HEMATOLOGY

Lymphoproliferative disorders

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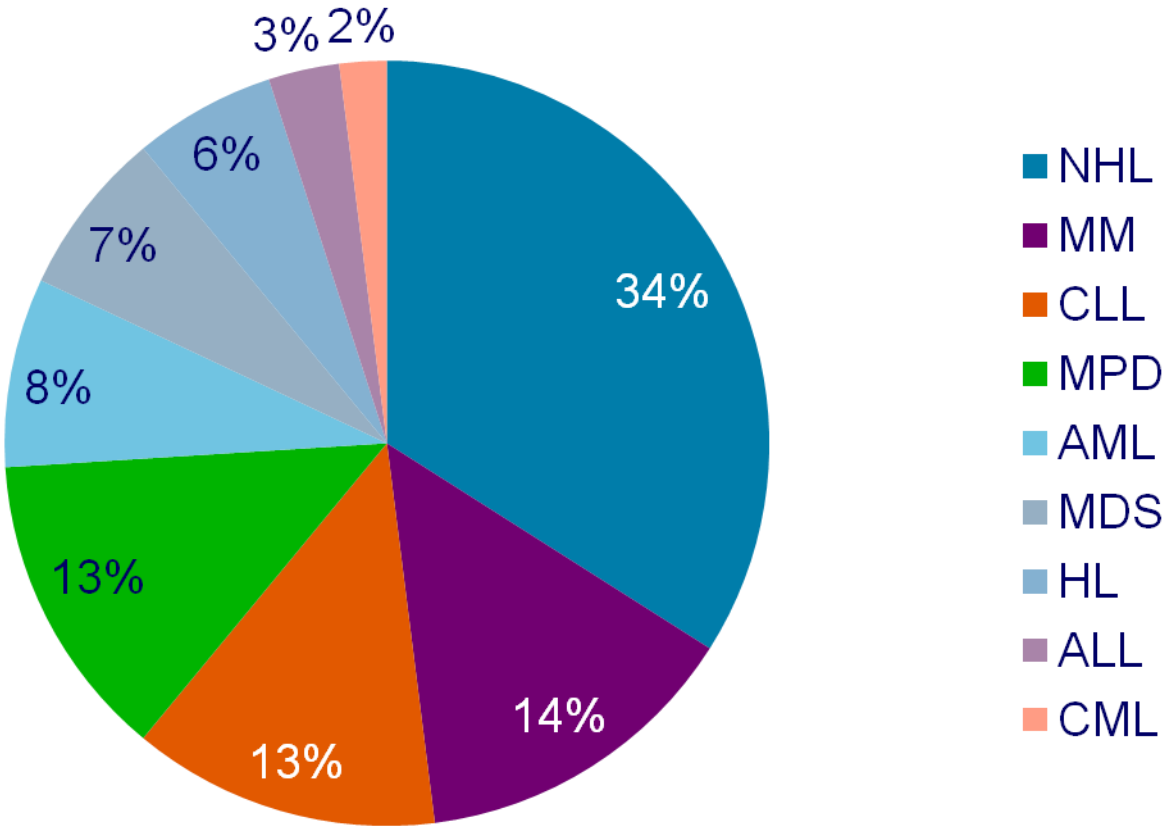
Hematologic malignancies

Incidence in the Czech Republic approx. 40/100.000/rok, tj. approx. 4.000 new patients per year in the Czech Republic. **Mature lymphoproliferative malignancies comprise approx. 2/3 of all hematologic malignancies !!!**



ALL = acute lymphocytic leukaemia; AML = acute myeloid leukaemia; CLL = chronic lymphocytic leukaemia; CML = chronic myeloid leukaemia; HL = Hodgkin lymphoma; MDS = myelodysplastic syndromes; MPD = myeloproliferative disorders; NHL = non-Hodgkin lymphoma.

Data from Leukaemia and Lymphoma Research. Facts about blood cancers; 2010. Available from: http://www.beatbloodcancers.org/facts-about-blood-cancers.

Hematopoiesis

Myelopoiesis

Process of "production" of mature blood cells: from hematopoietic stem cell level to mature blood elements (erythrocytes, neutrophiles, megakaryocytes, macrophages)

Early lymphopoiesis

Early stages of lymphocyte development in the bone marrow= from hematopoietic stem cell to pre-lymphocyte

Subsequent stages of lymphopoisis take place outside bone marrow (thymus → sec. lymph. organs)

Hematologic malignancies

1. **Disorders of hematopoietic stem cell (= disorders of hematopoiesis)**

-myelodysplasias (MDS)

-myeloproliferative syndromes (MPS)

-acute myelogeneous leukemias (AML)

-acute lymphoblastic leukemias / lymphoblastic lymphomas (ALL/LBL) (= **immature** or **precursor** lymphoproliferative disorders)

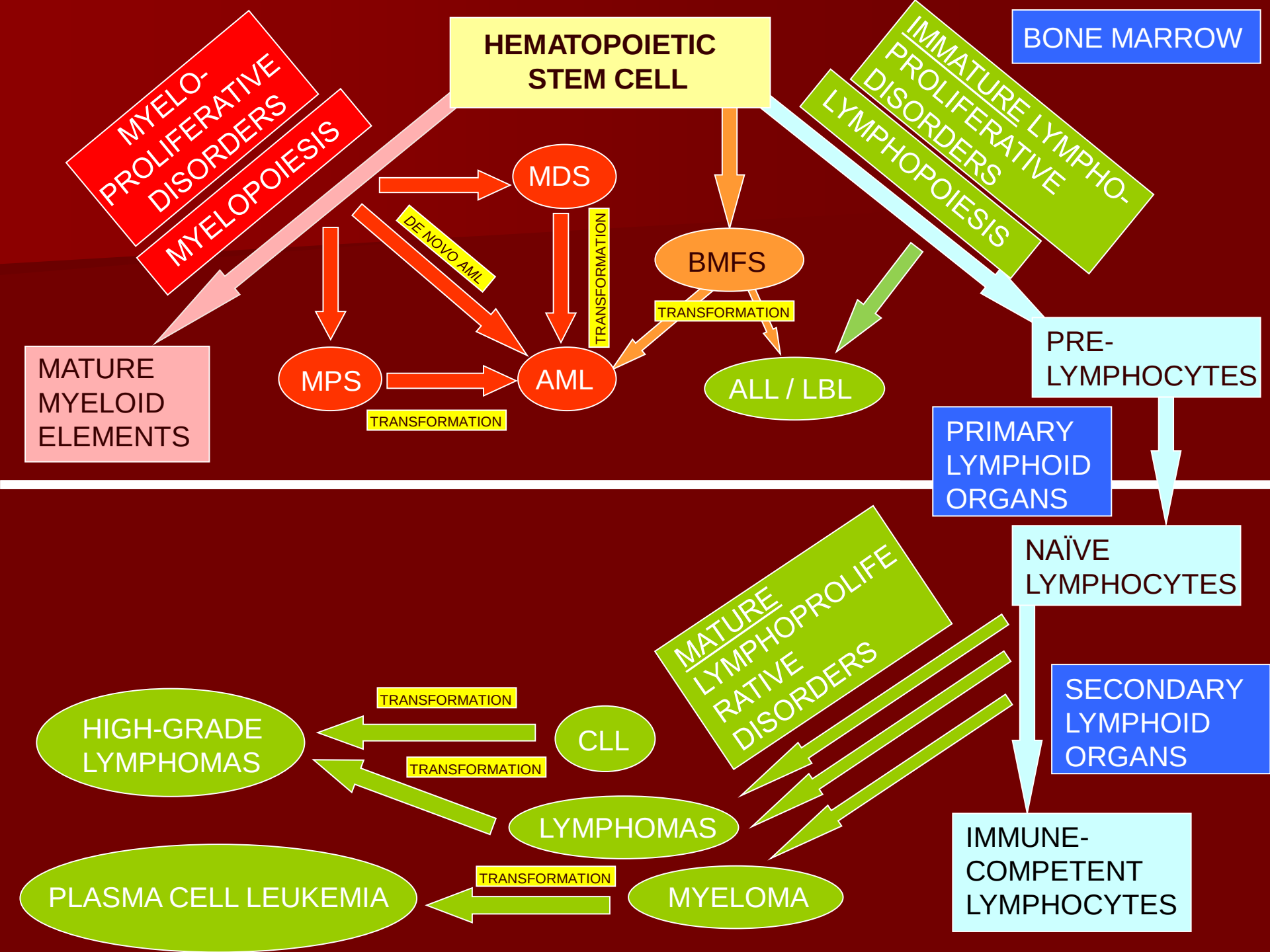
2. **Disorders of peripheral (mature) lymphocytes**

-**mature** lymphoproliferative disorders (Hodgkin and non-hodgkin lymphomas, multiple myeloma, chronic lymphocytic leukemia, amyloidosis etc.)

→ Mature lymphoproliferative disorders are not disorders of hematopoietic stem or progenitor cells, but disorders of mature (peripheral) lymphocytes that acquire critical mutations during the complex process of differentiation from the stage of naive lymphoid elements until the stage of immune-competent cells (plasma cells or cytotoxic T-cells). These events take place in secondary lymphoid organs (lymph nodes, spleen, tonsils, Peyer´s patches, mucosa-associated lymphoid tissue).

Hematologic malignancies

1. **Myeloproliferative disorders** (broadly speaking)= disorders of hematopoietic stem cell (MDS, MPS, AML).
2. **<u>Immature</u> lymphoproliferative disorders**= disorders of hematopoietic stem cell (ALL / LBL)
3. **<u>Mature</u> (peripheral) lymphoproliferative disorders**= disorders of mature lymphocytes (lymphomas, myeloma, CLL etc.). Hematopoietic stem cell is not affected.


HEMATOPOIETIC STEM CELL
BONE MARROW
MYELO-PROLIFERATIVE DISORDERS
MYELOPOIESIS
IMMATURE LYMPHO-PROLIFERATIVE DISORDERS
LYMPHOPOIESIS
MDS
DE NOVO AML
TRANSFORMATION
BMFS
TRANSFORMATION
MATURE MYELOID ELEMENTS
MPS
AML
ALL / LBL
TRANSFORMATION
PRE-LYMPHOCYTES
PRIMARY LYMPHOID ORGANS
NAÏVE LYMPHOCYTES
MATURE LYMPHOPROLIFE RATIVE DISORDERS
SECONDARY LYMPHOID ORGANS
TRANSFORMATION
HIGH-GRADE LYMPHOMAS
CLL
TRANSFORMATION
LYMPHOMAS
IMMUNE-COMPETENT LYMPHOCYTES
TRANSFORMATION
PLASMA CELL LEUKEMIA
MYELOMA

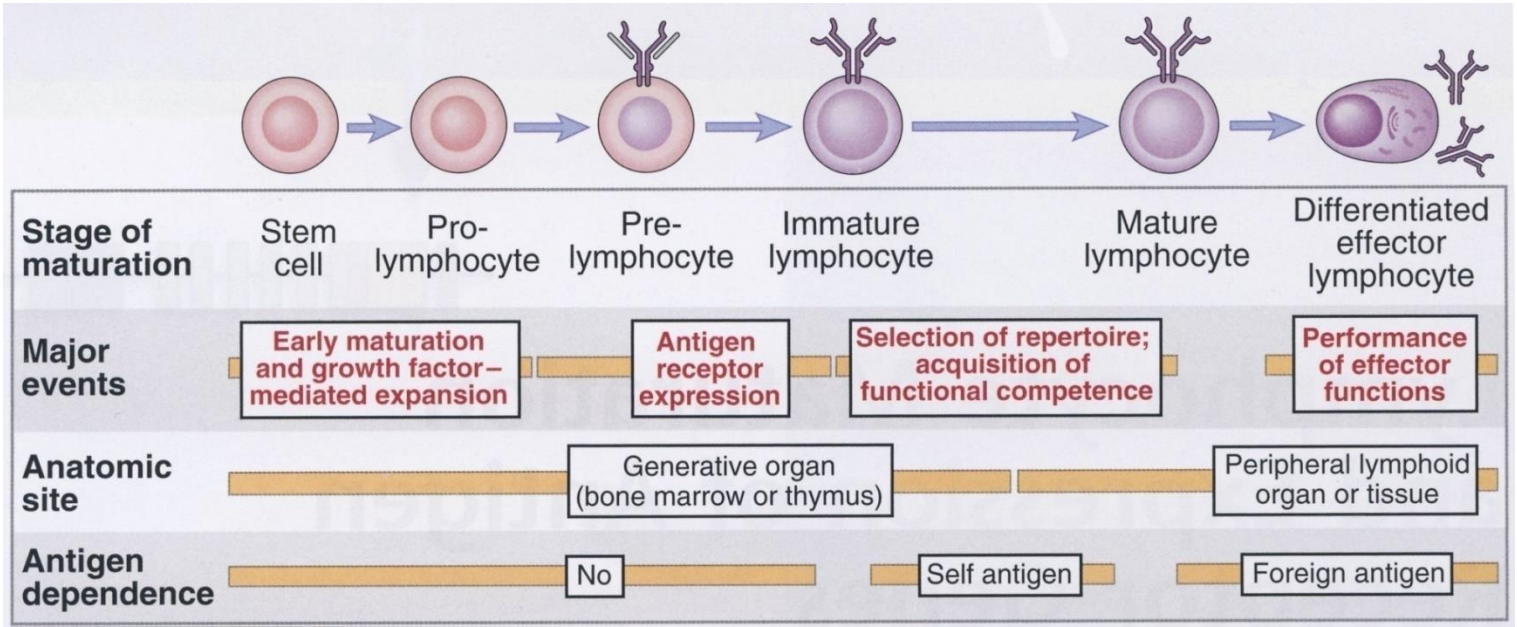
Lymphocyte development

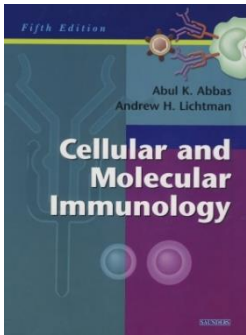
Development of lymphocytes: 3 different compartments

1. **Generative organ**= bone marrow
-hematopoietic stem cell → pre-lymphocyte
-acquisition of immune-competent receptors= B-cell receptors (BCR)= surface IgM/IgD, and T-cell receptors (TCR) by germ-line DNA recombination → ability to recognize antigen

2. **Primary lymphoid organs**= thymus and bone marrow (pre-lymphocyte → naïve lymphocyte)
-positive and negative selection in the thymus → elimination of non-functional or auto-reactive T-cells

3. **Secondary lymphoid organs**= lymph nodes, spleen, tonsils, Peyer´s patches, MALT (mucosa-associated lymphoid tissue)
-naïve B-cells → effector B-cells (plasma cells)
-encounter with antigen displayed on antigen-presenting cells (APCs) in complex with MHC molecules → differentiation and clonal expansion of B-cells
-B-cells secreting low-affinity IgM → plasma cells secreting high-affinity IgG antibodies





B-cell development and B-cell malignancies

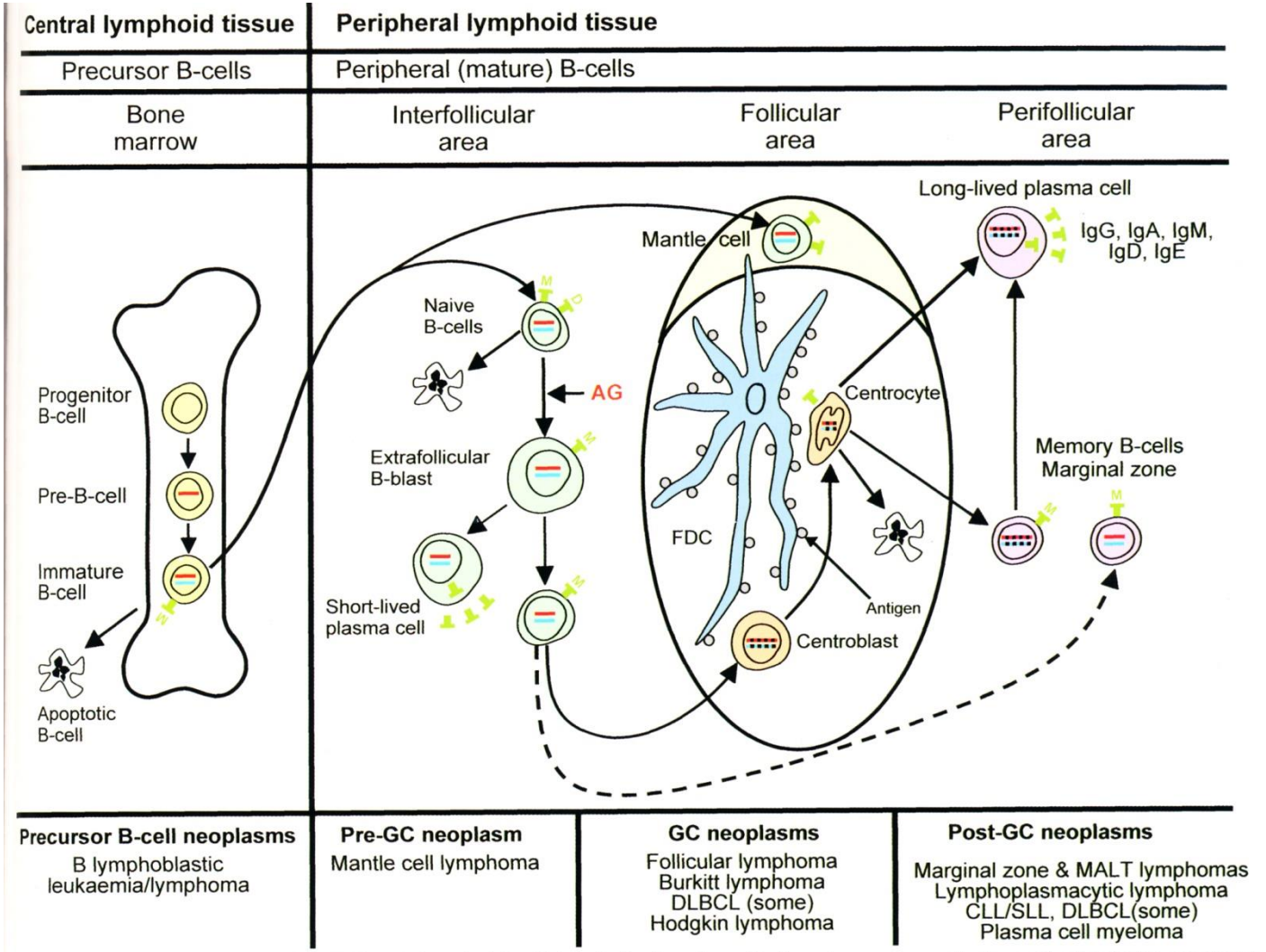


Fig. 8.02 Diagrammatic representation of B-cell differentiation and relationship to major B-cell neoplasms. B-cell neoplasms correspond to stages of B-cell maturation, even though the precise cell counterparts are not known in all instances. Precursor B-cells that mature in the bone marrow may undergo apoptosis or develop into mature naïve B-cells that, following exposure to antigen and blast transformation, may develop into short-lived plasma cells or enter the germinal centre (GC) where somatic hypermutation and heavy chain class switching occur. Centroblasts, the transformed cells of the GC, either undergo apoptosis or develop into centrocytes. Post-GC cells include both long-lived plasma cells and memory/marginal zone B-cells. Most B-cells are activated within the GC, but T-cell independent activation can take place outside of the germinal centre and also probably leads to memory type B-cells. Monocytoid B-cells, many of which lack somatic hypermutation, are not illustrated.
DLBCL, diffuse large B-cell lymphoma; CLL/SLL, chronic lymphocytic leukaemia/small lymphocytic lymphoma; MALT, mucosa-associated lymphoid tissue; AG, antigen; FDC, follicular dendritic cell. Red bar, immunoglobulin heavy chain gene *(IGH@)* rearrangement; blue bar, immunoglobulin light chain gene *(IGL)* rearrangement; black insertions in the red and blue bars indicate somatic hypermutation.

WHO Classification of Tumours of Haematopoietic and Lymphoid Tissues
Edited by Steven H. Swerdlow, Elias Campo, Nancy Lee Harris, Elaine S. Jaffe, Stefano A. Pileri, Harald Stein, Jürgen Thiele, James W. Vardiman

T-cell development and T-cell malignancies

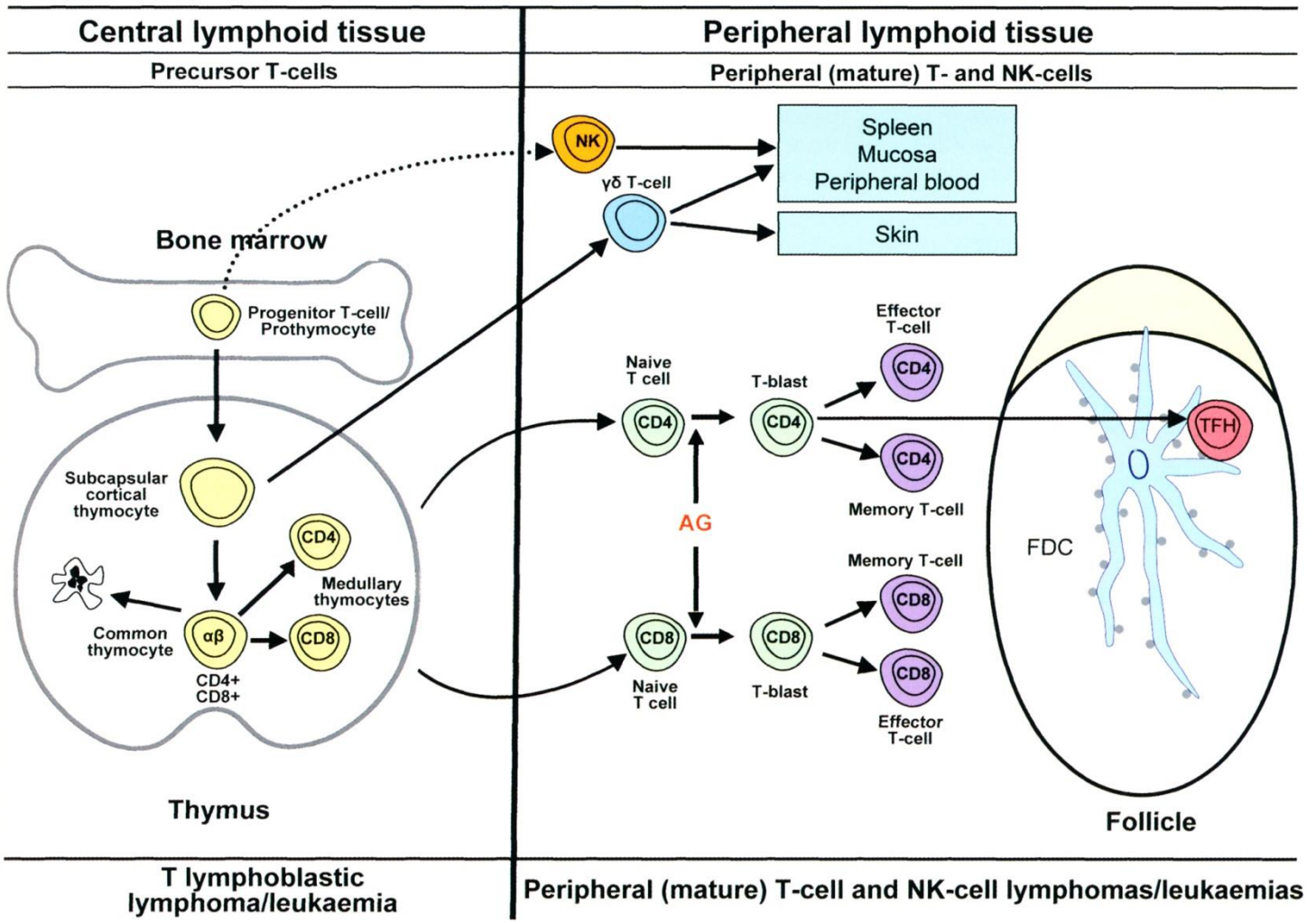


Fig. 8.04 Diagrammatic representation of T-cell differentiation. T-cell neoplasms correspond to different stages of maturation. Lymphoid progenitors enter the thymus where precursor T-cells develop into varied types of naïve T-cells. The precise maturational path of natural killer cells and γδ T-cells is not fully understood. The αβ T-cells leave the thymus where upon exposure to antigen they may undergo blast transformation and develop further into CD4+ and CD8+ effector and memory T-cells. T regulatory cells are the major type of CD4+ effector T-cells. Another specific type of effector T-cells is the follicular helper T-cell that is found in germinal centres. Upon antigenic stimulation, T-cell responses may occur independent of the germinal centre, or in the context of a germinal centre reaction. FDC, Follicular dendritic cells; AG, antigen.

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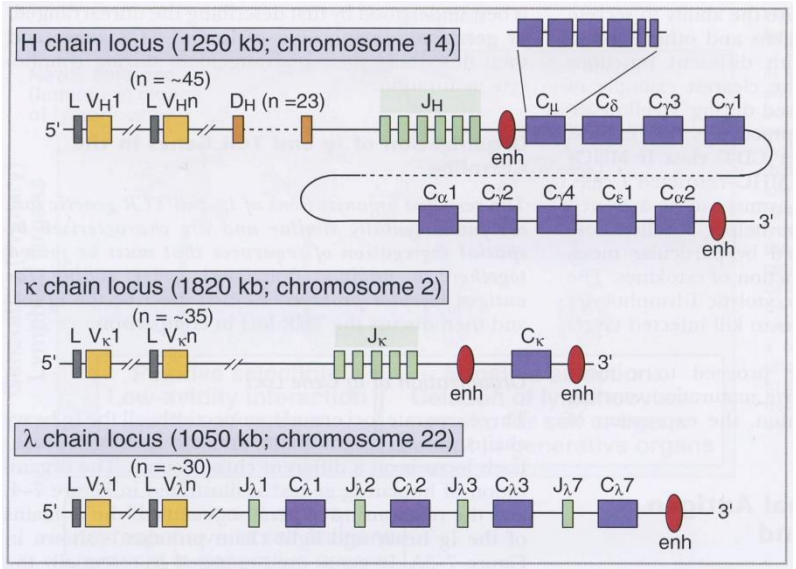
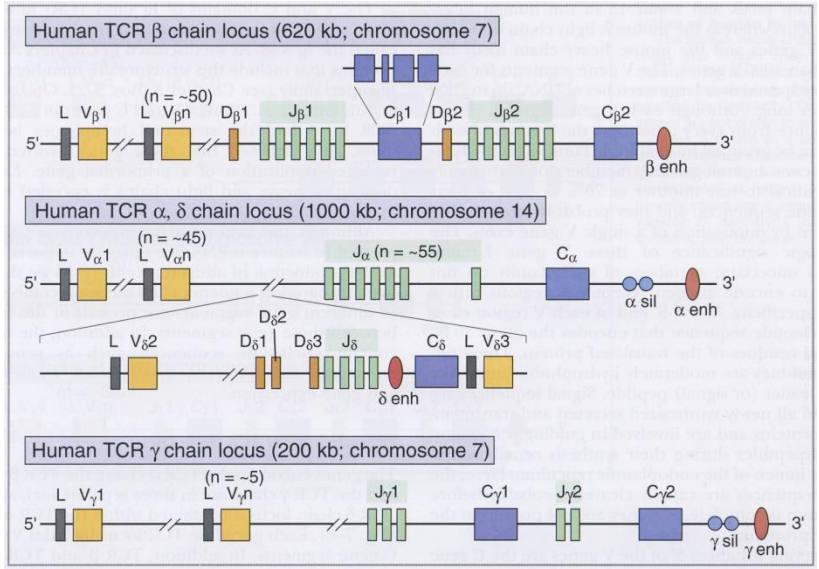
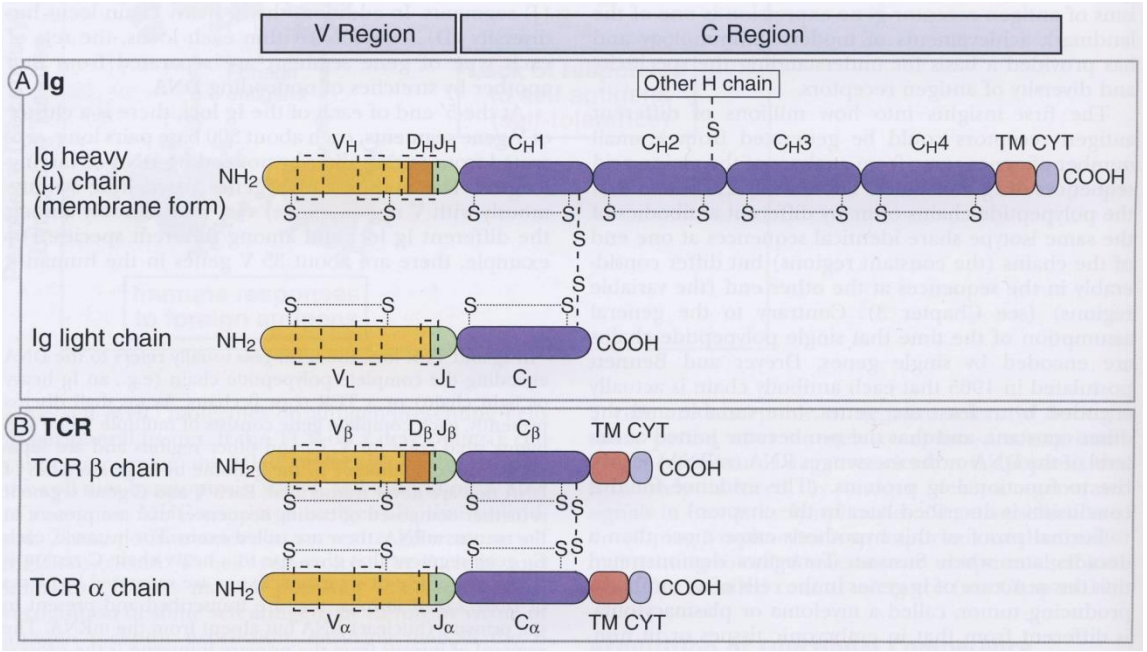
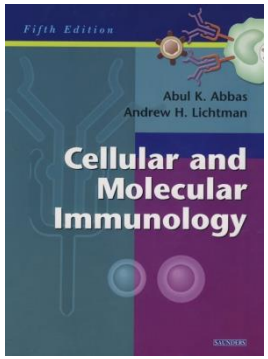
Ig and TCR gene loci- chrom. 2, 14, 22; 7, 14

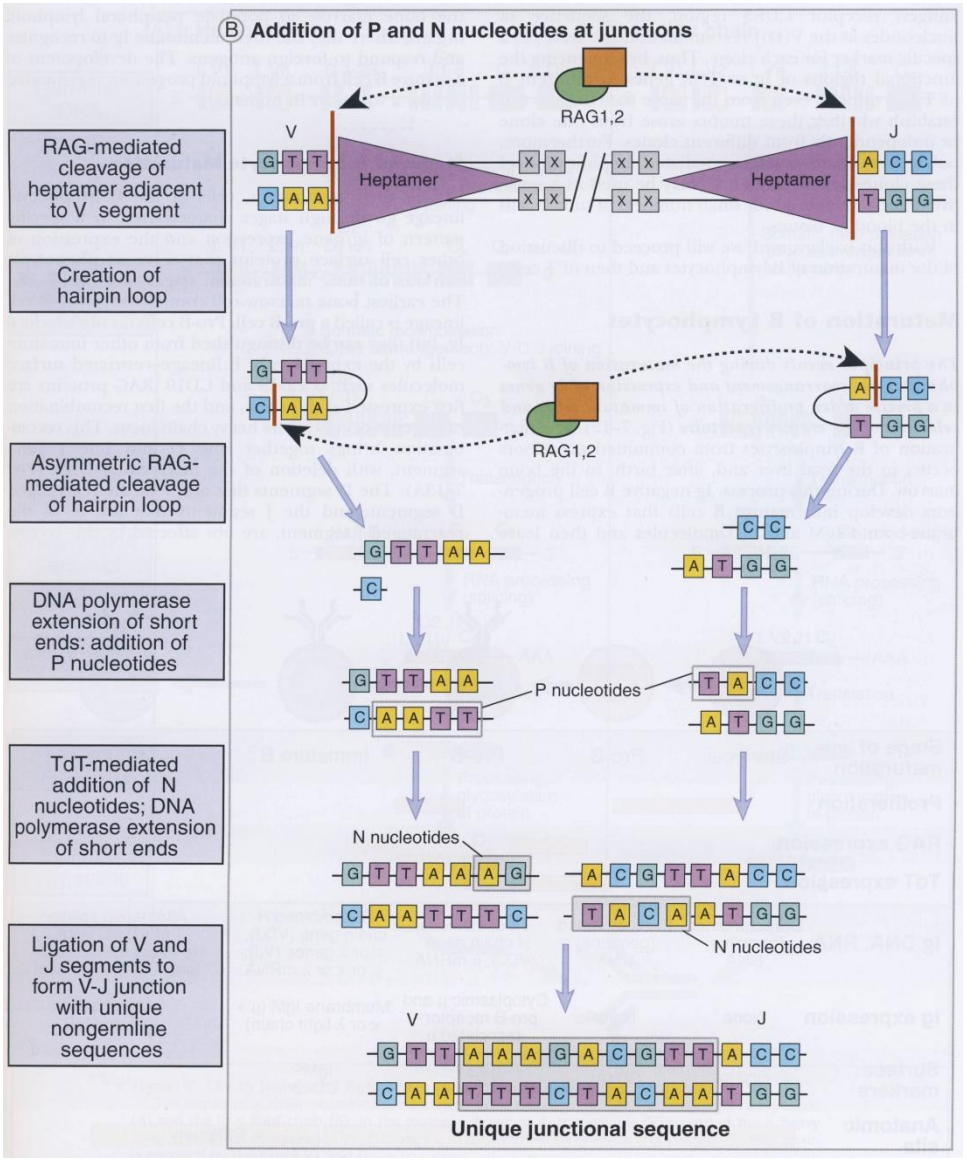


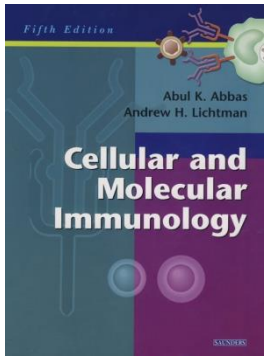




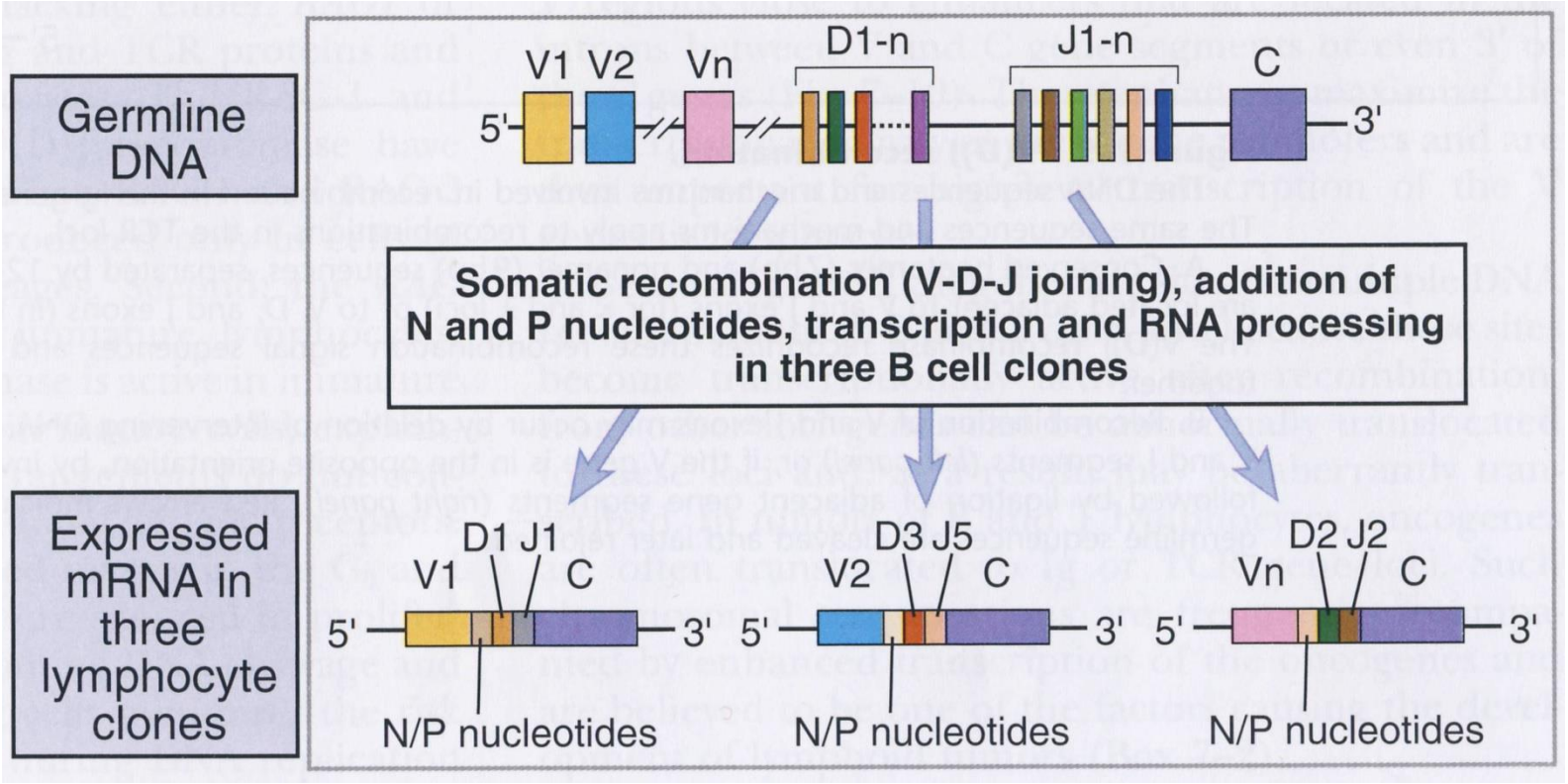


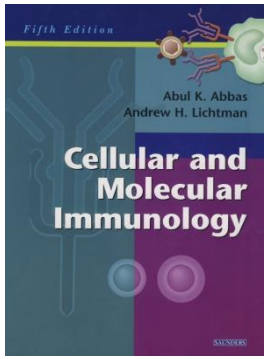
Recombination process: RAG-1 and RAG-2

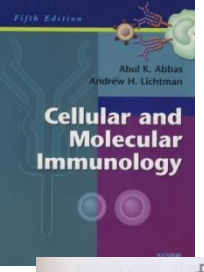




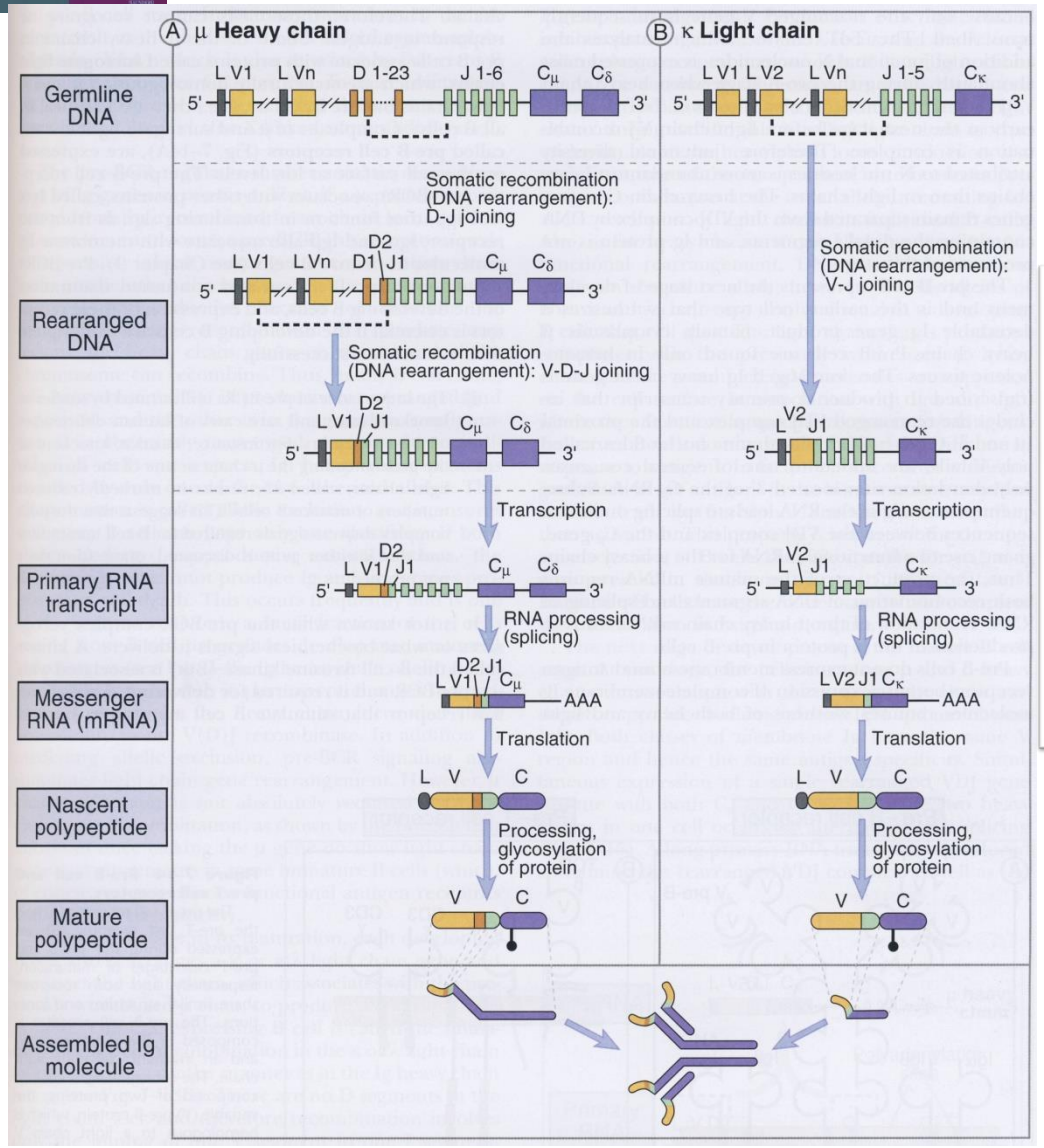
B-lymphocyte clone Ig somatic recombination



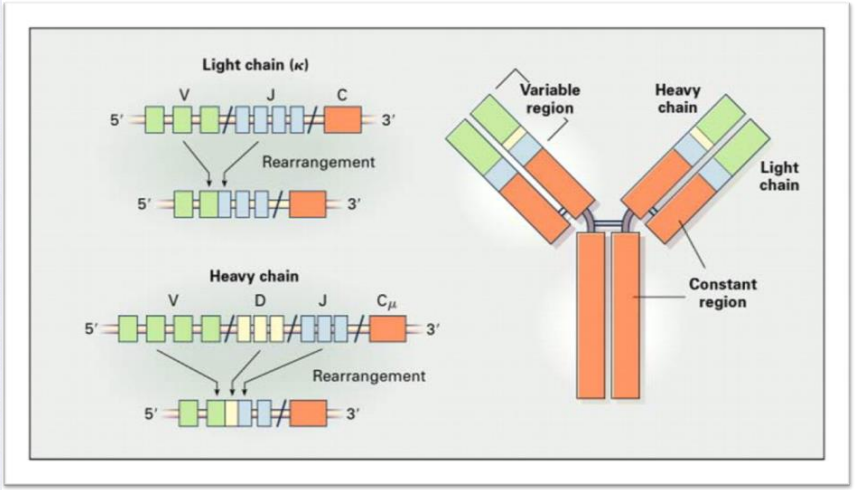




Ig recombination and assembly

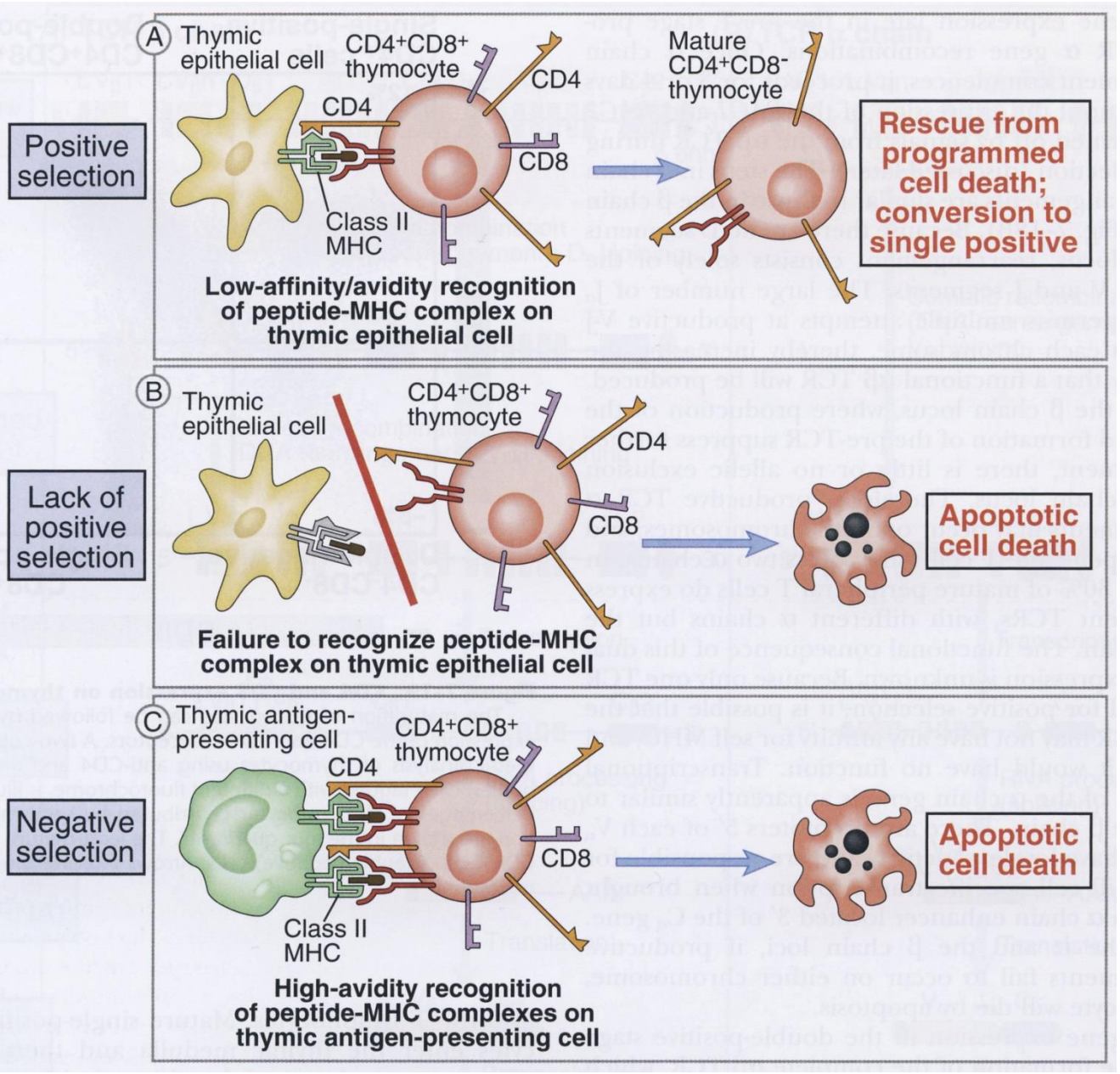


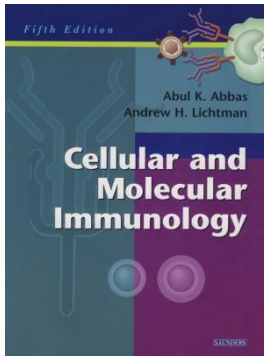
The immunoglobulin heavy chain genes (IgH) are assembled from various V (variable), D (diversity) and J (joining) elemenets, whereas the light chain is recombined from V and J elements.



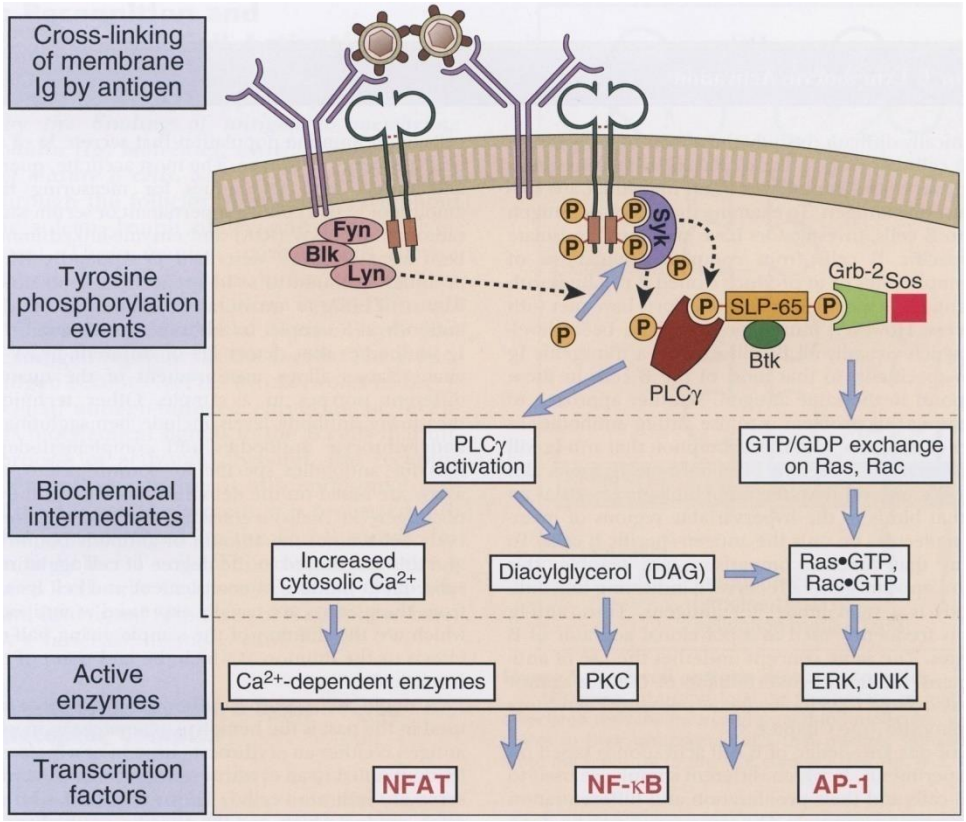
Only cells that have acquired heavy and light chain variable region genes that can be translated into protein (i.e. into functional B-cell receptor, BCR) will survive, whereas all other cells will undergo apoptosis.

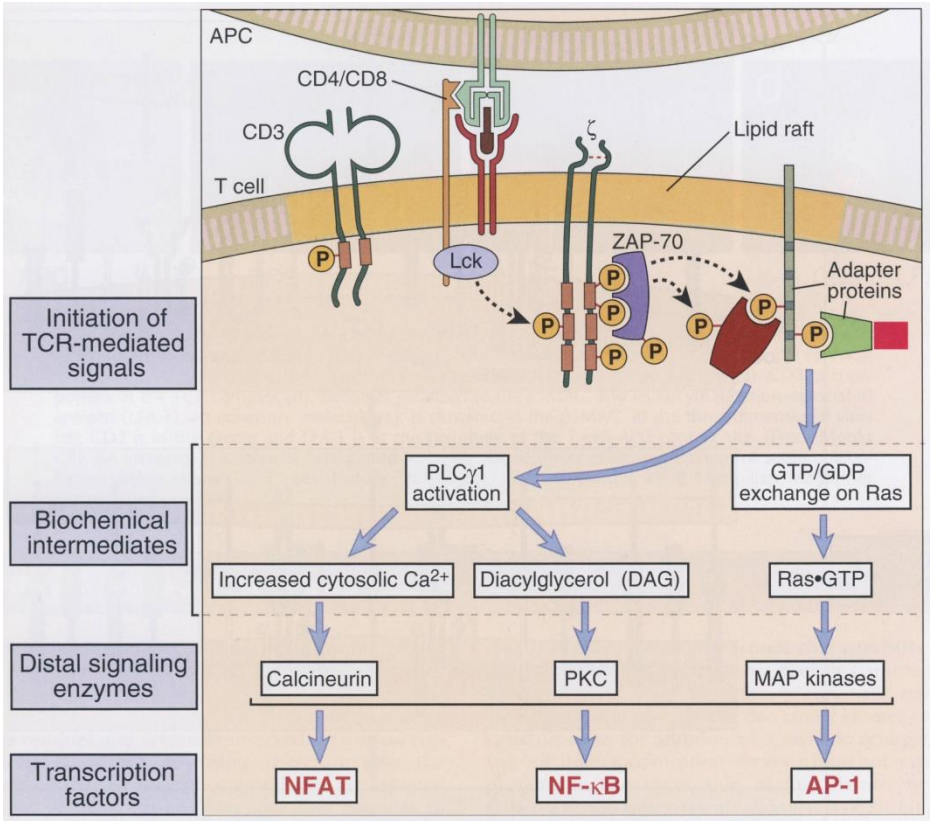
Positive and negative selection

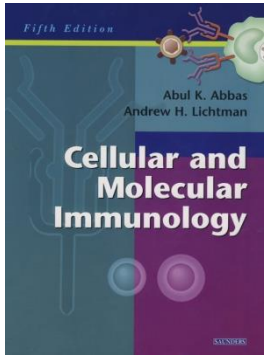




BCR and TCR signaling pathways



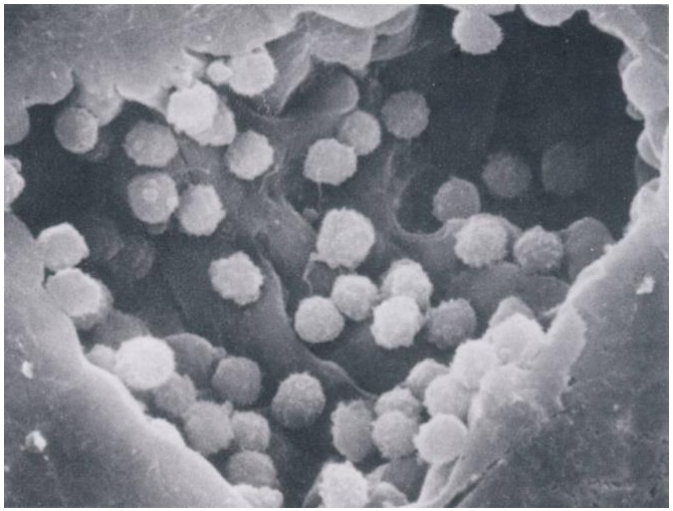


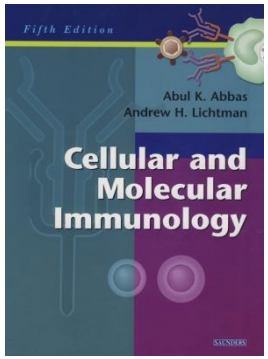


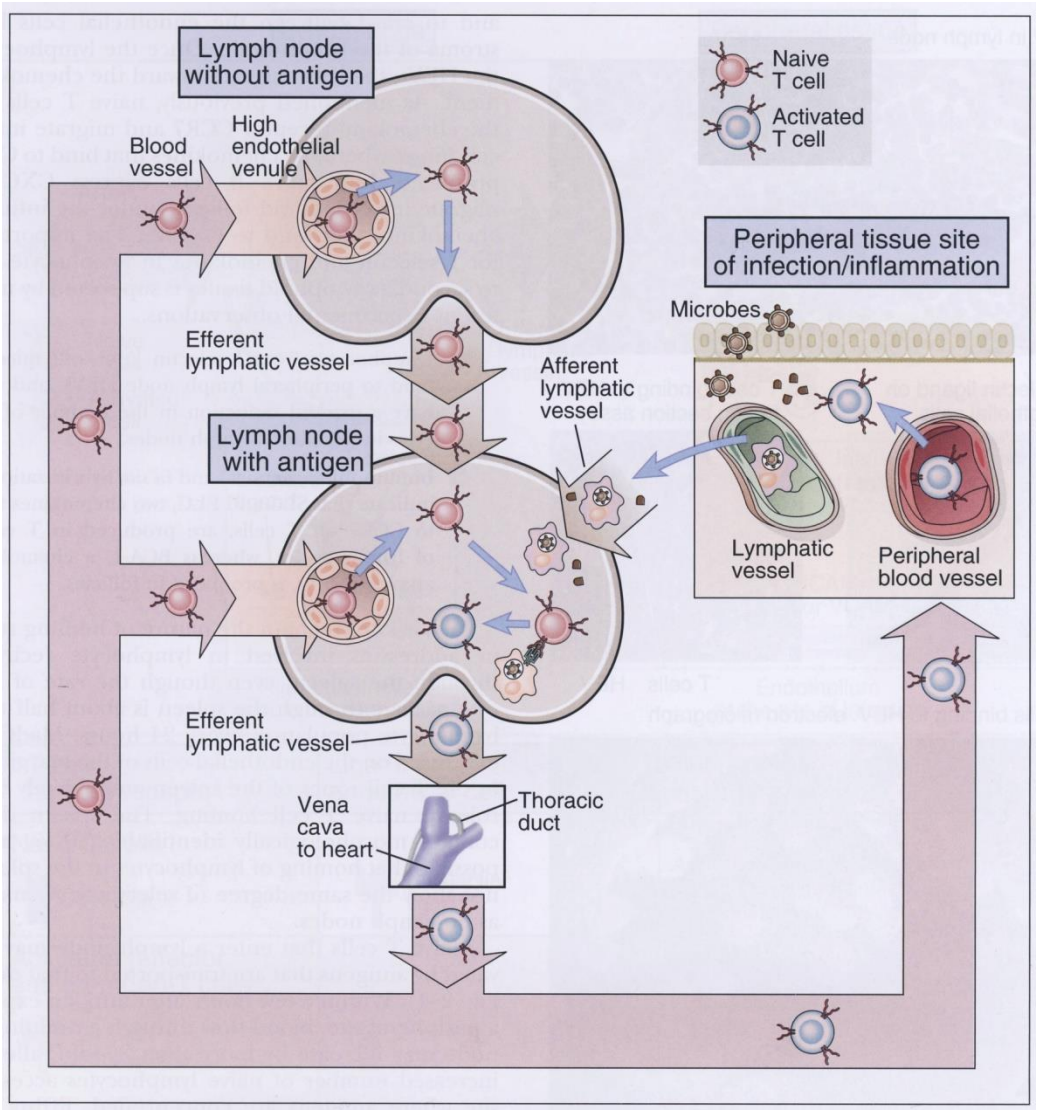
Functional BCR and TCR signaling plays crucial role in survival of B- and T-cells.

Aberrant BCR and TCR signaling is involved in survival of pathological lymphocyte clones (leukemia or lymphoma clones).

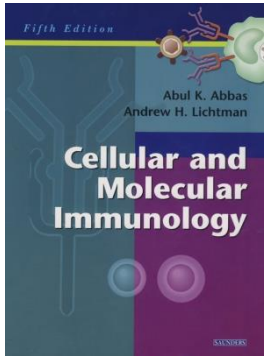
Lymphocyte activation upon encounter with receptor specific antigen

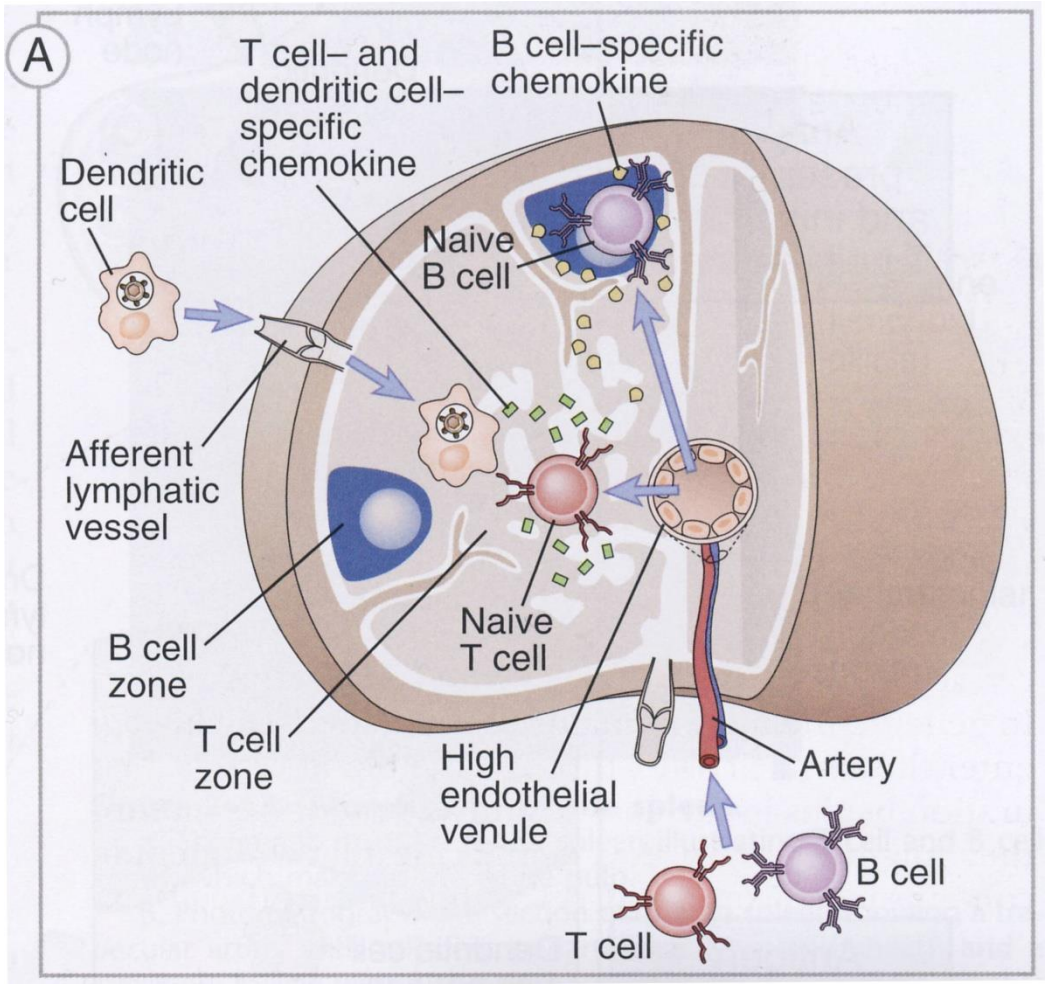


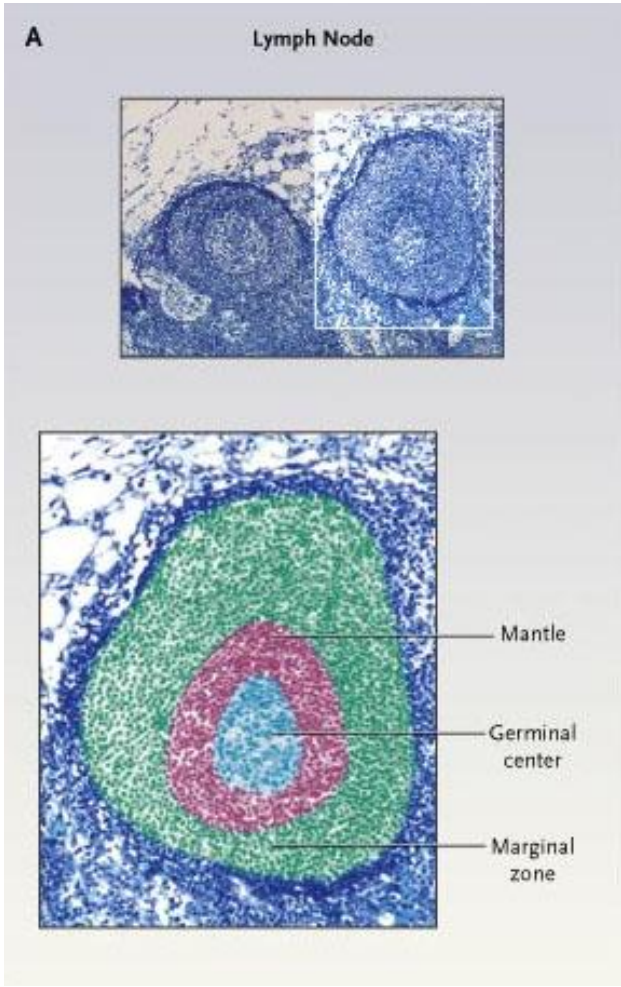




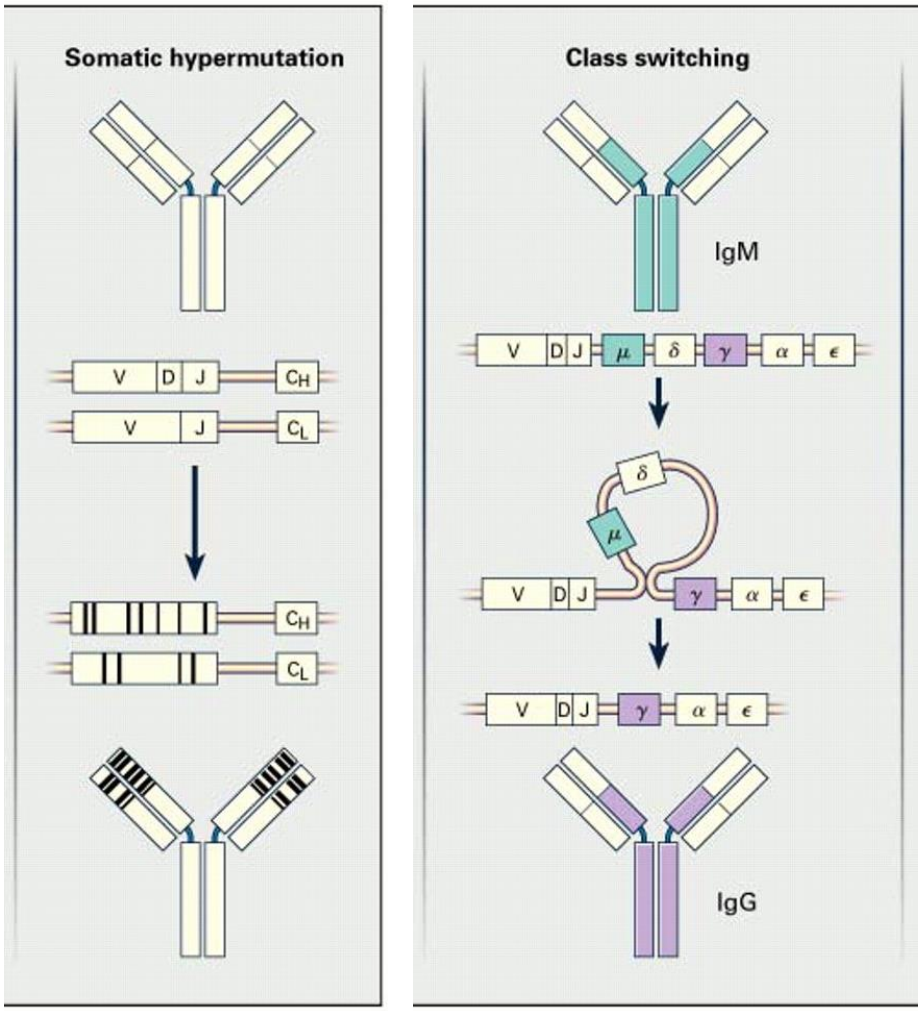
Lymphocyte cycling through lymph nodes in search for antigen







Somatic hypermutation and Ig heavy chain isotype switch



Somatic hypermutation carried out by enzyme called *activation-induced cytidine-deaminase* (AID) triggers random mutations into germ-line DNA coding for variable segments of Ig molecule → this leads to either increased or decreased affinity of BCR to antigen.

Clones with increased affinity are stimulated for higher mitotic activity.

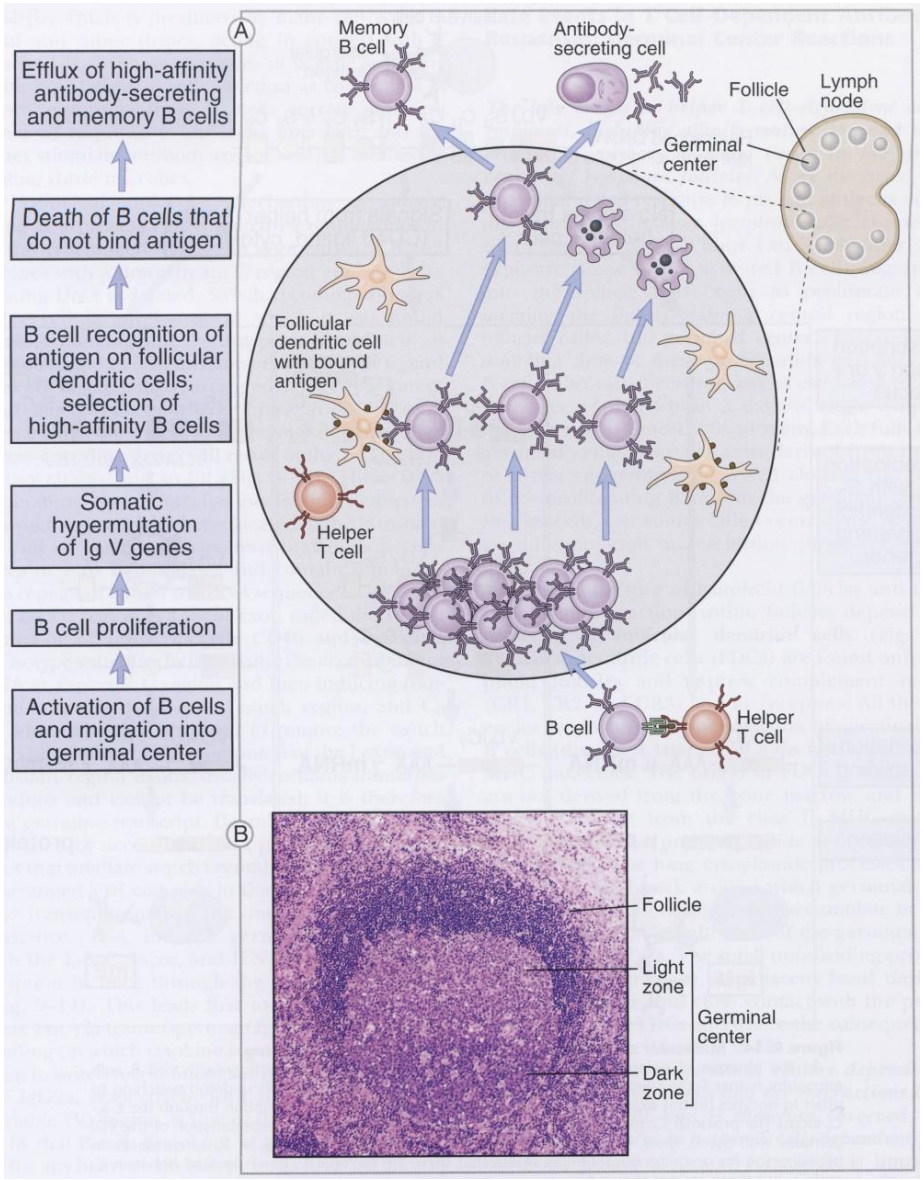
Other clones undergo apoptosis.

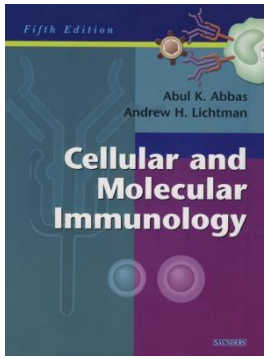
→ During clonal expansion in the secondary lymphoid follicule affinity to antigen is increased up to 10.000x.

→ Isotype switch (IgM → IgG) leads to changed immune functions (Fc fragment)

AID= activation-induced cytidine deaminase

Clonal expansion and maturation of naive B-lymphocyte into effector plasma cell

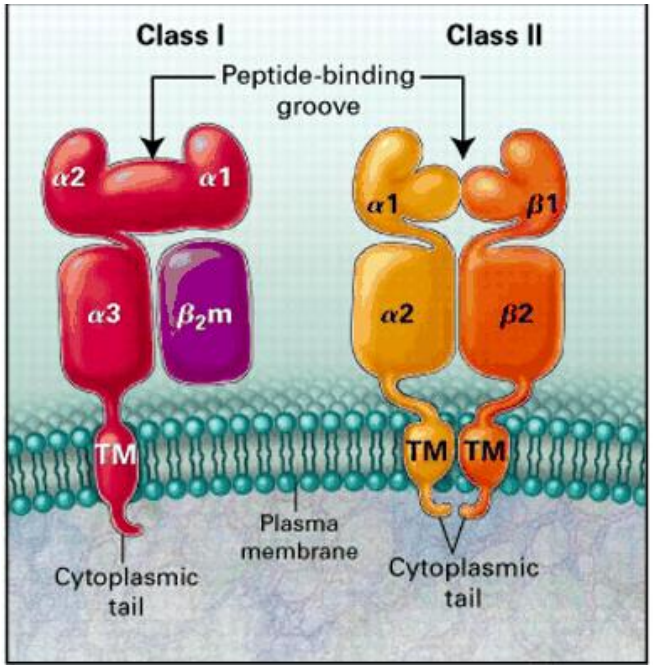




A window to immunology: MHC restriction and pathways of antigen presentation

MHC (major histocompatibility complex) restriction

Antigens can be presented only in complex with MHC molecules= MHC restriction.



MHC molecules= class I and class II HLA (human leukocyte antigen) molecules

Class I HLA molecules= single-chain molecules HLA-A, HLC-B, and HLA-C non-covalently bound to beta-2-microglobulin

→ 3 genes= 6 molecules only for each individual !!!

Class II HLA molecules= dimers (both homodimers and heterodimers) consisting of two HLA-DR, HLA-DQ, HLA-DP molecules

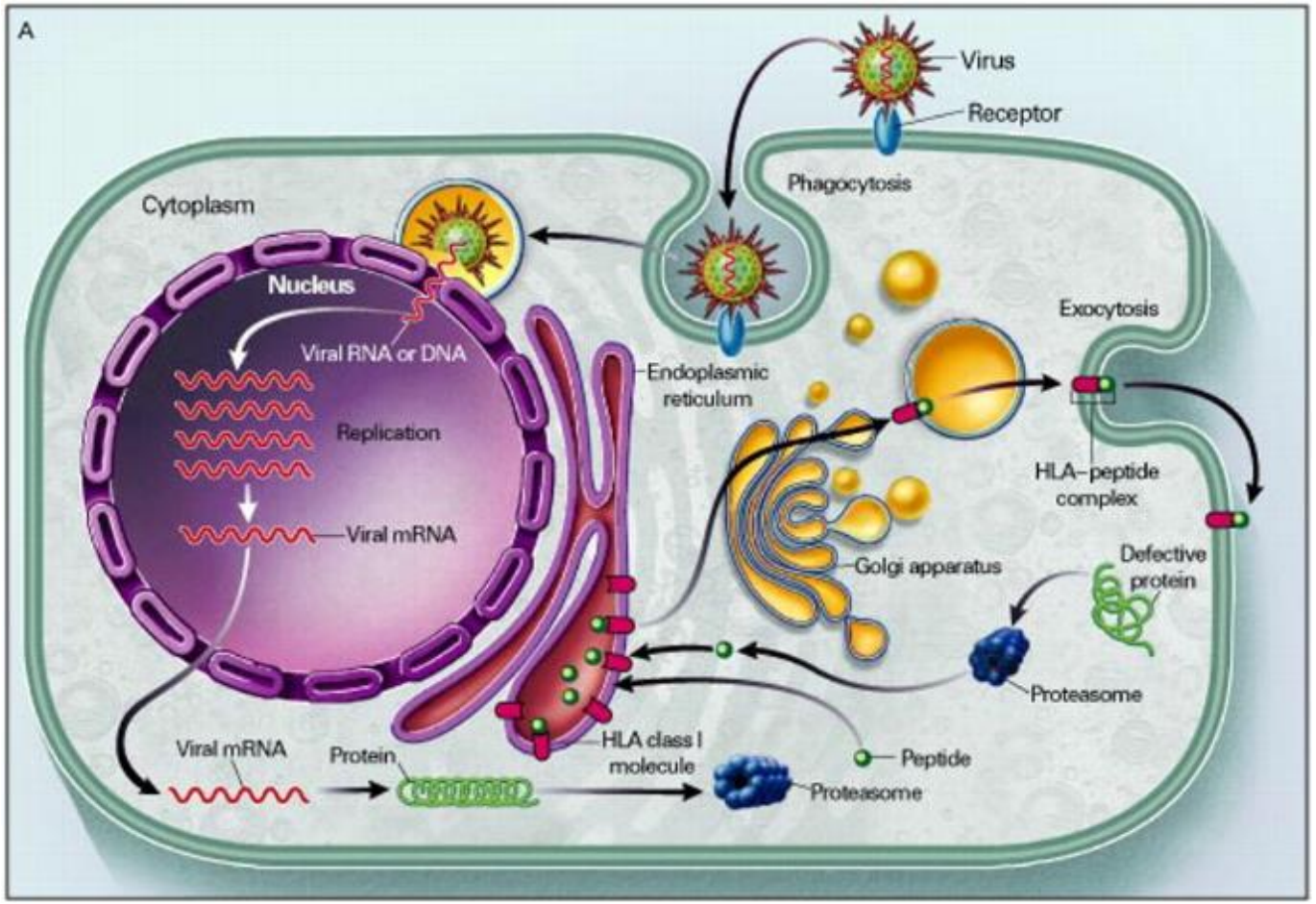
→ 9 genes= up to 20 different molecules for each individual

HLA class I molecules

Class I HLA molecules= single-chain molecules HLA-A, HLC-B, and HLA-C

→ expressed on all nucleated cells

→ presentation of cellular antigens (processed by proteolysis of cellular or viral proteins in proteasome)

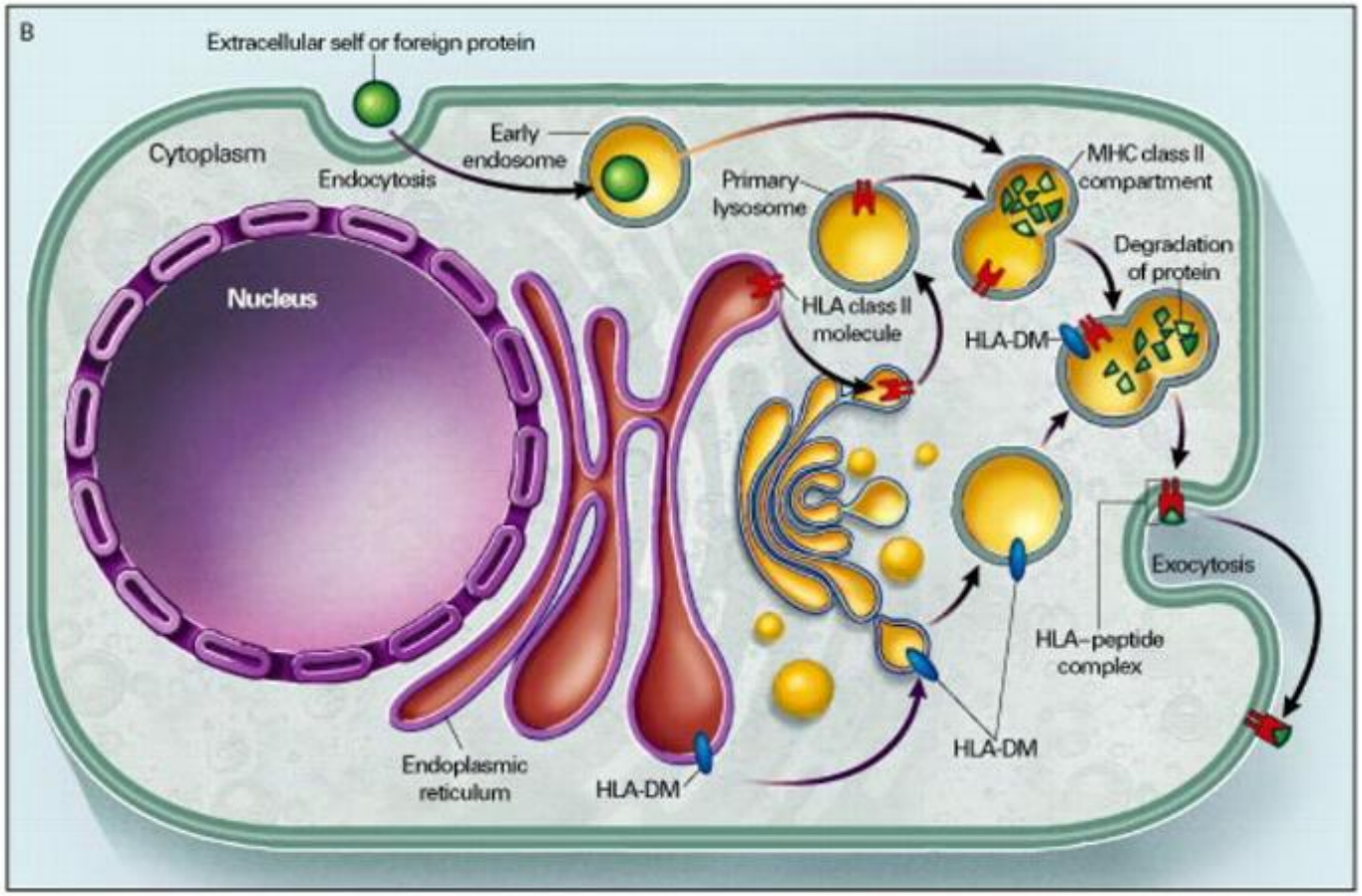


HLA class II molecules

Class II HLA molecules= dimers (both homodimers and heterodimers) consisting of two HLA-DR, HLA-DQ, HLA-DP molecules

→ expressed on antigen-presenting cells (APCs, i.e. macrophages, dendritic cells, B-cells)

→ presentation of foreign antigens (processed by endocytosis and proteolysis in lysozomes of macrophages)



HLA polymorphism

HLA genes belong to most polymorphic genes → great diversity of MHC molecules in the population.

HLA-B gene → over 1200 alleles

HLA-A → over 800 alleles

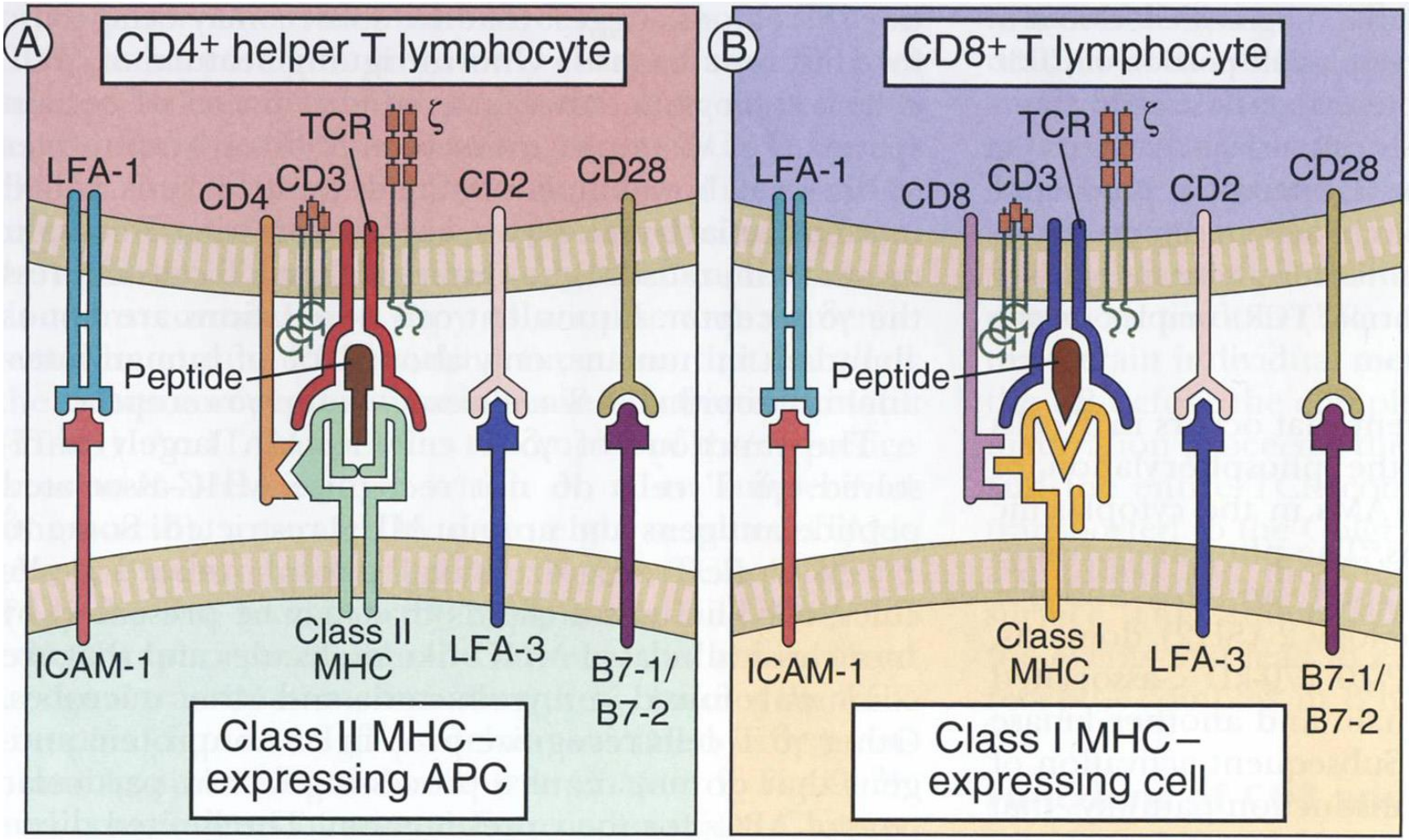
HLA-C → over 500 alleles

HLA-DRB1 → over 600 alleles

The great diversity of MHC molecules ensures that at least some individuals in the population will have at least some MHC molecules that will be able to display at least some antigens of any hypothetical pathogen that might invade human organism → at least some individual will always survive any imaginable lethal pandemy.

Immune synapsis

= complex of TCR and antigen displayed on MHC/HLA molecules + additional molecules (CD4, CD8, CD3, CD2)



HLA testing and organ transplantation

1. Serological typing of HLA antigens
2. Genetic analysis (sequencing) of HLA genes

<u>HLA system is inherited as a haplotyp</u> (Mendellian inheritance).

-one haplotyp from each parent → **25% chance of HLA identity between biologic siblings**

Registers of bone marrow donors.

Optimal bone marrow donor= HLA-identical AB0-identical young man.

Requirements: identical HLA-A/B/C and HLA-DR (= donor 8/8),

or even HLA-DQ (= donor 10/10).

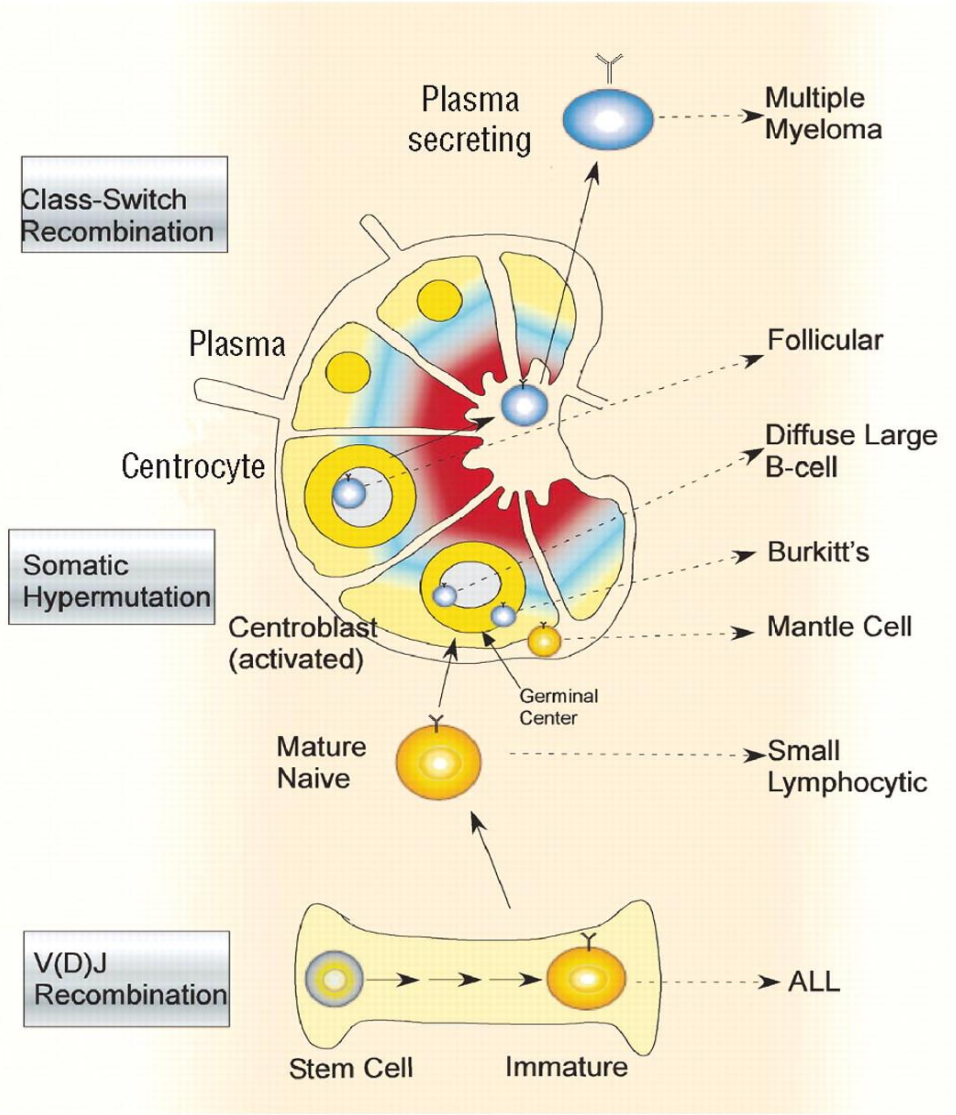
Lymphoproliferative neoplasms

Precursor lymphoid neoplasms= acute lymphoblastic leukemias (ALL) / lymphoblastic lymphomas (LBL)

ALL / LBL

Acute lymphoblastic leukemias / lymphoblastic lymphomas are highly aggressive clonal disorders of hematopoiesis (= **disorders of hematopoietic stem and progenitor cells**) characterized by hyper-prolieration of morphologically immature blasts of lymphoid origin.

Pathogenesis of ALL / LBL: critical role of mutations carried out by enzymes involved in DNA recombination (RAG1/2) → chromosomal translocations (BCR-ABL, TEL-AML1, MLL)



Skibola, C. F. et al. Haematologica 2007;92:960-969

haematologica the hematology journal

WHO classification of precursor lymphoid neoplasms

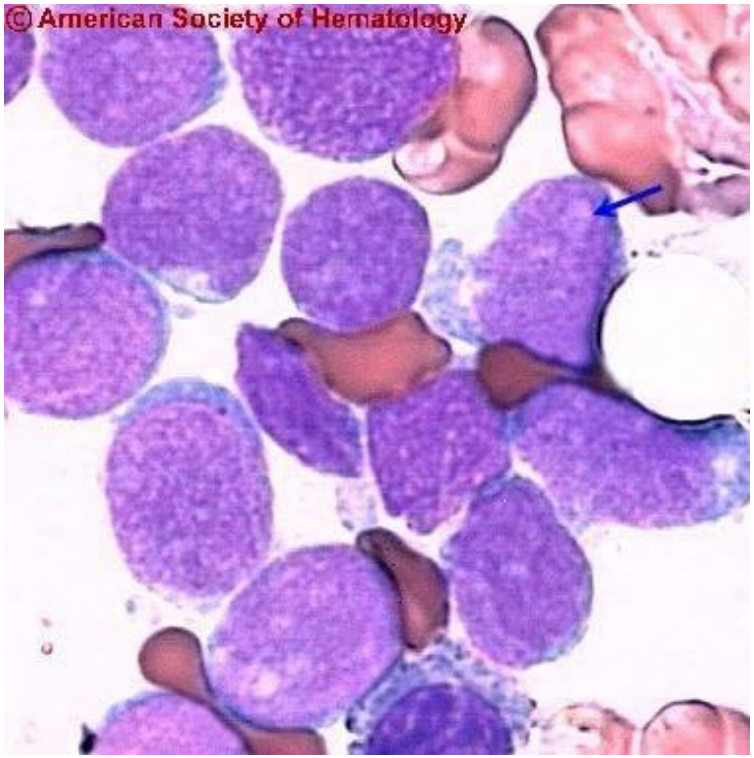
Precursor lymphoid malignancies are **clonal stem cell disorders** characterized by increased production and impaired differentiation of lymphoid lineage cells

→ presence of leukemic blasts in the bone marrow (>25%) = **ALL**

→ presence of leukemic blasts in extramedullar organs and/or tissues = **LBL**

<u>ALL / LBL cathegories:</u>

1. **B-lymphoblastic leukemia / lymphoma**
2. **T-lymphoblastic leukemia / lymphoma**



Epidemiology of precursor lymphoid malignancies

ALL is a **disease of the childhood** (=25% of all childhood malignancies, 75% ALL are diagnosed in children under 6 years of age).

ALL represents approx. **20% of acute leukemias of the adult** (80%= AML).

Incidence of ALL in the adult population (>50 years of age)= approx. 1 / 100.000.

B-ALL= 80%, T-ALL= 20%.

LBL is **the most common type of childhood lymphoma**.

T-LBL= 90%, B-LBL= 10%

Diagnostic proceedures in ALL

Blood and bone marrow smears are morphologically examined using a May-Grünwald-Giemsa or a Wright-Giemsa stain (at least 200 leukocytes on blood smears and 500 nucleated cells on bone marrow smears must be counted).

>25% blasts (blood or bone marrow examination) = diagnosis of ALL

Lineage identification relies on immunophenotyping (CD19, CD10, CD3, TdT), morphology, and cytochemistry.

Cytogenetic prognostic markers in ALL

• **t(9;22); BCR-ABL1 (p190 compared to p210 seen in CML)**
=Ph+ B-ALL
-accounts for approx. **25% of ALL in adults**, but only 2-4% of ALL in children.
-associated with adverse prognosis

• **t(12;21)**; ETV6-RUNX1 (alias TEL-AML1)
-**most common B-ALL in children** (25%)
-associated with favorable prognosis → more than 90% cures.

•**MLL rearrangements** (mixed leukemia / lymphoma, 11q23 locus), e.g. translocation t(4;11); MLL-AF4
=most common **infant ALL** (<1 year of age)
-these translocations may develop in utero; in twins it can be transferred in utero from one twin to another.
-associated with adverse prognosis

• **Hypodiploidy** (<46 chromosomes)
-associated with adverse prognosis (the fewer chromosomes, the worse prognosis)

• **Hyperdiploidy** (>55, but <66 chromosomes)
-associated with favorable prognosis → more than 90% cures.

Clinical picture of ALL

1. Expansion of leukemic clone in the peripheral blood → leukemia → leukostasis and hyperviscosity → dyspnoe, blurred vision, dizziness

2. Expansion of leukemic clone in the bone marrow → suppression of normal hematopoisis → anemia, thrombocytopenia, neutropenia → fatique, bruising, bleeding, infections

Aleukemic ALLs are not rare (are much more frequently seen in ALL compared to AML).

The reason for aleukemic ALL is the fact that the bone marrow is packed with ALL blasts, but there is no or very limited release of blasts into peripheral blood → pancytopenia without leukemia.

Involvement of extramedullary sites (i.e. site outside the bone marrow, including splenomegaly, hepatomegaly, lymphadenomegaly, infiltration of CNS, organ involvement) is also more common in ALL than in AML.

General principal of therapy of hematologic malignancies

In some types of hematologic malignancies therapy is risc-adapted, which means that the intesity of therapy depends on prognostic factors at diagnosis. In other types of hematologic malignancies therapy is the same regardless of prognostic factors.

INDUCTION

The major goal is to induce complete remission of the disease, i.e. disappearance of all signs of malignancy (response measured by CT, PET-CT and/or bone marrow examination)

Usually combined chemotherapy + immunotherapy (if available)

CONSOLIDATION

The major goal is to eradicate residual disease, which might underlie the relapse of the malignancy

E.g. Myeloablative chemo/radio-therapy followed by allogeneous stem cell transplantation in case of acute leukemias, or high-dose therapy followed by autologous stem cell transplant in lymphomas or myeloma

MAINTENANCE

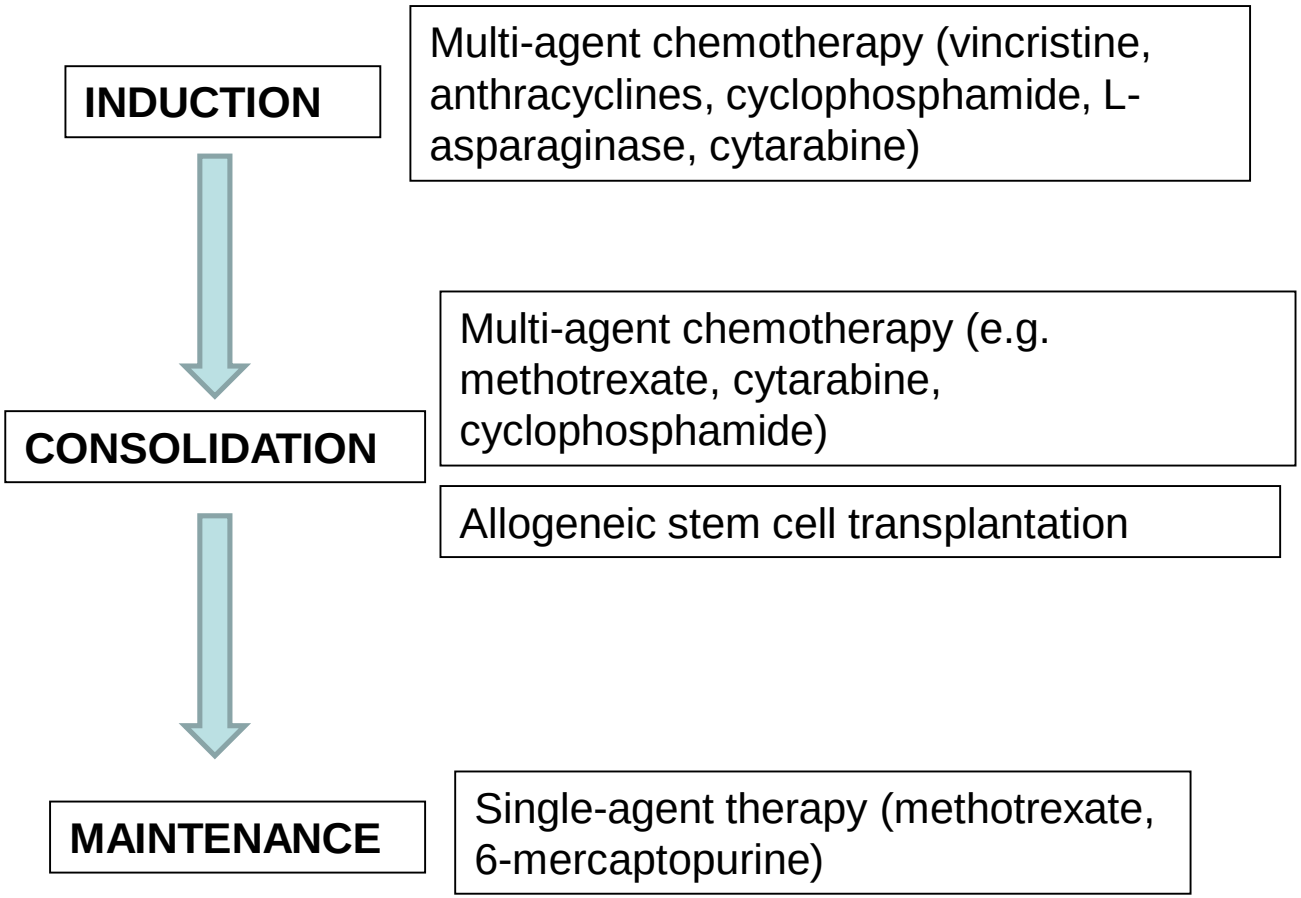
Major goal is to prevent the relaps of the malignancy

E.g. Monoclonal antibody anti-CD20 rituximab in some B-cell lymphomas, or 6-MP in ALL.

Therapy of ALL= risk-adapted treatment approach

Prognosis of acute leukemias depends on a wide array of factors including cytogenetics, age, performance status, comorbidities or extent of leukocytosis.

Adaptation of successful pediatric ALL treatment strategies into the therapeutic algorithms of adult ALL has resulted in complete response (CR) rates similar to those achieved in children.



Mature (peripheral) lymphoproliferative disorders

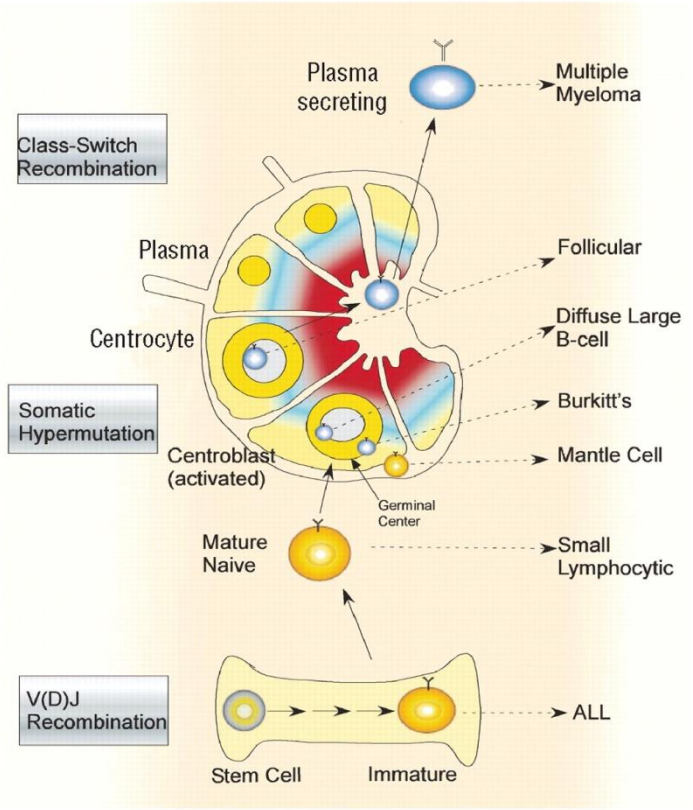
Mature lymphoproliferative disorders

Mature lymphoproliferative disorders are indolent, aggressive or highly-aggressive disorders of peripheral (mature) lymphocytes characterized by hyper-proliferation of morphologically mature or immature cells of lymphoid origin. In some types of disorders complete de-differentiation occured (e.g. Hodgkin lymphoma).

Incorrect application of the complex processes of the **somatic hypermutation** and **Ig heavy-chain isotype switch** play key roles in the pathogenesis of mature lymphoproliferative disorders.

→ Mutations → changed function of genes (MYC, BCL6, FAS).

→ chromosomal translocations → juxtapositioning of „unmutated“ genes to enhancers of transcription of Ig genes or T-cell receptor genes → **increased expression** of juxtapositioned genes (BCL2, MYC, cyklin D1, BCL6)

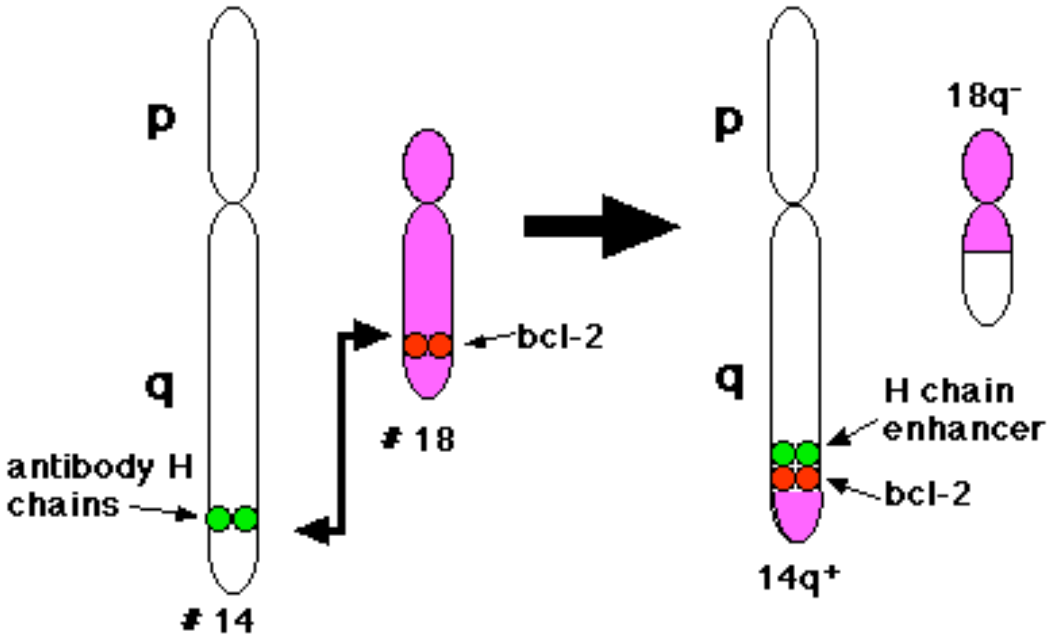


→Deregulation of apoptosis (BCL2, FAS), cell cycle progression (MYC, cyklin D1) and terminal differentiation of lymphocytes (BCL6)

BCL2 inhibits apoptosis

BCL2 is one of the key antiapoptotic proteins from the family of BCL2 proteins.

BCL2 translocation and resulting overexpression is the hallmark of follicular lymphoma.

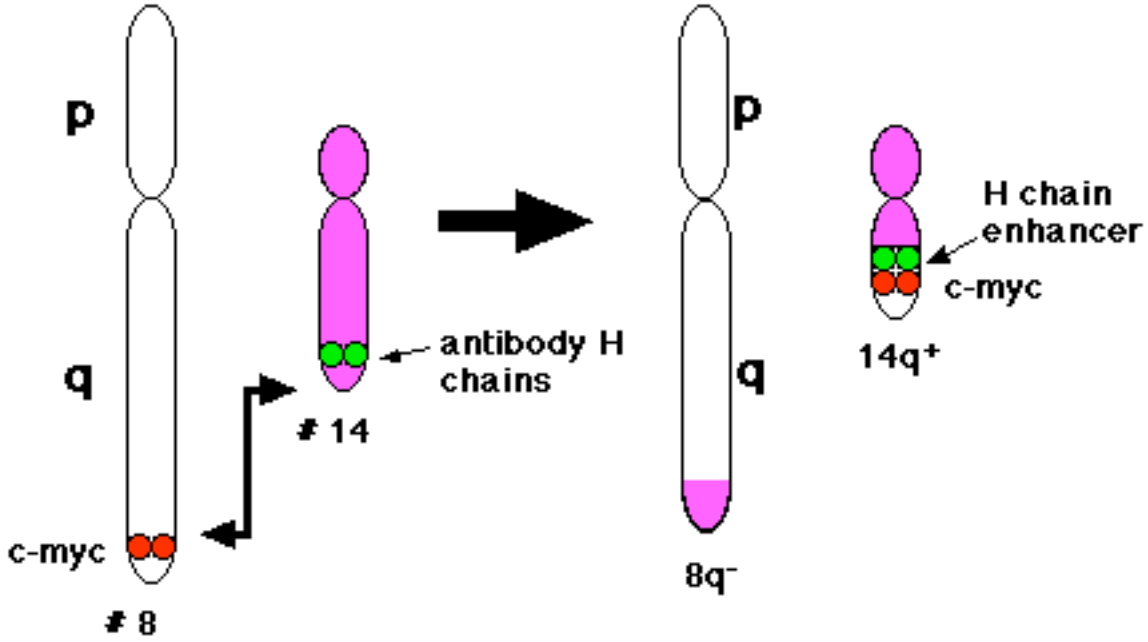


MYC promotes cell cycle progression

MYC is a transcription factor involved in many cellular processes including growth, proliferation and apoptosis.

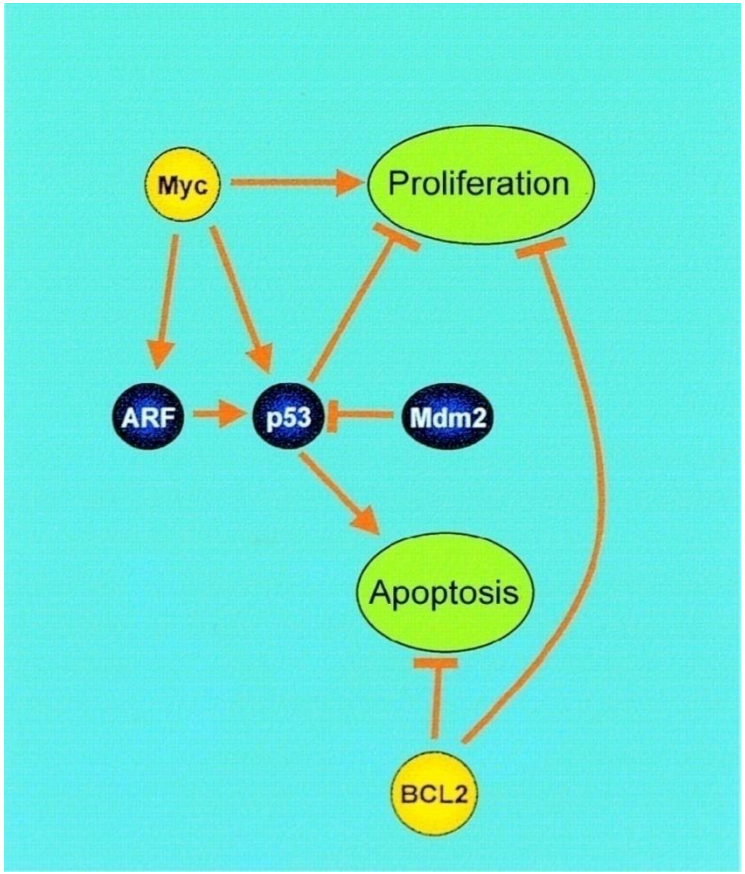
However, MYC overexpression activates the p53 apoptotic pathway through tumor suppressor ARF.

MYC translocation and resulting overexpression is a hallmark of Burkitt lymphoma.



Double-hit lymphomas (with BCL2 and MYC upregulation) are associated with adverse prognosis

The combination of concurrent deregulation of BCL2 and MYC results in a highly malignant phenotype that is usually resistant to combination chemotherapy.



Willis, T. G. et al. Blood 2000;96:808-822

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WHO classification of mature lymphoproliferative disorders

1. Mature B-cell neoplasms
2. Mature T-cell neoplasms
3. Hodgkin lymphoma
4. Immunodeficiency-associated lymphoproliferative disorders

Selected mature B-cell neoplasms

1. Hodgkin lymphoma
2. Non-hodgkin lymphomas

-Diffuse large B-cell lymphoma (DLBCL)

-Follicular lymphoma (FL)

-Mantle cell lymphoma (MCL)

-Burkitt lymphoma/leukaemia (BL)

3. Chronic lymphocytic leukaemia / small lymphocytic lymphoma (CLL/SLL)

4. Multiple myeloma / plasmocytoma

5. AL amyloidosis

Selected mature T-cell neoplasms

1. „Nodal“ lymphomas

-Peripheral T-cell lymphoma

-Anaplastic large cell lymphoma (ALCL)

2. „Leukemic“ lymphomas

-Adult T-cell leukaemia/lymphoma (HTLV-1, HTLV-2)

3. „Skin“ lymphomas

-Mycosis fungoides (MF)

-Sezary syndrome (SS)

Selected immunodeficiency-associated LPD

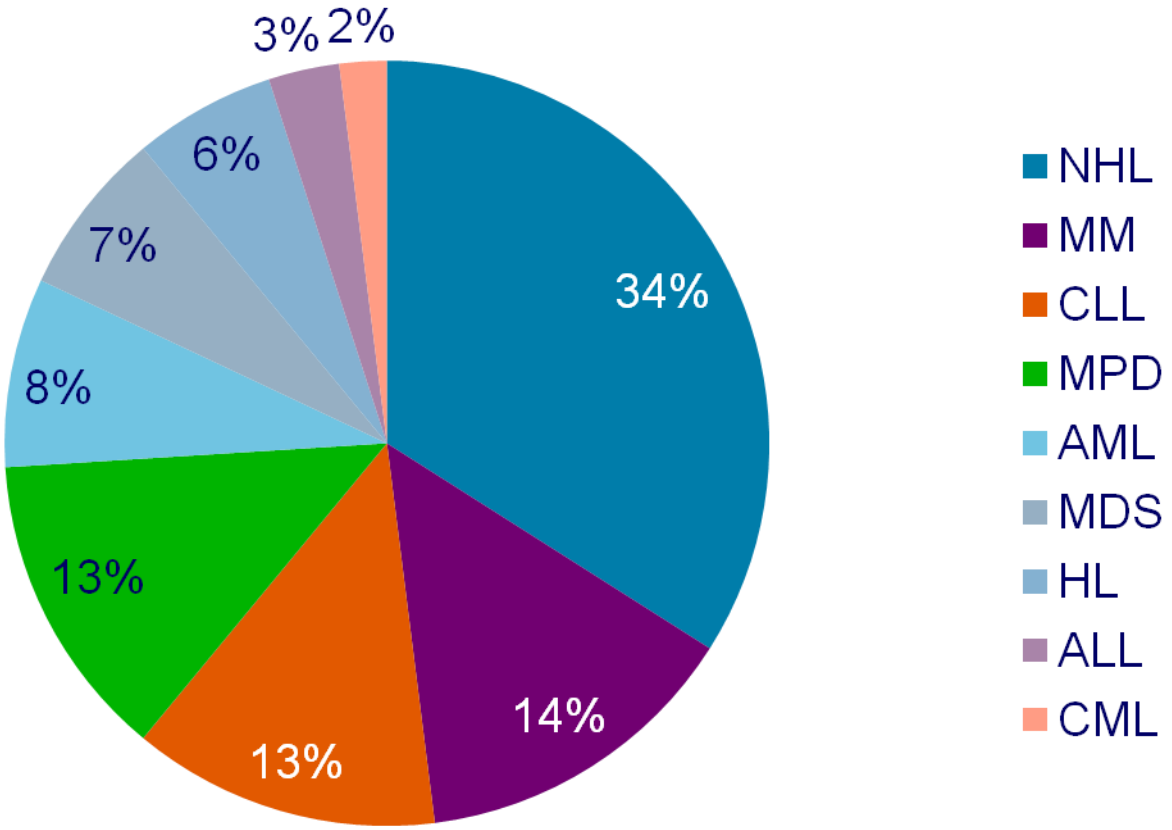
-LPD associated with primary immune diseases

-post-transplant LPD (usually after solid-organ transplantations)

-lymphomas associated with HIV

Mature lymphoproliferations account for 2/3 of all hematologic malignancies

Incidence of hematologic malignancies in Czech Republic is approx. 40/100.000/year, i.e. approx. 4.000 new patiens in Czech Republic per year.



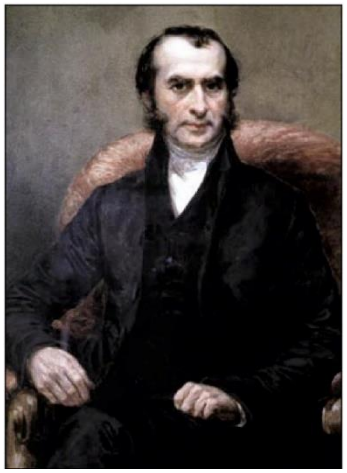
ALL = acute lymphocytic leukaemia; AML = acute myeloid leukaemia; CLL = chronic lymphocytic leukaemia; CML = chronic myeloid leukaemia; HL = Hodgkin lymphoma; MDS = myelodysplastic syndromes; MPD = myeloproliferative disorders; NHL = non-Hodgkin lymphoma.

Data from Leukaemia and Lymphoma Research. Facts about blood cancers; 2010. Available from: http://www.beatbloodcancers.org/facts-about-blood-cancers.

Pathogenesis, diagnosis and therapy of selected mature B-cell malignancies

Hodgkin lymphoma (HL)

Hodgkin lymphoma (HL)

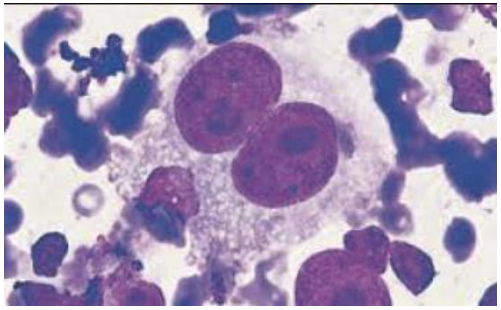


sir Thomas Hodgkin

Hodgkinův lymfom was described in 1832 by sir Thomas Hodgkin, the denomination „Hodgkin disease“ was coined in 1865 by dr. Samuel Wilks.

Incidence of HL= approx. 3/100.000, i.e. 300 new patients per year in the Czech Republic.

Hodgkin lymphoma (HL)



Patological (malignant) cells in HL are so called **Hodgkin and Reed-Sternberg cells (HRS cells)**, which arise as a result of malignant transformation of germinal-center B-cells.

HRS cells have lost most mature B-cell markers (CD20, CD19).

Typical expression of **CD30** antigenu (→ targeted therapy).

Dorothy Reed= American physician
Carl (von) Sternberg= Austrian pathologist

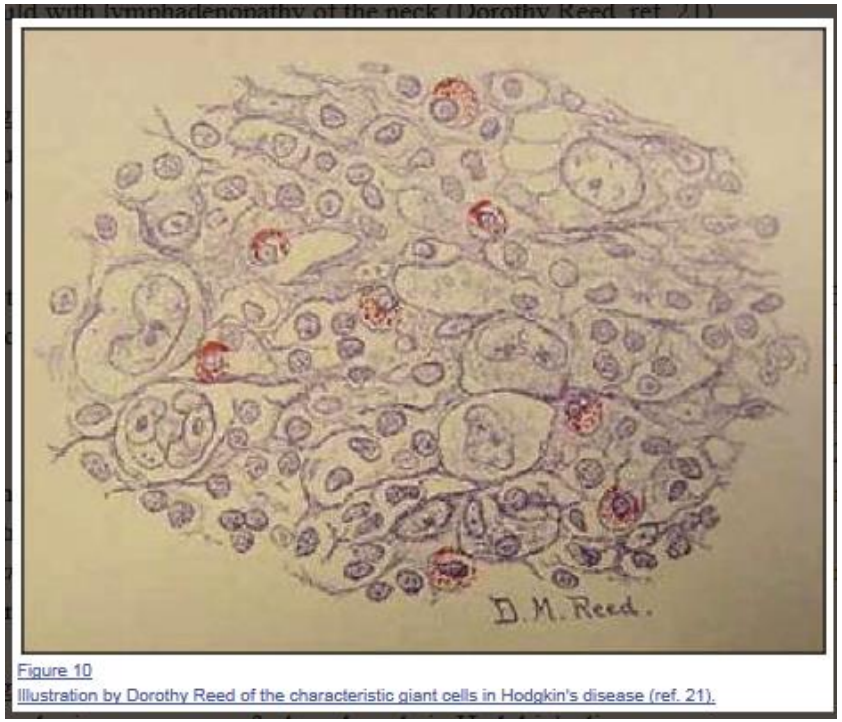
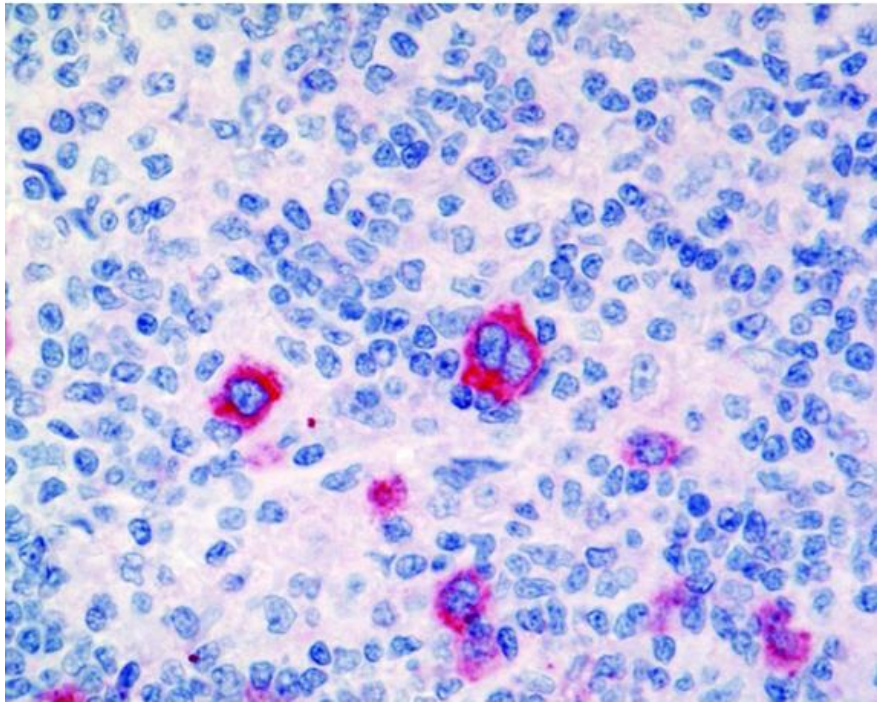


Figure 10
Illustration by Dorothy Reed of the characteristic giant cells in Hodgkin's disease (ref. 21).

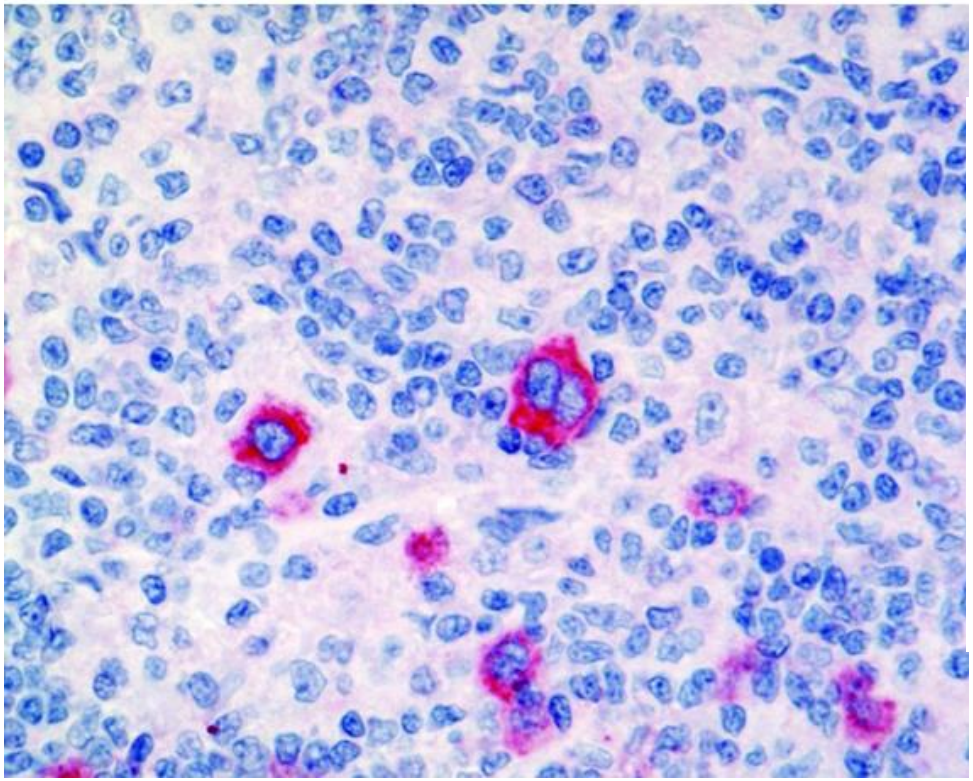


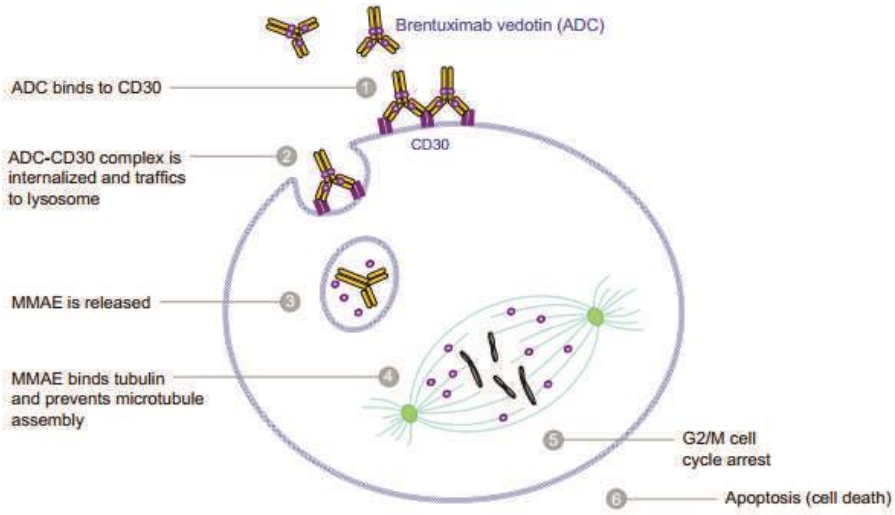
Dorothy Reed

Dorothy Reed Mendenhall with colleagues at Brooklyn Naval Hospital, 1898

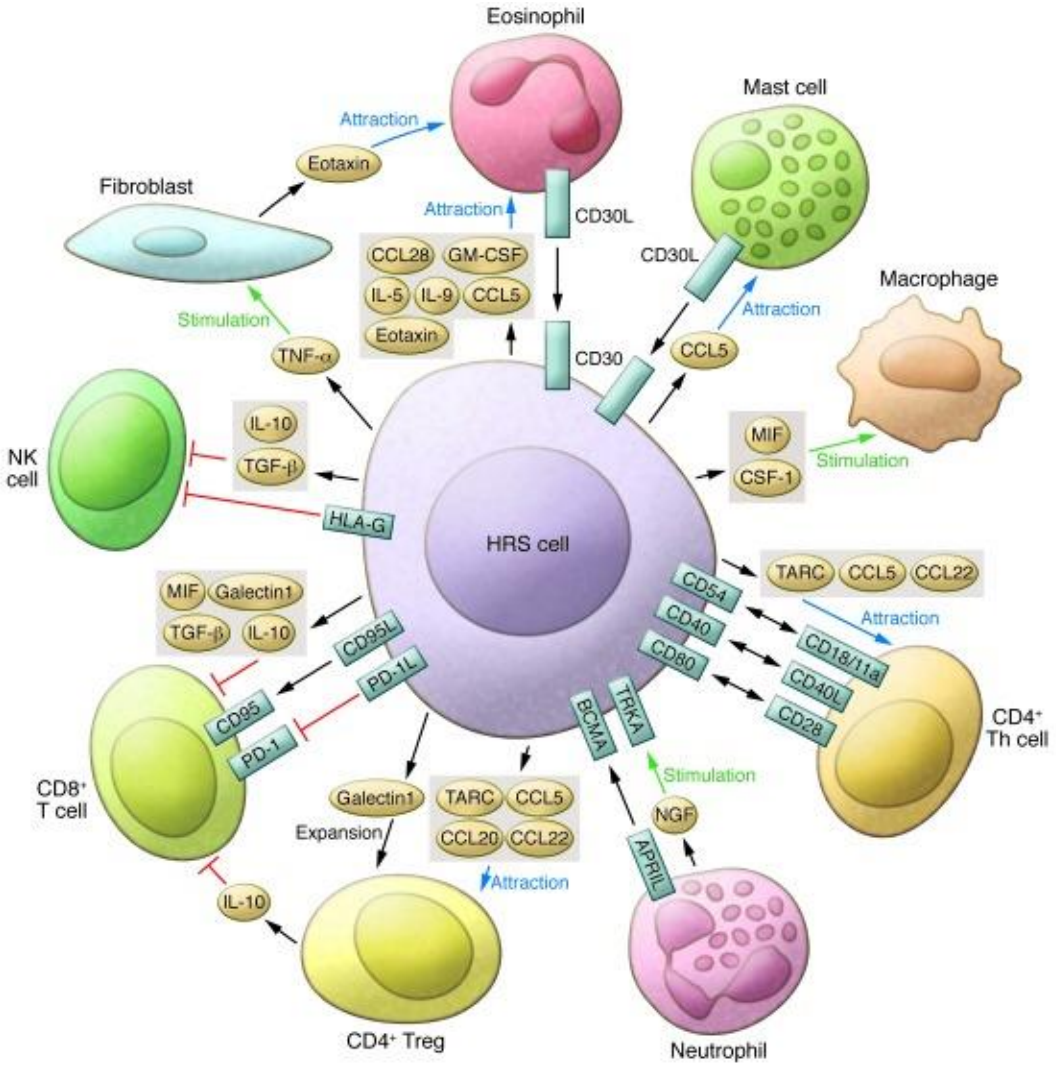
Parenchyma and stroma of Hodgkin lymphoma

HRS cells account only for 0.1-2% of lymphoma mass (!!!!!), while 98-99.9% of the lymphoma mass is formed by „non-malignant“ stroma comprising infiltrating cells of immune system (T-cells), fibroblasts and extracellular matrix.





HRS cells and „non-malignant“ cells of the lymphoma microenvironment



Küppers R, Engert A, Hansmann ML. Hodgkin lymphoma. J Clin Invest. 2012 Oct 1;122(10):3439-47.

Clinical picture of HL

Typical cervical and/or mediastinal lymphadenopathy

Common B-symtoms.

In case of bulky mediastinal involvement → dyspnoe, upper vena cava syndrome.

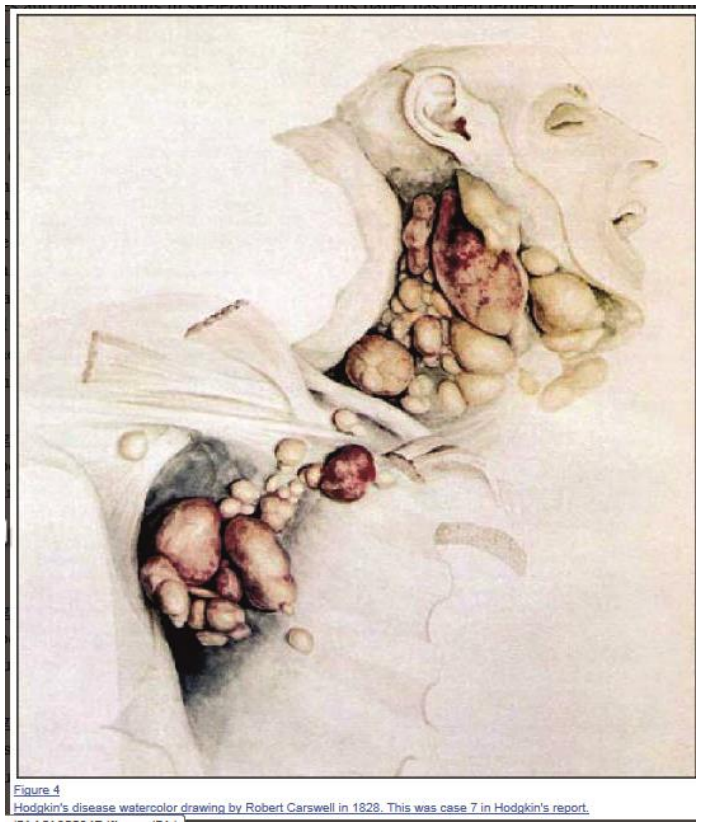


Figure 4
Hodgkin's disease watercolor drawing by Robert Carswell in 1828. This was case 7 in Hodgkin's report.

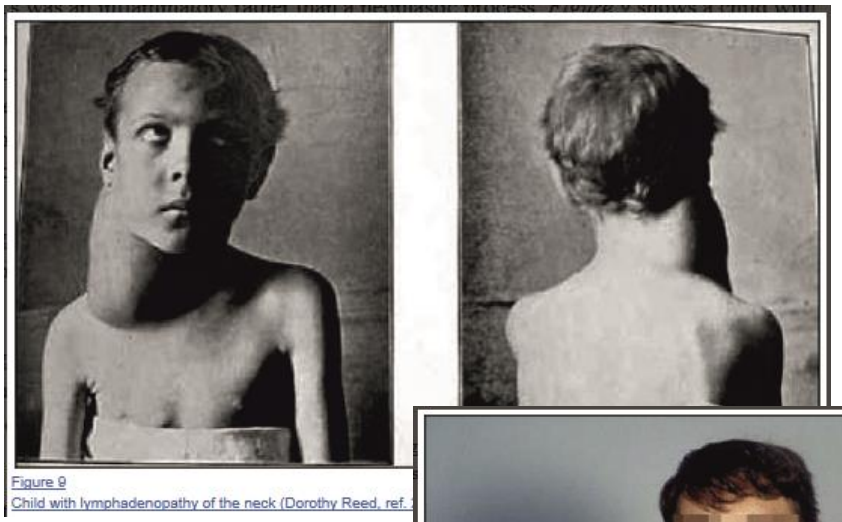


Figure 9
Child with lymphadenopathy of the neck (Dorothy Reed, ref.

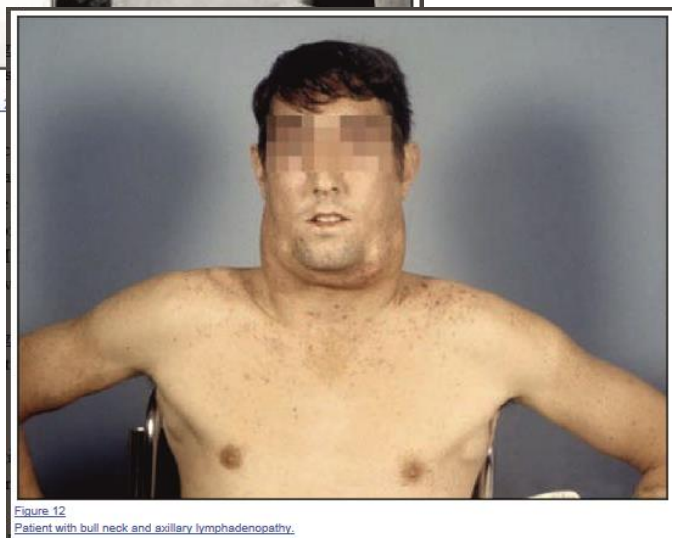


Figure 12
Patient with bull neck and axillary lymphadenopathy.

Overall survival of HL patients

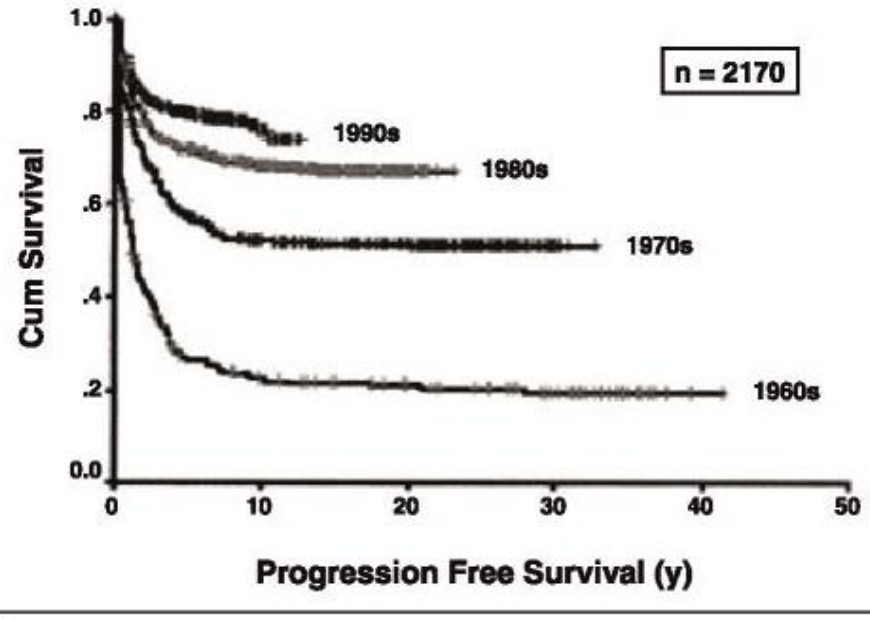


Figure 13
Improved survival of patients with Hodgkin's disease in British Columbia since the 1960s. Cum indicates cumulative.

Non-Hodgkin lymphomas (NHL)

Non-Hodgkin lymphomas (NHL)

Non-Hodgkin's lymphomas (NHL) comprise heterogeneous malignancies of B- and T-cell origin that involves their uncontrolled clonal expansion in the periphery.

B-cell lymphomas make up the majority of cases (approx. 90%) and, of these, diffuse large B-cell lymphoma (DLCL) and follicular lymphoma (FL) are the two major subtypes.

B-cell lymphomas arise during different septs of B-lymphocyte development and represent their malignant counterparts.

V(D)J recombination, somatic hypermutation and class-switch recombination represent critical molecular processes that predispose to the development of lymphomas.

Pathogenesis of B-NHL

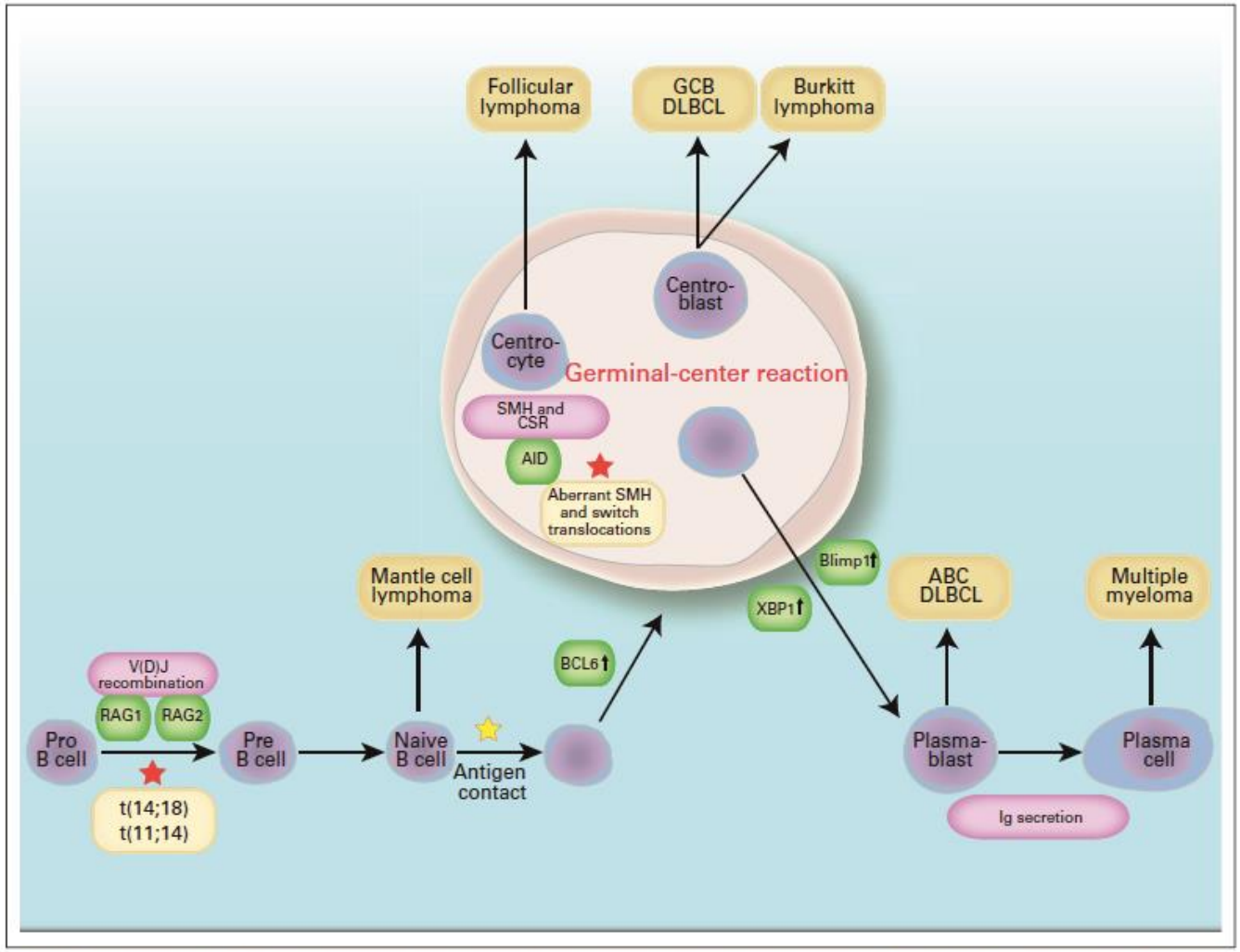
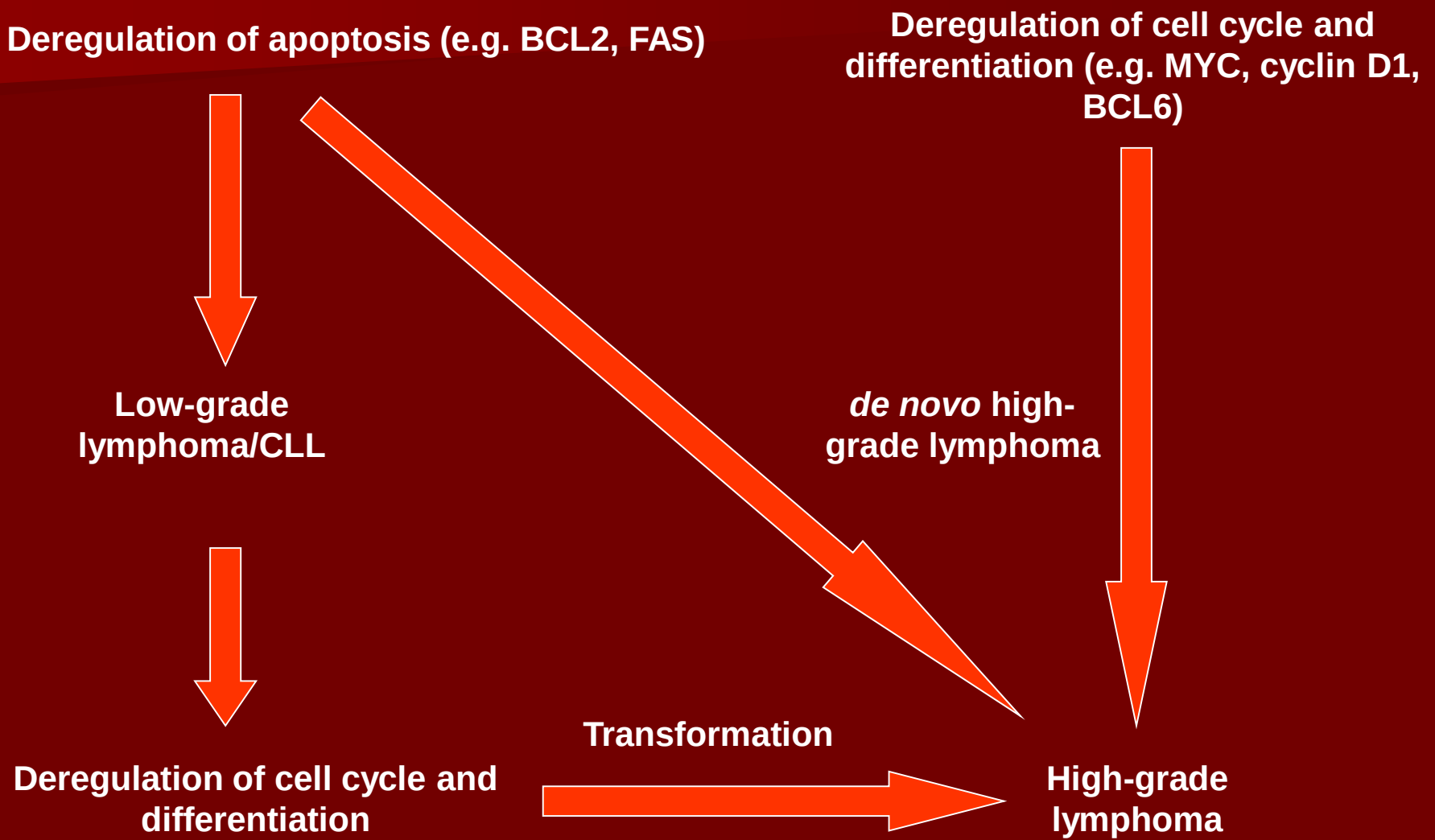


Fig 1. Lymphomas arise at different stages of B-cell differentiation. Specific recombination events are prone to the development of chromosomal aberrations. Recombination activating gene 1 (*RAG1*)-dependent and *RAG2*-dependent V(D)J recombination takes place in the bone marrow. The potentially resulting t(14;18) and t(11;14) represent critical first steps in lymphomagenesis of different lymphoma subtypes. After antigen contact, the stimulated B cells migrate to the lymph node and form the germinal center after upregulation of BCL6. The events during the germinal center reaction include activation-induced cytidine deaminase (AID) –mediated somatic hypermutation and class-switch recombination, which are critical events for lymphoma evolution. The germinal center reaction is terminated by the differentiation of B cells into plasma cells. XBP1 and Blimp-1 are key regulators for plasmacytic differentiation. GCB DLBCL, germinal center B-cell–like diffuse large B-cell lymphoma; SMH, somatic hypermutation; CSR, class-switch recombination; ABC DLBCL, activated B-cell–like diffuse large B-cell lymphoma; Ig, immunoglobulin.

Hendrik Nogai et al. Pathogenesis of Non-Hodgkin's Lymphoma. JCO.2011

Pathogenesis of non-Hodgkin lymphomas= deregulation of apoptosis and cell cycle control



Diagnostic algoritms in patients with NHL

1. **Histologically proven lymphoma from the <u>biopsy of the involved lymph node</u> is basic condition**

2. **Staging procedures**

-Ann Arbor staging system based on CT scan

-medical history, physical examination, performance status

-blood count + biochemistry (LDH)

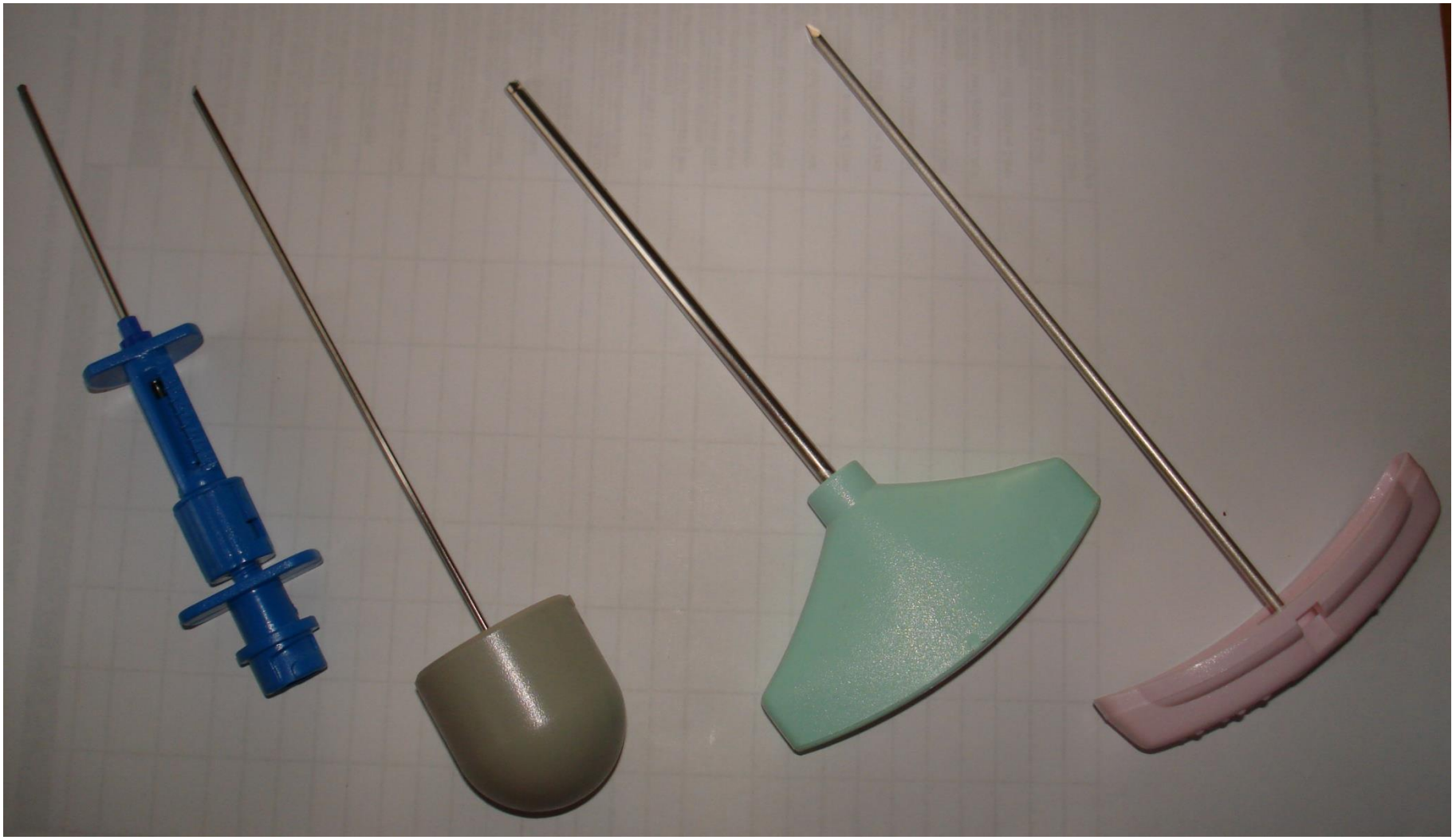
-evaluation of the bone marrow infiltration (trephine biopsy: uni- / bi-lateral)

-evalulation of CNS involvement (lumbar puncture) in selected cases only

3. **Prognosis**

-IPI (international prognostic index)= age + stage + LDH + performance status + number of extranodal sites

Sternal puncture vs Trephine biopsy

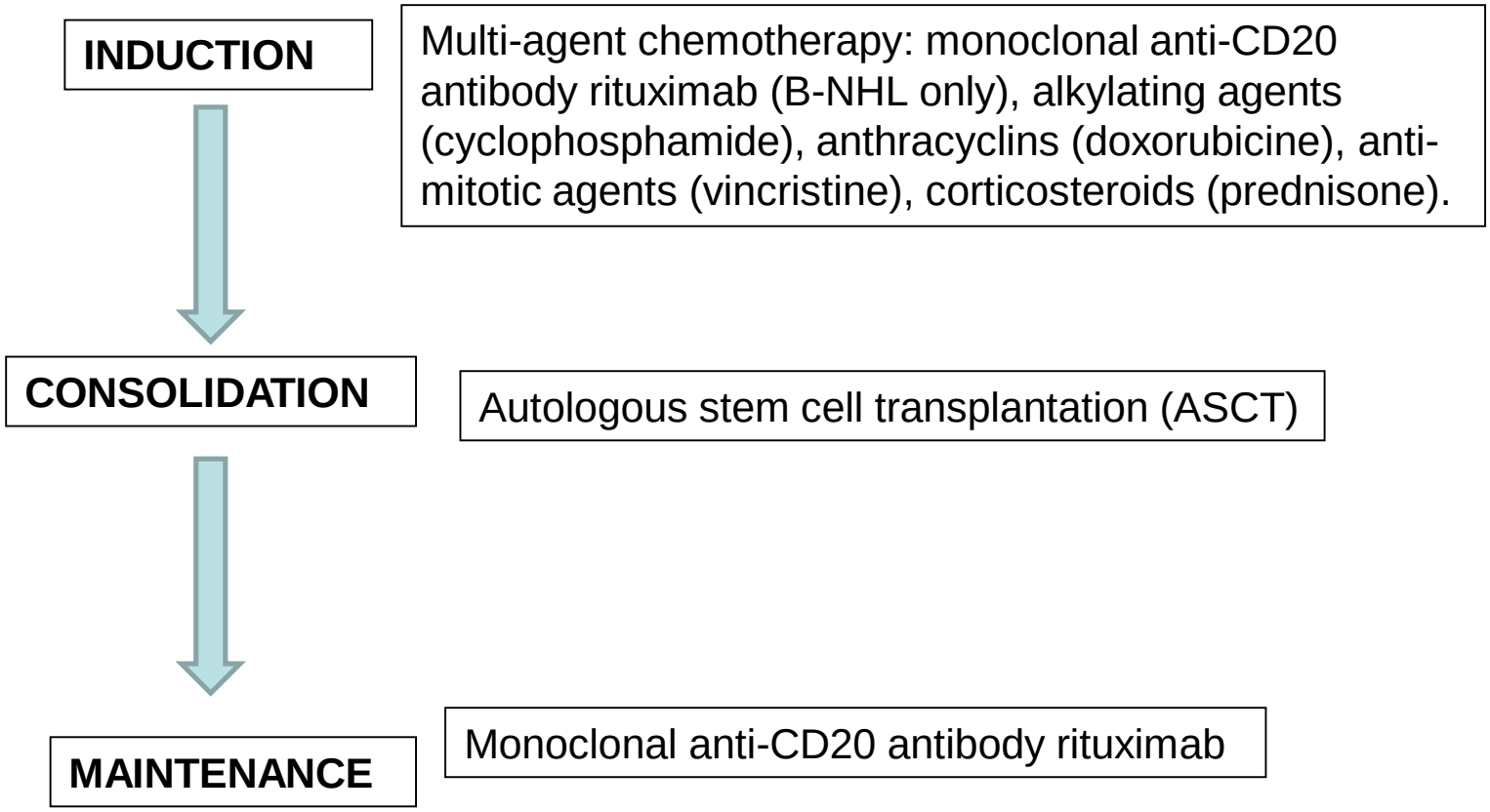


Therapy of NHL= risk-adapted treatment approach

Different NHL subtypes are treated differently (induction only, induction + ASCT, induction + ASCT + maintenance, induction + maintenance).

In general, indolent lymphomas (e.g. Follicular lymphoma) are not treated if asymptomatic.

Aggressive lymphomas are treated at any stage.



Autologous stem cell transplantation (ASCT)

High-dose therapy followed by autologous stem cell transplantation (ASCT)

-in selected NHL subtypes or under particular circumstances

-stem cell mobilization, collection and freezing is prerequisite

-high-dose therapy → stem cell rescue

-no graft-vs-lymphoma effect in case of ASCT= principal difference between auto-SCT and allo-SCT

-ASCT is associated with low morbidity and very low mortality (<2% mortablity in specialized centers)= another important difference compared to allogeneic stem cell transplantation.

Chronic lymphocytic leukemia (CLL)

Pathogenesis of CLL: BCR receptor

Patients with CLL use highly restricted sets of BCRs (e.g. VH1-69, VH4-34) → antigen-driven selection of lymphocytic clones.

Tonic stimulation by **specific auto- or allo-antigens** drives expansion of the malignant clone.

Unmutated IgH (<2% deviation from germline sequence) is associated with a more aggressive disease.

Mutated IgH is associated with more indolent disease.

Cell of origine (COO)= memory B-cell (gene expression profiling studies show both mutated and unmutated CLL are driven from the memory B-cell).

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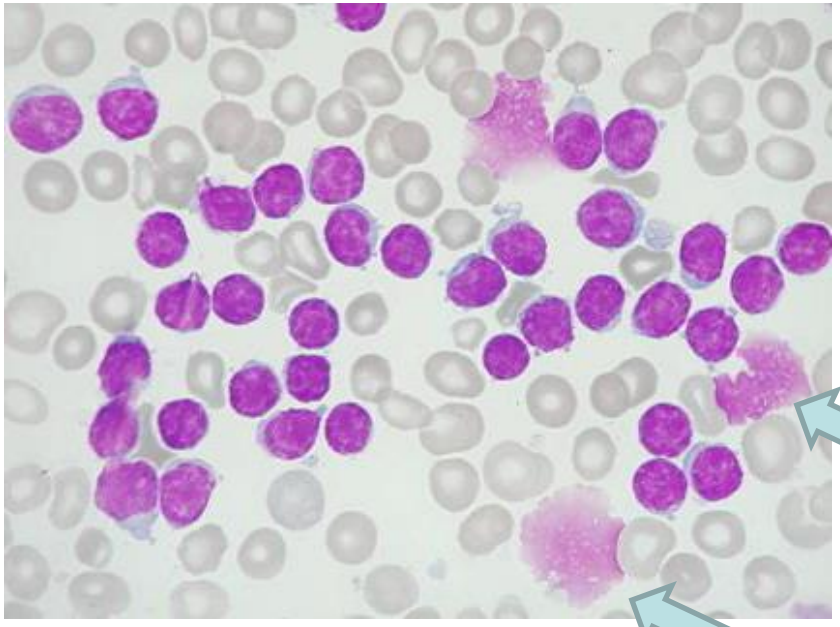
Diagnosis of CLL

70-80% patients are diagnosed incidentally from routine examination of blood cell count → **lymfocytóza**.

Typical morphology and typical imunofenotype (CD20+, CD19+, CD23+, CD5+, CD10-).



Gumprecht schadows

Course of CLL

Monoclonal lymphocytosis of uncertain significance (MLUS)

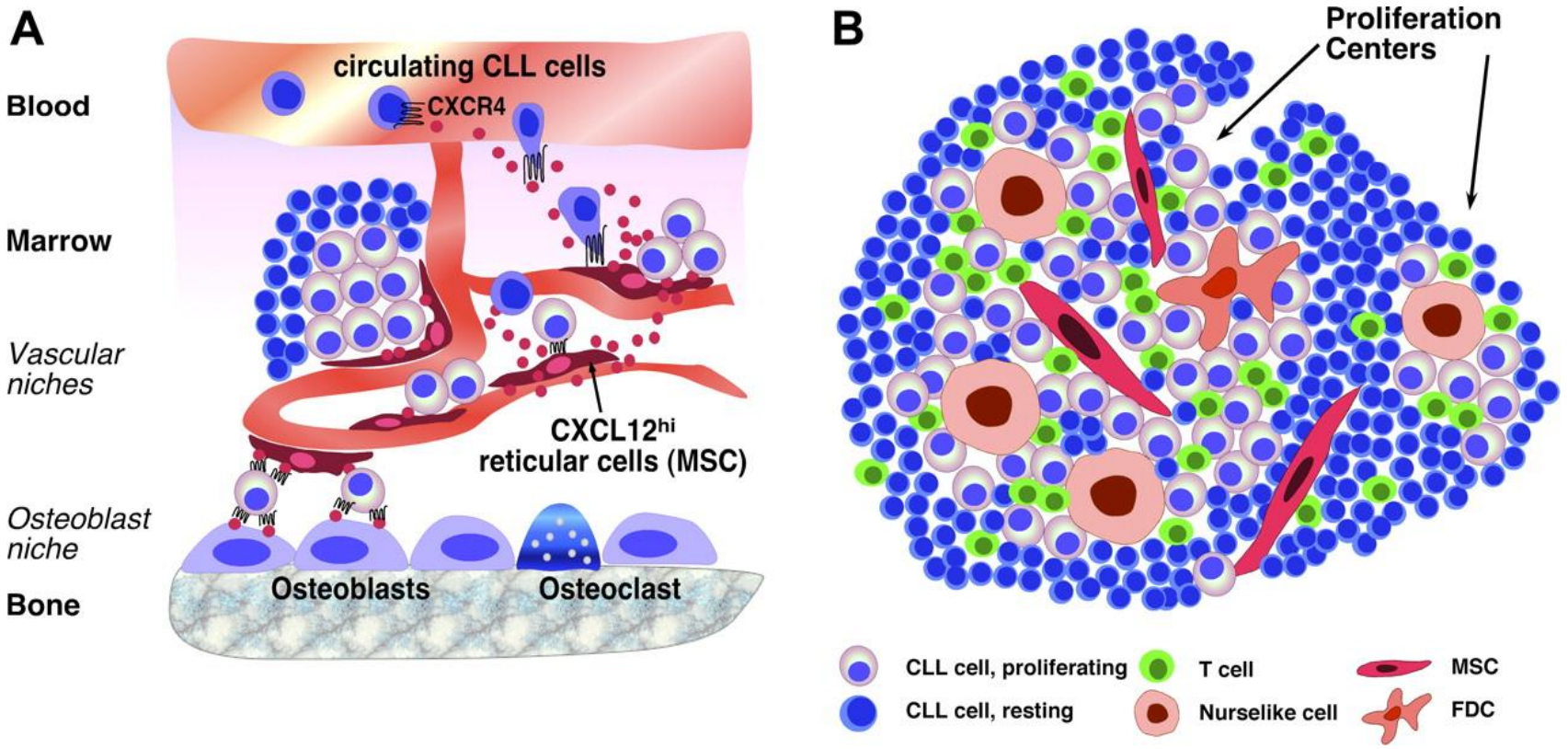
Asymptomatic CLL

Symptomatic CLL

High-grade lymphoma

Permissive niches in CLL

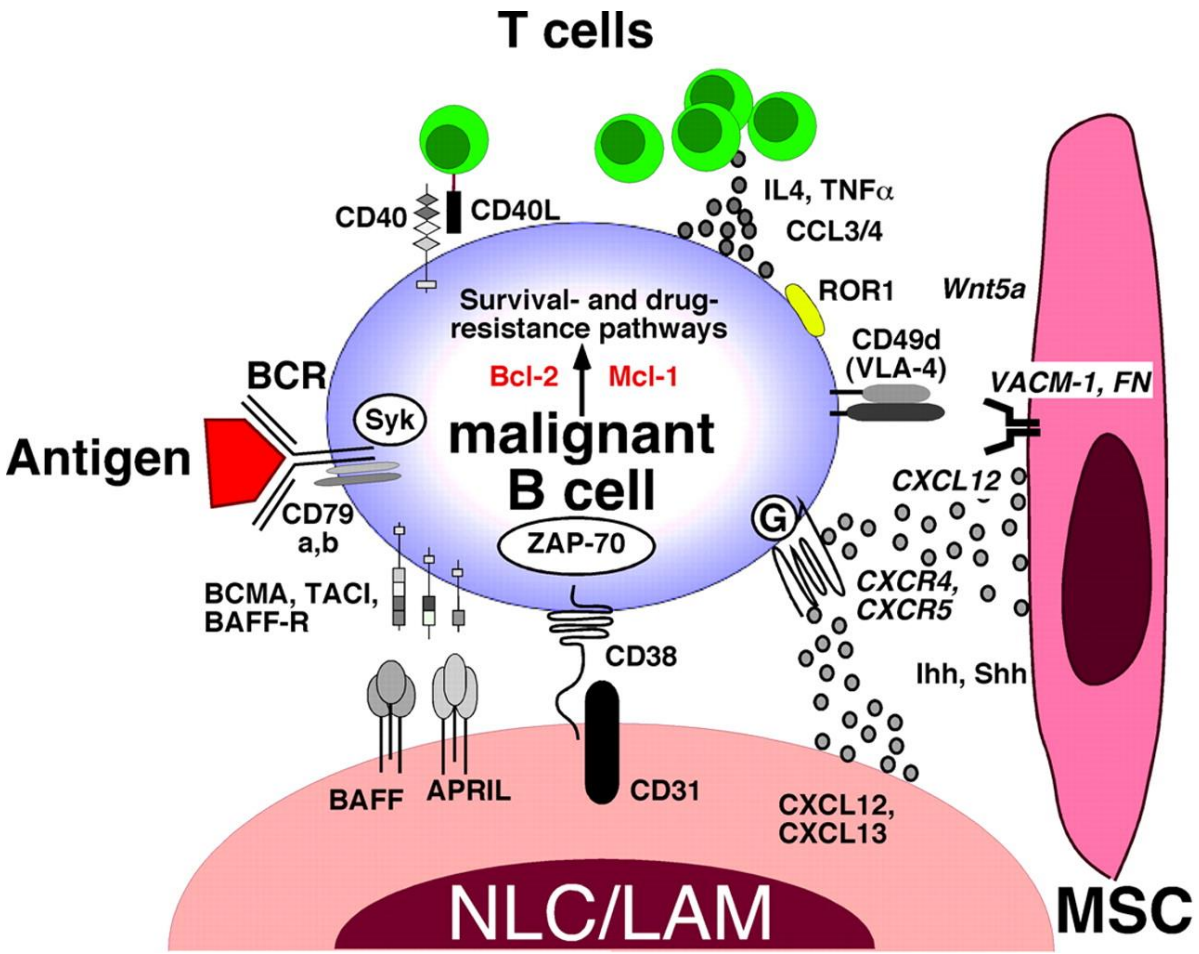
CLL survive in permissive niches in the bone marrow (A) and secondary lymphoid organs (lympho nodes) (B).



Burger J A et al. Blood 2009;114:3367-3375

CLL stem cell-like cells

Signals from the microenvironment stimulate survival, proliferation, mobilization and homing of CLL cells.



Burger J A et al. Blood 2009;114:3367-3375

Epidemiology and course of CLL

CLL is by far the most prevalent leukemia in the adult.

CLL has a strong genetic predisposition: the most important risk factor for the development of CLL is a family history of CLL. The relative risk of CLL among first-degree relatives = 7.0-8.5

Asymptomatic accumulation of CLL lymphocytes in the peripheral blood, lymph nodes and/or spleen → → → symptomatic disease.

Complications of CLL

1. secondary immunodeficiency

→ life-threatening infectious complications.

Many patients with CLL succomb to opportunistic infection, not to "CLL" as such.

2. autoimmune cytopenias

→autoimmune hemolytic anemia, immune thrombocytopenia.

3. transformation to high-grade lymphoma (most commonly to diffuse large B-cell lymphoma)

= Richter transformation

-as a consequence of secondary mutations that disrupt differentiation and cell cycle control of lymphocytes

Prognosis of CLL

Mutational status if IGHV:

1. Unmutated (<2% deviation from the germline sequence) → adverse prognosis
2. Mutated IGHV (>2% deviation from the germline sequence) → favorable prognosis

Immunophenotypic markers:

1. CD38 expression → adverse prognosis
2. ZAP70 expression → adverse prognosis

Cytogenetic prognostic markers:

-trisomy 12

-deletion of 13q (miR-15, miR-16) = negative regulators of BCL2

-deletion of 11q (ATM, ataxia teleangiectasia mutated)= adverse prognosis

-deletion of 17p (TP53 tumor suppressor)= adverse prognosis

Therapy of CLL

Therapy is iniated only in patients with a symptomatic disease !

Combination immunochemotherapy:

-anti-CD20 monoclonal antibody (rituximab)

-alkylating agent (cyclophosphamide)

-antinucleotides (fludarabine)

Multiple myeloma (MM)

Multiple myeloma (MM)

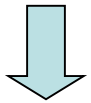
MM was first described by dr. Otto Kahler in Prager medizinische Woschenschrift in 1889 (Kahler´s disease).

Incidence of MM is approx. 6/100.000, i.e. 300-400 new patients per year in the Czech republic.

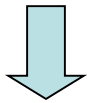
Dr. Otto Kahler

Course of MM

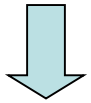
Monoclonal gamapathy of uncertain significance (MGUS)



Smouldering myeloma

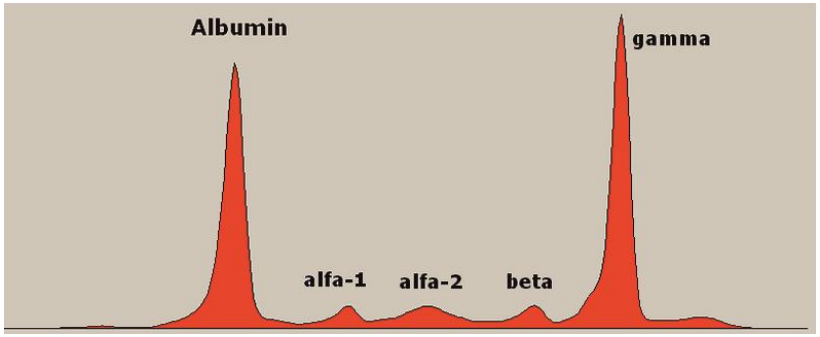


Multiple myeloma



Plasma cell leukemia

Diagnostic criteria



1. Detection of monoclonal protein (M-protein, M-komponenta, **paraprotein**) by immuno-electrophoresis of plasma proteins (iELFO).

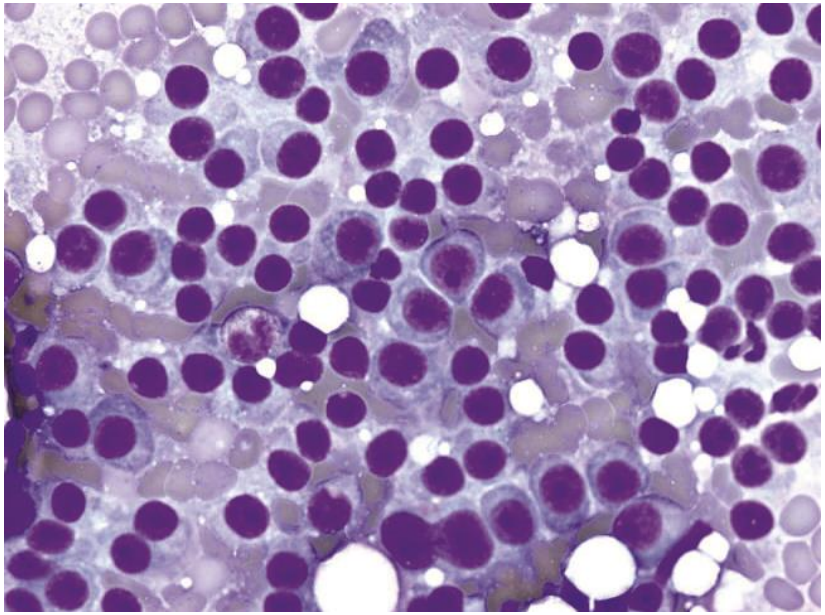
Monoclonal IgG, IgA or their parts (**light chains kappa or lambda**= **Bence-Jones protein**).

2. Detection of **monoclonal population of plasma cells**

-in the bone marrow by trephine biopsy (usually > 10% atypical plasmocytes)

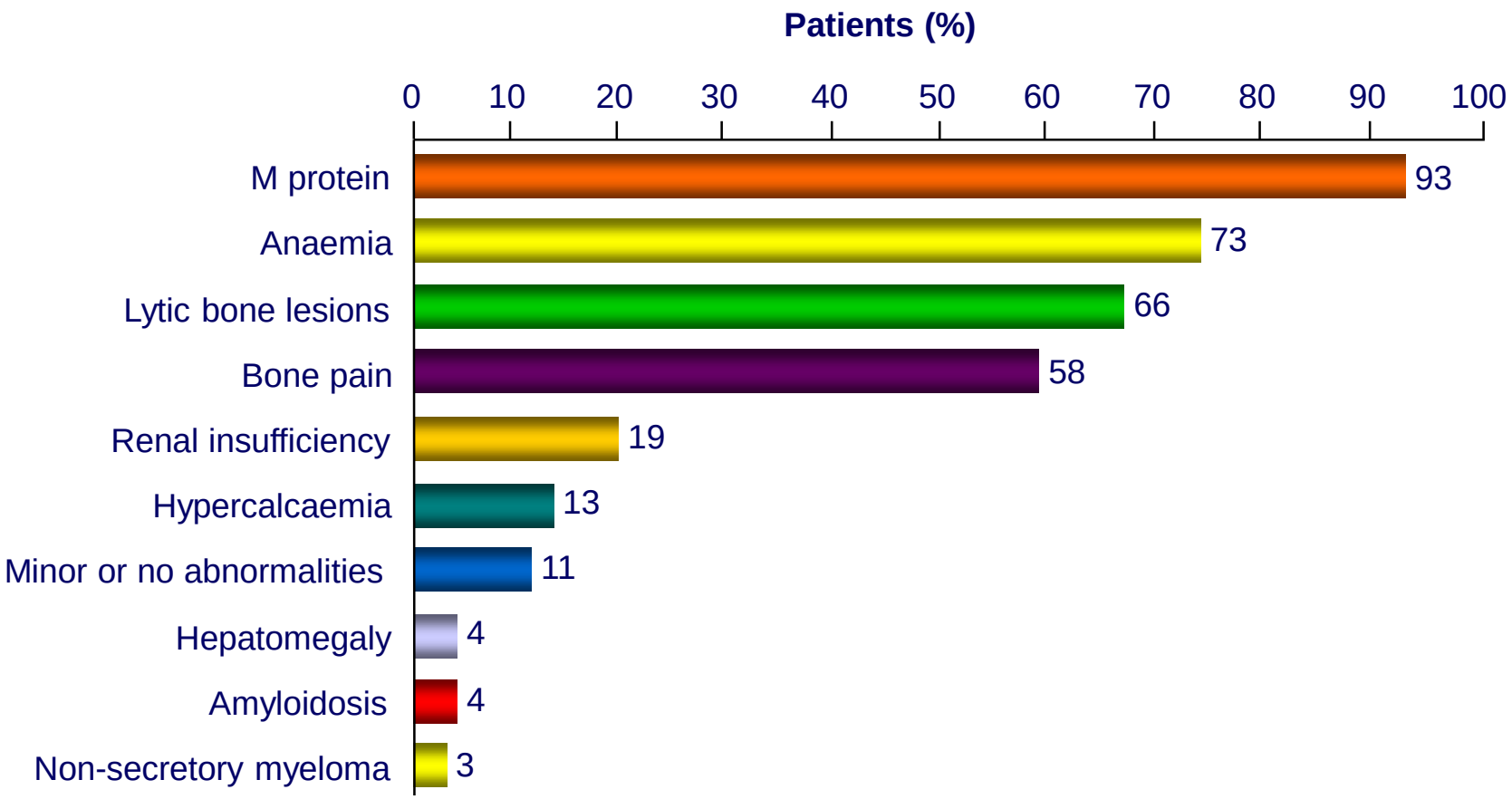
or

-histologically proven plasmocytoma.



3. **Symptoms associated with MM**

Symptoms



Kyle RA, et al. Mayo Clin Proc. 2003;78:21-33.

Symptomatic MM: CRAB

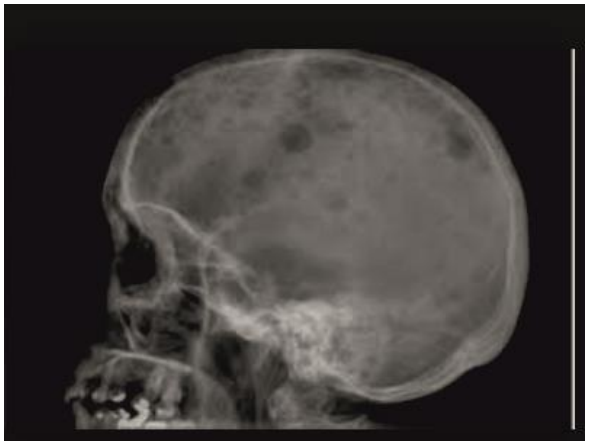
CRAB symptoms:

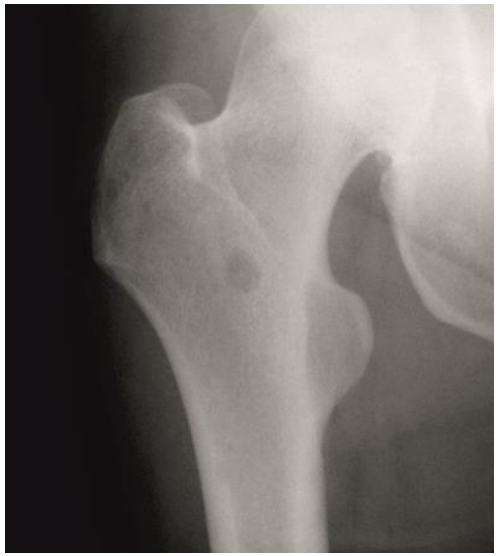
C= calcium, i.e. hypercalcemia

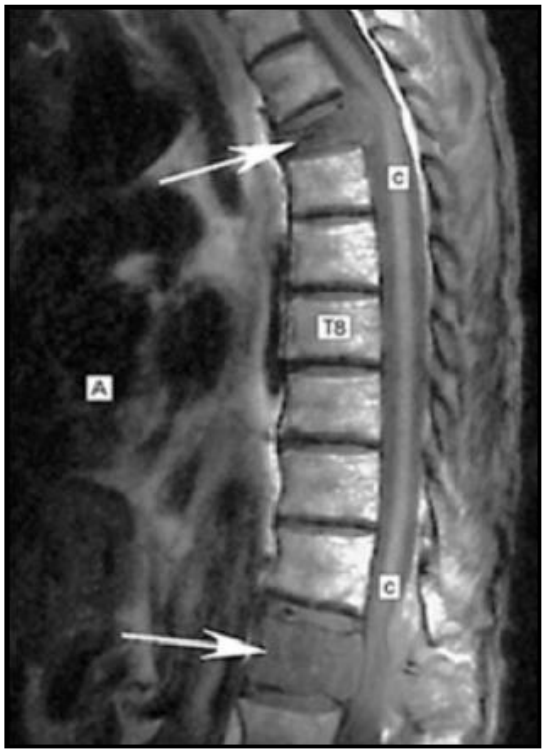
R= renal, i.e. acute renal failure

A= anemie

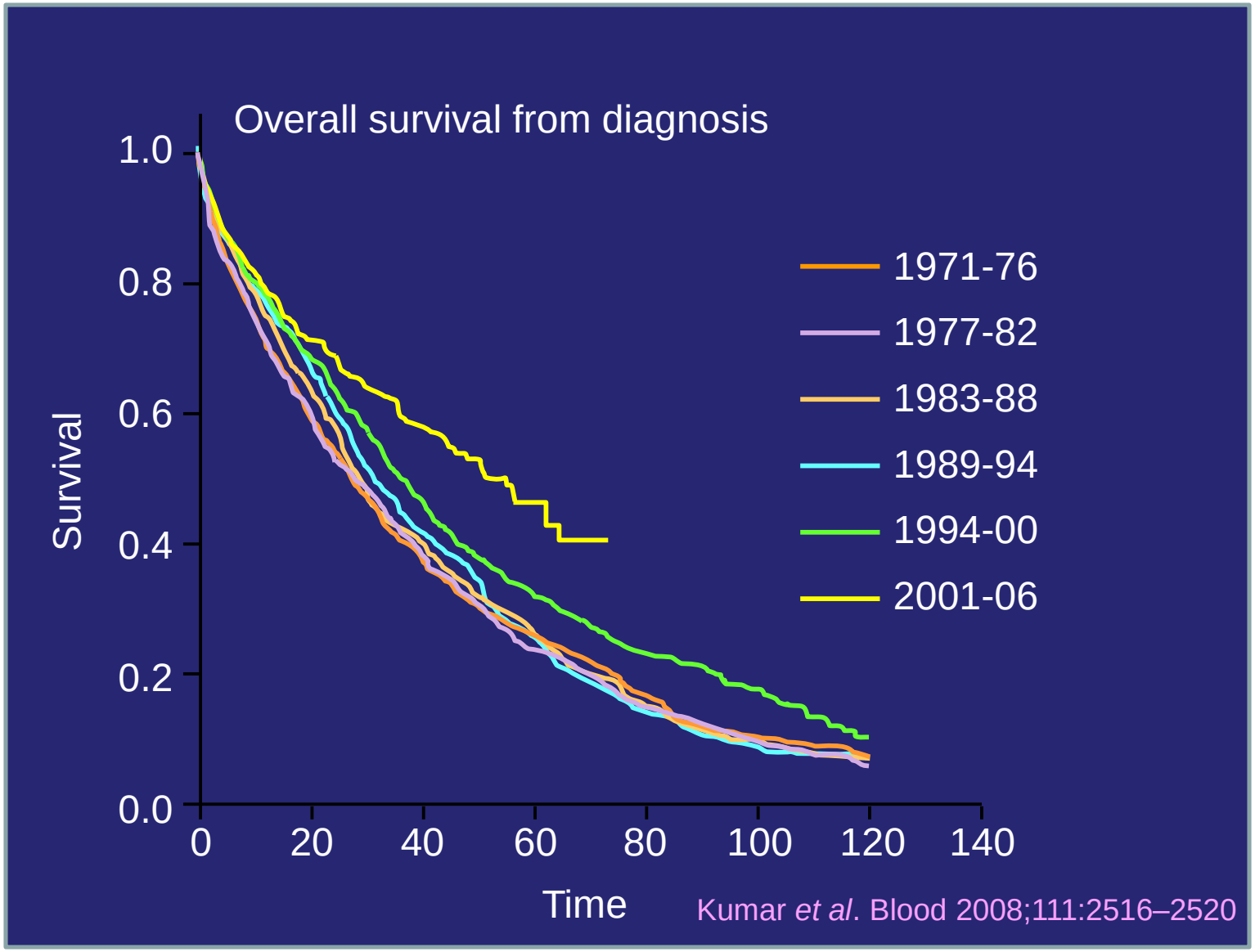
B= bone lesions







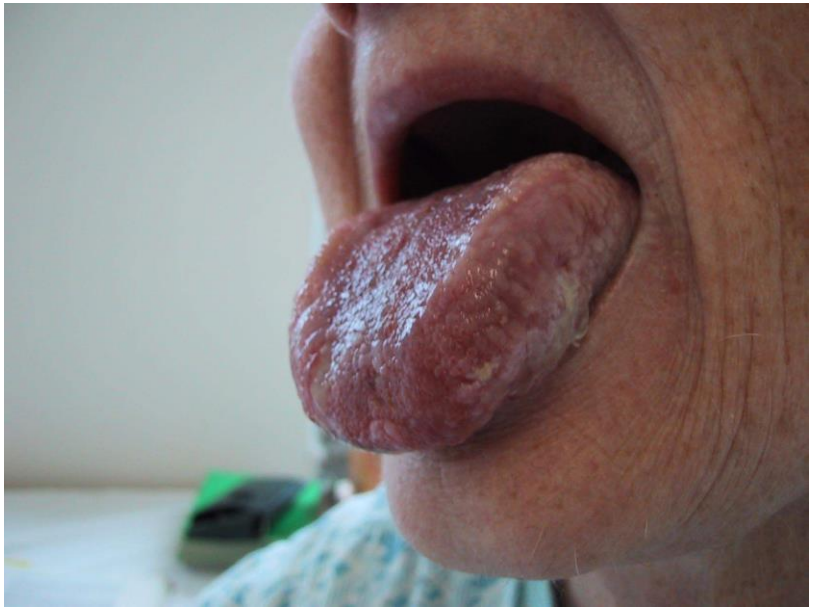
Overall survival of patients with MM

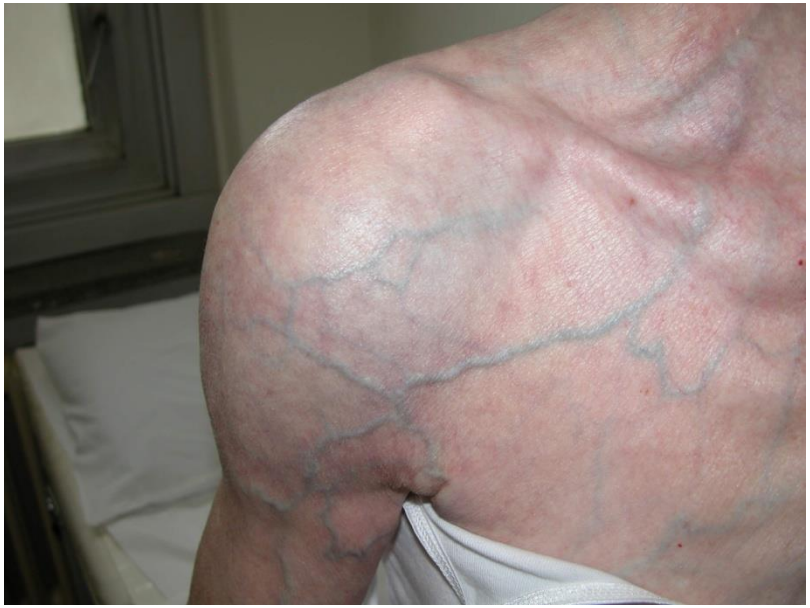


Primary AL amyloidosis

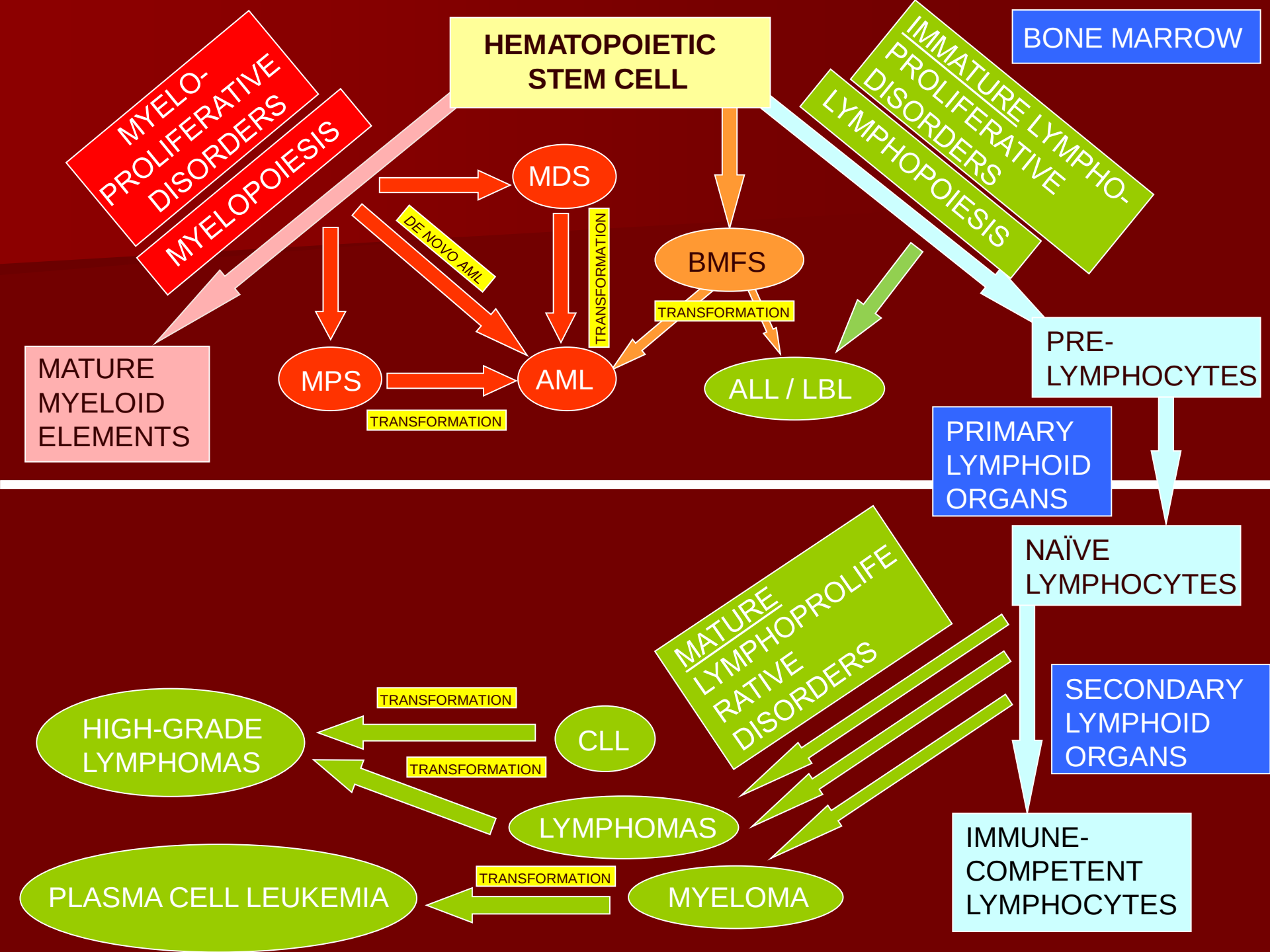
Low incidence (0.9/100 000), i.e. approx. 100 patients per year in the Czech Republic

Men > Women (2:1) with medain age 60-70 years
2 forms of AL amyloidosis: primary (w/o MM), secondary (as a complication of MM)
Symptoms:
- Weakness and dizziness, weight loss (> 50%)
- **organomegaly**: hepatomegaly (25%), splenomegaly, makroglosy
- **Organ syndroms**: nephrotic syndrom (30-40%), heart failure (25-50%), GIT (malabsorption, dysfagia)
- carpal tunnel syndrome (20%), peripheral neuropathy (paresthesias)
- purpura (fragility of capillaries)





Conclusions


HEMATOPOIETIC STEM CELL
BONE MARROW
MYELO-PROLIFERATIVE DISORDERS
MYELOPOIESIS
IMMATURE LYMPHO-PROLIFERATIVE DISORDERS
LYMPHOPOIESIS
MDS
DE NOVO AML
TRANSFORMATION
BMFS
TRANSFORMATION
MATURE MYELOID ELEMENTS
MPS
AML
ALL / LBL
TRANSFORMATION
PRE-LYMPHOCYTES
PRIMARY LYMPHOID ORGANS
NAÏVE LYMPHOCYTES
MATURE LYMPHOPROLIFE RATIVE DISORDERS
SECONDARY LYMPHOID ORGANS
TRANSFORMATION
HIGH-GRADE LYMPHOMAS
CLL
TRANSFORMATION
LYMPHOMAS
IMMUNE-COMPETENT LYMPHOCYTES
TRANSFORMATION
PLASMA CELL LEUKEMIA
MYELOMA

Fin