Diffuse Large B-Cell Lymphoma

DLBCL: Definition

- Diffuse proliferation of large neoplastic B lymphoid cells
- Nuclear size equal to or exceeding normal macrophage nuclei or more than twice the size of a normal lymphocyte

DLBCL: Synonyms

- *Rappaport*: diffuse histiocytic, diffuse mixed lymphocytic and histiocytic
- *Kiel*: centroblastic, B-immunoblastic, B-large cell anaplastic
- *Lukes-Collins*: large cleaved follicular center cell (FCC), large noncleaved FCC, B-immunoblastic
- *Working Formulation*: diffuse large cell, large cell immunoblastic, diffuse mixed small and large cell
- *REAL*: diffuse large B-cell lymphoma

DLBCL: Epidemiology

- 30-40% of adult non-Hodgkin lymphomas in western countries; higher proportion in developing countries
- Broad age range (median: 7th decade) including children
- Slightly more common in man
- Increasing incidence, independent of HIV

DLBCL: Site of Involvement

- Nodal or extra-nodal
- Up to 40% are at least initially confined to extranodal sites
- Most common extranodal site: GI (stomach or ileo-coecal region)
- Virtually any extranodal location
- Primary tumor in BM and/or PB is rare
- Certain morphologic variants are more prevalent at particular extranodal sites (eg, tumor in bone often exhibit multilobated nuclei)

DLBCL: Clinical Features

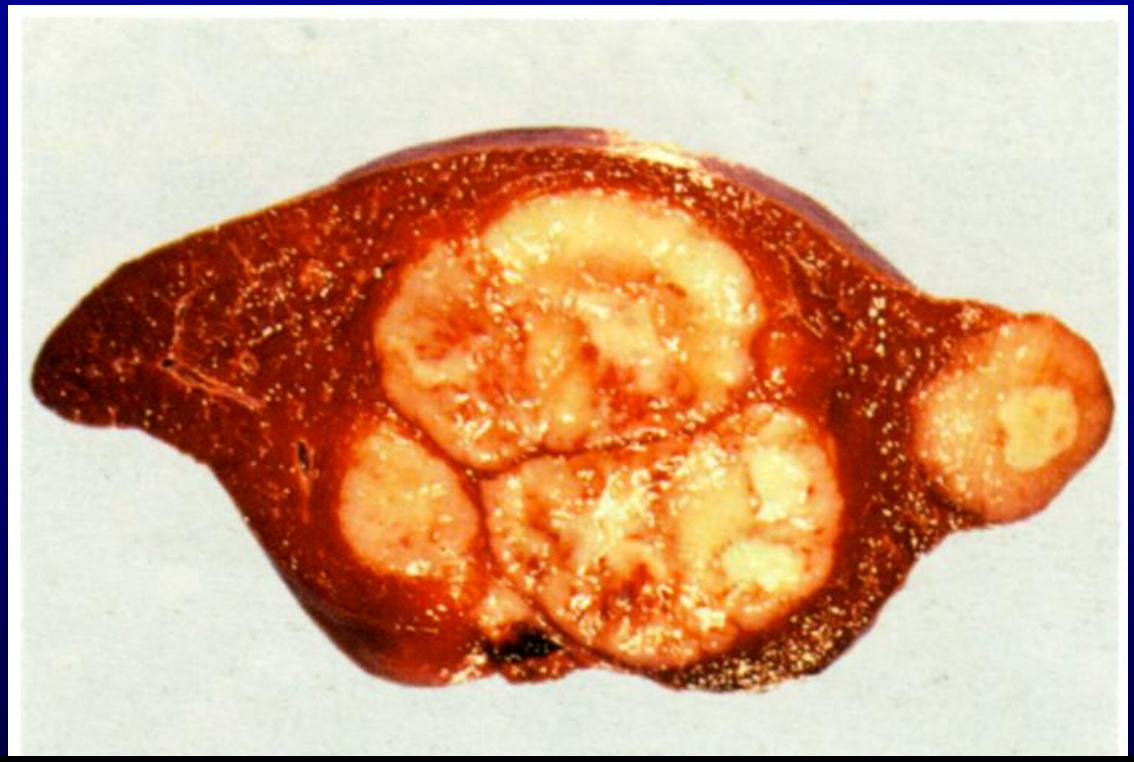
- A rapidly enlarging, often symptomatic mass at a single nodal or extranodal site
- With staging evaluation, many patients have disseminated disease

DLBCL: Etiology

- Unknown
- Usually *de novo* but can represent progression / transformation of a less aggressive lymphoma
- Immunodeficiency is a significant risk factor
- DLBCL in the setting of immunodeficiency are more often EBV-positive than sporadic DLBCL

DLBCL: Macroscopy

- Homogeneous fish-flesh replacement of most if not all of the structure
- The appearance of the lesion can be modified by hemorrhage or necrosis
- In extranodal sites, usually form a tumor mass with or without fibrosis



Spleen with DLBCL

DLBCL: Morphology

- Typically replaces the normal architecture in a diffuse pattern
- LN involvement may be complete, partial, interfollicular, or, less commonly sinusoidal
- The perinodal soft tissue is often infiltrated; broad or fine bands of sclerosis may be observed

DLBCL: Morphology

- Composed of large transformed lymphoid cells. Cytologically, they are diverse and can be divided into morphologic variants
- Distinction among these variants has generally met with poor intraobserver and interobserver reproducibility

DLBCL: Morphology

- Immunophenotypic and genotypic parameters have not helped to delineate distinctive morphologic subtypes, with rare exceptions
- Pathologists have the choice to use only the term DLBCL or to use one of the specific morphologic variants

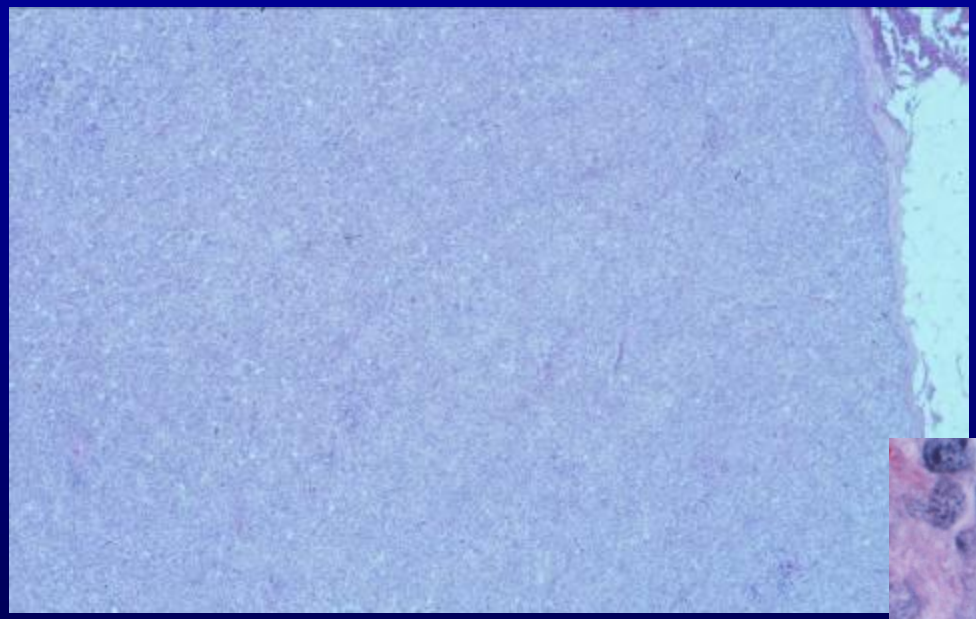
DLBCL: Morphology

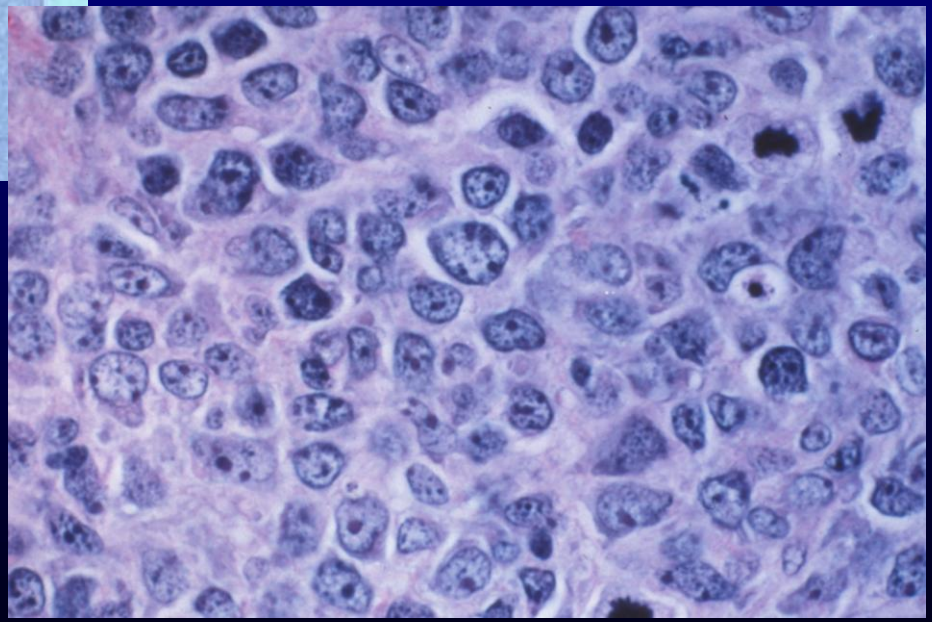
- Most cases will conform to one of the morphologic variants, with centroblastic being the most common
- Unusual variants have been described with myxoid stroma, a fibrillary matrix, pseudo-rosettes, spindle cells, signet ring cells, cytoplasmic granules, microvillous projections, and intercellular junctions

DLBCL: Morphology

- Cases of lymphomatoid granulomatosis with sheets of malignant cells represent progression to a variant of DLBCL
- Prominence of medium-sized cells may require special studies to exclude extramedullary leukaemias and Burkitt lymphoma variants

Diffuse Large Cell Lymphoma

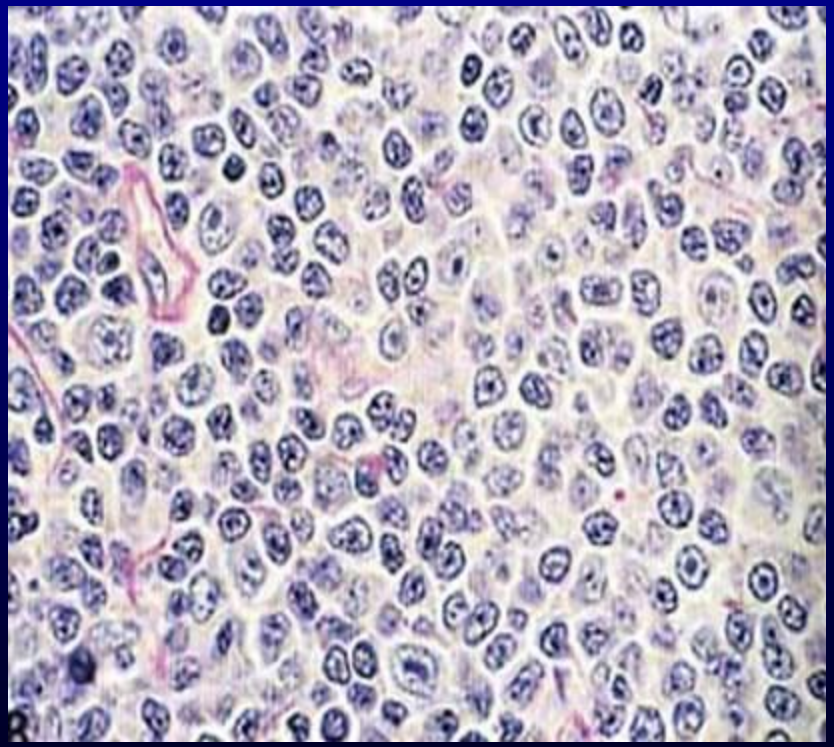




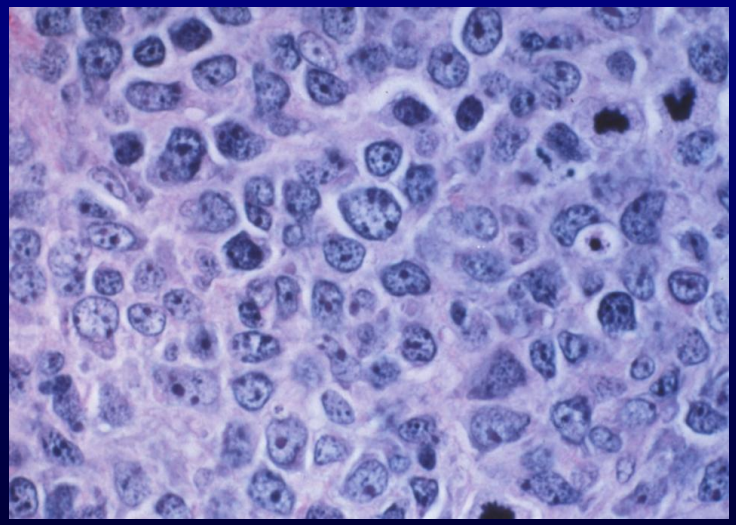
Small Lymphocytic Lymphoma vs Large Cell Lymphoma

Comparison of size

Diffuse Small lymphocytic lymphoma

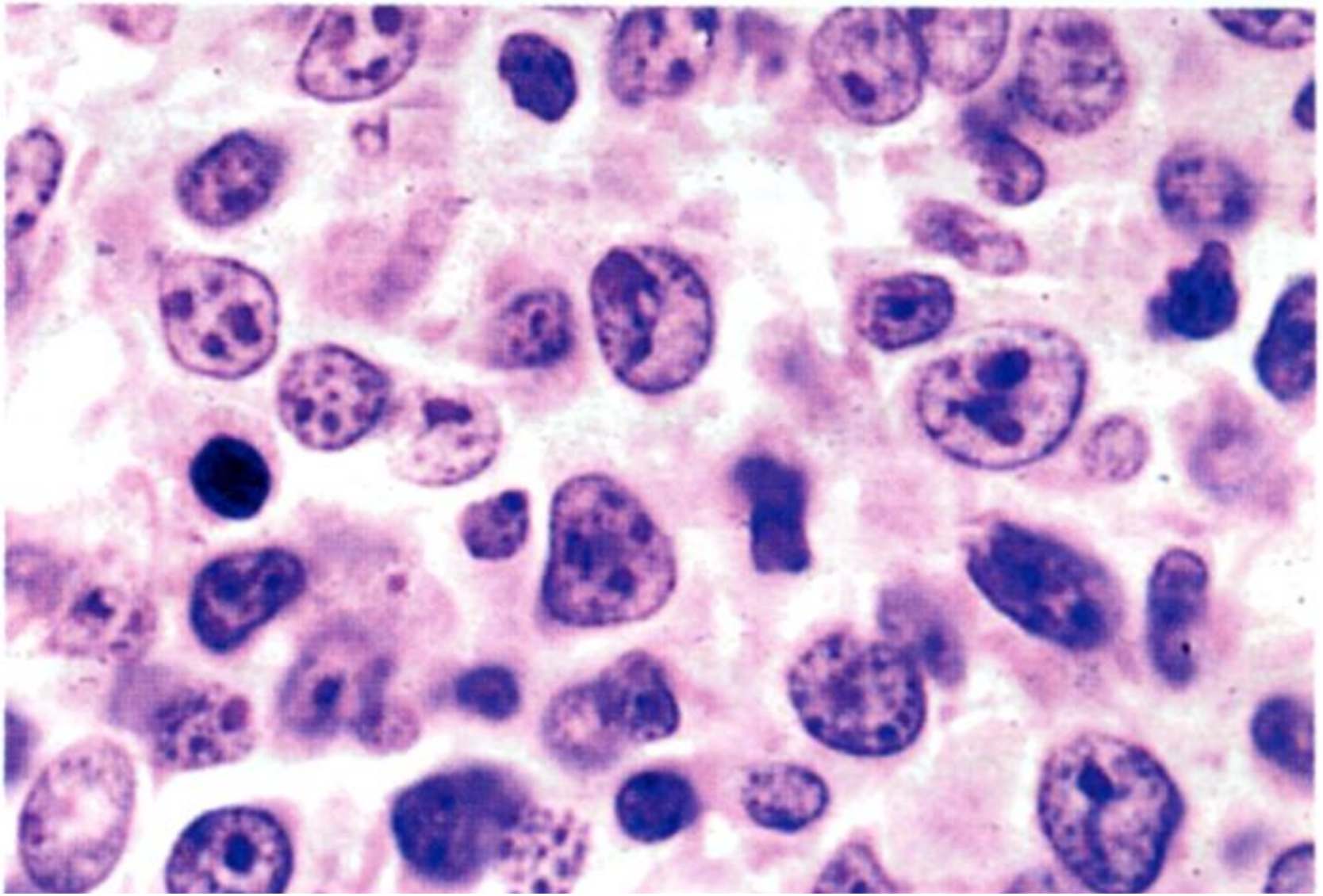


Diffuse Large B Cell Lymphoma

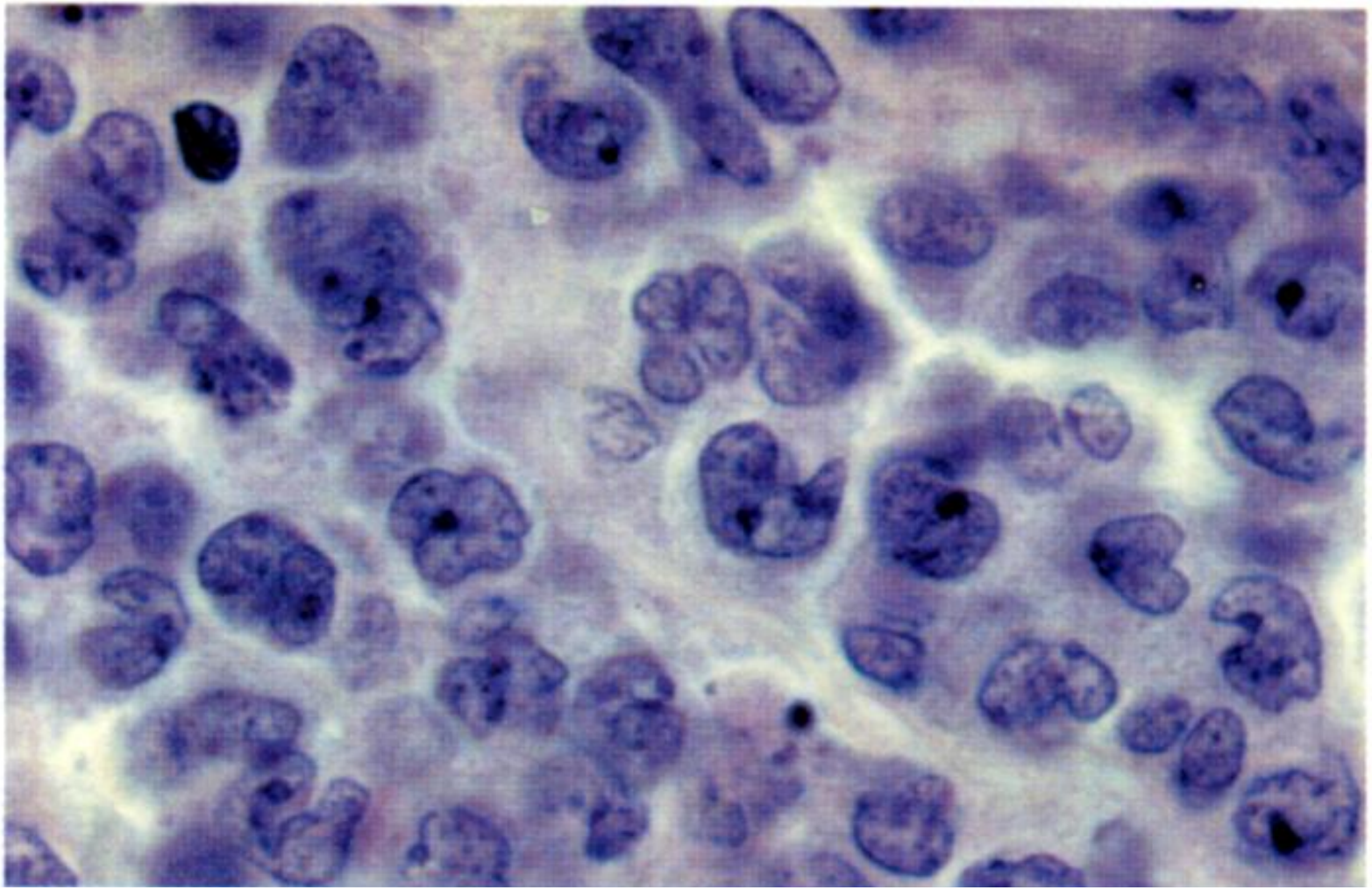


Morphologic Variants
Centroblastic

- Medium to large cells with oval to round, vesicular nuclei with fine chromatin and 2-4 nucleoli. The cytoplasm is generally scanty and amphophilic to basophilic
- May have a monomorphic or polymorphic appearance. It includes both the monomorphic and polymorphic variants of centroblastic lymphoma, as defined in the Kiel classification
- Cells may be multilobated. Centroblast-like cells may be admixed with multilobated cells and up to 90% immunoblasts

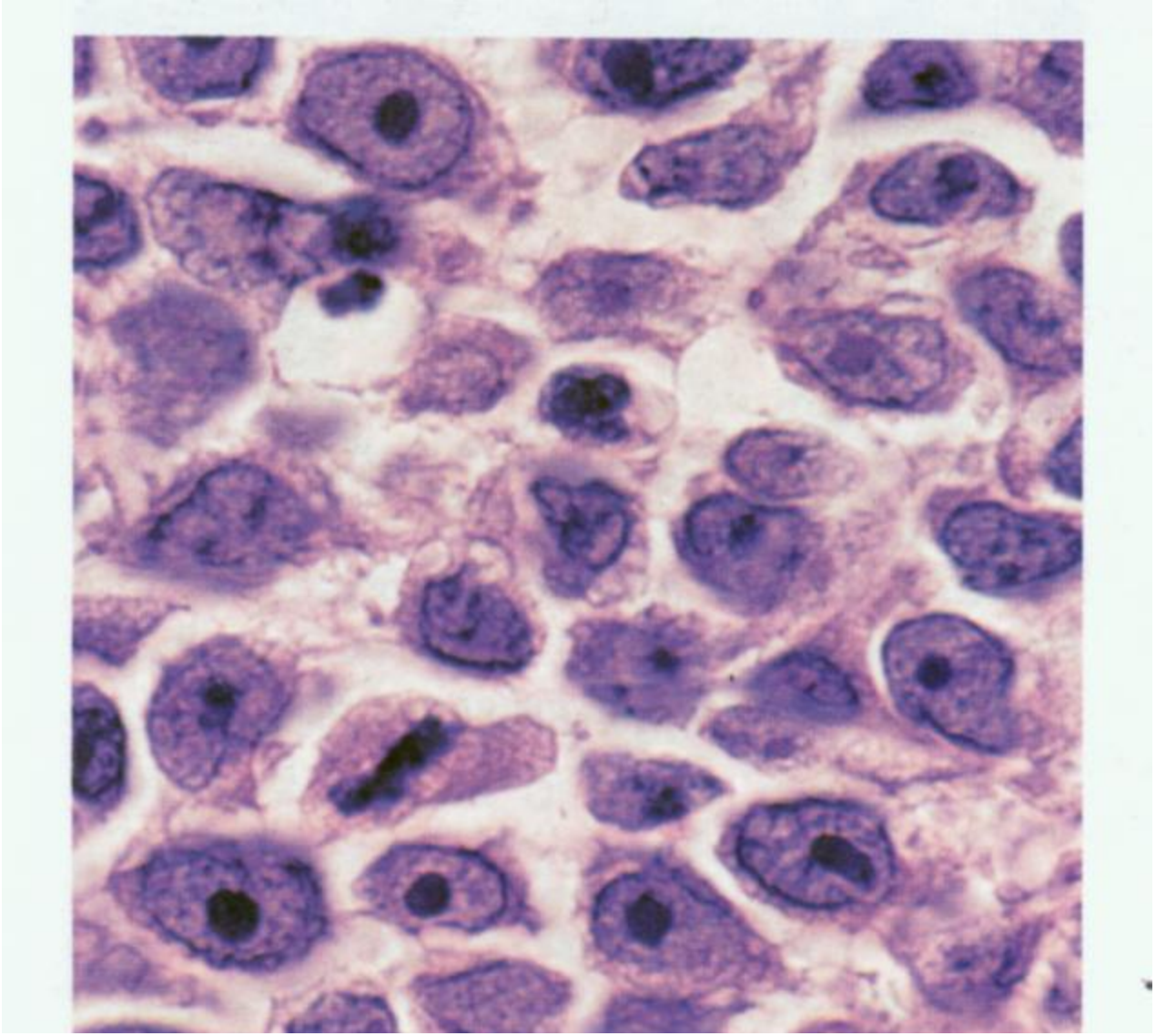


DLBCL, Centroblastic variant

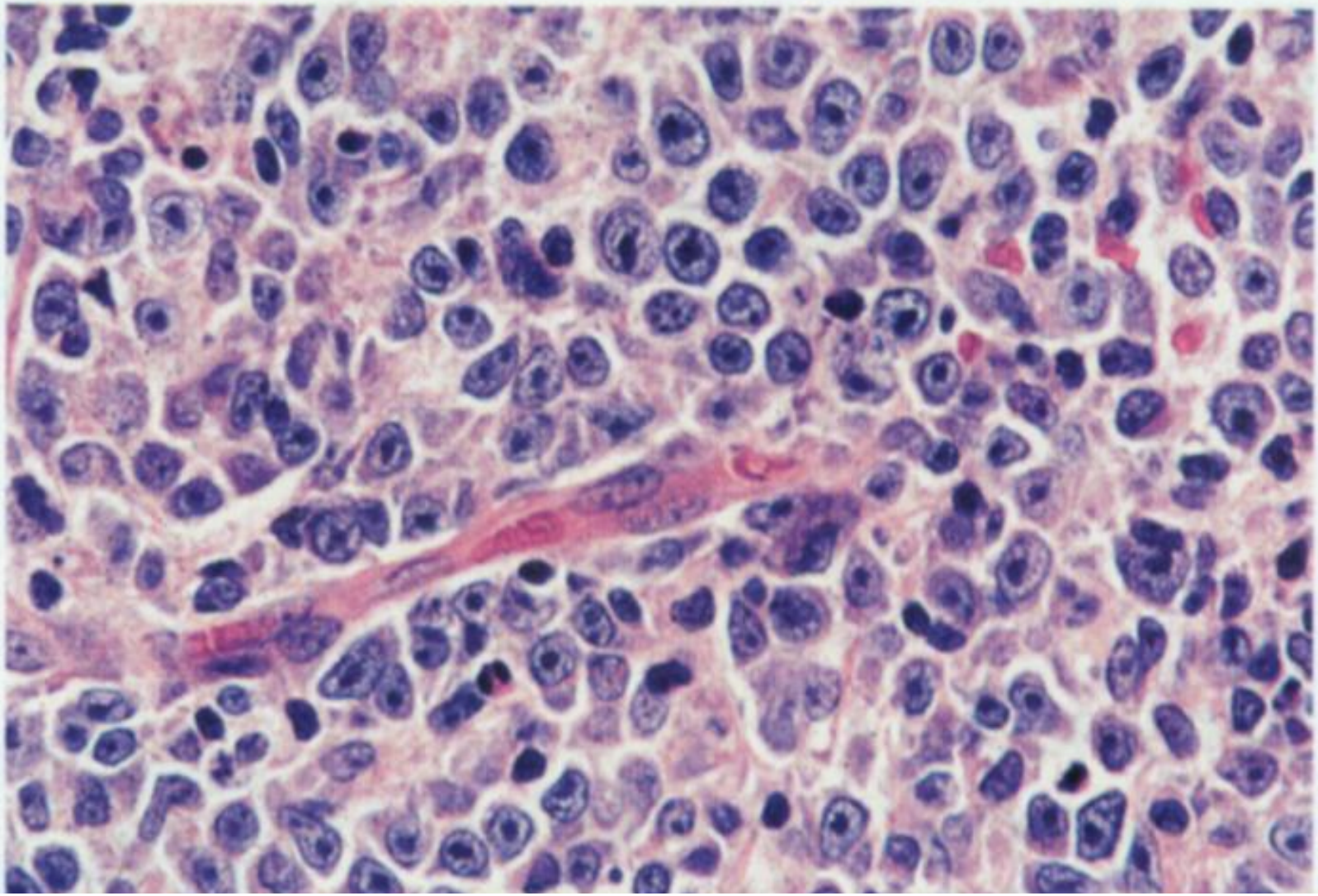


Morphologic Variants
Immunoblastic

- Immunoblasts > 90%, with a single centrally located nucleolus and an appreciable amount of basophilic cytoplasm
- Centroblasts <10%
- Plasmacytoid differentiation may be present
- Clinical and/or immunophenotypic findings may be essential in differentiating from extra-medullary involvement by a plasmablastic variant of plasma cell myeloma

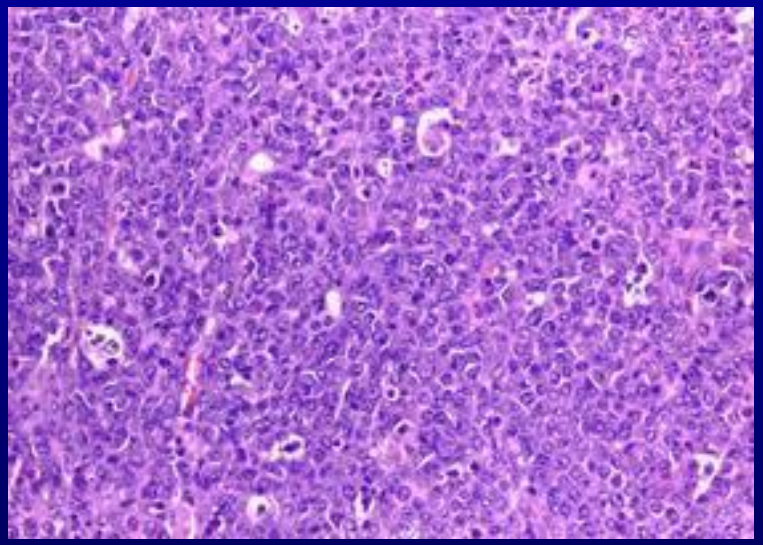


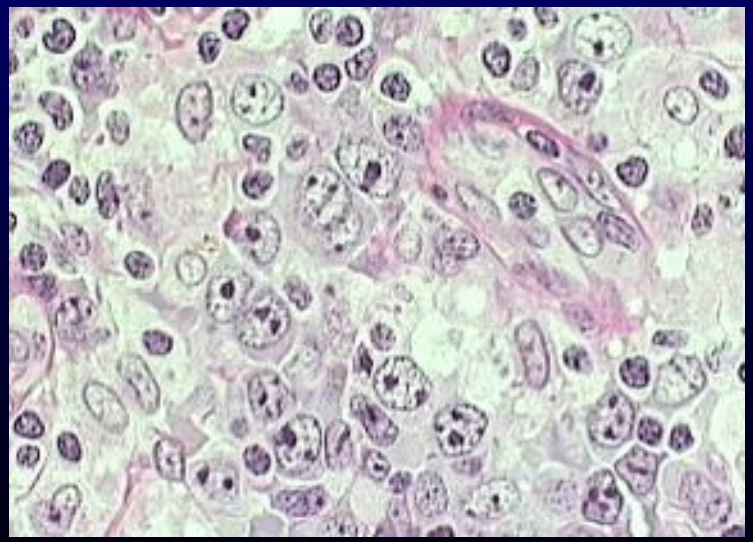
DLBCL, Immunoblastic variant



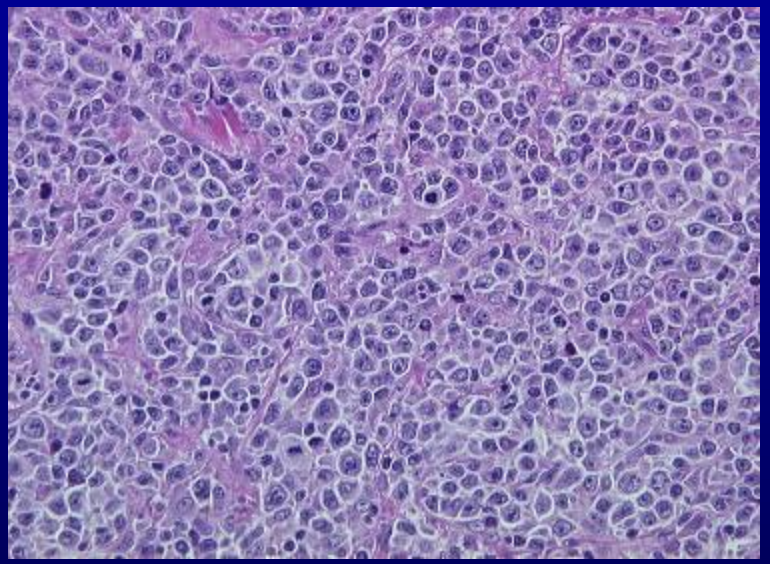
Diffuse Large B-Cell Lymphoma

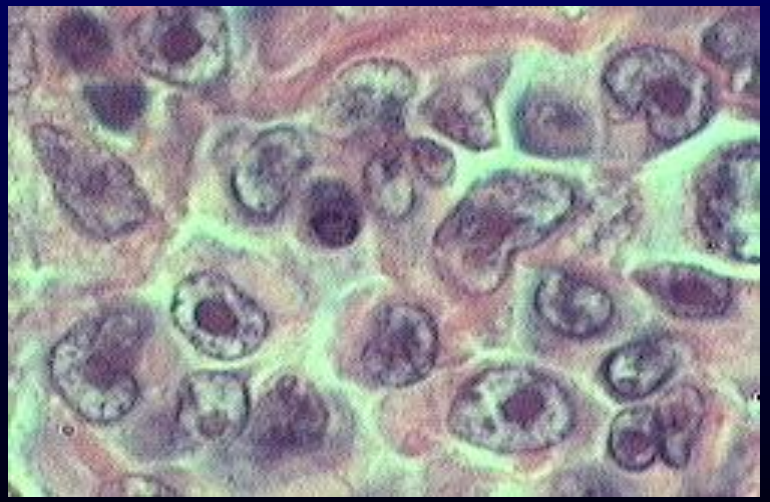
Centroblastic





Immunoblastic

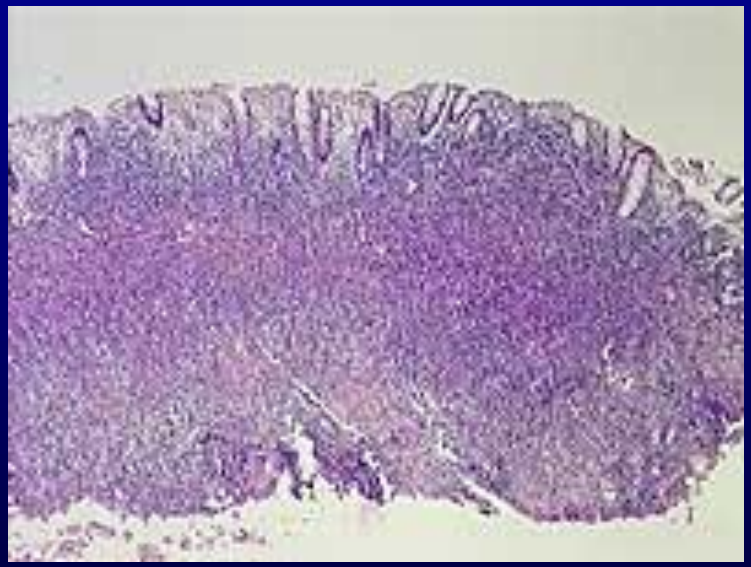


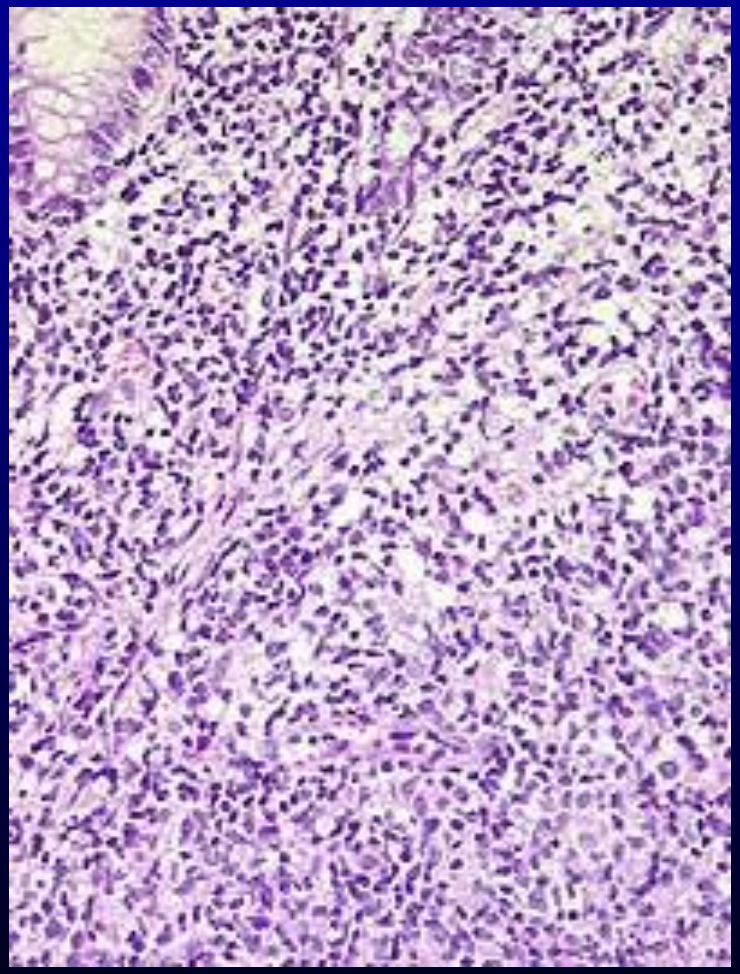


Morphologic Variants
T-Cell / Histiocyte Rich

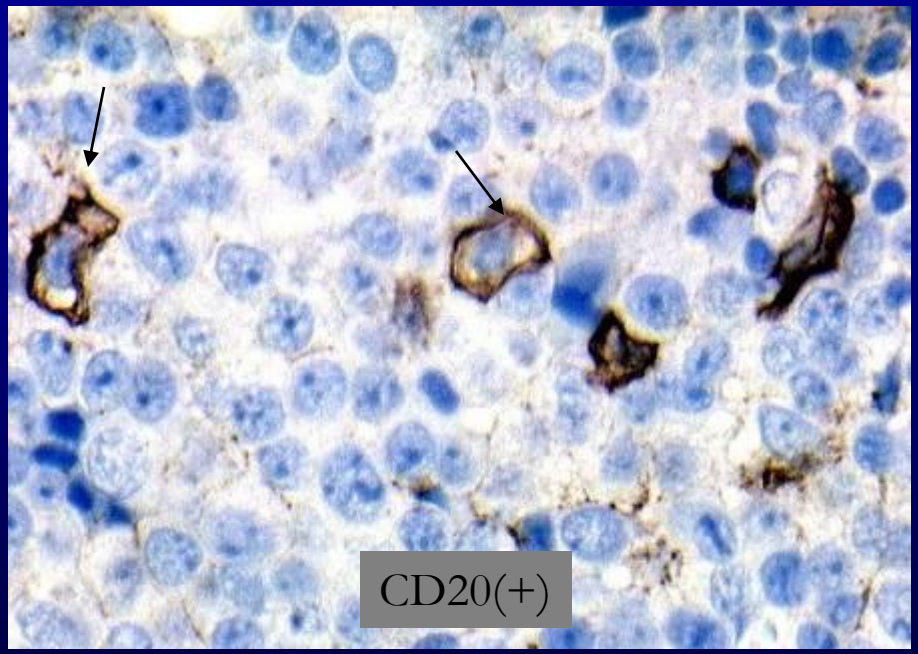
- Majority of cells are T-cells with or without histiocytes; <10% large neoplastic B-cells
- Histiocytes may or may not be epithelioid. The large cells may resemble L&H cells, centroblasts, immunoblasts, or Reed-Sternberg cells
- B-cells are rare to infrequent. Increased B-cells: possibility of NLPHL (especially vaguely nodular growth pattern)
- Immunophenotypic studies may be essential in the differential diagnosis with classical HD. Many diffuse mixed lymphoma cases in the Working Formulation represent the this variant of DLBCL

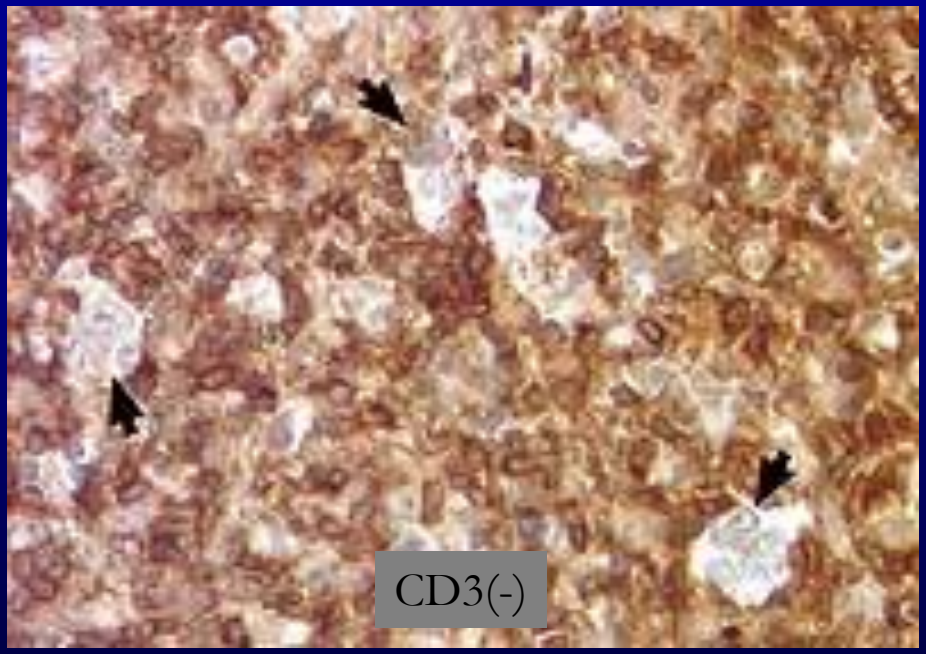
T-Cell Rich Large B-Cell Lymphoma

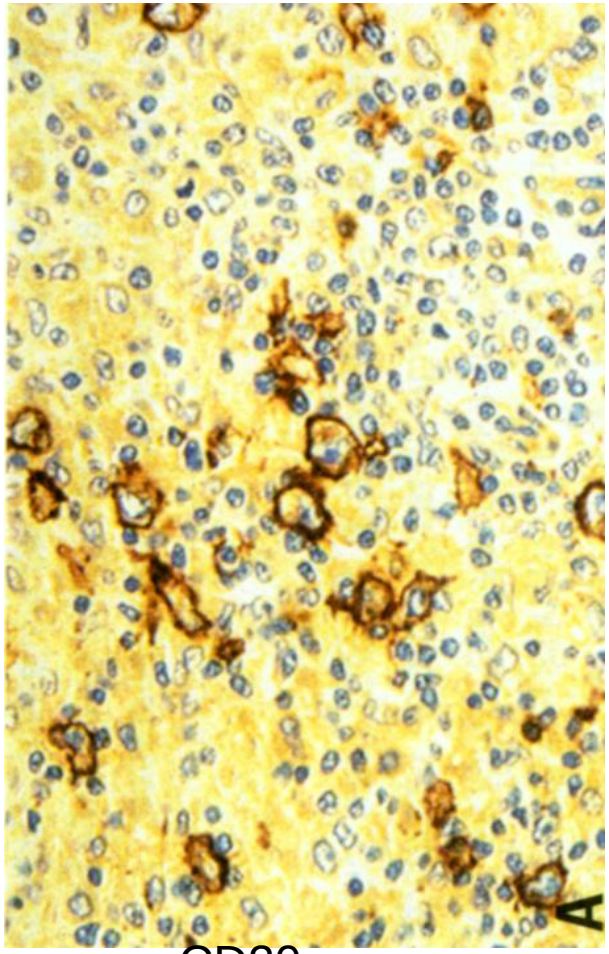




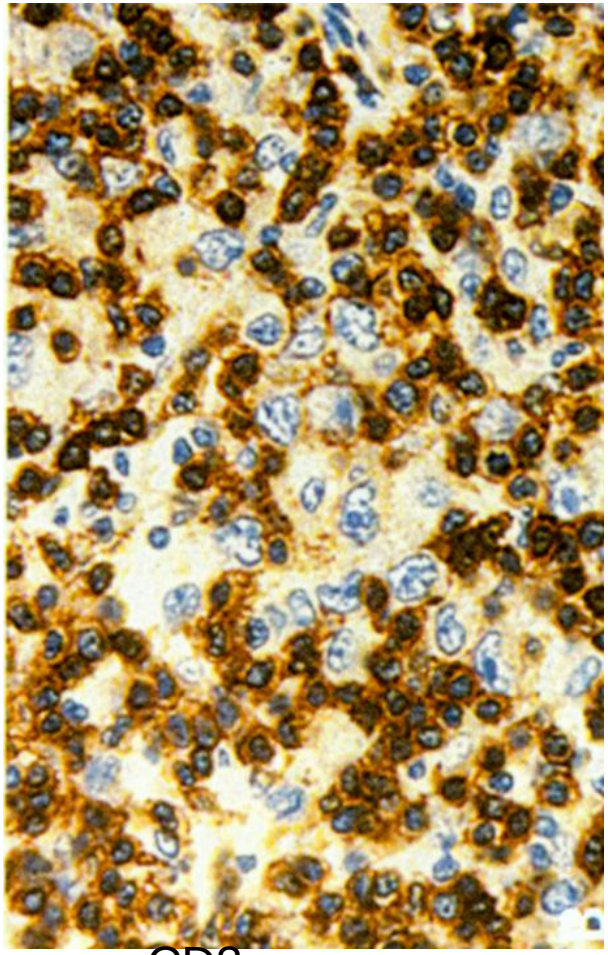
T-Cell Rich B-Cell Lymphoma



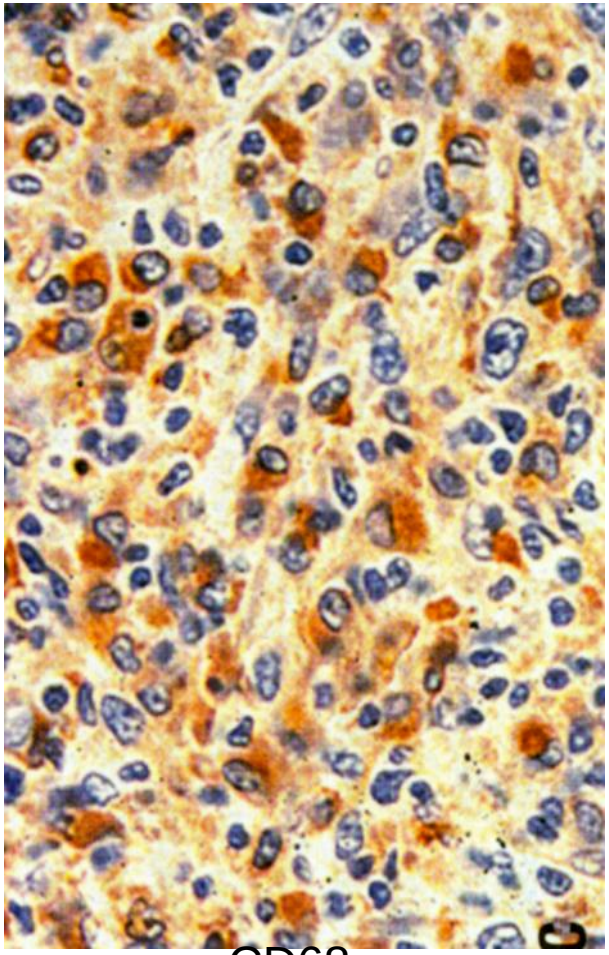




CD20



CD3



CD68

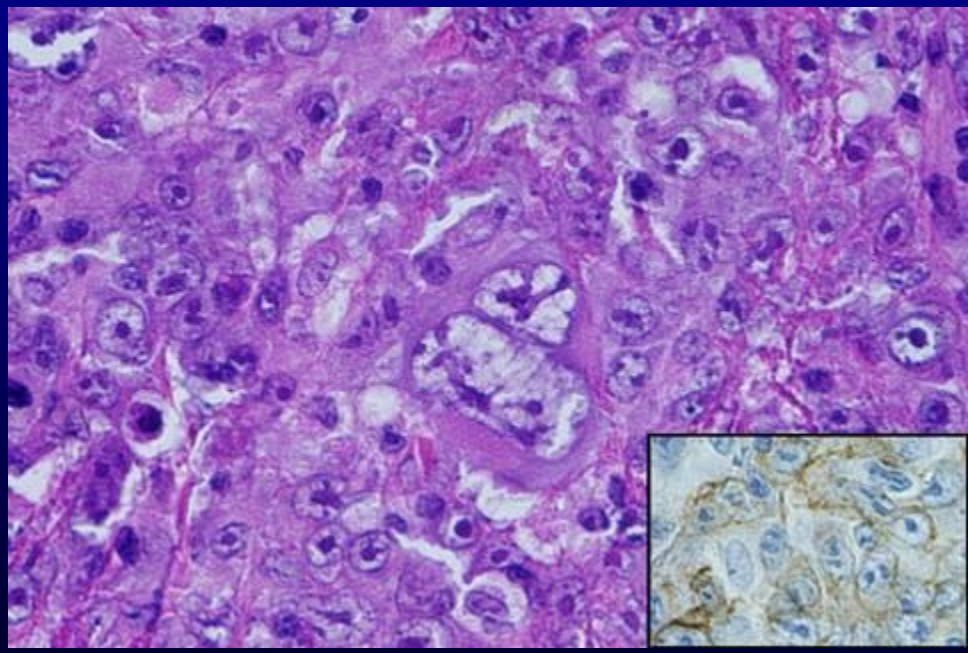
DLBCL, T cell/histiocytic rich variant

Morphologic Variants
Anaplastic

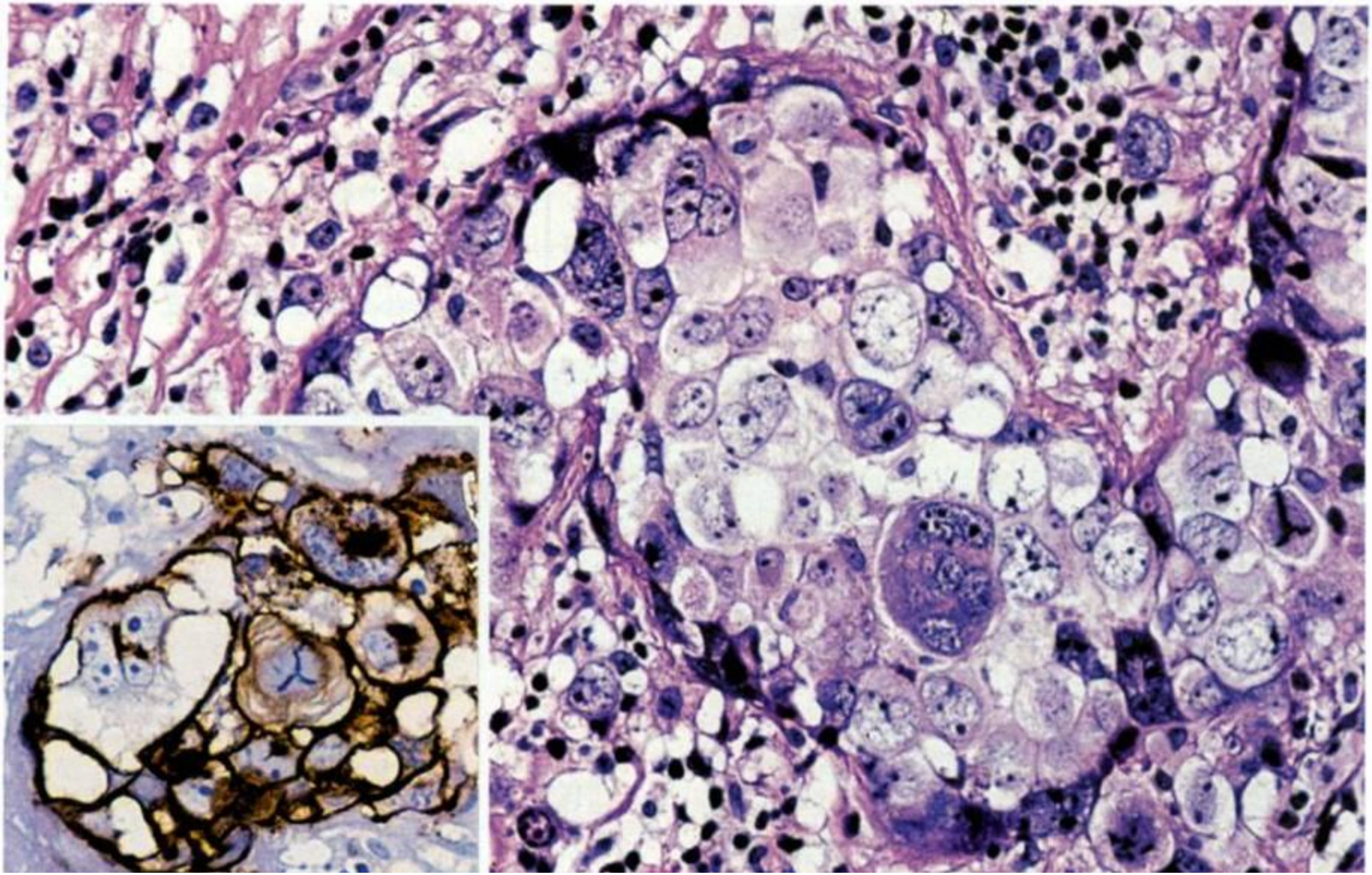
- Very large round, oval, or polygonal cells with bizarre pleomorphic nuclei which may resemble RS cells
- The cells may grow in a cohesive pattern mimicking carcinoma and may show a sinusoidal pattern of growth
- These cases are biologically and clinically unrelated to ALCL of cytotoxic T-cell derivation

Anaplastic Diffuse Large B-Cell Lymphoma

- Unrelated to T-cell large cell anaplastic lymphoma
- ALK negative
- May mimic carcinoma by cohesive growth pattern



CD20



DLBCL, anaplastic variant

DLBCL: Immunophenotype

- Express pan-B markers (CD19, CD20, CD22, and CD79a), but may lack one or more
- Surface/cyto Ig (lgM> IgG>lgA): 50-75%. Cyto Ig is often seen in cases with plasmacytic differentiation
- CD30: vast majority of anaplastic LBCL and occasional non-anaplastic cases
- CD5+ in 10% and CD10+ 25-50%. CD5+ DLBCL are negative for cyclin D1 (vs blastoid MCL). CD5+ DLBCL may arise *de novo* rather than as progression of SLL/CLL

DLBCL: Immunophenotype

- BCL2+ in 30-50%
- BCL6+ in a very high proportion of cases
- P53 expression, usually associated with *TP53* mutations, in a minority of cases
- Plasma cell-associated markers such as syndecan (CD 138) in a minority of cases
- Ki-67+ is usually high (>40%) and may be greater than 90%

DLBCL: Genetics

- Most cases have rearranged Ig H and L chain genes and show somatic mutations in the variable regions
- t(14:18) occurs in 20-30%
- Up to 30% show abnormalities of the 3q27 involving BCL6
- *MYC* rearrangement is uncommon
- Many cases exhibit complex cytogenetic abnormalities
- EBV+ is more common in cases associated with immunodeficiency

DLBCL: Genetics

- DNA microarrays identified two major molecular categories with gene expression patterns suggestive of different stages of B-cell development
- One type had an expression profile characteristic of germinal center B-cells, whereas the other type had a profile similar to that of *in vitro* activated peripheral blood B-cells

DLBCL: Postulated Cell of Origin

Peripheral B-cells of either germinal center or post germinal center origin

DLBCL: Prognosis and Predictive Factors

- Aggressive but potentially curable
- IPI is strongly predictive of outcome
- Adverse prognostic indicators: high proliferative rate, BCL2 and P53 overexpression
- Possibly worse prognosis for immunoblastic over centroblastic variants
- Better prognosis indicator: BCL6 translocation
- Germinal center B-like DLBCL had a significantly better overall survival than activated B-like.

Distinct types of diffuse large B-cell lymphoma identified by gene expression profiling

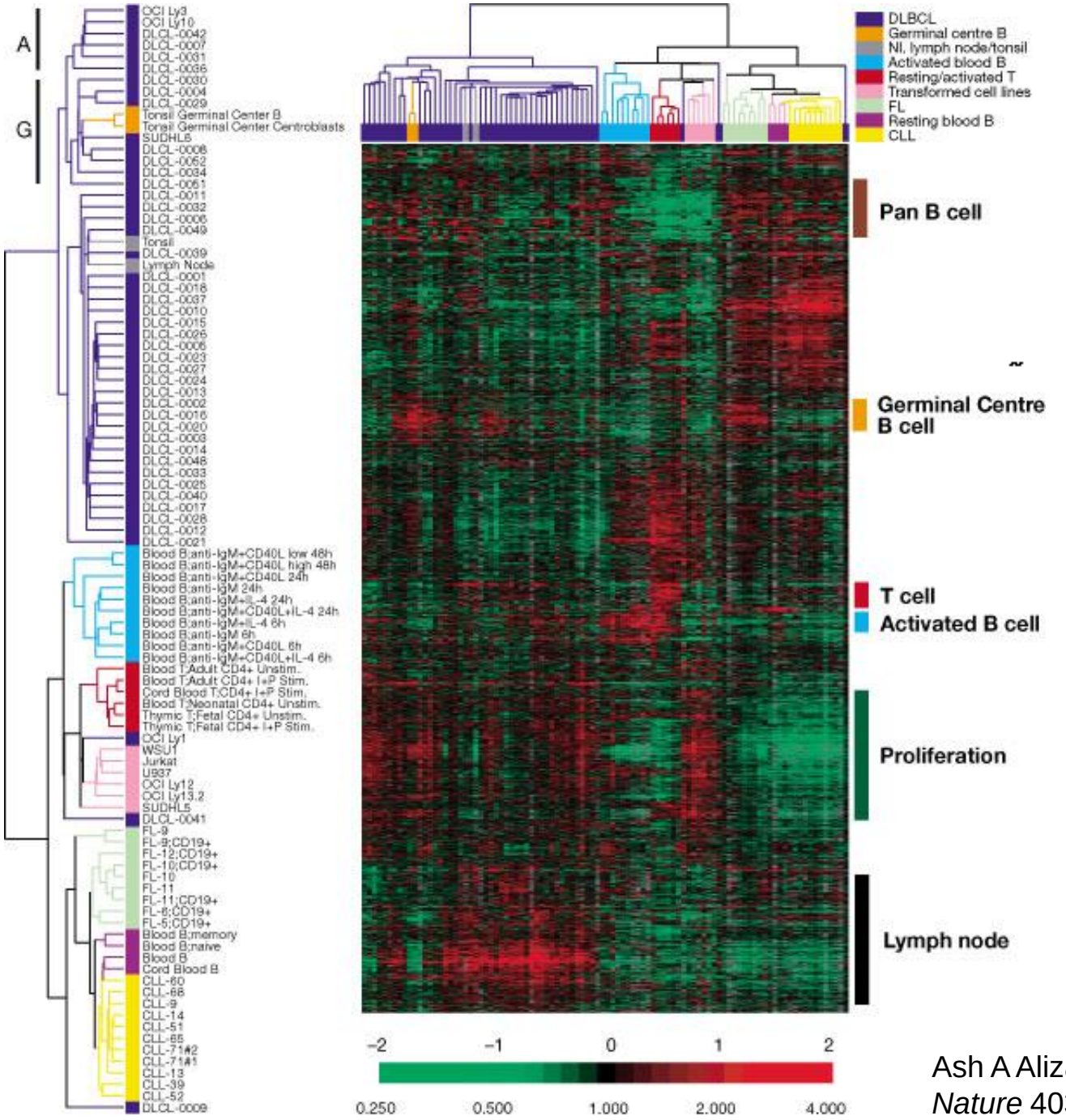


Figure 1. Hierarchical clustering of gene expression data. Depicted are the 1.8 million measurements of gene expression from 128 microarray analyses of 96 samples of normal and malignant lymphocytes. The dendrogram at the left lists the samples studied and provides a measure of the relatedness of gene expression in each sample. The dendrogram is colour coded according to the category of mRNA sample studied (see upper right key). Each row represents a separate cDNA clone on the microarray and each column a separate mRNA sample. The results presented represent the ratio of hybridization of fluorescent cDNA probes prepared from each experimental mRNA samples to a reference mRNA sample. These ratios are a measure of relative gene expression in each experimental sample and were depicted according to the colour scale shown at the bottom. As indicated, the scale extends from fluorescence ratios of 0.25 to 4 (-2 to +2 in log base 2 units). Grey indicates missing or excluded data.

Ash A Alizadeh, Michael B Eisen, et al.
Nature 403, 503 – 511, 2000

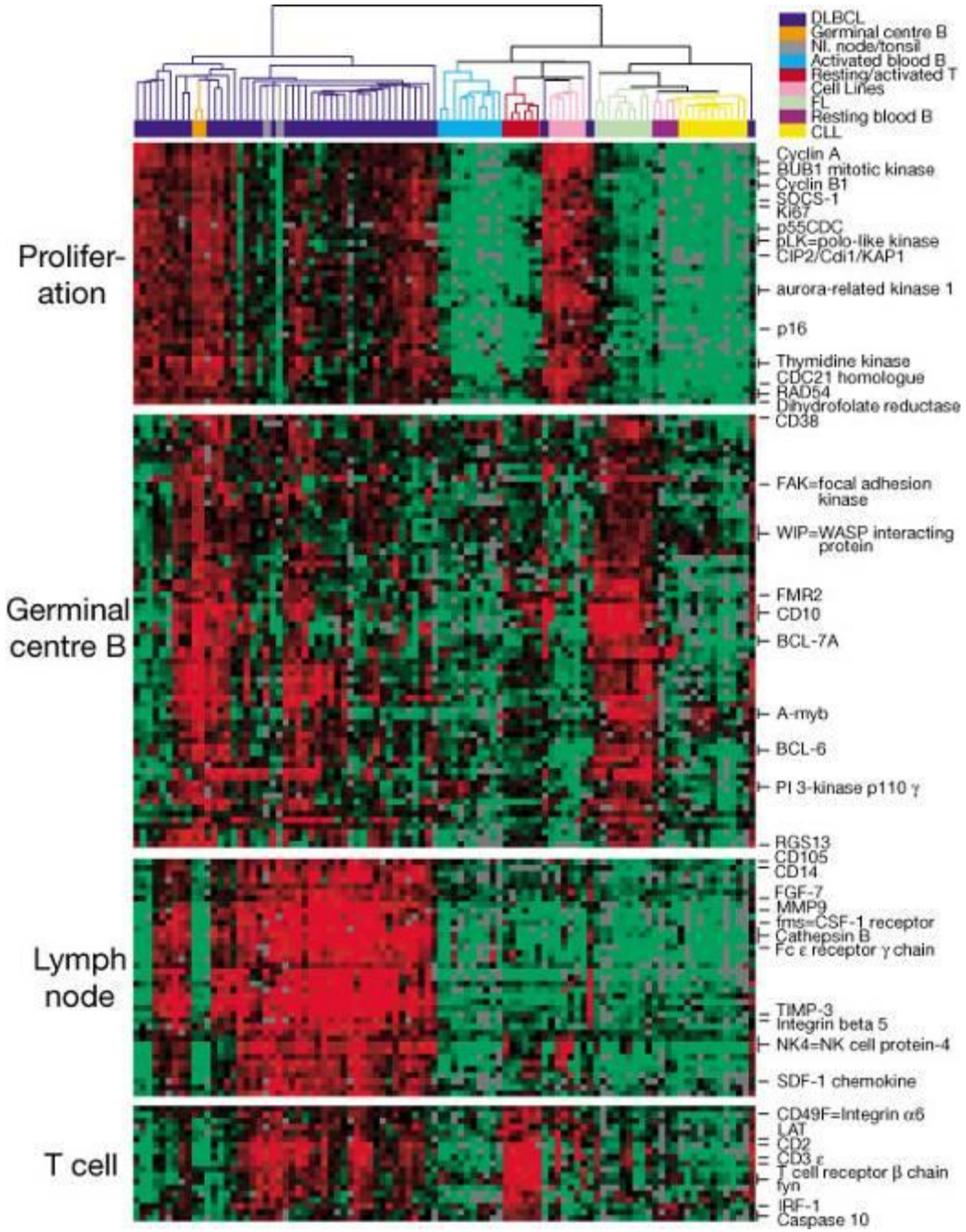


Figure 2 Expanded view of biologically distinct gene expression signatures defined by hierarchical clustering. Data are the same as in Fig. 1. Most genes without designations on the right are new genes of unknown function derived from various lymphoid cDNA libraries.

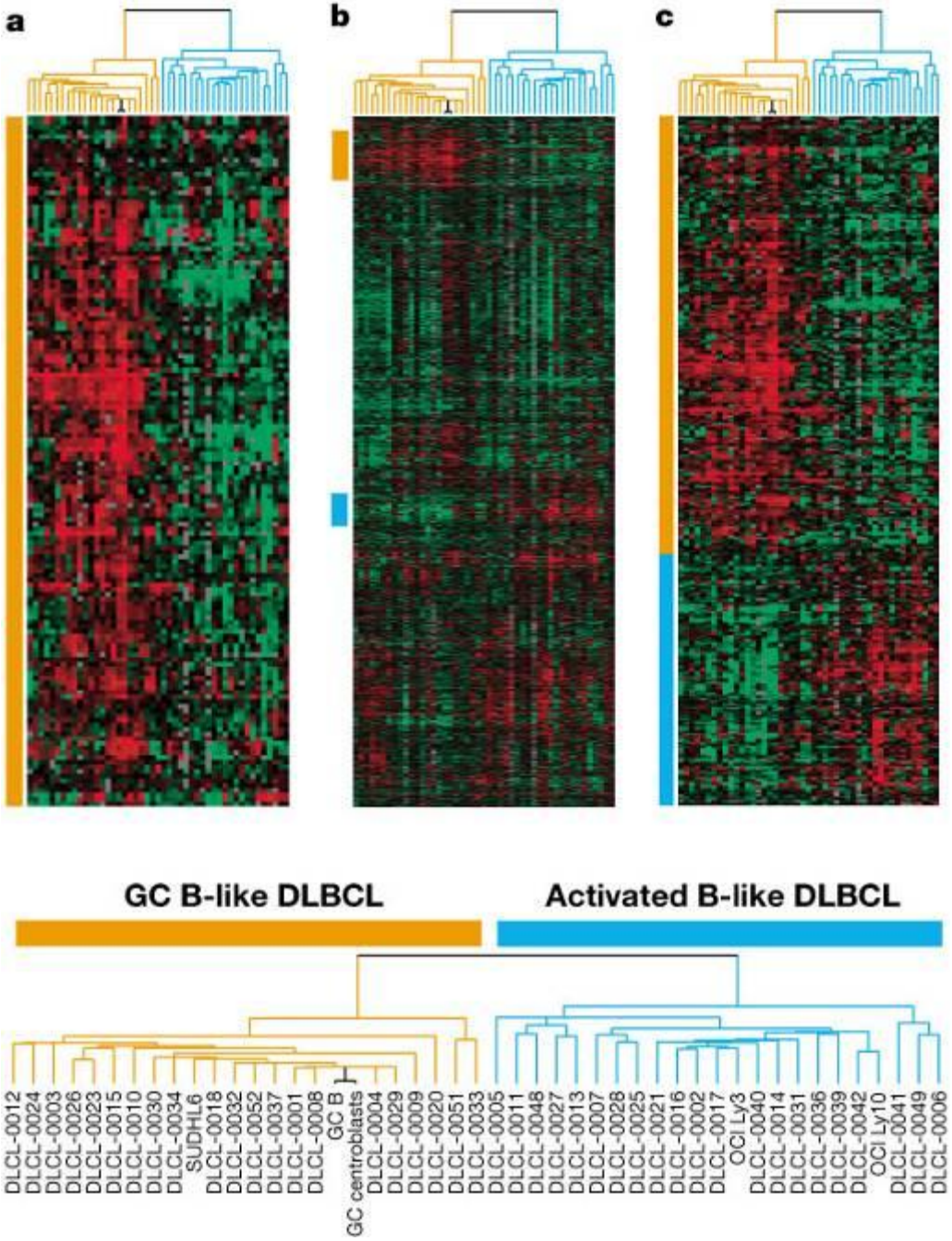


Figure 3. Discovery of DLBCL subtypes by gene expression profiling. The samples used in this clustering analysis are shown at the bottom. **a**, Hierarchical clustering of DLBCL cases (blue and orange) and germinal centre B cells (black) based on the genes of the germinal centre B-cell gene expression signature shown in Figs 1 and 2. Two DLBCL subgroups, GC B-like DLBCL (orange) and activated B-like DLBCL (blue) were defined by this process. **b**, Discovery of genes that are selectively expressed in GC B-like DLBCL and activated B-like DLBCL. All genes from Fig. 1, with the exception of the genes in the proliferation, T-cell and lymph-node gene expression signatures, were ordered by hierarchical clustering while maintaining the order of samples determined in Fig. 3a. Genes selectively expressed in GC B-like DLBCL (orange) and activated B-like DLBCL (blue) are indicated. **c**, Hierarchical clustering of the genes selectively expressed in GC B-like DLBCL and activated B-like DLBCL, which was determined from Fig. 3b.

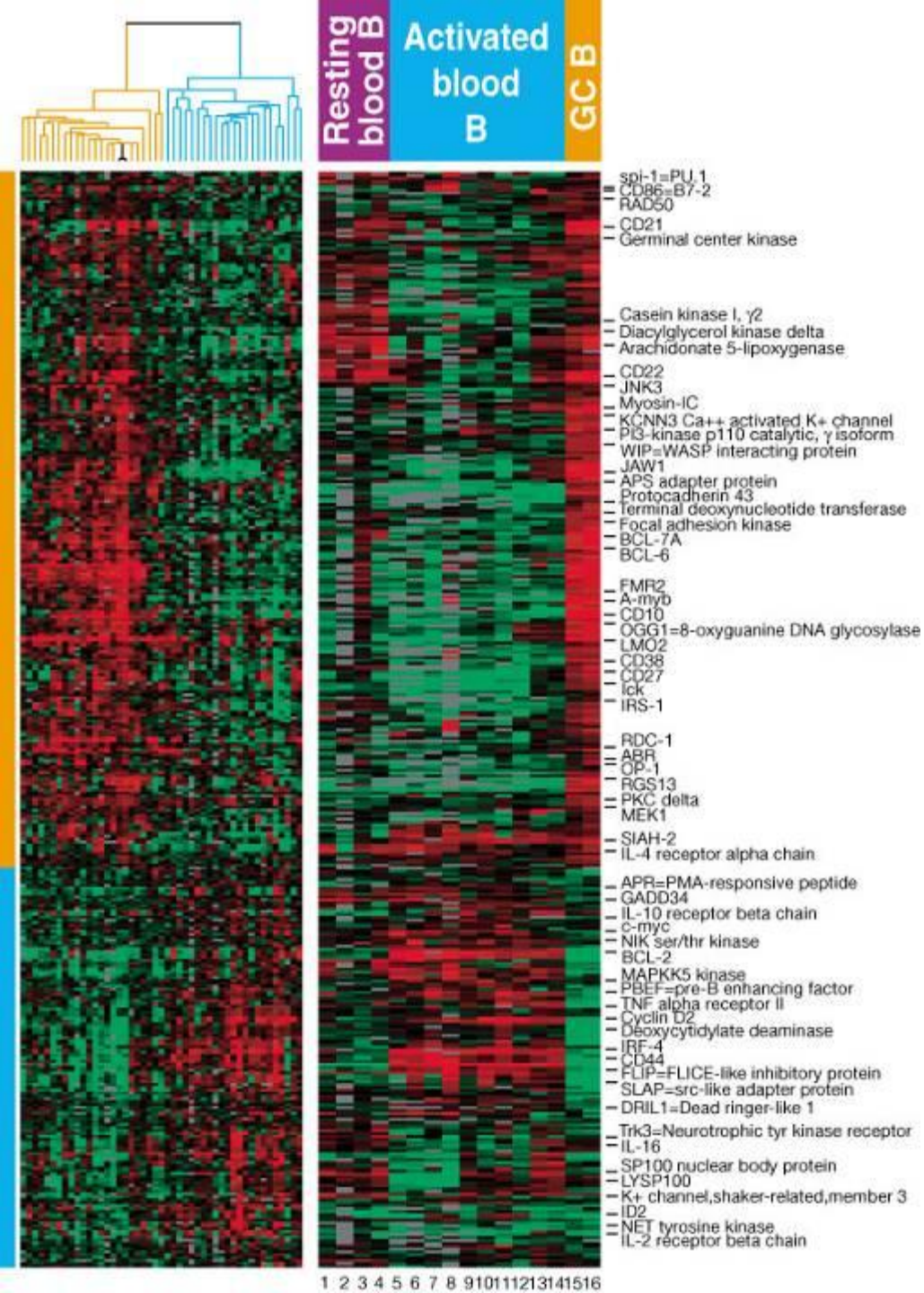


Figure 4 Relationship of DLBCL subgroups to normal B-lymphocyte differentiation and activation. The data in the left panel are taken from Fig. 3c. The right panel depicts gene expression data from the following normal B-cell samples: (1) Total $CD19^+$ blood B cells; (2) Naive $CD27^-$ blood B cells; (3) Memory $CD27^+$ blood B cells; (4) cord blood $CD19^+$ B cells; (5) blood B cells; anti-IgM 6 h; (6) blood B cells; anti-IgM + IL-4 6 h; (7) blood B cells; anti-IgM + CD40 ligand 6 h; (8) blood B cells; anti-IgM + CD40 ligand + IL-4 6 h; (9) blood B cells; anti-IgM 24 h; (10) blood B cells; anti-IgM + IL-4 24 h; (11) blood B cells; anti-IgM + CD40 ligand 24 h; (12) blood B cells; anti-IgM + CD40 ligand + IL-4 24 h; (13) blood B cells; anti-IgM + CD40 ligand (low concentration) 48 h; (14) blood B cells; anti-IgM + CD40 ligand (high concentration) 48 h; (15) tonsil germinal centre B cells; (16) tonsil germinal centre centroblasts.

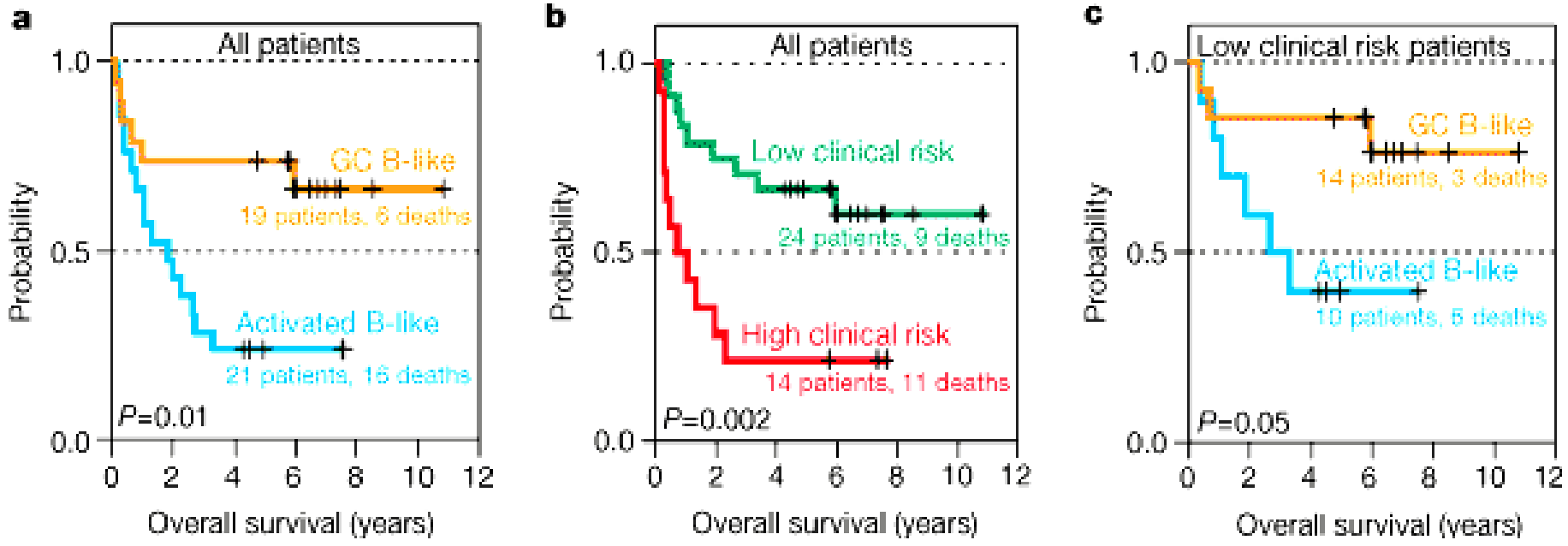
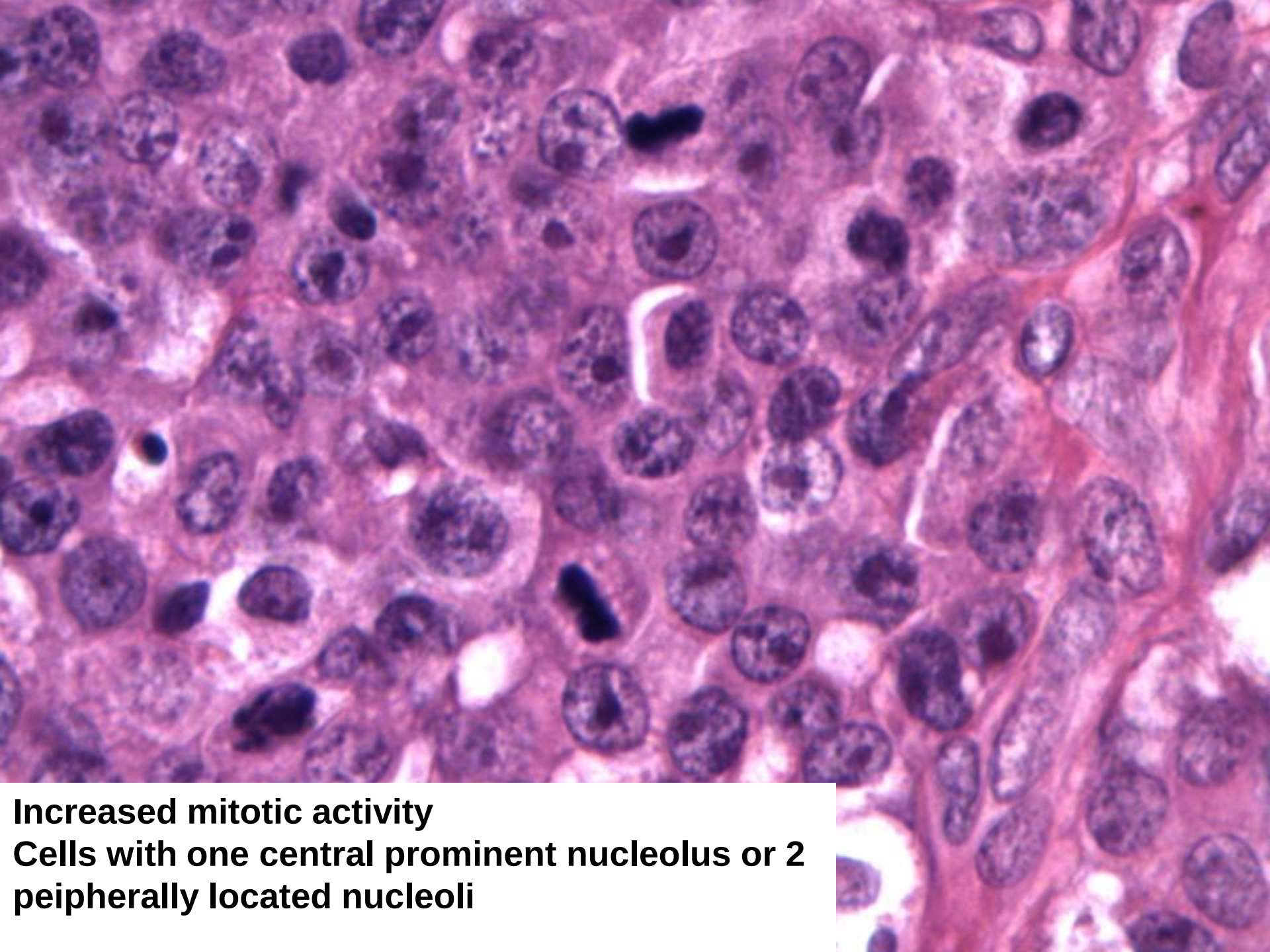


Figure 5. Clinically distinct DLBCL subgroups defined by gene expression profiling. **a**, Kaplan–Meier plot of overall survival of DLBCL patients grouped on the basis of gene expression profiling. **b**, Kaplan–Meier plot of overall survival of DLBCL patients grouped according to the International Prognostic Index (IPI). Low clinical risk patients (IPI score 0–2) and high clinical risk patients (IPI score 3–5) are plotted separately. **c**, Kaplan–Meier plot of overall survival of low clinical risk DLBCL patients (IPI score 0–2) grouped on the basis of their gene expression profiles.

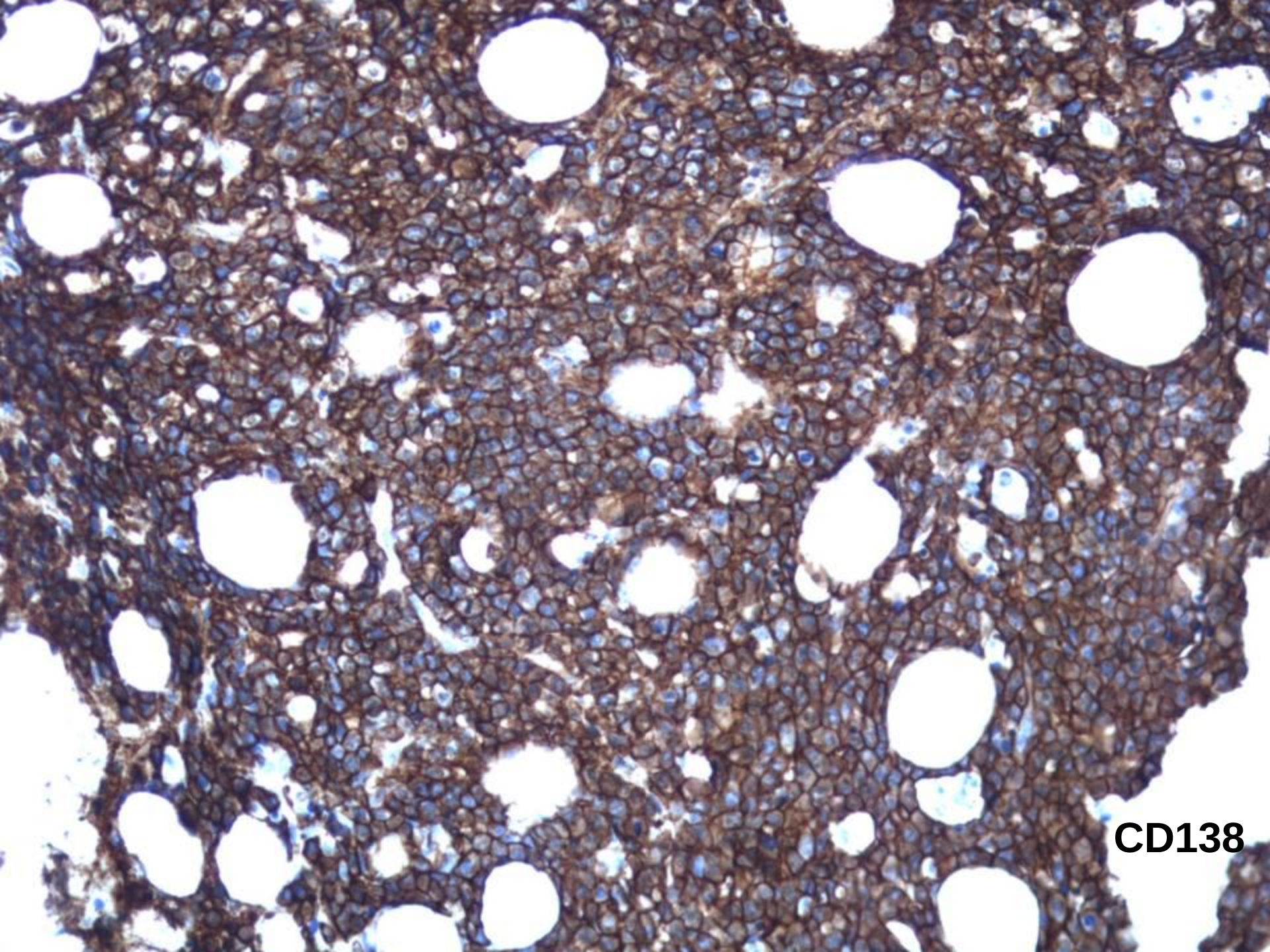
DLBCL: Other Rare Variants/Subtypes with Distinct Immunophenotypic Features

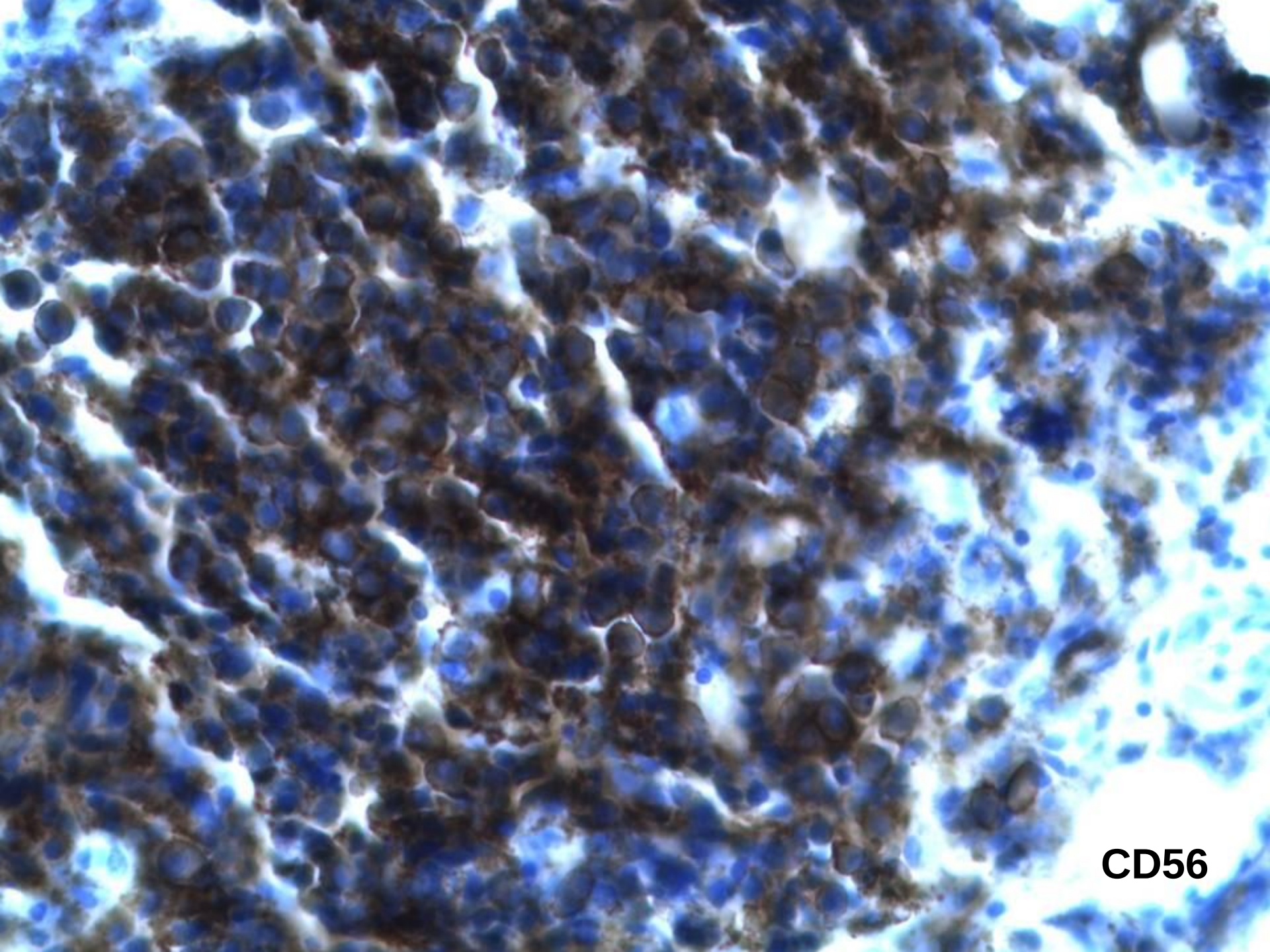
Plasmablastic

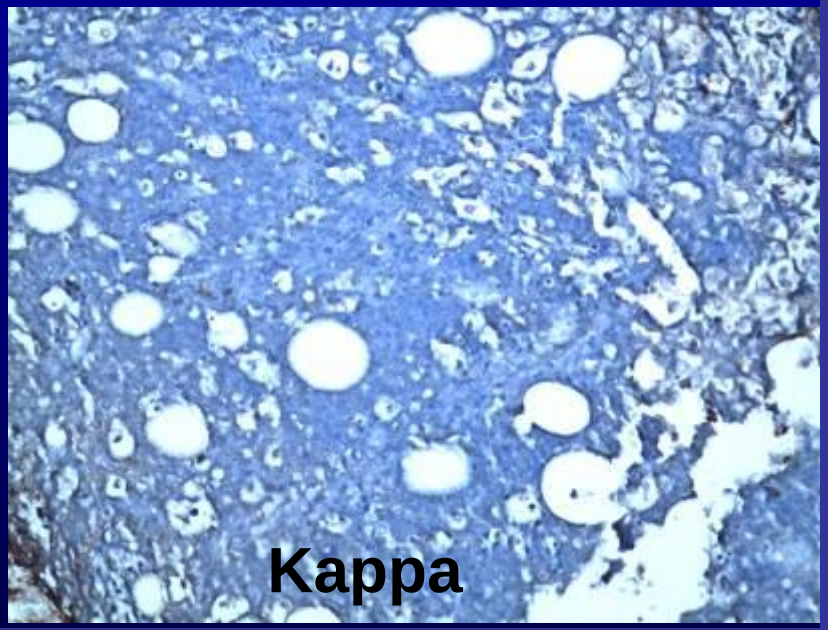
- Typically presents in the oral cavity in the setting of HIV infection
- EBV+ in 60% of cases
- Although these lymphomas are indistinguishable from some immunoblastic lymphoma on morphologic grounds, few if any of the lymphoma cells stain for CD20 and CD45 but they do express plasma cell markers such as vs38c and CD138
- High growth fraction, absence of mature monoclonal plasma cells, and the characteristic clinical features help to distinguish this variant from plasma cell myeloma

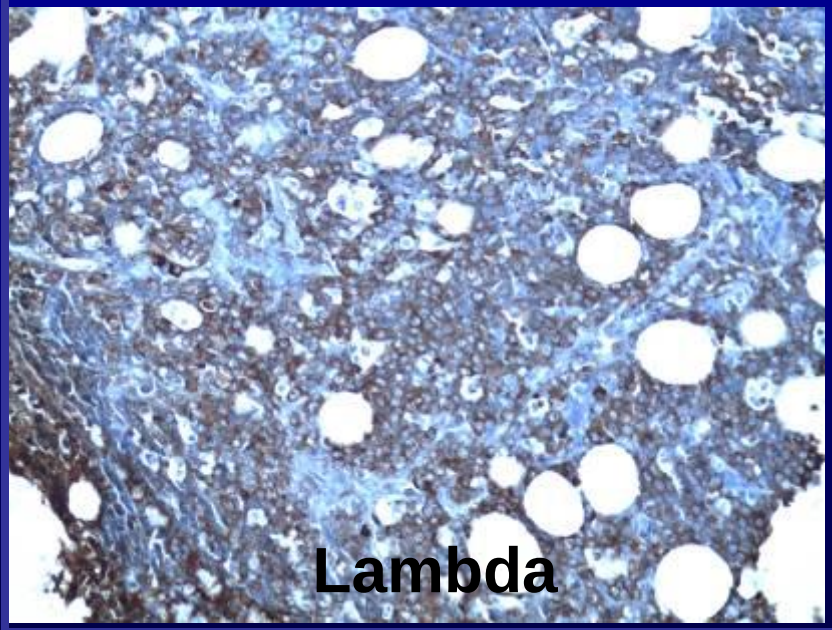


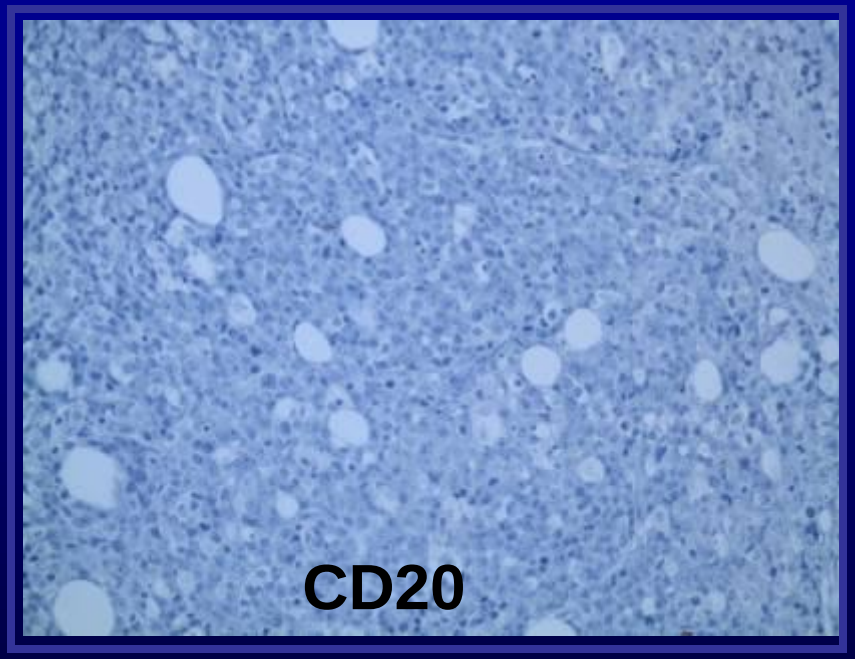
Increased mitotic activity
Cells with one central prominent nucleolus or 2 peipherally located nucleoli

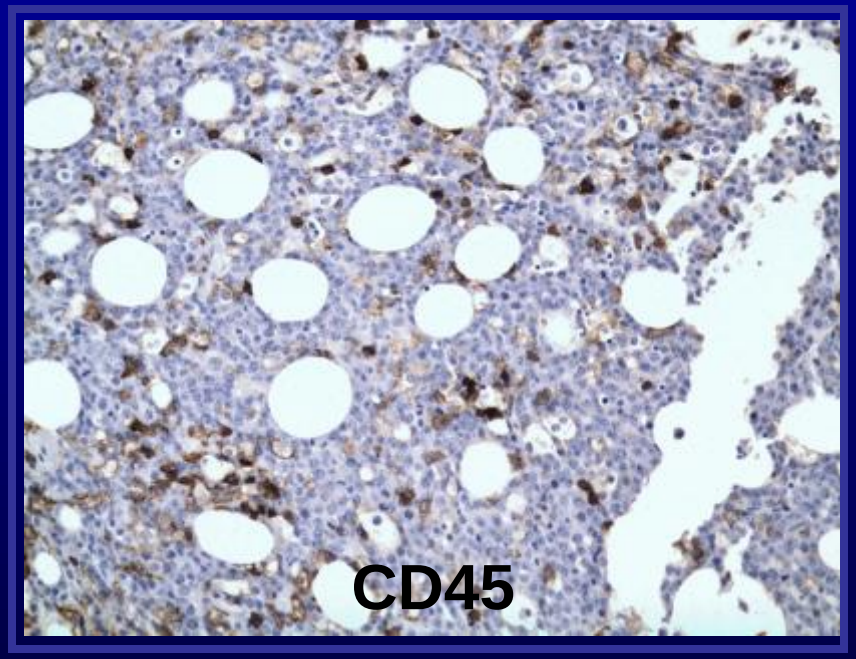

CD138


CD56

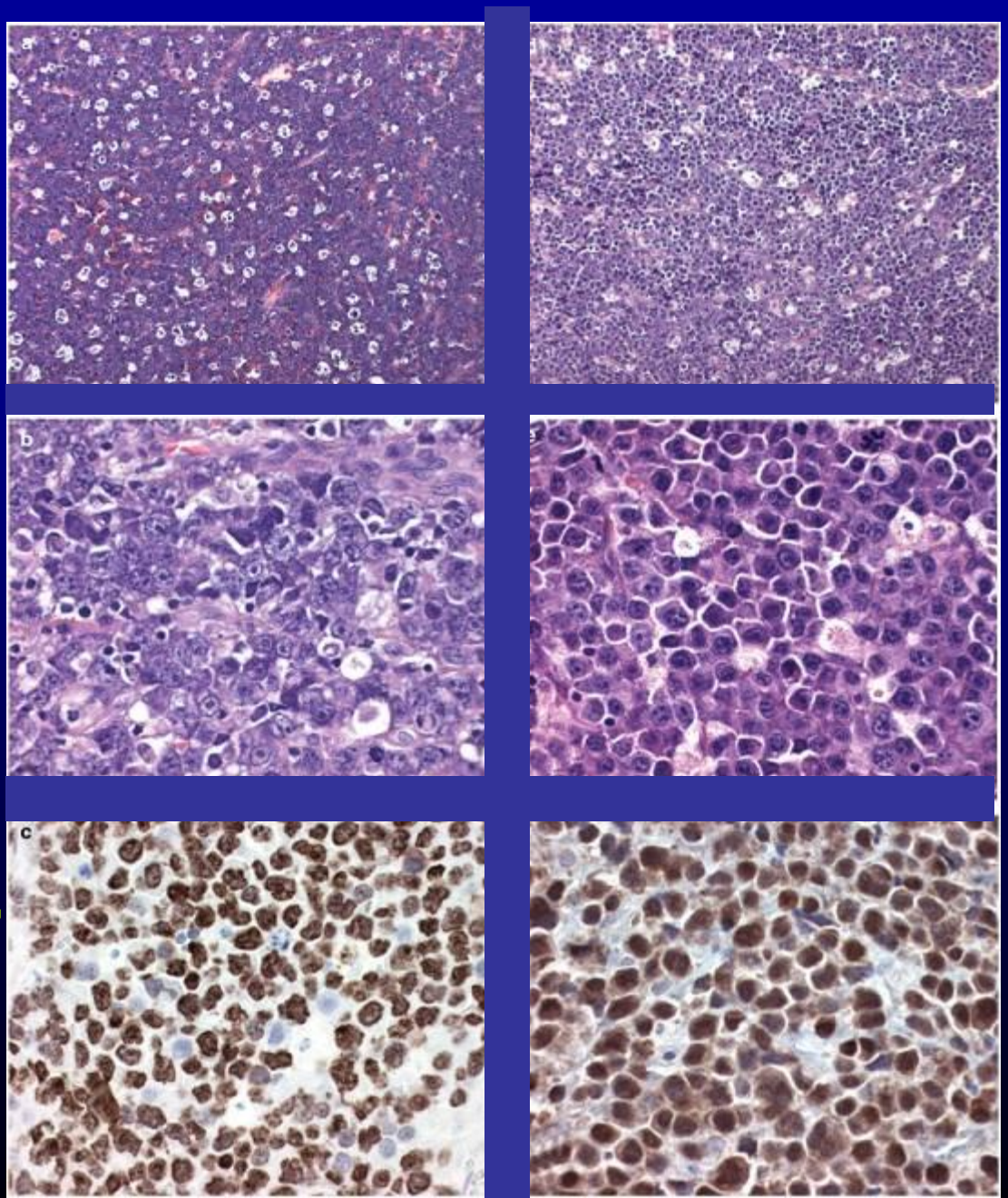

Kappa


Lambda


CD20


CD45

Plasmablastic lymphoma



Ki-67

DLBCL: Other Rare Variants/Subtypes with Distinct Immunophenotypic Features

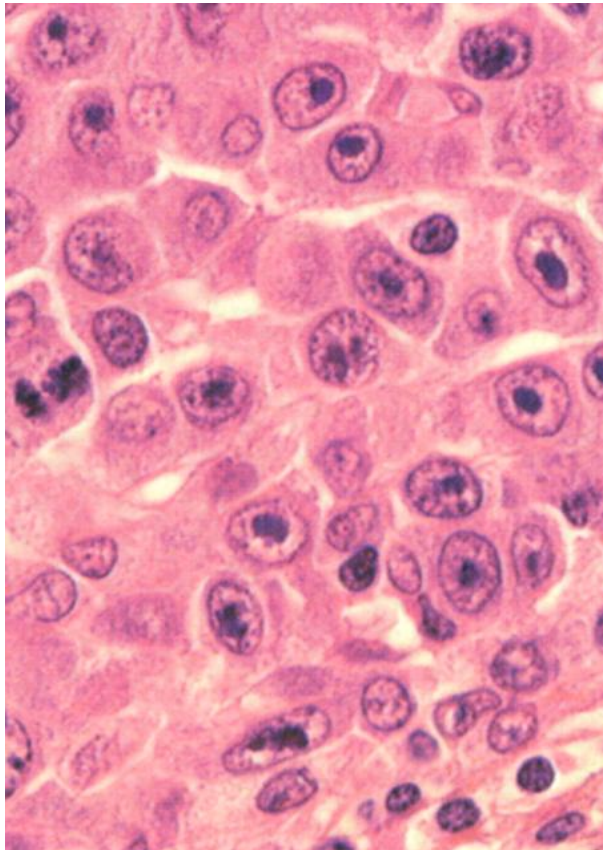
DLBCL with expression of full-length ALK

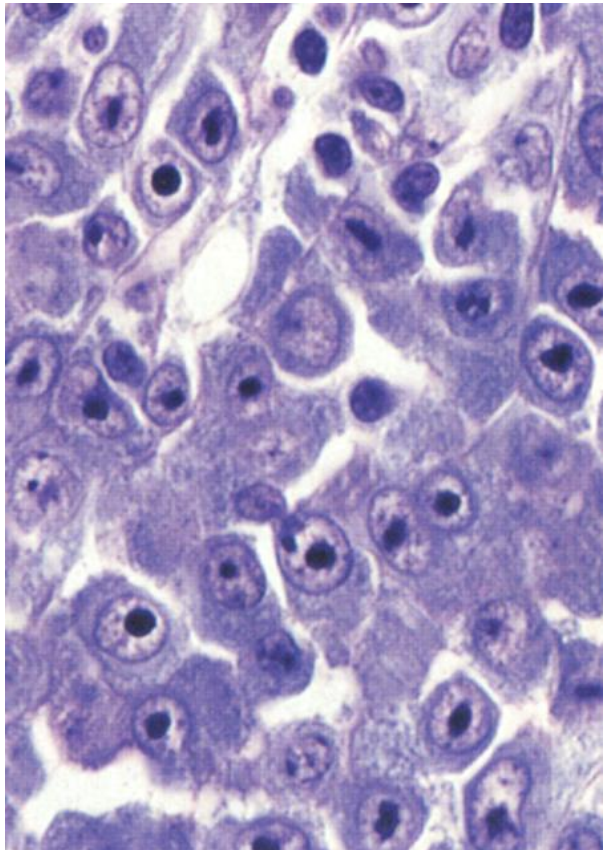
- Composed of monomorphic large immunoblast-like cells, with round pale nuclei containing large central nucleoli and an abundant amphophilic cytoplasm (basophilic with the Giemsa stain) with sometimes plasmablastic differentiation. Some Reed-Sternberg-like cells are often seen. Lymph nodes are massively infiltrated with invasion of the sinuses.

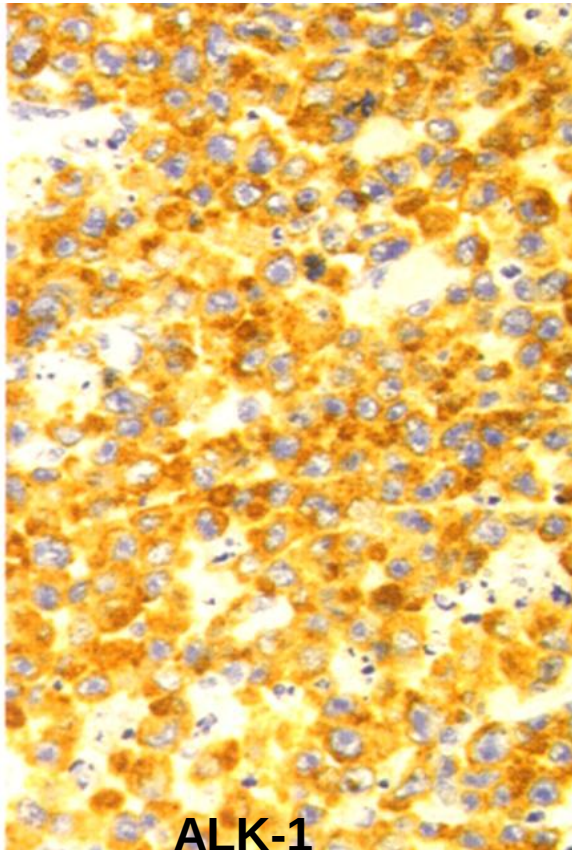
DLBCL: Other Rare Variants/Subtypes with Distinct Immunophenotypic Features

DLBCL with expression of full-length ALK

- CD30(-) but express CD45 (weakly), EMA (strongly), and VS38 (ER-associated marker).
- Cyto IgA+ with light chain restriction.
- Lack other B or T markers with the exception of CD4 and CD57.
- ALK-1 show a granular cytoplasmic and dot-like positivity in the Golgi area.
- No t(2;5). The mechanism of ALK upregulation is unknown.
- More frequently in adults and in males.
- Aggressive course.







DLBCL with expression of full-length ALK