HEMATOLOGY

Disorders of hematopoiesis and hematologic malignancies

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Normal adult hematopoiesis

Major aims of the presenation

-to gain insight into the differences between disorders of hematopoiesis and hematologic malignancies

-to explain pathophysiological aspects of disorders of hematopoiesis

Two separate presentations will later focus on hematologic malignancies (i.e. lymphoproliferative and myeloproliferative disorders)

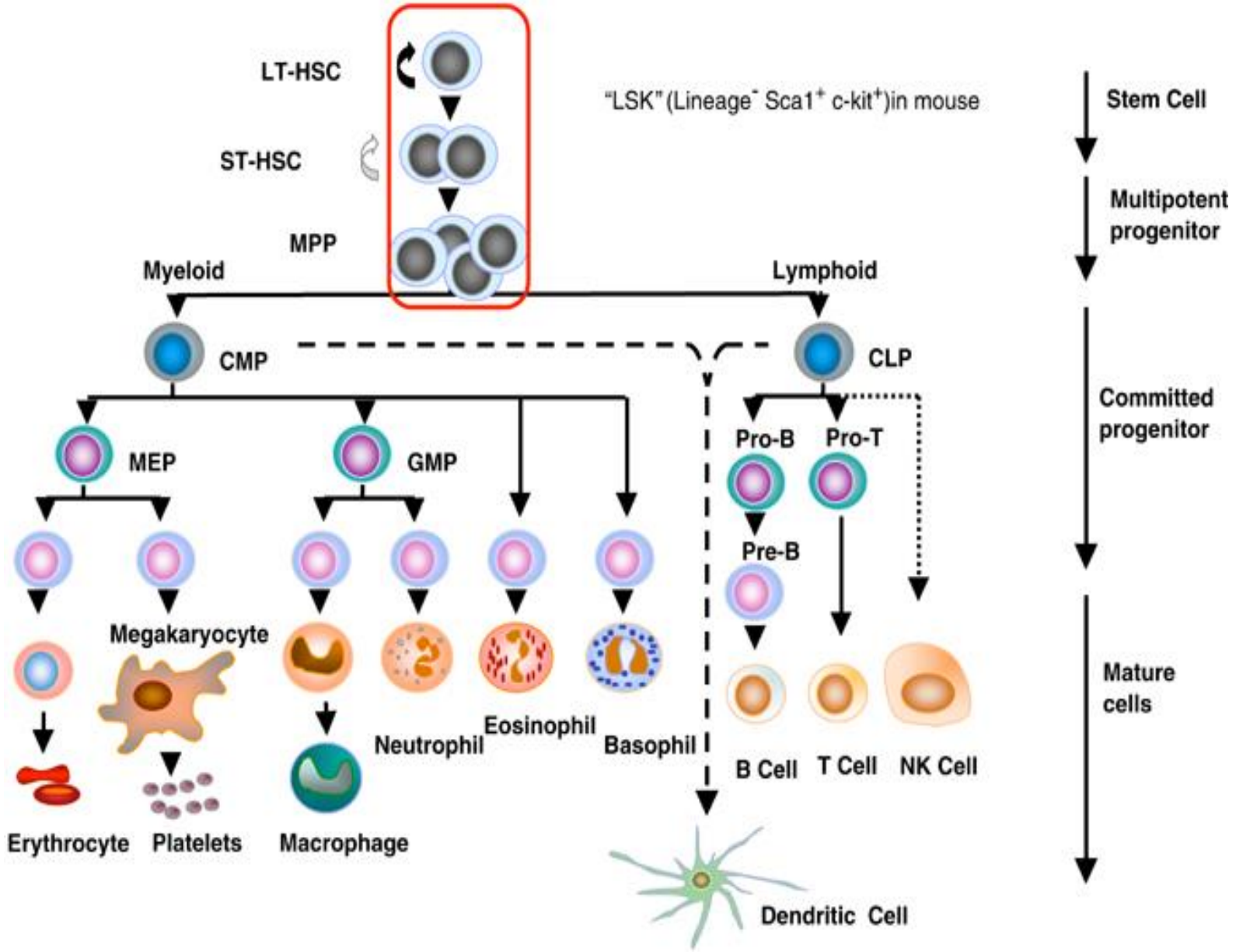
Major hitch is that the bone marrow is the site of two different processes: **myelopoiesis** and **<u>early stages</u> of lymphopoiesis**

<u>Later stages</u> of lymphopoiesis take place **outside the bone marrow**

→ Disorders of hematopoiesis and hematologic malignacies are **overlapping, yet separate categories**.

Understanging basic principles of regulation of hematopoiesis and division of hematologic malignancies will enable and facilitate further study of hematology and hemato-oncology.

Adult bone marrow (BM) hematopoiesis



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

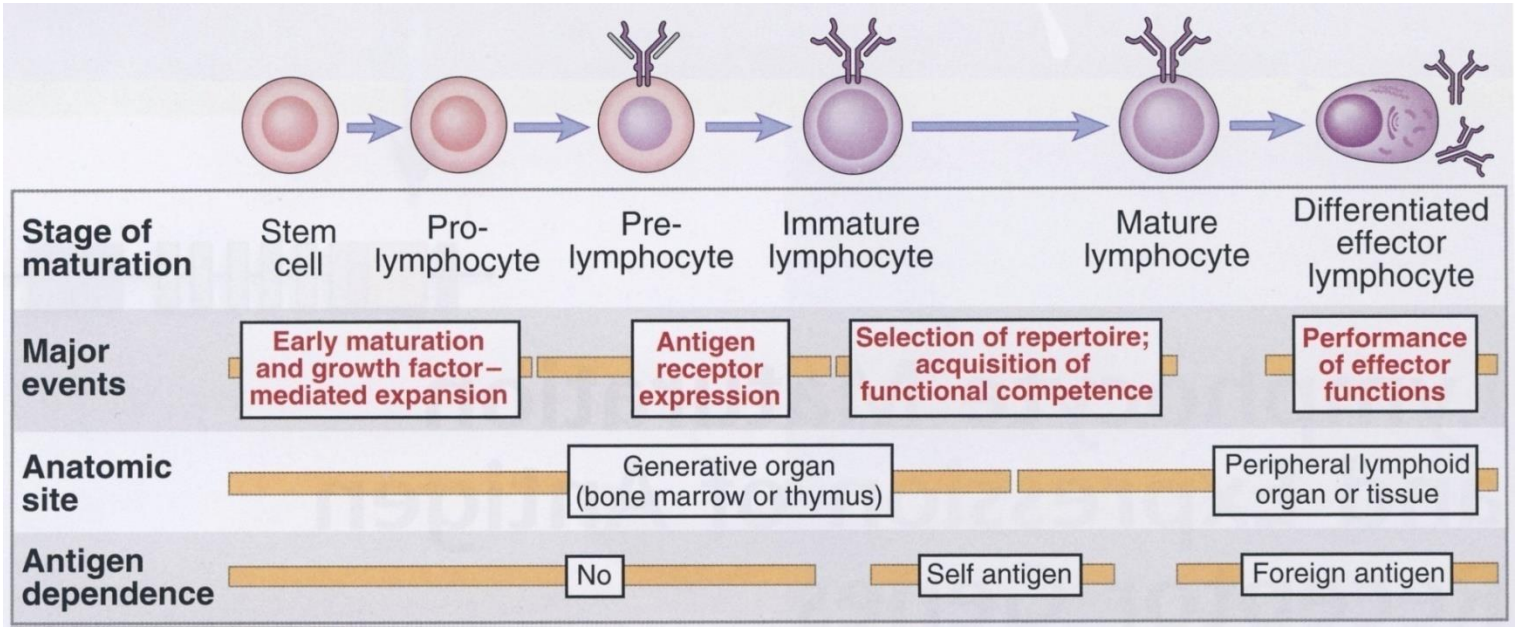
Subsequent stages of lymphopoisis take place outside bone marrow (thymus → sec. lymph. organs)

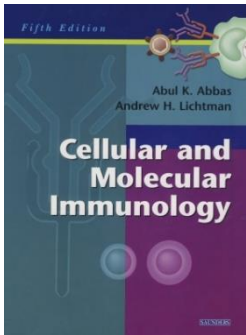
Development of lymphocytes: 3 different compartments

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



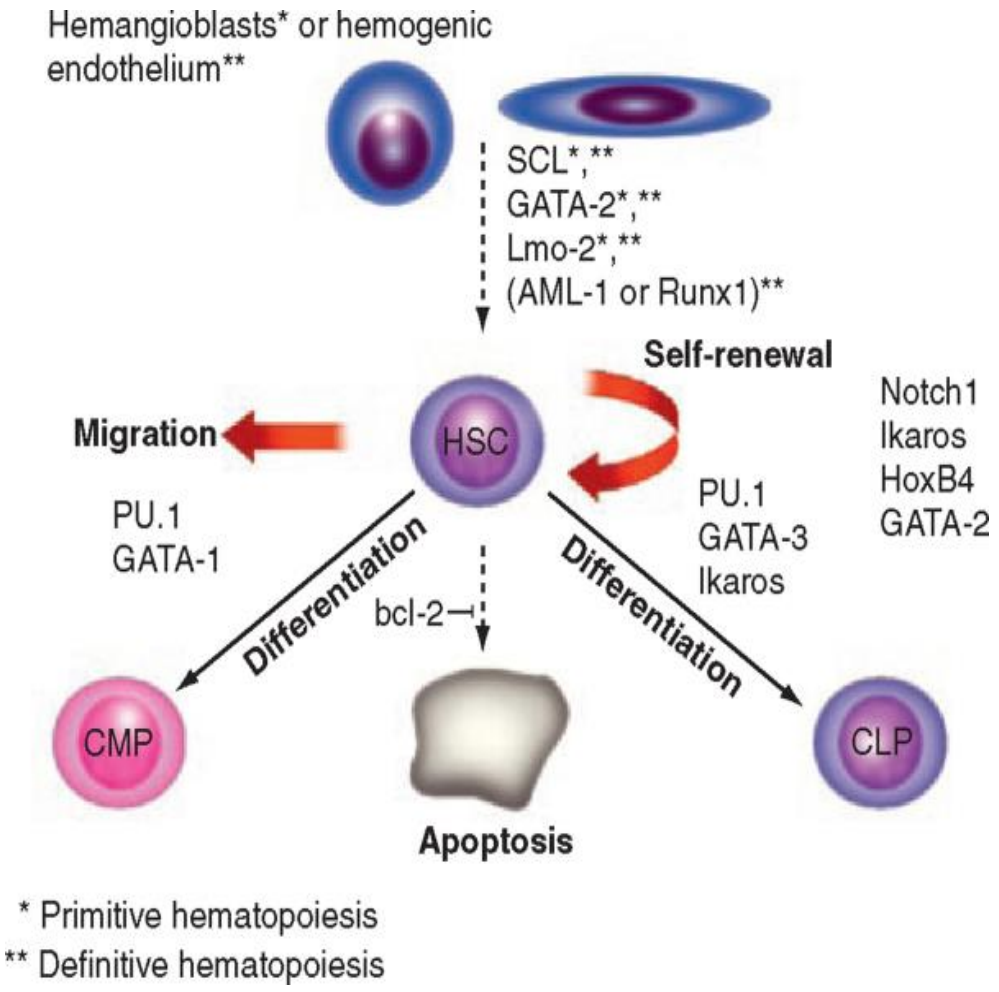


Hematopoietic stem cell (HSC)

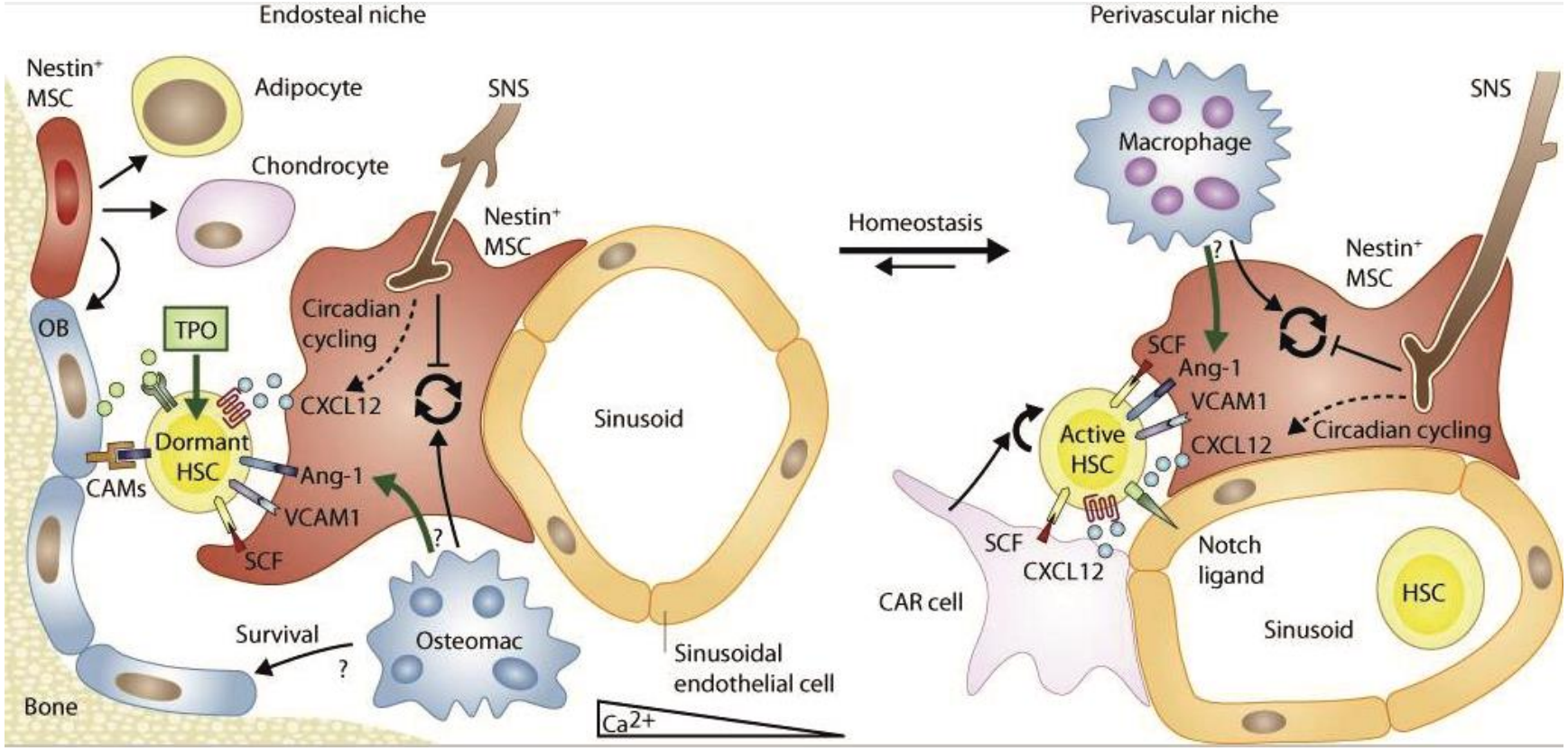
-**unlimited capability of self-renewal**.

-**pluripotent**, can differentiate into all types of mature blood cells, as different as a lymphocyte, erythrocyte and megakaryocyte.

-bone marrow **microenvironment** / stem cell **niches**.



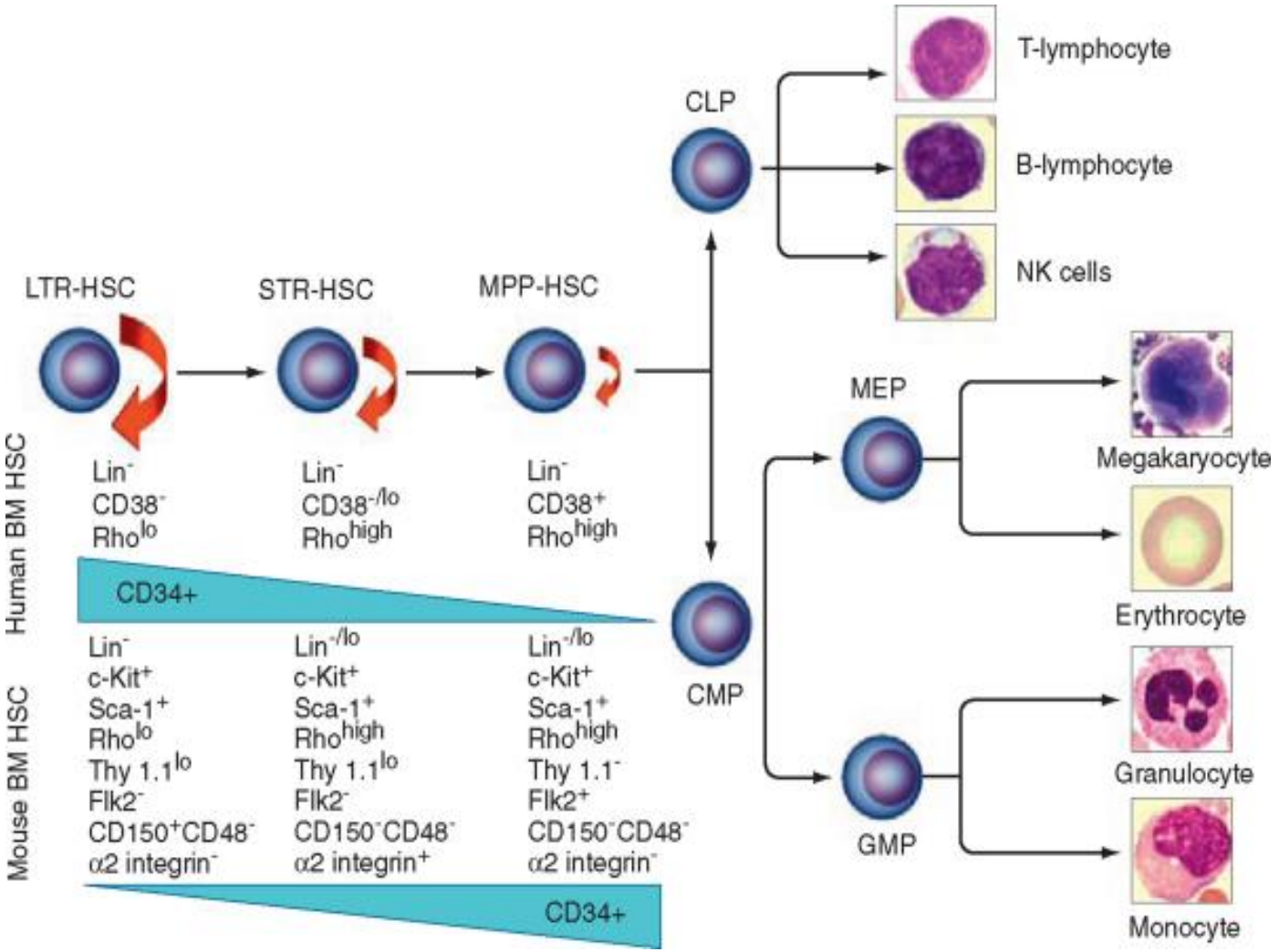
Endosteal and perivascular niches



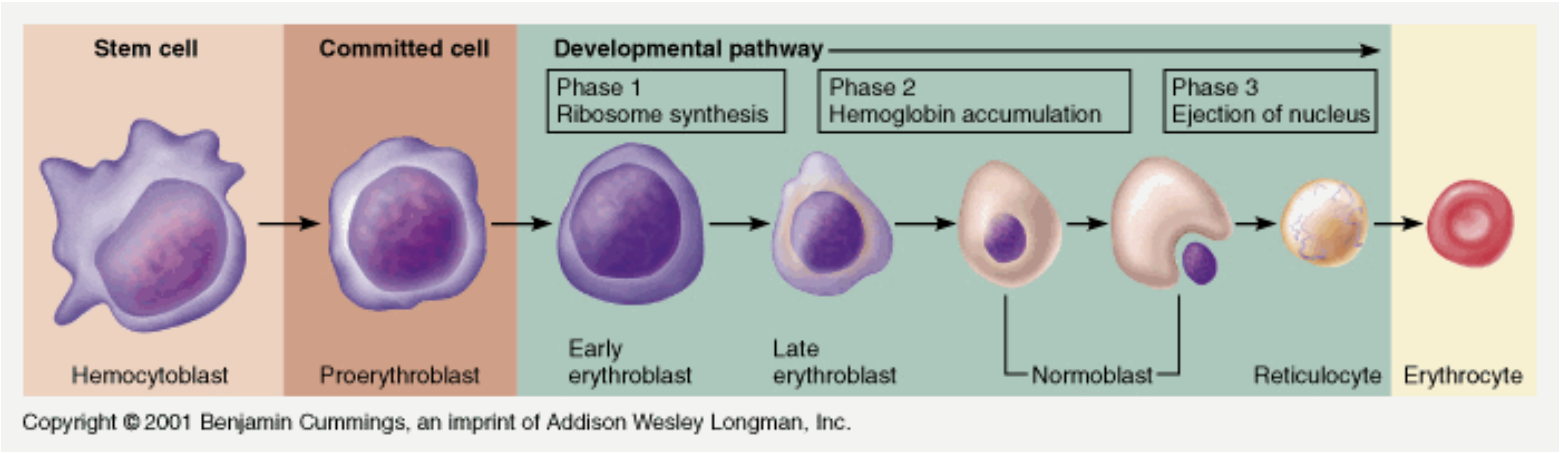
Armin Ehninger and Andreas Trumpp. The bone marrow stem cell niche grows up: mesenchymal stem cells and macrophages move in. *J Exp Med.* 2011 March 14; **208(3):** 421–428.

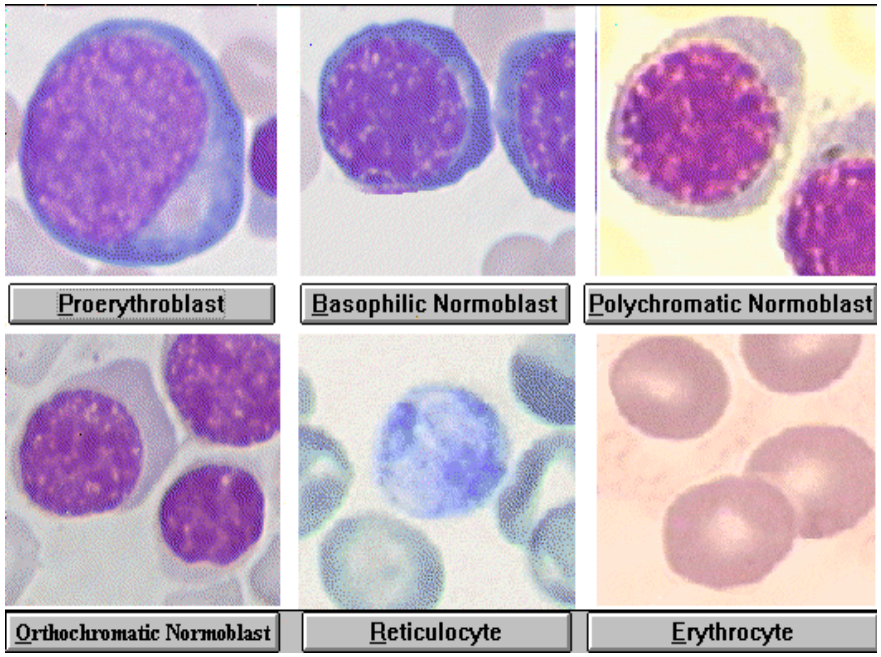
Hematopoietic stem cell

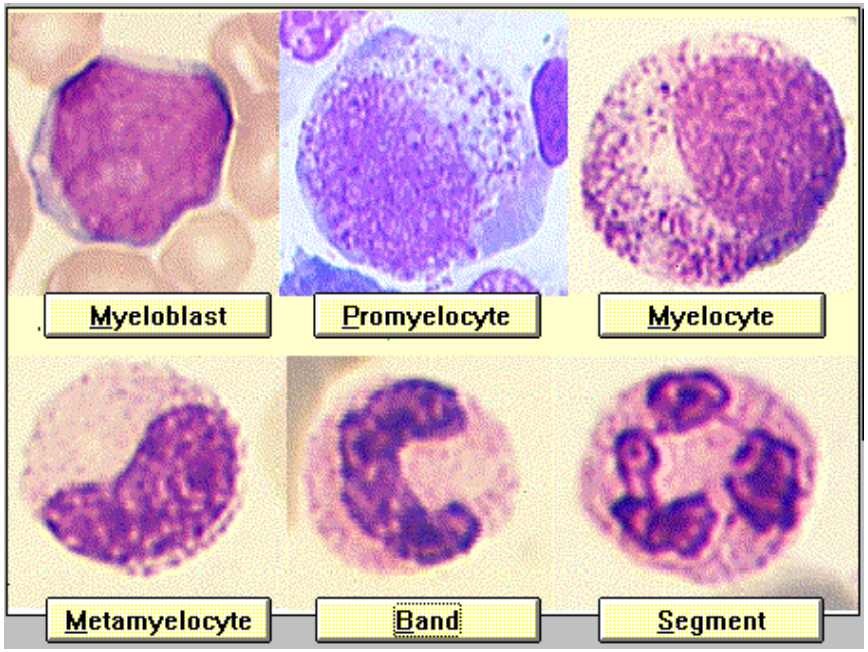
Flow cytometry – cell surface antigens (CD= cluster of differentiation)



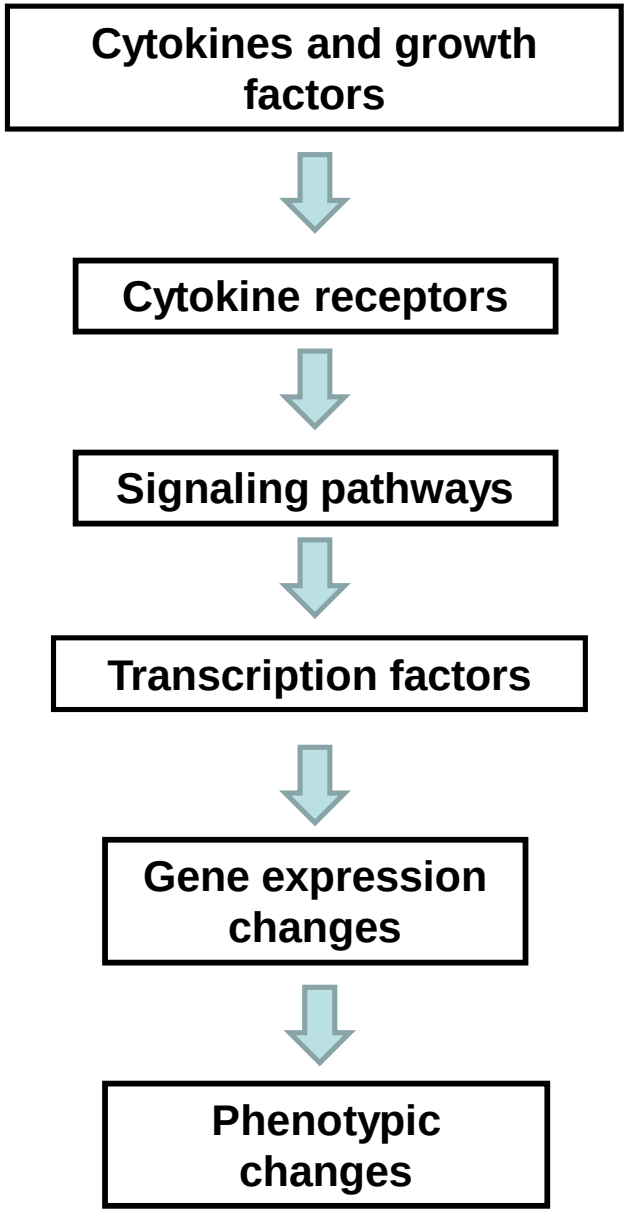
Comittment and differentiation





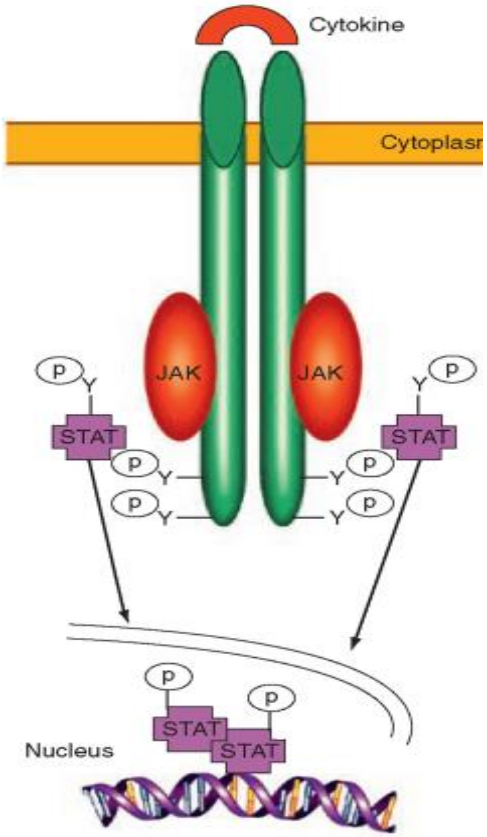


Cell fate decisions: cytokines and hematopoietic transcription factors

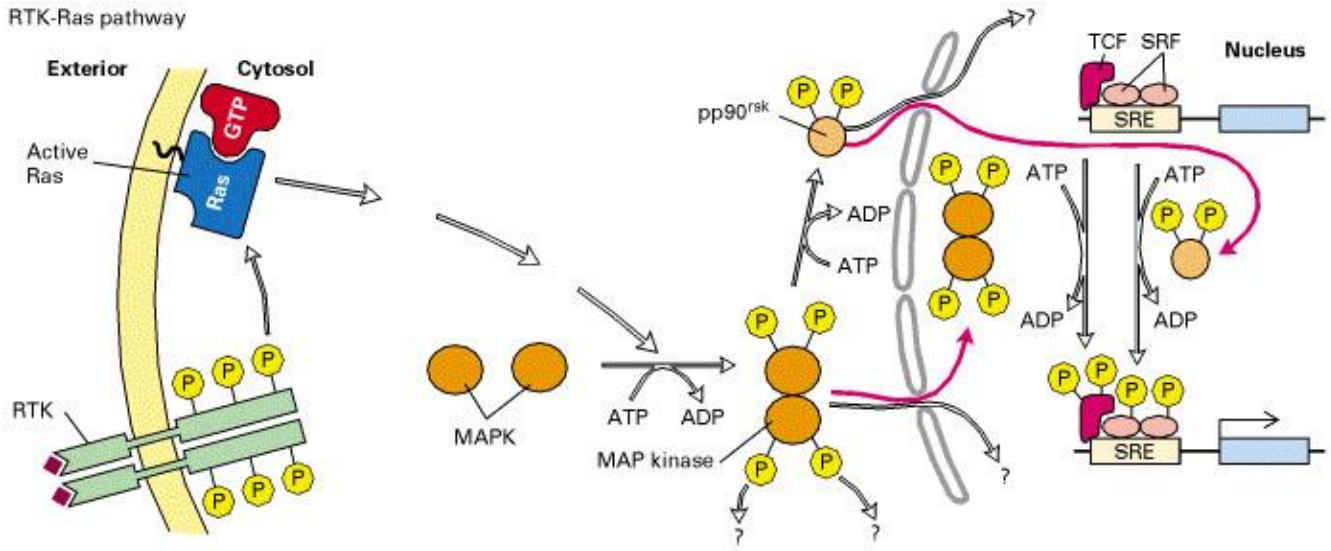


Cytokine receptors: type I and type II

Type I receptors = receptors associated with JAK/STAT
JAK – *Janus kinase*
STAT- *signal transducer and activator of transcription*



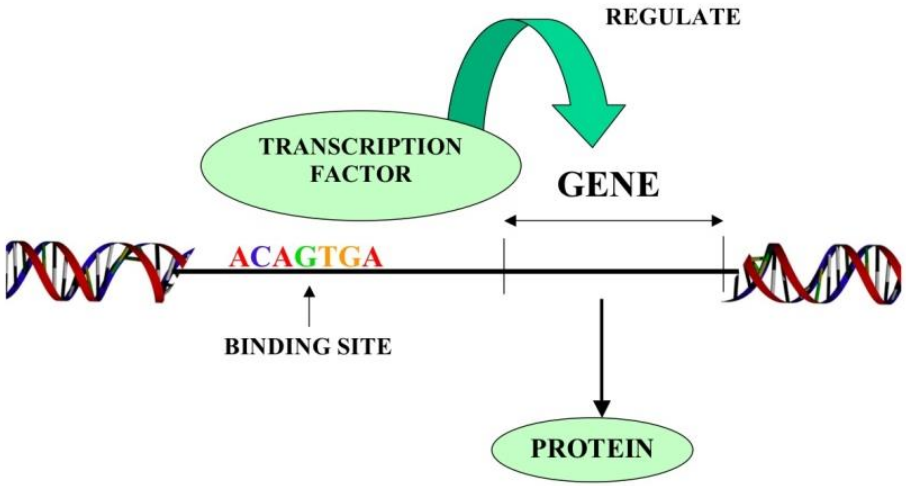
Type II receptors = receptors with intrinsic tyrosine-kinase activity
RTK= *receptor tyrosine-kinase*
MAPK= *mitogen-activated protein-kinase*

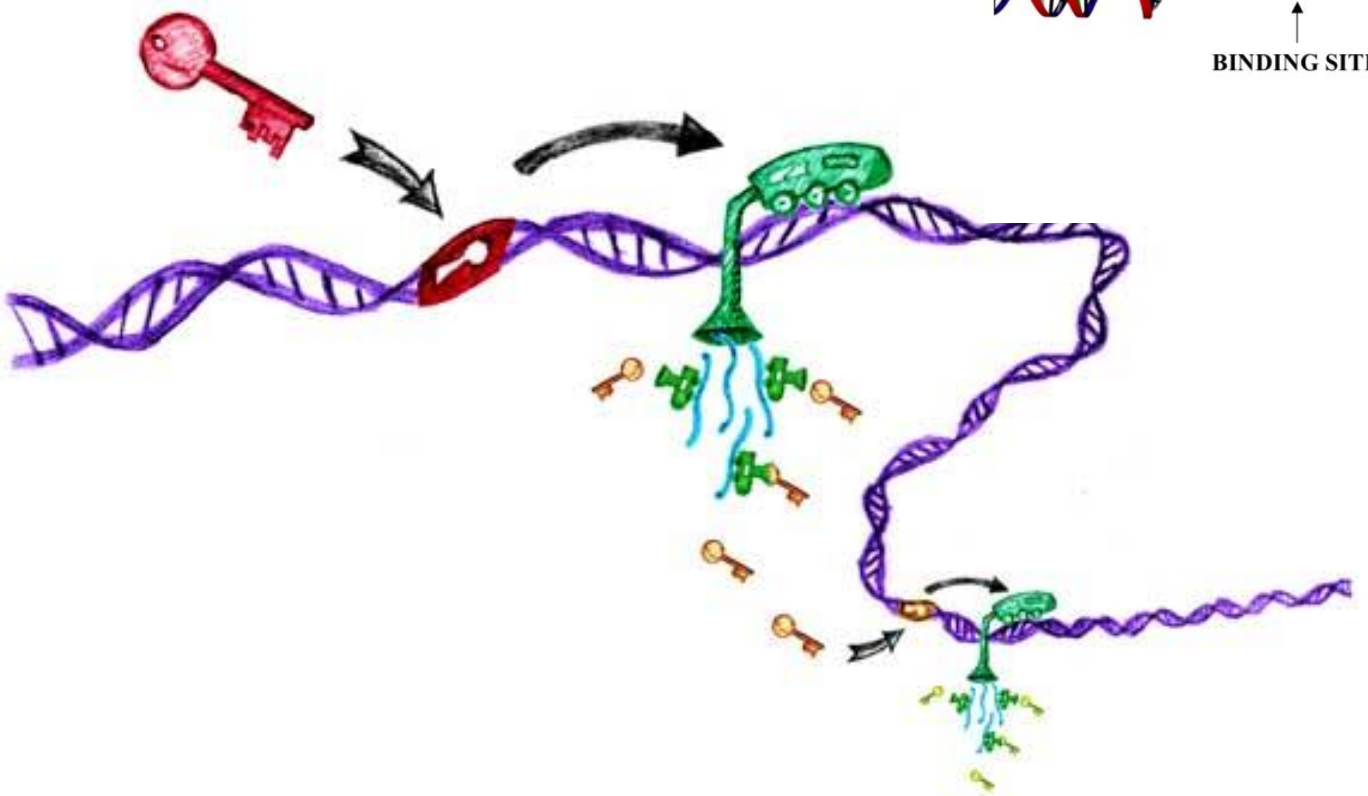


Transcription factors (TFs)

Transcription factors (TFs): proteins that bind DNA and regulate expression of target genes

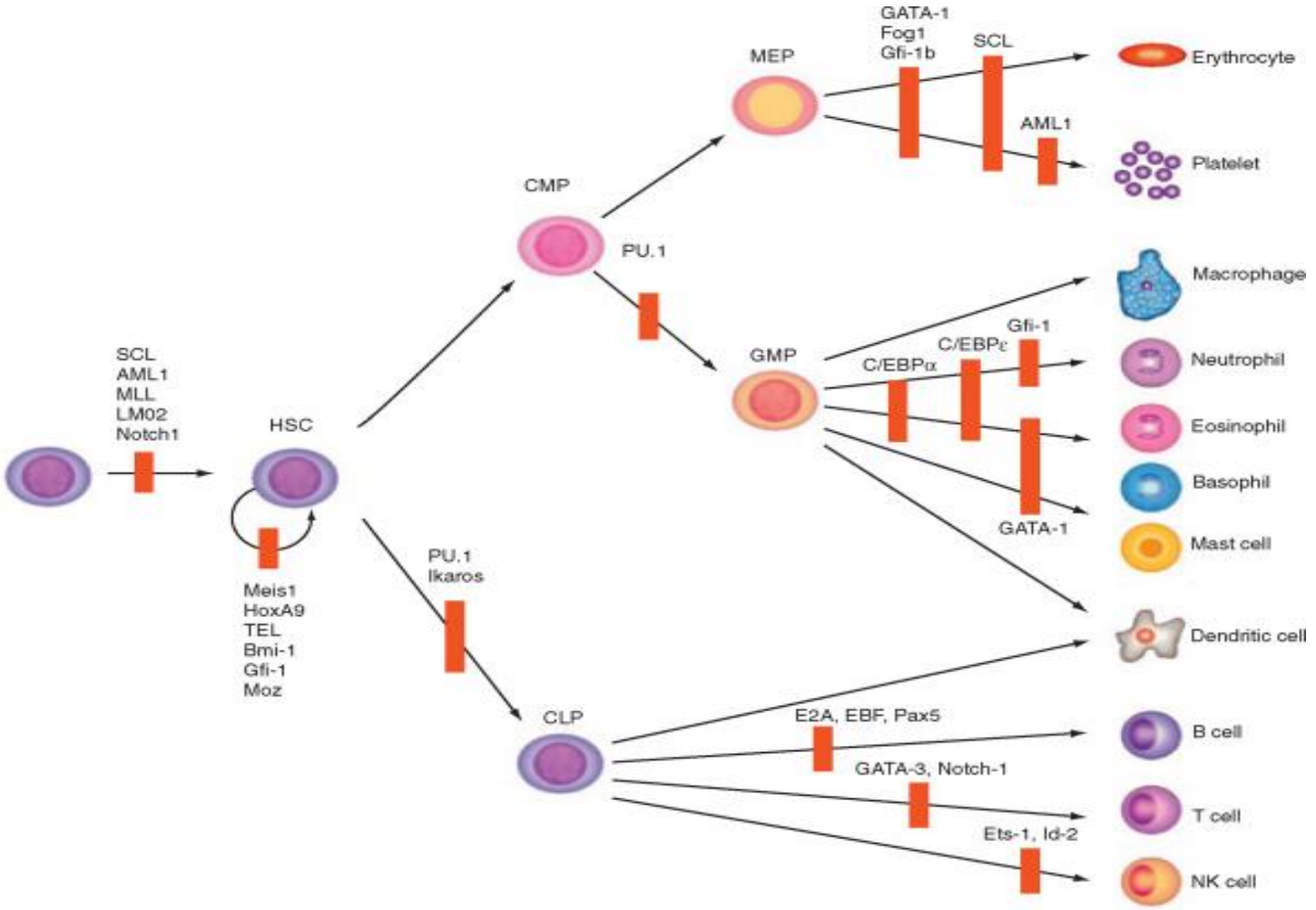
TFs can regulate expression of other TFs…..





Transcription factors regulate comittment and differentiation of myeloid and lymphoid cells

Loss-of-function of a particular TFs results in **block of differentiation (maturation arrest)**= typically seen in acute leukemias (hiatus leukemicus) and myelodysplatic syndromes

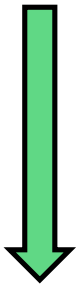


Disorders of hematopoiesis

Disorders of hematopoiesis:

A. Bone marrow failure syndromes

1. Inherited BMFS
2. Acquired aplastic anemias (AA)

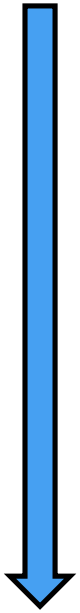


Myeloaplasia

Insufficient hematopoiesis→ severely decreased production of (normal) blood cells (AA, PNH)

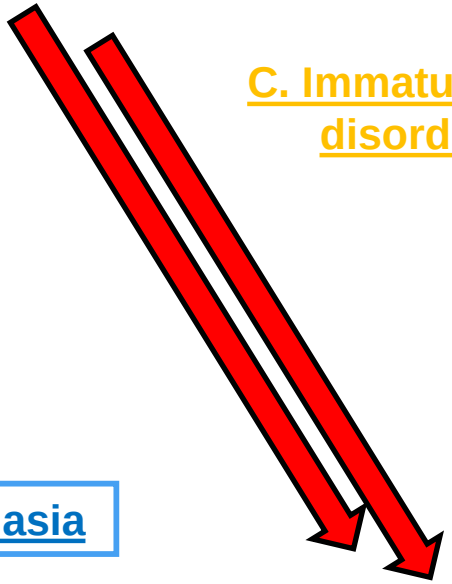
B. Myeloproliferative disorders

1. Myelodysplastic syndromes (MDS)
2. Myeloproliferative syndromes (MPS)
3. Acute myelogeneous leukemia (AML)



Myelodysplasia

Ineffective hematopoiesis→ (under)production of abnormal (dysplastic) cells (MDS)



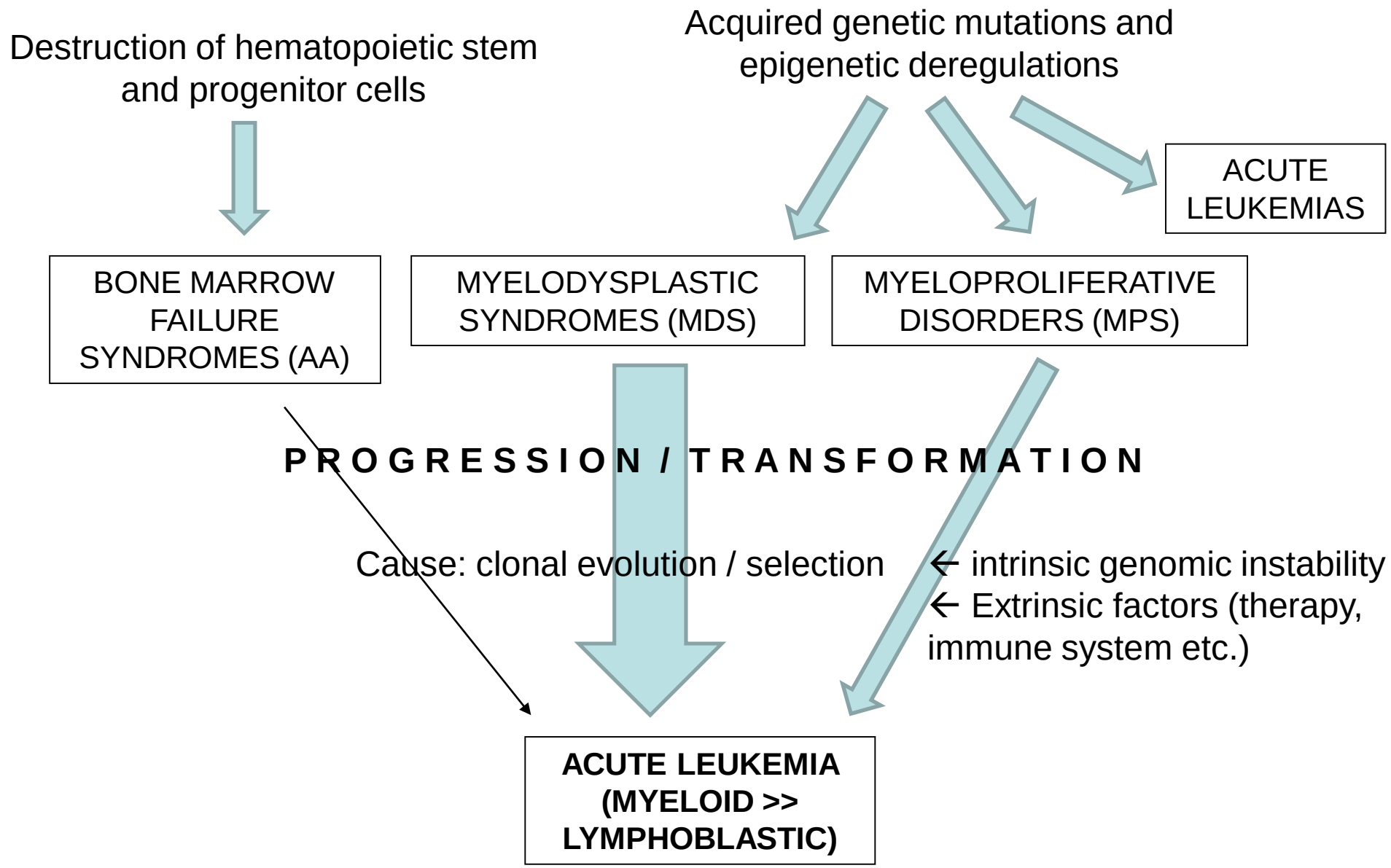
Myeloproliferation

Expansion of a pathological clonal hematopoiesis→ accumulation of morphologically mature (MPS) or immature (AML) blood cells

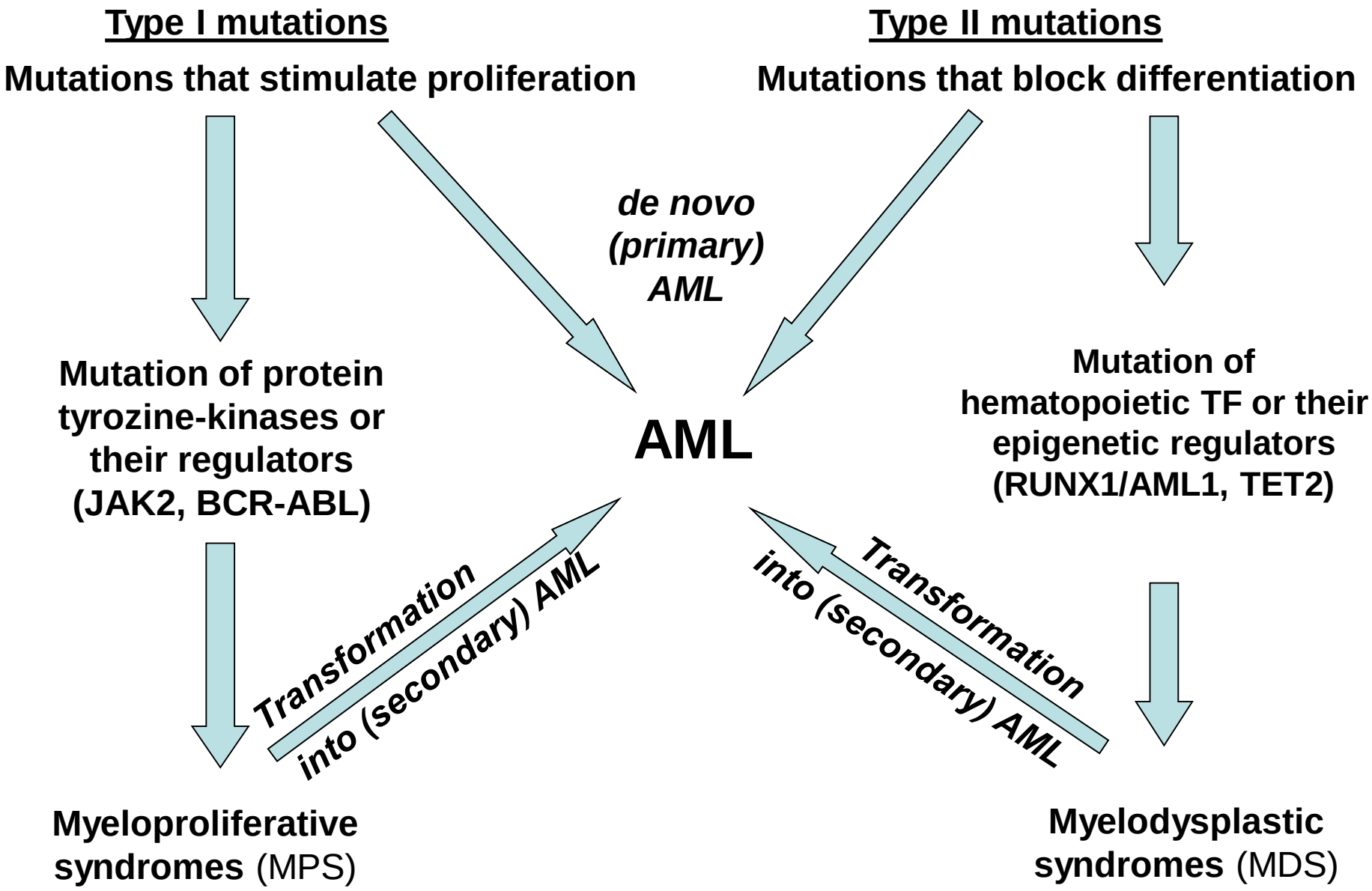
C. Immature lymphoproliferative disorders

= Acute lymphoblastic leukemia / lymphoblastic lymphoma

Pathogenesis and „evolution“ of disorders of hematopoiesis



Leukemogenesis: two-hit model



And where lymphoproliferative disorders (lymphoid malignancies) are classified ?

Lymphoproliferative disorders can be divided into immature (precursor) and mature (peripheral)

Immature (precursor) lymfoproliferative disorders

-represented by a one clinical unit: acute lymphoblastic leukemia / lymphoblastic lymphoma

-can be subclassified according to immunophenotype: B-ALL/LBL or T-ALL/LBL

-B-ALL and T-LBL represent **the most common hematologic malignancies of the childhood**

-ALL/LBL is (similar to AML) a **disorder of hematopoietic stem cell**

Mature (peripheral) lymphoproliferative disorders

= <u>the most common</u> hematologic malignancies (approx. 2/3 of all hematologic malignancies !!!)

=e.g. Hodgkin lymphoma, non-Hodgkin lymphoma, chronic lymphocytic leukemia, monoclonal gamapathies (multiple myeloma, amyloidosis) etc.

= heterogeneous group of diseases that can be classified according to different criteria:

-“historical criteria“ (Hodgkin versus non-Hodgkin lymphomas)

-“immunophenotype“ (B-cell lymphoma, T-cell lymphoma, presence / absence of typical cell surface or intracellular antigens)

-“histology“ (follicular vs diffuse growth)

-“pathological-anatomical features“ (cuteanous lymphomas, lymphomas of CNS, lymphoma of the stomach, testicular lymphomas etc.)

-“evolution“ of the malignancy (MGUS → smouldering myeloma → symptomatic myeloma→ plasma cell leukemia)

-“biological behavior“ (indolent, agressive, highly aggressive lymphomas)

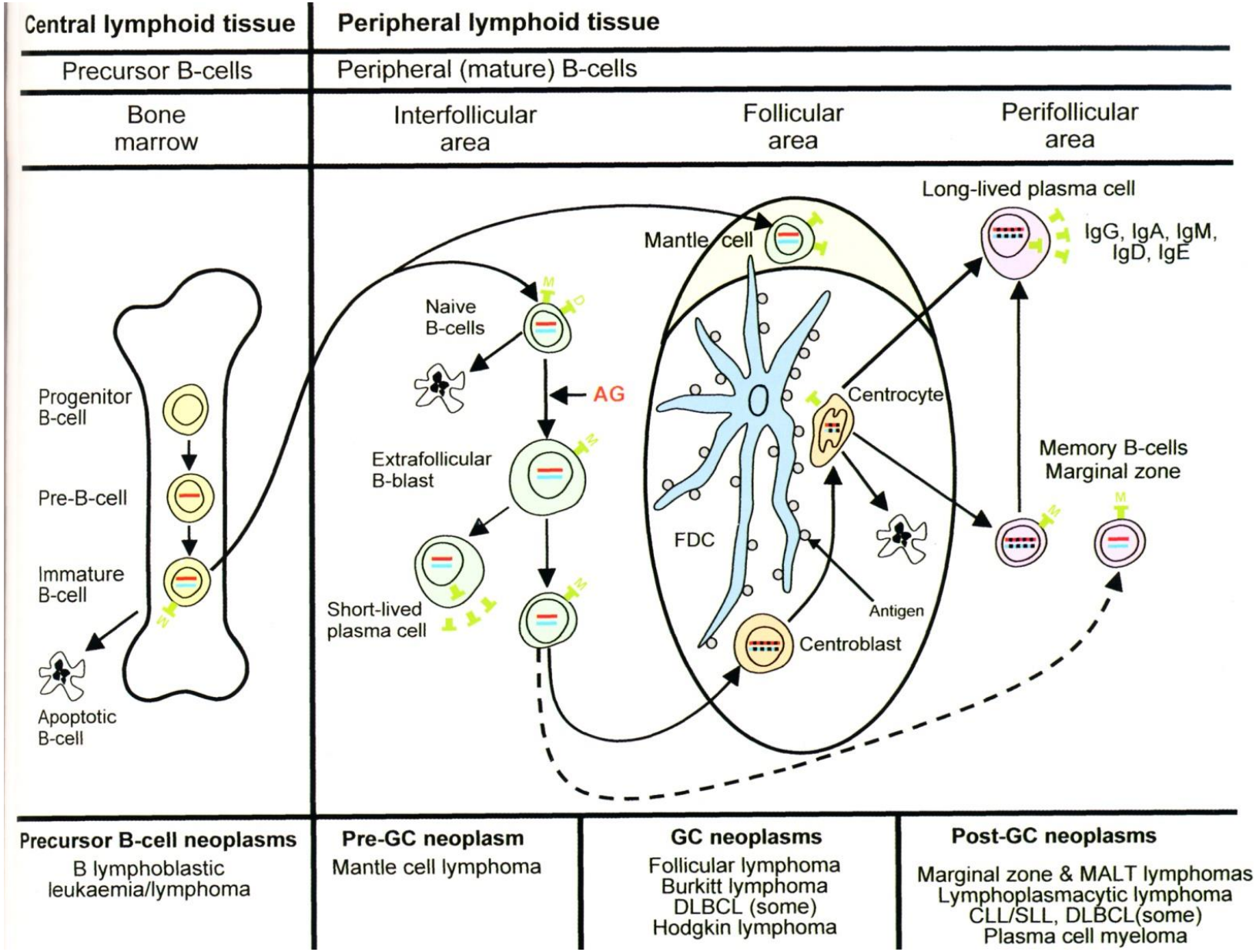
-and more and more „molecular / cytogenetic markers“ (e.g. translocations, somatic mutations etc.)

Mature (peripheral) lymphoproliferative disorders

-do not belong to disorders of hematopoiesis, as they do not arise as a results of mutation of hematopoietic stem cell, but as a results of mutation of peripheral lymphocyte during development and maturation outside the bone marrow (i.e. lymph nodes, spleen).

-**mutations of the peripheral lymphocyte** (somatic mutations, chromosomal translocations) are caused by erroneous application of processes of **somatic hypermutation** and **heavy Ig chain isotype switch** during differentiation and maturation of lymphomcytes from naive cells into immunocompetent effector cells

Precursor and mature B-lymphoproliferative neoplasms



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

Disorders of hematopoiesis

versus

Hematologic malignancies

Disorders of hematopoiesis versus hematologic malignancies

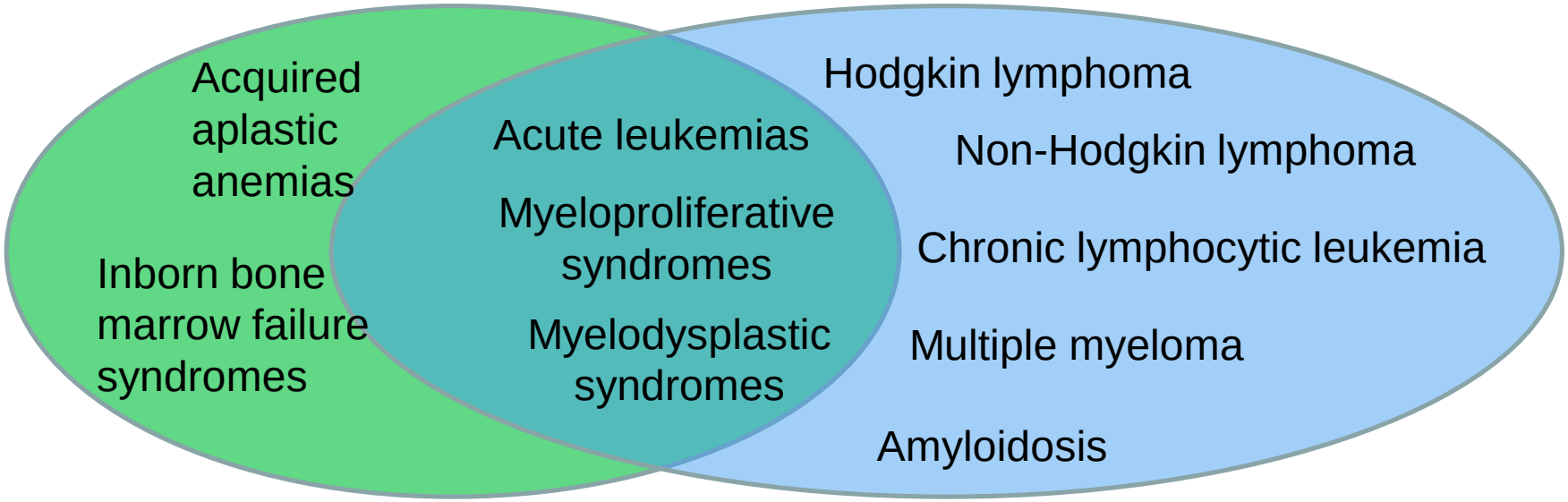
=partially overlapping categories of diseases

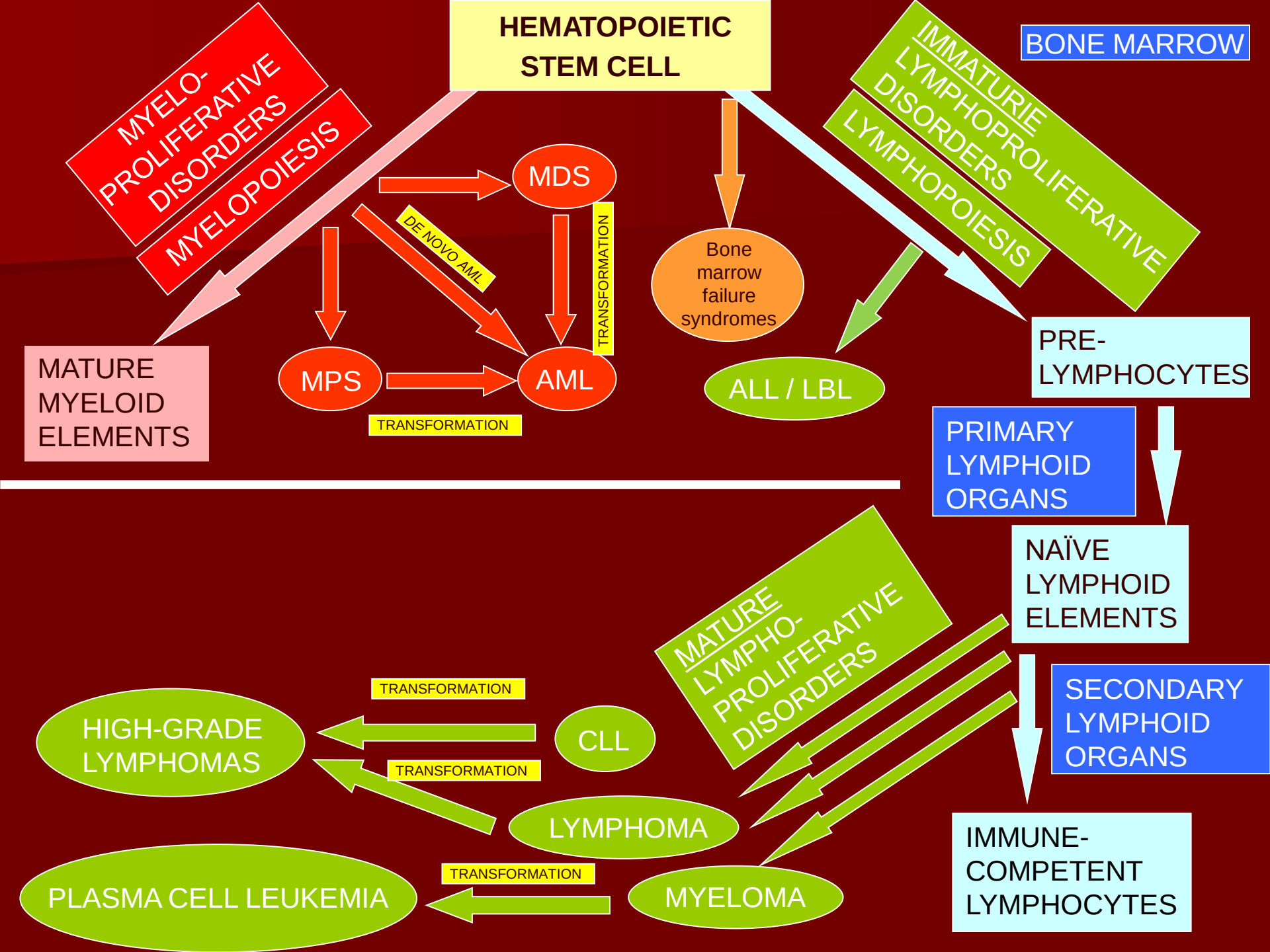
Disorders of hematopoiesis comprise only **disorders of „hematopoietic stem cell“** (i.e. do not comprise mature / peripheral lymphoproliferative neoplasms)

Hematologic malignancies comprise **all myeloproliferative and lymphoproliferative disorders** (but not inborn syndromes of bone marrow failure or acquired aplastic anemias)

Disorders of hematopoiesis=
Disorders of hematopoietic stem cell

Hematologic malignancies =
Myelo + lymphoproliferative neoplasms




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

Bone marrow failure syndromes (BMFS)

Pathogenesis of bone marrow failure syndromes

1. **<u>Acquried</u> bone marrow failure syndromes**
    1. PNH
    2. Aplastic anemias (AA) – secondary
                                - idiopathic

2. **<u>Inherited</u> bone marrow failure syndromes**
    1. Fanconi Anemia (FANC)
    2. Schwachman-Diamond syndrome (SBDS)
    3. Dyskeratosis congenita (DKC1, TERC)

Patogenesis of inherited bone marrow failure syndromes: inborn mutations of genes that regulate stability of chromosomes or length of telomeres.

Patogenesis of acquired bone marrow failure syndromes: destruction or suppression of hematopoietic stem and progenitor cells (chemotherapy, radiotherapy, immune-mediated, carcinomatosis, fibrosis etc.)

Paroxysmal nocturnal haemoglobinuria (PNH)

Paroxysmal nocturnal hemoglobinuria (PNH)

PNH is acquired clonal disorder of hematopoiesis characterized by:

1. **Hemolytic anemia**
2. **Bone marrow failure** (=secondary aplastic anemia)
3. **Thromboembolism** (PNH= hypercoagulable state, just like many other hemolytic anemias).

Acquired mutation of PIG-A gene in hematopoietic stem cells → complete absence of GPI-ancored membrane proteins (GPI= glycosylphosphoinositol), including inhibitors of complements **CD55/DAF (decay accelerating factor) and CD59/MIRL (membrane inhibitor of reactive lysis).**

Absence of GPI-dependent molecules can be detected on all blood cells (besides erythrocytes also on monocytes and granulocytes)

Intravascular hemolysis is a direct consequence of impaired inhibition of complement cascade. Free hemoglobin binds nitric oxid, which triggers smooth muscle spasms (oesophageal) and is associated with decreased blood flow through the kidneys with potential acute renal failure.

Clinical picture

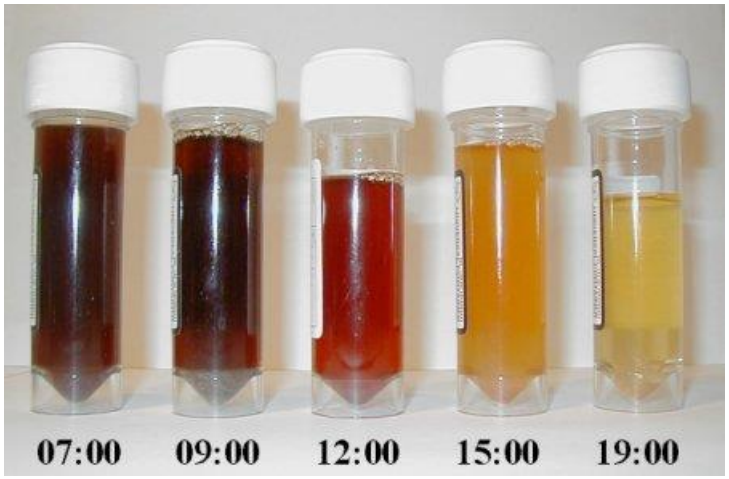
1. Hemoglobinuria immediately after the attact of intravascular hemolysis.

2. Hemosiderinuria 3-5 days after the attack of hemolysis.

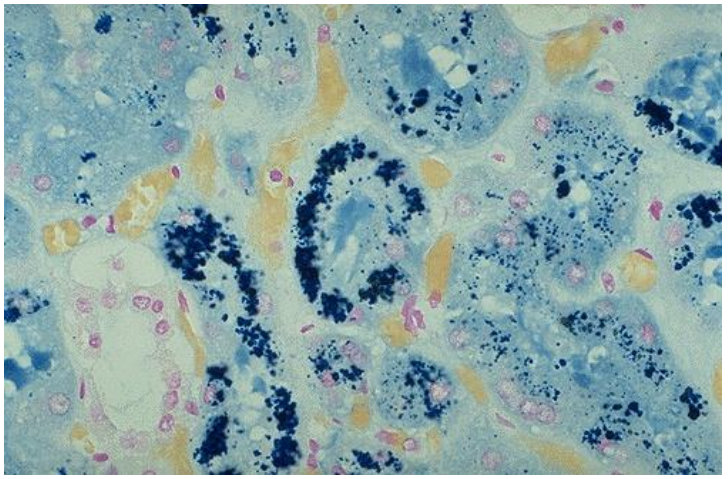
3. Trombosis as a result of hemolysis and smooth muscle spasms.

4. Acute renal failure as a result of decreased blood flow through the kidneys (prerenal component) and toxic effects of free Hb (renal component).

Hemoglobinuria in a patient with PHN



Hemosiderin in renal tubules



Laboratory findings in PNH

1. Normocytic normochromic anemia

2. Decreased plasmatic iron as a result of repeated blood losses during hemoglobinuria

3. Decreased (unmeasurable) haptoglobin as a result of intravascular hemolysis

4. Increased reticulocyte count as a result of accelerated erythropoiesis in the bone marrow

5. Direct Coombs test **negative** (there is no autoantibody !!!)

6. Increased bilirubin and LDH as a result of hemolysis

For the diagnosis of PNH, the percentage of GPI-deficient erythrocytes must be >15%. PNH clones <15% rarely cause clinical symptoms or require therapy.

PNH and bone marrow failure

Aplastic anemia develops probably as a result of T-cell-mediated immune destruction of hematopoietic progenitors with PIG-A mutation.

Frequently, in patients with idiopathic aplastic anemia small PNH clones can be detected (usually <10%).

Differential diagnosis of bone marrow failure syndromes (aplastic anemias) must include PNH !!!

PNH and thrombosis

The most common cause of death in patients with PNH is **thrombosis (!).**

Other causes of death:

-bone marrow failure

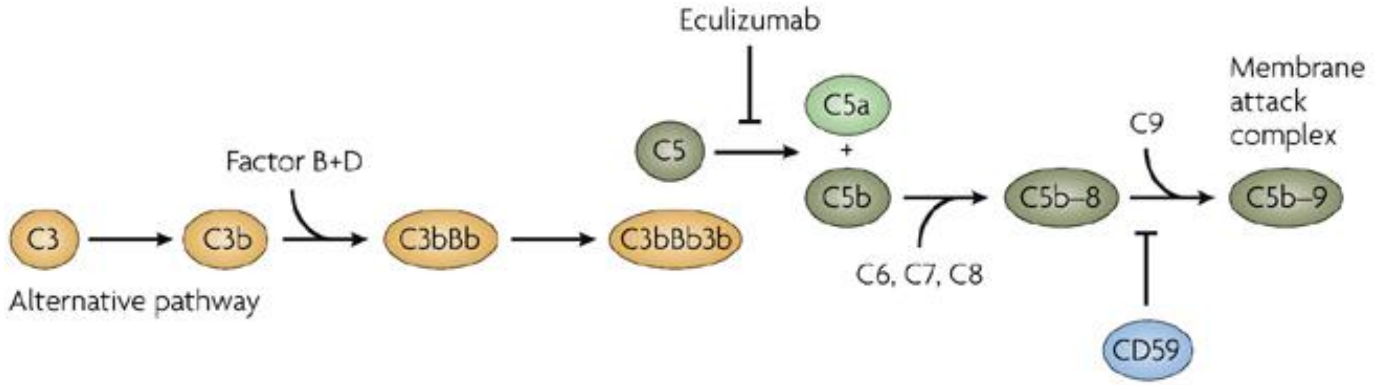
-acute renal failure

-rarely transformation to MDS or AML.

As a result, the overall survival of PNH patients is variable (10-15 years).

Principles of therapy

1. The only curative method is **allogeneic stem cell transplantation** (graft vs PNH effect).
2. **Immunosuppression** in patients with bone marrow failure
3. **Symptomatic therapy** with monoclonal antibody against C5 of complement (eculizumab) → blockage of complement cascade activation.



Nature Reviews | Drug Discovery

Aplastic anemias (AA)

Aplastic anemias (AA)

Aplastic anemias are characterized by bone marrow failure → rather should be designated aplastic pancytopenias (because the whole hematopoiesis is affected).

Aplastic anemias can be divided into:

1. **Inherited** bone marrow failure syndromes
2. **Acquired** AA
    1. **Idiopathic** AA
    2. **Secondary** AA

Aplastic anemia = pancytopenia

Acquired aplastic anemias are characterized by acquired failure of hematopoiesis or severe bone marrow hypoplasia with pancytopenia in peripheral blood count.

Diagnostic criteria of **severe** aplastic anemia:

1. **Reticulocyte** count $<20x10^9/L$
2. **Platelet** count $<20x10^9/L$
3. Absolute **neutrophil** count $<0,5x10^9/L$
4. Bone marrow **cellularity** <30%

Acquired secondary aplastic anemia

Caused by other disease:

1. AA associated with a viral infection (hepatitis, mononucleosis)
2. AA after exposure to drugs, chemical compounds or radiation
3. Paroxysmal nocturnal hemoglobinuria
4. Hypoproliferative MDS
5. Aleukemic ALL/AML/APL
6. Lymphoproliferative malignancies (lymphoma, hairy cell leukemia)
7. Bone marrow fibrosis (myeloproliferative neoplasm)
8. Infiltration of bone marrow with solid tumor (carcinomatosis)
9. Inherited bone marrow failure syndromes

Secondary pancytopenia

= typical examples of secondary pancytopenia is bone marrow suppression as a results of chemotherapy

Blood cell cound of a patient 5 days after administration of high-dose therapy (transplant unit, 1st dept. of medicine- hematology, General University Hospital)

Blood cell count: **Leu: 0,04**, Ery: 2,66, **HB: 81**, HTC: 0,245, MCV: 92,1, MCH: 30,5, MCHC: 331, RDW: 12,1, **Plt: 14**

What risks are associated with long-term bone marrow suppression ?

Acquired idiopathic aplastic anemia

Acquired **idiopathic** AA: **immune-mediated destruction** of hematopoietic stem and progenitor cells by pathological T-cell clones.

The reasons for the destruction remain largely unknown.

Idiopathic AA is a **dg per exclusionem** → to establish a definitive diagnosis it is necessarry to exclude all potential causes of bone marrow destruction / opression, which could lead to acquired **secondary** AA

= a very rare disease (1-2 / 1 million)

Cause: unknown (idiopathic)

Principles of therapy:

In older patients (>45 years) the therapy is based on **immunosuppression** (e.g. ATG= antithymocyte globuline, CSA= cyclosporin A).

In younger patients (<45 years) **allogeneic stem cell transplant** is a therapy of choice.

Case Report- a patient with pancytopenia

Patient (female), 77 years, admitted for dyspnoe, pancytopenia in peripheral blood count:

Blood Ceůů Count: **Leu: 1,09**, Ery: 2,46, **HB: 68**, HTC: 0,208, MCV: 84,6, **Plt: 34**

Laboratory: Na: 137, **K: 3,7**, Cl: 99, Ca: 2,19, P: 0,89, Mg: 0,54, Urea: 11,4, **Creat.: 155**, CB: 64,0, **CRP: 198,0**, Prokalcitonin: 0,24, Prealb.: 0,15, **Glycemie: 8,0**

History: a polymorbid patient, no exposure to new drugs, chemotherapy, radiotherapy

Symptoms: Fatigue, bruising, anorexia, dyspnoe

What dg. procedures would you recommend?

Inherited bona marrow failure syndromes (IBMFS)

Inherited bone marrow failure syndromes (IHMFS)

Inherited aplastic anemias= inherited bone marrow failure syndromes- IHMFS.

IHMSF include:

1. **Fanconi anemia (FA)**
2. **Dyskeratosis congenita (DC)**
3. **Schwachmann-Diamond syndrome (SDS)**

Inherited bone marrow failure syndromes (IBMFS)

Background information:

1. IBMFS are genetically determined diseases caused by inherited mutations of genes involved in maintenance of stability of chromosomes (FA) or telomeres (DC, SDS).
2. Bone marrow failure is usually only one of symptoms of these disorders that tend to be accompanied by developmental abnormalities (mainly skeletal), small stature and other anomalies (skin, mucosa).
3. Bone marrow failure is usually manifested in postnatal period, typically in the first decade, sometimes during the second or event third decade of life.
4. IBMFS are associated with increased risc of secondary malignancies (leukemias, solid tumors) compared to healthy population.

Principles of therapy:

1. **Stimulation of hematopoiesis** (androgenes, growth factors)
2. **Substitution of hematopoiesis** (allogeneic stem cell transplant)

Fanconi anemia (FA)

FA is inherited disorder characterized by mutation of genes (FANCA, FANCC, BRCA2 aj.) involved in maintenance of integrity of chromosomes → FA is characterized by increased fragility of chromosomes

Clinical presentation:

1. **Developmental disorders** (abscence of thumbs, absence of radii, short stature, hyperpigmentation of skin (café-au-lait), hypogonadism, microcefalia (facies Fanconica).
2. **Early bone marrow failure** (aplastic anemia between 3rd to 14th year of age)
3. **Extremely increased risc of secondary malignancies** (4.000x increased risc of carcinoma of genital tract in women, 800x increased risc of AML, 700x increased risc of solid tumors of head-and-neck)

Principles of therapy: stimulation of hematopoiesis (androgenes), substitution of hematopoiesis (alloSCT)

Dyskeratosis congenita (DC)

DC is inborn disorder characterized by mutated genes (TERT, TERC, DKC1 aj.) involved in the maintenance of telomeres.

Clinical symptoms:

1. Hyperpigmentation, leucoplakia, nail dystrophy
2. Bone marrow failure (progressive aplastic anemia typically manifests during the second decade of life).
3. Increased risc of secondary malignancies

Deregulated telomere maintenance results in their extreme shortening (under 1 percentil of healthy population). Shortened telomeres probably induce extinction of hematopoietic stem cell compartment.

Prognosis: patients die around 20 years of age.

Principles of therapy: stimulation of hematopoiesis (androgenes), substitution of hematopoiesis (alloSCT)

Schwachman-Diamond syndrome (SDS)

SDS is inborn disase characterized by mutation of genes (e.g. SBDS) that induce abnormal shortening of telomeres.

Clinical symptoms:

1. Exocrine pancreatic insufficiency
2. Bone marrow failure (most frequently neutropenia, thrombocytopenia, or aplastic anemia)
3. Developmental disorders (short stature, skeletal abnormalities)
4. Oral cavitiy pathologies (caries, infections, gingivitis, defects) as a result of neutropenia
5. Increased risc of AML

Prognosis: patients die aged 30-40 years.

Principles of therapy: stimulation of granulopoiesis (G-CSF, granulocyte colony stimulating factor), substitution of hematopoiesis (allogeneic stem cell stransplant)

Conclusions

Disorders of hematopoiesis versus hematologic malignancies

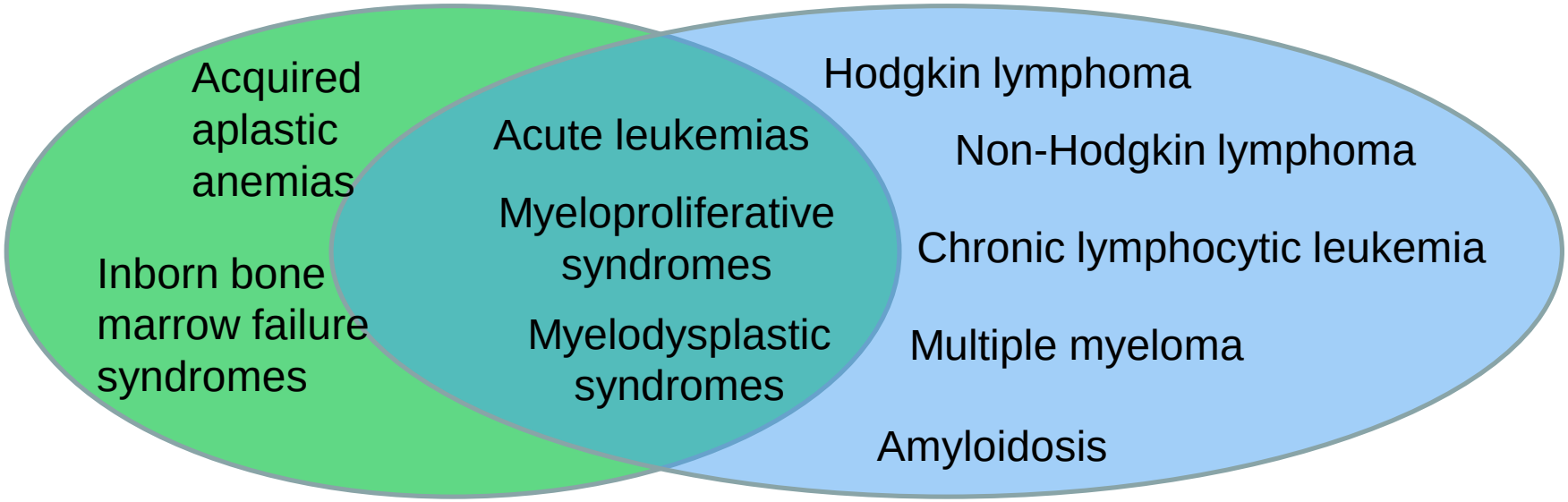
=partially overlapping categories of diseases

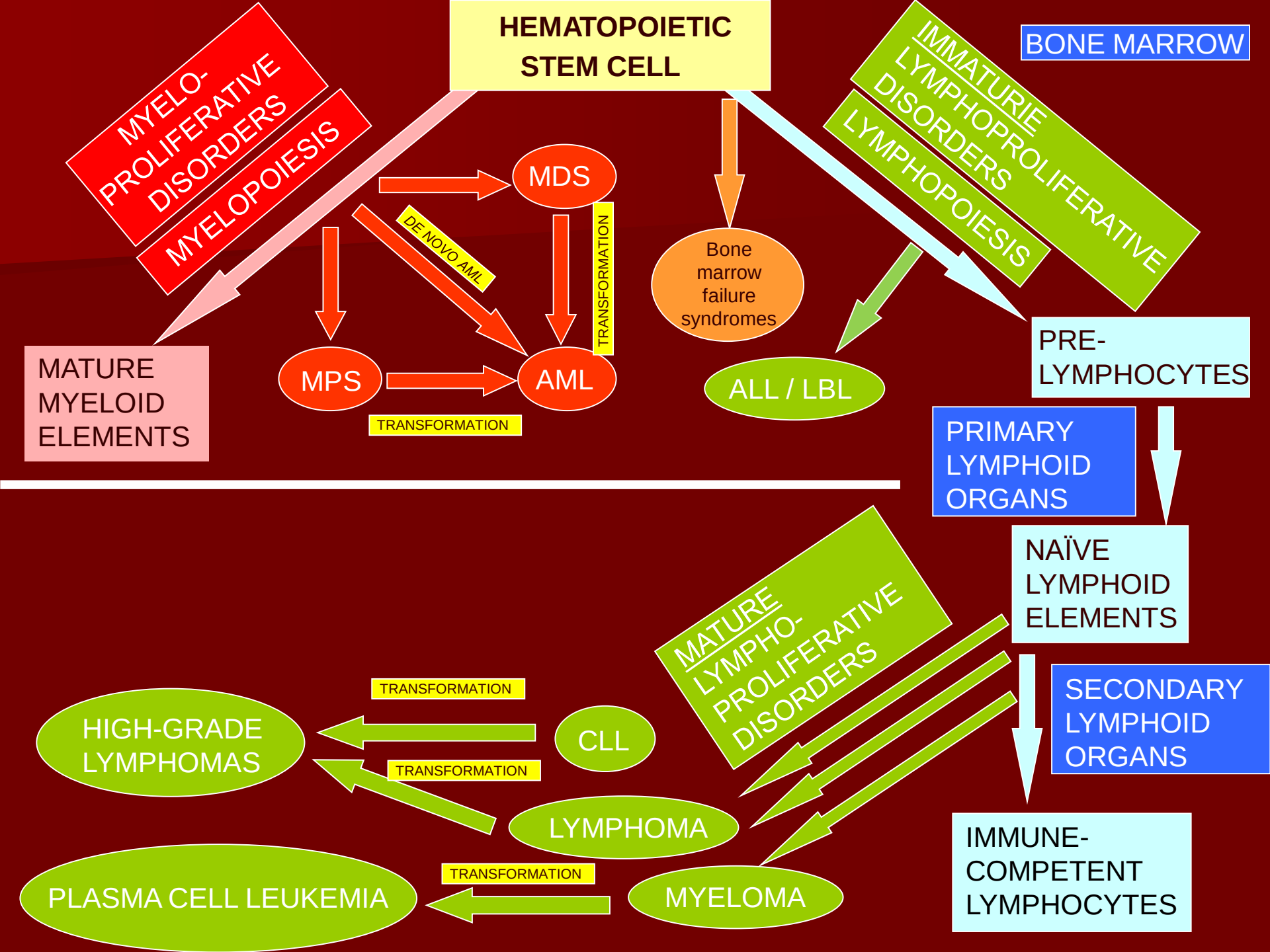
Disorders of hematopoiesis comprise only **disorders of „hematopoietic stem cell“** (i.e. do not comprise mature / peripheral lymphoproliferative neoplasms)

Hematologic malignancies comprise **all myeloproliferative and lymphoproliferative disorders** (but not inborn syndromes of bone marrow failure or acquired aplastic anemias)

Disorders of hematopoiesis=
Disorders of hematopoietic stem cell

Hematologic malignancies =
Myelo + lymphoproliferative neoplasms




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

Thank you !!

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