Lymphoma

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Objectives

- Introduction of some important terminologies.
- Know the major subtypes of lymphoma.
- Know the typical presentation of the major subtypes.

Lymphomas

Not only they all look the same but also…

Table 21–3 2008 World Health Organization classification of B-cell and T-cell neoplasms

B-cell neoplasms	T-cell neoplasms
Precursor B-cell neoplasms*	**Precursor T-cell neoplasms***
B-lymphoblastic leukemia/lymphoma NOS	T-lymphoblastic leukemia/lymphoma
B-lymphoblastic leukemia/lymphoma with recurrent genetic abnormalities	
Mature B-cell neoplasms	**Mature T-cell neoplasms**
Aggressive lymphomas	*Leukemic or disseminated*
Diffuse large B-cell lymphomas: variants, subgroups, and subtypes/entities	T-cell large granular lymphocytic leukemia†
Diffuse large B-cell lymphoma, NOS	Chronic lymphoproliferative disorders of NK cells†
Common morphologic variants: centroblastic, immunoblastic, anaplastic	T-cell prolymphocytic leukemia
Rare morphologic variants	Aggressive NK-cell leukemia
Molecular subgroups: germinal center B-cell like (GCB) and activated B-cell like (ABC)	Adult T-cell leukemia/lymphoma
Immunohistochemical subgroups: CD5+ DLBCL, GCB, and non-GCB	Systemic EBV-positive T-cell lymphoproliferative disorders of childhood
Diffuse large B-cell lymphoma subtypes	*Extranodal*
T-cell/histiocyte-rich large B-cell lymphoma	Extranodal NK/T-cell lymphoma, nasal type
Primary DLBCL of the CNS	Enteropathy-type T-cell lymphoma
Primary cutaneous DLBCL, leg type	Hepatosplenic T-cell lymphoma
EBV-positive DLBCL of the elderly	
Other lymphomas of large B cells	*Cutaneous*
Primary mediastinal large B-cell lymphoma	Mycosis fungoides†
Intravascular large B-cell lymphoma	Sézary syndrome†
DLBCL associated with chronic inflammation	Primary cutaneous CD30+ T-cell lymphoproliferative disorder†
Immunodeficiency-associated lymphoma	Primary cutaneous CD4+ small/medium T-cell lymphoma†
Lymphomatoid granulomatosis	Primary cutaneous anaplastic large cell lymphoma
ALK-positive large B-cell lymphoma	Lymphomatoid papulosis
Plasmablastic lymphoma	Subcutaneous panniculitis-like T-cell lymphoma
Large B-cell lymphoma arising in HHV-8–associated multicentric Castleman disease	Primary cutaneous γδ T-cell lymphoma
Primary effusion lymphoma	Primary cutaneous CD8+ aggressive epidermotropic cytotoxic T-cell lymphoma
B-cell lymphoma, unclassifiable, with features intermediate between DLBCL and Burkitt lymphoma	Hydroa vacciniforme–like lymphoma
B-cell lymphoma, unclassifiable, with features intermediate between DLBCL and classical Hodgkin lymphoma	*Nodal*
Burkitt lymphoma	Peripheral T-cell lymphoma, NOS
Mantle cell lymphoma	Angioimmunoblastic T-cell lymphoma
	Anaplastic large-cell lymphoma, ALK positive
Indolent lymphomas	Anaplastic large-cell lymphoma, ALK negative
Follicular lymphoma	
Primary cutaneous follicle center lymphoma	
Extranodal marginal zone lymphoma of mucosa-associated lymphoid tissue (MALT)	
Nodal marginal zone lymphoma	
Splenic marginal zone lymphoma	
Splenic B-cell lymphoma/leukemia, unclassifiable	
Lymphoplasmacytic lymphoma	
Heavy chain disease	
Plasma cell neoplasms	
CLL/SLL	
B-cell prolymphocytic leukemia	
Hairy cell leukemia	

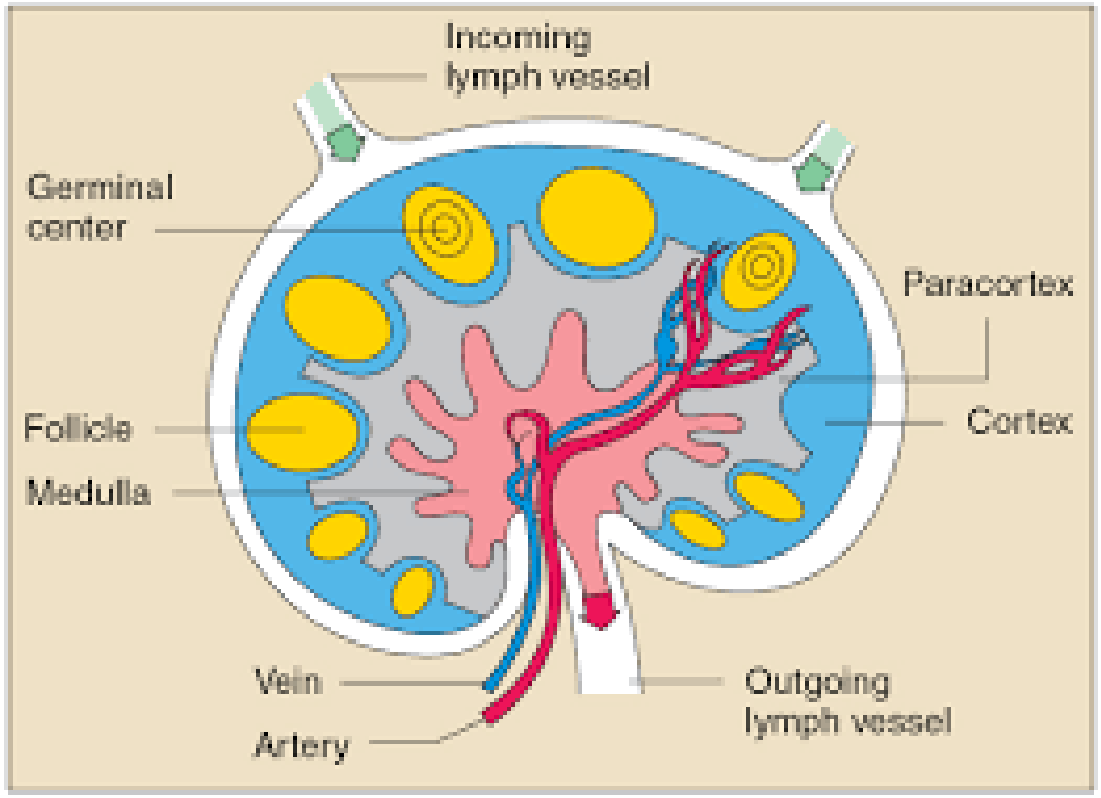
CLL = chronic lymphocytic leukemia; CNS = central nervous system; DLBCL = diffuse large B-cell lymphoma; HHV-8 = human herpesvirus 8; NK = natural killer; NOS = not otherwise specified; SLL = small lymphocytic lymphoma.

* All precursor neoplasms are considered aggressive.

† Indolent T-cell neoplasms; all other T-cell neoplasms are considered aggressive.

> 65 types !!!

Lymph node structure



How do we classify lymphomas?

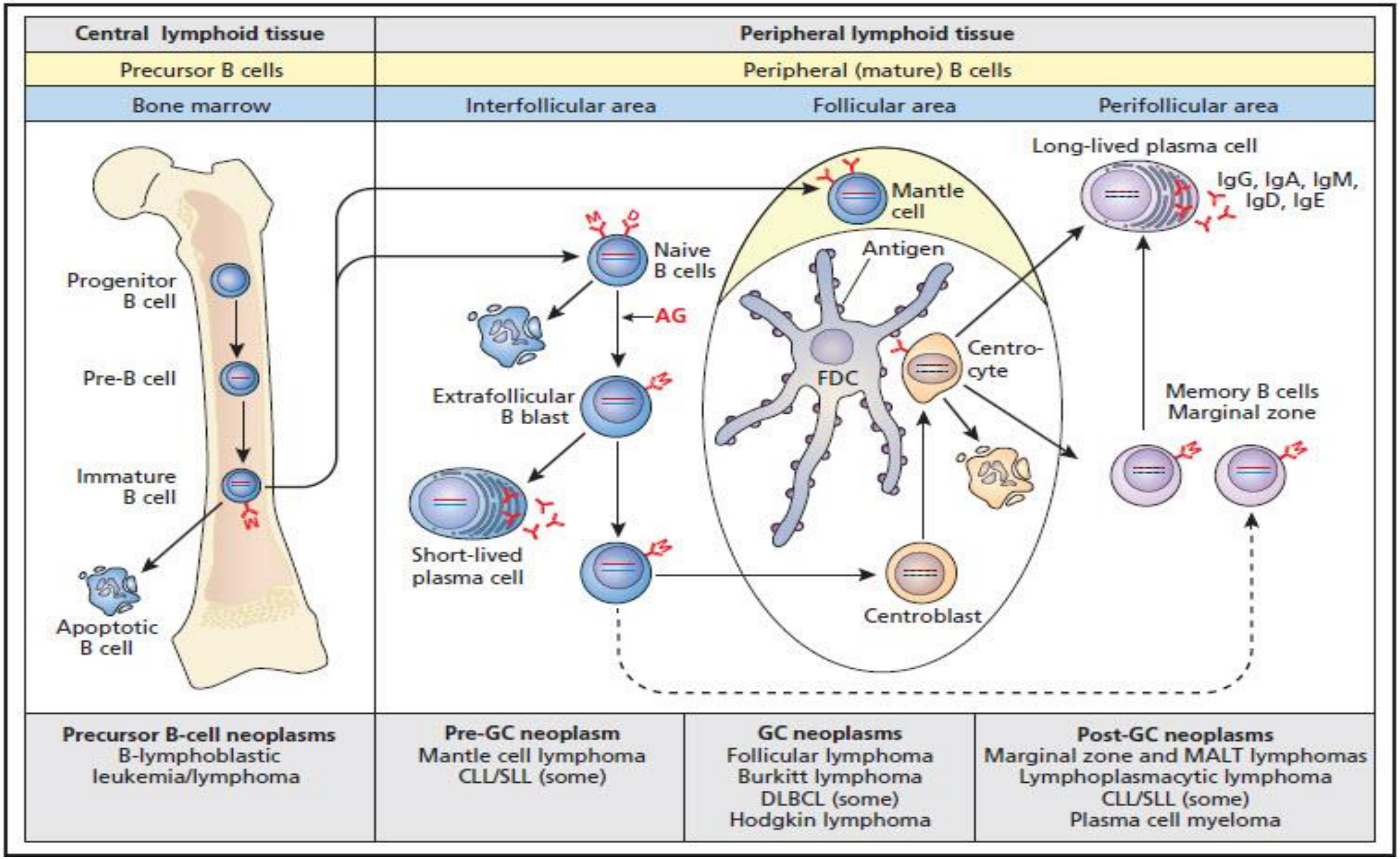
- Knowledge of B- and T-cell development is important
- Lymphomas are derived from their normal B- and T-cell counterparts

B-cell development

- Lymphocytes are derived from hematopoietic stem cells in the bone marrow.
- B-cell maturation occurs in the bone marrow.
- Begins with recombination of the *V*, *D*, *J* gene segments of the immunoglobulin heavy chain (IgH) followed by the light chain
- To generate a functional immunoglobulin that is expressed on the cell surface as B-cell receptor (BCR).

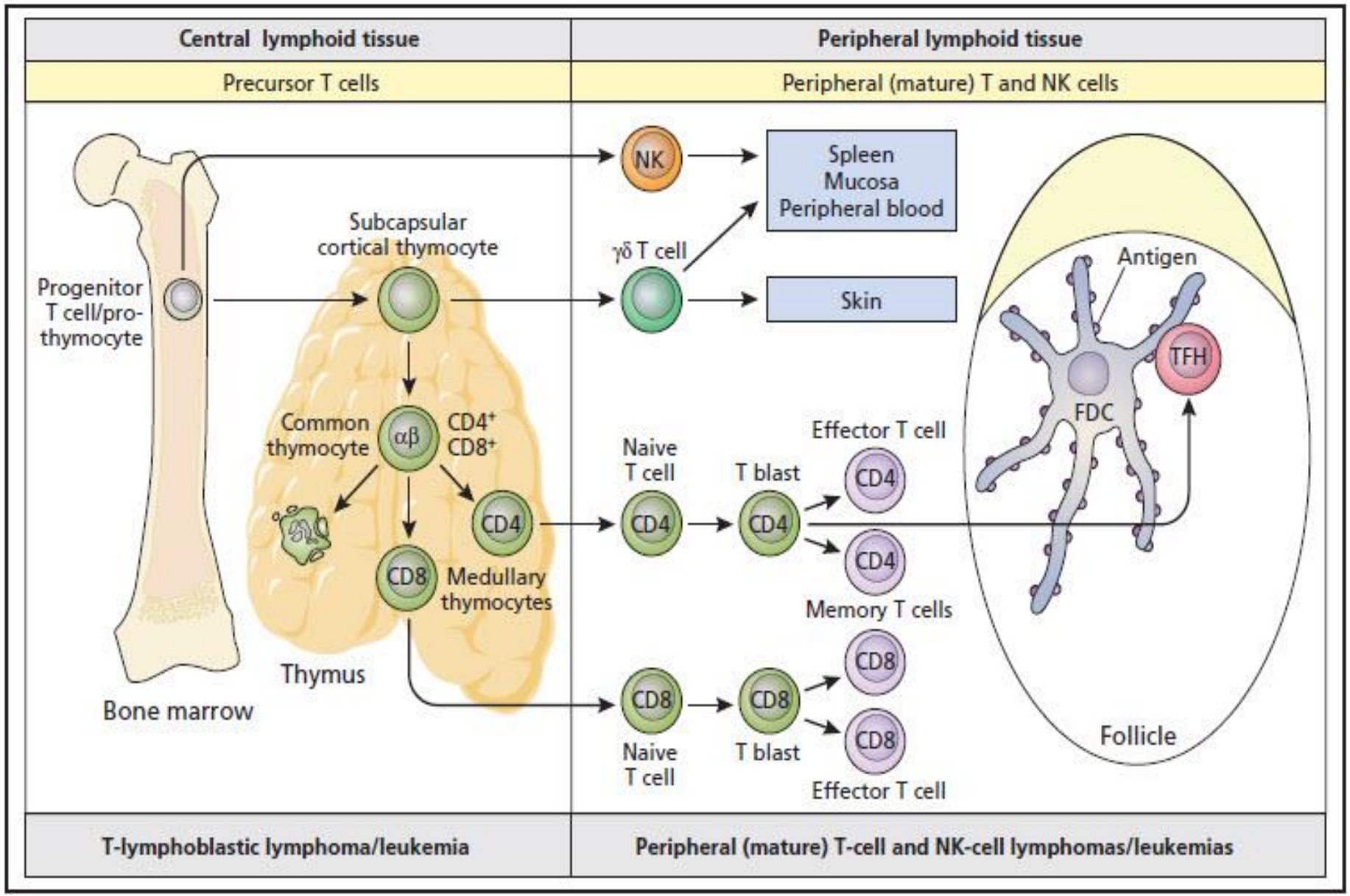
B-cell development

- The primary function of B-cells is to produce a vast diversity of immunoglobulins.
- Diversity come from **random** *V*, *D*, and *J* rearrangements.
- Rearrangement results in expression of IgM and IgD on the surface mature B-cells that exit the marrow.
- These are mature but (naïve B-cells).
- Then they get exposed to antigen in the germinal centers of secondary lymphoid organs:
  - such as lymph nodes
  - mucosa associated lymphoid tissue (MALT)
  - or the spleen
- Here, somatic hypermutation occurs in the V genes of the heavy and light chains:
  - Multiple antibodies with different affinity to the antigen.
  - Those with good affinity differentiate to memory B-cells or plasma cells.
  - The others undergo apoptosis.
- Finally, class switching occurs in the germinal center and involves changing the heavy chain to produce IgG, IgA, or IgE.


Central lymphoid tissue
Peripheral lymphoid tissue
Precursor B cells
Peripheral (mature) B cells
Bone marrow
Interfollicular area
Follicular area
Perifollicular area
Progenitor B cell
Pre-B cell
Immature B cell
Apoptotic B cell
Naive B cells
AG
Extrafollicular B blast
Short-lived plasma cell
Mantle cell
Antigen
FDC
Centro-cyte
Centroblast
Long-lived plasma cell
IgG, IgA, IgM, IgD, IgE
Memory B cells Marginal zone
Precursor B-cell neoplasms
B-lymphoblastic leukemia/lymphoma
Pre-GC neoplasm
Mantle cell lymphoma
CLL/SLL (some)
GC neoplasms
Follicular lymphoma
Burkitt lymphoma
DLBCL (some)
Hodgkin lymphoma
Post-GC neoplasms
Marginal zone and MALT lymphomas
Lymphoplasmacytic lymphoma
CLL/SLL (some)
Plasma cell myeloma

T-cell development

- In contrast to B-cell, T-cell exit the marrow and develop in the thymus.
- Similar to B cells, each T cell recognizes a specific antigen, but through a T-cell receptor (TCR) rather than BCR.
- Similar to BCRs, diversity of TCRs is generated through recombination of *V*, *D*, and *J* gene segments of the four TCR genes, *alpha* (α), *beta* (β), *gamma* (γ) and *delta* (δ).
- Mature T-cells express either αβ TCR or γδ TCR.


Central lymphoid tissue
Peripheral lymphoid tissue
Precursor T cells
Peripheral (mature) T and NK cells
Progenitor T cell/pro-thymocyte
Subcapsular cortical thymocyte
Common thymocyte
αβ
CD4+ CD8+
CD4
CD8
Medullary thymocytes
Thymus
Bone marrow
NK
γδ T cell
Spleen
Mucosa
Peripheral blood
Skin
Antigen
TFH
FDC
Follicle
Naive T cell
T blast
Effector T cell
Memory T cells
Naive T cell
T blast
Effector T cell
T-lymphoblastic lymphoma/leukemia
Peripheral (mature) T-cell and NK-cell lymphomas/leukemias

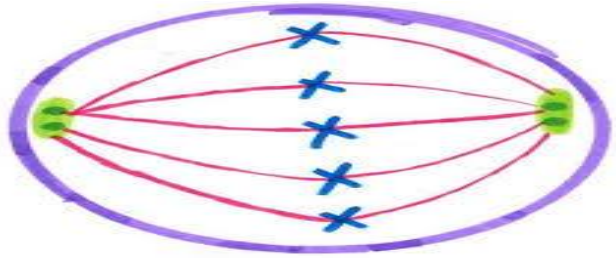
Cytogenetic techniques

- Cytogenetic techniques:
  - Conventional cytogenetic
  - FISH

- Molecular:
  - PCR

Conventional cytogenetics

- Metaphase **cytogenetics** = chromosomal analysis = Conventional cytogenetic techniques = karyotyping
  - Numeric chromosomal abnormalities (too many or too few chromosomes)
  - Deletion
  - Translocation.
- Bone marrow or tissue → maintained in culture → exposed to a mitotic inhibitor → blocks formation of the spindle → arrests cell division at the metaphase stage.
- Thus, cytogenetic studies require dividing cells.



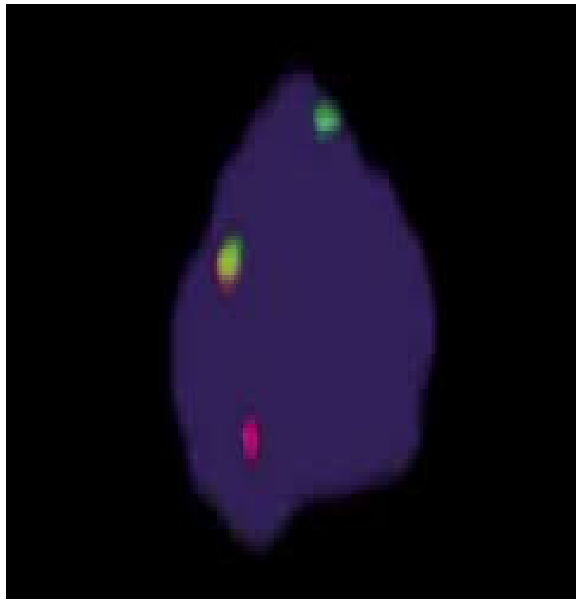
Limitations

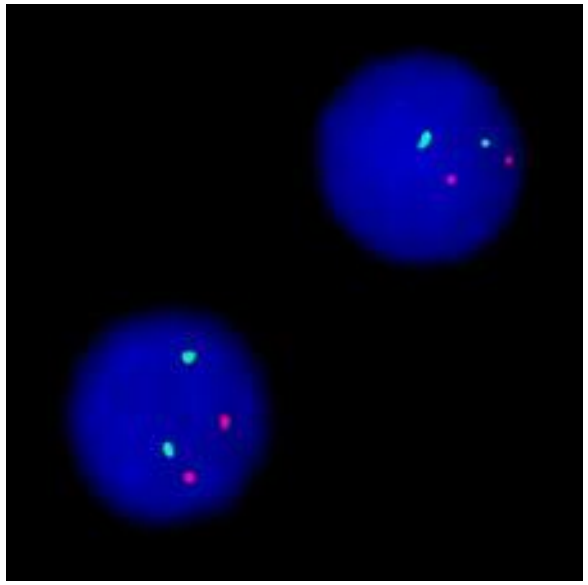
- Require active cell division.
- Insensitive to submicroscopic abnormalities
- Small number of cells are analyzed

Fluorescence in situ hybridization (FISH)

- A specific single-stranded DNA probe corresponding to a gene or chromosomal region of interest is labeled for fluorescent detection.
- Probes are then incubated with the sample and examined by microscopy.

FISH



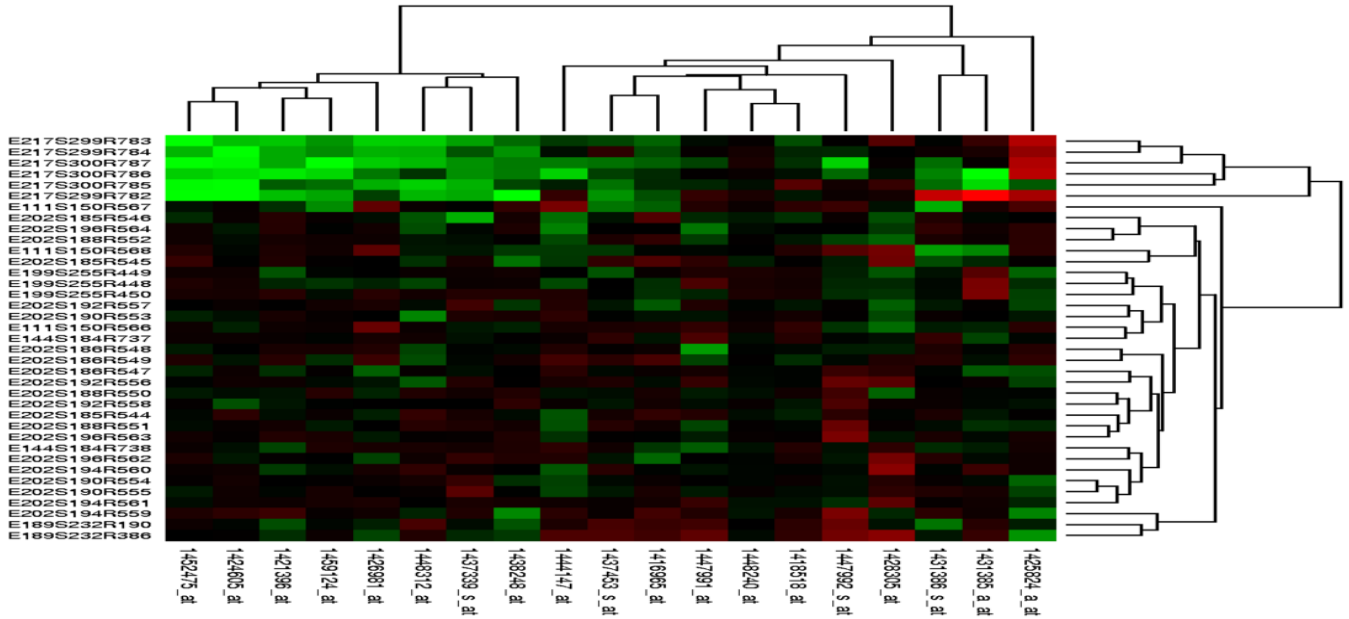


FISH

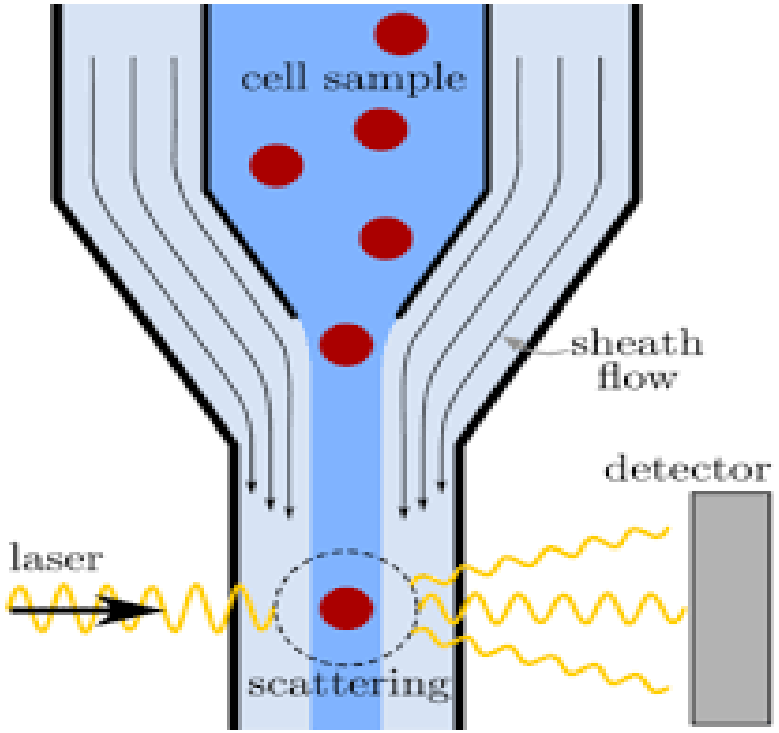
- Abnormalities can be seen in non-dividing cells (interphase nuclei)
- Peripheral blood slides can be directly processed.
- Specific molecular abnormalities
- More sensitive than conventional cytogenetic.

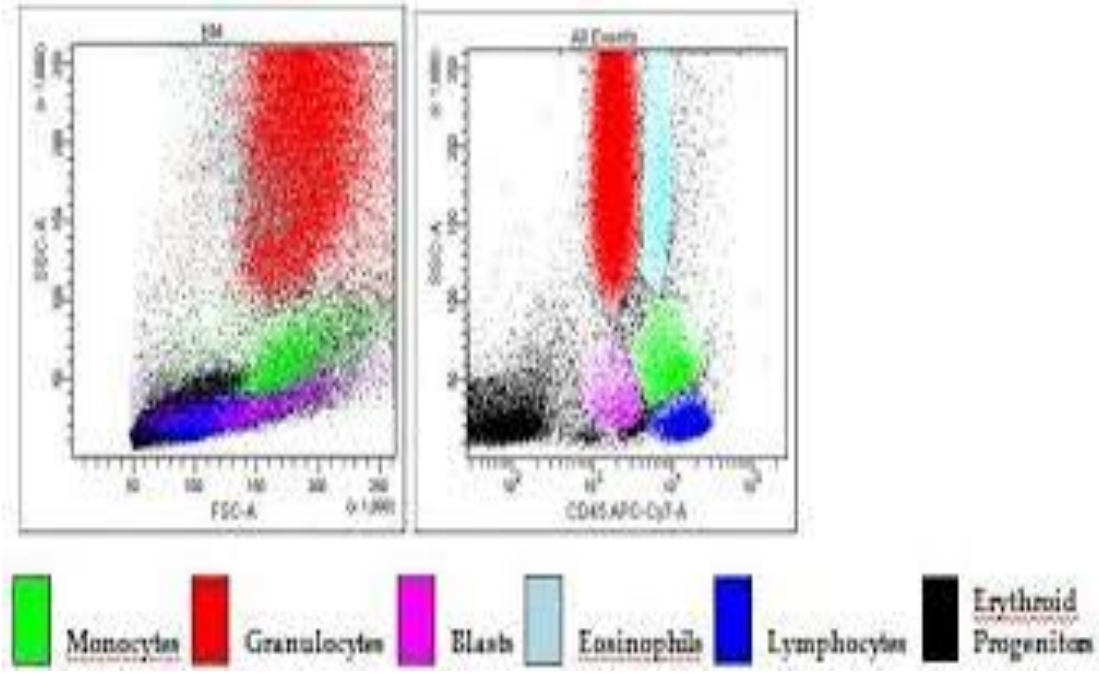
RNA expression arrays

- Characterization of the gene expression patterns within the cells of interest
- Referred to as a **gene expression profile** (GEP)

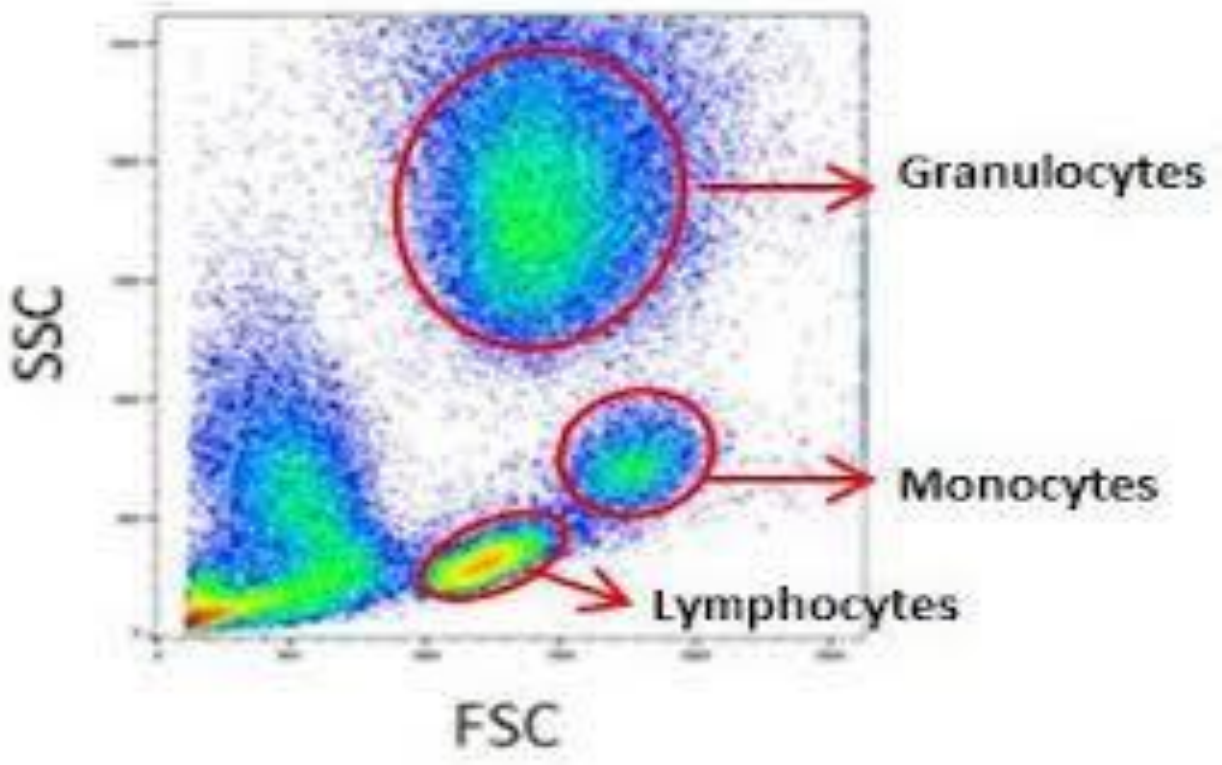


Flow cytometry

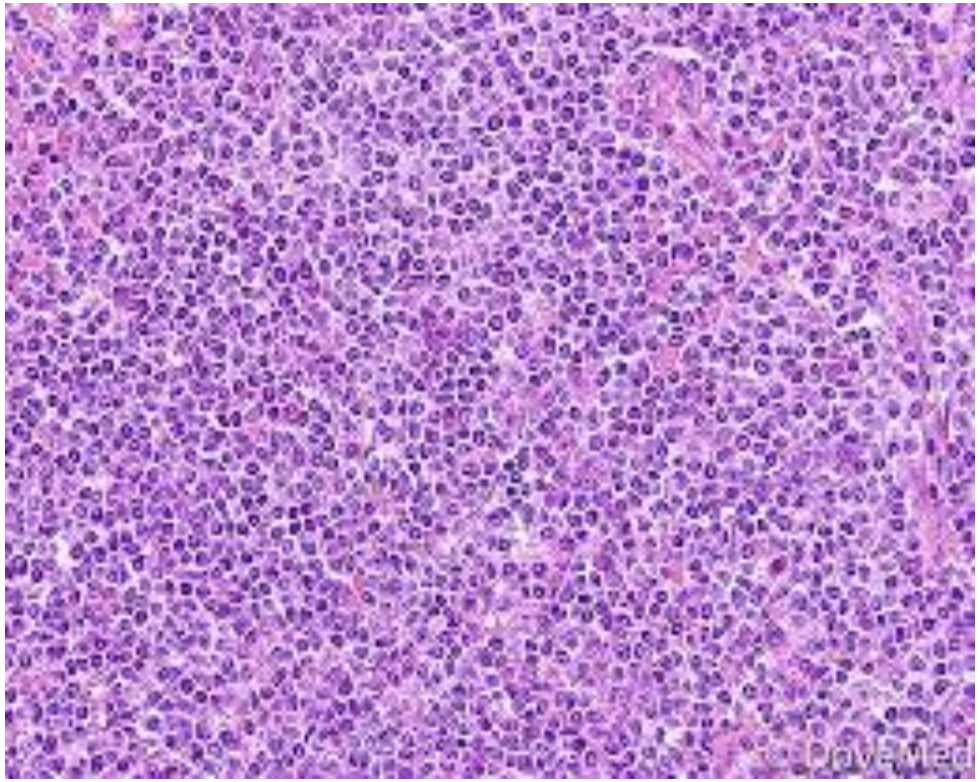




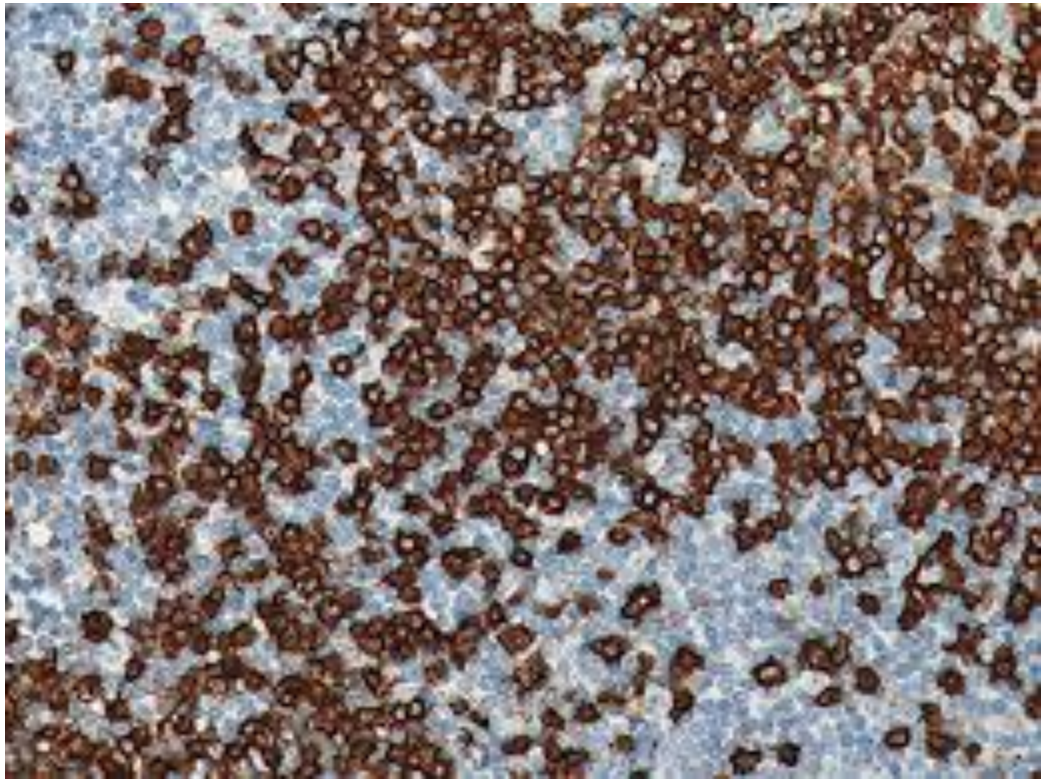
Forward vs side scattered

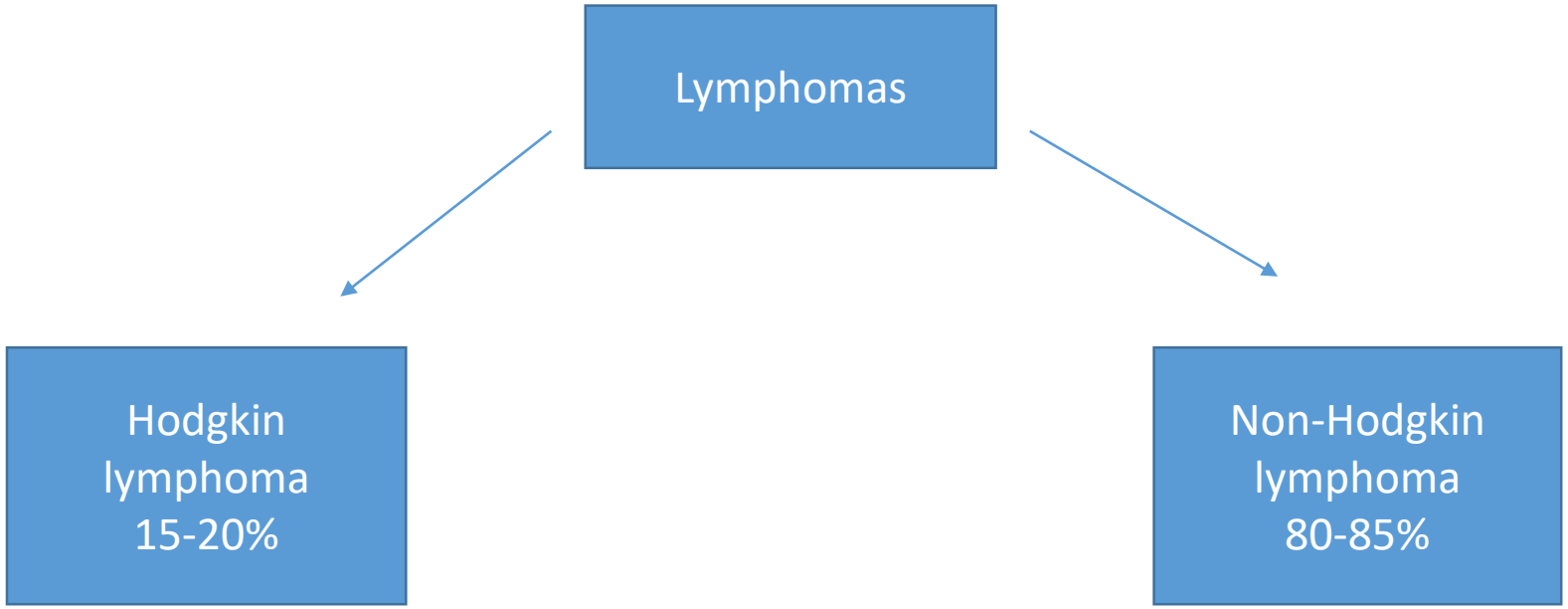


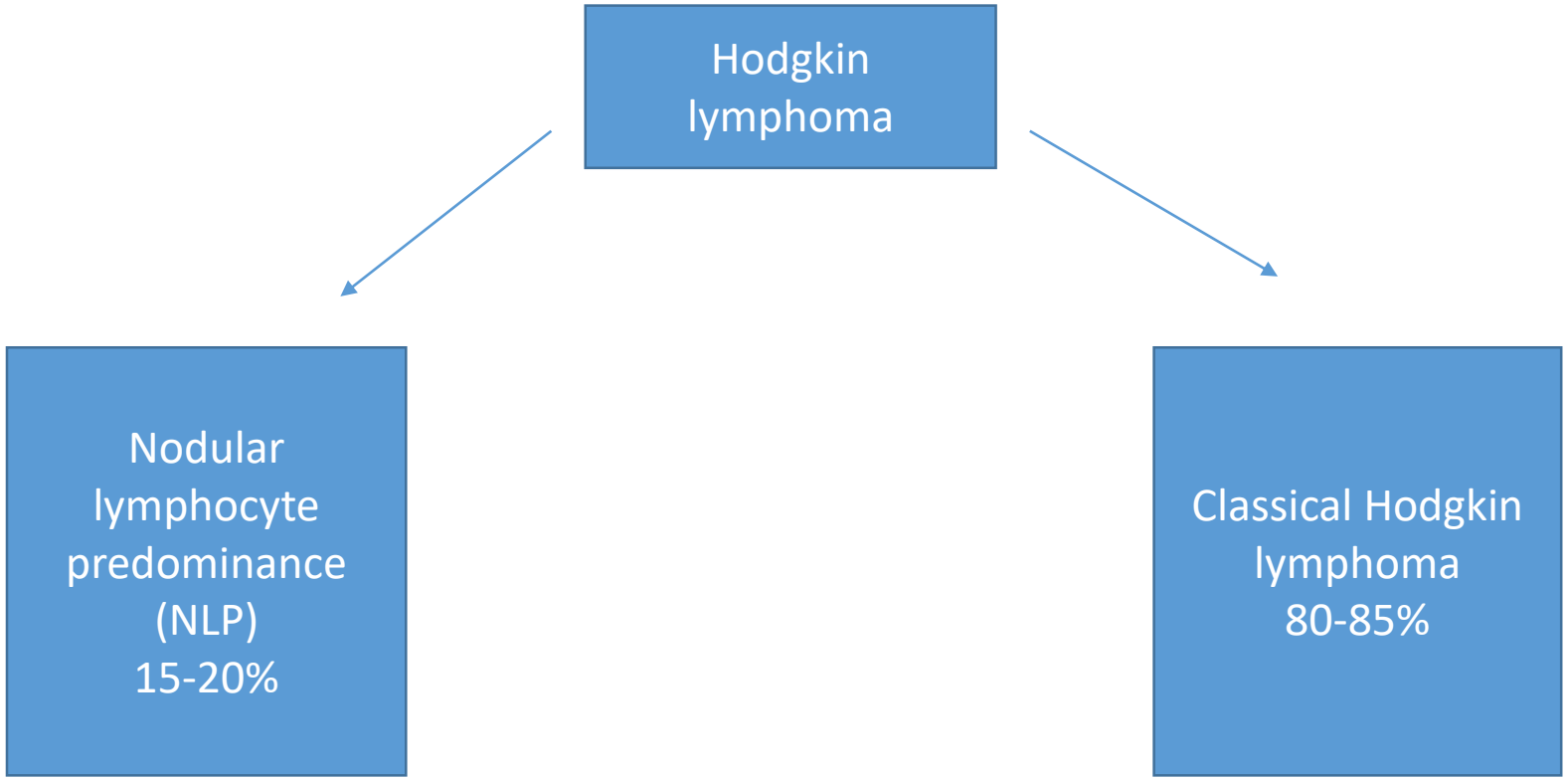
Morphology

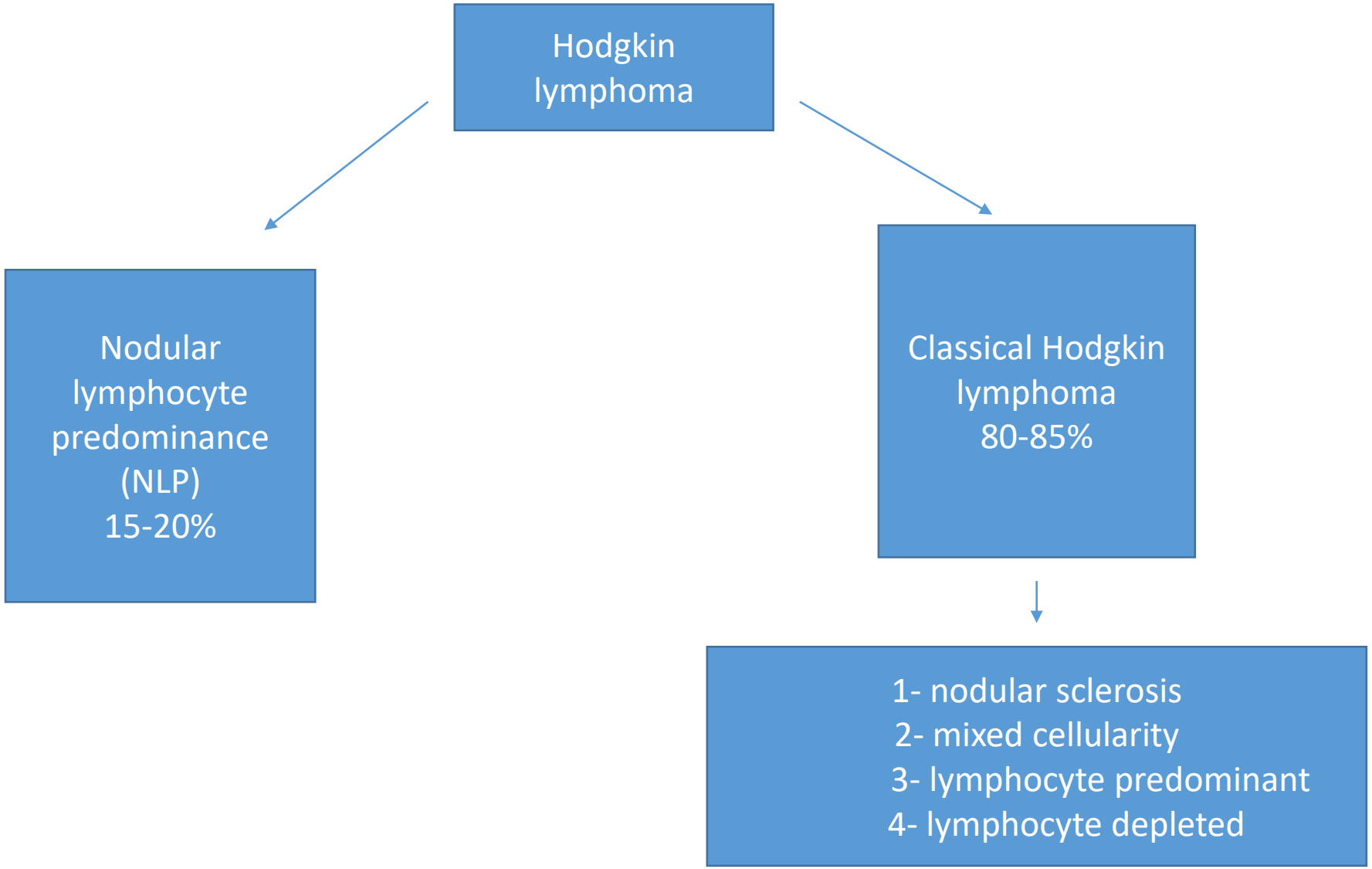


Immunohistochimestry




Lymphomas
Hodgkin lymphoma 15-20%
Non-Hodgkin lymphoma 80-85%


Hodgkin lymphoma
Nodular lymphocyte predominance (NLP) 15-20%
Classical Hodgkin lymphoma 80-85%

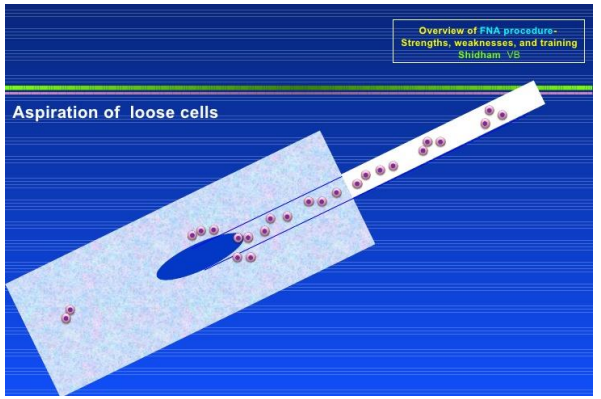

Hodgkin lymphoma
Nodular lymphocyte predominance (NLP) 15-20%
Classical Hodgkin lymphoma 80-85%
1- nodular sclerosis
2- mixed cellularity
3- lymphocyte predominant
4- lymphocyte depleted

How to diagnose lymphomas?

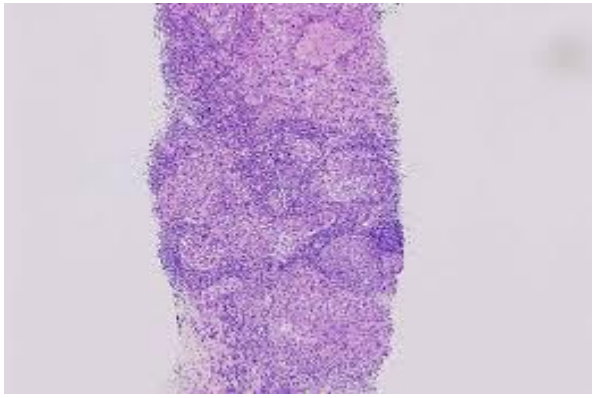
- A tissue biopsy!

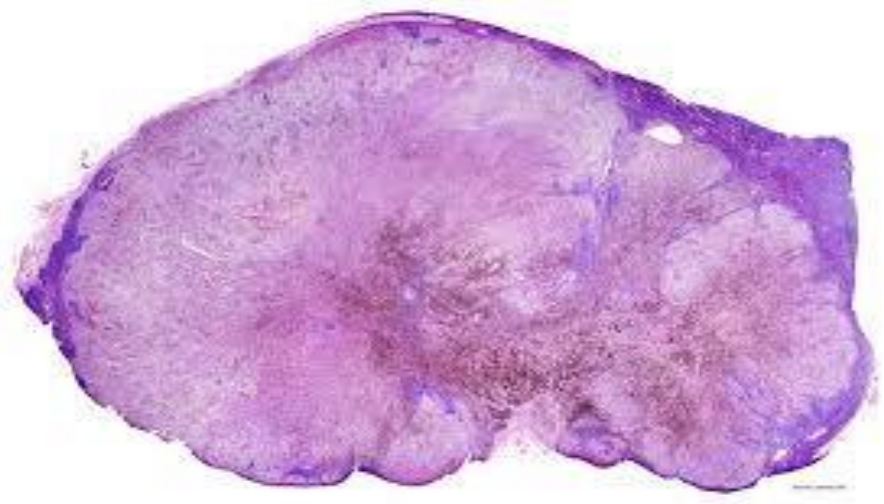
Types of LN biopsies

- FNA: Looks at cells not tissues.
- Incisional (tissue of lymph node)
- Excisional (whole lymph node out)



Cytology:
+/-





How to stage lymphoma?

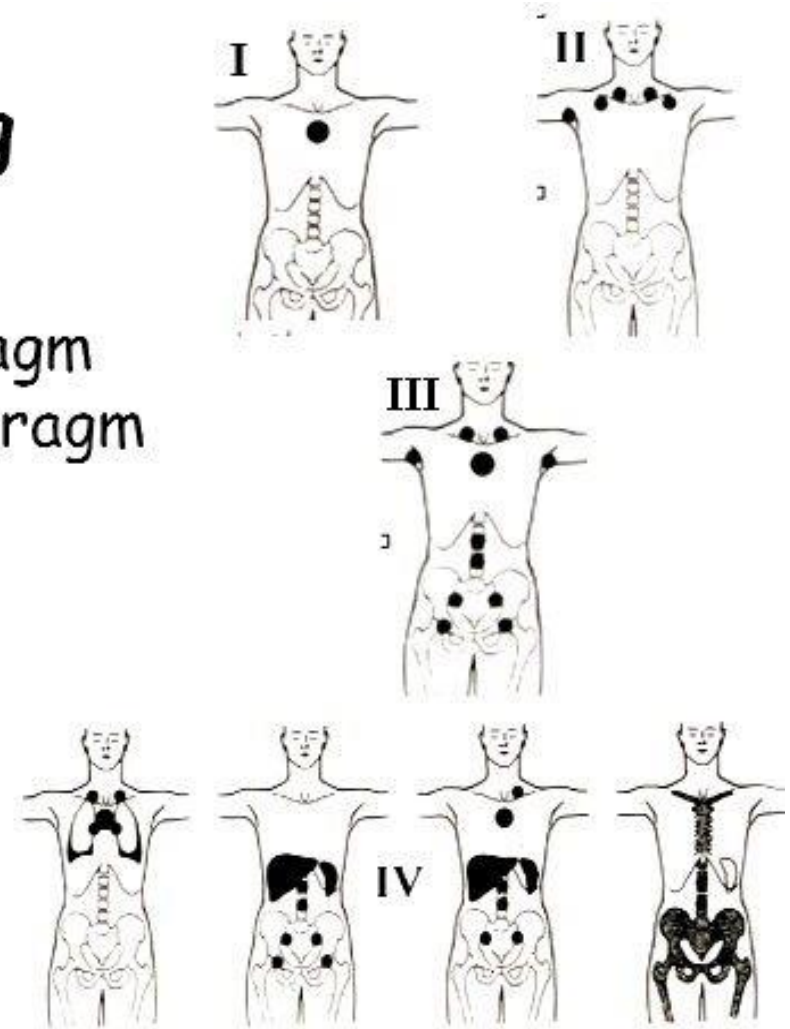
- CT scan: neck, chest abdo and pelvis.
- PET scan: in curable lymphomas:
  - Hodgkin lymphoma.
  - Aggressive NHL: DLBCL, Peripheral T-cell lymphoma.
  - Less role in indolent lymphomas.
- Bone marrow biopsy:
  - No need in PET staged Hodgkin.
  - We still do it in all NHL although the role in DLBCL is fading out of favor.
- Investigations:
  - CBCD, LDH, LFTs, Hepatitis serology (Hb C, Hb B core and surface antigen, HIV)
  - Quantitative immunoglobulins

Ann Arbor Staging

I	Single LN region
II	One side of diaphragm
III	Both sides of diaphragm
IV	Disseminated

A No systemic symptoms
B Fever, night sweats, weight loss

E Extralymphatic site
S Splenic disease



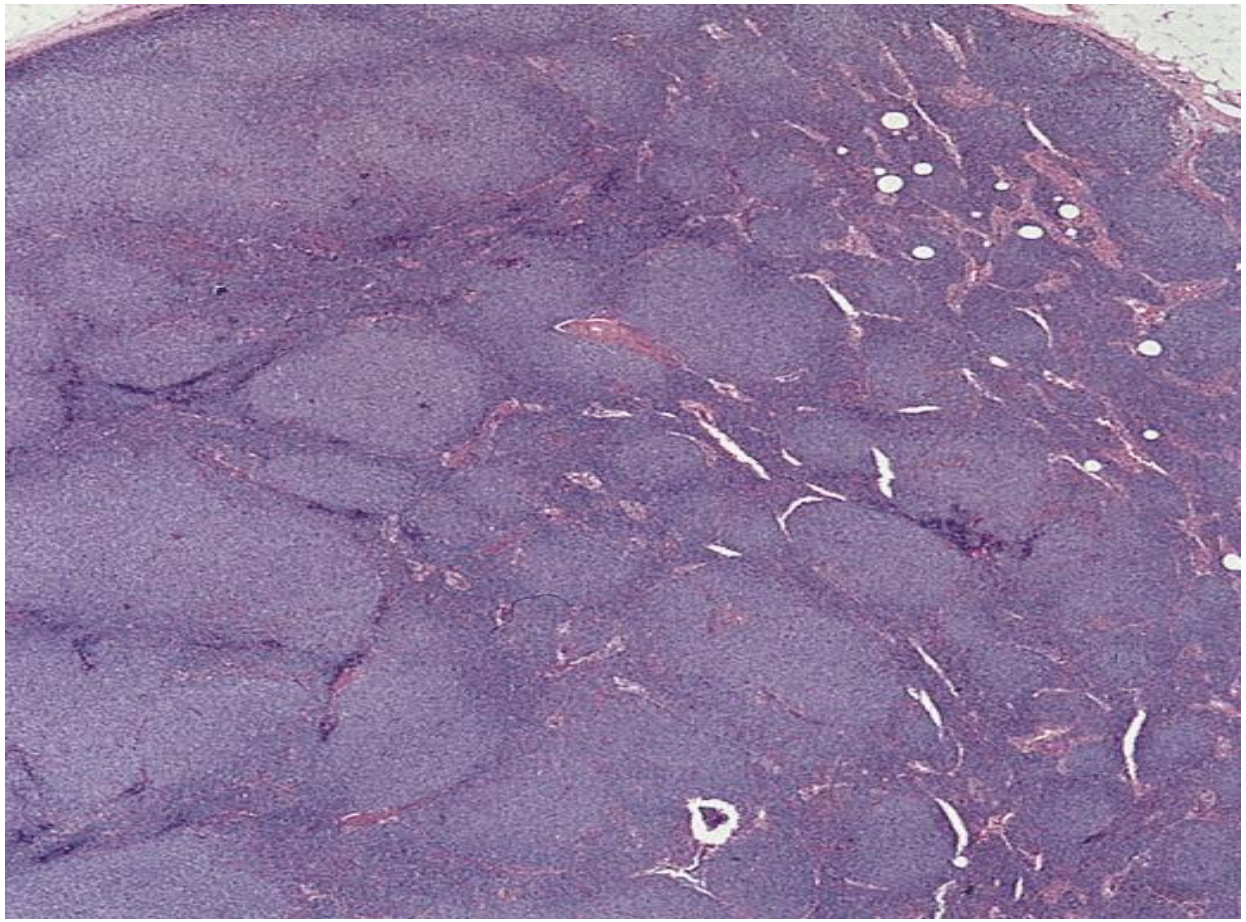
B-cell types

Indolent (kills in yrs)	Aggressive (kills in months-weeks)	Highly aggressive (kills in days to weeks)
Follicular lymphoma (FL) Lymphoplasmacytic lymphoma (LPL) Marginal zone lymphoma (MZL): - Nodal MZL - Extra nodal MZL Small lymphocytic lymphoma (SLL)/CLL Hairy cell leukemia (HCL) Mantle cell lymphoma	**Diffuse large B-cell lymphoma (DLBCL)** Mantle cell lymphoma High grade B-cell lymphoma (previously called Burkiit's like)	**Burkitt's lymphoma (BL)** B-Lymphoblastic lymphoma (LBL)

T-cell types

Indolent	Aggressive	Highly aggressive
T-cell large granular lymphocytic leukemia (T-LGL) Mycosis Fungoides Primary cutaneous types	PTCL Angioimmunoblastic T-cell lymphoma (AITCL) Anaplastic T-cell lymphoma: - ALK negative (bad prognosis) - ALK + (good prognosis) Aggressive NK-cell leukemia Extranodal NK/T-cell lymphoma, nasal type Enteropathy associated T-cell lymphoma (celiac) Adult T-cell leukemia/lymphoma (ATLL) Hepatosplenic T-cell lymphoma Sezary syndrome	T-Lymphoblastic lymphoma (LBL)

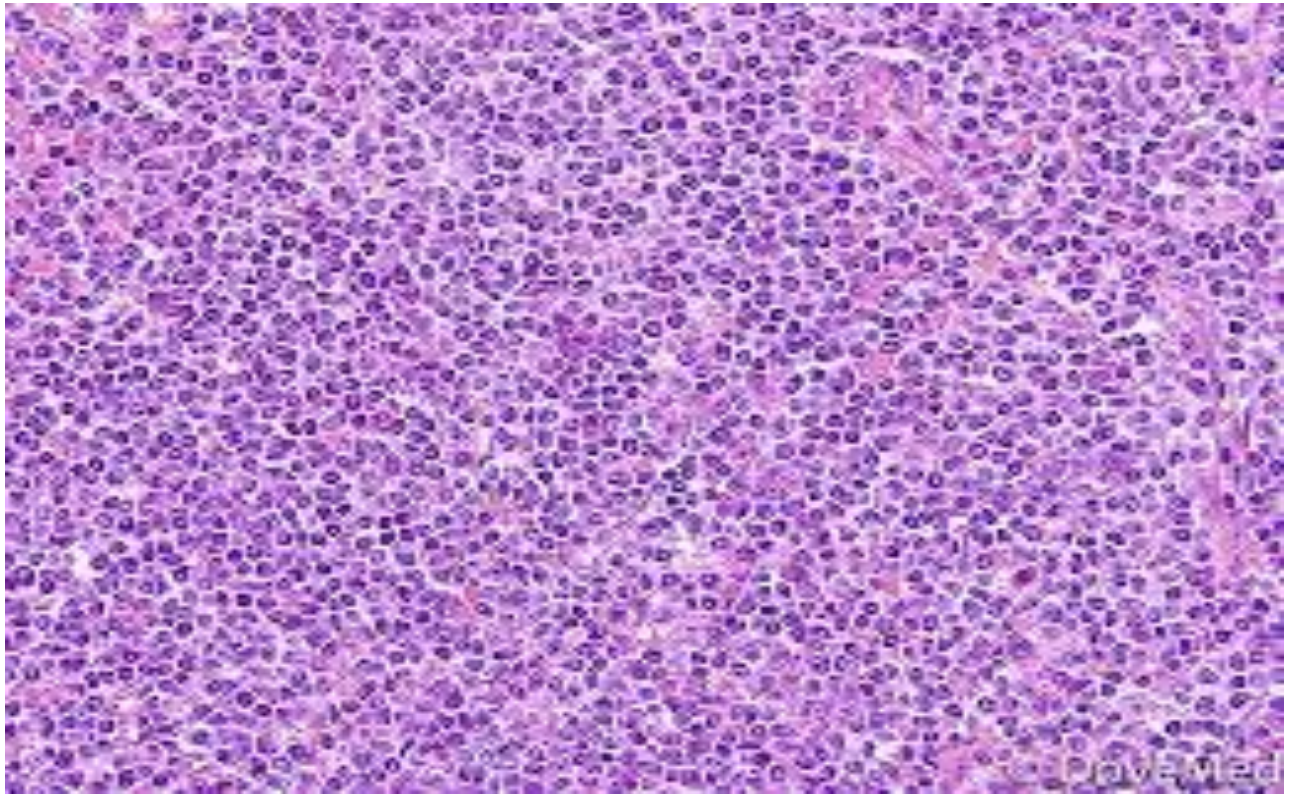
Follicular lymphoma



Follicular lymphoma

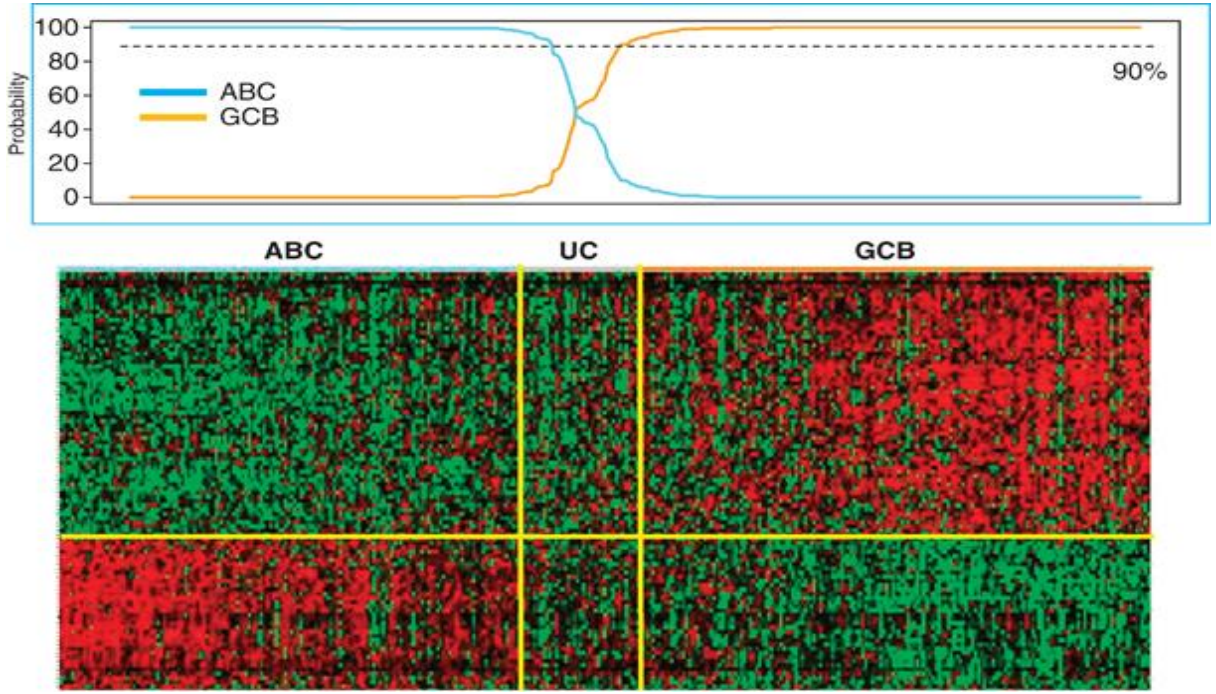
- Germinal center lymphoma (+ CD10, BCL6)
- t(14:18) results in overexpression of the anti-apopototic protein bcl-2.
- The translocation moves the bcl-2 gene on 18 to near the enhancer element of the immunoglobulin heavy chain on chromosome 14.
- The problem is cells don’t die (not a problem of hyperproliferation)
- The proliferative rate is low (proliferative index Ki67 20-40%)
- Incurable but responsive to treatment

Diffuse large b-cell lymphoma (DLBCL)



DLBCL

- Morphological diagnosis is a key (large cells & diffuse infiltration).
- The problem is with high proliferative rate (not with apoptosis)
- The proliferative index is very high (Ki67 = 80-90%)
- Curable (>60% cure rate).
- Many clinical forms:
  - DLBCL-NOS (most common)
  - Mediastinal Large B-cell lymphoma
  - Primary CNS DLBCL
- 2 molecular forms:
  - Activated B-cell type (ABC) poor risk
  - Germinal center B-cell (GCB) better risk



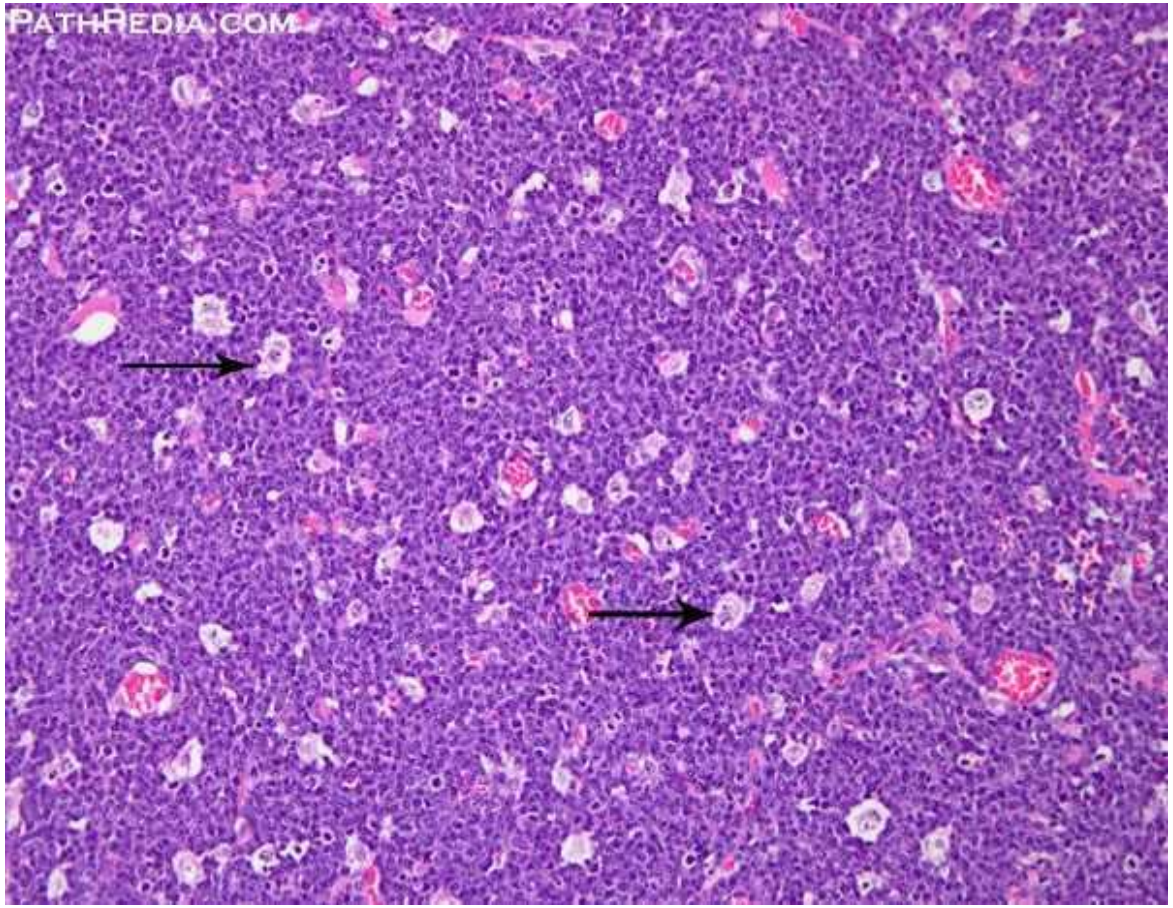
Mantle cell lymphoma

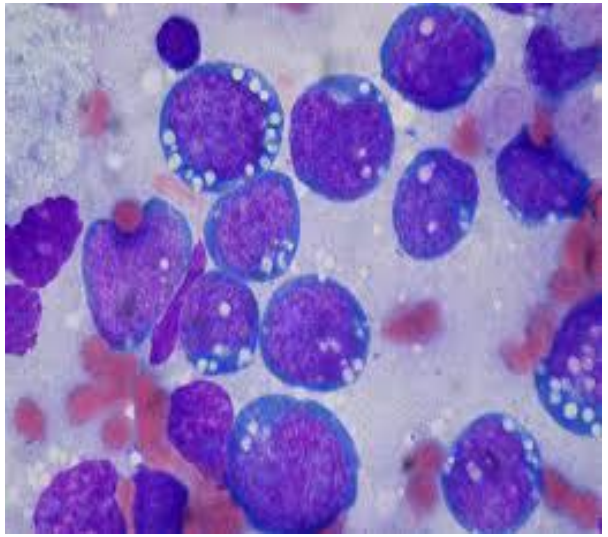
- Has the 2 worse features of both DLBCL and FL
- t(11:14) Cyclin D1
- Cells don’t die and they also proliferate !!!
- Bad bad type!
- Incurable and poor prognosis.
- Treated differently:
  - Young <60: R-chemo then ASCT
  - Elderly: R-chemo (BR, RCHOP, RCVP)
  - Ibrutinib (BTK inhibitor) in relapsed refractory cases.

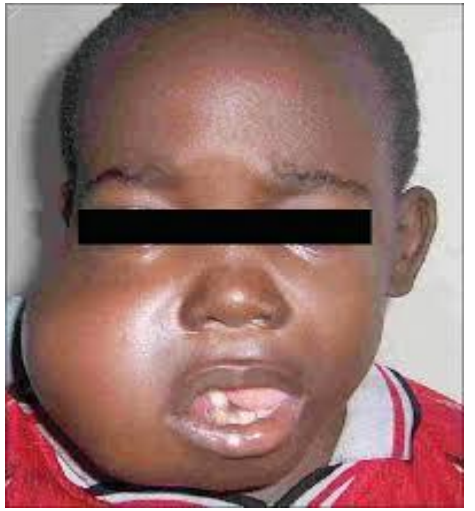
Burkitts lymphoma

- t(8:14) c-myc on chromosome 8 is translocated near the enhancer of IGH gene on chromosome 14.
- Cells have a very high proliferate rate (100% ki67)
- High rate of apoptosis (cells die easily)
- Very sensitive to chemotherapy
- Highly curable (>90%) but fatal if you don’t treat
- High risk for tumor lysis syndrome (like ALL, in fact ALL-3=BL)

Burkitts lymphoma



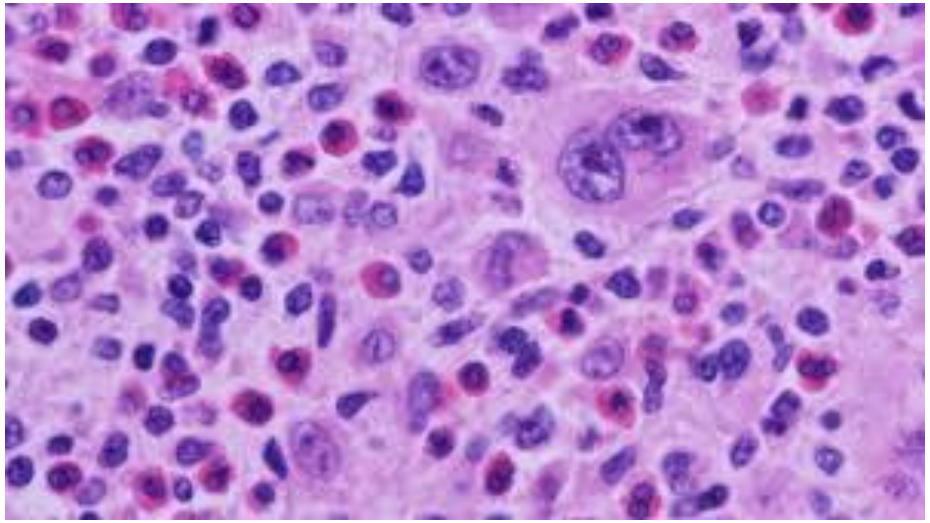


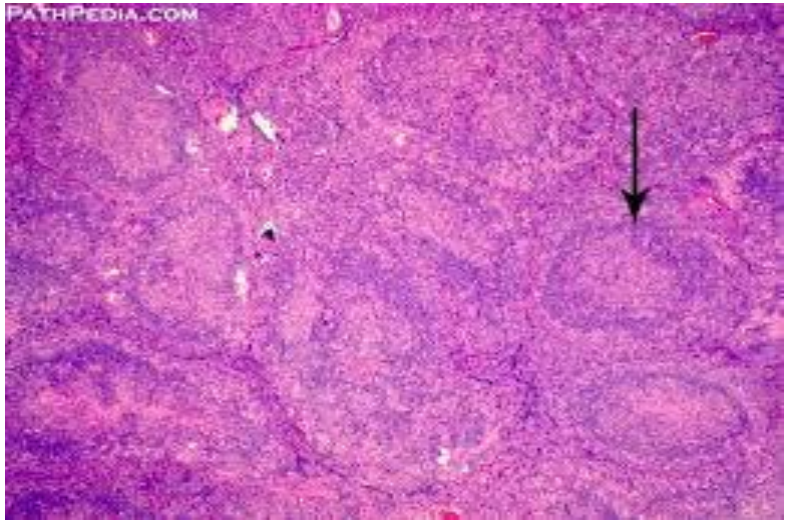


Double Hit lymphoma

- Imagine a lymphoma with: 2 translocations:
  - Anti-apoptotic BCL-2
  - C-myc
  - Double hit (poor prognosis)
- Triple HIT: BCL-2 and BCL-6 and c-myc (poor prognosis)
- Treated with dose adjusted EPOCHR
  - Infusional chemotherapy to target continuous cell kill
  - Adjusted to ANC nadir
  - Adding Etoposide (compared to RCHOP)

Hodgkin lymphoma





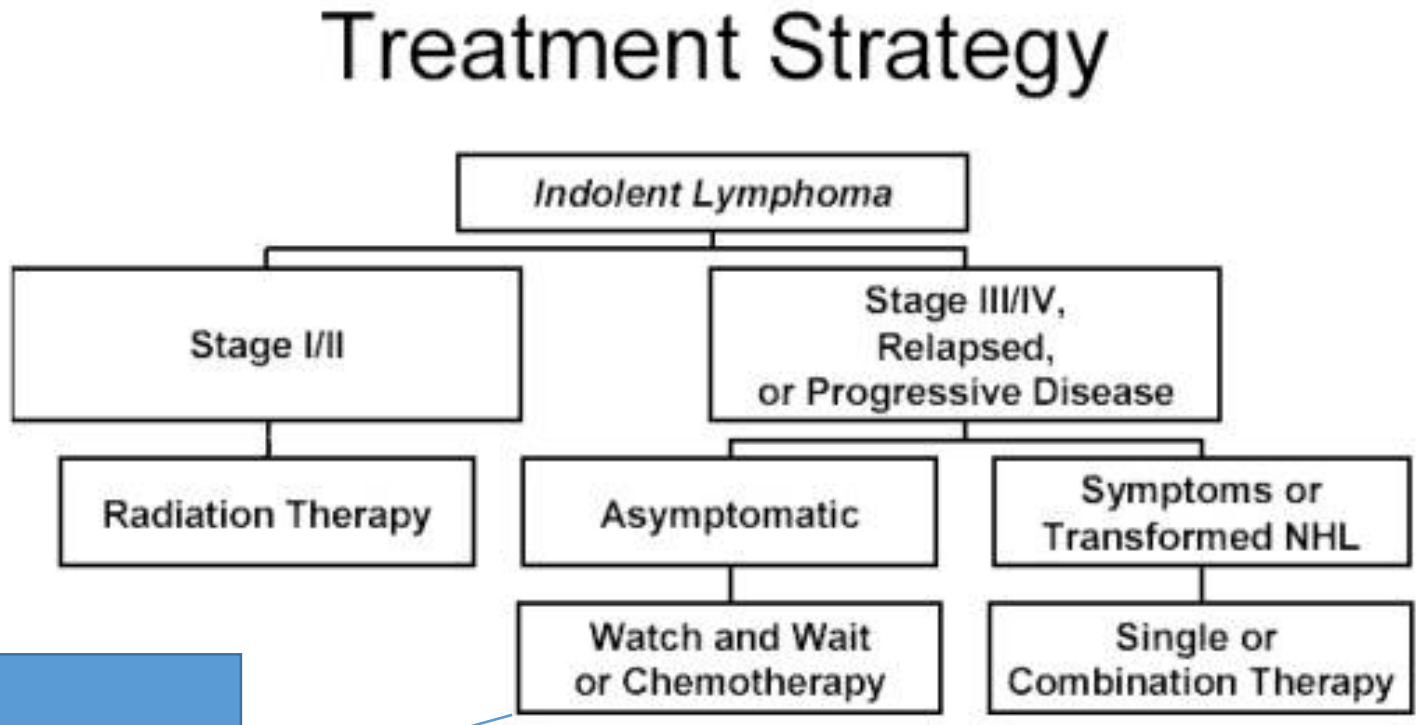
Hodgkin lymphoma

- B-cell origin
- Contiguous spread (typically neck → mediastinum →abdo/spleen)
  - NHL spread non-contiguously
- Highly Curable >90%
- Typical presentation is with a young pt with cervical and or mediastinal LN.

Treatment Hodgkin

- Limited stage:
  - ABVD x 2 then PET can if in CR then 1-2 cycles then stop.
- Advanced:
  - ABVD x 2 then PET if in CR then 4 more cycles of AVD
  - If not in CR: consider escalation (escalated BEACOP) or continue ABVD x 4

Treatment of indolent lymphoma



1. Bendamustine Rituximab (BR)
2. RCHOP
3. RCVP

R-chemo

Rituximab weekly
x 4 in FL

Treatment of aggressive types

- DLBCL:
  - Limited: RCHOP x 3 cycles then PET if –ve 1 last cycle then stop. If + RT.
  - Advanced: RCHOP x 6 cycles
- PTCL:
  - Limited: CHOP x 3 cycles then PET if –ve 1 last cycle then stop. If + RT.
  - Advanced: CHOP x 6
- High grade B-cell:
  - Double HIT: DA-EPOCHR x 6
- Highly aggressive Burkitts:
  - Aggressive short course chemotherapy: (CODOX-MR/IVACR)

Relapsed/refractory lymphoma

- Salvage chemotherapy.
- Multiple regimens exist with equal efficacy but different side effect profile:
  - ESHAP, EHAP, DHAP, GDP, RICE etc
  - If in CR: proceed to auto stem cell transplant
  - In not in CR: palliatiation
- Targeted therapies exist and may be helpful in relapsed diseases
  - Brentuximab (anti CD30) in HL and some T-cell lymphomas

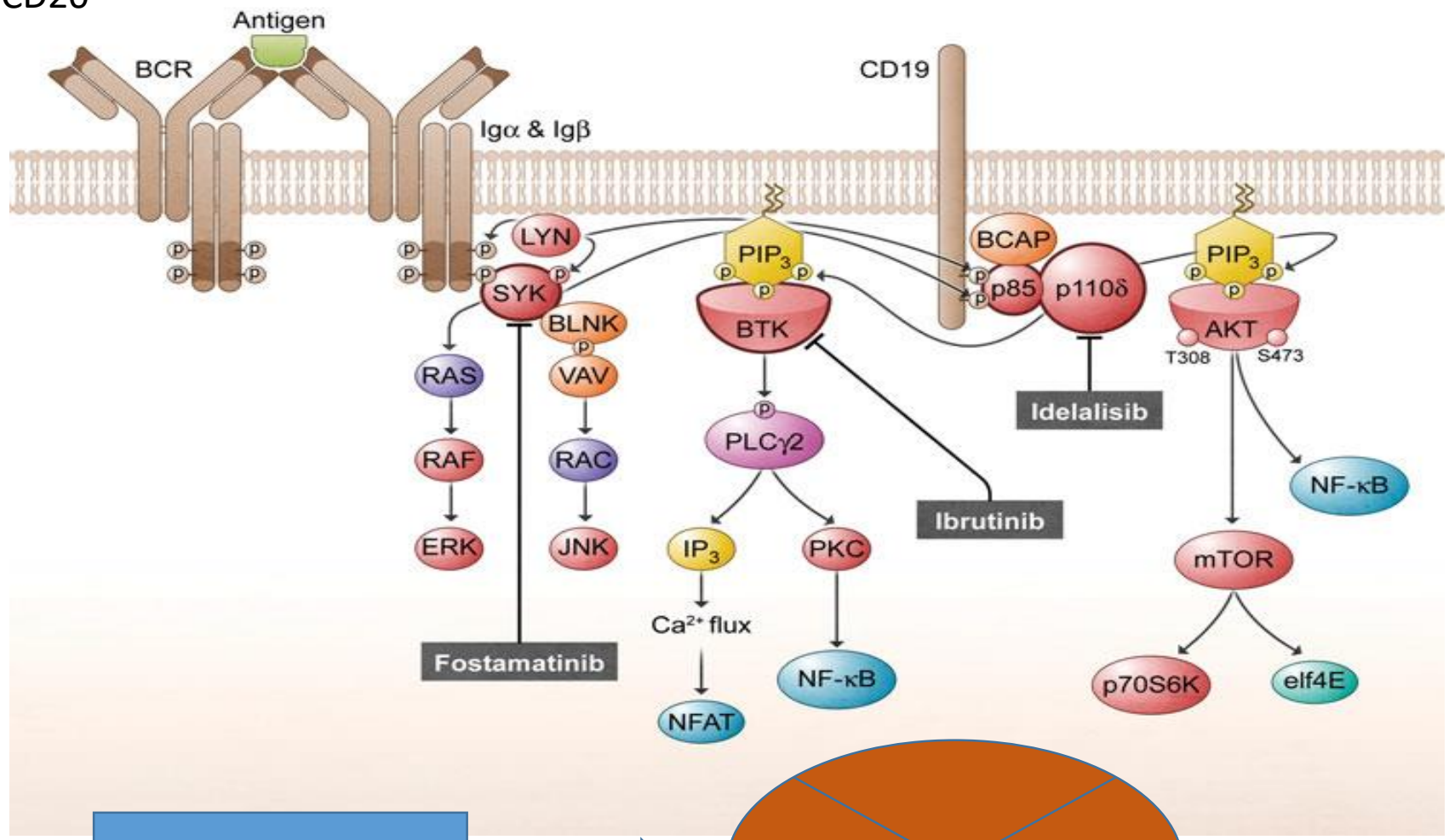
New novel therapies

- Plethora of new therapies in the past few years
- Very effective even in high risk pts
- E.g. BCL-2 inhibitors (venetoclax in CLL)
- Other small molecule inhibitors (targeting BCR signalling)

B-cell receptor (BCR) signalling

Rituximab

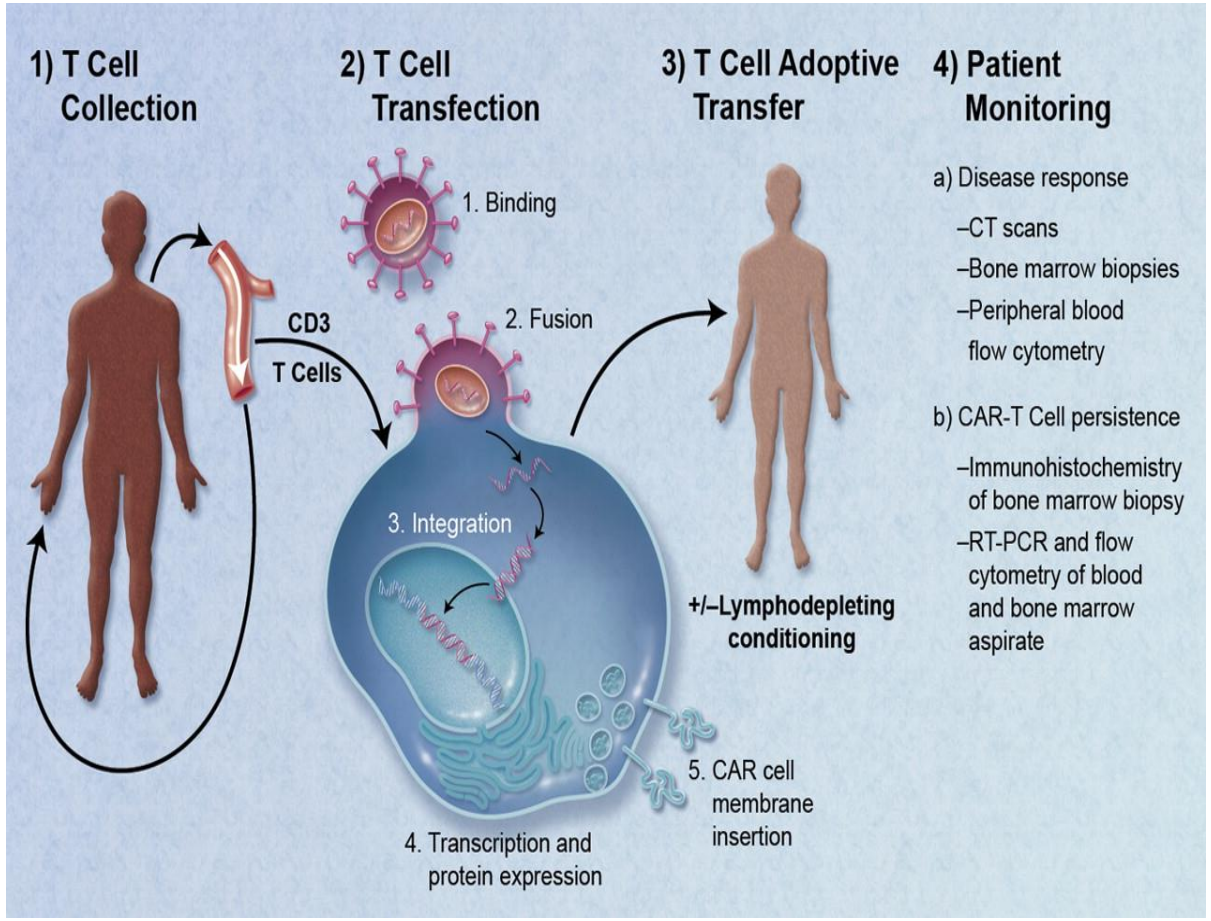
CD20

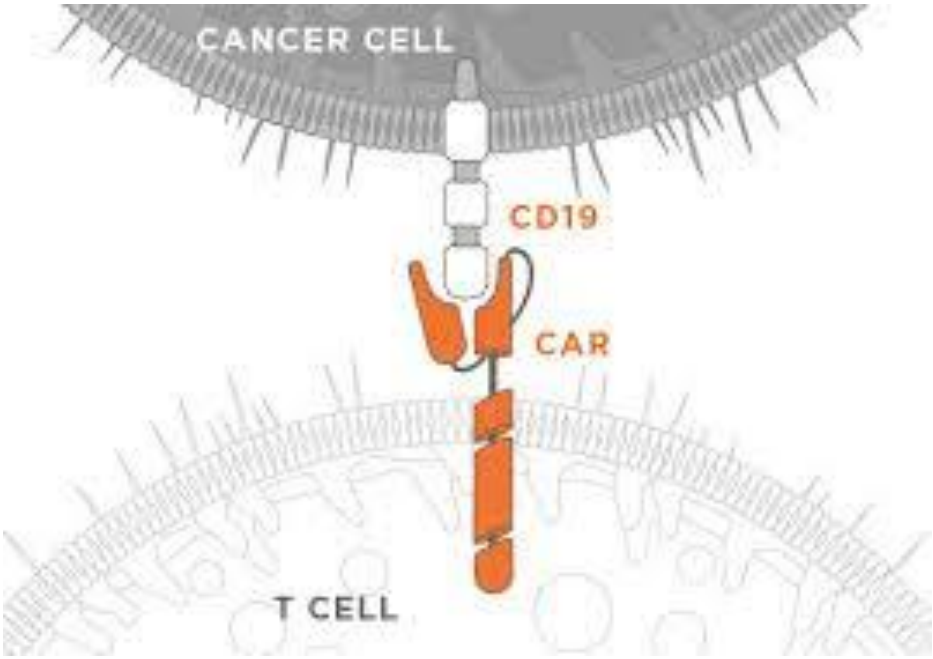


Chemotherapy

Nucleus

CAR T cells


1) T Cell Collection
2) T Cell Transfection
3) T Cell Adoptive Transfer
4) Patient Monitoring
1. Binding
CD3
T Cells
2. Fusion
3. Integration
4. Transcription and protein expression
5. CAR cell membrane insertion
+/–Lymphodepleting conditioning
a) Disease response
–CT scans
–Bone marrow biopsies
–Peripheral blood flow cytometry
b) CAR-T Cell persistence
–Immunohistochemistry of bone marrow biopsy
–RT-PCR and flow cytometry of blood and bone marrow aspirate


CANCER CELL
CD19
CAR
T CELL

Thanks

- questions?