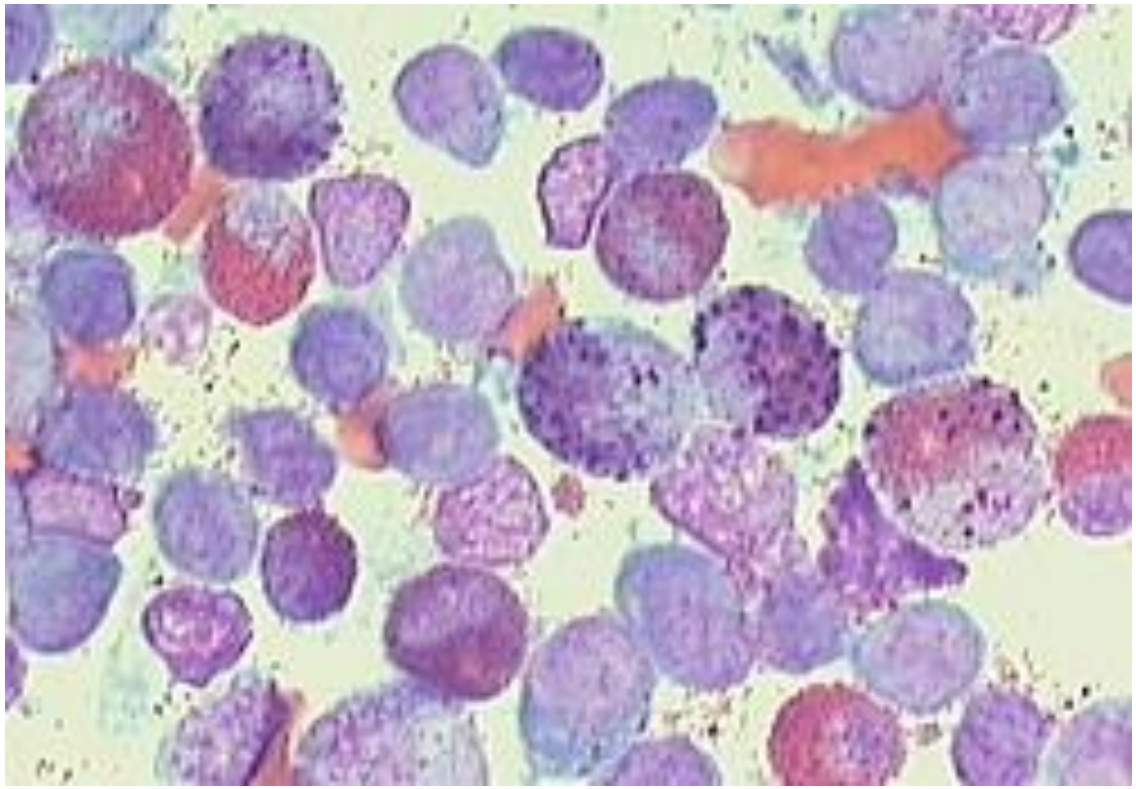
  Acute myelomonocytic leukemia comprises approximately 15-25% of all cases of AML. Some patients have a history of CMML. It occurs in all age groups, but is more common in older adults. The median age is 50 years of age. The male to female ratio is 1.4 to 1.

Morphology

- Monoblasts are large cells with abundant cytoplasm which can be moderately to intensely basophillic.
- There may be scattered azurophillic granuoles and vacuoles. The monoblasts will have lacy nuclear chromatin and one or more predominant nucleoli.



- A variant of AML-M4 has an increase in eosinophils and is classified as AML-M4e.



Cytochemistry:

- More than 20% of the blasts should be MPO + and more than 20% should be NSE + .
- **Genetics:** Chromosome abnormalities: t(4;11), t(9;11), 8+ and -7. Variant: M4e variant in which eosinophils (> 5%) are increased in number and abnormal associated with abnormalities of chromosome 16.
- *Diagnosis Aides:*

-High serum lysozyme (3x normal)

-A peripheral monocytosis of > $5x^{10}$/L in an otherwise M2 marrow and increased lysozyme

-A peripheral monocytosis of >5 x^{10}/L in M2 marrow and >20% NSE + marrow blasts.

IPH:

- Myeloblasts are **CD45** +ve and express **CD13**, **CD33**, **CD34, CD117, HLA Dr**.
- Monocytoid cells (purple) including monoblasts and promonocytes are**CD45** bright. These cells express **CD11b**, **CD11c, CD13, CD14, CD33, CD64** and**HLA Dr**.

AMoL (Acute Monoblastic/Monocytic Leukemia) (M5)

- **Definition:** a patient must have greater than 20% blasts in the bone marrow, and of these, greater than 80% must be of the monocytic lineage. A further subclassification (M5a versus M5b) is made depending on whether the monocytic cells are predominantly monoblasts (>80%) (acute monoblastic leukemia) or a mixture of monoblasts and promonocytes (<80% blasts).

Epidemiology

- **Epidemiology**

Acute monoblastic leukemia comprises 5-8% of cases of AML. It may occur at any age but is most common in young individuals. In infancy, it is frequently associated with abnormalities of 11q23. Extramedullary lesions may occur. Acute monocytic leukemia conmprises 3-6% of cases; the male to female ratio is 1.8 to 1.0. It is more common in adults. The median age is 49 years.

Morphology

- Monoblasts can be distinguished by having a roughly circular nucleus, delicate lacy chromatin, and abundant, often basophilic cytoplasm. These cells may also have pseudopods.

- ***Cytochemistry*:** Monoblasts are typically MPO negative and promonocytes are MPO variable. Both monoblasts and promonocytes stain positive for non-specific esterase (NSE), however NSE may often be negative.
- ***Genetics*:** There is a strong association with AMoL and deletions and translocations involving chromosome 11 band 23. Translocation t(8:16)(p11;p13)may be associated with AMoL and AMML.

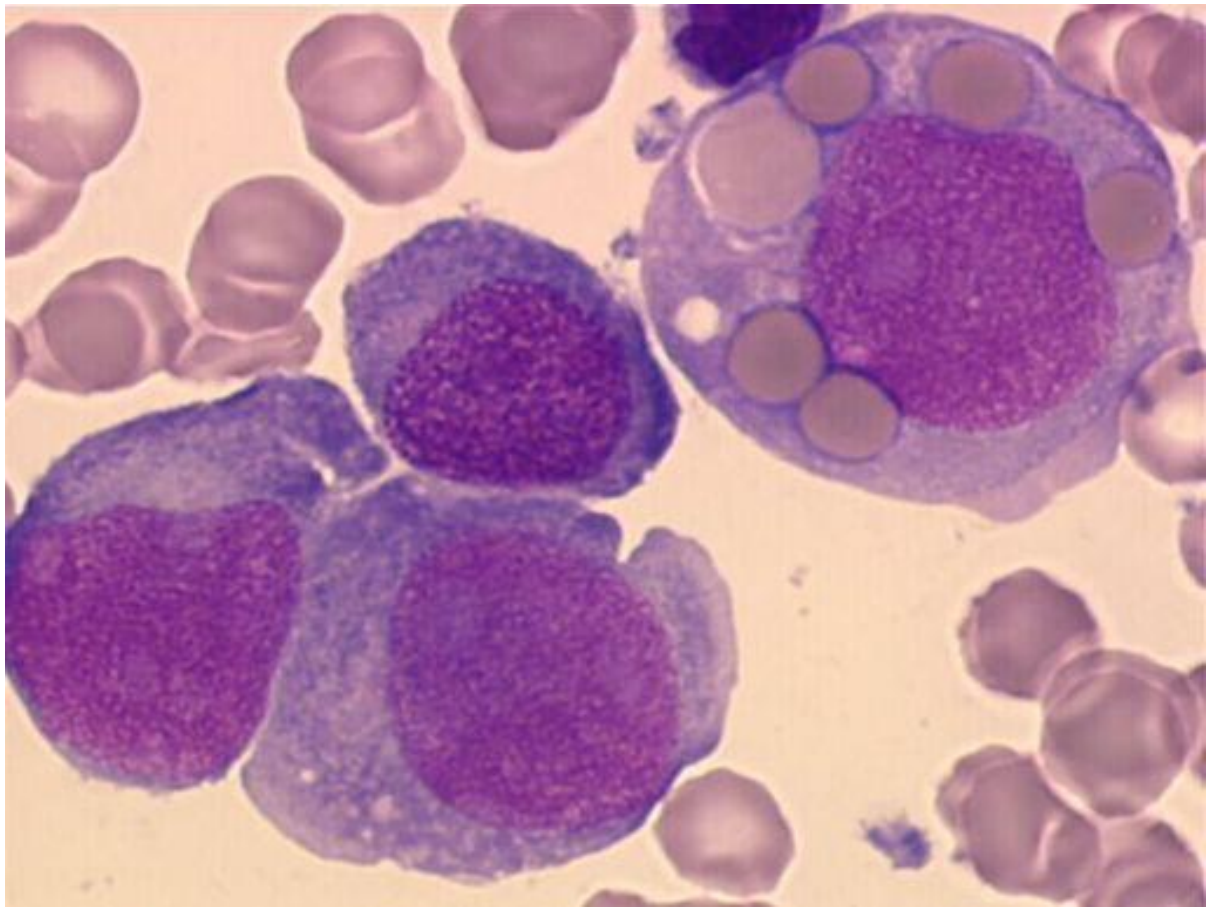
IPH

- Acute monoblastic/monocytic leukemia (M5) blasts express characteristically express **CD4, CD11b,CD11c,CD13** (dim), **CD33**(bright), **CD45** (bright-mod), **CD56, CD64** and **HLA Dr**. A subset of these cases may also express **CD2, CD7,CD10,CD16, CD23**, **lysosome** and **CD117**.
- **CD34** is predominantly negative.
- Though normal monocytes express **CD14**, in AMoL, this marker is variably expressed (predominantly negative).

Criteria for diagnosis

80% or more nonerythroid bone marrow cells are monocyte lineage (monoblasts, promonocytes and monocytes)

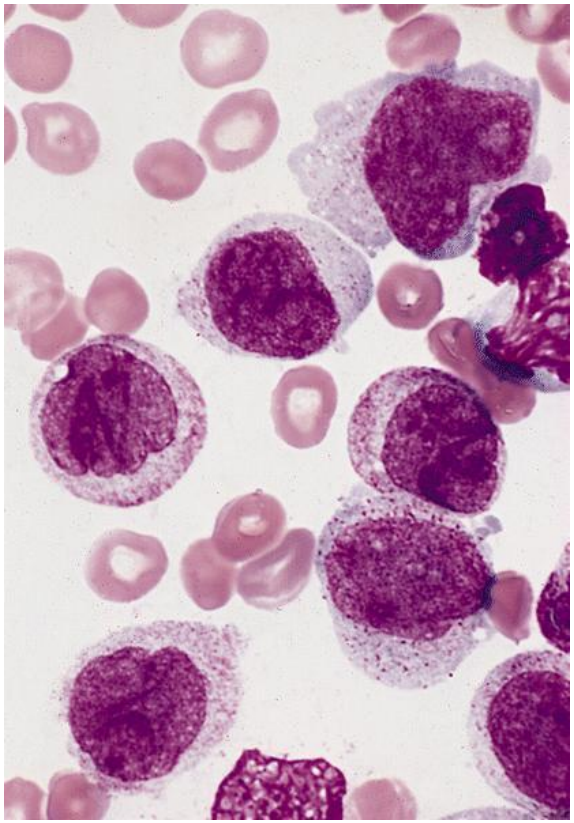
- A minor neutrophil component < 20%



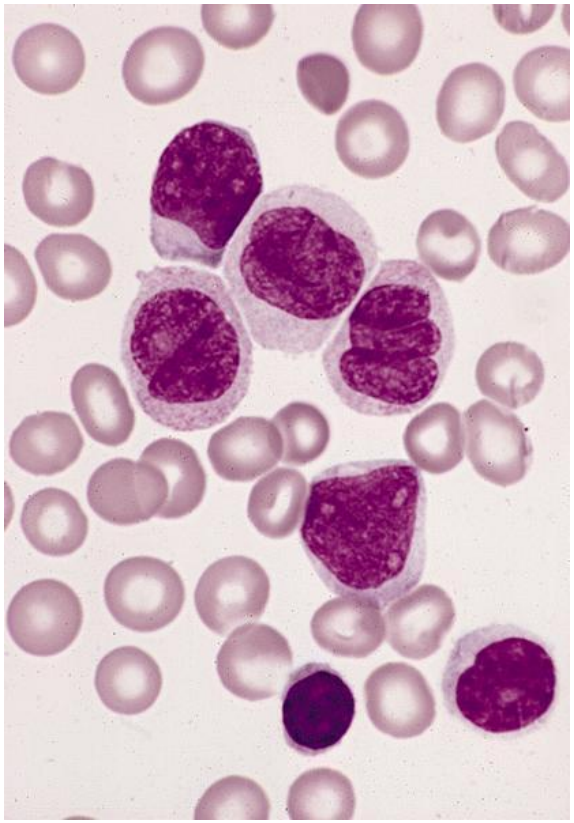
Acute monocytic leukemia (M5b)

- 3-6% of AML
- Affects all ages
- Mature monocytes or promonocytes predominate in peripheral blood (< 80% of monocyte lineage cells are monoblasts, usually < 20%)
- Treatment may cause tumor lysis syndrome, DIC and falsely elevated platelet counts

M5b



M5b



Molecular description

- 30% have cytogenetics abnormalities, including 11q23 in 12% (these cases should be classified as a recurrent genetic abnormality)
- FLT3 mutations in 30%

Prognosis of AML M5

Prognosis of the M5 subtype is found to be poor when compared to other AML subtypes.

AML M5 patients tend to have Flt3 gene mutations than those with other subtypes, reflecting the often unfavorable prognosis for patients with this subtype.

M6 Erythroblastic leukemia

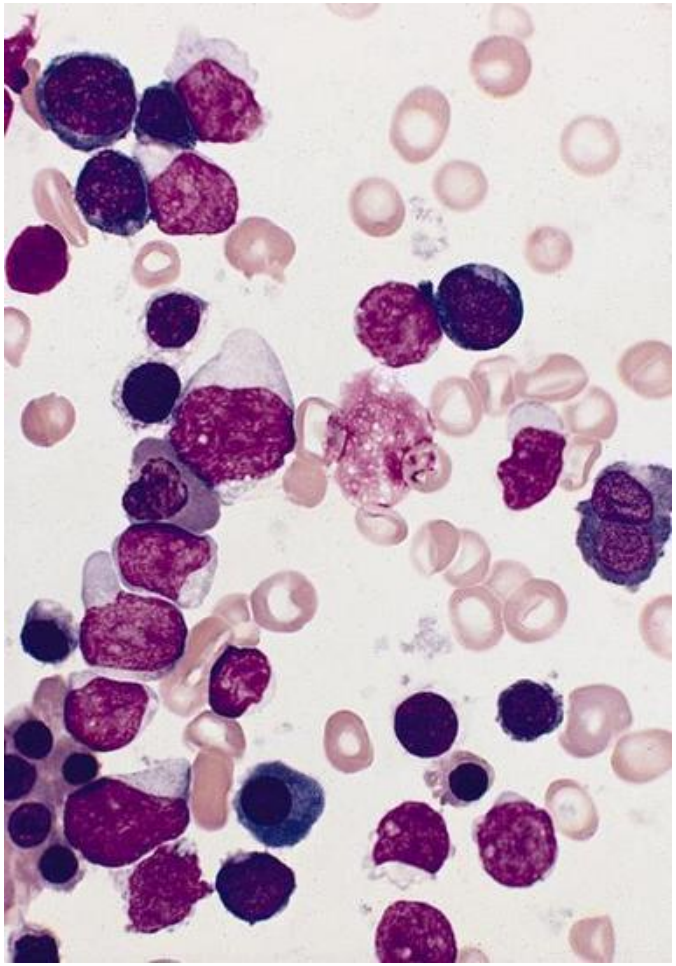
- **Definition**
- Erythroleukemia is a rare form of acute myeloid leukemia (AML) where the myeloproliferation is of erythrocyte precursors.
- It is defined at type "M6" under the FAB classification. M6 or erythroleukemia is rare and difficult to diagnose.
- More than 30-50% of the nucleated marrow cells are abnormal nucleated red blood cells.

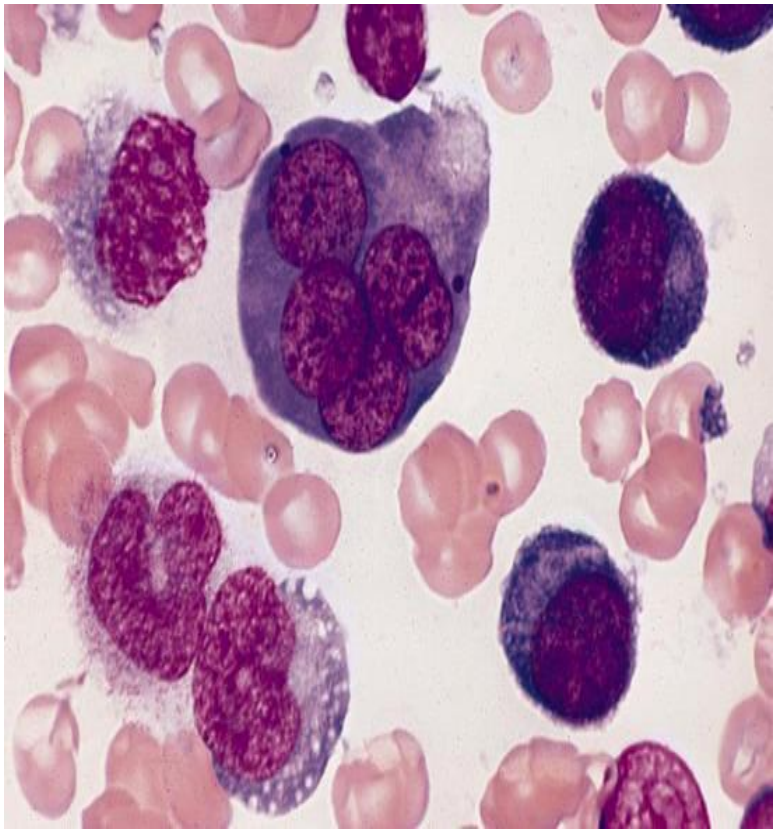
Epidemiology

- Erythroleukemia is predominantly a disease of adults.
- It comprises 5-6% of cases of AML. Pure erythroid leukemiais extremely rare and can occur at any age.
- Occasional cases of CML will transform in to AML-M6.

Morphology

The leukemic red cells are frequently bizarre with extreme dysplastic features including: giant forms, multinucleation, cytoplasmic vacuolization, cytoplasmic buds, and megaloblastoid changes. Cytoplasmic pseudopods similar to those in (M7) may be present. Nuclei are round or irregular with lobulation and multinucleation.





- WHO recognizes two subtypes of AML-M6: erythroleukemia (erythroid/myeloid leukemia) and pure erythroid leukemia. Erythroleukemia is defined by ≥ 50% erythroid precursors and ≥ 20% myeloblasts. Pure erythroid leukemia is defined by ≥80% erythroid precursors.

Cytochemistry: The blasts are MPO negative, but often positive for NSE. The malignant red cells are PAS positive, (forming PAS positive lakes or containing coarse chunks of PAS positive material).

Genetics: Chromosome Abnormalities: 8+, -5, del(5q), and -7.

IPH

- Erythroblasts are positive for **Glycophorin A (GPHA)** and **CD71**. The erythroid component lacks **MPO**, **CD34**, **CD45** and pan myeloid markers. **CD117** and **CD33** are often positive, when myeloblasts are present.

Acute Megakaryoblastic Leukemia (AMKL) (M7)

Definition

- Acute megakaryoblastic leukemia (AMKL) is one form of acute myelogenous leukemia (AML). It is classified as M7 according to the FAB system. AMKL is defined as an AML with ≥20% blasts, of which 50% or more are of the megakaryocyte lineage.

Morphology

- Medium- to large-sized megakaryoblasts with round or indented nucleus and one or more nucleoli.
- Agranular, basophilic cytoplasm with pseudopod formation.
- Lymphoblast-like morphology (high nuclear-cytoplasmic ratio) in some cases.
- Circulating micromegakaryocytes, megakaryoblastic fragments, dysplastic large platelets, and hypogranular neutrophils.
- Stromal pattern of marrow infiltration mimicking a metastatic tumor in infants.
- The blast cells vary considerably in size in this aggressive form of leukemia and there is frequent budding of cytoplasmic margins. Auer rod bodies may be found within the neoplastic cells.
- M7 blasts are often resemble lymphoblasts, although M7 leukemias may be accompanied by atypical megakaryocytes.
  The marrow is often fibrotic.

Immunophenotyping

- Platelet glycoproteins expression: **CD41** (glycoprotein IIb/IIIa) and/or **CD61** (glycoprotein IIIa).
- Myeloid markers **CD13** and **CD33** may be positive. Blasts are negative with the **anti-MPO** antibody .

The differential diagnosis includes

- minimally differentiated AML,
- acute panmyelosis with myelofibrosis,
- ALL,
- pure erythroid leukemia,
- blastic transformation of chronic myeloid leukemia or idiopathic myelofibrosis , and metastatic tumors in the bone marrow (particularly in children).

Biphenotypic Acute Leukemia

- Biphenotypic acute leukaemia (BAL) is an uncommon disease.
- Incidence approximately 5% of all acute leukaemias.
- BAL can be de novo or secondary to previous cytotoxic th

Clinical

- As with other types of acute leukaemia, BAL presents with the symptoms resulting from cytopenias. The blast count at diagnosis does not tend to differ from that in acute myeloid leukaemia (AML) or acute lymphoid leukaemia (ALL). BAL can present at any age, including children, although it is more common in adults.

Morphology

- The morphology of the blasts in BAL is not consistent. The cells may display myeloid differentiation features such as azurophilic granules or Auer rods, or have lymphoid/undifferentiated morphology.
- From those cases with myeloid features, the most common FAB subtype is M1 and M5. In some cases, there appears to be two blast populations Ð one larger population resembling myeloid blasts and another, with smaller lymphoid appearing blasts.

- The blasts co-express myeloid and lymphoid markers.
- The markers considered to be most specific areB-lymphoid lineage: CD79a, CD22, cytoplasmic immunoglobulin,T-lymphoid lineage: CD3, anti-TCR, andmyeloid lineage: myeloperoxidase by cytochemistry or flow cytometry. Most cases express early haemopoietic markers such as CD34.
- The score allows four groups to be identified. The most common group, accounting for 60-70% of cases, are those which co-express myeloid and B-lymphoid antigens. Less commonly the blasts co-express myeloid and T-lymphoid antigens. Co-expression of T and B-lymphoid markers and those with trilineage differentiation are rare.

Scoring	B-lymphoid	T-lymphoid	Myeloid
2	cCD79a, CD22, cIgM	cCD3	cMyelo Peroxidase
1	CD19, CD10	CD2, CD5	CD33, CD13
0.5	TdT	TdT, CD7	CD11b, CD11c CD14, CD15