Introduction to Hematolymphoid malignancies

Alireza Torabi, MD PhD

Associate Professor

Hematopathology Division

How to make the diagnosis of hematolymphoid neoplasms?

- Clinical History/physical examination
- Morphology (BM biopsy, PB smear, lymph node)
- Immunophenotyping:
  - Immunohistochemistry
  - Flow cytometry
- Genetics:
  - Karyotyping and FISH
  - Molecular methods such as gene sequencing

Clinical History/physical examination (myeloid neoplasm)

- Fatigue and weakness, fever, infection, abnormal bleeding.
- Abnormal CBC: Cytosis (reactive, AML or MPN) or cytopenia (infection, nutritional, drug effect, AML, MDS).
- Physical exam:
  - Pale conjunctiva (anemia).
  - Splenomegaly, hepatomegaly (possible MPN).
  - Blood clot/thrombosis (high platelet count).
  - Ecchymoses, purpura, or petechiae.

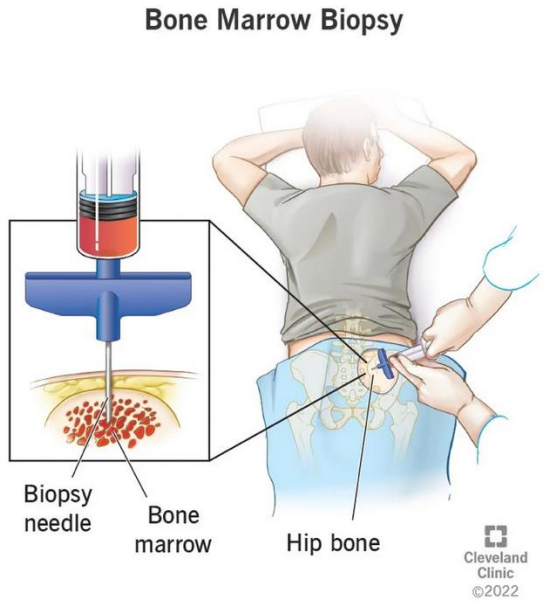
Clinical History/physical examination (lymphoid neoplasm)

- Lymphadenopathy
- Mediastinal mass
- Splenomegaly
- Soft tissue/extranodal mass: Expanding MALT, brain or skin lesions.
- B symptoms (fever, night sweats, weight loss).
- If BM involved: weakness, fatigue, infection, bleeding.
- Plasma cell neoplasm can cause bone lesion and pathologic fracture.
- Secretion of Ig fragments or antibodies: autoimmune cytopenia or organ damage.

When and how do we do BM biopsy?

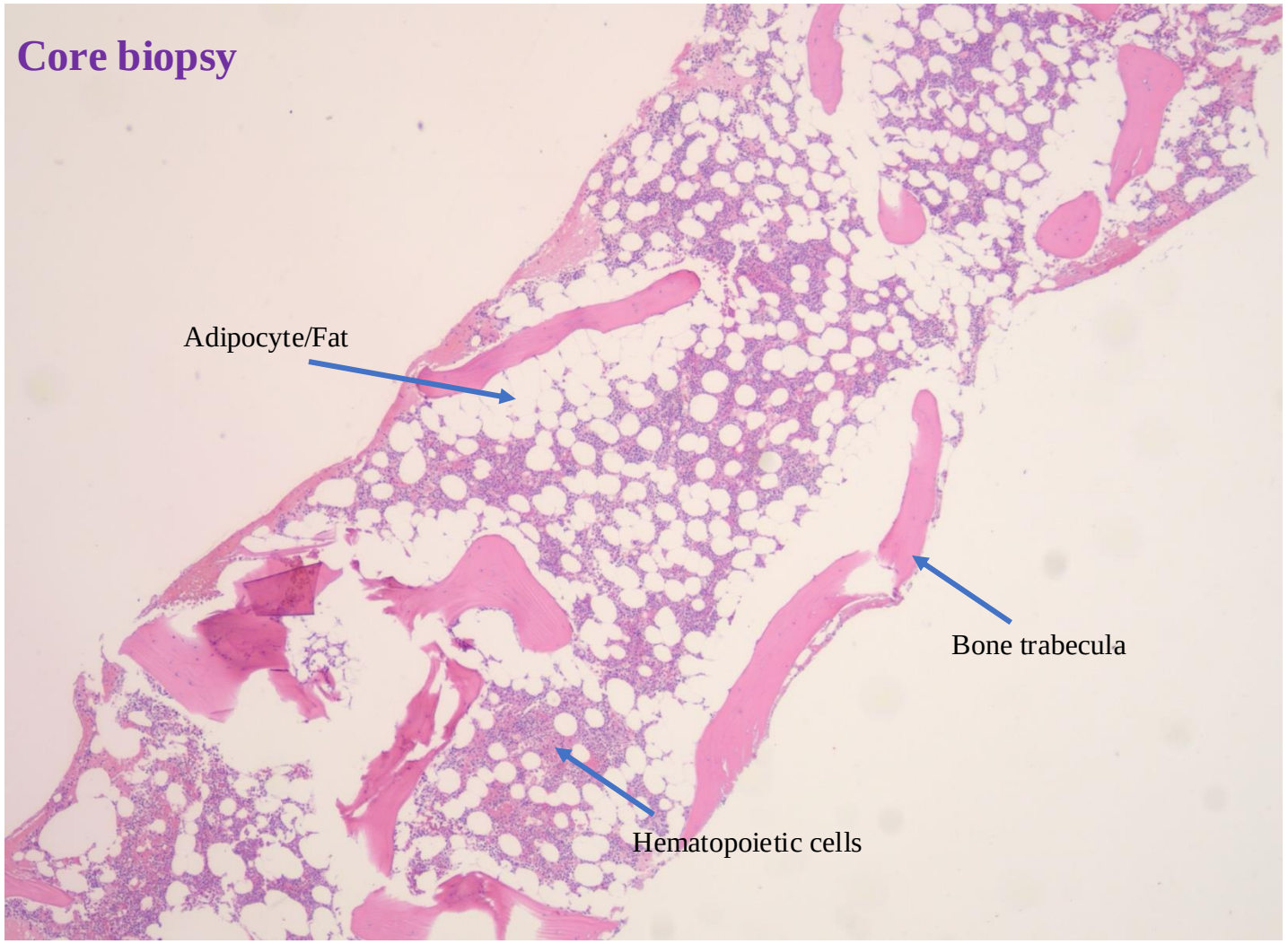
- Evaluation of hematologic/myeloid neoplasm.
- Staging lymphomas.
- BM failure.
- Monitoring therapy for minimal residual disease.

- It's been done from posterior iliac crest, using Jamshidi needle.
- Obtain core biopsy and aspirate smears.



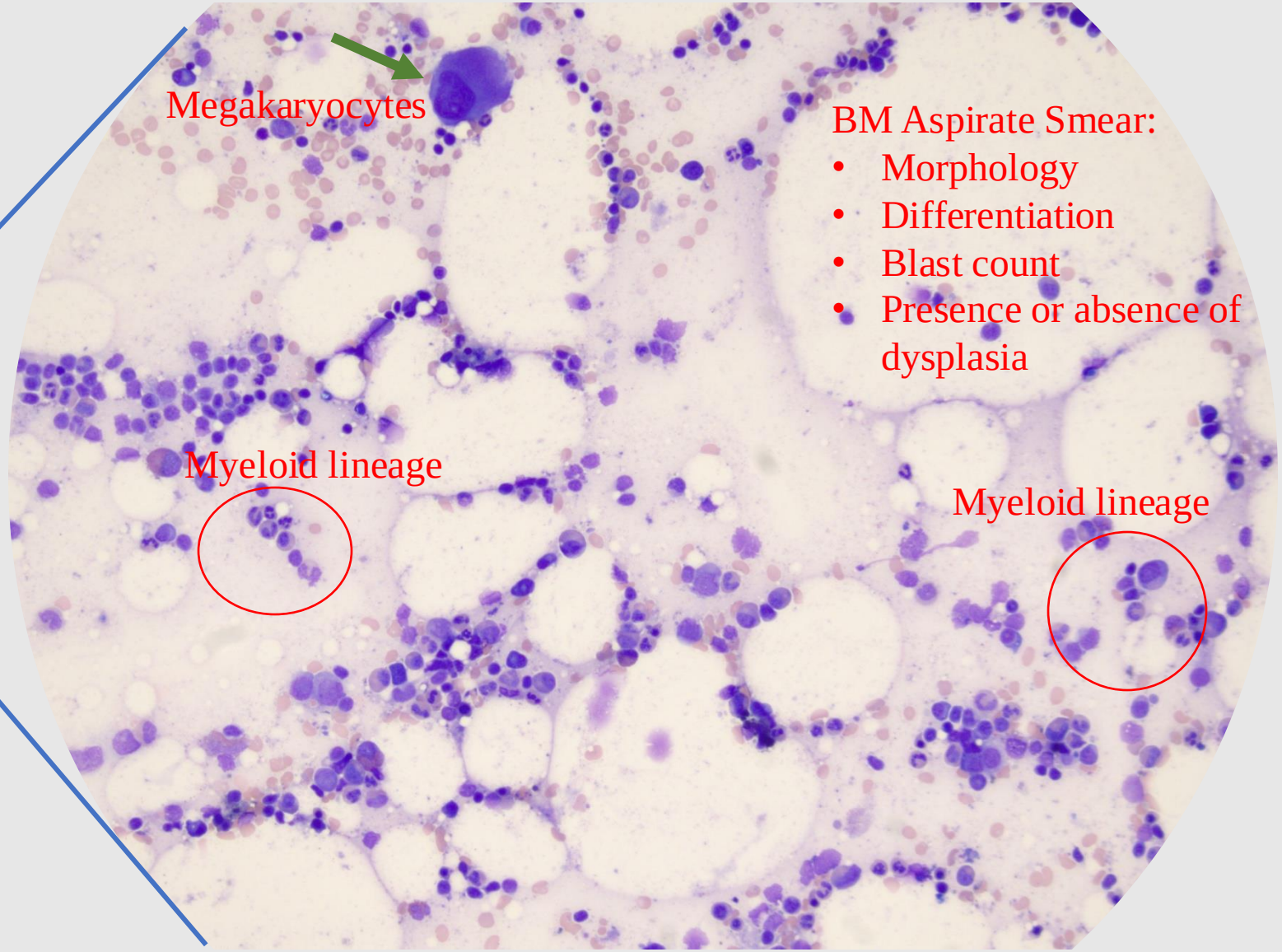
Morphology

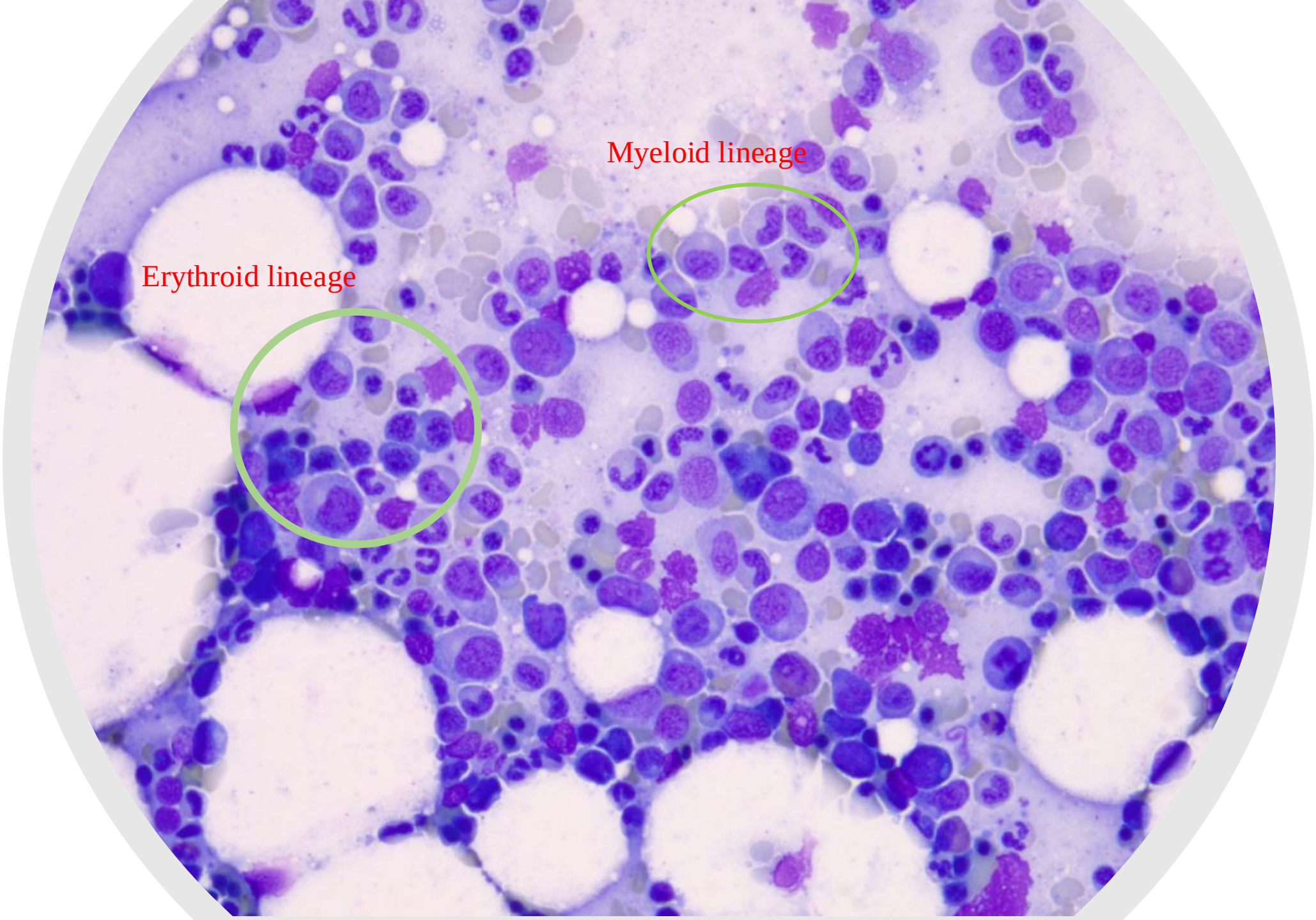
Normal BM cellularity %:
20 y/o: 20% fat/ 80% cells
50 y/o: 50% fat/ 50% cells
80 y/o: 80% fat/ 20% cells



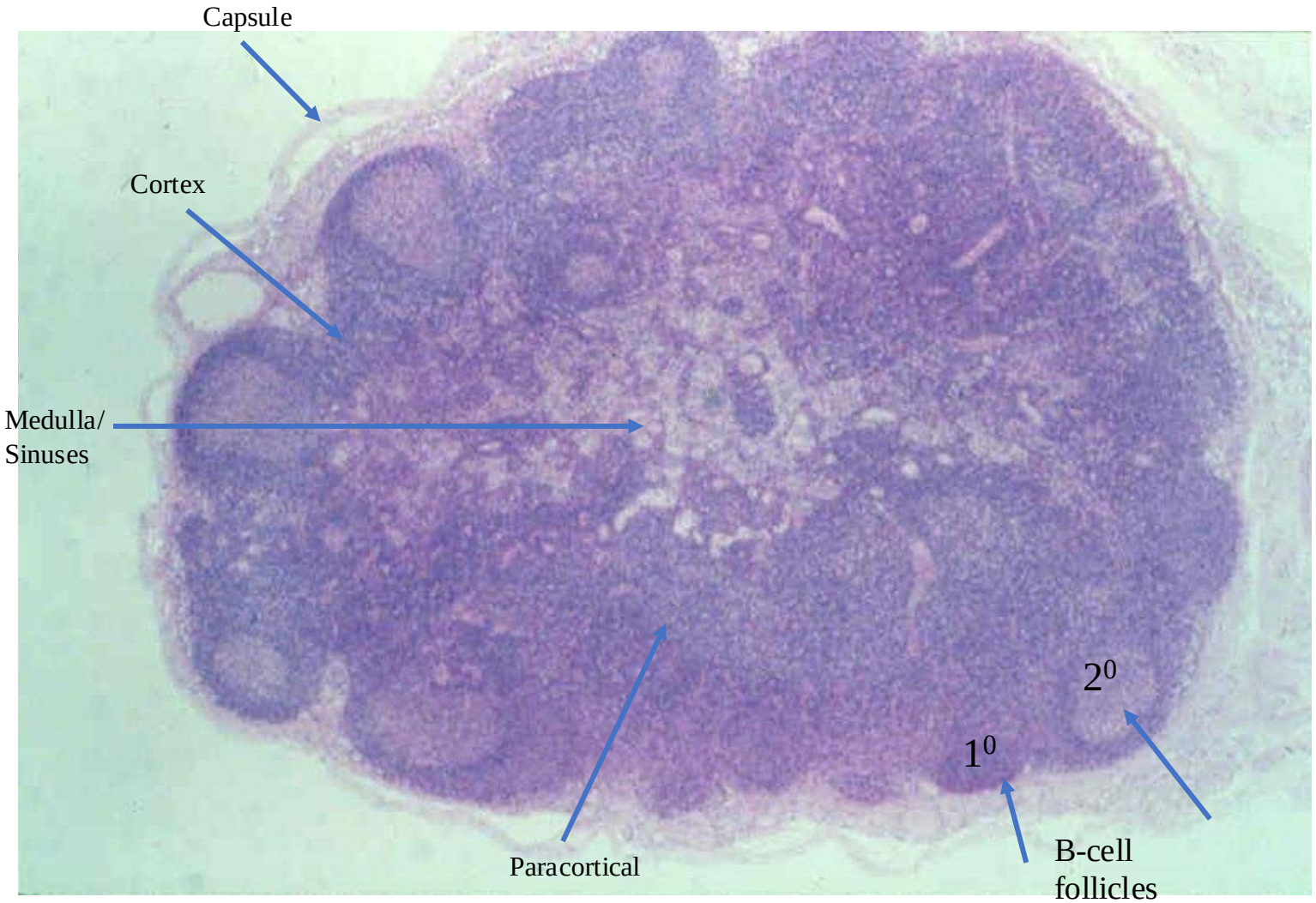
Bone Marrow Cellularity

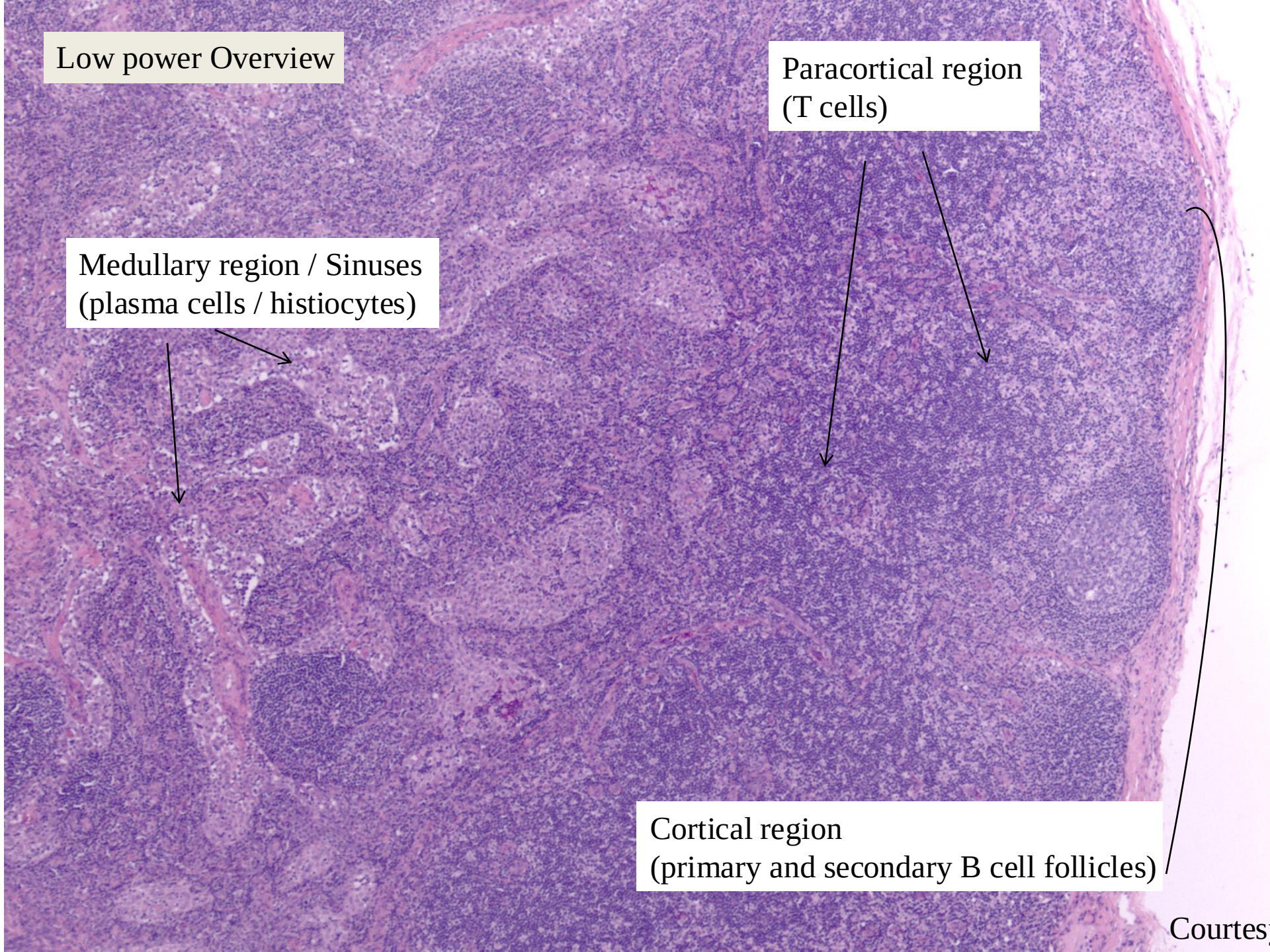
- Hypercellular bone marrow for age:
  - Acute leukemia
  - Myeloproliferative neoplasm
  - Myelodysplastic neoplasm/syndrome
  - BM involvement by plasma cell neoplasm/lymphoma/carcinoma
  - Reactive cases such as nutritional deficiency
- Hypocellular bone marrow for age:
  - Aplastic anemia
  - Hypoplastic MDS
  - Paroxysmal nocturnal hemoglobinuria
  - Genetic disorders (such as Fanconi anemia)
  - Fibrotic bone marrow
  - Post treatment


Megakaryocytes
BM Aspirate Smear:
• Morphology
• Differentiation
• Blast count
• Presence or absence of dysplasia
Myeloid lineage
Myeloid lineage

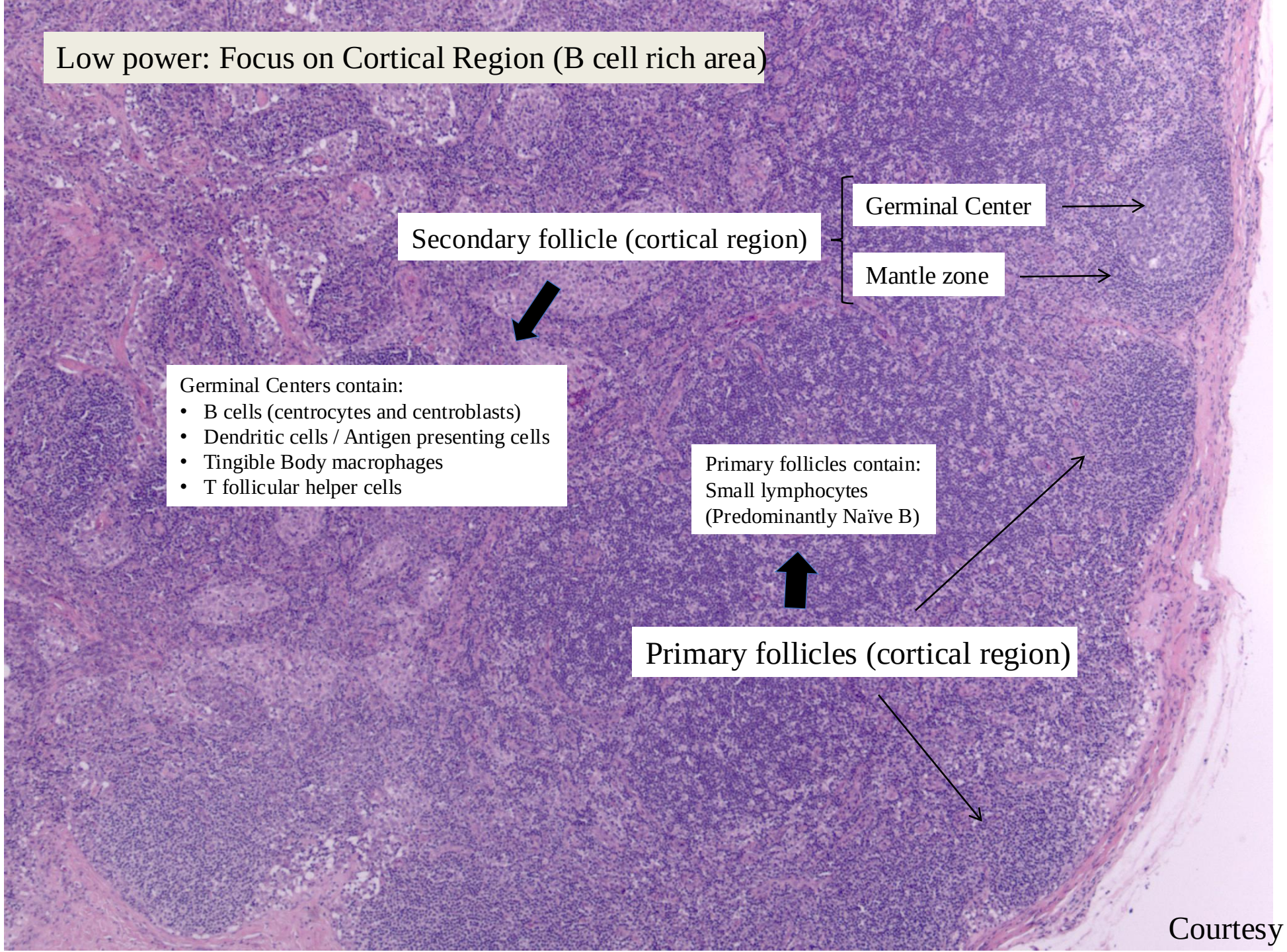

Myeloid lineage
Erythroid lineage

Normal lymph node architecture





Courtesy Dr. Lori Soma



Courtesy Dr. Lori Soma

Phenotyping

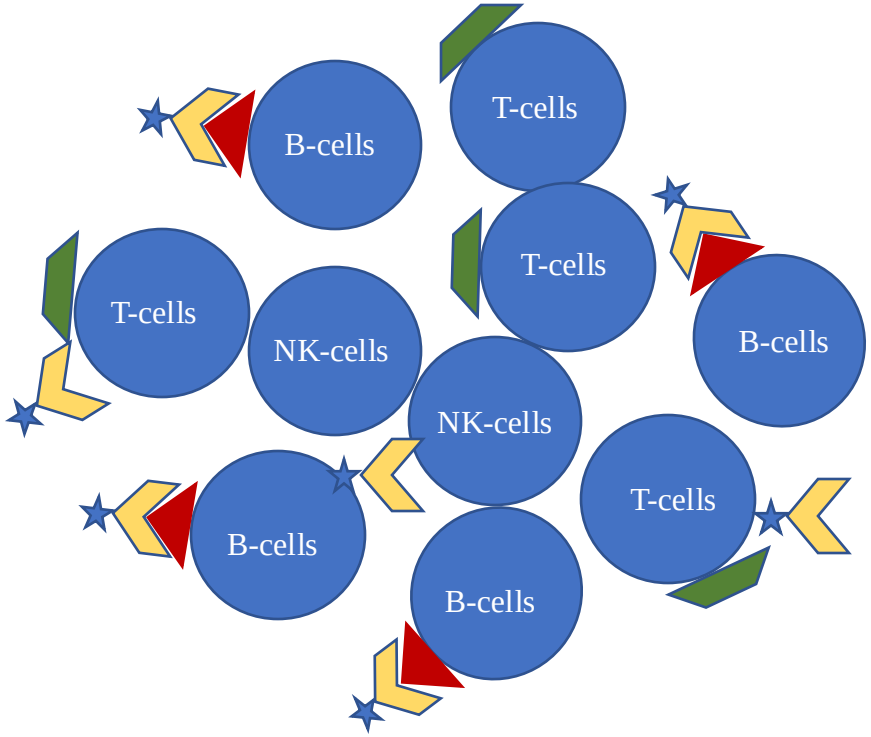
- Immunohistochemistry: can be done on formalin fixed paraffin embedded tissue
- Flow cytometry:
  - Fresh tissue
  - Rapid turn-around time
  - Very sensitive
  - Semi-quantitative method

Phenotyping

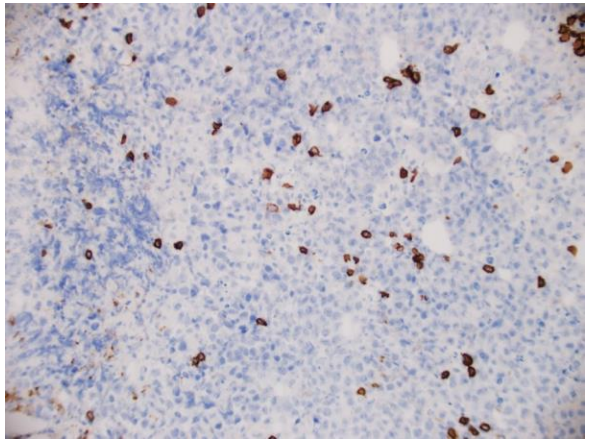
Add the antibody

Wash

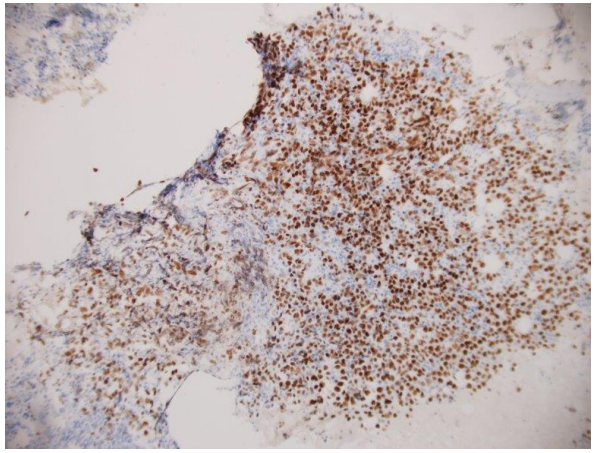
Add chromogen

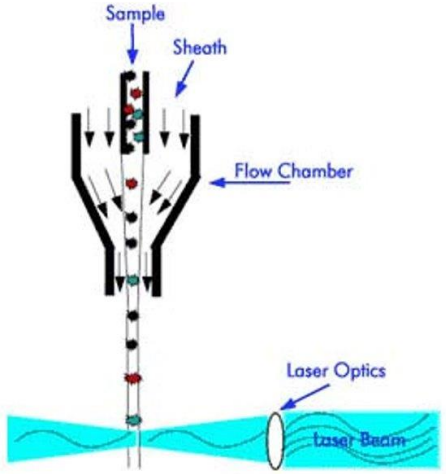


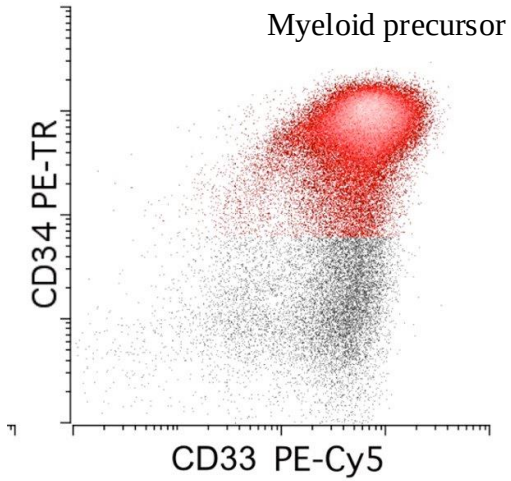
CD138

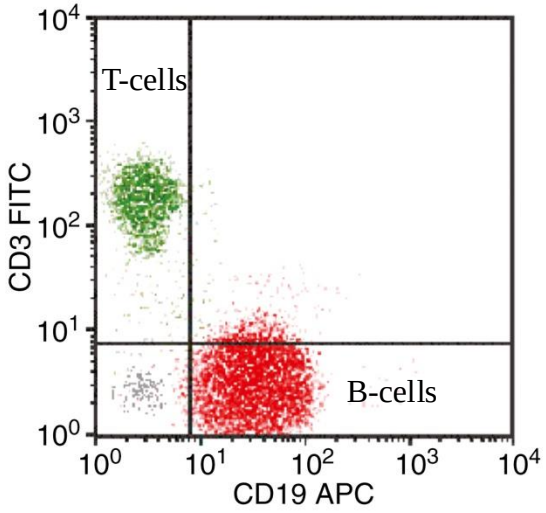


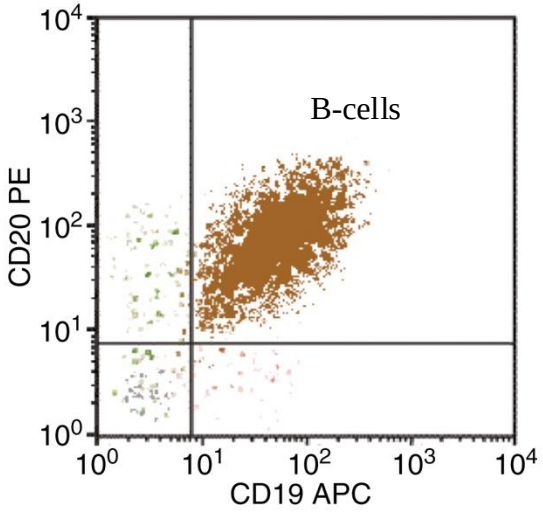
cMyc, Nuclear stain











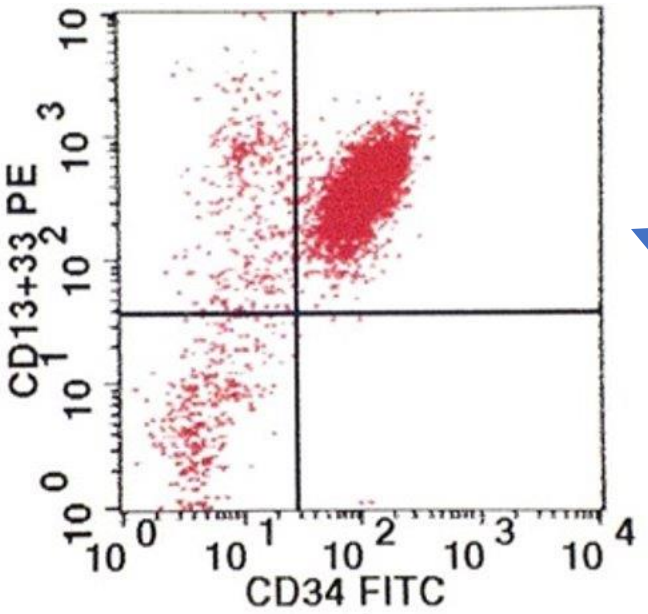
Flow Cytometry

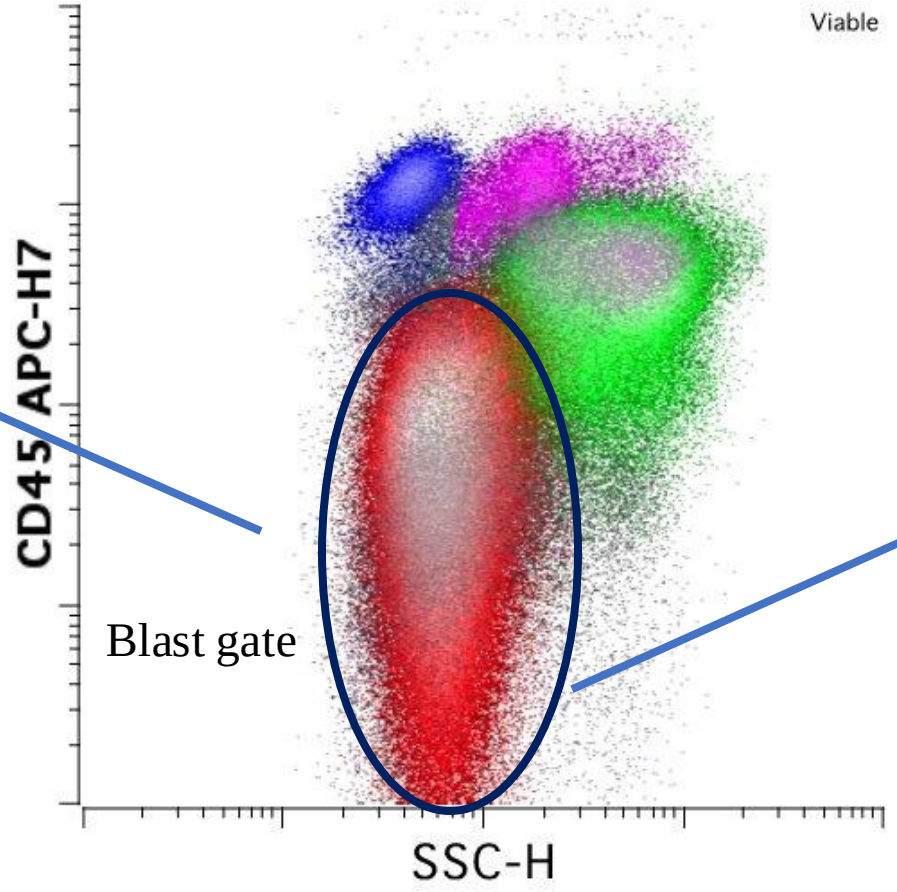
Flow cytometry

- Pattern of antigen expression in leukemia and lymphomas is well established.
- Leukemia:
  - Markers of immaturity: CD34, TdT.
  - Myeloid markers: CD117, CD13, CD15, CD33, MPO.
- Lymphoma:
  - B-cell markers: CD19, CD20.
    - Clonality: Kappa versus lambda.
  - T-cell markers: CD2, CD3, CD4, CD5, CD7, CD8.

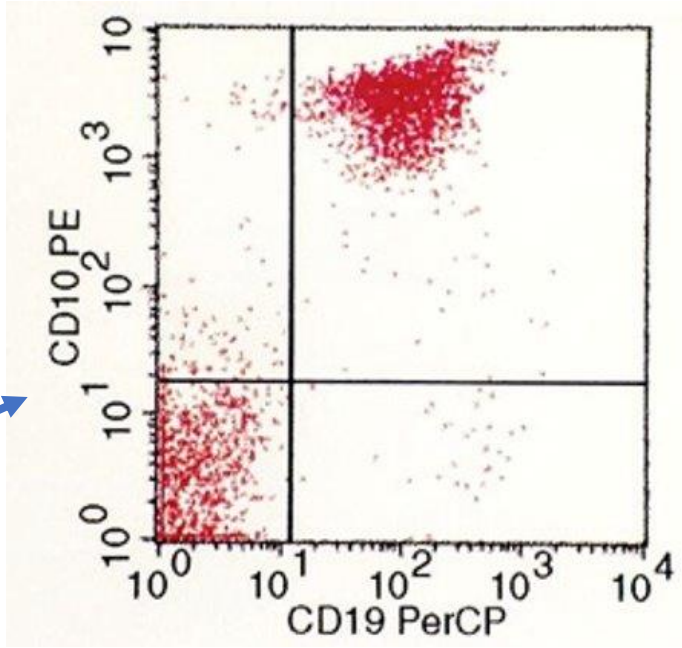
Flow Cytometry

AML





ALL



Markers usually used in myeloid neoplasm:

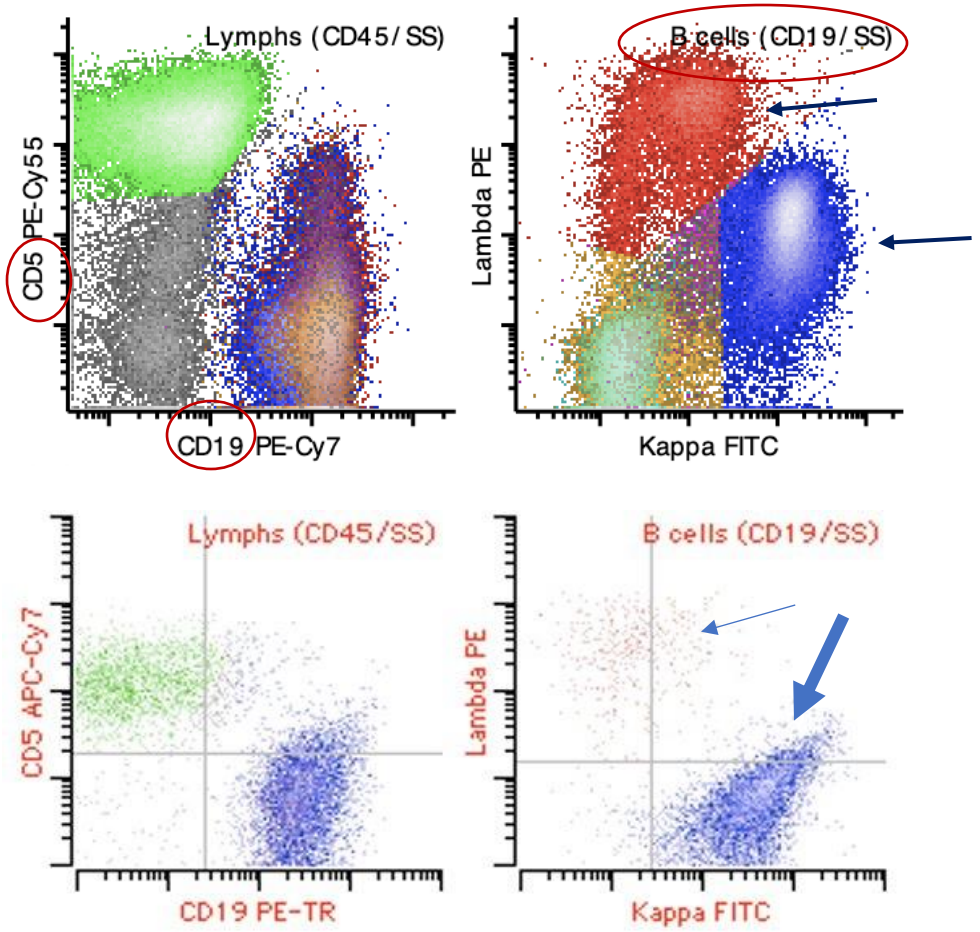
- CD34: Blast/stem cell marker
- CD117, CD33, CD13, MPO: Myeloid markers
- CD14, CD64: Monocytic marker
- Glycophorin A: Erythroid marker
- CD41, CD61: Meg marker

Markers usually used in immature lymphoid neoplasm:

- CD34, TdT, HLADR, CD10
- B cells: CD19, CD22, CD20
- T cells: CD3, CD2, CD5, CD7

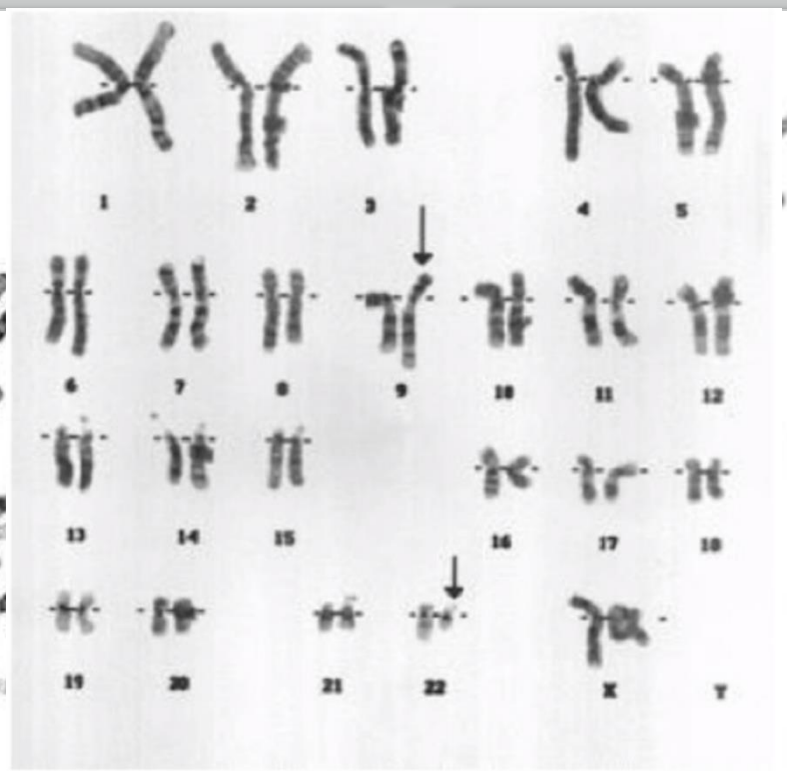
Immunophenotype

- B cells: CD19, CD20, Pax5
- B-cell clonality: Kappa or lambda light chains
- T cells: CD3, CD2, CD4, CD7, CD8
- **CD5+ abnormal B-cell: CLL, mantle cell lymphoma, diffuse large B-cell lymphoma.**
- **CD10+ abnormal B-cell: Follicular lymphoma, Burkitt's lymphoma, diffuse large B-cell lymphoma.**
- **CD5 and CD10 negative abnormal B-cell: marginal zone lymphoma, Hairy cell leukemia, diffuse large B-cell lymphoma.**

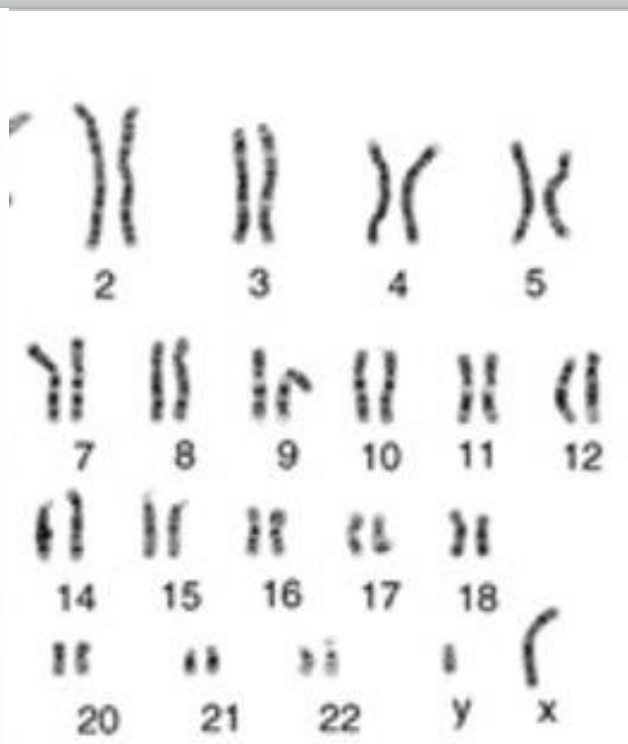


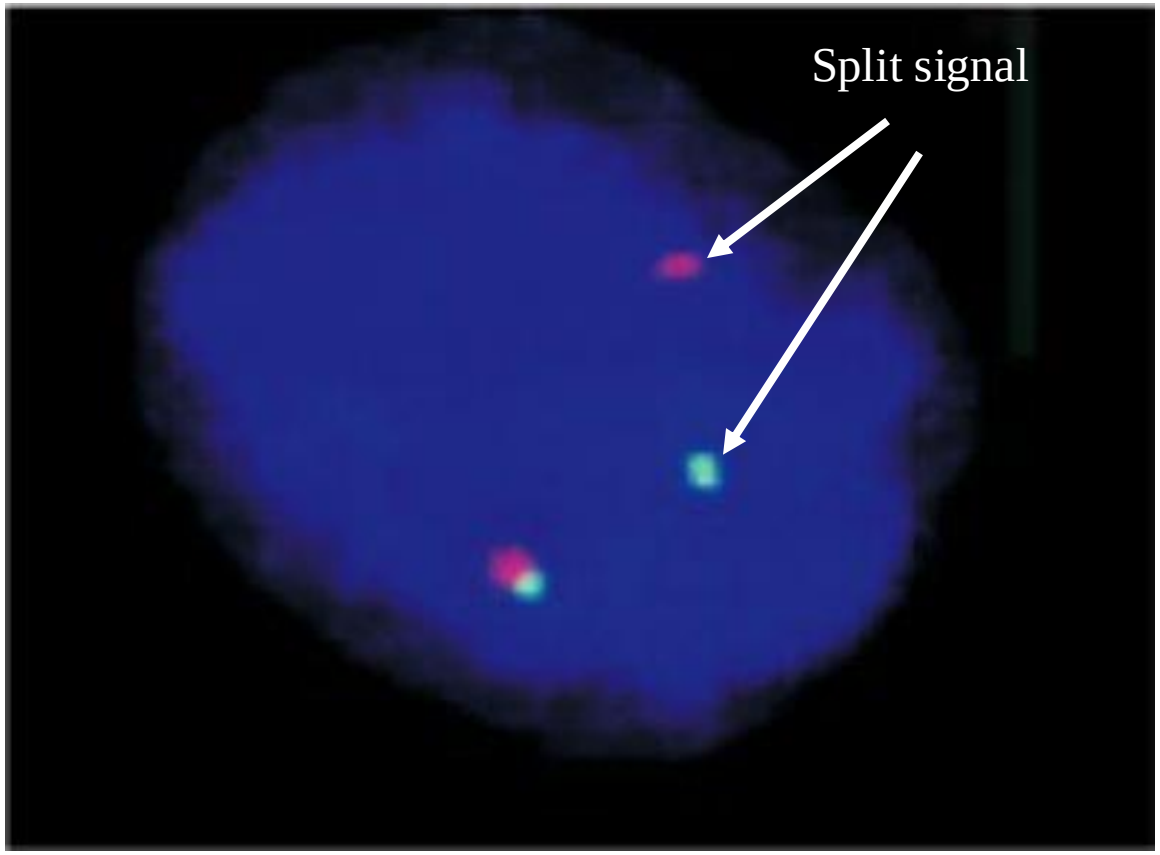
Genetics

- Karyotyping:
  - Requires dividing cells (culture 24-48 h) and review of metaphase spread
  - Identification of numeric and structural abnormalities
  - Low resolution and sensitivity



The abnormality seen by Nowell & Hungerford on chromosome 22, Now known as the Philadelphia Chromosome.





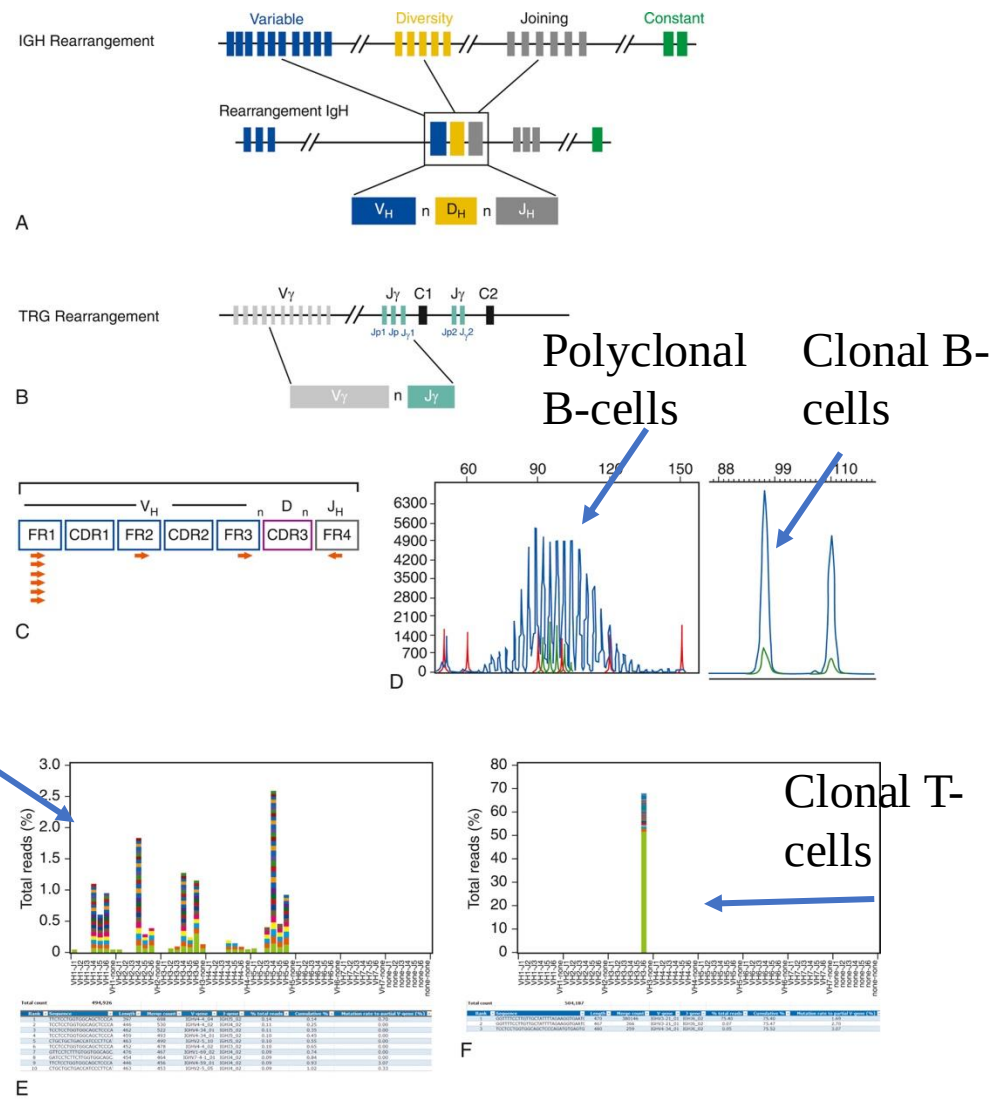
Genetics

- Fluorescent in situ hybridization (FISH)
  - Design probes for abnormal chromosomal structure (rearrangement, deletion, etc).
  - Faster than conventional karyotyping
  - Must know what you are looking for.

Genetic/Molecular

- Some of the non-Hodgkin B-cell lymphomas harbor translocation between IgH enhancer/promoter on Chr 14 and an oncogene/anti-apoptosis, resulting in overexpression of the gene:
  - Burkitt lymphoma: t(8;14)
    cmyc
  - Follicular lymphoma: (14;18)
    Bcl2
  - Mantle cell lymphoma: (11;14)
    cyclin D1

Clonality



Henry's Clinical Diagnosis

Genetics

- Molecular studies (often PCR based):
  - Fast TAT
  - Very sensitive
  - Recent large panels (myeloid or lymphoid) allow for screening of many genes using high throughput sequencing.