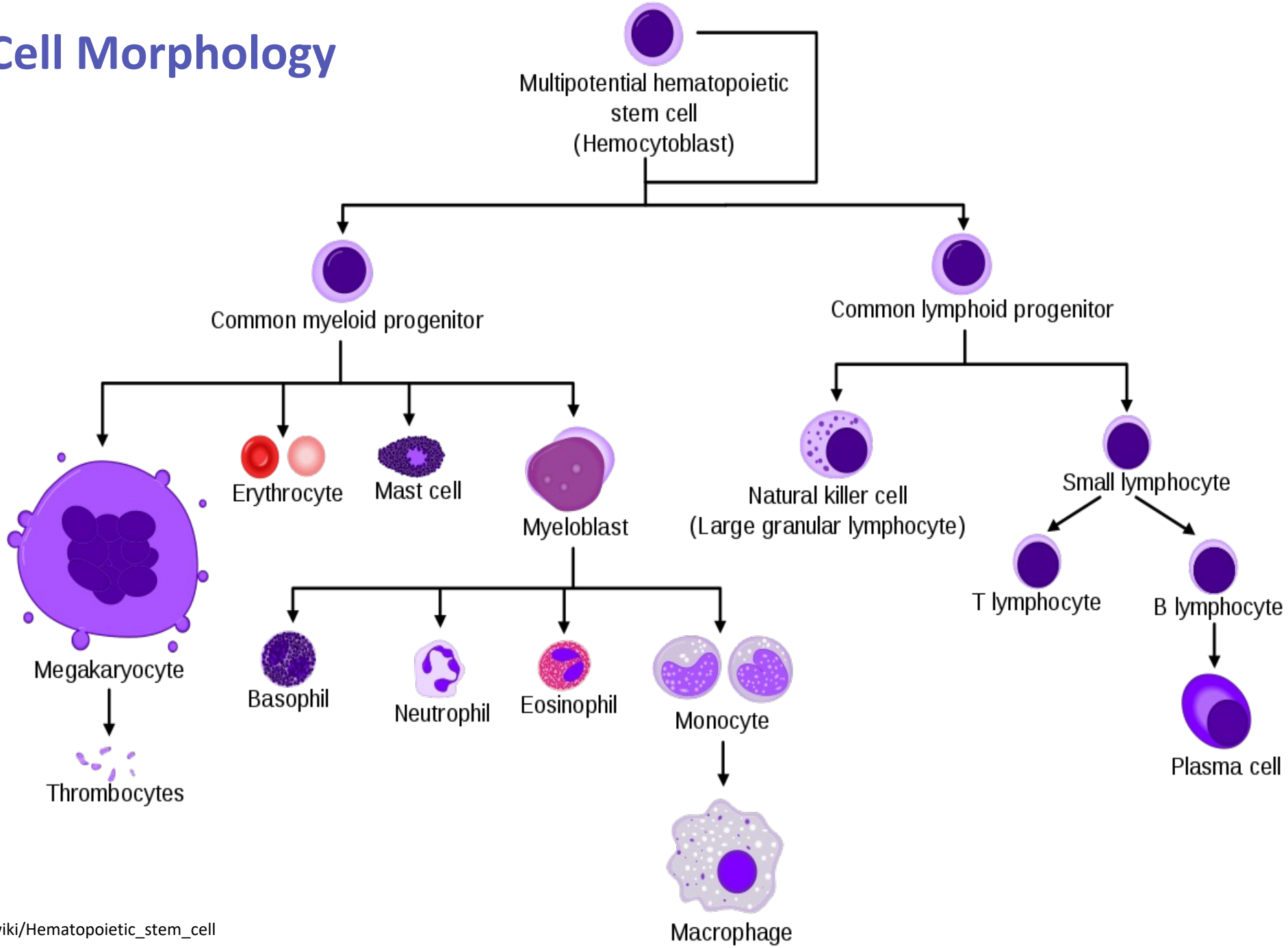
ACUTE LYMPHOID LEUKEMIA
Introductory Lecture

OVERVIEW

- Blood Cell Morphology
- Lab Techniques
- ALL
- Review Handouts

Blood Cell Morphology

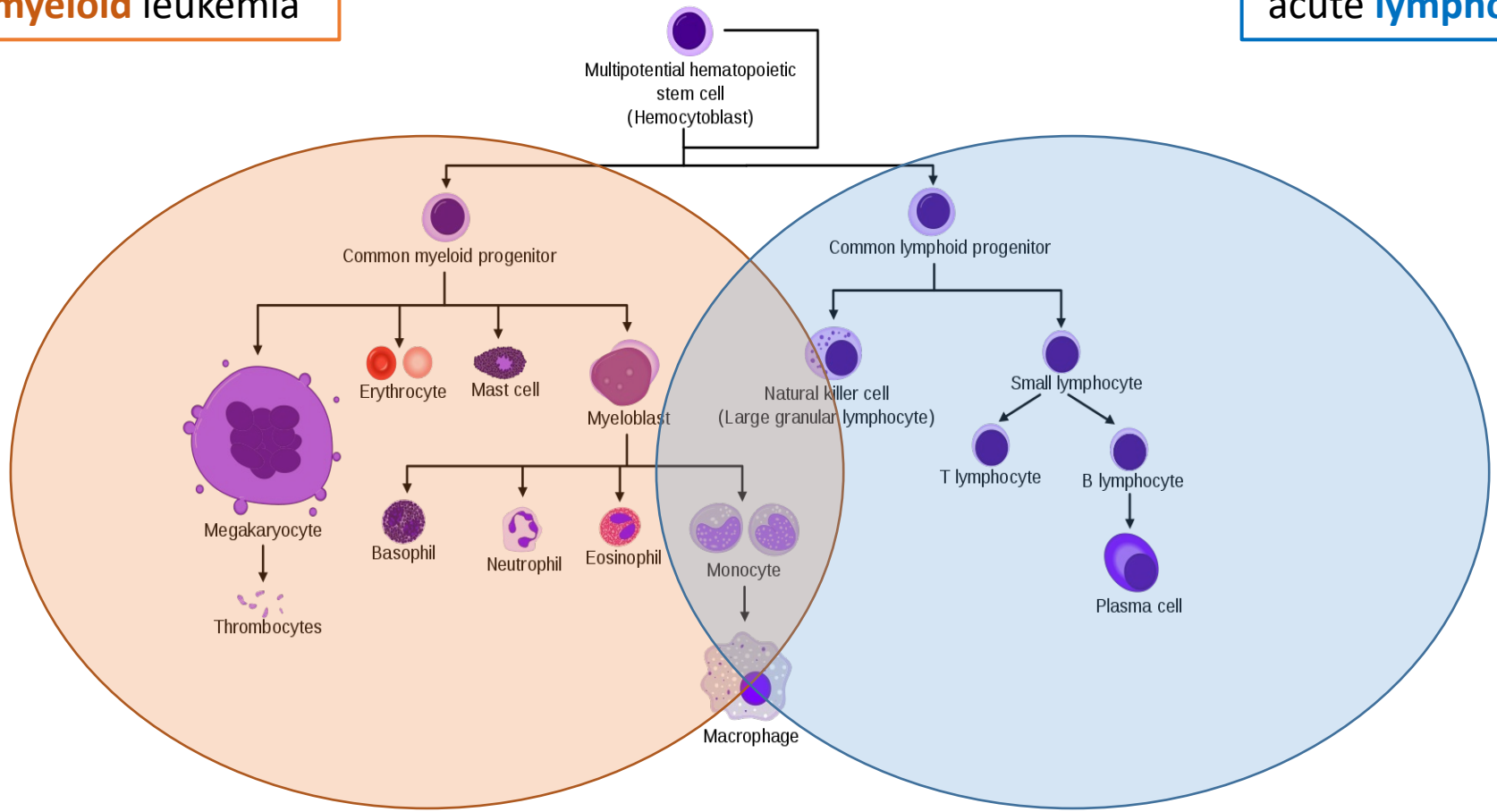


https://en.wikipedia.org/wiki/Hematopoietic_stem_cell

Types of Acute Leukemia

acute **myeloid** leukemia

acute **lymphoid** leukemia



Types of Acute Leukemia

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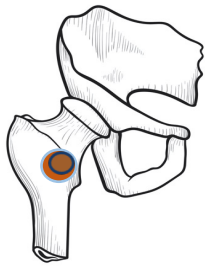
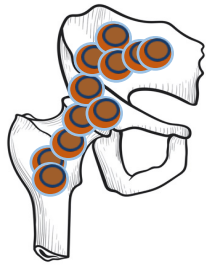
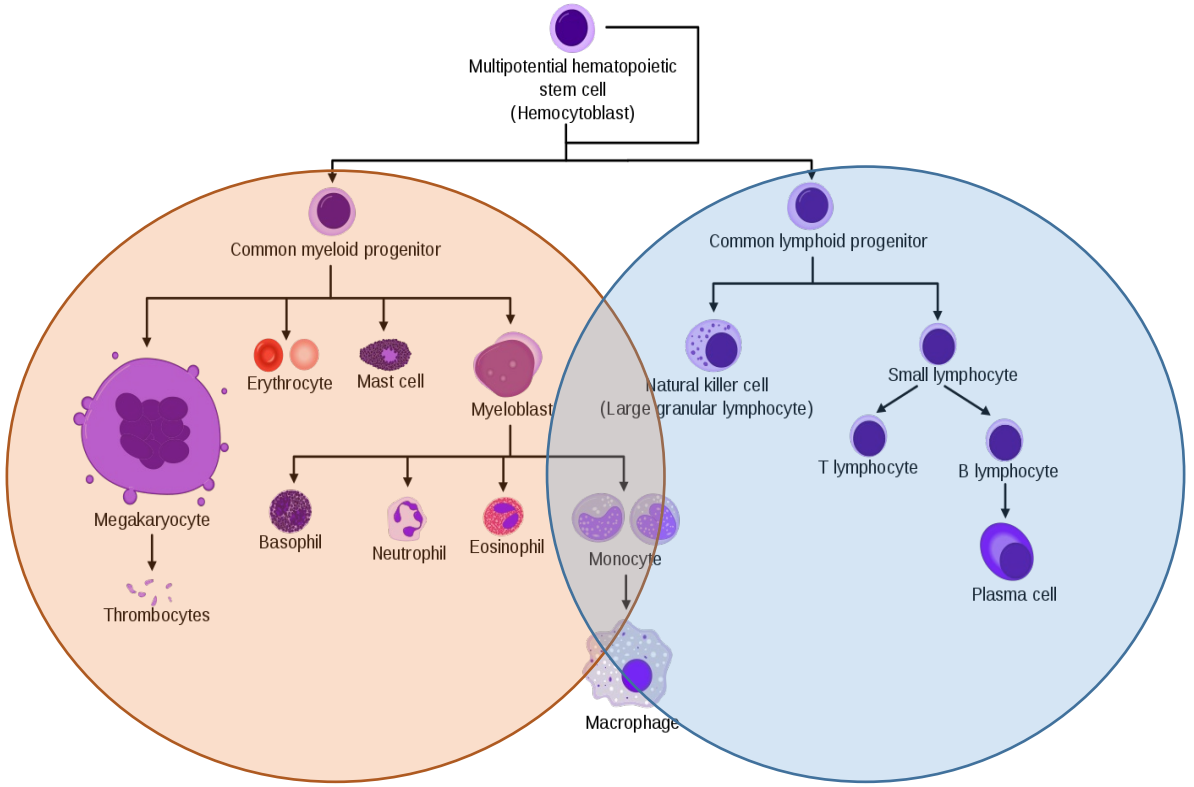


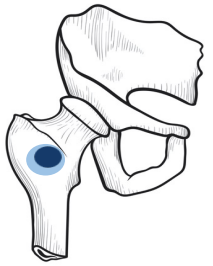
normal bone marrow



excess proliferation of myeloid cells



acute **lymphoid** leukemia



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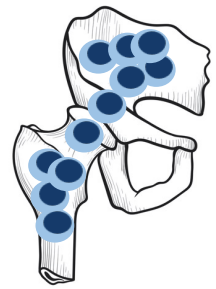
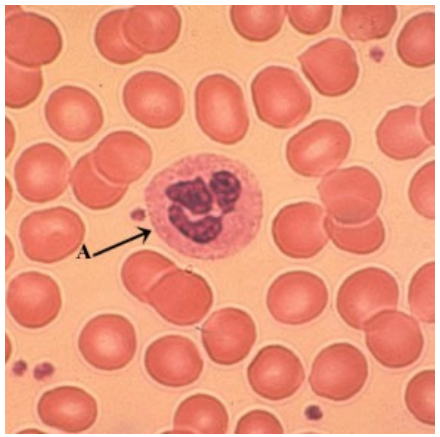


excess proliferation of lymphoid cells

PERIPHERAL BLOOD SMEARS

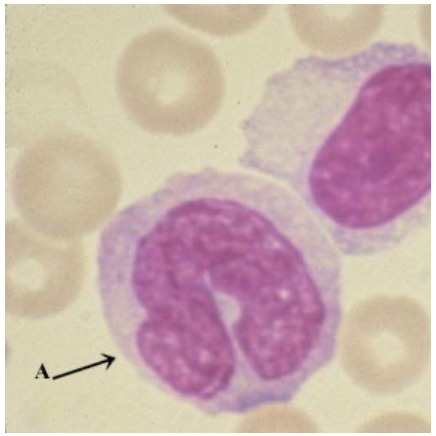
Mature Myeloid Cells

(A) Neutrophil

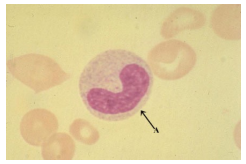


- Most common peripheral WBC
- Average 3-lobed nucleus
- >6 lobes = hyper-segmented
- Thin chromatin strand between lobes

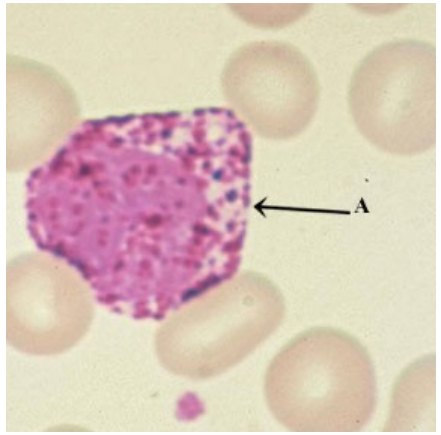
(A) Monocyte



- Horse-shoe/kidney shaped nucleus
- Gray-blue cytoplasm
- Sometimes vacuoles
- Can look like a band

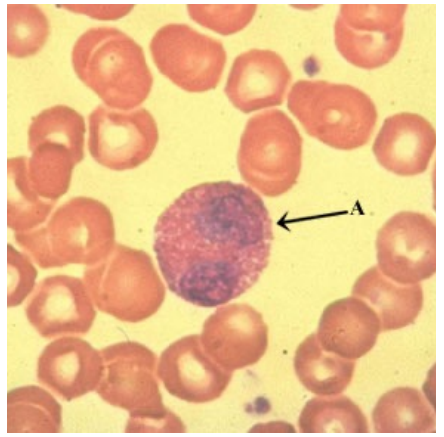


(A) Basophil



- Bi-lobed nucleus
- Large purple/black granules

(A) Eosinophil

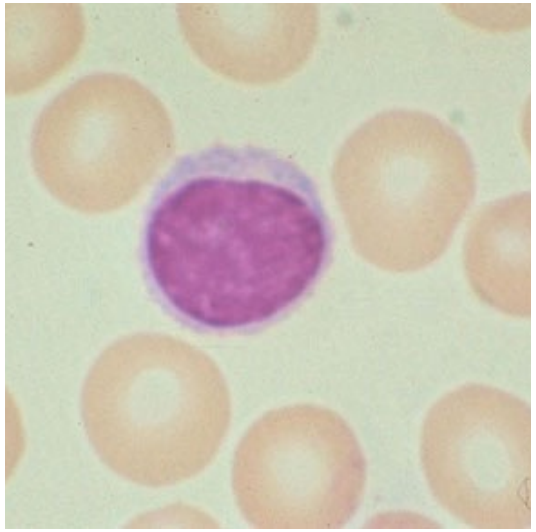


- Bi-lobed nucleus
- Red/orange cytoplasmic granules

Courtesy of Dr. Scigliano's Online Morphology Tutorial

Mature Lymphoid Cells

Mature Lymphocyte

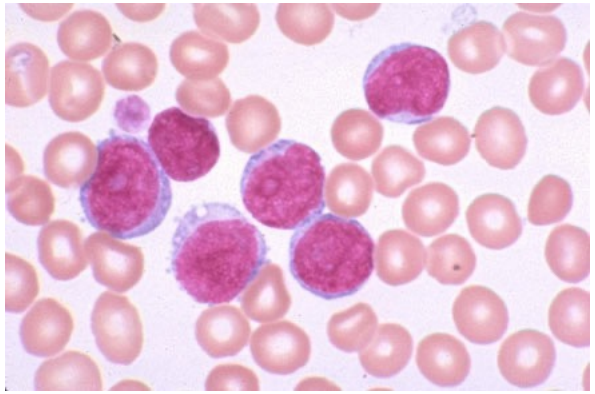


- Size slightly bigger than surrounding RBCs
- Large round/oval nucleus
- Slightly eccentric nucleus
- Thin rim blue cytoplasm

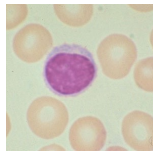
Courtesy of Dr. Scigliano's Online Morphology Tutorial

Immature Blasts

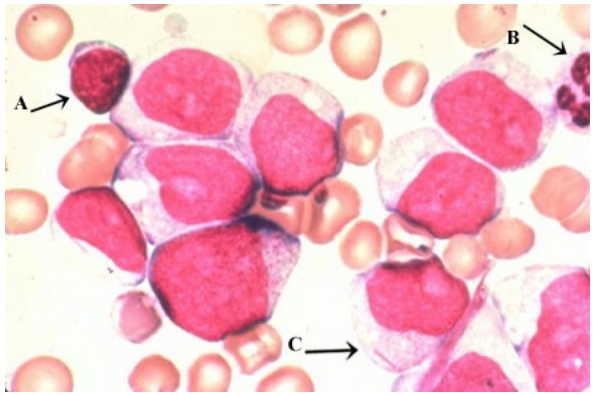
Blasts (AML or ALL)



- Larger than mature lymphocyte/RBC
- High nuclear:cytoplasmic ratio
- Nucleoli
- Fine chromatin
- Basophilic cytoplasm

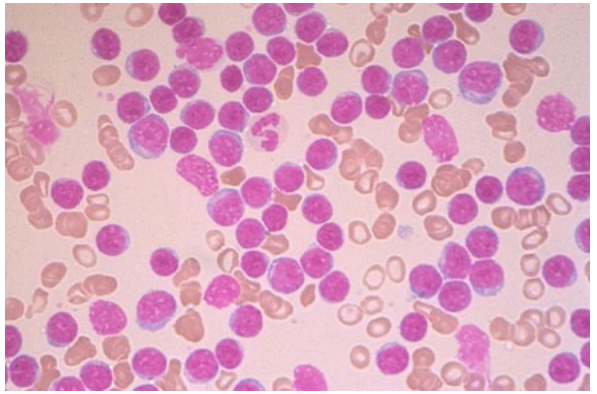


Myeloblasts (APML)



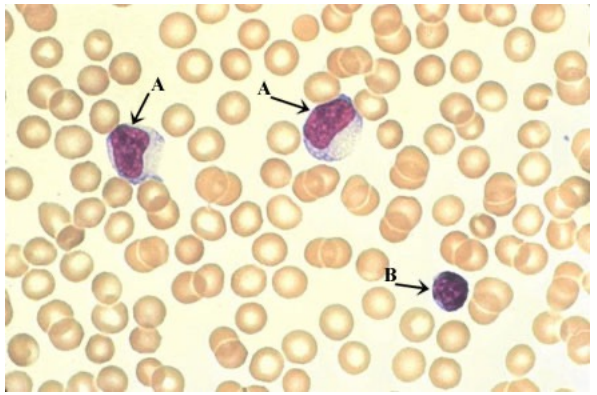
- (c) = auer rods
- Linear granules in cytoplasm of myeloblasts
- NOT seen in lymphoblasts
- Cannot distinguish between ALL and AML on peripheral smear UNLESS auer rods present

Mature Lymphocytes (CLL)



- Increased number of mature, small lymphocytes
- "Soccer ball" nuclear pattern

Atypical Lymphocytes (viral infection)



- Larger than mature lymphocyte/RBC
- Cytoplasm appears idented by RBCs
- Nucleus immature, large, convoluted
- Sometimes azurophilic granules

Courtesy of Dr. Scigliano's Online Morphology Tutorial

LABORATORY TESTS

Lab Techniques

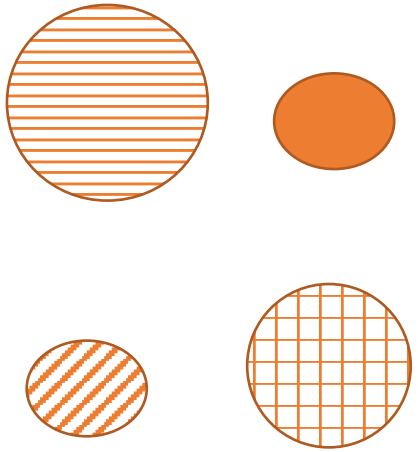
Flow Cytometry

Karyotyping

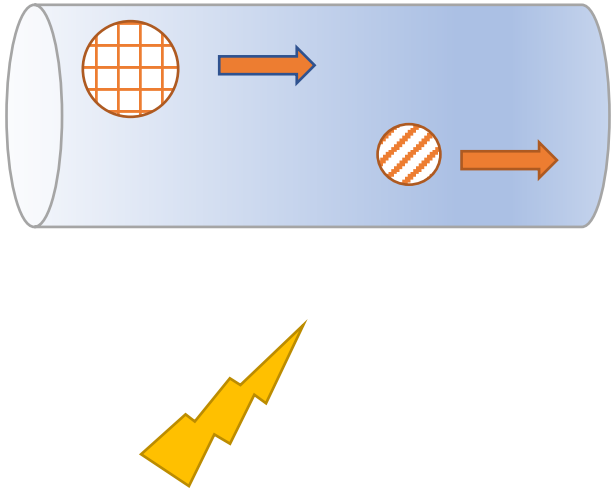
FISH

Genetic Sequencing

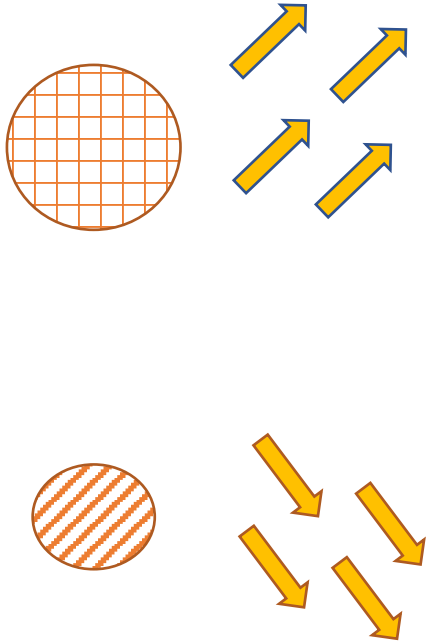
Flow Cytometry



Different blood cell types have unique sizes and granularities

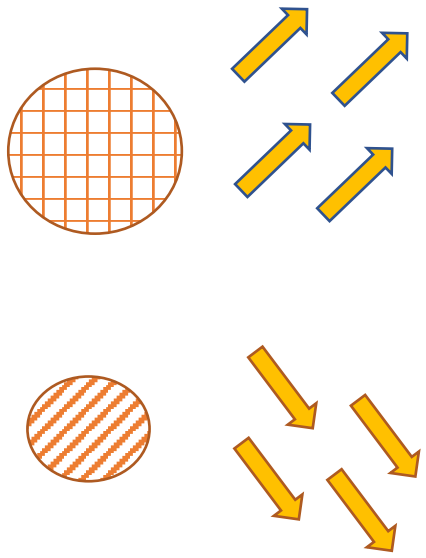


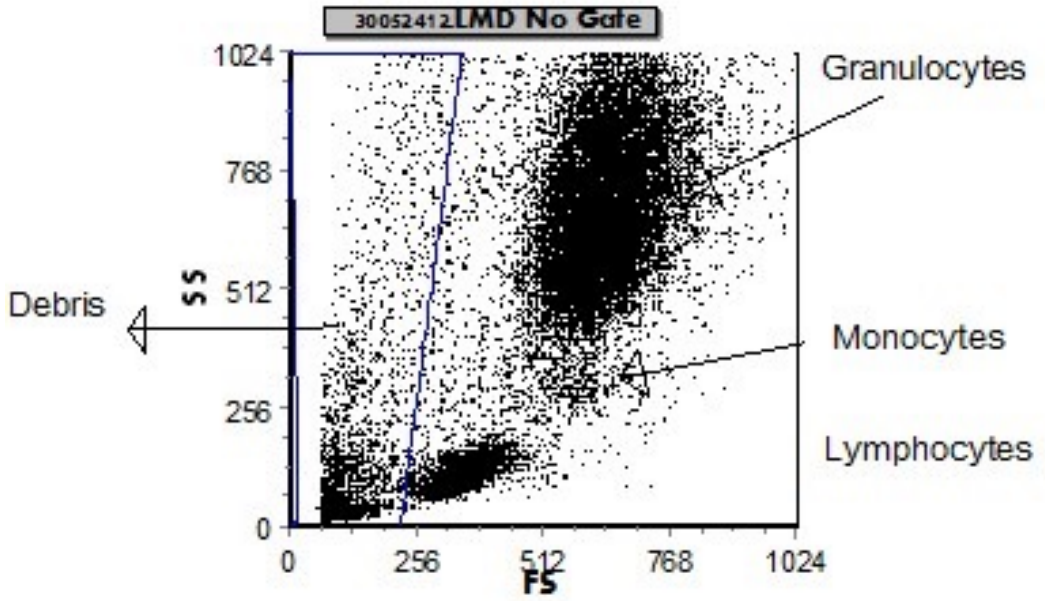
these cells travel through a flow cytometry machine which shoots a laser beam at the cells



Unique cell sizes and granularities causes unique patterns of light scatter from the laser beam

Flow Cytometry



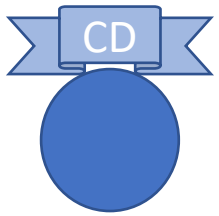


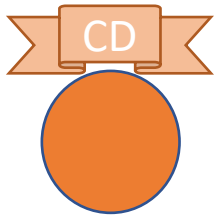
flow cytometry scatter data output is mapped by:

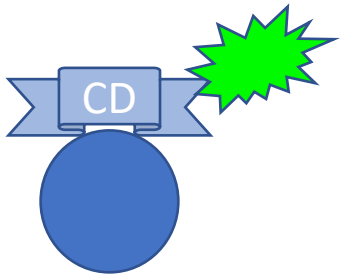
FS “forward light scatter”
SS “side light scatter”

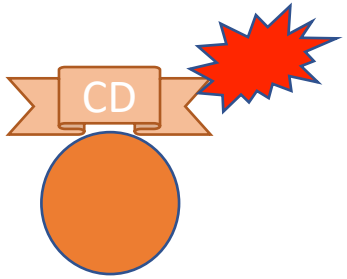
FS and SS data can be graphed to identify different cell populations

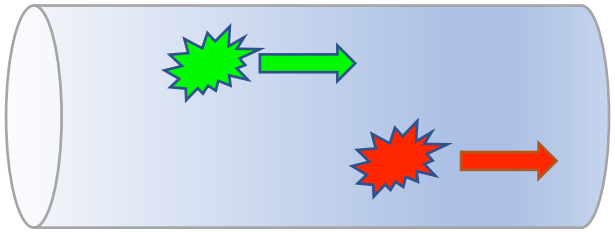
Flow Cytometry









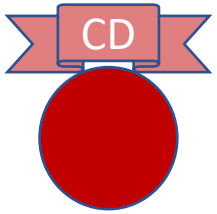


blood cells have identifying markers on their cell surface = CD “cluster of differentiation”

these CD markers can be tagged with fluorescently labeled monoclonal antibodies

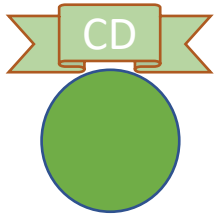
These fluorescent tags can be picked up on flow cytometry

Flow Cytometry



some Myeloid CD Markers

- CD11
- CD13
- CD14
- CD33
- CD64



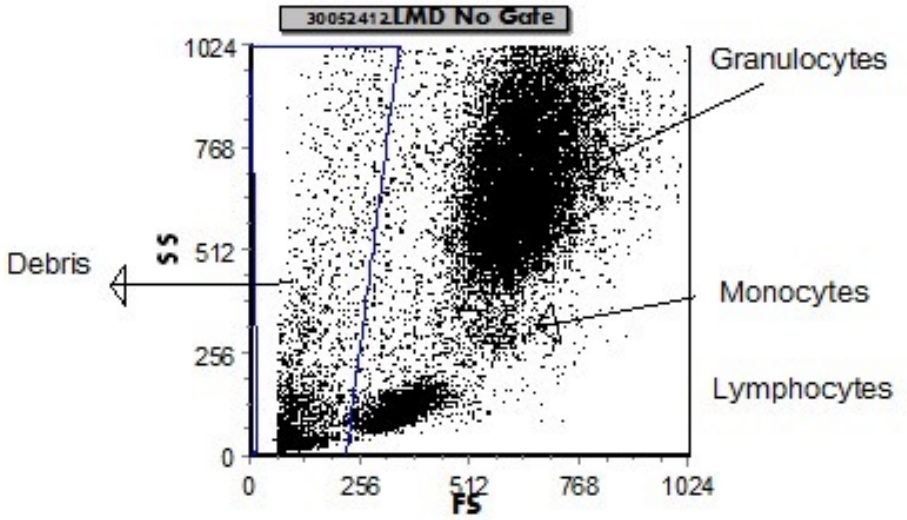
some B Lymphoid CD Markers

- CD10
- CD19
- CD20
- CD22

some T Lymphoid CD Markers

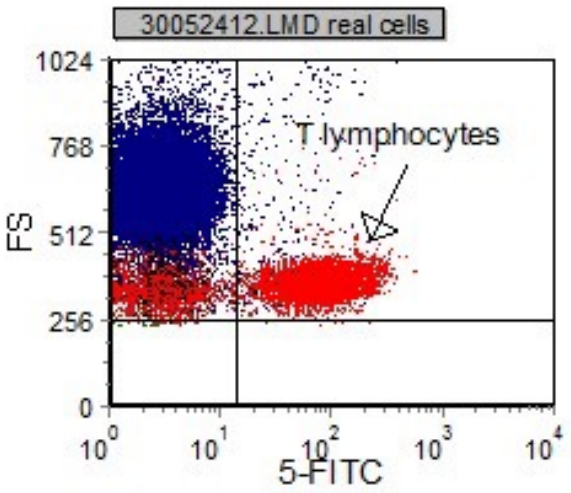
- CD3
- CD4
- CD7
- CD8

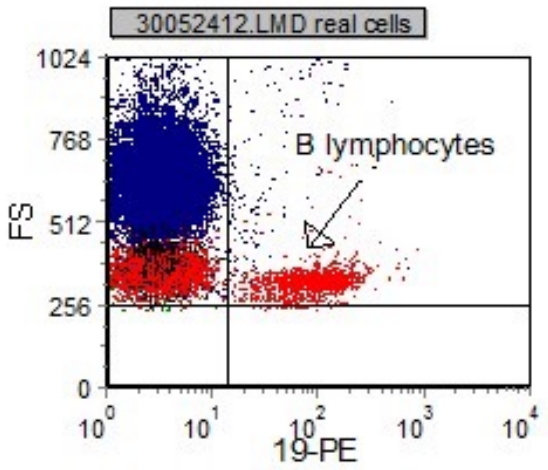
Flow Cytometry



CD5+ T cells

CD19+ B cells





Flow cytometry data tells you what cell type you are looking at: granulocyte, monocyte, lymphocyte…

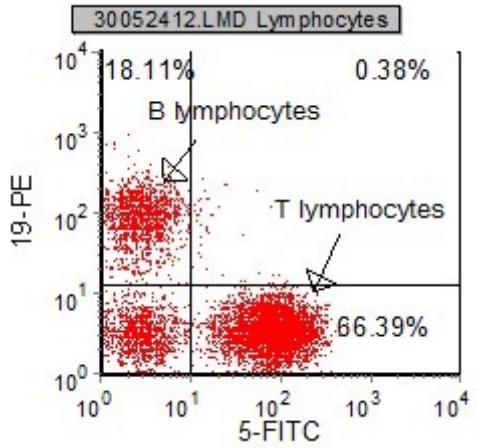
As well as what CD markers each cell type has: B cell markers, T cell markers, myeloid cell markers…

Taken together, can tell you what percentage of lymphocytes are B cells and identify monoclonal cell populations

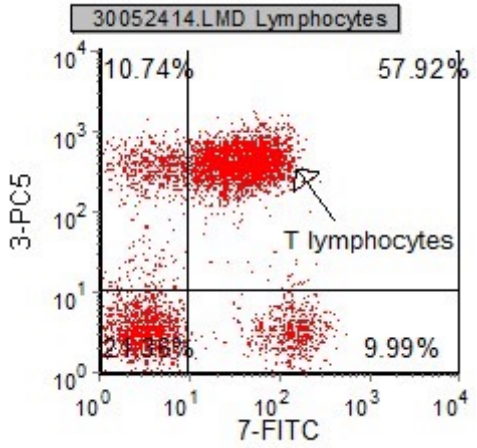
https://wiki.clinicalflow.com/introduction-flow-cytometric-analysis

Flow Cytometry

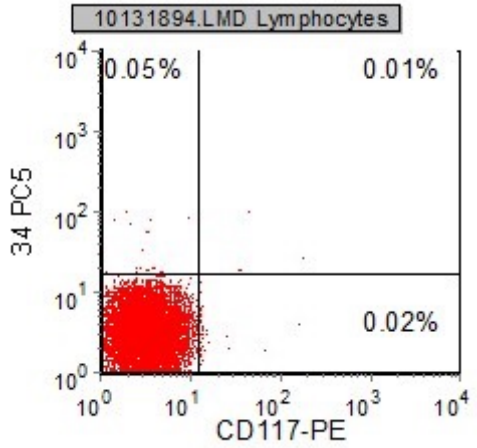
Multiple CD markers can be tagged and analyzed at one time



Mutually Exclusive Markers
CD5 and CD19 stain different populations



Co-Expression Markers
CD7 and CD3 stain the same population

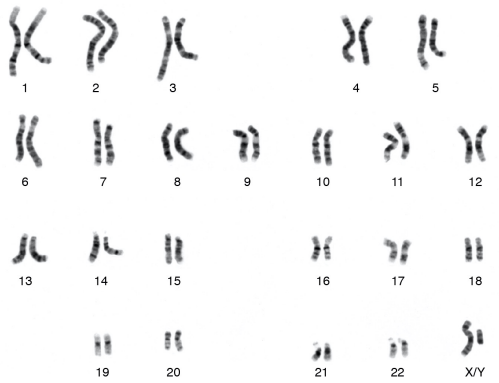


Non-Expression Markers
CD117 and CD34 stain neither population

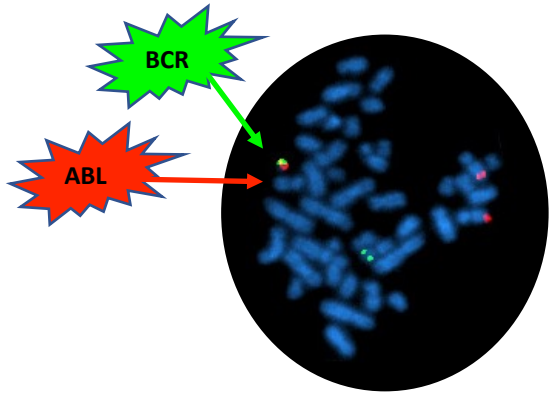
https://wiki.clinicalflow.com/introduction-flow-cytometric-analysis

Cytogenomics

1. Karyotyping

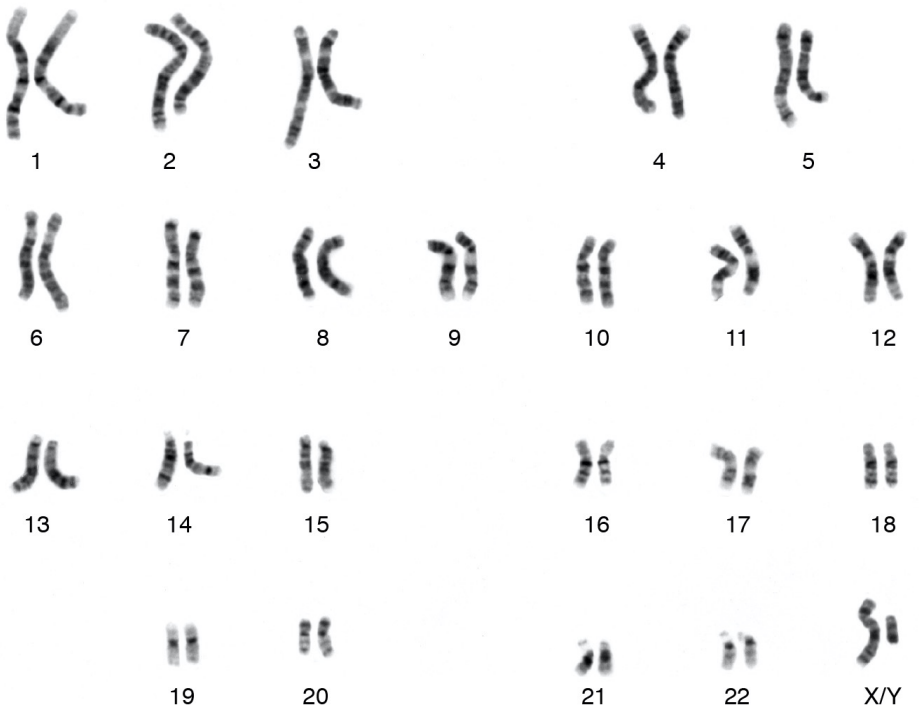


2. FISH



https://www.genome.gov/genetics-glossary/Karyotype

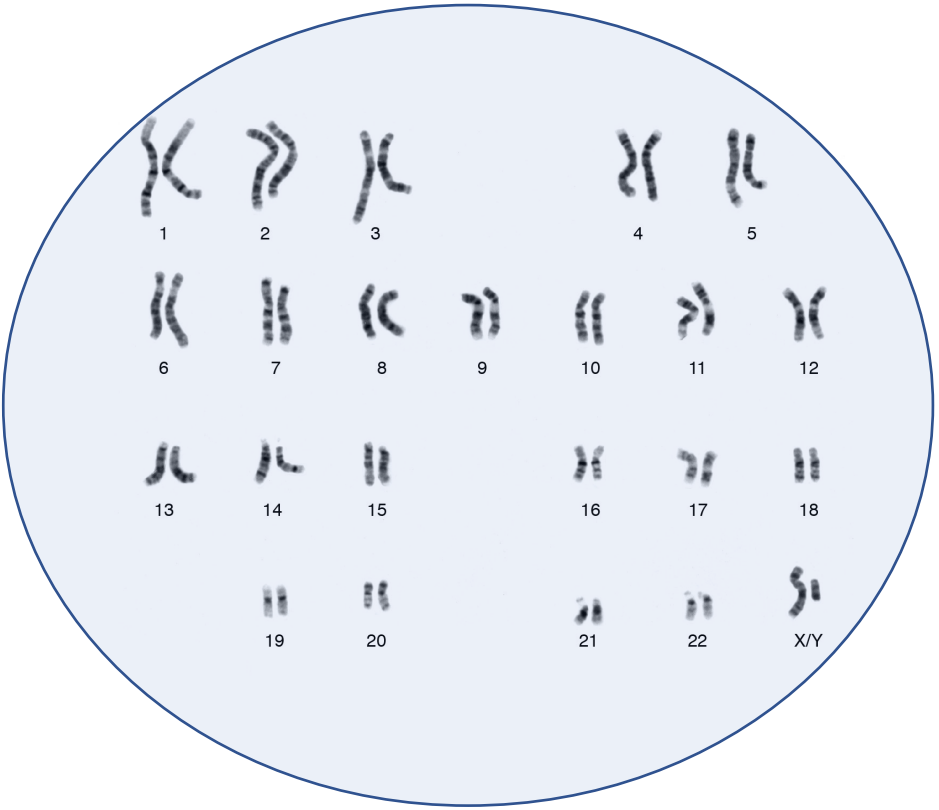
Karyotyping



Karyotyping = visual inspection of metaphase (condensed) chromosomes

https://www.genome.gov/genetics-glossary/Karyotype

Karyotyping

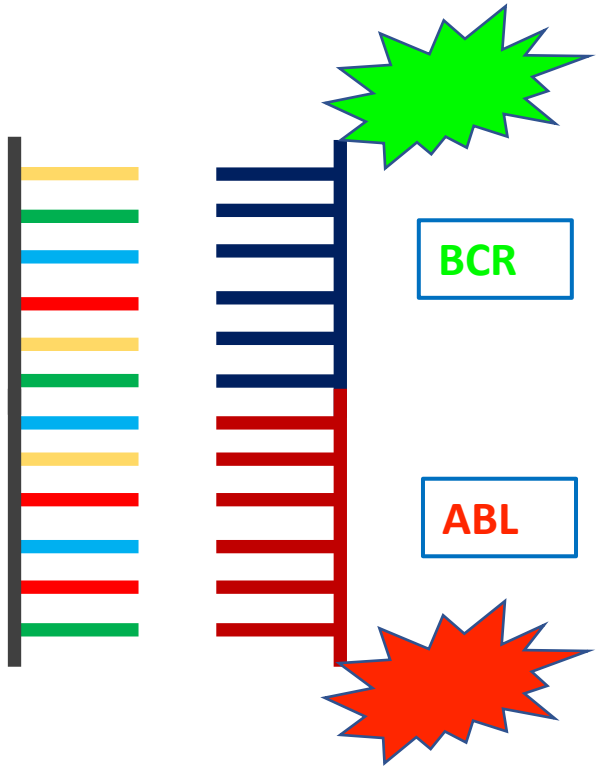


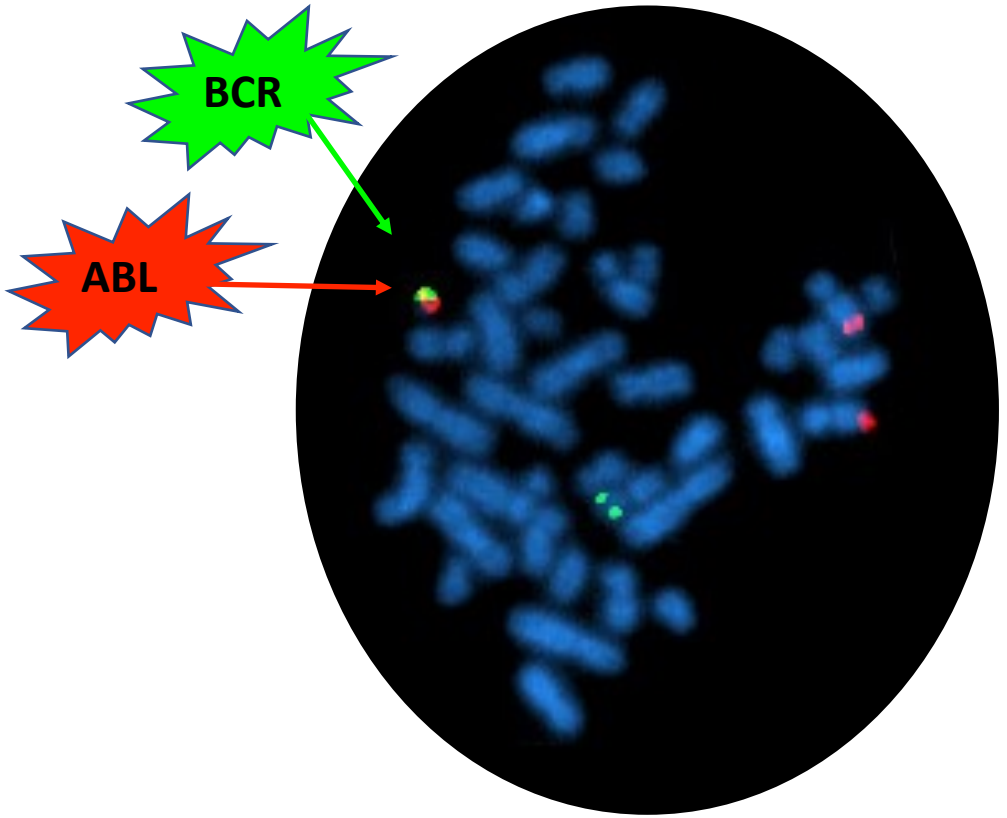
Monosomy	Absence of a single chromosome
Trisomy	Gain of a single chromosome
Deletions	Deletion of part of a chromosome
Duplications	Duplication of a piece of a chromosome
Translocation	Movement of a piece of a chromosome to another area

46 Chromosomes:
22 autosomal chromosome pairs
1 sex chromosome pair (XX or XY)

https://www.genome.gov/genetics-glossary/Karyotype

Fluorescence In-Situ Hybridization = FISH

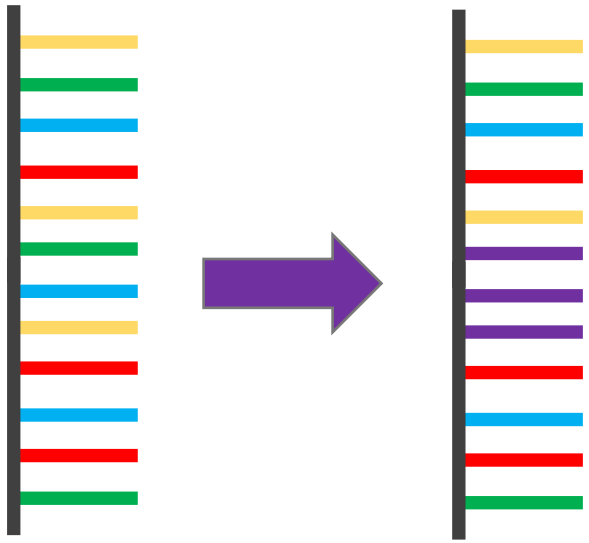




Fluorescently tagged DNA probes can detect targeted DNA sequences

FISH can only detect KNOWN targets with KNOWN DNA probes

Next Generation Sequencing = NGS



Gene X

Substitution in Gene X

Gene Sequencing sequences specific genes and detects ANY gene mutations

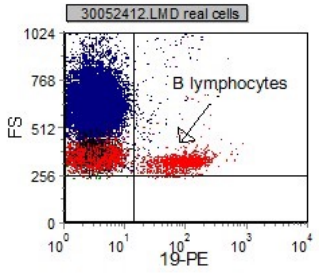
TCGACTA → CGTCCTA

Substitution	Substitution of one nucleotide for another
Insertion	Insertion of additional nucleotide
Deletion	Deletion of additional nucleotide

** **Germline mutation = INHERITED:** present at birth, present in gametes
** **Somatic mutation = NOT INHERITED:** acquired during lifetime, present in certain cells

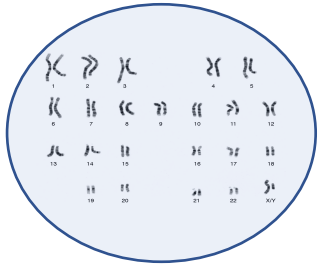
Lab Technique Review

Flow Cytometry



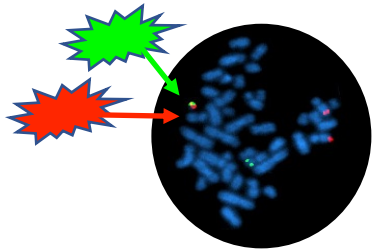
Cell shape/size and CD marker identifies the cell population

Karyotyping



Visual inspection of metaphase chromosomes reveals large gene changes

FISH



Fluorescently tagged DNA probes can detect target DNA sequences

Genetic Sequencing

GGCCTAA → GGTCCAA

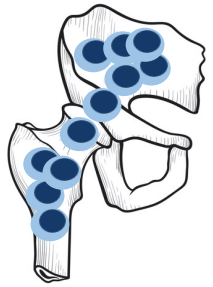
NGS sequences specific genes and detects ANY gene mutations

ACUTE LYMPHOCYTIC LEUKEMIA

ALL Pathology

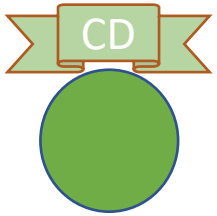
1) RISK FACTORS
- Age (childhood, > 50)
- Radiation Exposure
- Chemical Exposure
- Prior Chemo
- Congenital/Inherited

2) GENETIC MUTATION
a genetic abnormality causes proliferation of lymphoid progenitor cells



3) ABNORMAL PROLIFERATION
These lymphoid progenitor cells take over the bone marrow, crowding it and inhibit production of other BM produced cells (RBCs, platelets)

ALL can be B-cell or T-cell



some B Lymphoid CD Markers

CD10
CD19
CD20
CD22

some T Lymphoid CD Markers

CD3
CD4
CD7
CD8

ALL Presentation

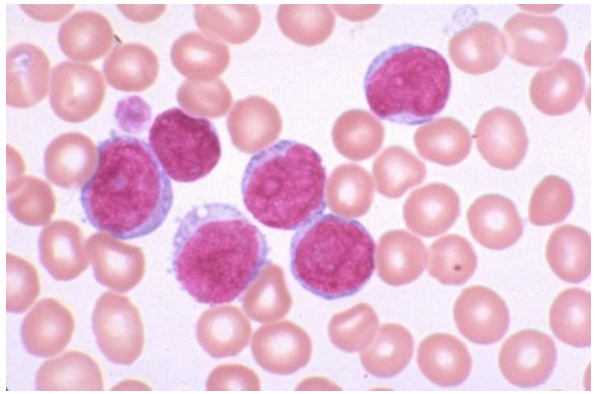
Presentation = Similar to AML →

ALSO can present as primary:
(1) Mediastinal Mass
(2) Testicular Mass

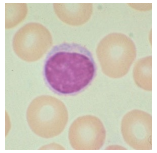
PRIMARY DISORDER	SYMPTOM/LAB FINDING
Leukocytosis/Leukopenia	**Infections/Fever** **Fatigue** **Peripheral blasts**
Anemia Myelophthisic	**Fatigue** **Pallor** **SOB** **Peripheral teardrop RBCs**
Thrombocytopenia Myelophthisic	**Petechiae** **Mucocutaneous bleeding**
Leukemic Cell Organ Infiltration	**Bone infiltration → Pain** **Skin infiltration → Rash/Leukemia cutis** **Liver/Kidney → liver/kidney dysfunction** **CNS → HA, neuropathy**
DIC Activation of the clotting cascade	**INR/PTT, D-dimer elevation** **Thrombocytopenia, Low fibrinogen** **Low Factor levels (including F8)** **Increased bleeding/clotting**
TLS Increased leukemic cell turnover	**Hyperkalemia** **Hyperuricemia** **Hyperphosphatemia** **Hypocalcemia**
Leukostasis Increased viscosity, endothelial damage, cytokine release	**HA, neuropathy, visual changes, tinnitus** **SOB/respiratory failure, MI**

ALL Peripheral Smear

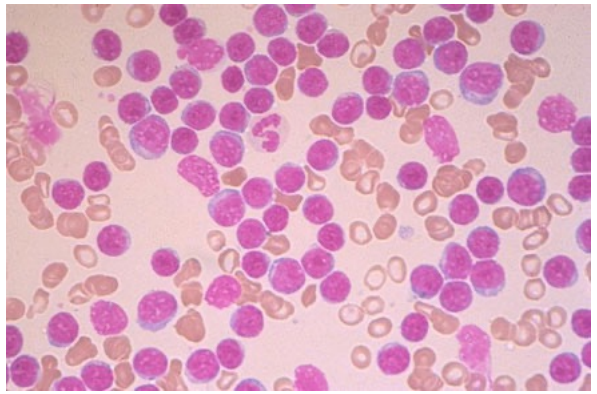
Blasts
(AML or ALL)



- Larger than mature lymphocyte/RBC
- High nuclear:cytoplasmic ratio
- Nucleoli
- Fine chromatin
- Basophilic cytoplasm



Mature Lymphocytes
(CLL)



- Increased number of mature, small lymphocytes

CBC DIFF on PERIPHERAL BLOOD

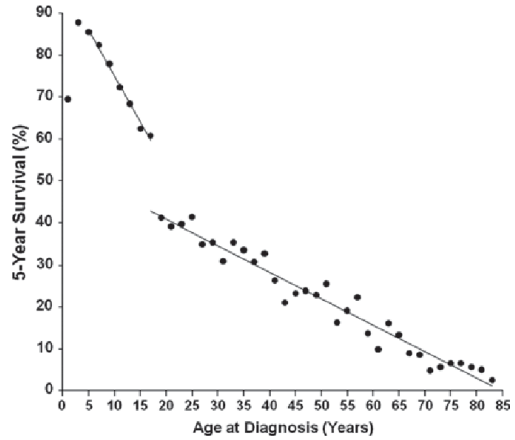
- Peripheral blasts
  * may be read as “other”
- Lymphocytosis

ALL Pathology

Bad Clinical Prognostic Factors

Age

- Worse survival for older adults



WBC

B cells > 30K

T cells > 100K

Early T/B Cells

CNS disease

MRD at end of induction

Bad Genetic Prognostic Factors

Philadelphia chromosome t(9;22)

Ph-Like

t(4;11)

Complex cytogenetics

Prognosis By Ph-Status:

Ph- ALL: 5Y EFS 50%

Ph+ ALL: 5Y EFS 20%

ALL Treatment

INDUCTION

Goal = achieve **remission**

* remission = no leukemia cells in bone marrow

Chemotherapy

+ CNS prophylaxis

CONSOLIDATION

Goal = maintain **remission**

Low Risk

Chemotherapy

Intermediate or High Risk

Allogeneic SCT

ALL Treatment

Treatment is Stratified by Philadelphia Chromosome

Ph-

CHEMOTHERAPY

Ph+

CHEMOTHERAPY + TYROSINE KINASE INHIBITOR

* in older patients can consider TKI/steroids alone

ALL Chemotherapy

MULTI-AGENT CHEMOTHERAPY REGIMENS:
there are many institution-dependent chemotherapy regimens, a few examples below

- CALGB-10403
- HyperCVAD
- GRAALL-2003
- EWALL
- ECOG-2993
- GMALL
- BFM

CALGB-10403
* commonly used at MSH

- Peg-asparaginase
  * **Monitor fibrinogen/AT3 level**
  * **Can't use Posaconazole with PEG**
- Vincristine
- Daunorubicin
- Prednisone
- IT Methotrexate
- IT Cytarabine

HyperCVAD
* commonly used at MSH

- **C**yclophosphamide
- **V**incristine
- Doxorubicin (**A**driamycin)
- **D**examethasone
- IT Methotrexate
- IT Cytarabine

*** Regimens include IT Chemo: Intrathecal CNS prophylaxis**

ALL Other Agents

Tyrosine Kinase Inhibitors

Ph+

ADD TKI

Antibodies

CD19, CD20, or CD22

ADD CD-specific Antibody

ALL TKI

TYROSINE KINASE INHIBITORS: inhibit the Philadelphia chromosome, only in Ph+

First Generation TKI

Imatinib	QTC Rash Diarrhea Muscle cramps Fluid Retention

Second Generation TKI

Dasatinib * **Penetrates CNS**	QTC Pleural Effusion Pericardial Effusion Pulmonary HTN Thrombocytopenia
Nilotinib	QTC Pancreatitis Hyperglycemia Hyperlipidemia GI/Liver toxicity
Bosutinib	Rash Diarrhea GI/Liver toxicity

Third Generation TKI

Ponatinib	QTC Thrombosis CHF Liver toxicity Pancreatitis Fluid retention

* These TKIs also used in CML

ALL Antibodies

Rituximab = CD20+ antibody, in CD20+ B-cell ALL

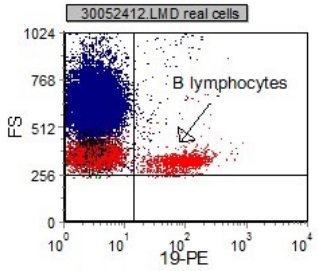
Blinatumomab = Bi-specific T-cell Engager (BiTE), targets CD3/CD19
* Toxicities include cytokine release syndrome

Inotuzumab = CD22+ (in relapse)

ALL Review Handout

Lab Techniques

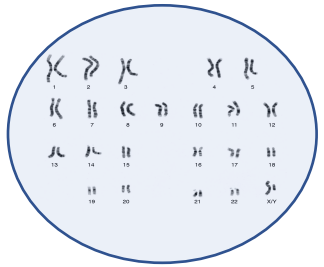
Flow Cytometry



Cell shape/size and CD marker identifies the cell population

Blood or Bone Marrow Flow Cytometry Result:
There is a 20% abnormal CD20+ population

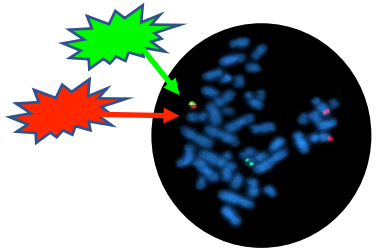
Karyotyping



Visual inspection of metaphase chromosomes reveals large gene changes

Karyotype result:
There is trisomy 21

FISH



Fluorescently tagged DNA probes can detect target DNA sequences

FISH result:
There are X copies of a BCR-ABL translocation

NGS

GGCCTAA → GGTCCAA

Gene Sequencing sequences specific genes and detects ANY gene mutations

NGS result:
There is a FLT3 mutation

ALL Diagnosis/Prognosis

B cell	T cell
CD10	CD3
CD19	CD4
CD20	CD7
CD22	CD8

Bad Clinical Prognostic Factors	Bad Genetic Prognostic Factors
Age	Philadelphia chromosome T(9,22)
B cells > 100K T cells > 30K	Ph-Like
Early B/T cells	T(4,11)
CNS disease	Complex cytogenetics
MRD	

ALL Treatment

Ph- = Chemotherapy

CALGB-10403

- Peg-asparaginase
  * Monitor fibrinogen/AT3 level
  * Can't use Posaconazole with PEG
- Vincristine
- Daunorubicin
- Prednisone
- IT Methotrexate
- IT Cytarabine

Ph+ = Chemotherapy + TKI

HyperCVAD

- **C**yclophosphamide
- **V**incristine
- Doxorubicin (**A**driamycin)
- **D**examethasone
- IT Methotrexate
- IT Cytarabine

TKIs

Ph+ = Chemotherapy + TKI

Imatinib (1st TKI)	QTC Rash Diarrhea Muscle cramps Fluid Retention
Dasatinib (2nd TKI) * Penetrates CNS	QTC Pleural Effusion Pulmonary HTN Thrombocytopenia
Nilotinib (2nd TKI)	QTC Pancreatitis Hyperglycemia Hyperlipidemia GI/Liver toxicity
Bosutinib (2nd TKI)	Rash Diarrhea GI/Liver toxicity
Ponatinib (3rd TKI)	QTC Thrombosis CHF Liver toxicity Pancreatitis Fluid retention