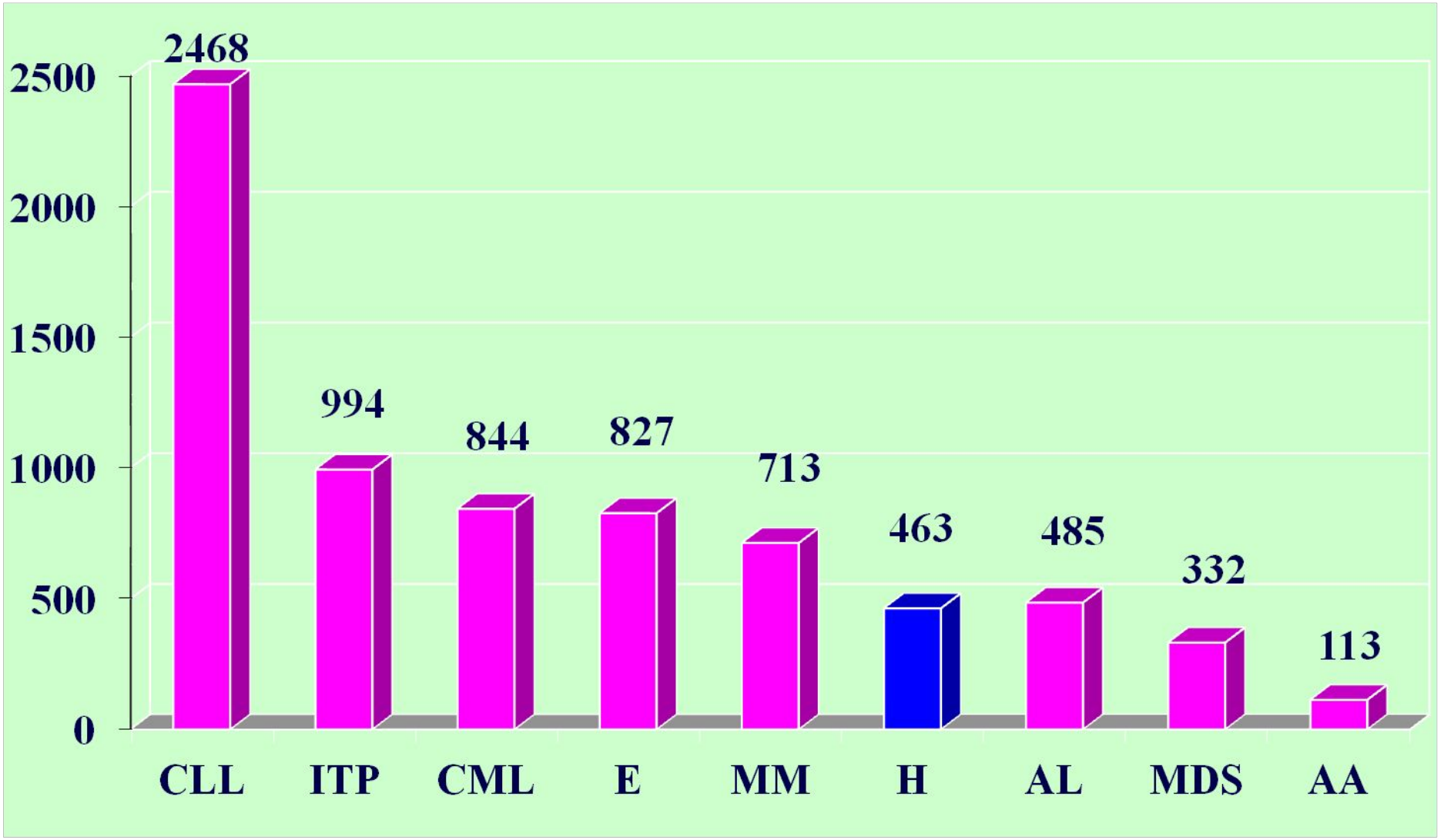
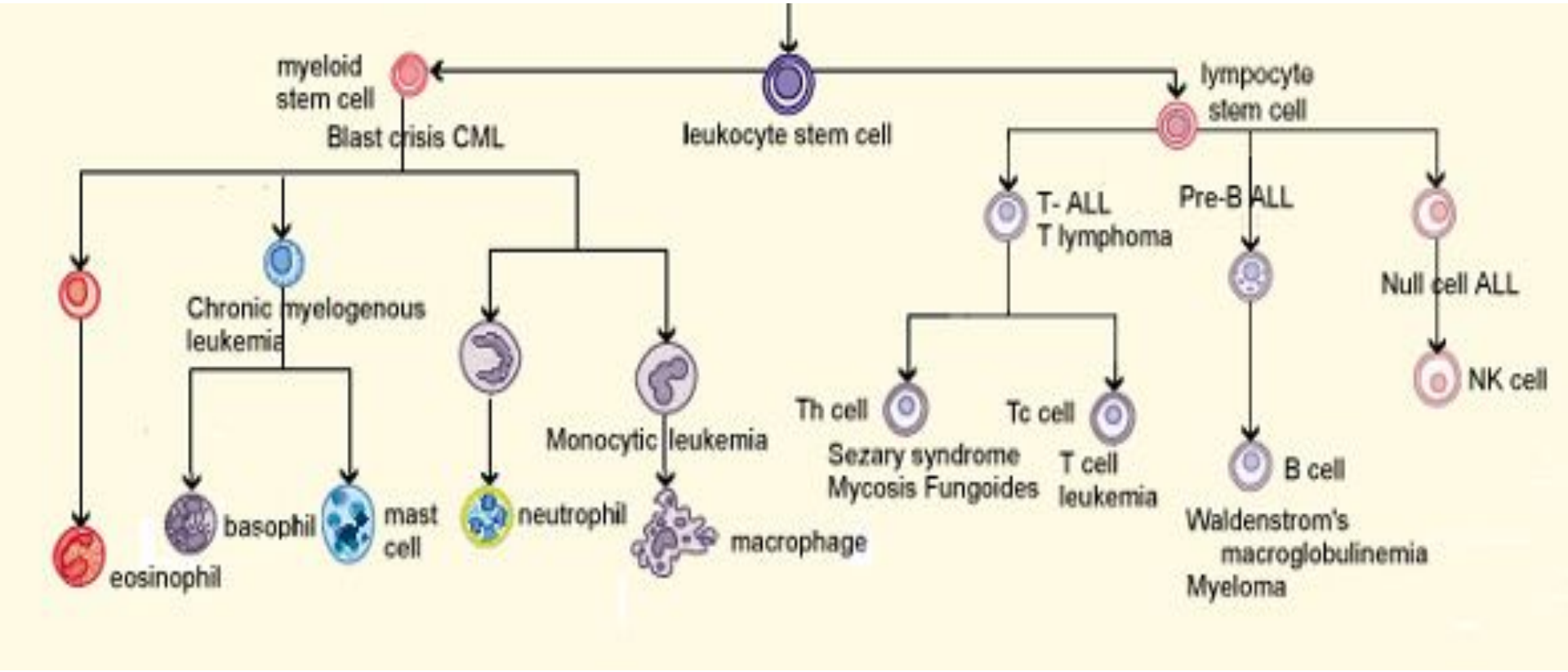
Лимфопролиферативные заболевания.

Проф. М.П.Потапнев

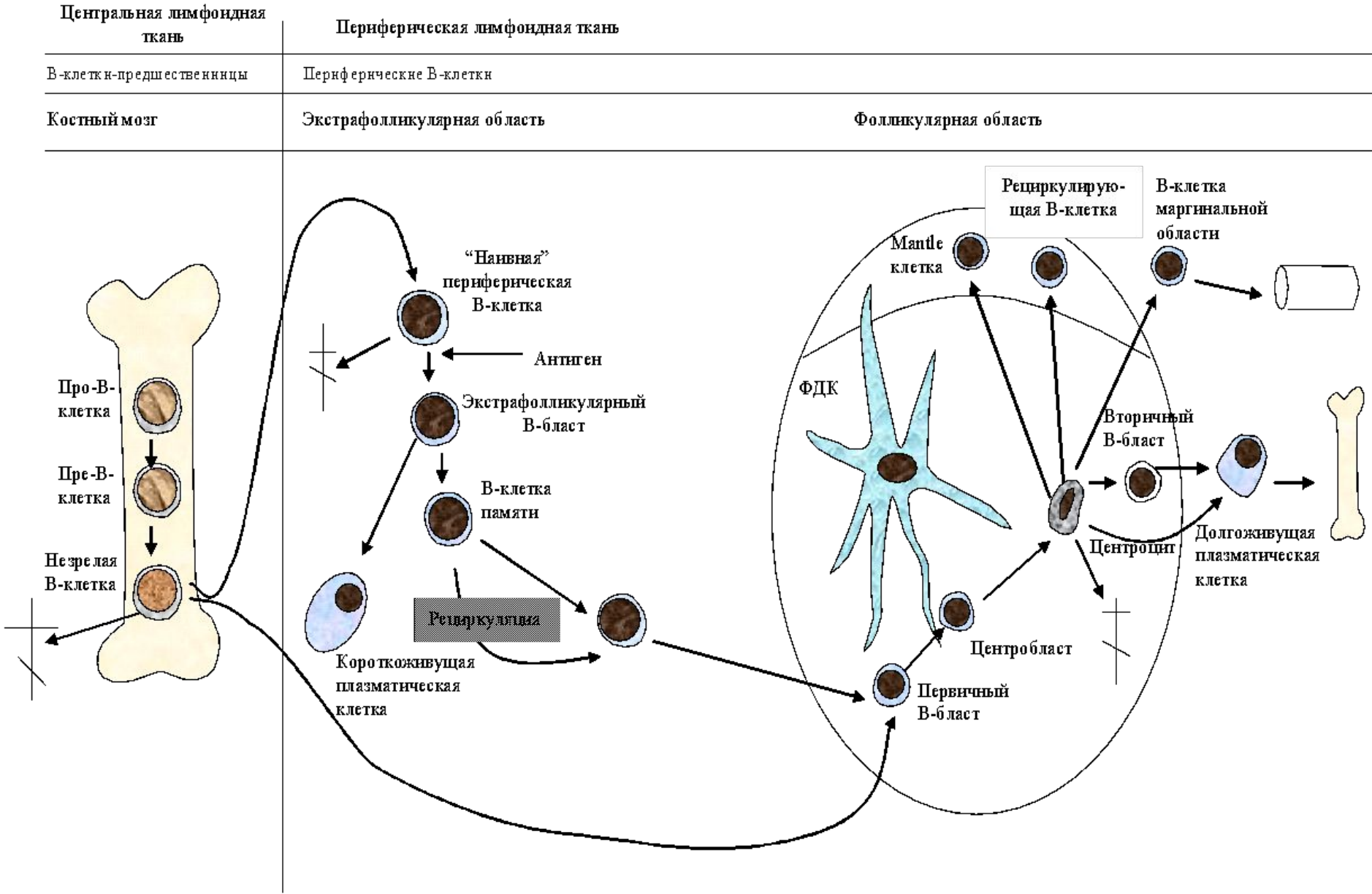
Patients with hematological malignancies in Belarus (adults) (2007).

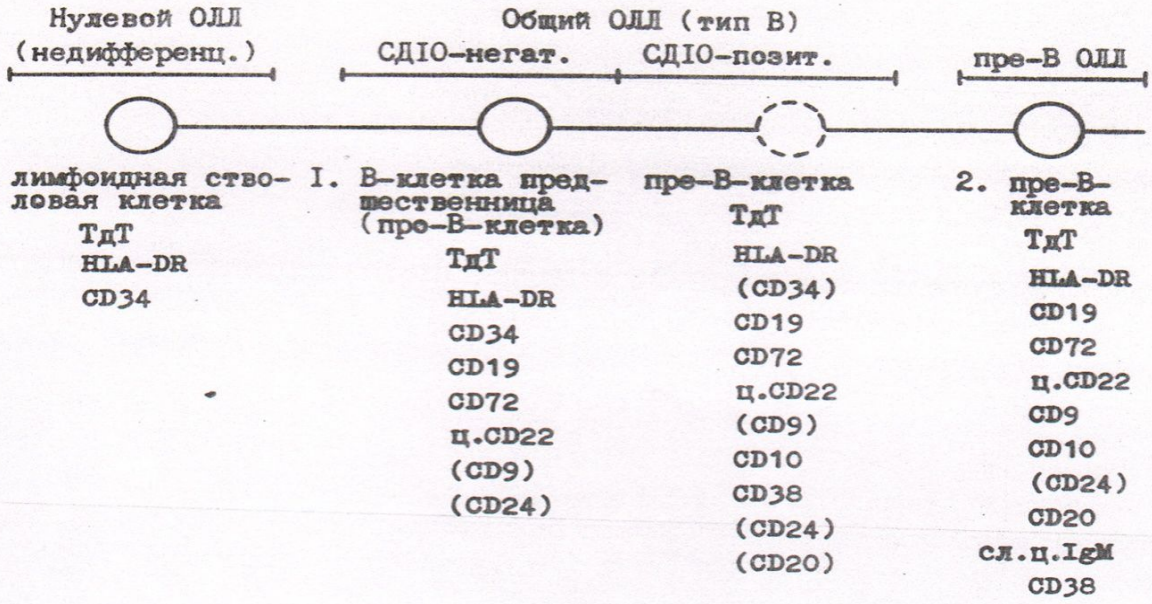


Limphoproliferative diseases

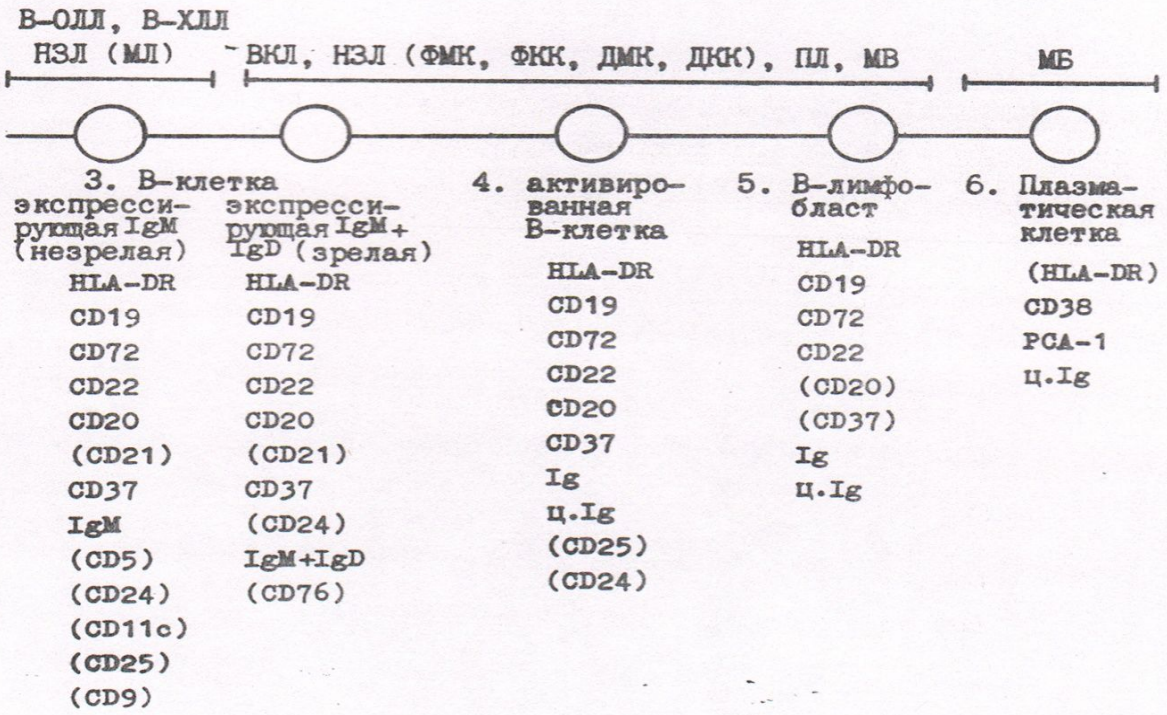


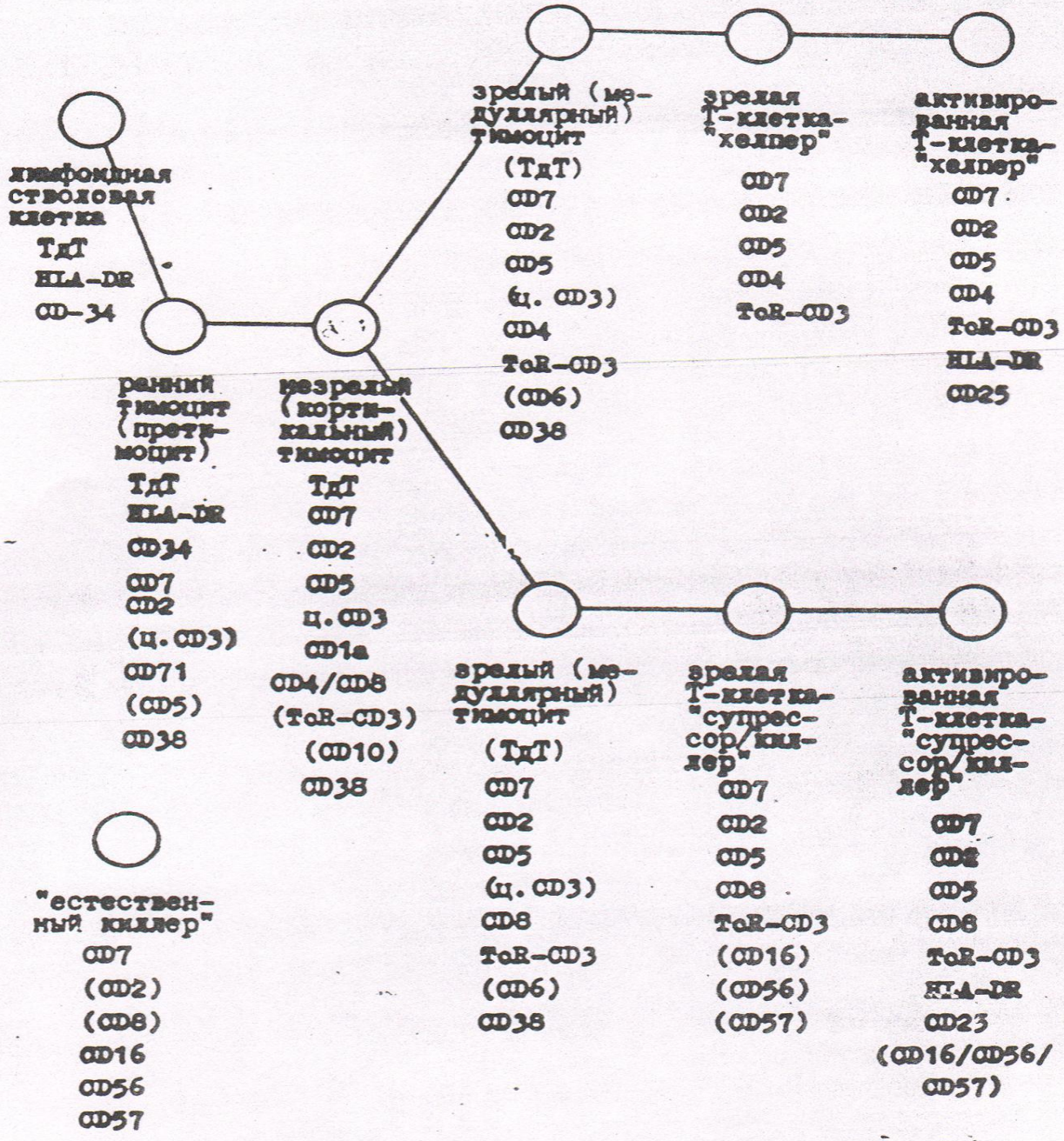
B-cell lymphopoiesis





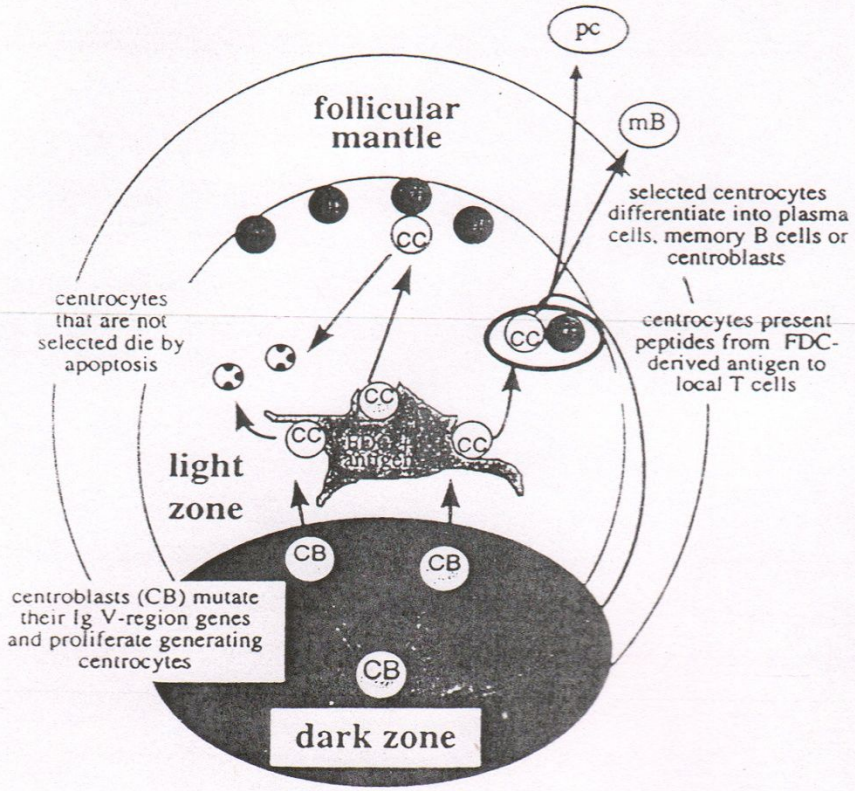
B-cell malignancies



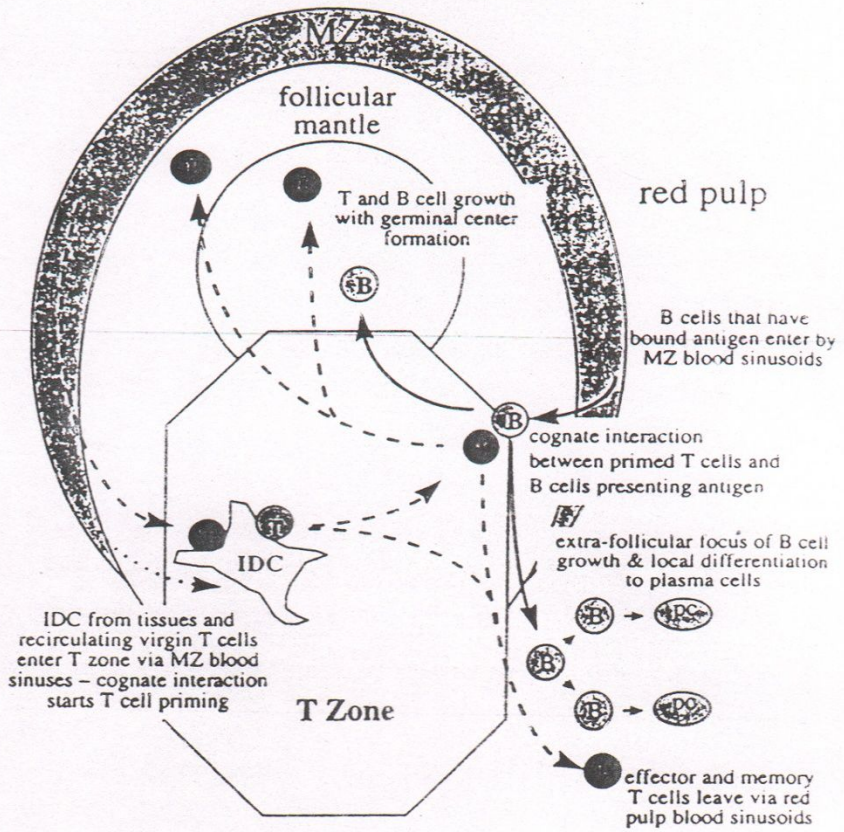


T-cell differen-ti ation stages

Lymphopoiesis in lymph nodes.

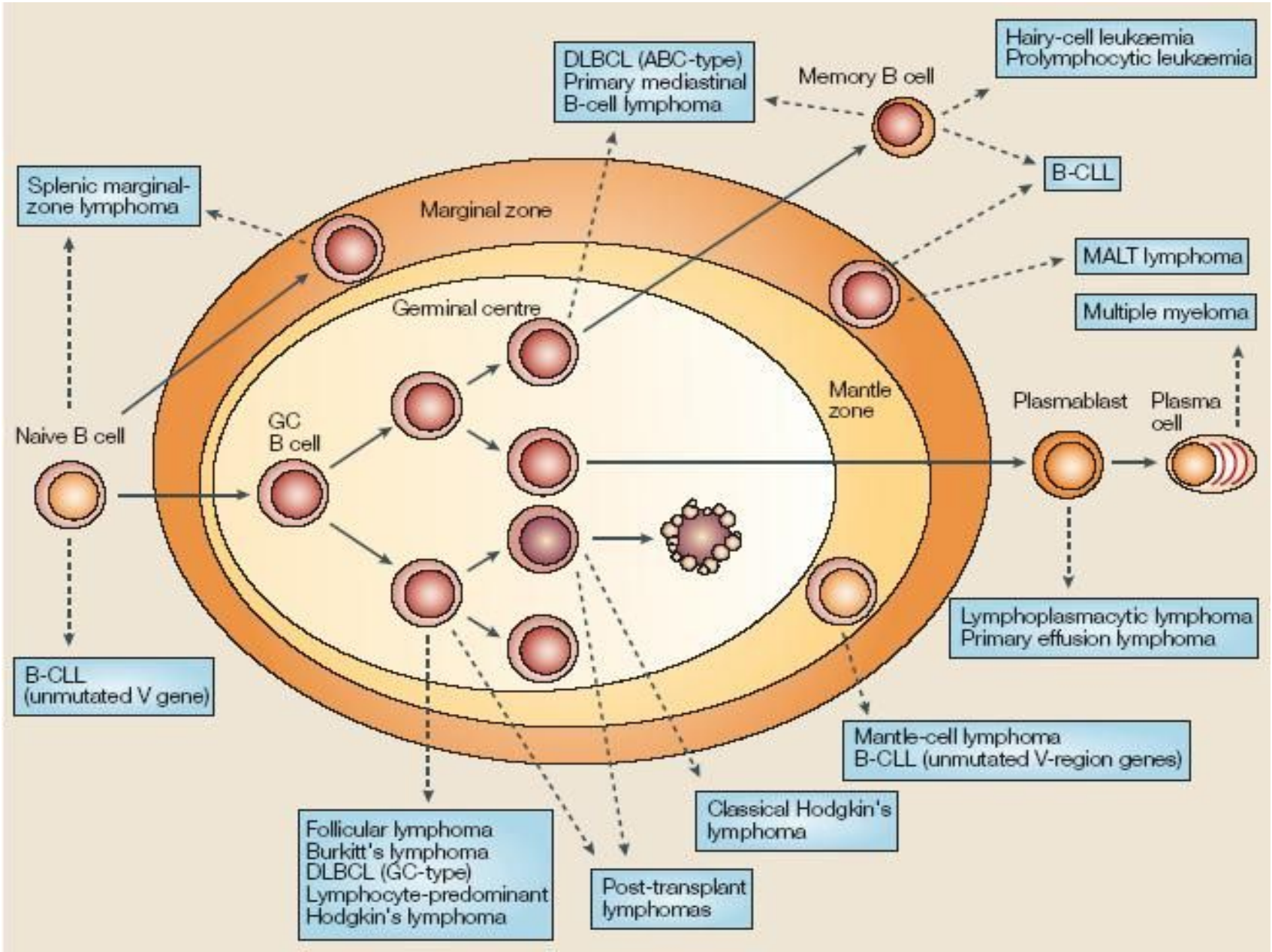


Hypermutation and selection of germinal centre B cells.
CB - centroblasts; cc - centrocytes; T - CD4+ T cell; pc - plasma cell and mB - memory B cell. The red circle denotes successful cognate interaction between the encompassed T and B cell.

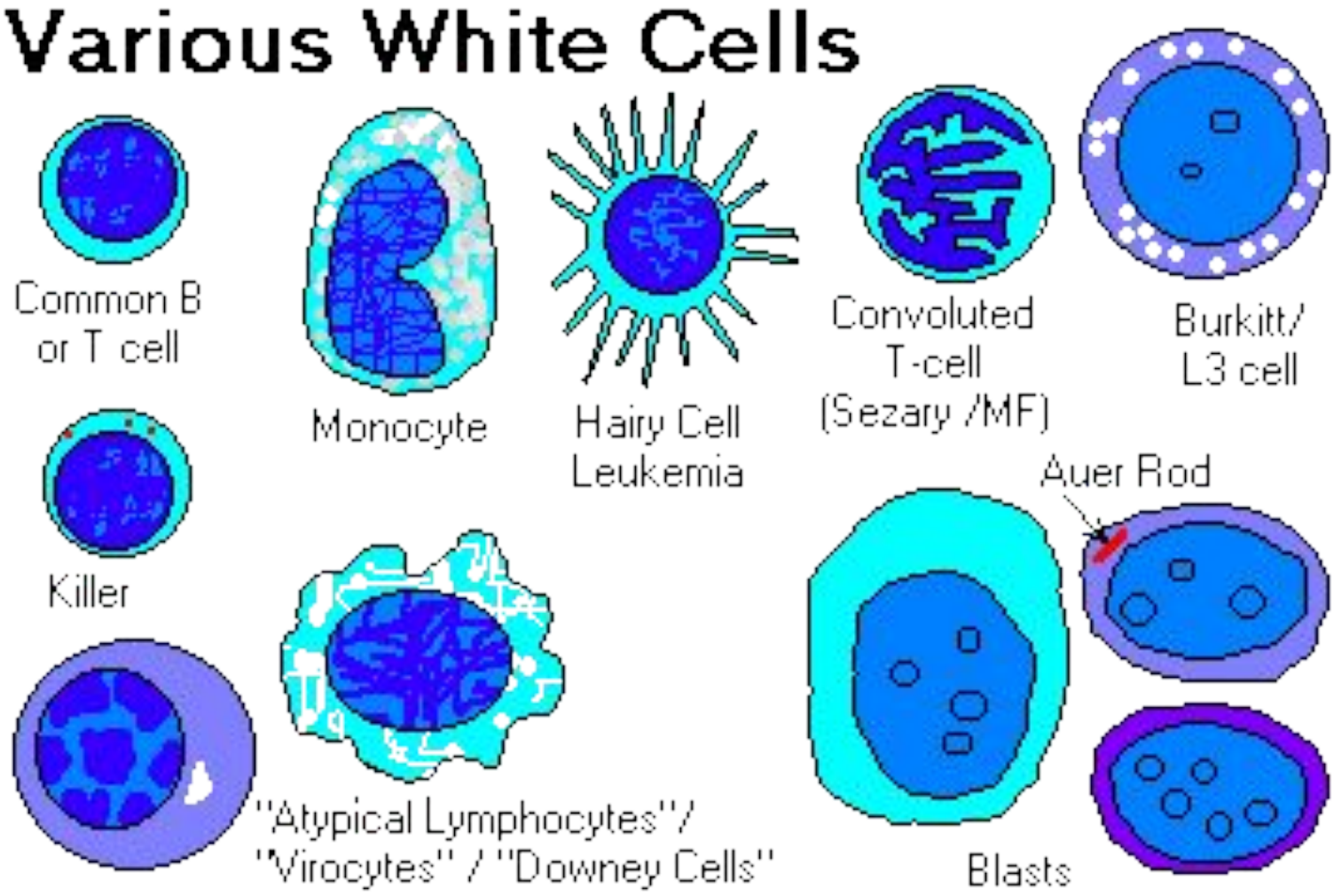


Cells involved in cognate interactions in the T zone of the spleen.
MZ - marginal zone; T - T cell; B - B cell; pc - plasma cell; IDC - interdigitating dendritic cell.

B-cell malignancies



Morphology of leukocytes



Acute leukemia.

- **Originated from bone marrow (>25% blasts).**
- **Usually monoclonal disease.**
- **Lineage committed morphology (FAB classif.)**
- **B and T or myeloid malignant cells are estimated by immunophenotyping (FAB classif. 1996 classif.)**
- **Cytogenetic abnormalities (WHO classif. 2001,2008).**
- **Fusion genes as markers of disease diagnosis and prognosis.**

Acute leukemia

(WHO classification, 2008).

- **Mixed phenotype acute leukemia (T or B- myeloid, NK-cell…)**
- **B lymphoblastic leukemia/lymphoma with t(9:22)(q34;q11.2); *BCR-ABL1*.**
- **B lymphoblastic leukemia/lymphoma with t (v;11q23); *MLL* rearranged.**
- **B lymphoblastic leukemia/lymphoma with t(12;21)(p13;q22) TEL-AML1 (ETV6-RUNX1)**
- **B lymphoblastic leukemia/lymphoma with hyperdiploidy.**
- **B lymphoblastic leukemia/lymphoma with hypodiploidy.**
- **B lymphoblastic leukemia/lymphoma with t(5;14)(q31;q32); *IL-3-IgH***
- **B lymphoblastic leukemia/lymphoma with t (1;19)(q23;p13.3); *TCF-PBX1***
- **T lymphoblastic leukemia/lymphoma.**

Cytogenetic and genetic features of ALL.

Fusion oncogene	Translocation	Clinical Frequency	Prognosis
BCR-ABL	t(9;22)(q34;q11)	>95% in adult CML, 30% in adult ALL	favorable
MLL-AF4	t(4;11)(q21;q23)	5% ALL	poor
TEL-AML1	T(12;21)(p13;q22)	25% in pediatric B-ALL	favorable
E2A-PBX1	T(4;11)(p13;q22)	3-5% ALL	favorable
IgH, IGL	-	>95% B-ALL	n.d.(diagn.)
TCRδ, TCRγ	-	>95% T-ALL	n.d.(diagn.)

Chronic lymphocytic leukemia

(WHO classification, 2008).

- **Mature B-cell neoplasms**

- **Chronic lymphocytic leukemia/small lymphocytic lymphoma,**
- **B-cell prolymphocytic leukemia,**
- **Splenic marginal zone lymphoma,**
- **Hairy cell leukemia,**
- **Lymphoplasmacytic lymphoma,**
- **Waldenstrom macroglobulinemia,**
- **Heavy chain diseases,**
- **Plasma cell myeloma,**
- **-MALT lymphoma,**
- **Follicular lymphoma,**
- **Diffuse large B-cell lymphoma,**
- **Plasmablastic lymphoma,**
- **Burkitt lymphoma.**

Chronic lymphocytic leukemia

(WHO classification, 2008).

- **Mature T-cell and NK-cell neoplasms:**

- **T-cell prolymphocytic leukemia,**
- **T-cell large granular lymphocytic leukemia,**
- **Aggressive NK-cell leukemia,**
- **Adult T-cell leukemia/lymphoma,**
- **Mycosis fungoides,**
- **Sezary syndrome,**
- **Primary cutaneous CD30+ T cell lymphoproliferative disorders,**
- **Peripheral T-cell lymphoma,**
- **Anaplastic large cell lymphoma…**

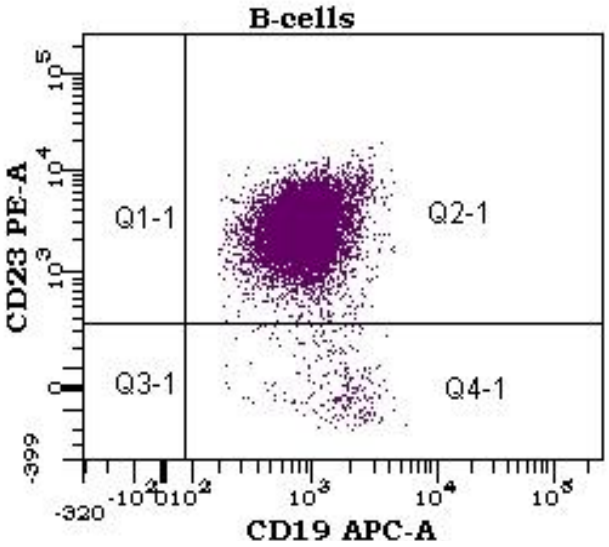
Adverse prognostic factors of CLL

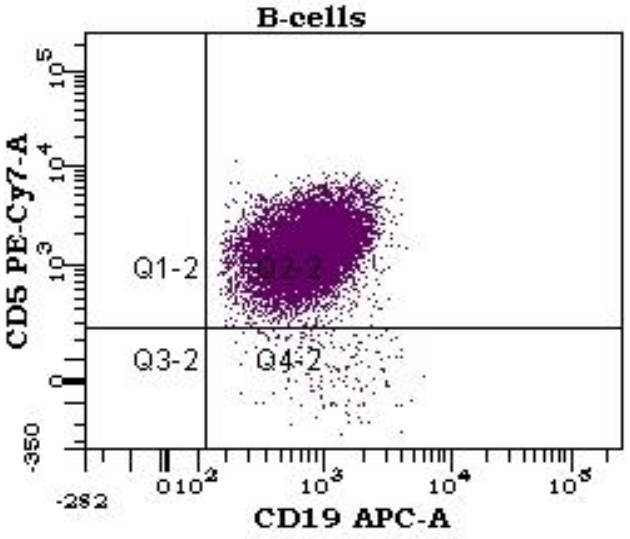
- **Diffuse infiltration of bone marrow by lymphocytes;**
- **Advanced age;**
- **Male gender;**
- **Deletions in chr.17p (p53!) or 11q (ATM !) (5-10% of pts for each) ;**
- **High serum level of beta-2 – microglobulin;**
- **Increased fraction of prolymphocytes in PB;**
- **>20% of ZAP-70-positive cells, >30% CD38+ cells;**
- **No rearangement in IgH V region.**

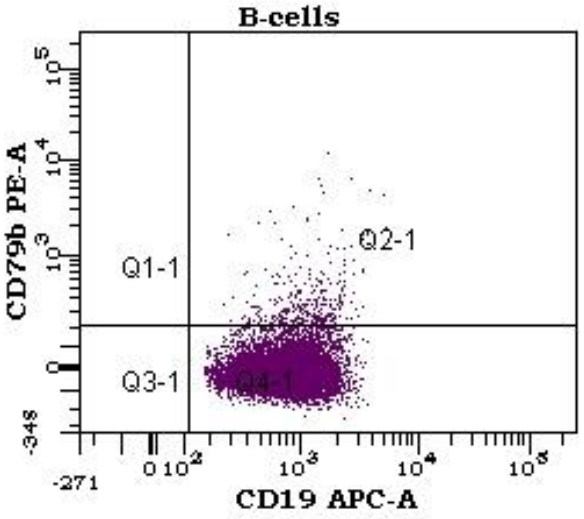
Favorable prognostic factors

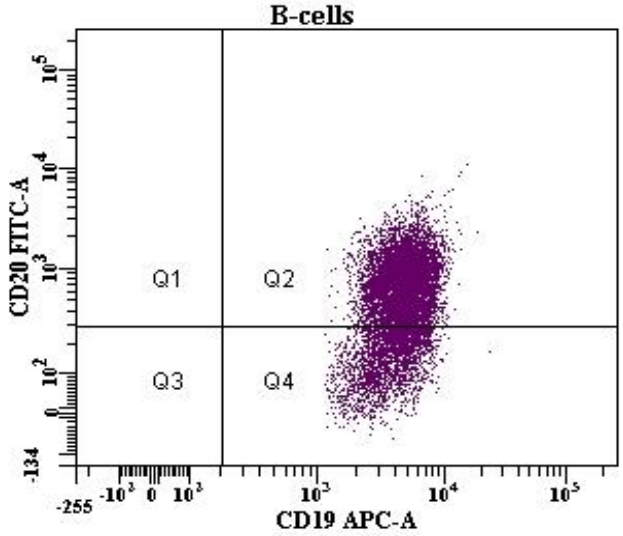
- **No diffuse infiltration of bone marrow by lymphocytes;**
- **Deletion in chr.13 q (50% of pts);**
- **<20% of ZAP-70-positive cells, <30% CD38+ cells;**
- **Mutations in IgH V region.**

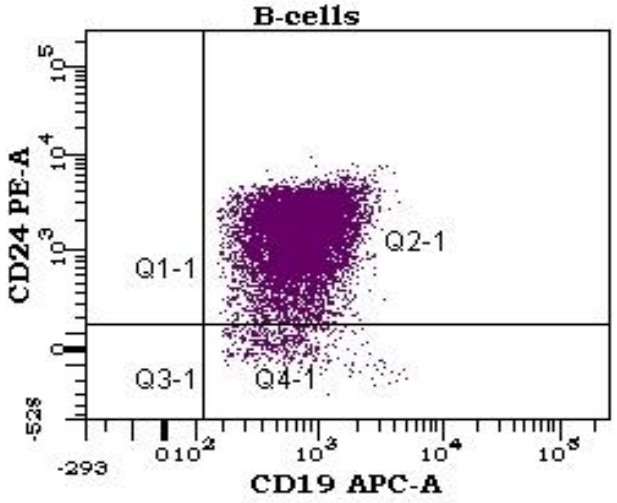
Typical B cell phenotype in CLL

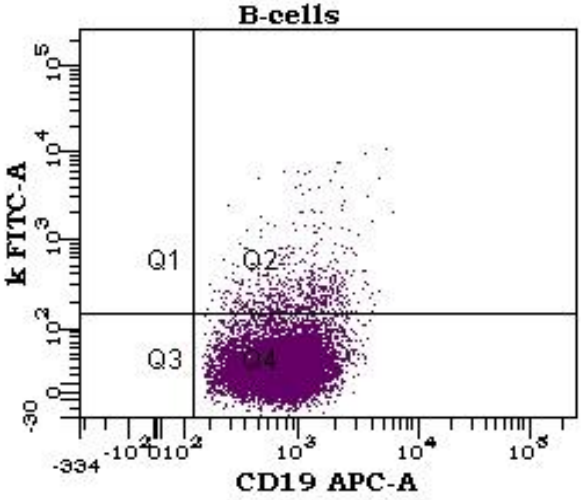












Strategy for CLL therapy.

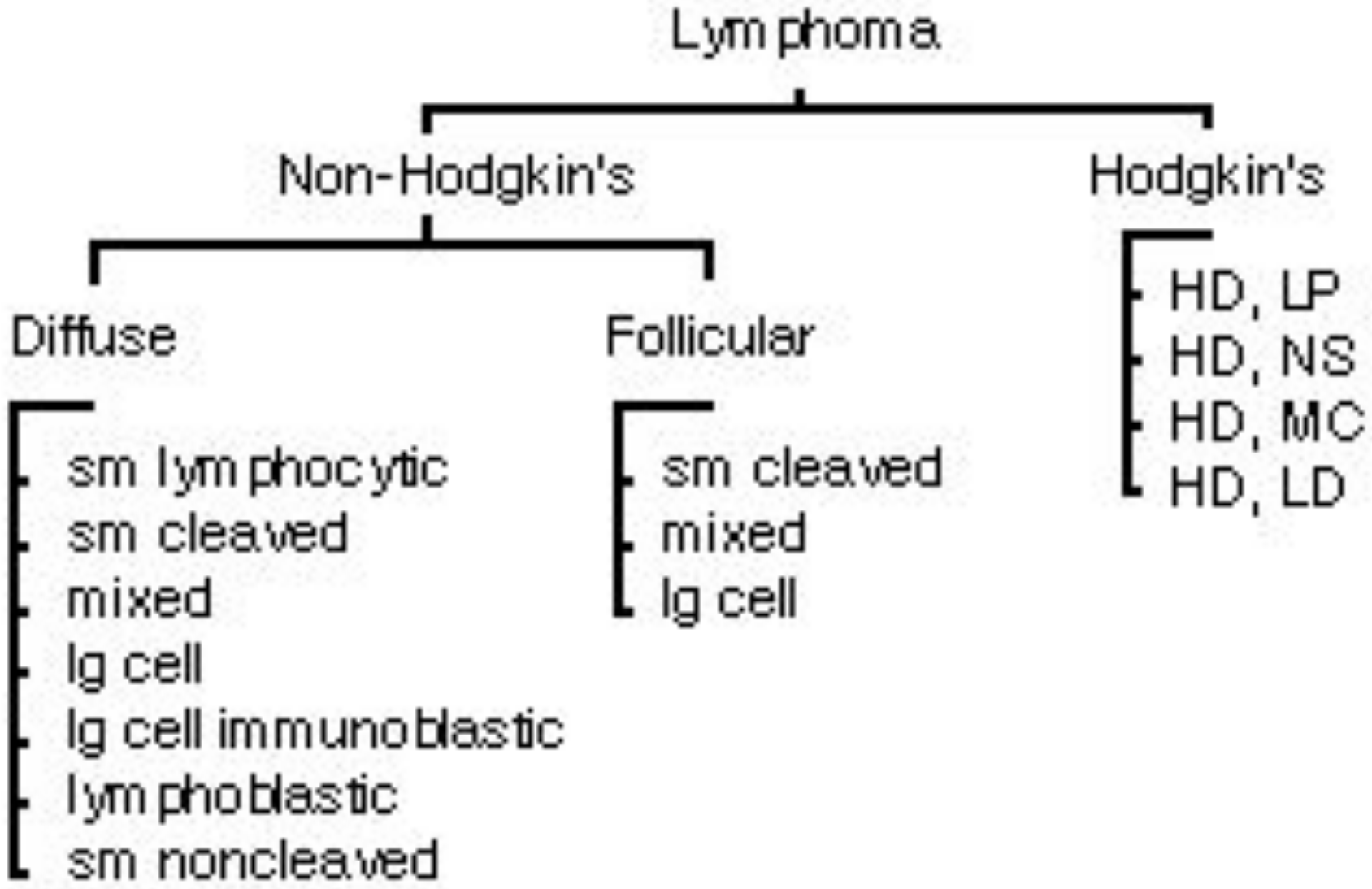
First line of therapy: Fludarabine, Cyclophosphamine, Rituximabe (FCR).

Chemotherapy, MABs such as alemtuzumab (directed against CD52) and ofatumumab (directed against CD20) are also used.
Stem cell transplantation – rare.

Survival:
Subclinical "disease" can be identified in 3,5% of normal adults and up to 7% of individuals over the age of 70.

Survival rate depends on subtypes (6-8 years to 22 years).

Types of lymphomas.



Hodgkin Lymphoma et al. (WHO, 2008).

- **Hodgkin lymphoma:**
  - **classical Hodgkin lymphoma,**
  - **Lymphocyte-rich classical Hodgkin lymphoma, …**
- **Histiocytic and dendritic cell neoplasms:**
  - **histiocytic sarcoma,**
  - **Langerhans cell histiocytic,**
  - **Follicular dendritic cell sarcoma,…**
- **Posttranplantation lymphoproliferative disorders:**

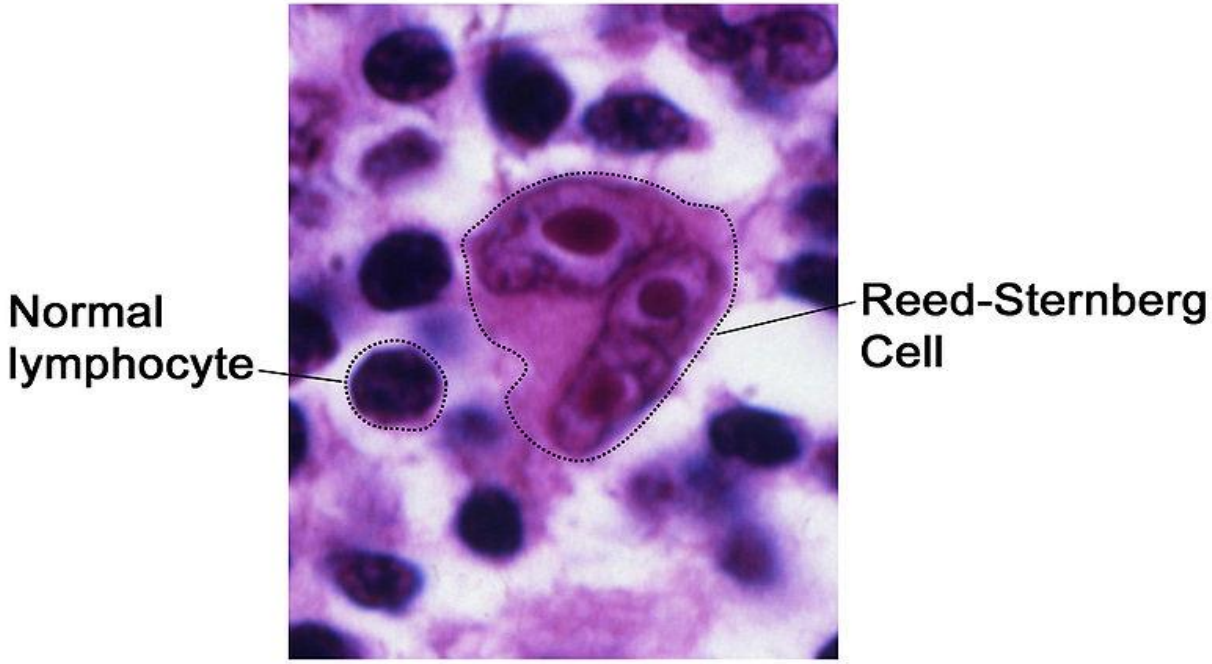
-plasmacytic hyperplasia,

-Infectious mononucleous-like PTLD,

-polymorphic PTLD,

- monomorphic PTLD (B- and T/NK-cell types),…

Histological diagnosis of HD.



The Reed–Sternberg cells are identified as large often bi-nucleated cells with prominent nucleoli and an unusual CD45-, CD30+, CD15+/- immunophenotype. In approximately 50% of cases, the Reed–Sternberg cells are infected by the Epstein–Barr virus.

The adverse prognostic factors for HD

Age ≥ 45 years

Stage IV disease

Hemoglobin < 105 g/l

Lymphocyte count < 600/μl or < 8%

Male gender

Albumin < 40 g/l

White blood count ≥ 15,000/μl

Stages and Therapy of HD

Stage I is involvement of a single lymph node region (I) (mostly the cervical region) or single extralymphatic site (IIe);
Stage II is involvement of two or more lymph node regions on the same side of the diaphragm (II) or of one lymph node region and a contiguous extralymphatic site (IIe);
Stage III is involvement of lymph node regions on both sides of the diaphragm, which may include the spleen (IIIs) and/or limited contiguous extralymphatic organ or site (IIIe, IIIes);
Stage IV is disseminated involvement of one or more extralymphatic organs

Therapy strategy: radiation therapy +/- chemotherapy.

Prognosis: The 5-year survival rate for those patients with a favorable prognosis was 98%, while that for patients with worse outlooks was at least 85%

Non-Hodgkin lymphoma

Causes

The many different forms of lymphoma likely have different causes. These possible causes and associations with at least some forms of NHL include:

Infectious agents like Epstein-Barr virus, Human T-cell leukemia virus, Helicobacter pylori, HHV-8 and HIV infection.

Chemicals, like diphenylhydantion, dioxin, and phenoxyherbicides.

Medical treatments like radiation therapy and chemotherapy.

Genetic diseases, like Klinefelter ‘s syndrome, Chediak-Higashi syndrome, ataxia-telangiectasia syndrome

Autoimmune diseases, like Sjogren’s syndrome, celiac sprue, rheumatoid arthritis and systemic lupus erythematosis

TABLE 2: Immunophenotypic and histochemical markers of B-cell lymphomas/leukemias

	sIg	cIg	CD5	CD10	CD20	CD23	CD43	CD103	Cyclin D1
Follicular	+	–	–	+	+	–(+)	–	–	–
CLL/SLL	dim+	–(+)	+	–	dim+	+	+	–	–
Mantle	+	–	+	–	+	–(+)^	+	–	+
MZL/MALT	+/+	–(+)/(+)	–/–	–/–	+/+	–/–	–(+)/–(+)	+	–/–
B-cell-PLL*	+	–	–(+)	–	+	+(–)	+	+	–
DLBCL#	+(–)	–(+)	–(+)	–(+)	+	–	–	–	–
HCL	+	–	–	–	+	–	+	–	+(–)
BL/BLL	+	–	–	+	+	–	+	NA	–
LPL	+	+	–	–	+	–	–(+)	–	–

+ = > 90% positive; +(–) = > 50% positive; –(+) = < 50% positive; – = < 10% positive; BL/BLL = Burkitt lymphoma/Burkitt-like lymphoma; cIg = cytoplasmic immunoglobulin; CLL = chronic lymphocytic leukemia; B-cell PLL = B-cell prolymphocytic leukemia; DLBCL = diffuse large B-cell lymphoma; HCL = hairy cell leukemia; LPL = lymphoplasmacytic lymphoma; MZL/MALT = splenic marginal zone/mucosa-associated lymphoid tissue; sIg = surface immunoglobulin; SLL = small lymphocytic leukemia

* = A T-cell variant is present in approximately 20% to 30% of PLL cases.

= A T-cell histiocyte-rich B-cell lymphoma variant is present in approximately 1% to 3% of DLBCL cases.

^ = 20% to 25% of cases are CD23+ by flow cytometric immunophenotyping; testing for *bcl-1* is essential.

TABLE 3: Immunophenotypic and histochemical markers of T-cell lymphomas/leukemias

Histology	CD3	CD5	CD7	CD4	CD8	CD30	NK16/56	Cytotoxic granules	TCR
T-PLL	+	–	+	+(–)	–(+)	–	–	–	α/β
T-LGL disease*	+	–	+	–	+	–	+/–	+	α/β >> γ/δ
Mycosis fungoides	+	+	+	+	–(+)	–(+)	–	–	α/β
Cutaneous ALCL	+	+(–)	+(–)	+(–)	(–)	++	–(+)/–(+)	+/–	α/β
Primary systemic ALCL^	+(–)	+(–)	+(–)	–(+)	–(+)	++	–	–	α/β
Peripheral T-cell lymphoma, unspecified	+(–)	+(–)	–(+)	+(–)	–(+)	–(+)	–(+)/–(+)	–(+)	α/β > γ/δ
Subcutaneous panniculitis-like T-cell	+	+	+	–(+)	+(–)	–(+)	–/–(+)	+	γ/δ >> α/β
Hepatosplenic T-cell lymphoma	+	–	+	–	–	–	+/+(–)	+	γ/δ >> α/β
Angioimmunoblastic T-cell lymphoma#	+	+	–	+(–)	–(+)	–	–	–	α/β#
Extranodal NK/T-cell lymphoma	S –, C +	–	–(+)	–(+)	–	–	–/+	+	–
Enteropathy-associated T-cell lymphoma	+	+	+	–(+)	+(–)	+(–)	–	+	α/β >> γ/δ
Adult T-cell leukemia/lymphoma&	+	+	–	+(–)	–(+)	+(–)	–	–	α/β

+ = > 90% positive; +(–) = > 50% positive; –(+) = < 50% positive; – = < 10% positive; ALCL = anaplastic large cell lymphoma; C = cytoplasmic; LGL = large granular lymphoproliferative; NK = natural killer; PLL = prolymphocytic leukemia; S = surface; TCR = T-cell–rearranged (molecular)

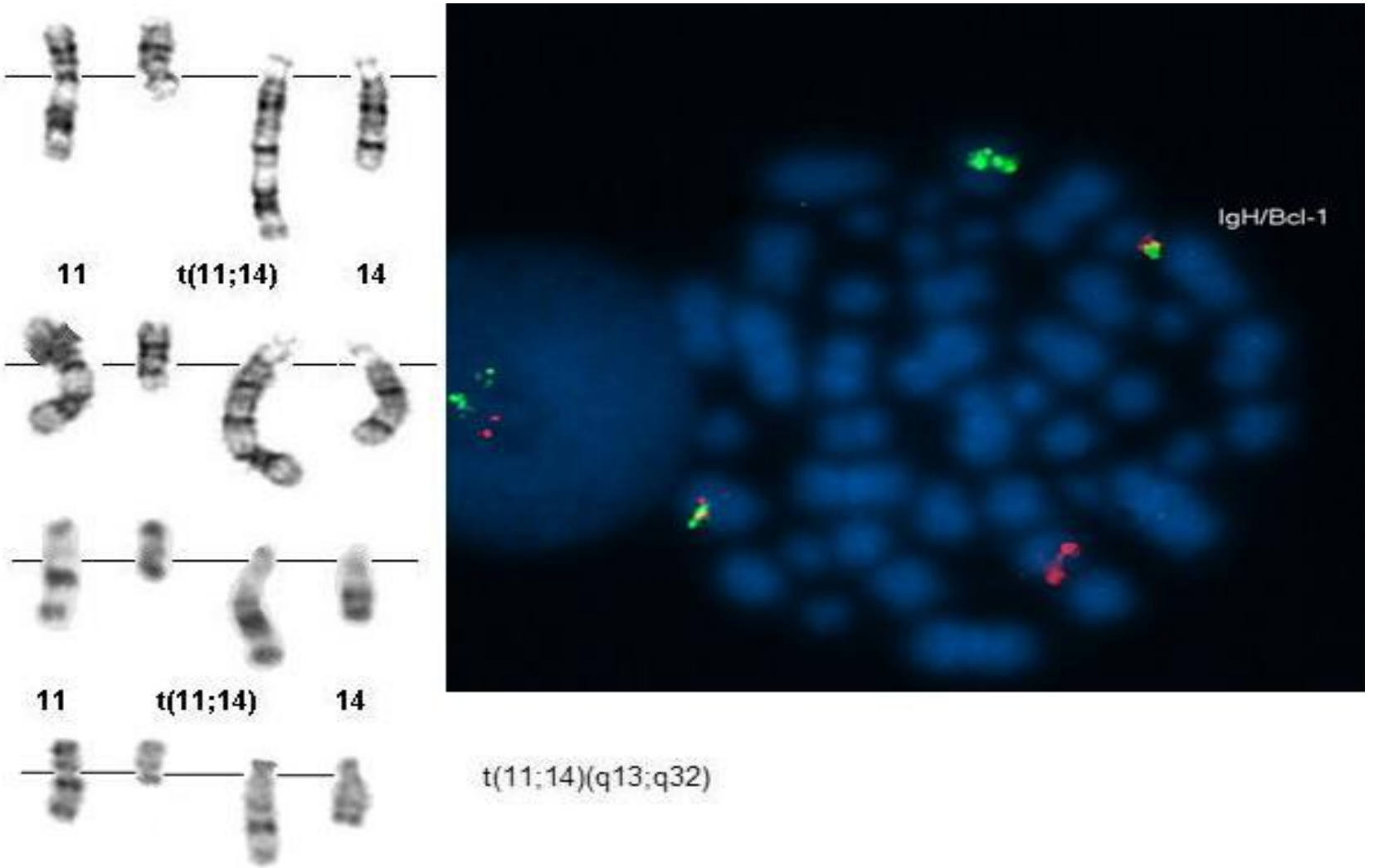
*Approximately 15% to 20% of LGL cases arise from a NK lineage; they are typically CD56+ and CD16-negative.

^The anaplastic lymphoma kinase (ALK) protein is expressed in 50% to 60% of cases.

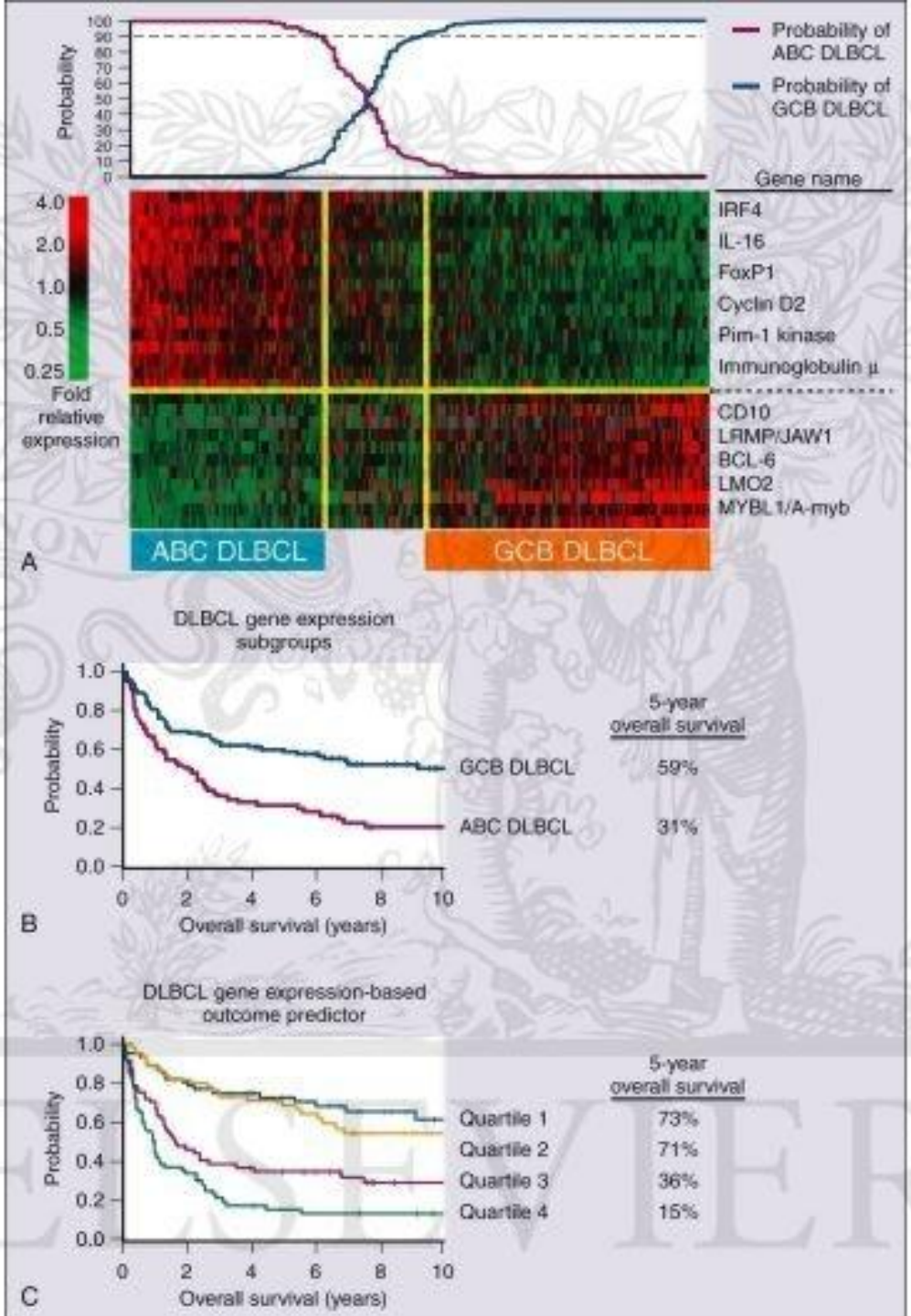
#Expanded follicular dendritic cell clusters (CD21+) are present around proliferated venules; Epstein-Barr virus (EBV) genomes are detected in most cases (eg, EBER) and may be present in either T or B cells; in addition, TCR may be negative or oligoclonal in 20% to 25% of cases, whereas B-cell immunoglobulin may be rearranged in 10% of cases.

&Adult T-cell leukemia/lymphoma cases are always associated with the presence of HTLV-I; further, CD25 is expressed in the majority of cases.

Cytogenetic analysis for B-cell malignancies



t(11;14) is mainly found in mantle cell lymphoma, but also in B-prolymphocytic leukaemia, in plasma cell leukaemia, in splenic lymphoma with villous lymphocytes, in chronic lymphocytic leukaemia, and in multiple myeloma, herein briefly described; all these diseases involve a B-lineage lymphocyte



Diagnosis of DLBCL by MicroArray technique:

Germinal center B cell DLBCL *vs* activated (post-germinal center) B cell DLBCL

Burkitt's lymphoma (rare type of NHL)

(endemic= EBV positive)

Table 1

Distinction Between Burkitt Lymphoma and Diffuse Large B-Cell Lymphoma

	Burkitt Lymphoma	Diffuse Large B-Cell Lymphoma
Epidemiology	1%–2% of adult NHL cases, much more prevalent in children than adults	30% of adult NHL cases, much more prevalent in adults than children
Morphology	Uniform round to ovoid medium sized cells with round nuclei that contains coarse chromatin and multiple nucleoli. Starry-sky appearance	Large lymphoid cells, similar in size or larger than tissue macrophages, in diffuse growth pattern
Immunophenoype	CD10+, CD19+, CD20+, CD22+, CD79a+, monotypic sIg+, CD5–, and TdT–	CD19+, CD20+, CD22+, CD45+, CD79a+, PAX5, monotypic sIg±, CD5±, and CD10±
Proliferation fraction by Ki-67	Nearly to 100%	53%
Genetics	c-*myc*+, Bcl-6+, Bcl-2–	Bcl-2 (15%–30%), Bcl-6 (20%–40%), c-*myc* (5%–15%)
Treatment	Short course of intensive chemotherapy (CODOX-M/IVAC±R, HyperCVAD±R)	CHOP-R

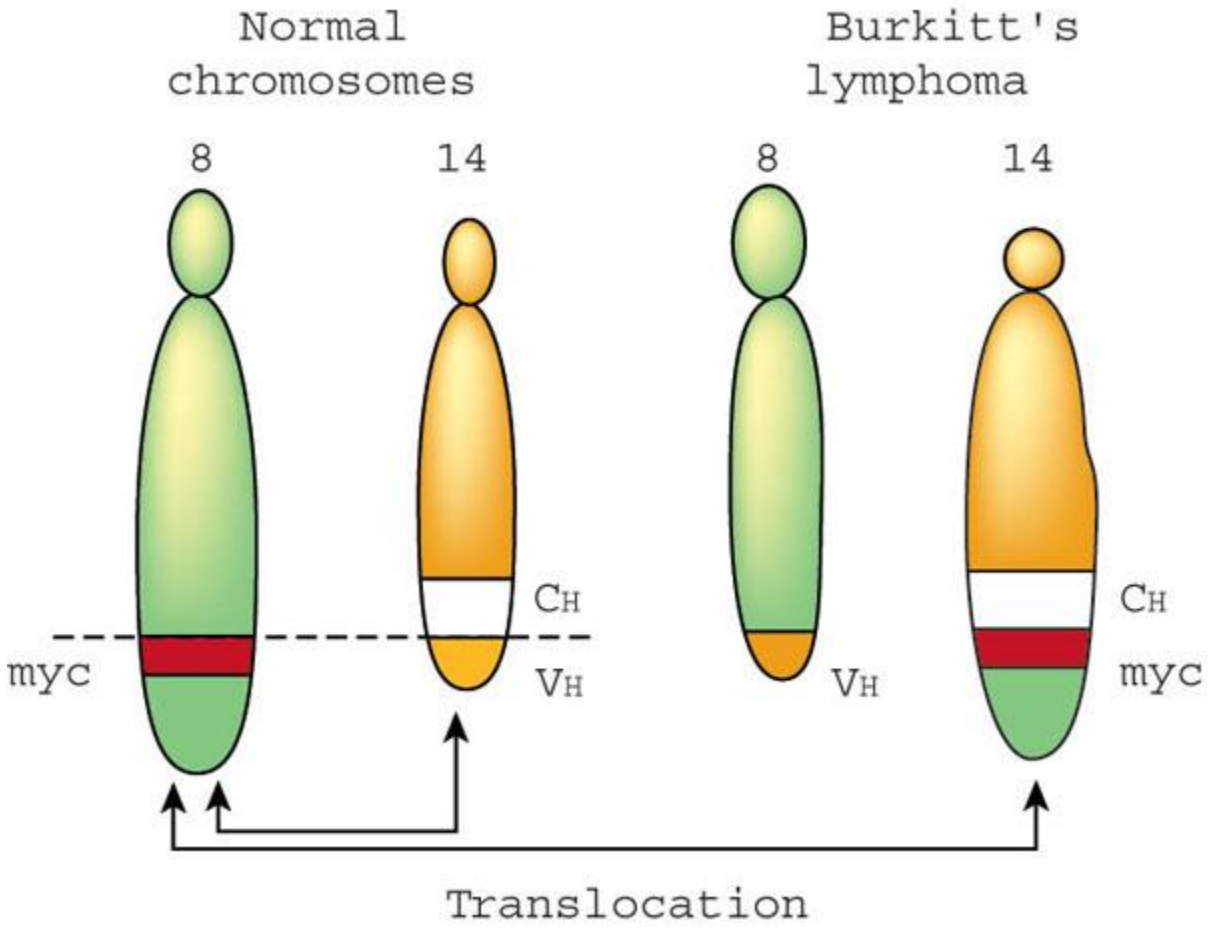
HyperCVAD = hyperfractionated cyclophosphamide, vincristine, doxorubicin (Adriamycin), dexamethasone; CHOP = cyclophosphamide, doxorubicin, vincristine, prednisone; CODOX-M/IVAC = cyclophosphamide, vincristine, doxorubicin, methotrexate, ifosfamide, etoposide, cytarabine; NHL = non-Hodgkin lymphoma; R = rituximab.

Immunophenotypic diagnosis of Burkitt's lymphoma

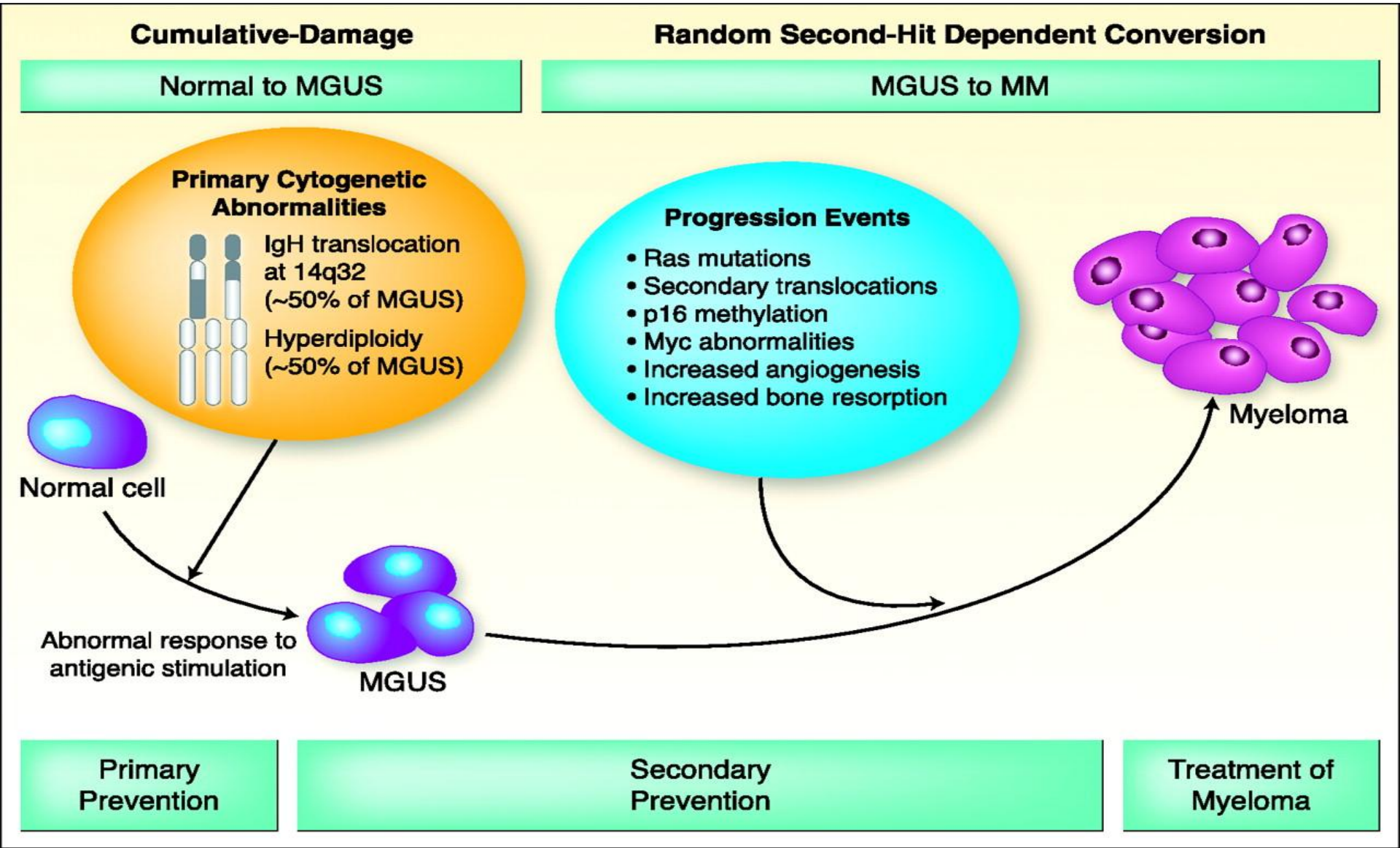
The cells of BL typically express monotypic surface IgM, CD19, CD20, CD22, CD10, Bcl-6, and CD79a, and are negative for CD5, CD23, Bcl-2, and nuclear terminal deoxyribonucleotide transferase (TdT).Lack of surface immunoglobulin has been reported in a few cases. The presence of CD10 and Bcl-6 expression supports the germinal center-cell stage of differentiation.

A remarkable feature of BL is the high growth fraction (> 95%) as demonstrated by Ki-67. The leukemic cells of BL express a mature immunophenotype that distinguishes it from precursor B-cell acute lymphoblastic leukemia (ALL).

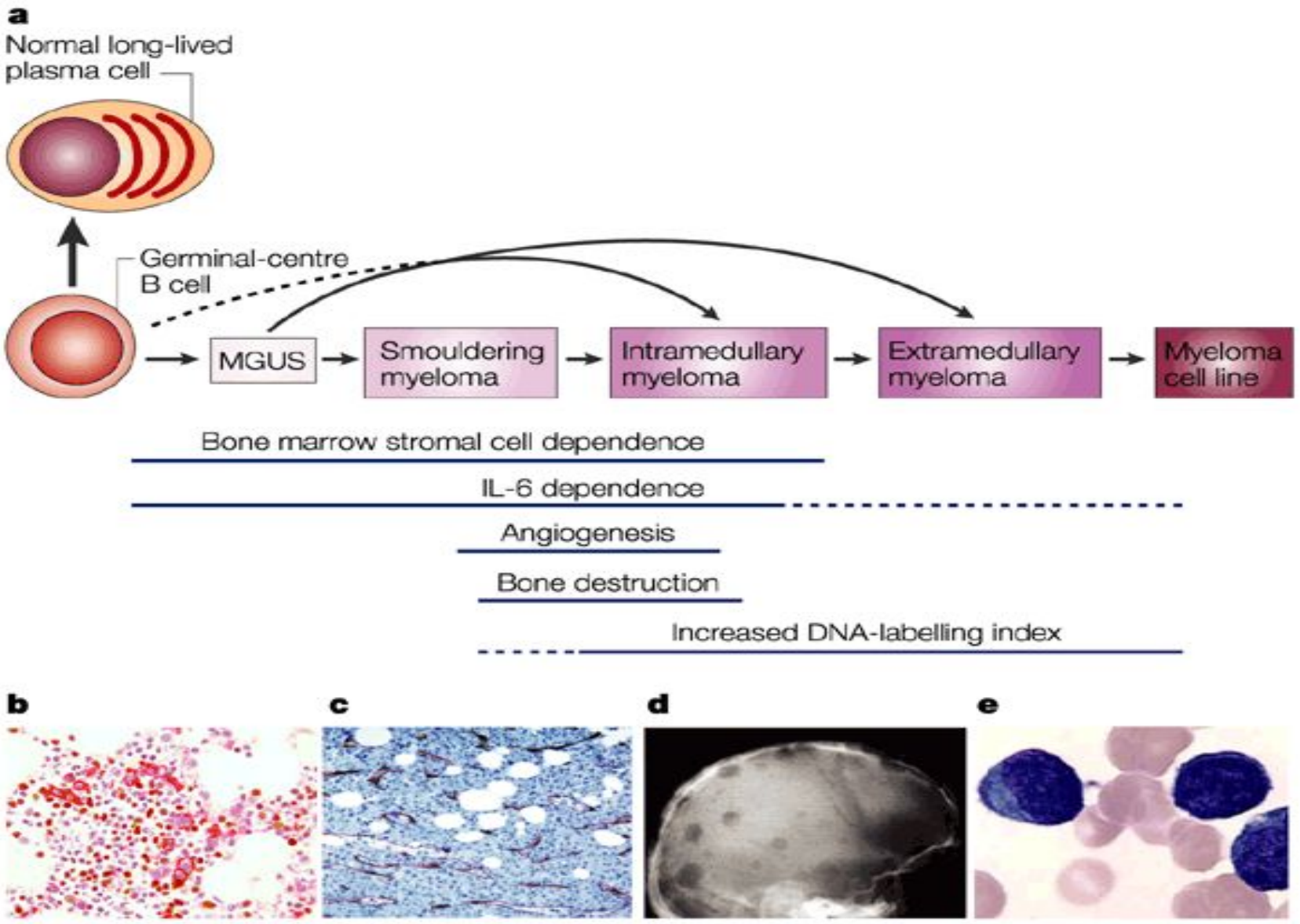
T (8,14) in Burkitt's lymphoma



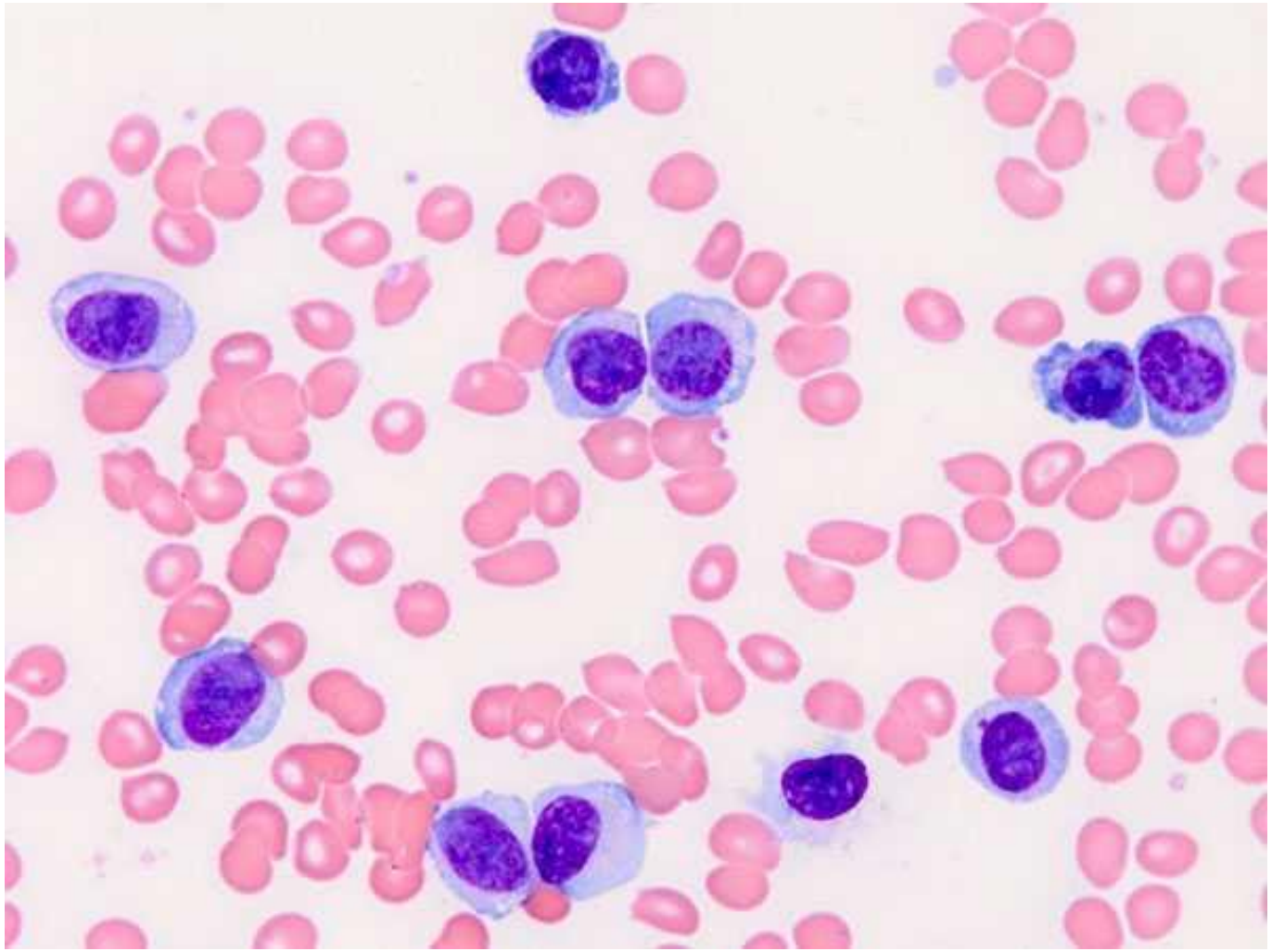
Path from Normal plasma cells through Monoclonal Gammopathy of Undetermined Significance to Multiple Myeloma.



Plasma cell malignancies



Morphology of malignant plasma cells in blood **(H&E staining)**



Immunophenotyping of Plasma Cells

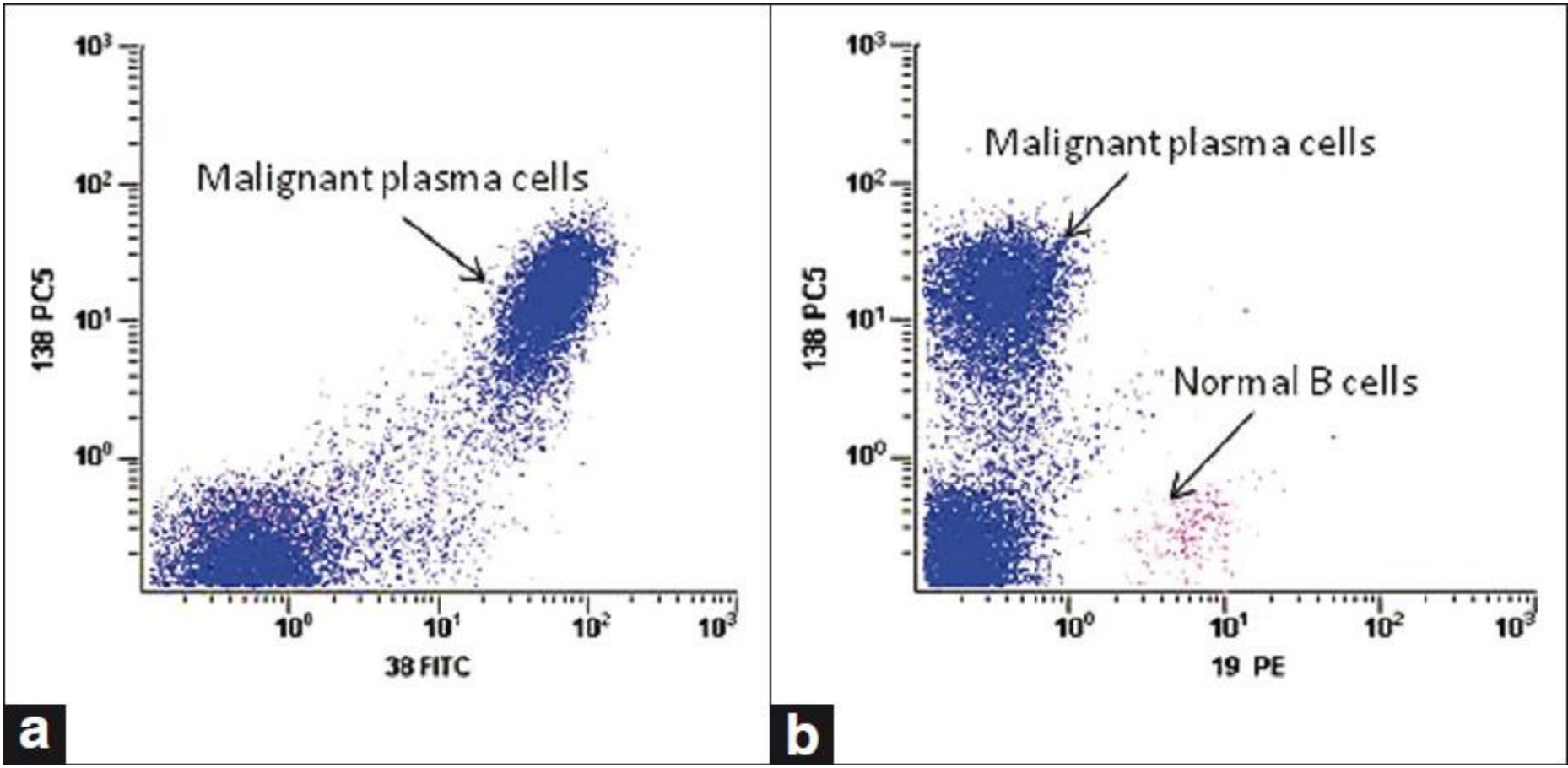


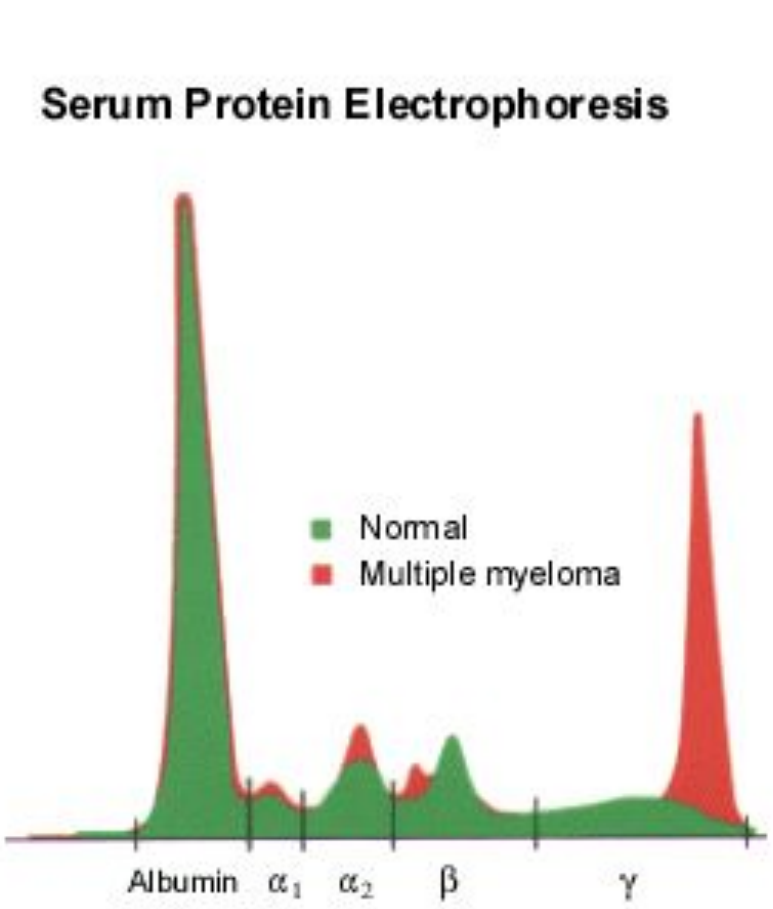
TABLE 1: The location and oncogenes involved in multiple myeloma

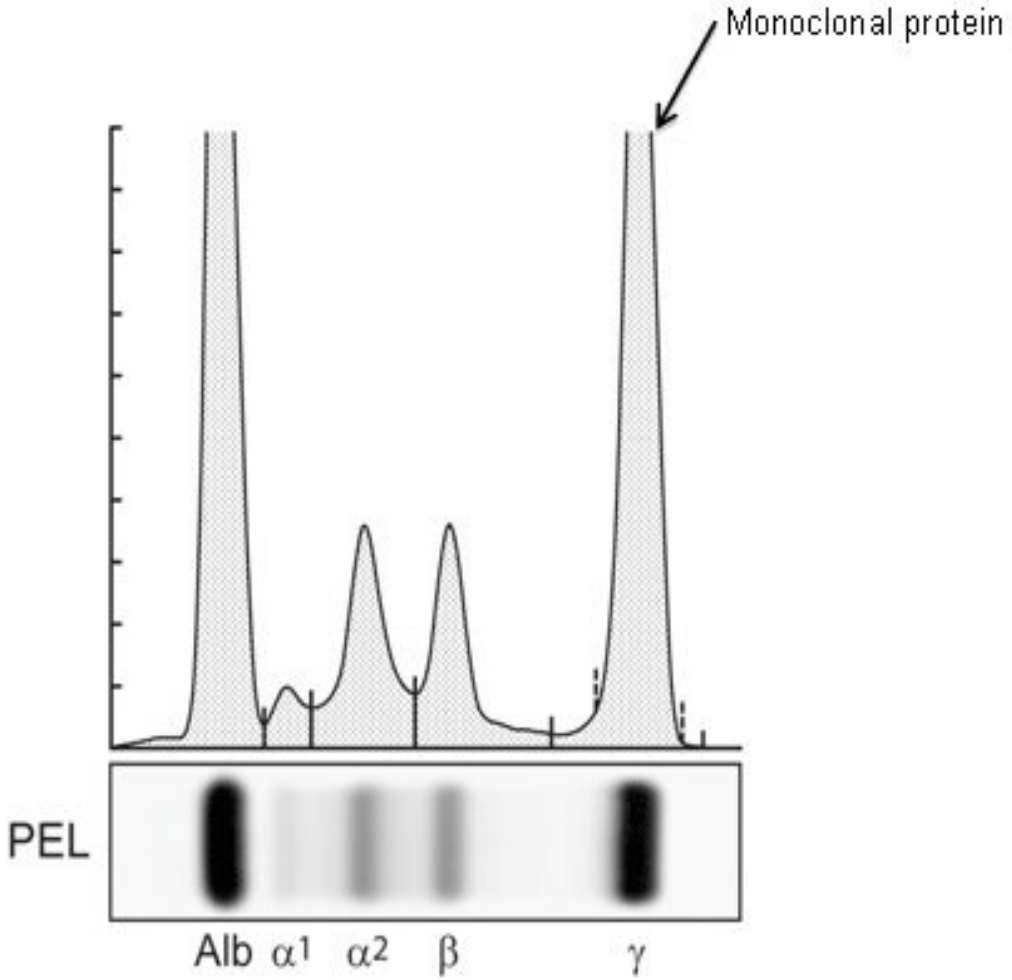
Locus	Oncogene	Incidence
11q13	*CCND1*	15%–20%
6p21	*CCND3*	5%
4p16.3	*FGFR3* and *WHSC1*	12%
16q23	*MAF*	5%–10%
8q24	*MYC*	< 10%
6p25	*MUM1/IRF4*	5%
20q11	*MAFB*	5%
1q21-34	*BCL9, IL6R, MCL1*	Frequent

Multiple Myeloma diagnosis and therapy.

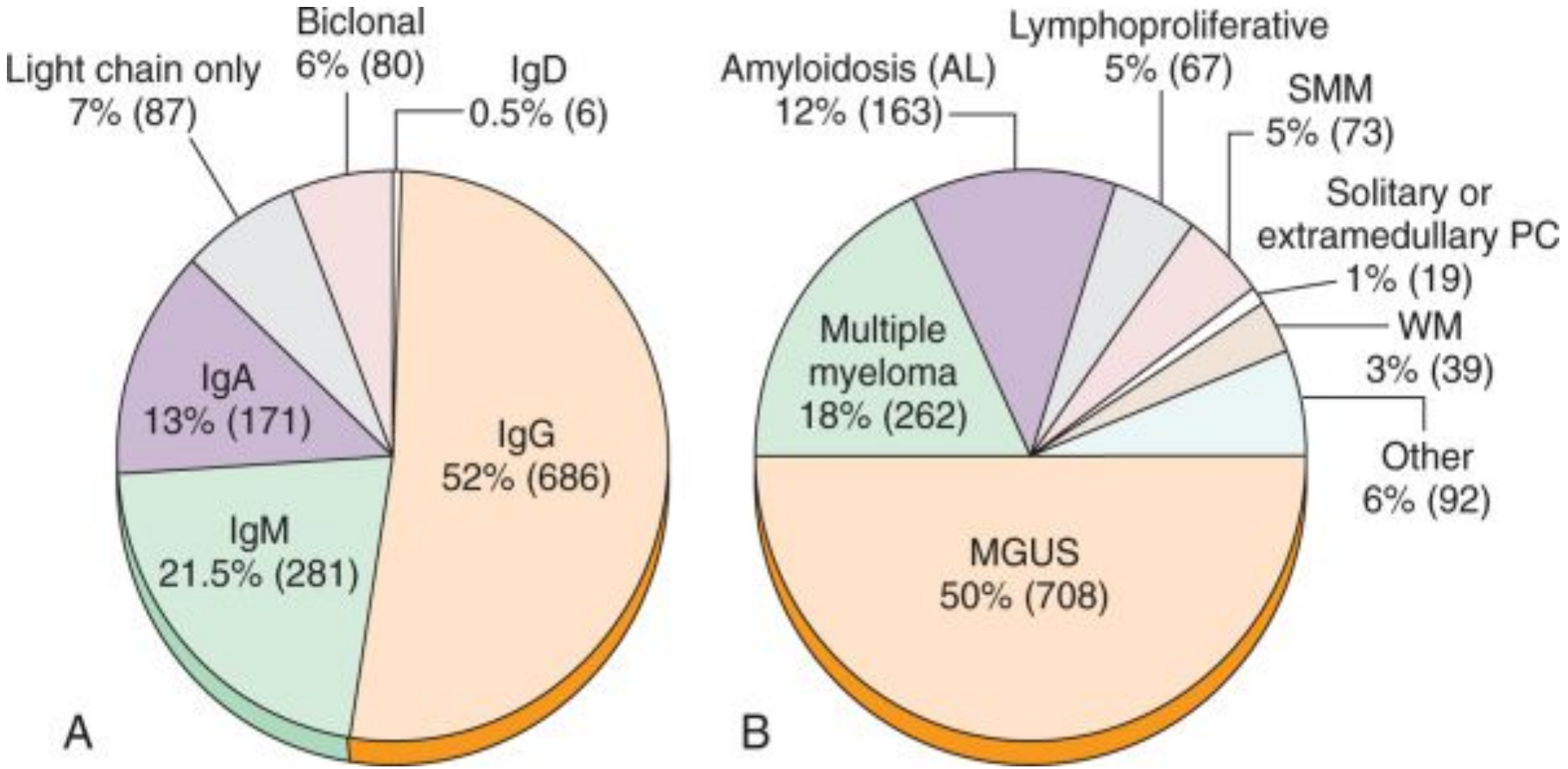
- Diagnosis: Roentgen + BM biopsy+..
- Therapy: chemotherapy, BMT.
- Survival: 5-8 years.

Serum paraprotein detection



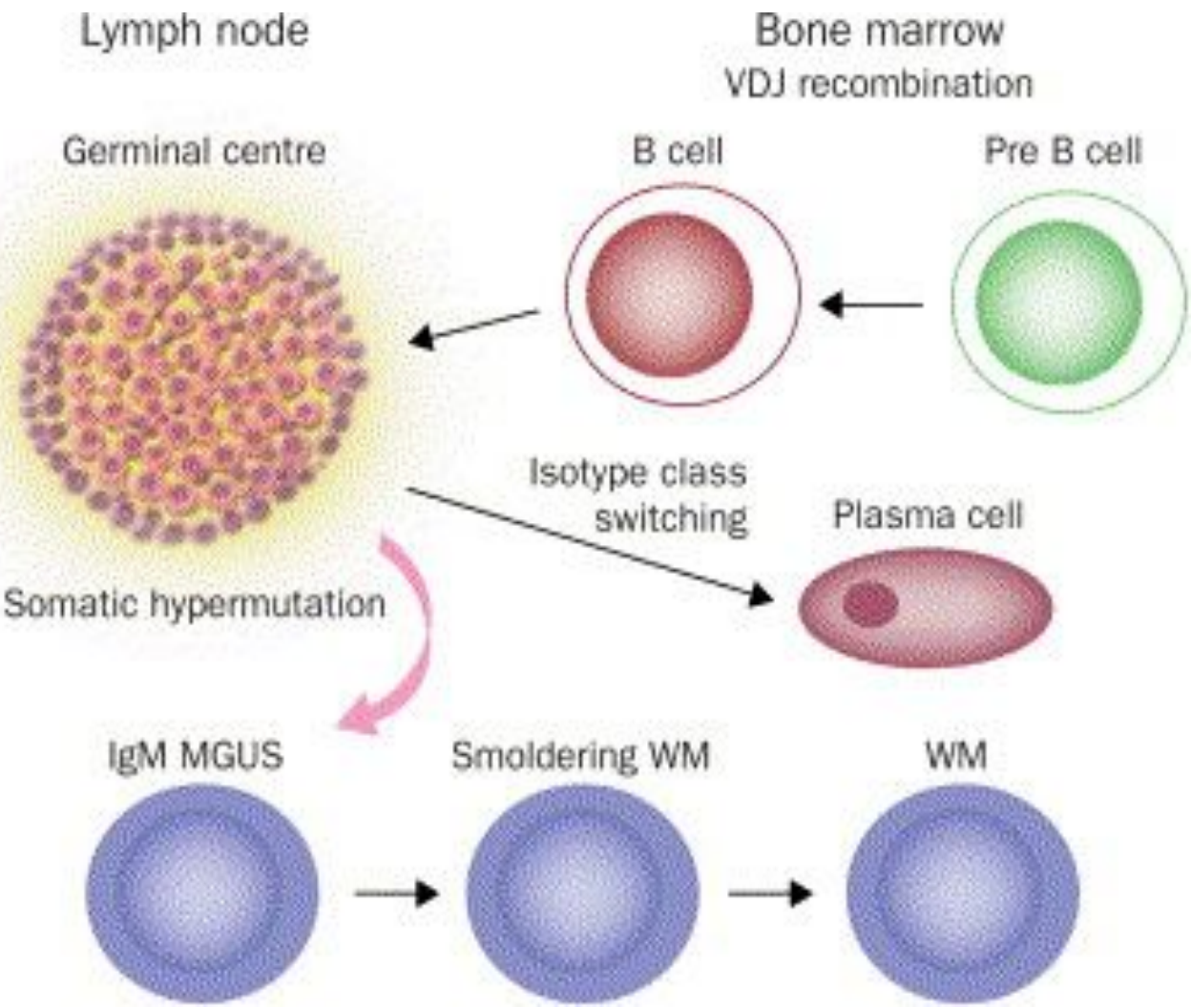


M-protein and diseases.



More than 50% of patients with serum M protein have an initial clinical diagnosis of MGUS (M protein <30g/l in serum, +10% plasma cells in BM). The prevalence of MGUS increases with age, from approximately 1% in patients 50 to 60 years old to greater than 5% in those older than 70 years. The age-adjusted prevalence is higher in males than in females and is twice as high in patients of African descent as in patients of European descent

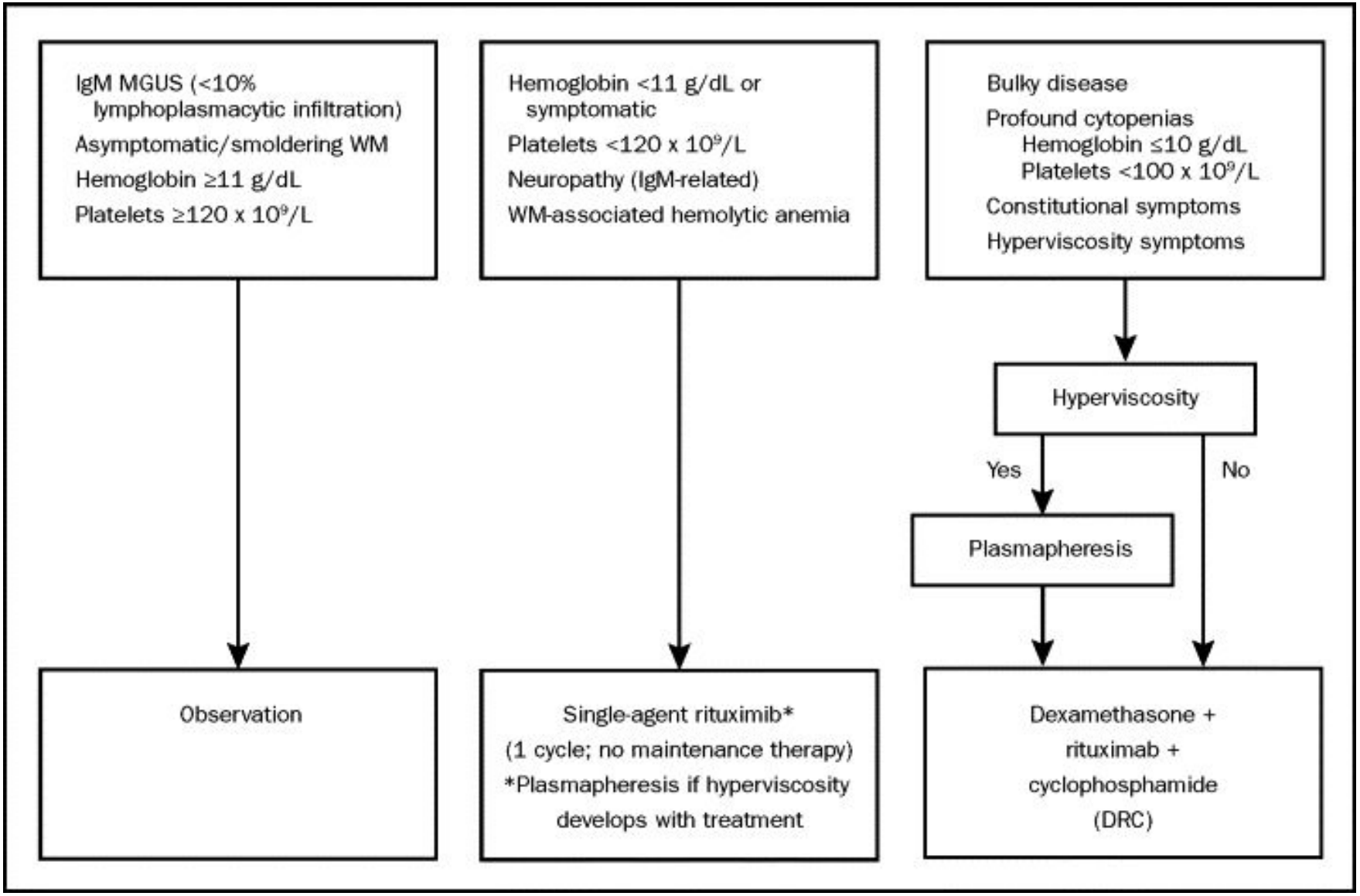
Waldenstrom macroglobulinemia: pathogenesis



Immunophenotype of BM cells in WM

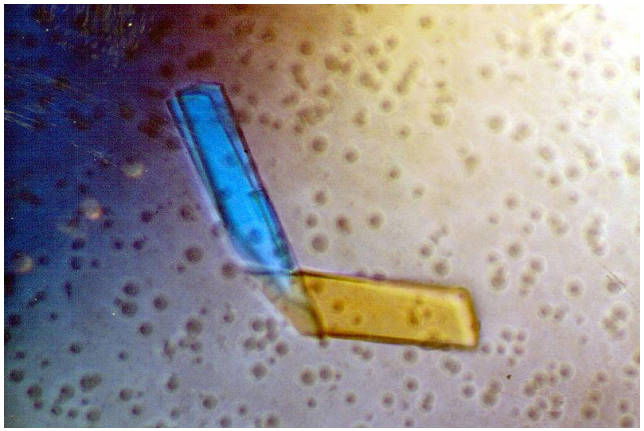
Ig light chain	- Positive
CD19	- Positive
CD20	- Positive
CD52	- Positive
Surface IgM	- Positive
CD79b	- Positive
CD11c	- Usually negative
CD25	- Positive
CD23	- Usually negative
CD38	- Dim positive
FMC7	- Usually dim positive
CD22	- May be positive
CD5	- Negative
CD10	- Negative
CD27	- Dim positive
CD75	- Usually negative
CD138	- Usually negative
Bcl2	- Dim positive
Bcl6	- Usually absent
PAX5+	- Dim positive
CD45 (RA)	- Usually positive

Diagnosis and Therapy of WM.



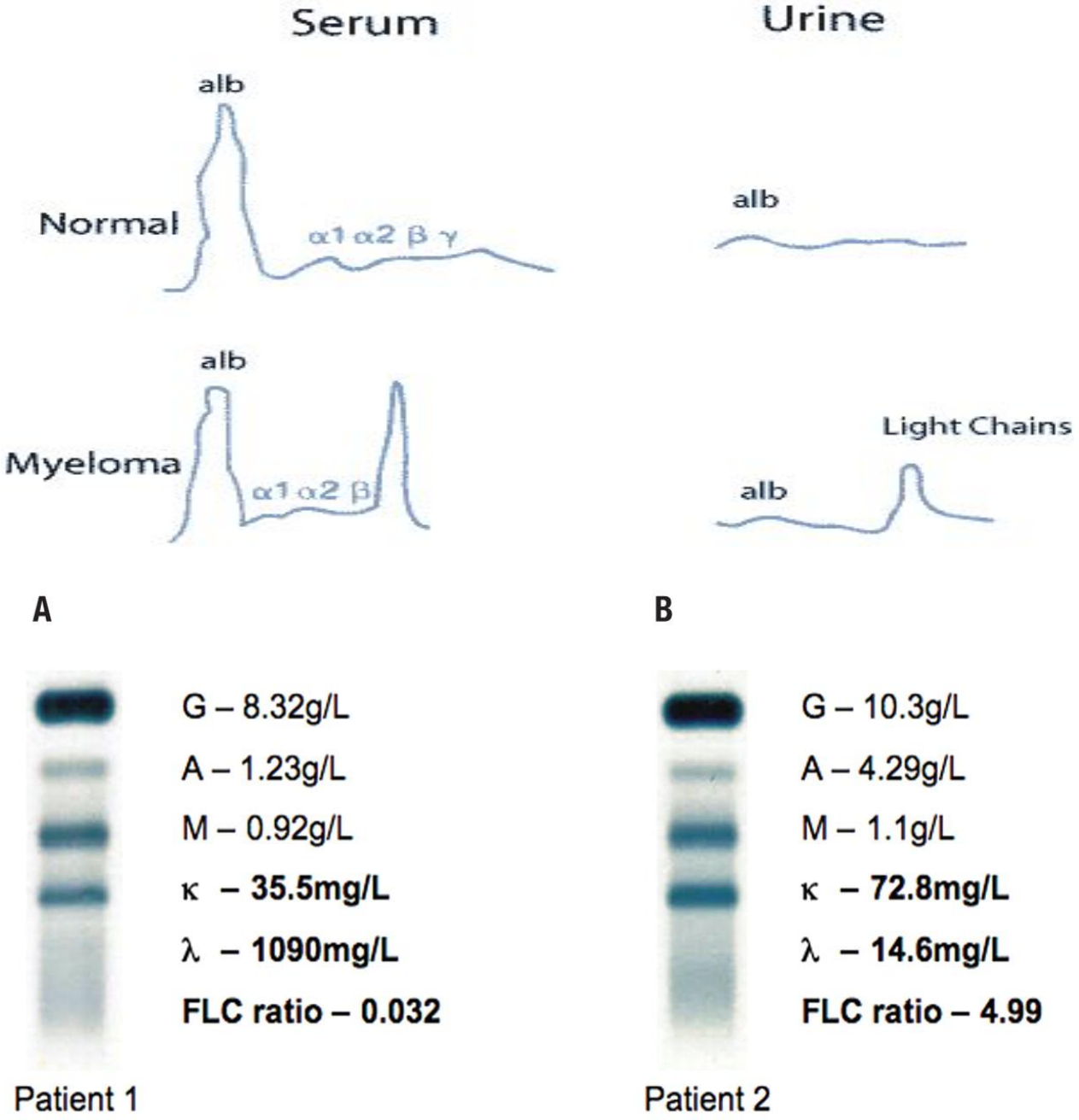
Light chain Disease

(Bence-Jones proteins).



A **Bence Jones protein** is a monoclonal globulion protein or immunoglobulin light chain found in the urine, with a molecular weight of 22-24 kDa. Detection of Bence Jones protein may be suggestive of Multiple Myeloma or Waldenstrom's macroglobulinemia.

(Bence-Jones protein in serum/urine (up) and serum (down))



HEAVY CHAIN DISEASE

Heavy chain disease is a form of paraproteinemia with a proliferation of cells producing immunoglobulin heavy chains

There are <u>four forms</u>:
alpha chain disease (Seligmann's disease)
gamma chain disease (Franklin's disease)
mu chain disease
delta chain disease

Secondary immunodeficiency in lymphoproliferative diseases.

- 1. Lymphoadenopathy (decreased lymphocyte proliferation to mitogens, T cell subpopulation imbalance).
- 2. Autoimmunity (autoantibodies, amyloidosis, renal and liver failure, coagulopathy, vasculitis).