Hematology Unit
Lab 2 Review Material - 2018

Objectives

Laboratory Instructors:

1. Assist students during lab session

Students:

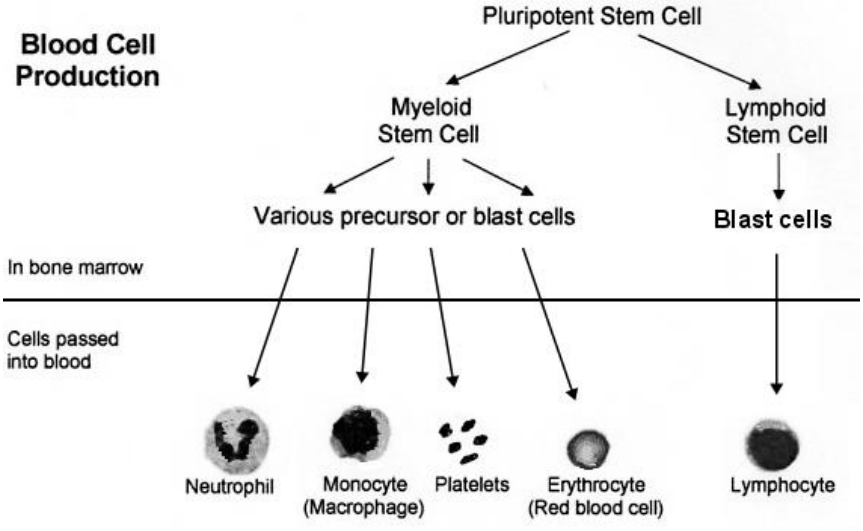
1. Review the introductory material
2. Study the case histories provided for MD Lab 2
3. Examine the pathological material related to each case using virtual microscopy
4. Answer the questions related to each case

Acute Leukemia: Pathophysiology

- Defined by the presence of ≥ 20% blasts in the blood or bone marrow
- Develops when acquired defects result in clonal expansion without significant maturation beyond the blast stage
- Blasts rapidly accumulate in the marrow
- The expansion of blast cells compromises normal hematopoiesis resulting in bone marrow failure:
  - Anemia
  - Neutropenia
  - Thrombocytopenia
- Infiltration of lymph nodes by leukemic cells leading to lymphadenopathy is commonly seen in acute lymphoblastic leukemia

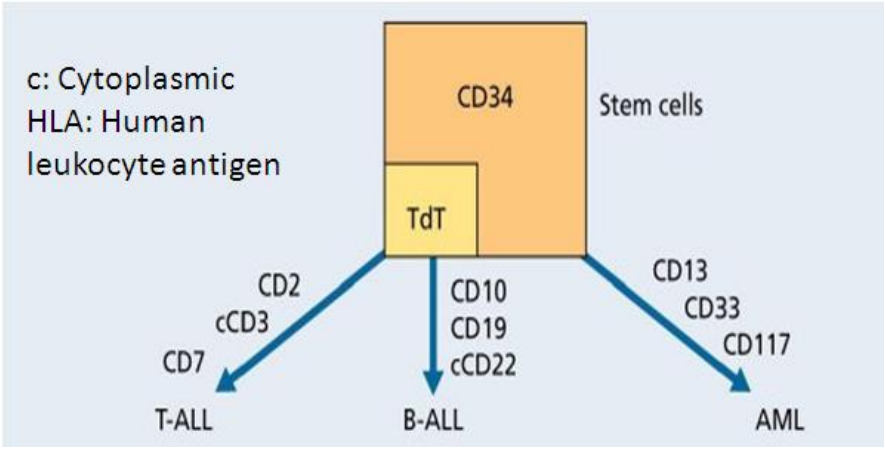
Consequences of leukemic transformation:

- Increased proliferation (at the blast level, diagram below)
- Blocked differentiation
- Decreased cell death (decreased apoptosis)
- Bone marrow failure
- Infiltration of organs by leukemic cells



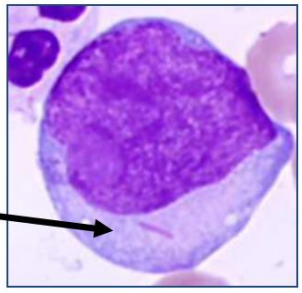
Acute Leukemia: Classification

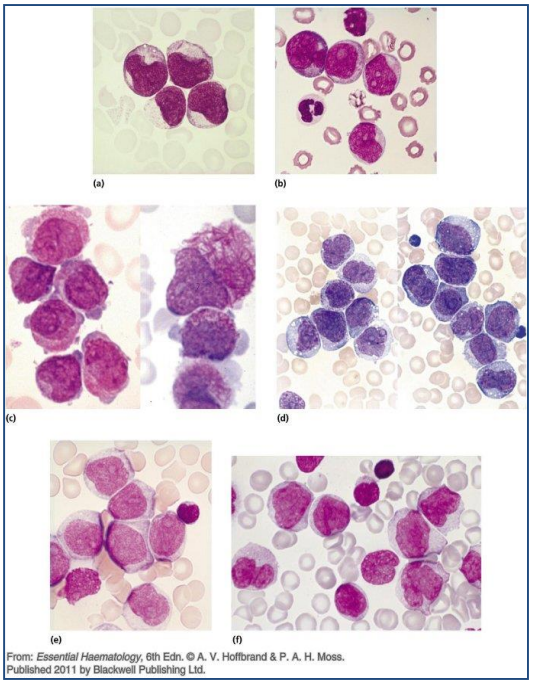
- Acute leukemia is divided into two broad categories:
  - Acute lymphoblastic leukemia (ALL): consists of blasts of either T cells or B cells
  - Acute Myeloid leukemia (AML) consists of blasts with characteristics of myeloid cells (granulocytes, monocytes, megakaryocytes, erythrocytes)
- Further subclassification is according to World Health Organization (WHO) system which incorporates morphology, cytochemistry, flow cytometry, genetic markers and clinical features



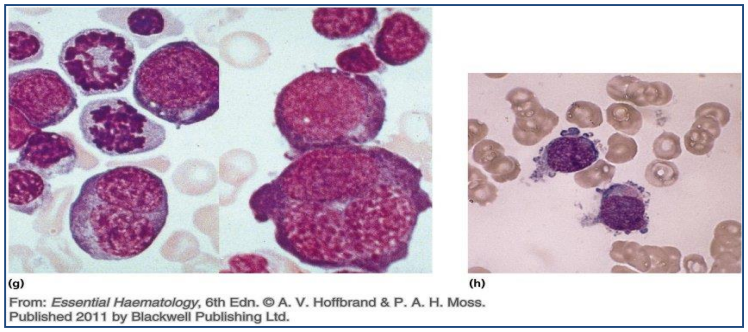
AML Morphology

Auer Rod:
Diagnostic of AML



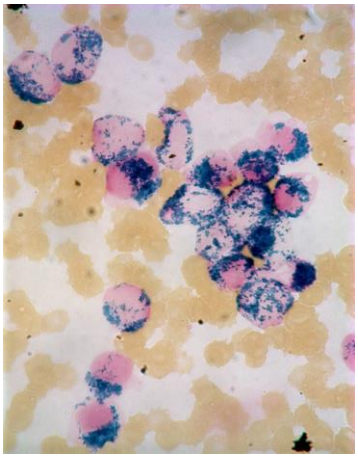


Morphological examples of acute myeloid leukemia. (a) Blast cells without differentiation show few granules but may show auer rods, as in this case; (b) cells in differentiation show multiple cytoplasmic granules or (c) M_3 blast cells contain prominent granules or multiple Auer rods; (d) myelomonocytic blasts have some monocytoid differentiation; (e) monoblastic leukemia in which >80% of blasts are monoblasts; (f) monocytic with <80% of blasts monoblasts

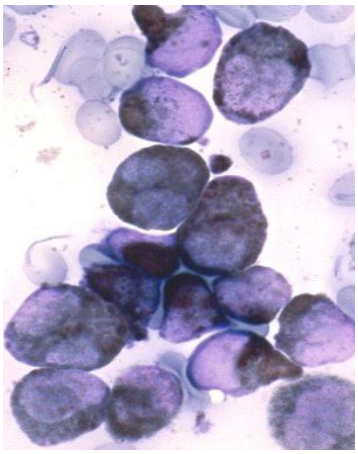


(g) Erythroid showing preponderance of erythroblasts (h) megakaryoblastic showing cytoplasmic blebs on blasts

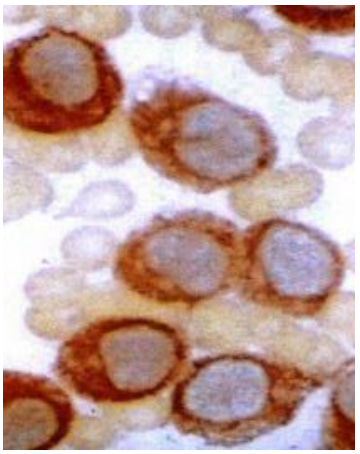
AML: Cytochemistry



Myeloperoxidase (MPO): specific for myeloid differentiation



Sudan Black: positive in myeloid cells (Courtesy of: www.hmds.org.uk/aml.html)

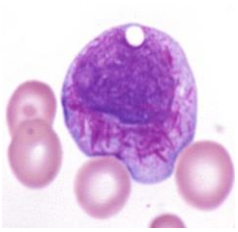


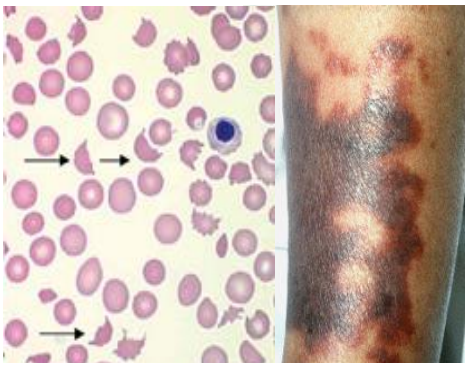
Non-specific esterase (NSE): Positive in monocyte lineage

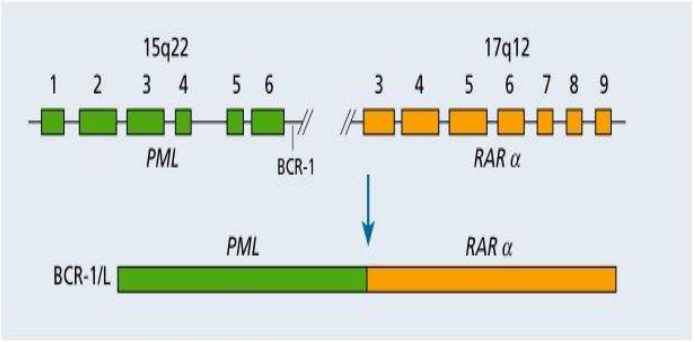
Acute Promyelocytic Leukemia (APL)

Characterized by:

- Characteristic morphology
- t (15;17) chromosomal translocation
- Disseminated intravascular coagulation
  - Microangiopathic blood picture (schistocytes)
  - Microvasculature thrombosis (tissue







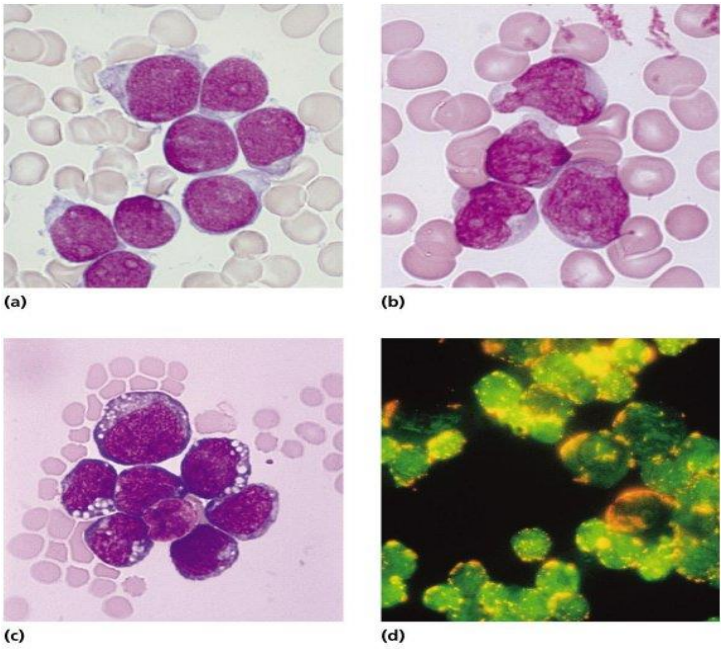
Generation of the t(15;17) translocation. The PML gene at 15q22 may break at one of three different breakpoint cluster regions (BCR-1, -2 and -3) and join with exons 3-9 of the RARα gene at 17q12. Three different fusion mRNAs are generated (termed long (L), variable (V) or short (S)) and these give rise to fusion proteins of different size. In this diagram only the long version resulting from a break at BCR-1 is shown.

Acute Lymphoblastic Leukemia

Morphology, cytochemistry and immunophenotyping of acute lymphoblastic leukemia (ALL).

(a) Lymphoblasts show scanty cytoplasm without granules.
(b) Lymphoblasts are large and heterogeneous with abundant cytoplasm.
(c) Lymphoblasts are deeply basophliic with cytoplasmic vacuolation.
(d) Indirect immunofluorescence reveals nuclear terminal deoxynucleotidyl transferase (TdT) (green) and membrane CD10 (orange).

Courtesy of Professor G Janossy



Acute Lymphoblastic Leukemia: Fast Facts

- The most common malignant disease of childhood. 75% of cases occur before age 6
- Eighty-five percent of the cases are of B-cell lineage, the rest are T-cells
- The chromosome number in leukemic cells is prognostic: higher is favorable (>50= hyperdiploidy)
- Overall, 85% of children are now expected to be cured of ALL; adults with ALL have worse prognosis

Myeloproliferative Neoplasms

Definition: Hematopoietic stem cell disorders characterized by proliferation of one or more of the myeloid lineages

- Granulocytic
- Erythroid
- Megakaryocytic
- Mast cells

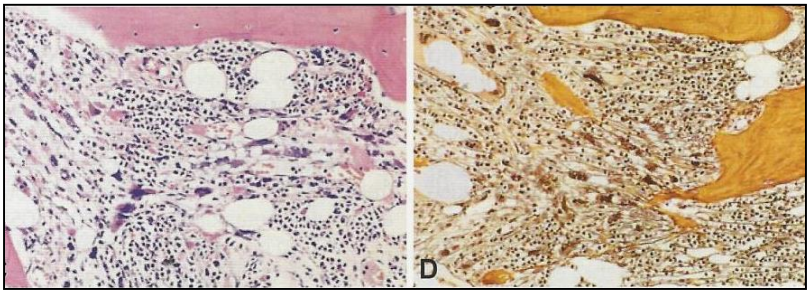
MPN's typically present with:

- Hypercellular bone marrow with maturation of cells
- Increased numbers of peripheral blood neutrophils, red blood cells and/or platelets
- Hepatosplenomegaly
- Each has the potential to progress, resulting in marrow fibrosis and/or acute leukemia

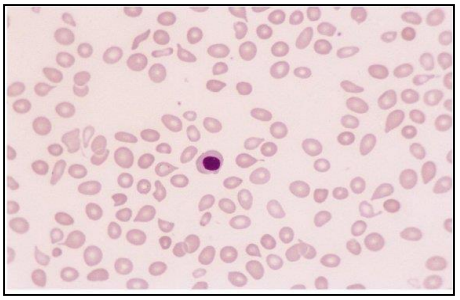
Classification:

- Chronic Myelogenous Leukemia
- Polycythemia Vera
- Primary Myelofibrosis
- Essential Thrombocythemia
- Mastocytosis

Primary Myelofibrosis

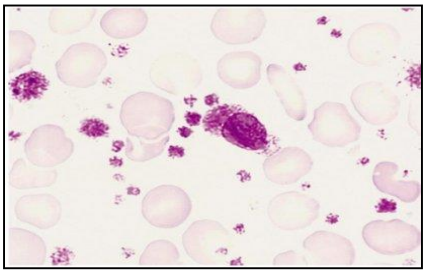


Above: Bone marrow biopsy showing loss of normal architecture. Hematopoietic cells surrounded by increased fibrous tissue (right: sliver staining)



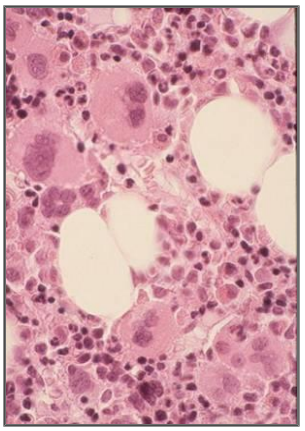
Peripheral blood film showing tear-drop RBCs characteristic of this diagnosis and a normoblast (as part of leukoerythroblastic picture)

Essential Thrombocythemia (ET)



Left: Peripheral blood film in essential thrombocythemia showing increased numbers of platelets and a nucleated megakaryocytic fragment.

Right: Bone marrow with increased Megakaryocytes in ET



MDS – General Summary

- Clonal disorder of marrow stem cell
- Occurs mainly in older patients
  - Median age is 70 years
- Symptoms are secondary to cytopenias
- Hallmark is dysplastic features in the hematopoietic cells.
- Increased risk of blastic transformation
- Bone marrow is normocellular or hypercellular in >90%, but there is failure to produce mature cells
- Prognostic variables:
  - Blast percent
  - Number and degree of cytopenias
  - Cytogenetic Abnormalities

MDS: WHO Classification

- MDS with Single Lineage Dysplasia(Refractory cytopenia with single lineage Dysplasia)
- Refractory Anemia with ring sideroblasts (RARS)
- Refractory Cytopenia with Multilineage dysplasia (RCMD)
- Refractory anemia with excess blasts (RAEB-1, RAEB-2)
- MDS with isolated 5q del
- MDS Unclassified

Diagnosis of MDS

- Unequivocal evidence of dysplasia in one or more cell lineages
- Abnormality should involve ≥ 10% of the affected lineage
- Careful assessment of percentage of blasts

MDS: Morphological Features

Appearances of the peripheral blood and bone marrow.

(a) Multinucleate polychromatic erythroblasts.

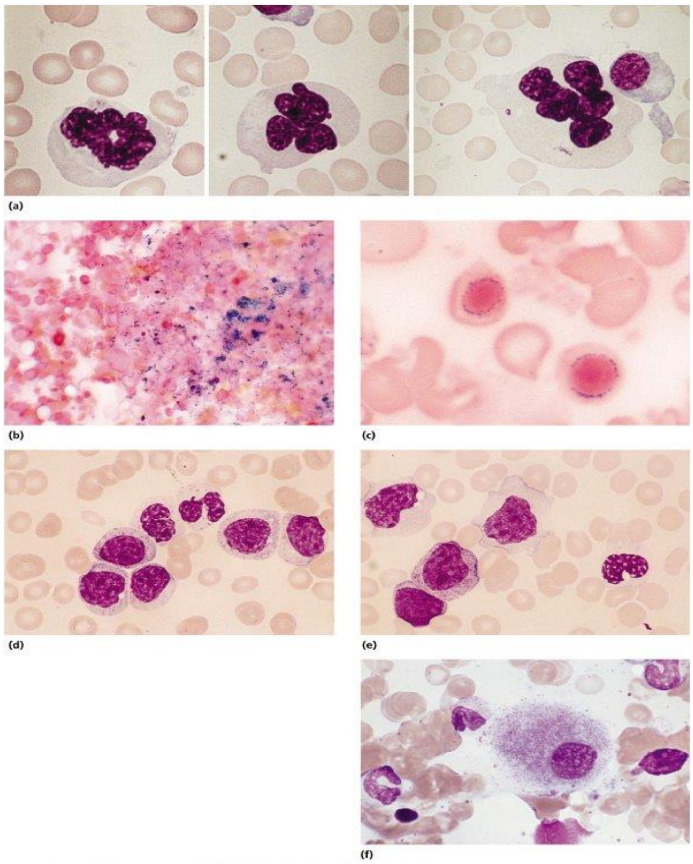
(b) Perls' stain showing iron overload in macrophages of a bone marrow fragment.

(c) Ring sideroblasts.

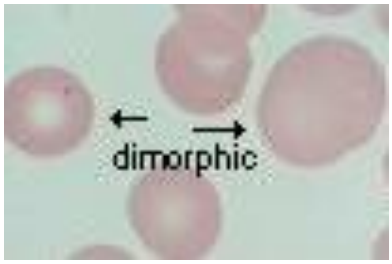
(d) White cells showing pseudo-Pelger cells, agranular myelocytes and neutrophils.

(e) Monocytoid cells and an agranular neutrophil.

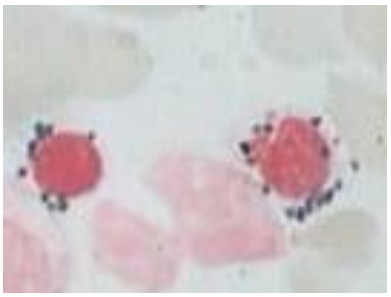
(f) Mononuclear megakaryocyte



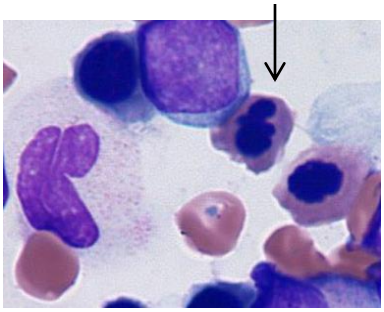
MDS: Dysplastic Erythropoiesis



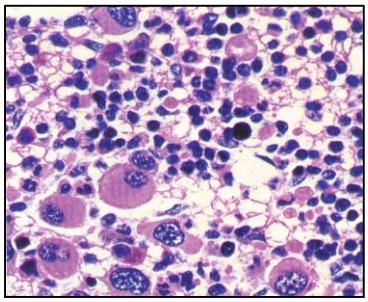
Ring sideroblast

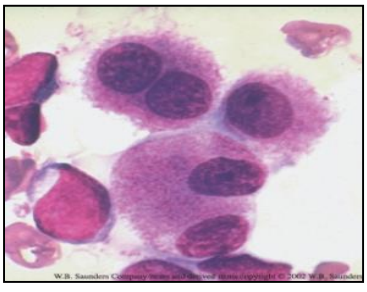


Nuclear irregularity, nuclear budding



Dysplastic Megakaryocytes





Mononuclear or binucleated megakaryocytes, some with hypogranular cytoplasm

Refractory Anemia with Excess Blasts (RAEB)

Cytopenias with uni- or multilineage dysplasia

- **Type 1**:
  - <5% blasts in peripheral blood
  - 5-9% blasts in bone marrow
- **Type 2**:
  - 5-19% blasts in peripheral blood
  - 10-19% blasts in bone marrow

Risk of progression to AML:

- **Type 1** - 25%
- **Type 2** - 33%