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Pathophysiology of hematopoietic system I– hematological malignancies

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I. Hematopoiesis

Hematopoiesis

process of creation of cell components of blood

adult human produces 4 – 5 x 10^{11} of hematopoietic cells daily

highly regulated, highly responsive system

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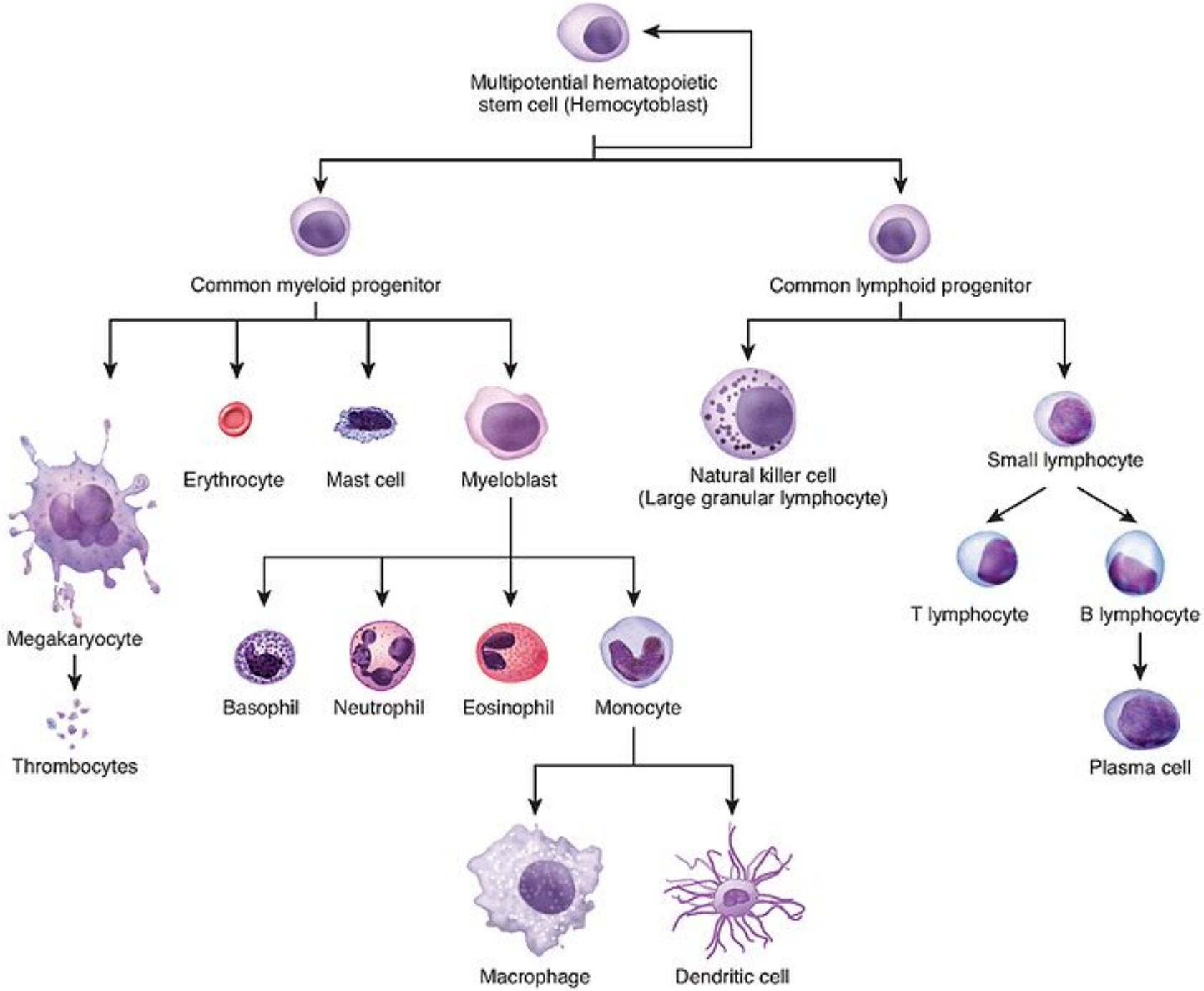
Multipotential hematopoietic stem cell (Hemocytoblast)
Common myeloid progenitor
Common lymphoid progenitor
Erythrocyte
Mast cell
Myeloblast
Megakaryocyte
Thrombocytes
Basophil
Neutrophil
Eosinophil
Monocyte
Macrophage
Dendritic cell
Natural killer cell
(Large granular lymphocyte)
Small lymphocyte
T lymphocyte
B lymphocyte
Plasma cell

Production and destruction of blood

- **Production of blood**
  - the liver creates protein components of blood
  - the endocrine glands produce hormones
  - the GI tract and kidneys maintain water fraction

- **Destruction of blood**
  - Spleen – destruction of blood cells
  - Liver – destruction of blood cells, proteins and amino acids collected
  - Kidneys – proteins collected; amount of water regulated

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Hematopoietic stem cells - HSC

- Multipotent - capable of generating entire hematopoietic system
- Embryogenesis - aorto-gonado-mesonephros region, fetal liver
- Adults - bone marrow
- highly specialized rare cells
  - self renewal
  - differentiation into functional progenitors

- important for renewal after transplantation, infection, wound
- balance between differentiation and self renewal
- Intracellular factors
  - Regulators of transcription and epigenetics, metabolic pathways
- Extracellular factors
  - Humoral and neural signals, signals from the bone marrow niche

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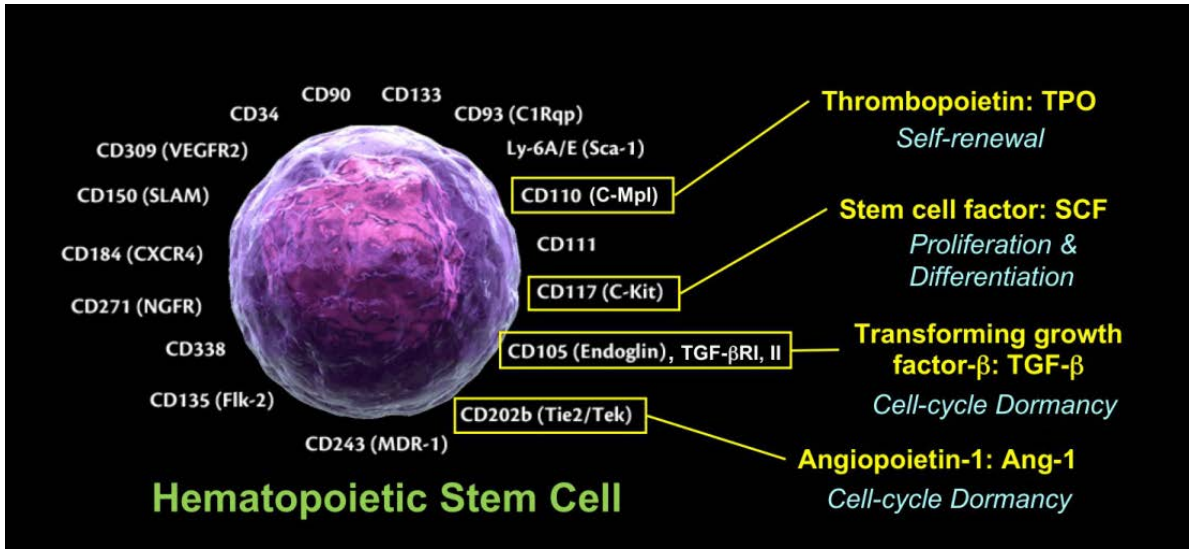
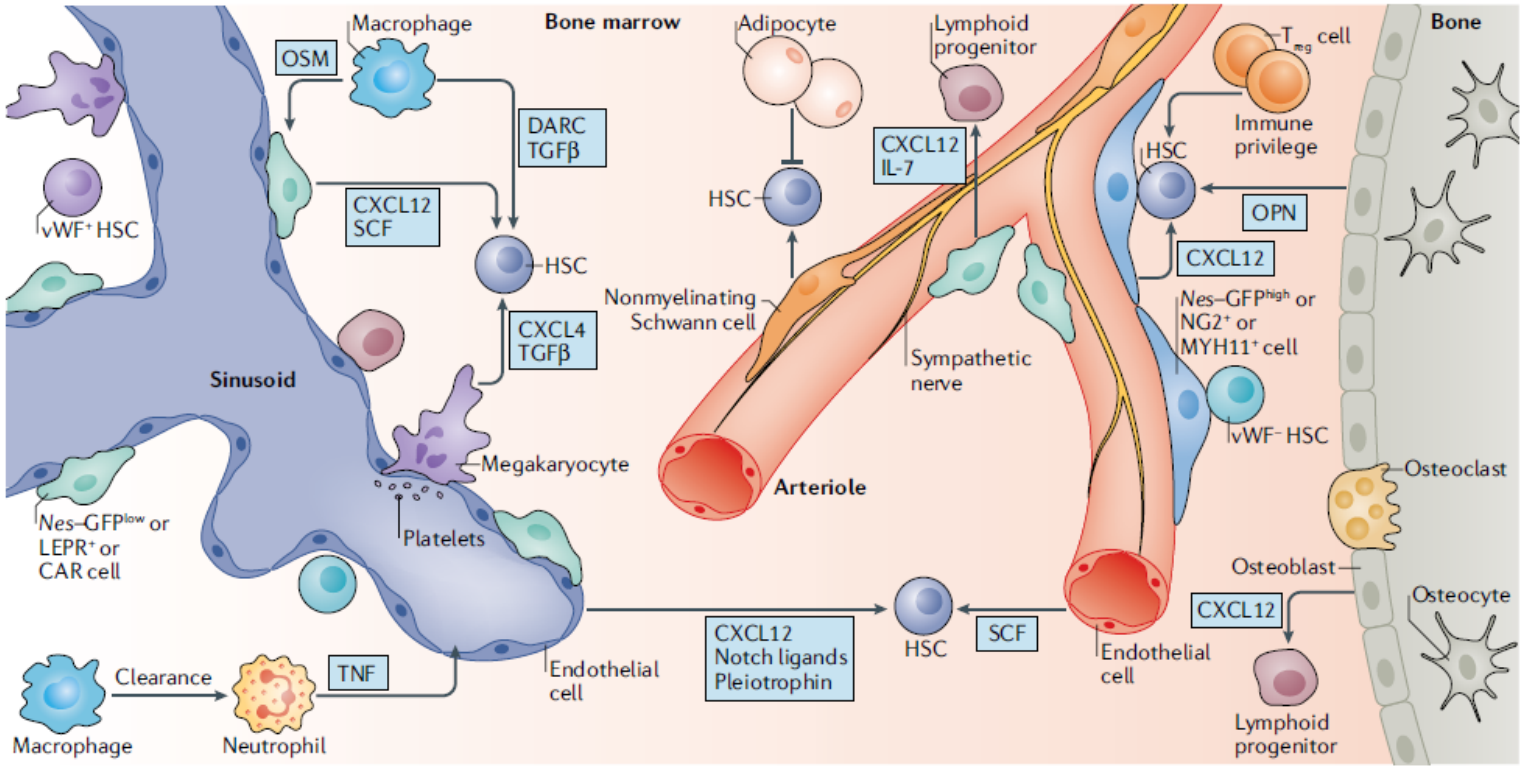
Hematopoietic stem cells - HSC

- 1:10 000 cells in the bone marrow
- Isolated based on Hoescht dye exclusion, resistance to 5-fluorouracil or ϒ irradiation
- Flow-cytometry – lack of CD markers of mature cells, expression of c-Kit (receptor for cytokine stem cell factor)
- Reside in specific niche in the bone marrow



Tsuruta 2012

Adult bone marrow in homeostasis



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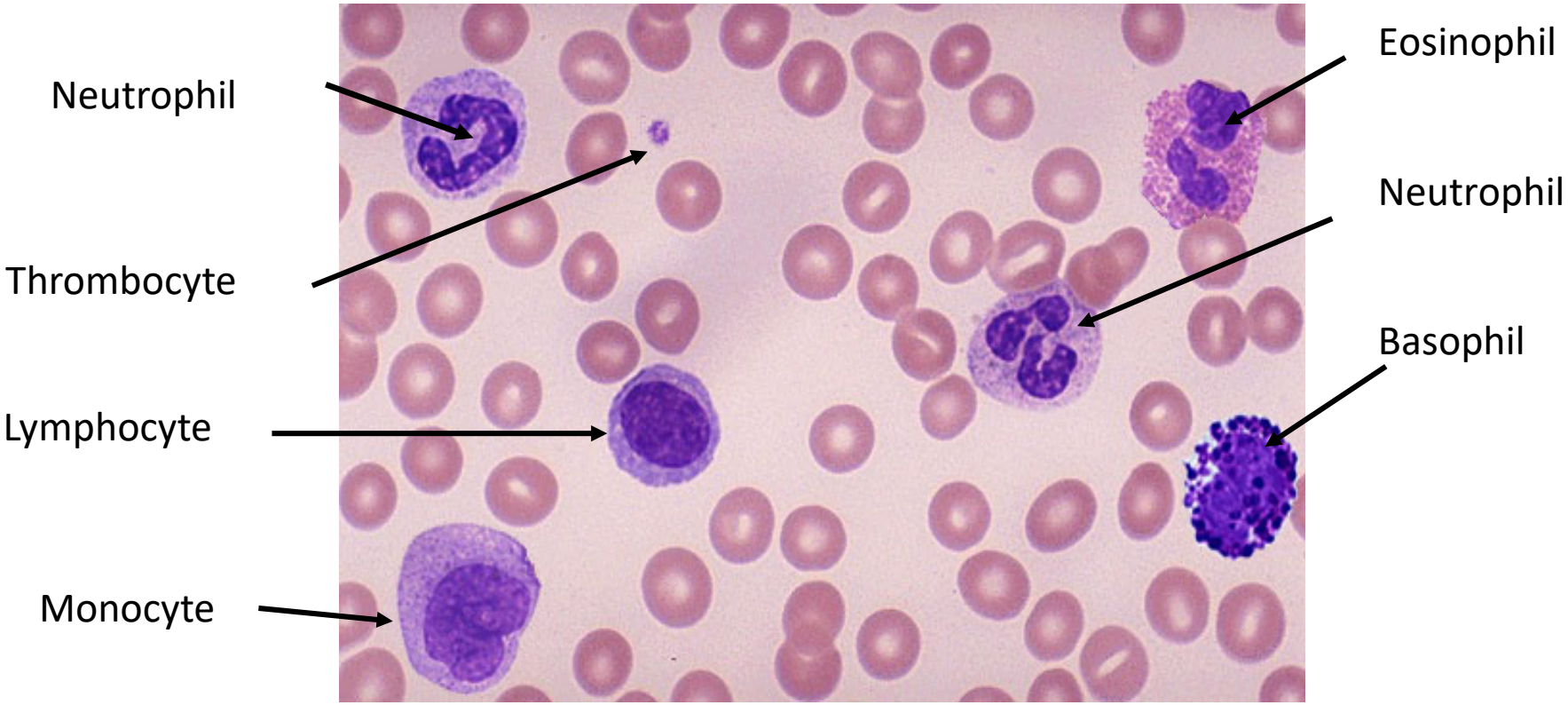
II. Basic overview of blood cells

Blood smear

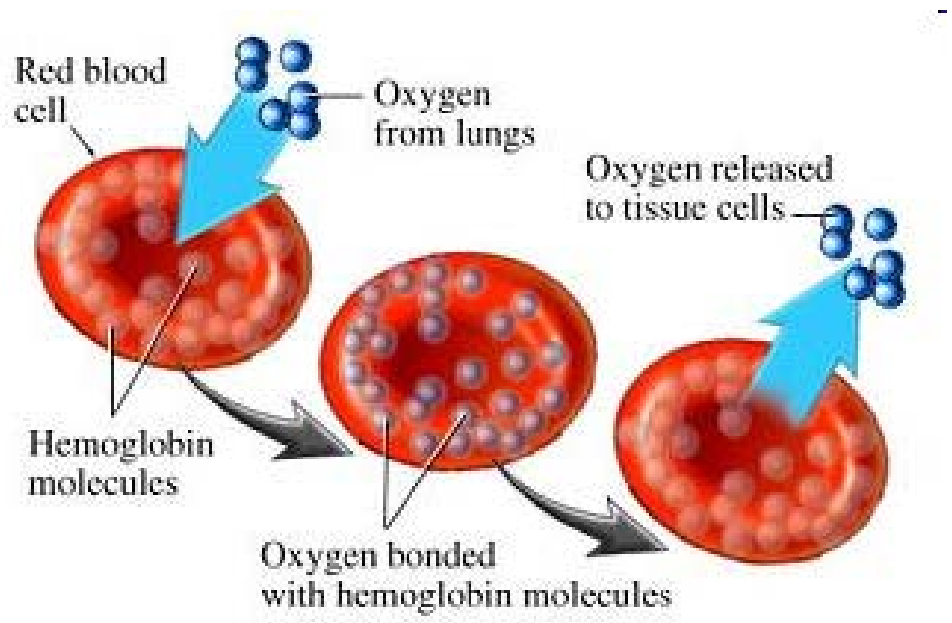

Neutrophil
Thrombocyte
Lymphocyte
Monocyte
Eosinophil
Neutrophil
Basophil

Erythrocytes

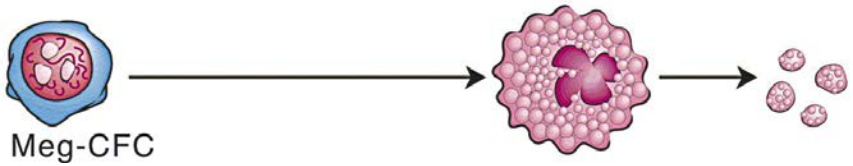
- Round, binconcave (larger area for gas exchange)
- no cell nucleus or organelles

Function

- transport of gases that are bound to hemoglobin inside erythrocytes
- transport of oxygen from lungs to the tissues, of CO2 from tissues to lungs and out of the body



Thrombocytes



- small cells, oval shape, survive for four days, do not contain cell nucleus
- created by fragmentation of cytoplasm of large cells called megakaryocytes

- **Function**
  - ability to adhere and congregate
  - involved in coagulation, every time a blood vessel is injured
  - involved in the production of the thrombus that protects from large loss of blood

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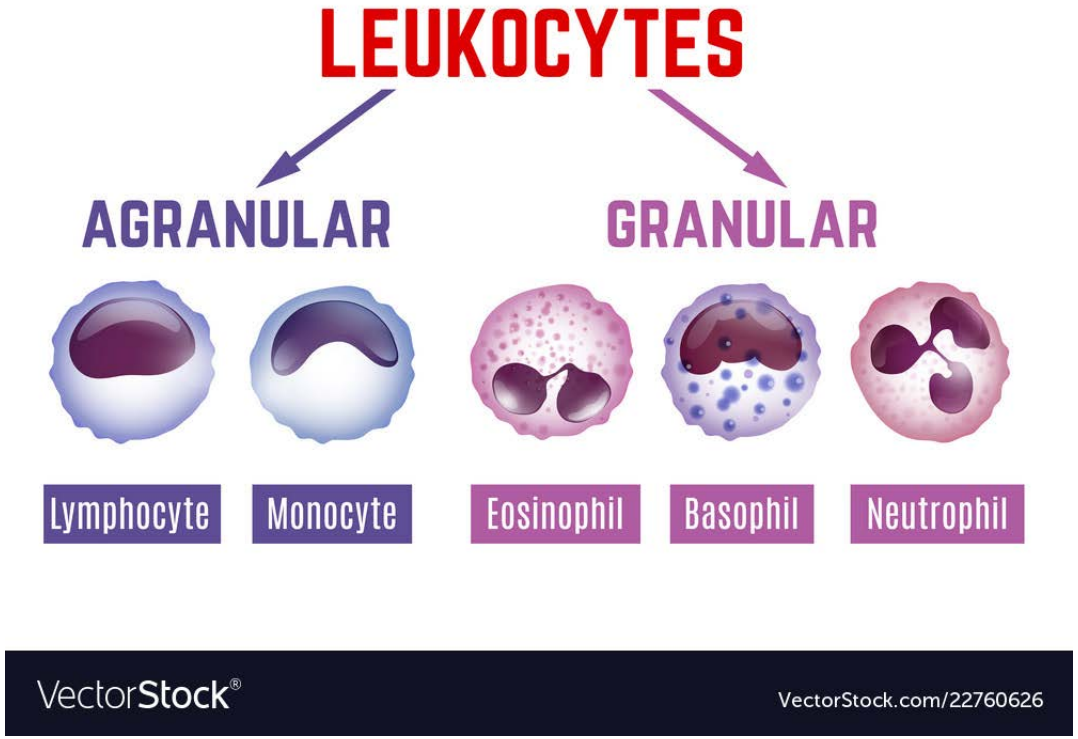
Leukocytes

- blood cells that are lighter in color and contain nucleus in comparison to erythrocytes
- divided based on size, shape of nucleus and function



Leukocytes

- **Function**
  - cells with ability to adhere, perform diapedesis and phagocytosis
  - part of the immune system
  - involved in a protective mechanism of the organism
  - numbers increase in infections and inflammation

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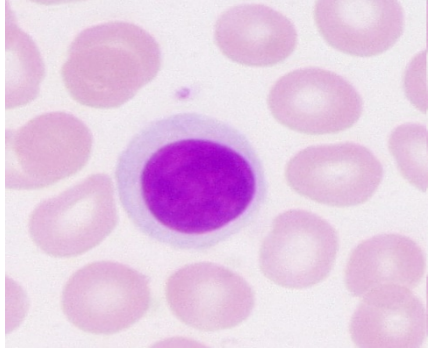
Lymphocytes

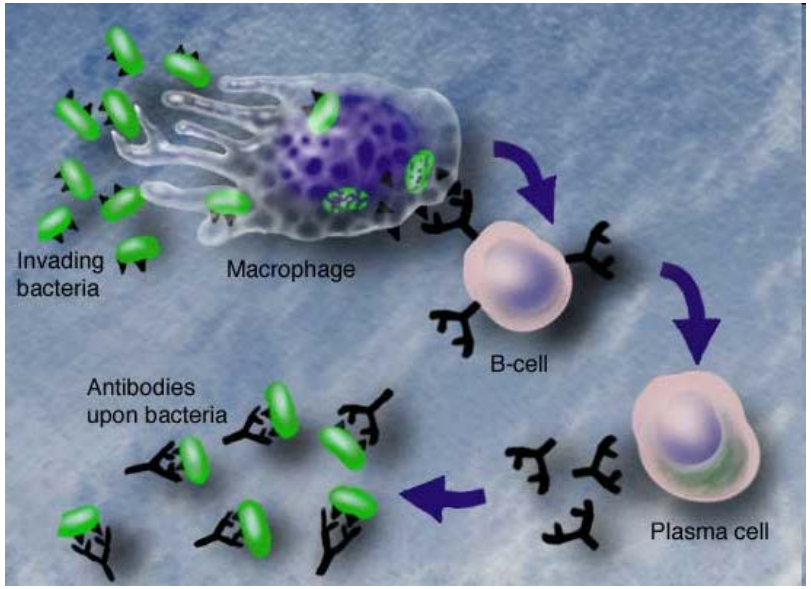


- round cells with a small amount of cytoplasm and one round nucleus
- two basic groups differing in function
  - T – lymphocytes (direct destruction)
  - B – lymphocytes (production of antibodies)

- **Function**
  - involved in specific immunity of the organism- antigen specific receptors
  - small fraction of lymphocytes - in peripheral blood, most are in the bone marrow, spleen, lymph nodes
  - after recognizing a foreigner particle, they start the protective reaction of the organism leading to destruction of the foreign particle

B-lymphocytes

- Originate and mature in the bone marrow, then migrate to lymph nodes, spleen and intestines
- after recognizing an antigen, they turn into plasma cells - production of antibodies (immunoglobulins - Ig)
- plasma cells migrate to peripheral blood, intestines, breast milk, tears etc



B-lymphocytes – production of antibodies

- to recognize and destroy foreign objects in the organism
- specific recognition of antigen based on a principle of a lock and key
- once an antibody reacts to specific antigen, a cascade is started leading to elimination of that pathogen
- Function of antibodies: opsonization, neutralization, complex formation
- 5 classes of antibodies:
  - IgG, IgA, IgM, IgE and IgD

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Antibodies

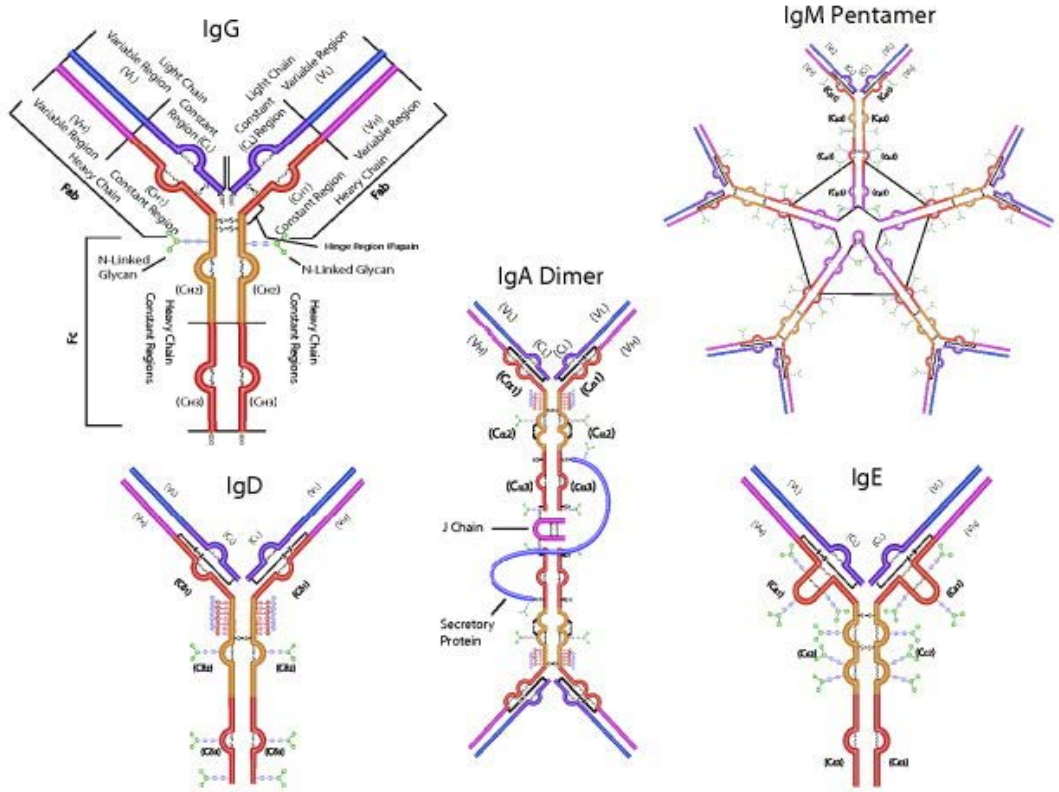
IgG antibodies are able to get into tissues and are the only ones that can enter the fetus through the placenta.

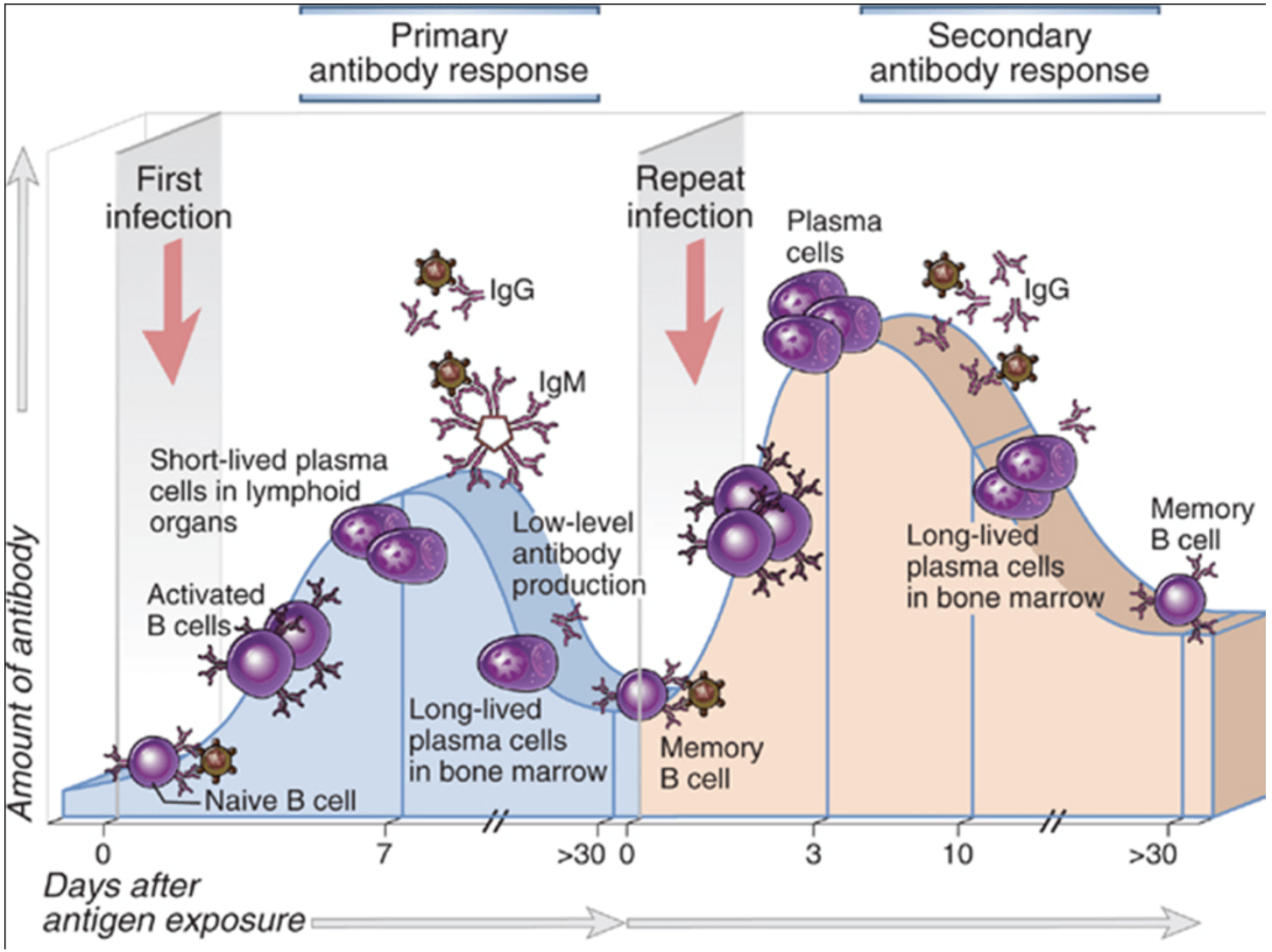
IgA antibodies are produced mainly in the mucous membranes of the intestine and breathing tube and protect the body from microorganisms entering the body

IgM antibodies are produced first during infection. They protect the organism within the first few days before other types of antibodies are produced

IgE antibodies are produced as a protection against parasites and are involved in allergic reactions

IgD antibodies are rare and are involved in histamine release




Primary antibody response
Secondary antibody response
First infection
Repeat infection
IgG
IgM
Plasma cells
IgG
Short-lived plasma cells in lymphoid organs
Activated B cells
Low-level antibody production
Long-lived plasma cells in bone marrow
Memory B cell
Naive B cell
Long-lived plasma cells in bone marrow
Memory B cell
Amount of antibody
0
7
>30
0
3
10
>30
Days after antigen exposure

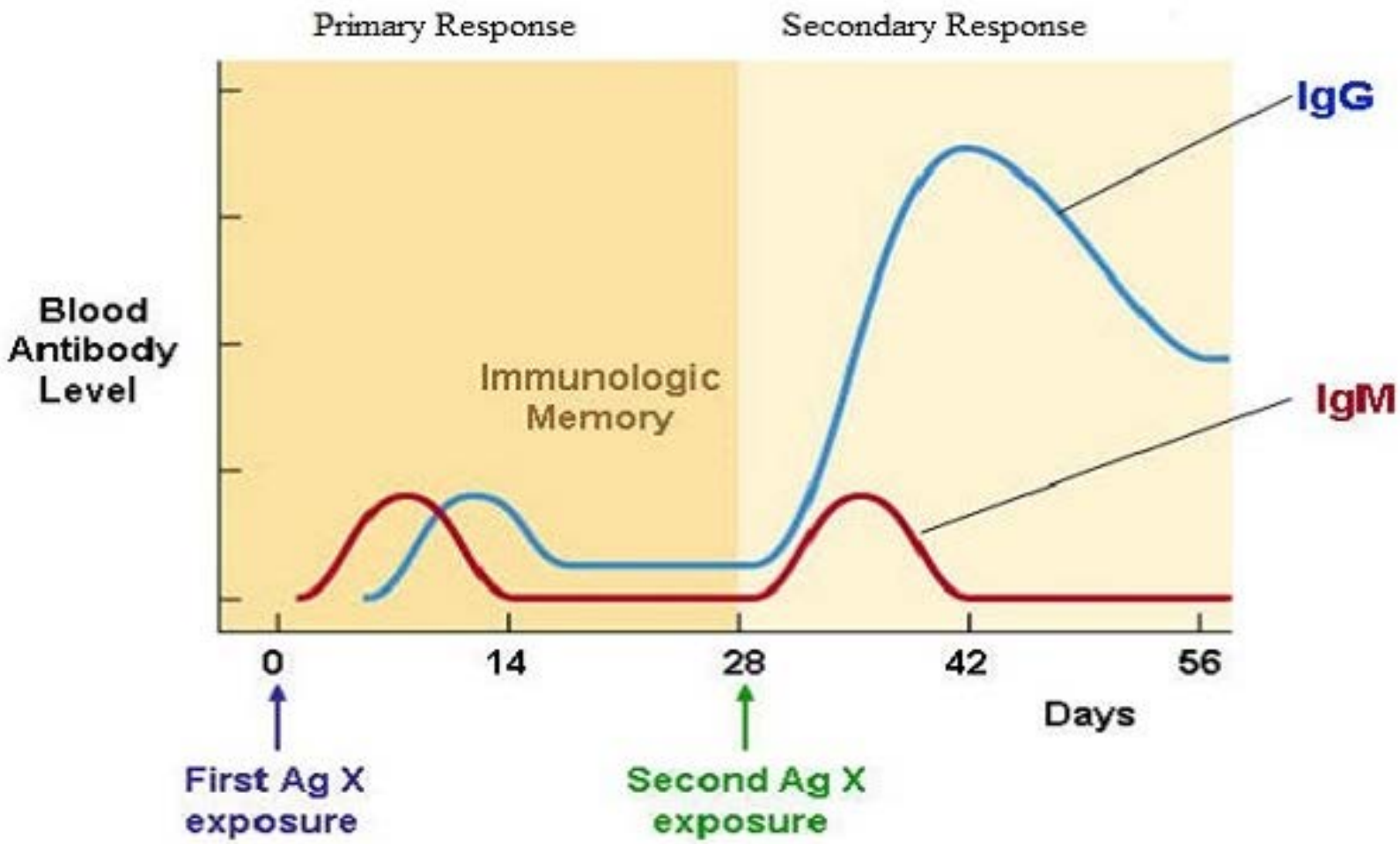


Fig. Immune Response and Secretion of antibodies

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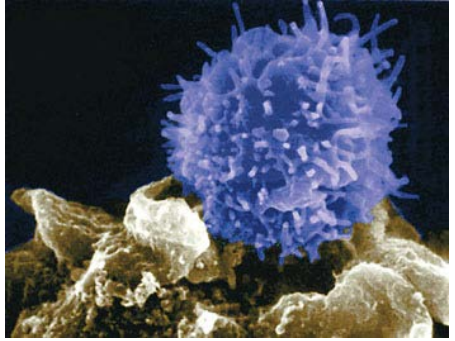
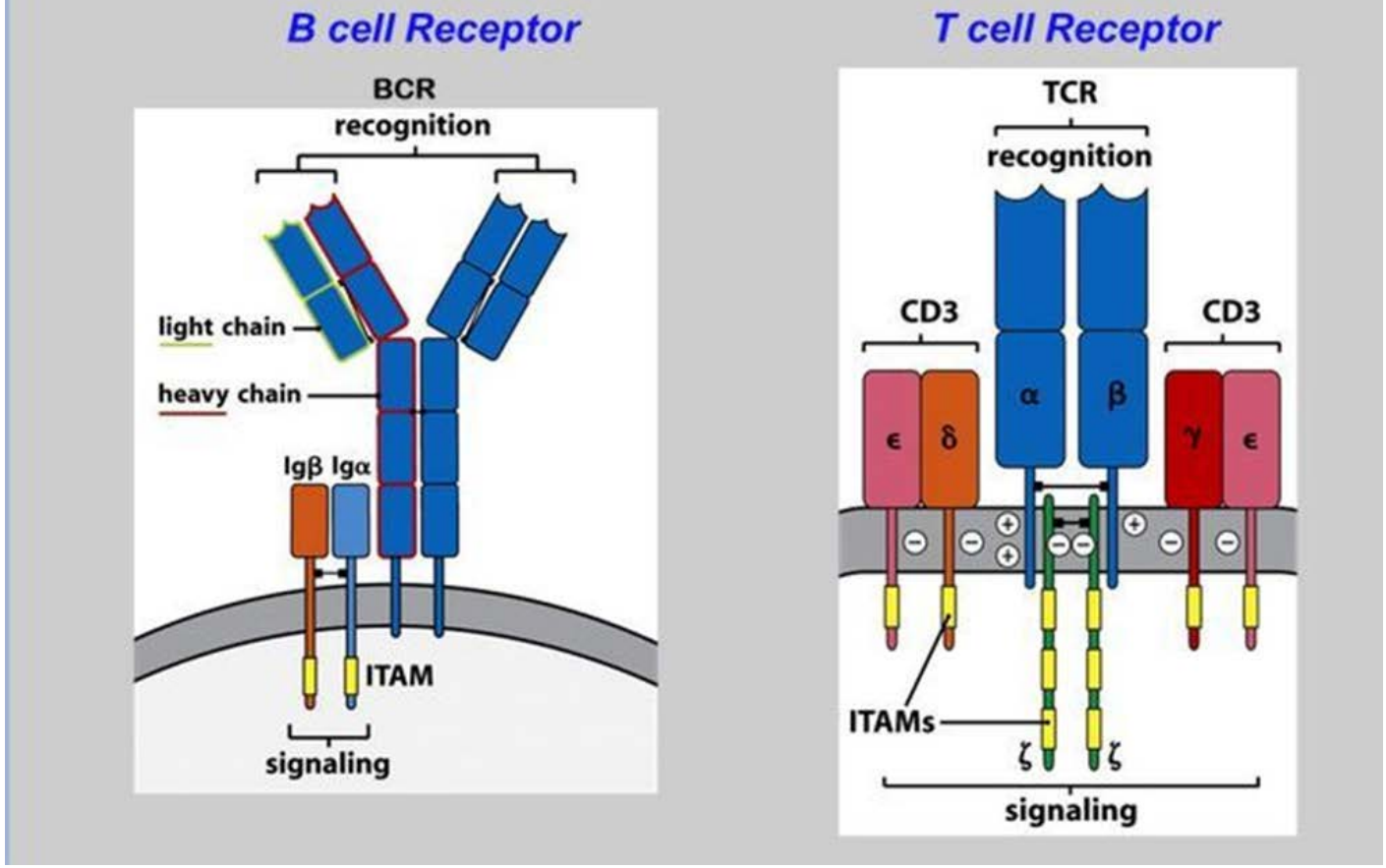
T lymphocytes



- Originate in bone marrow, thymus (if no thymus, no mature T cells)
- Mature T cells migrate to lymphoid organs, especially lymph nodes, spleen, bone marrow and peripheral blood
- Bind antigens using TCR receptors
- Unable to produce antibodies
- destroy cells that had been attacked by microorganisms
- regulate function of other immune cells

T Cell and B Cell Antigen Receptors
(TCR and BCR)



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Classes of T cells

- **Cytotoxic** (Tc)
  - directly kill cells (some viruses are able to survive and duplicate inside cells. Infected cells need to be destroyed so that the infection does not spread)

- **Helper** (Th)
  - support the function of other cells of the immune system (Tc, B cells, macrophages)

T – cells are target cells of HIV virus

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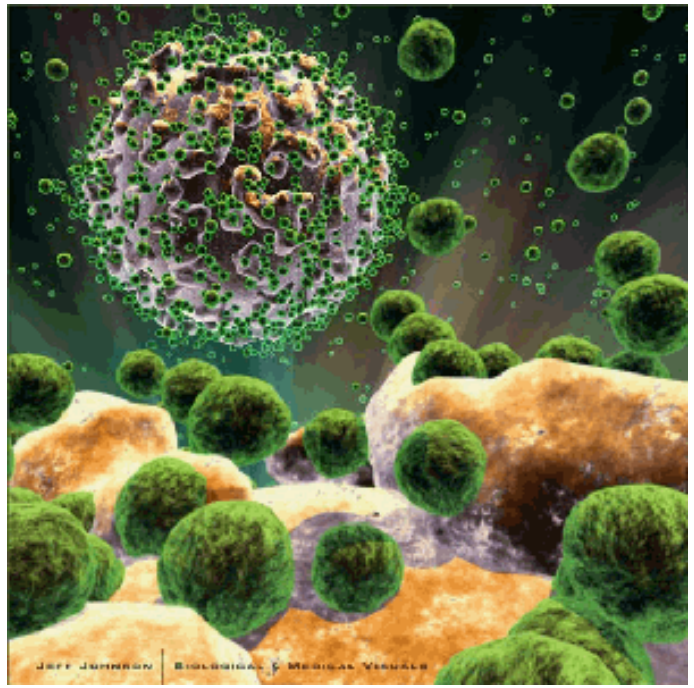
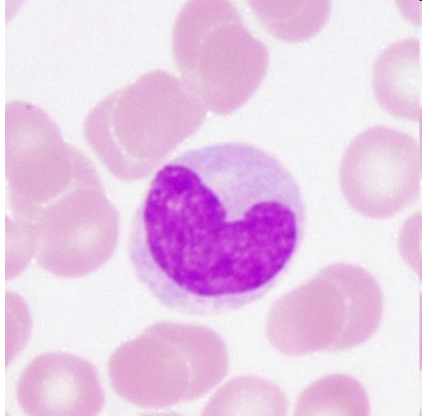
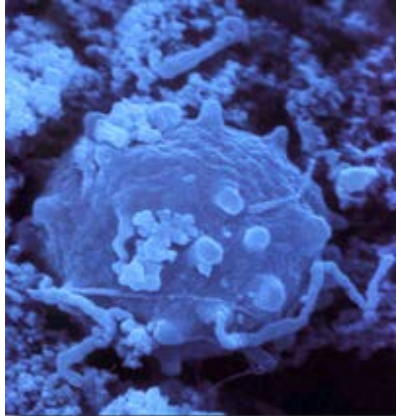
HIV

- acquired immune deficiencies - immune system effected during the lifetime of an individual
- acquired immune deficiency syndrome (AIDS)
- HIV infects Th lymphocytes, macrophages and CNS cells
- after initial infection, virus survives in the body for several years without any symptoms
- then virus replicates - Th cells drastically decrease
- insufficient amount of Th cells leads to opportunistic infections (Kaposi sarcoma…)



Monocytes

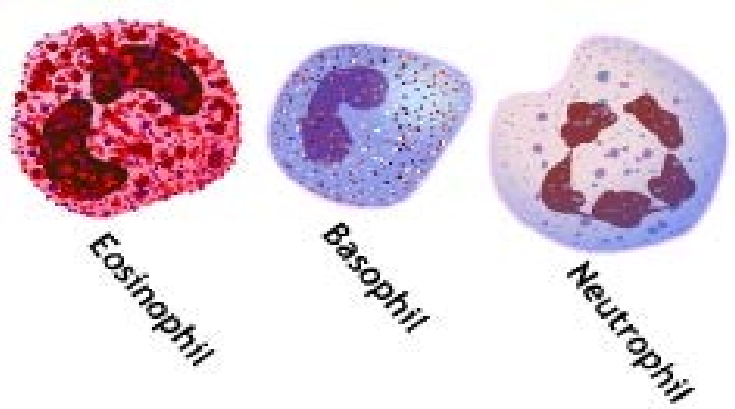




- large cells with a round or kidney shaped nucleus
- created in the bone marrow, migrate to peripheral blood where they circulate for about 8 hours
- then they enter tissues and change into macrophages

- **Function**
- monocytes and macrophages are part of the immune system
- the basic function of macrophages is the phagocytosis of bacteria, foreigner bodies or dead cells

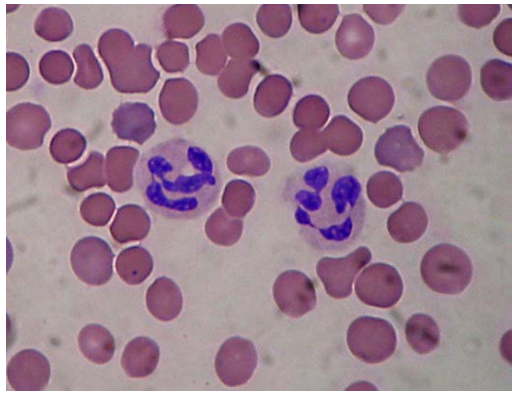
Granulocytes



- Polymorphous nucleus - two to five segments
- cytotoxic granules in the cytoplasm
    - Neutrophil - pinkish purple granules
    - Eosinophil – orange-red granules
    - Basophils - dark blue granules

- **Function**
    - granulocytes are part of the non-specific immunity
    - involved in destruction of bacteria and parasites

Neutrophils



- Most common type of white blood cells with the shortest half life (12 hrs in blood, 1-2 days in tissues)
- Professional phagocytes - inflammation
- **Function:**
  - Phagocytosis (if opsonization, phagocytosis is easier)
    - Opsonization – process increasing effectivity of phagocytosis
  - Chemotaxis – ability to migrate to a place with highest concentration of bacteria
  - Diapedesis – ability to migrate from peripheral blood into the place of inflammation through the wall of the vein
- Perform phagocytosis only once, then they die

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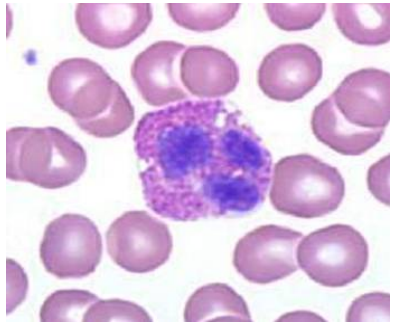
Eosinophils



- weak phagocytes
- main function is protection against parasites
  - Accumulate in places where parasites enter body (lungs, GIT)
  - Release granules that contain chemicals attacking the parasites

- involved in allergic reaction

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Basophils

- Least common of all granulocytes and leukocytes
- Receptors for IgE on membrane
- Their granules contain heparin and histamine- inflammation and allergies
- Mast cells – in tissues and connecting tissues

- **Histamine**
  - Effects muscles, increases permeability of blood vessels
  - Massive release during allergic reaction

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III. Hematological malignancies

Important definitions

- Incidence - number of new cases of a disease diagnosed each year

- Prevalence - a measure of the total number of people in a specific group who have (or had) a certain disease, condition, or risk factor at a specific point in time or during a given period of time.

- Overall survival - length of time from either the date of diagnosis or the start of treatment for a disease, such as cancer, that patients diagnosed with the disease are still alive.

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Important definitions

- Remission – a decrease or disappearance of signs and symptoms of cancer, including normalization of lab values (blood count) and imaging methods (X ray, ultrasound, CT) in response to treatment.

- Complete remission - The disappearance of all signs of cancer in response to treatment. This does not always mean the cancer has been cured. Also called complete response.

- In hematological malignancies (leukemias), the total number of leukemic cells in blood is observed. Partial remission means decrease of leukemic cells by at least 50%.

- Relapse - return of a disease or the signs and symptoms of a disease after a period of improvement. Reaching remission does not mean cure as there might be lesions that are impossible to detect and may become the source of new return of the disease.

https://www.cancer.gov/publications/dictionaries/cancer-terms/search?contains=false&q=overall+survival

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Minimal residual disease - MRD

- Tumor cells not eradicated by the treatment
- Usually results in growth of these cells – resistance to treatment
- Emerging component of CR assessment in MM patients

- MRD negativity - associated with significantly longer OS in MM patients

Paiva et al, 2008; Rawston et al., 2013

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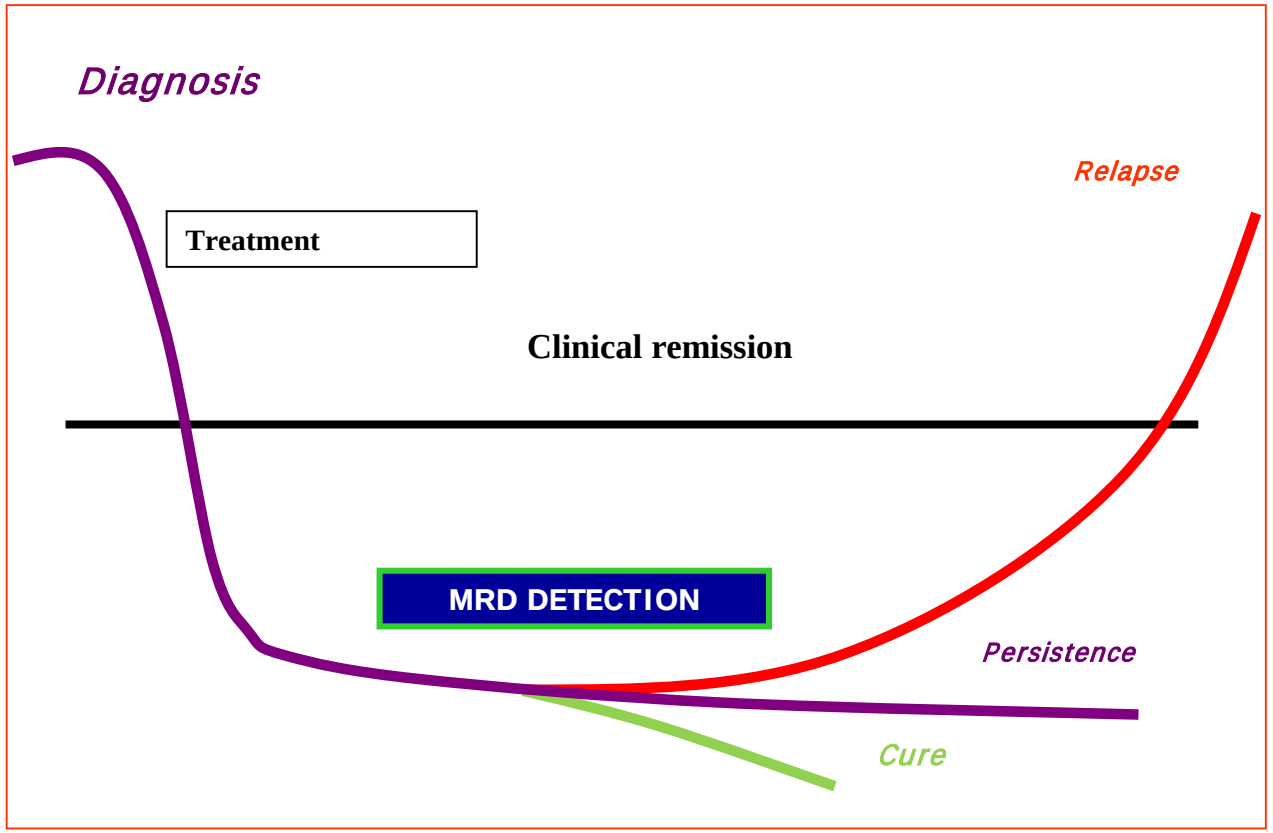
Minimal residual disease



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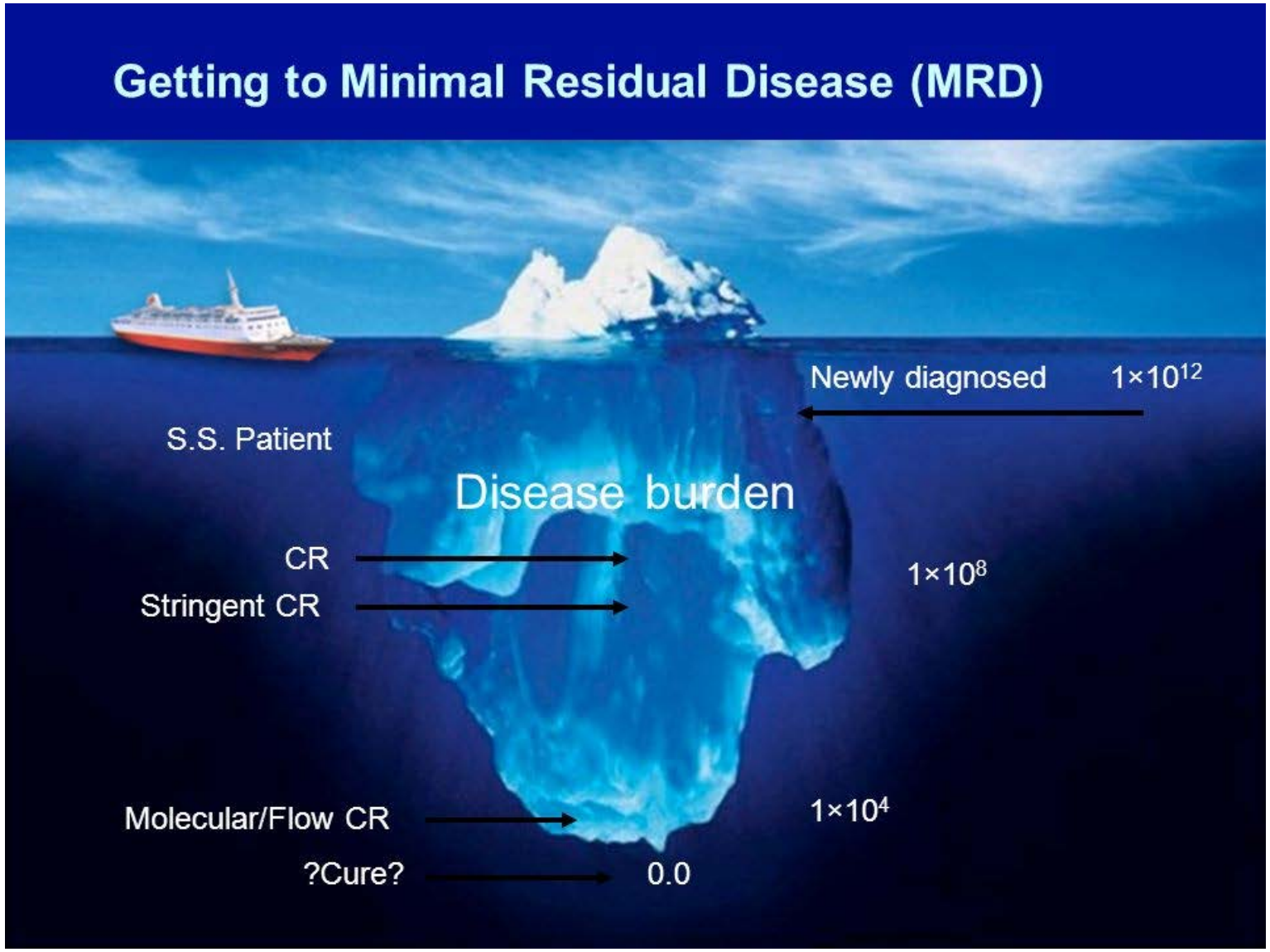
Getting to Minimal Residual Disease (MRD)
Newly diagnosed
1×10^12
S.S. Patient
Disease burden
CR
Stringent CR
1×10^8
Molecular/Flow CR
1×10^4
?Cure?
0.0

Hematological malignancies

Leukemia

Lymphoma

Multiple myeloma

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Hematological malignancies

Leukemia

Lymphoma

Multiple myeloma

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Leukemia

- From Greek – leukos-white, hemos-blood
- Symptoms known in the era of Hippokrates (460 - 370 BC)
- R. Wirchow described in 1839 – 1845, when microscopy was used
- R. Wirchow named leukemia
- „Omnis cellula e cellula“

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Leukemia

- heterogeneous group of diseases
- most common tumors in children
- leukemic cells lose the ability to differentiate, high proliferation potential
- two cell populations in the body - mature cells and immature cells = blasts

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Clinical features

- Erythropenia – anemia
- Thrombocytopenia – bleeding
- Leukocytopenia – infections

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Prognosis of leukemia

Morphology

Chromosomal aberrations

Age – worse prognosis

B cells - worse prognosis

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Treatment of leukemia

- Induction – treatment given with intent to induce complete remission
- Consolidation – repetition of induction in a patient with induced complete remission to increase cure rate
- Maintenance – long-term, low-dose treatment to delay regrowth of residual tumor cells

- radiation and chemotherapy (combination)

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After chemotherapy

- biopsy of bone marrow
- further treatment if 5-10% of blasts
- bone marrow transplantation

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Leukemia

Acute

Chronic

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Leukemia

Acute

Chronic

Myeloid

Lymphoid

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Acute leukemia

- fast proliferation of immature cells
- bone marrow does not produce enough healthy cells
- leukemic cells get into peripheral blood and infiltrate other organs (even CNS)
- fast treatment needed – „medical emergency“
- most common in children

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Chronic leukemia

- proliferation of relatively mature but abnormal cells
- lasts for months or years
- treatment not necessary at once in comparison to acute leukemia
- mostly in older people

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ALL – more common in children

AML – more common in elderly

CLL – most common in adults

CML – mostly in adults

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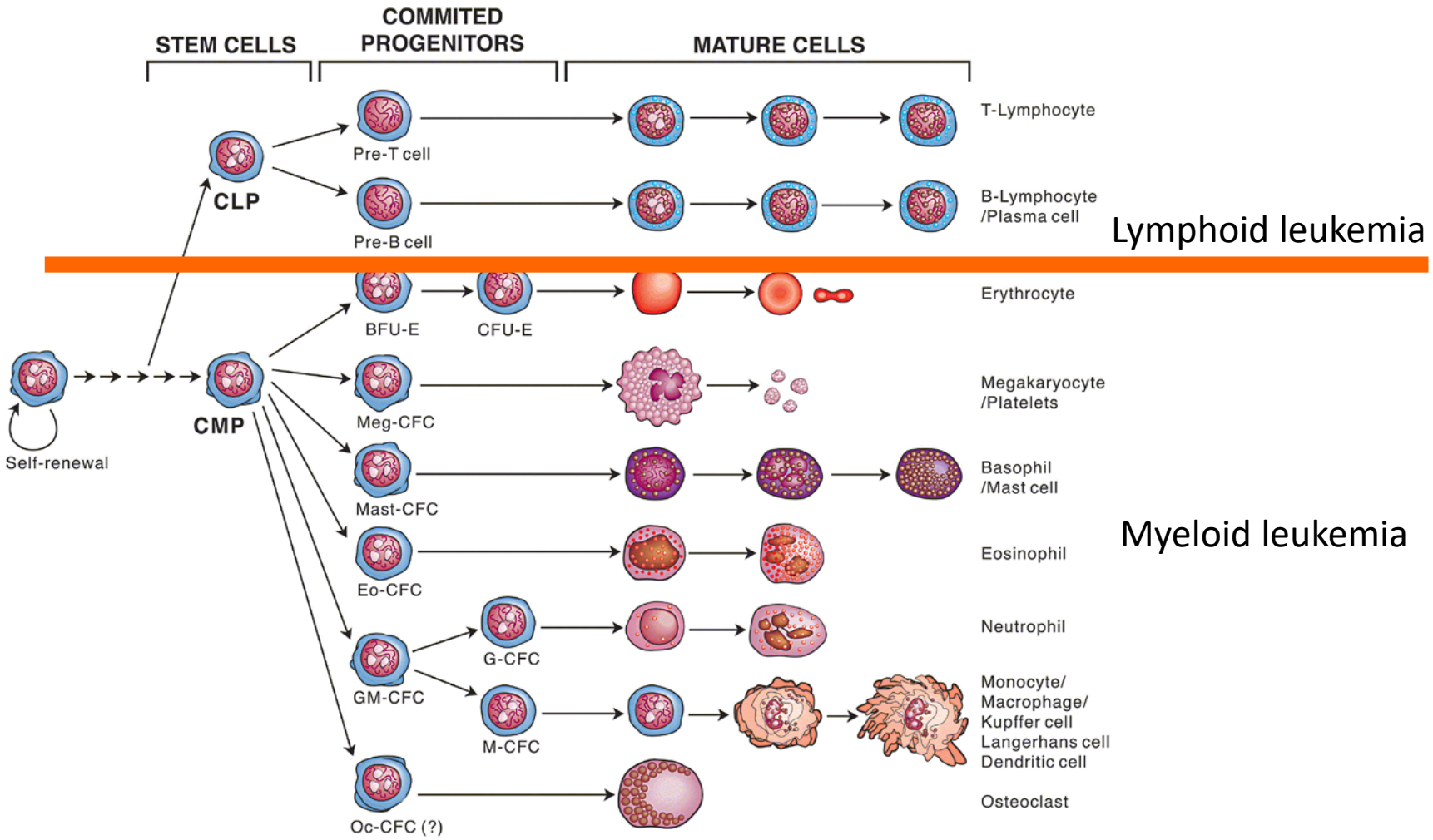
Hematopoesis



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Risk factors for leukemia development

- ionizing radiation
- chemicals – benzene, cytostatics, alkylators and carcinogens
- syndrome: Down (trisomy 21), Klinefelter (47, XXY)
- viruses – HTLV-1 causes development of leukemia from T cells in adults
- secondary leukemia - common after treatment for other malignancies

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Acute myeloid leukemia - AML

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Acute myeloid leukemia - AML

- Fatigue, fever, bleeding
- accumulation of blasts in bone marrow (> 20 %), bone marrow failure
- Blasts in peripheral blood
- Differentiation block at various stages of development
- Most common leukemia in adults over 65 (80%)
- about 20,000 of newly diagnosed patients in a year
- Incidence 1.3/100 000 until 65, 12.5/100 000 over 65
- 70% of patients die within one year after diagnosis

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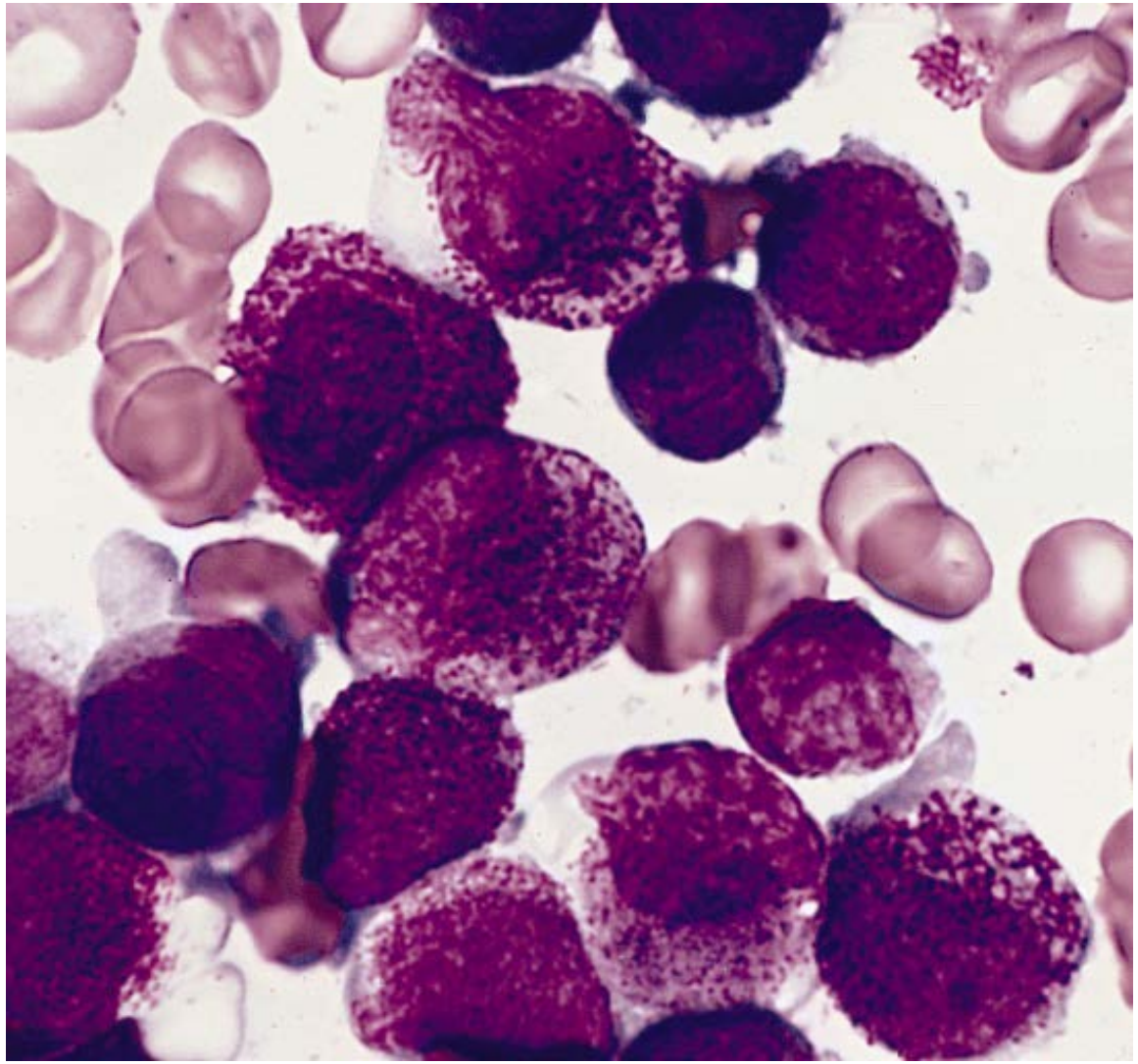

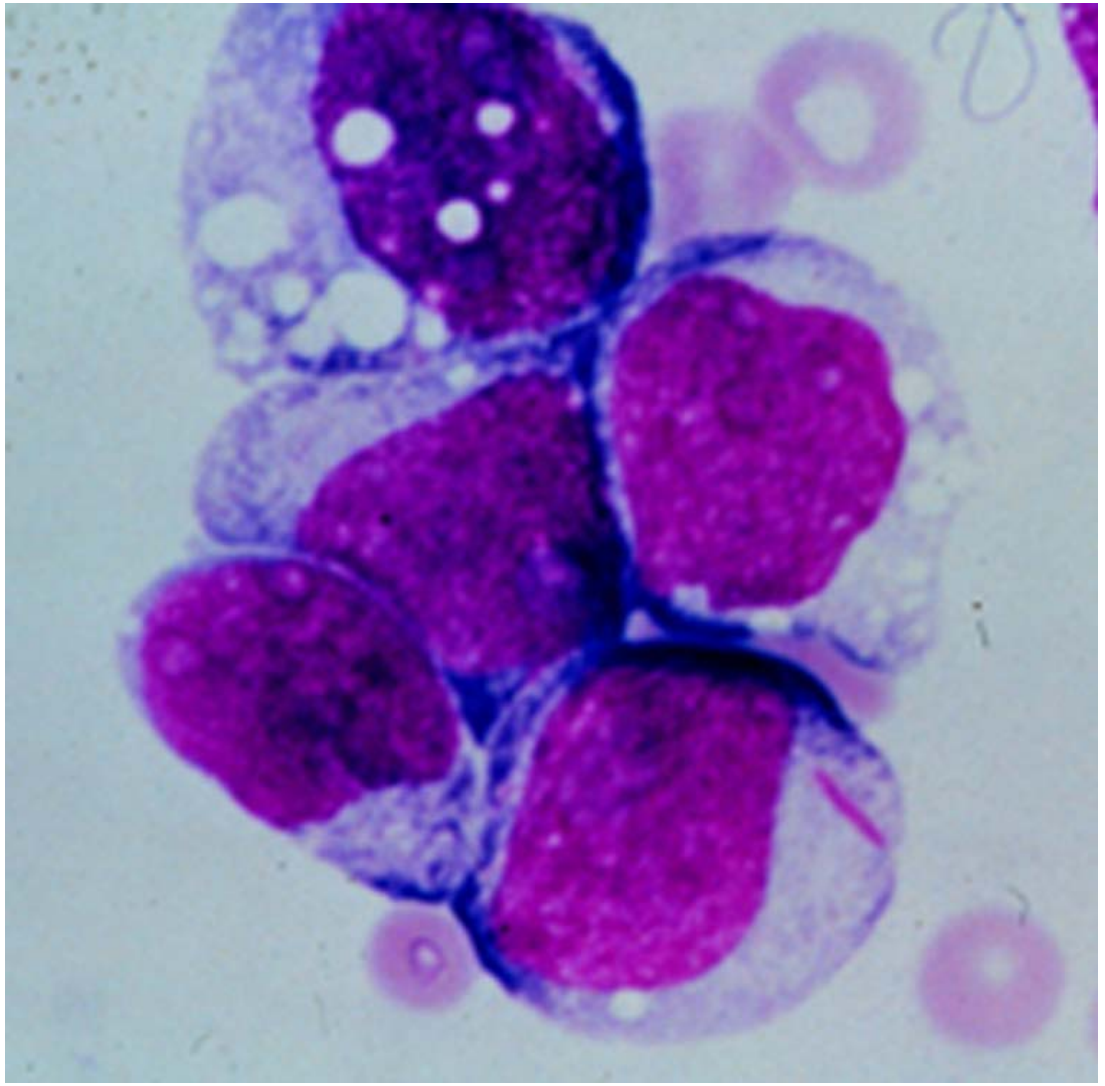

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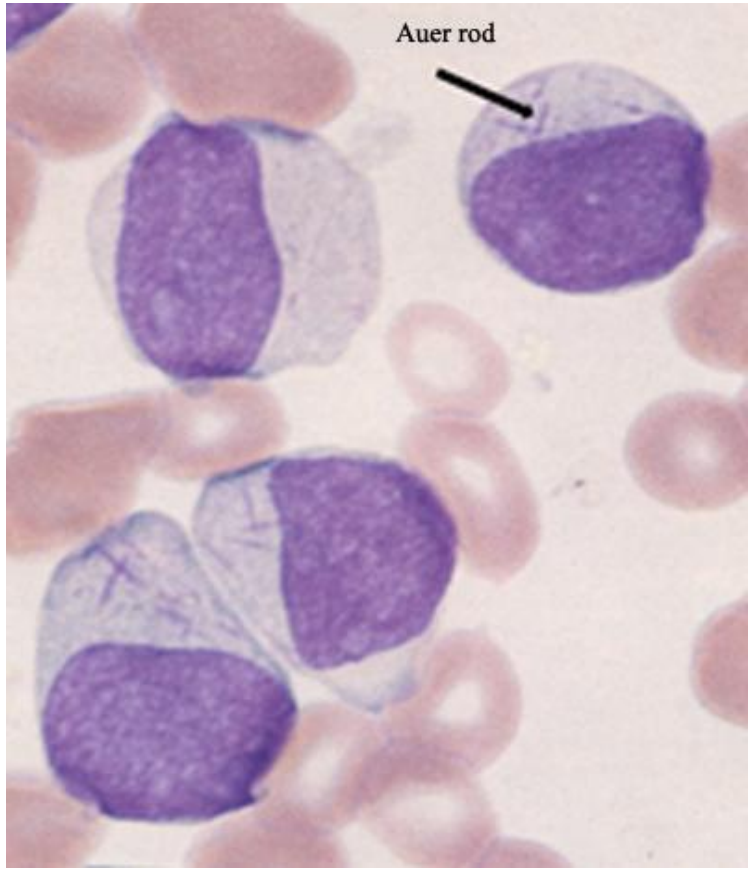
Auer rods

- typical feature of AML
- in cytoplasm of myeloblasts
- negative prognostic marker
- abnormal fusion of primary granules
- Identified in 1905



Prognosis of AML

Morphology

Chromosomal aberrations

Age at diagnosis

Number of leukocytes at diagnosis
FAB classification

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Classification of AML

FAB -

French American British classification

- 8 subtypes
- based on morphology and cytochemistry

WHO classification

- based on molecules, morphology and clinics

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FAB Classification

Classification of AML

AML w/o maturation	M0	no azurophil granules	-
AML	M1	few Aeur rods	del(5); del(7); +8
AML w/ differentiation	M2	maturation beyond promyelocytes; Auer rods	t(8:21) t(6:9)
Acute Promyelocytic Leukemia	M3	hypergranular promyelocytes; Auer rods	t(15:17)
Acute Myelomonocytic Leukemia	M4	> 20% monocytes; monocytoid cells in blood	inv(16) del(16) t(16:16) t(4:11)
Acute Monocytic Leukemia	M5	monoblastic; promonocytic	t(9:11) t(10:11)
Acute Erythroleukemia	M6	predominance of erythroblasts; dyserythropoiesis	-
Acute Megakaryocytic Leukemia	M7	dry' aspirate; biopsy dysplastic with blasts	-

Table 1. 2016 WHO classification of mature lymphoid, histiocytic, and dendritic neoplasms

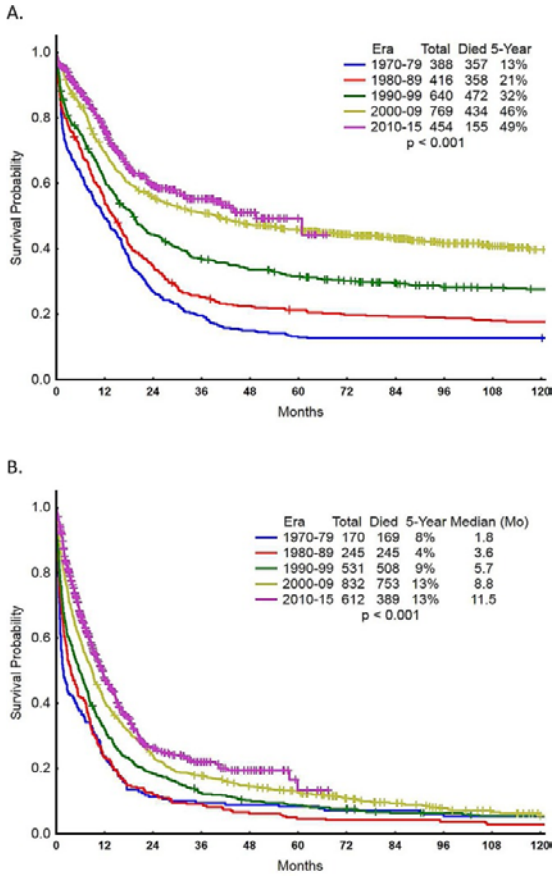
Mature B-cell neoplasms
Chronic lymphocytic leukemia/small lymphocytic lymphoma
Monoclonal B-cell lymphocytosis*
B-cell prolymphocytic leukemia
Splenic marginal zone lymphoma
Hairy cell leukemia
Splenic B-cell lymphoma/leukemia, unclassifiable
Splenic diffuse red pulp small B-cell lymphoma
Hairy cell leukemia-variant
Lymphoplasmacytic lymphoma
Waldenström macroglobulinemia
Monoclonal gammopathy of undetermined significance (MGUS), IgM*
μ heavy-chain disease
γ heavy-chain disease
α heavy-chain disease
Monoclonal gammopathy of undetermined significance (MGUS), IgG/A*
Plasma cell myeloma
Solitary plasmacytoma of bone
Extraosseous plasmacytoma
Monoclonal immunoglobulin deposition diseases*
Extranodal marginal zone lymphoma of mucosa-associated lymphoid tissue (MALT lymphoma)
Nodal marginal zone lymphoma
Pediatric nodal marginal zone lymphoma
Follicular lymphoma
In situ follicular neoplasia*
Duodenal-type follicular lymphoma*
Pediatric-type follicular lymphoma*
*Large B-cell lymphoma with IRF4 rearrangement**
Primary cutaneous follicle center lymphoma
Mantle cell lymphoma
In situ mantle cell neoplasia*
Diffuse large B-cell lymphoma (DLBCL), NOS
Germinal center B-cell type*
Activated B-cell type*
T-cell/histiocyte-rich large B-cell lymphoma
Primary DLBCL of the central nervous system (CNS)
Primary cutaneous DLBCL, leg type
EBV$^+$ DLBCL, NOS*
*EBV$^+$ mucocutaneous ulcer**
DLBCL associated with chronic inflammation
Lymphomatoid granulomatosis
Primary mediastinal (thymic) large B-cell lymphoma

Table 1. (continued)

Monomorphic epitheliotropic intestinal T-cell lymphoma*
*Indolent T-cell lymphoproliferative disorder of the GI tract**
Hepatosplenic T-cell lymphoma
Subcutaneous panniculitis-like T-cell lymphoma
Mycosis fungoides
Sézary syndrome
Primary cutaneous CD30$^+$ T-cell lymphoproliferative disorders
Lymphomatoid papulosis
Primary cutaneous anaplastic large cell lymphoma
Primary cutaneous γδ T-cell lymphoma
Primary cutaneous CD8$^+$ aggressive epidermotropic cytotoxic T-cell lymphoma
*Primary cutaneous acral CD8$^+$ T-cell lymphoma**
*Primary cutaneous CD4$^+$ small/medium T-cell lymphoproliferative disorder**
Peripheral T-cell lymphoma, NOS
Angioimmunoblastic T-cell lymphoma
*Follicular T-cell lymphoma**
*Nodal peripheral T-cell lymphoma with TFH phenotype**
Anaplastic large-cell lymphoma, ALK$^+$
Anaplastic large-cell lymphoma, ALK$^-$*
*Breast implant–associated anaplastic large-cell lymphoma**
Hodgkin lymphoma
Nodular lymphocyte predominant Hodgkin lymphoma
Classical Hodgkin lymphoma
Nodular sclerosis classical Hodgkin lymphoma
Lymphocyte-rich classical Hodgkin lymphoma
Mixed cellularity classical Hodgkin lymphoma
Lymphocyte-depleted classical Hodgkin lymphoma
Posttransplant lymphoproliferative disorders (PTLD)
Plasmacytic hyperplasia PTLD
Infectious mononucleosis PTLD
Florid follicular hyperplasia PTLD*
Polymorphic PTLD
Monomorphic PTLD (B- and T-/NK-cell types)
Classical Hodgkin lymphoma PTLD
Histiocytic and dendritic cell neoplasms
Histiocytic sarcoma
Langerhans cell histiocytosis
Langerhans cell sarcoma
Indeterminate dendritic cell tumor
Interdigitating dendritic cell sarcoma
Follicular dendritic cell sarcoma
Fibroblastic reticular cell tumor
Disseminated juvenile xanthogranuloma
Erdheim-Chester disease*

WHO classification
Swerdlow 2016

Differences in survival of young and older AML patients



- upper graph shows survival of younger patients from 1970 (<60 years)
- lower graph shows survival of older patients from 1970
- Kantarjian et al 2015 - MD Anderson

MUNI MED

Acute promyelocytic leukemia - APL

the most malignant human leukemia

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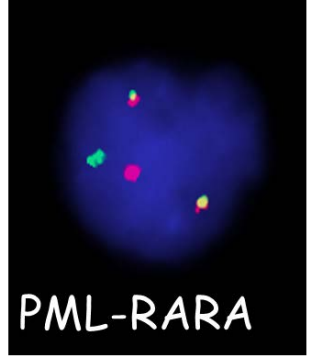
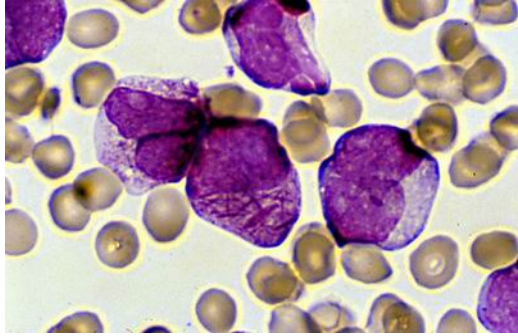
APL

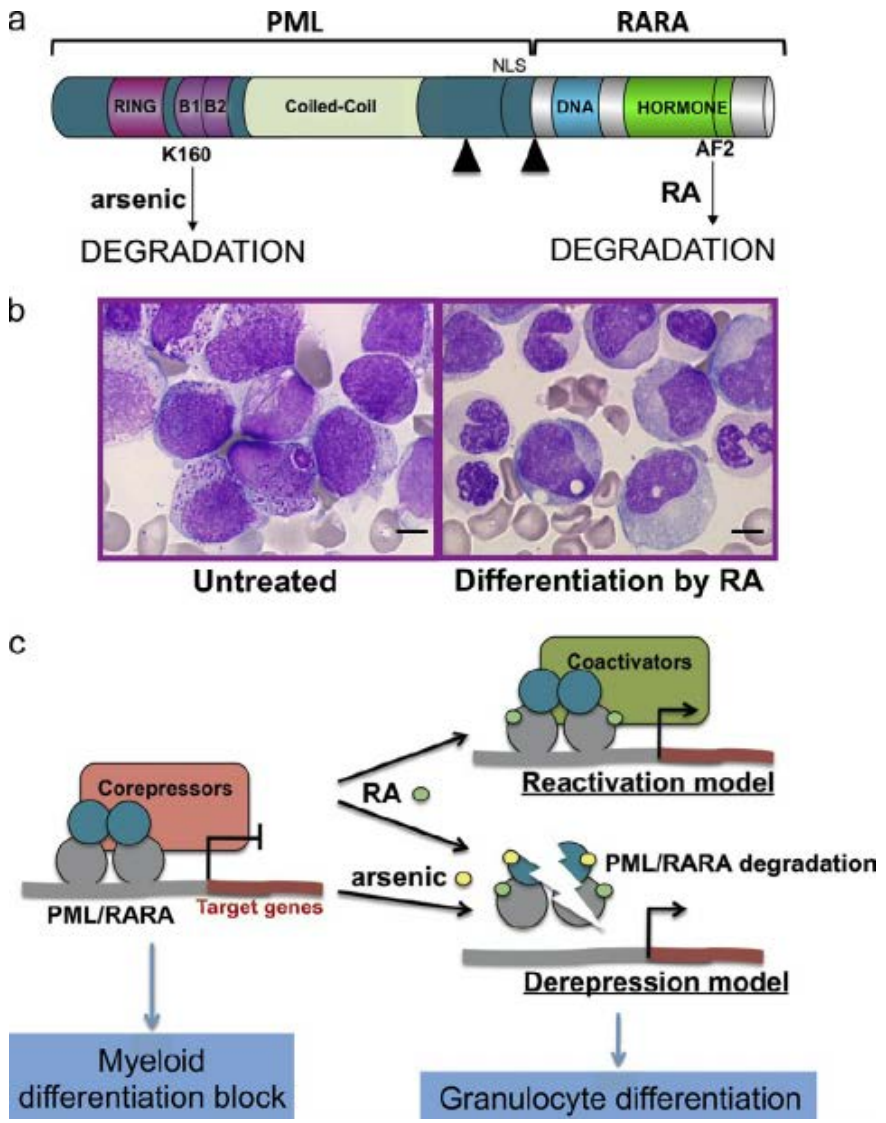




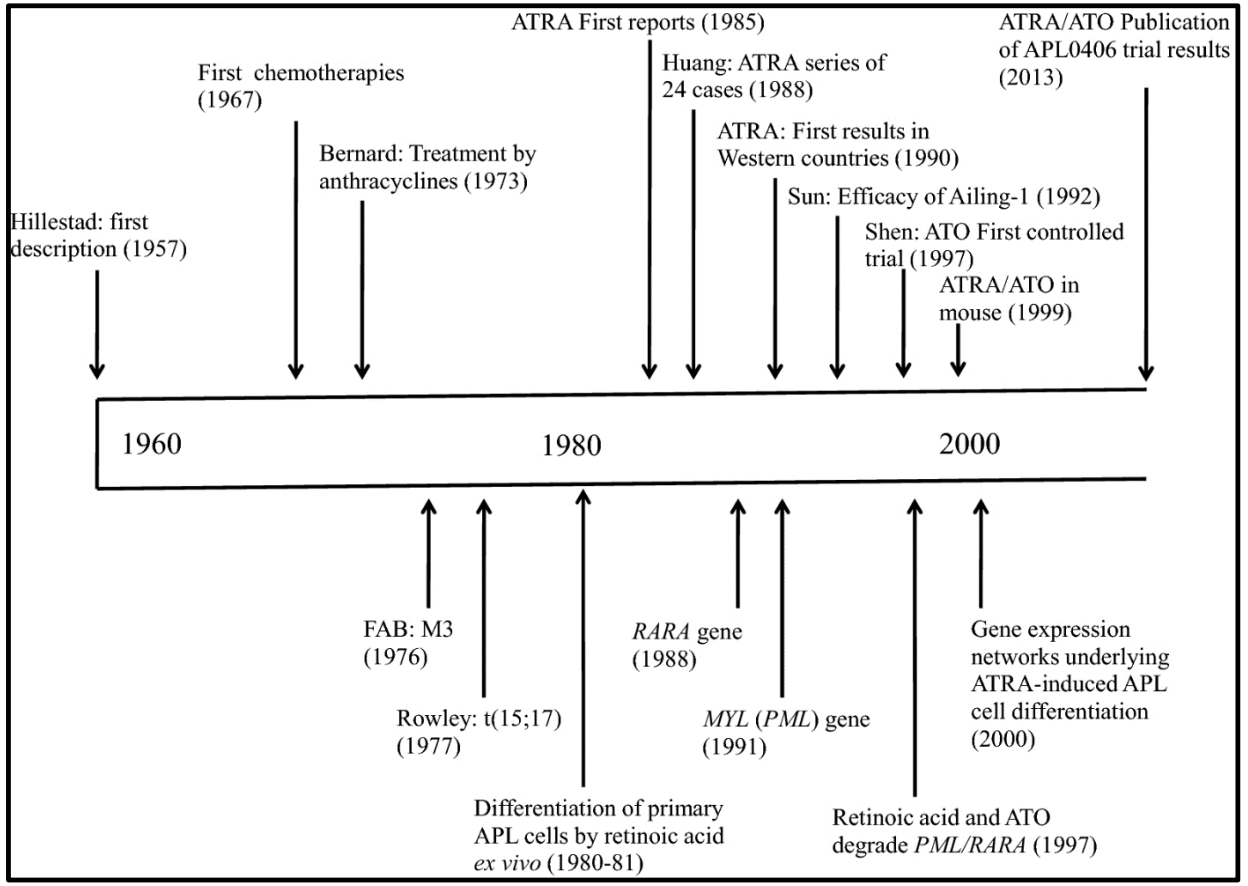
- accumulation of promyelocytes (differentiation stage of granulocytes)
- M3 classification based on FAB
- treatment commenced immediately – medical emergency
- for a diagnosis - detection of translocation necessary
- median age at diagnosis 40 - same risk throughout lifetime
- 1957 - subtype of leukemia
- 1970 – identification of translocation - Dr. J. Rowley

Molecular basis of APL

- RARα – receptor pro all-trans retinoic acid
- PML – promyelocytic gene
- Translocation t(15;17) – reciprocal translocation



APL treatment



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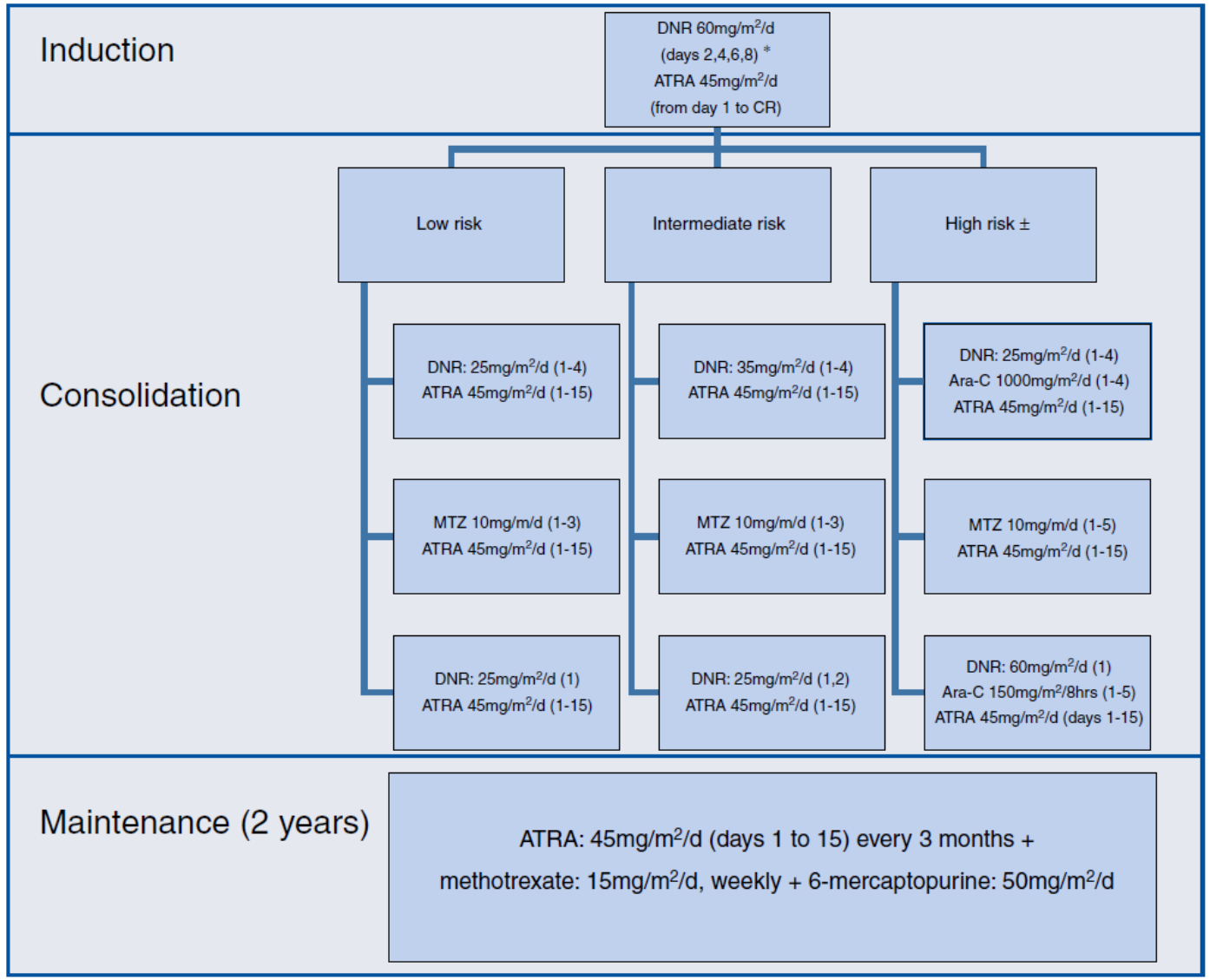
APL treatment



Crespo-Solis 2016

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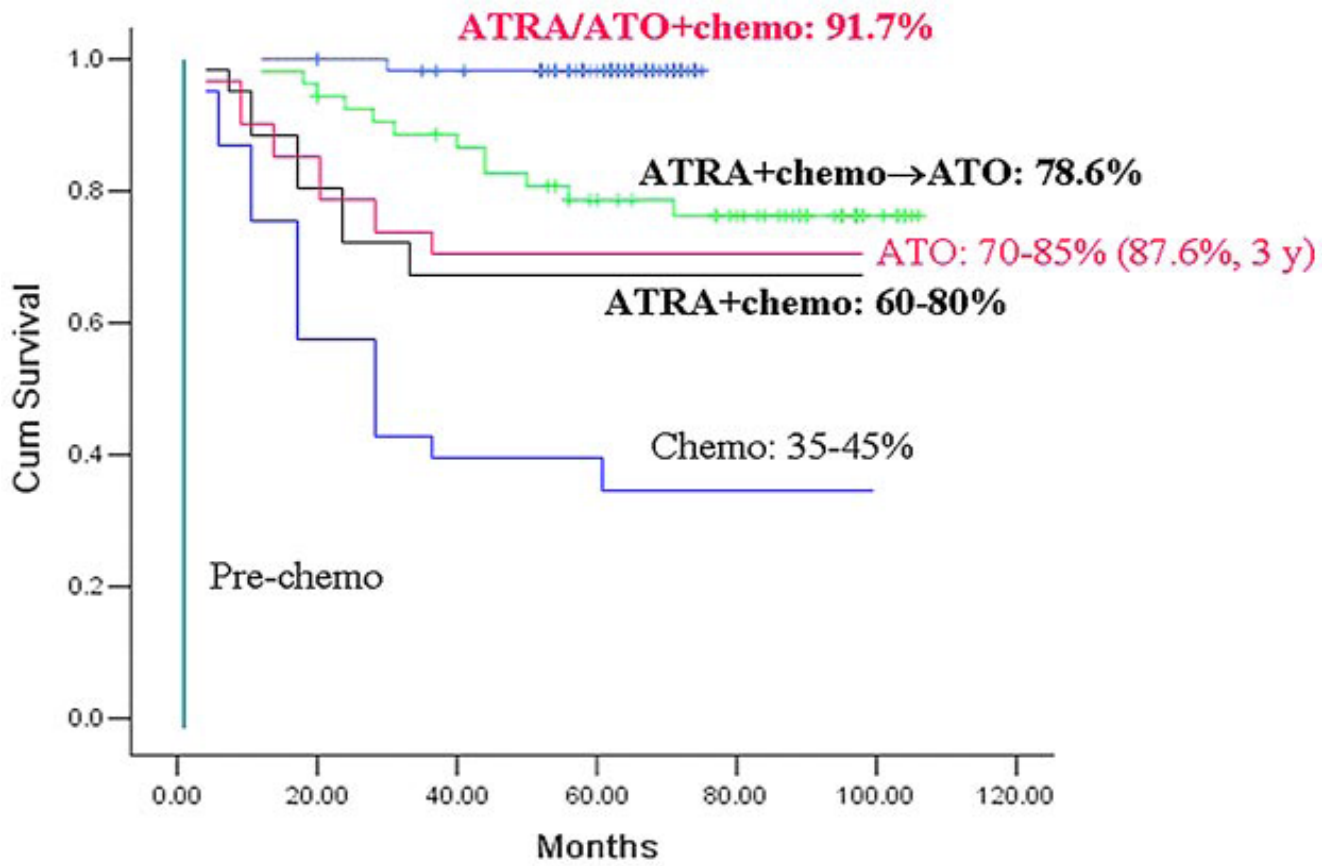
APL survival



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Acute lymphoid leukemia - ALL

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Acute lymphoid leukemia - ALL

- malignant transformation and proliferation of lymphoid progenitor in the bone marrow, peripheral blood and extramedullary sites
- 80% ALL in children
- Incidence 1.6/100 000 (USA)
- 2016 - 6590 of newly diagnosed patients, 1400 deaths
- bimodal distribution of incidence – children (4 years) and adults (50 years)
- In children – survival 90% but only about 30-40% of adults reach long-term remission

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ALL etiology

- significant correlation with Down syndrome, Fanconi anemia, Bloom syndrom, Ataxia Telangiectasia and Nijmegen breakdown syndrome
- ionizing radiation, pesticides, smoking
- Viruses - Epstein-Barr and HIV
- Often *de novo*
- Chromosomal aberrations t(12;21), t(1;19), t(9;22) and aberrations in MLL – not enough for ALL development – unknown origin

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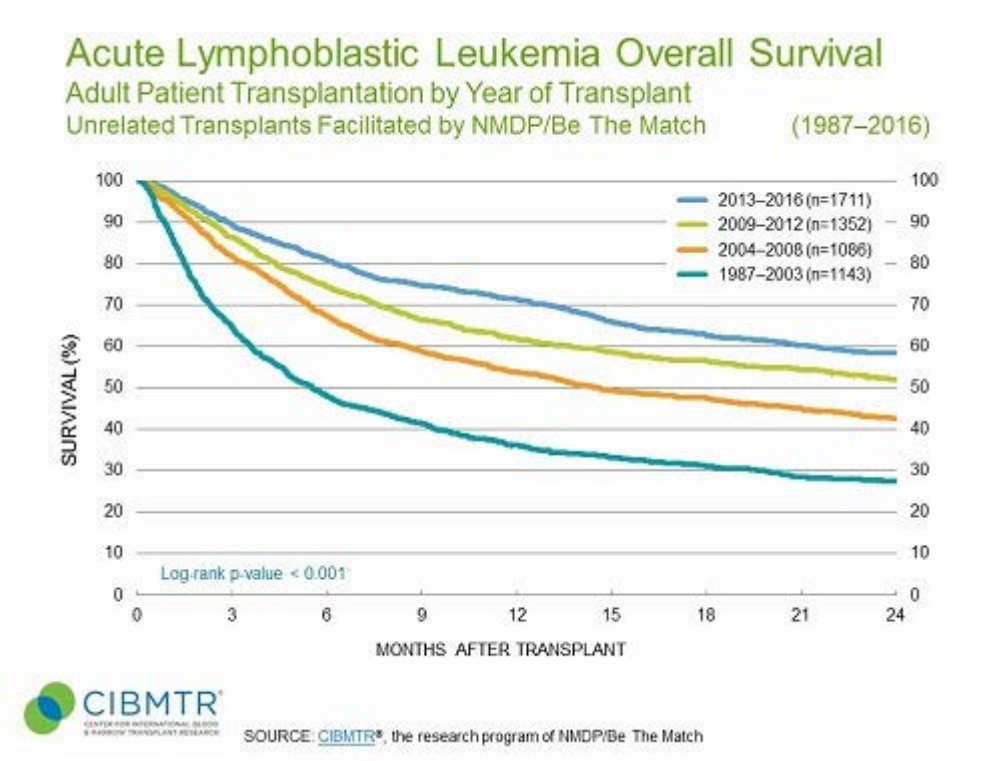
ALL treatment

- Induction (vincristin, corticosteroids, anthracyclins)
- Transplantation of bone marrow
- Or
- Consolidation
- Maintenance 2-3 years



Terwilliger 2017

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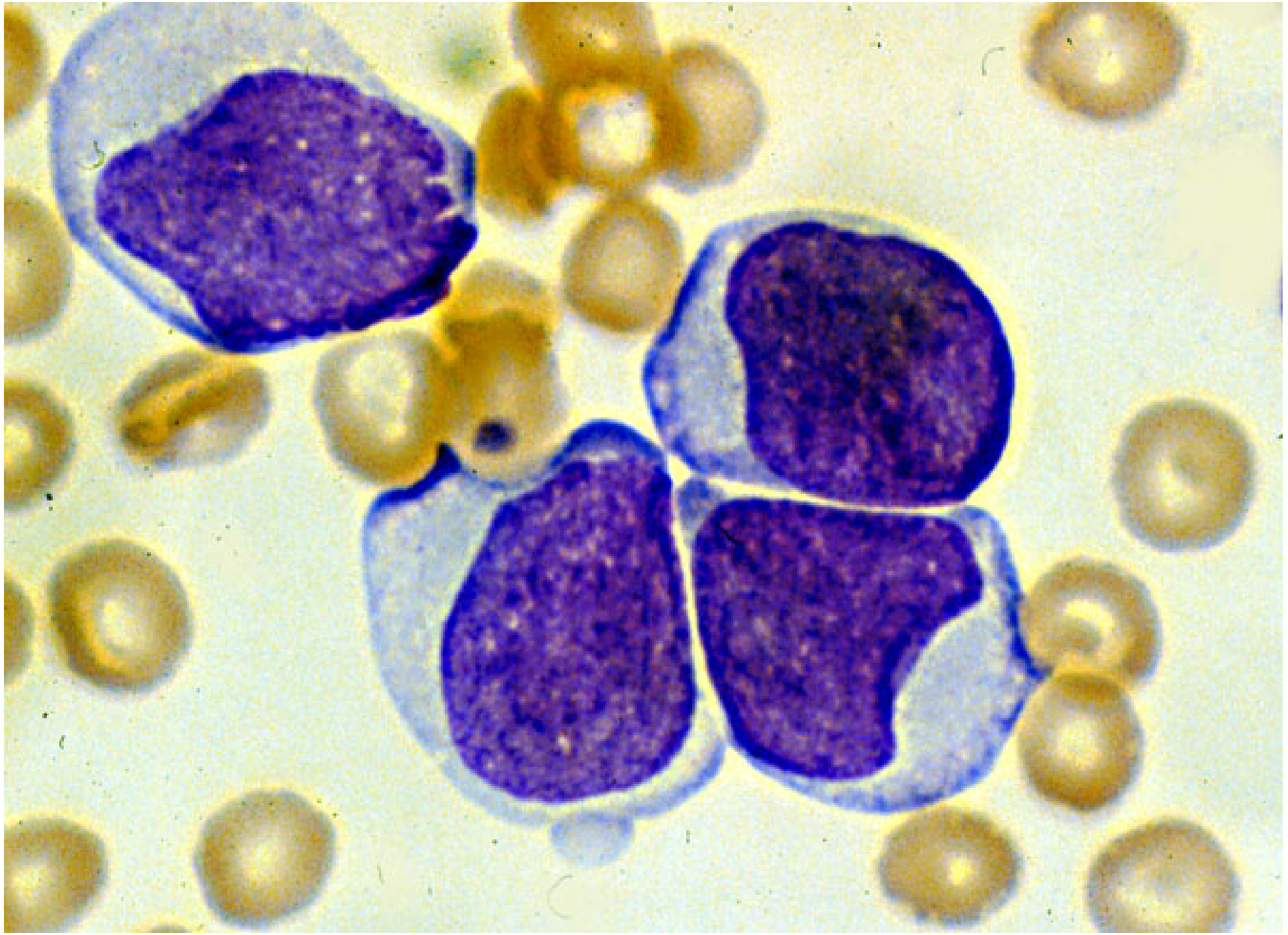

Chronic myeloid leukemia - CML

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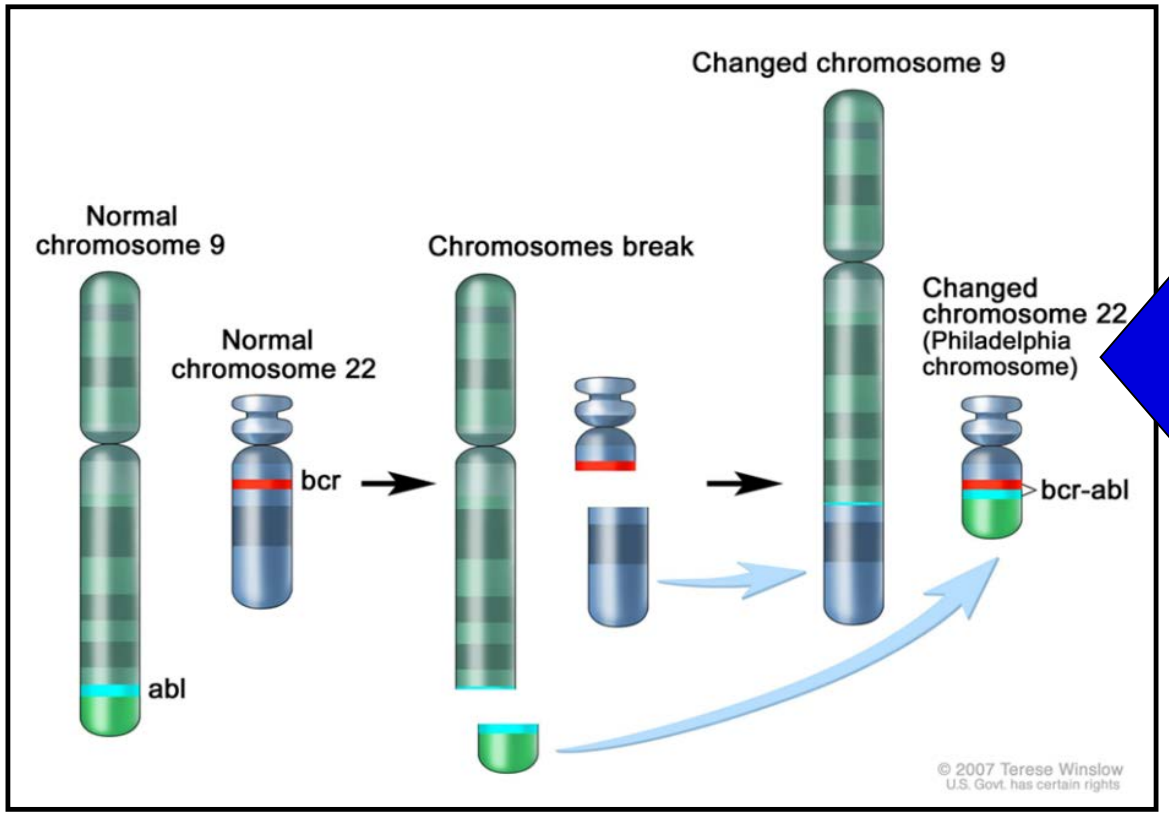
Chronic myeloid leukemia - CML



first tumor linked to specific translocation between chromosomes 9 and 22 t(9;22)

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Philadelphia chromosome

- 1960 – Peter Nowell and David Hungerford described an abnormal chromosome in CML
- First genetic cause of tumors
- 1972 – reason or consequence? Janet Rowley – t(9,22)

CML

- first tumor linked to specific aberration
- CML chromosome described in 1960 in Philadelphia – Philadelphia chromosome
- 1972 translocation described t(9;22) (Rowley)
- 1983 kinase abl described on chromosome 9 (Heisterkamp)
- 1984 bcr region described on chromosome 22 (Groffen)
- 1990 bcr-abl reason for CML (Daley)
- Bcr-abl- abnormal tyrosin kinase (Lugo, 1990)
- Chronic phase, accelerated phase, blast crisis
- Very bad prognosis (Less than 3 years)

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CML

- Incidence 1-2/100 000
- 15% newly diagnosed patients with leukemia
- 9000/year of new cases in USA
- 1000/year die (since Gleevec – annual mortality 1-2%)
- Prevalence – 25 000 (2000), 100 000 (2017), 180 000 (2030)

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CML treatment

- Until 2000 – hydroxyurea, IFNα
- Transplantation of bone marrow curative but high mortality
- Gleevac – 10 year survival 80-90 %

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Gleevec (1993) Novartis

- Imatinib mesylate
- Active against CML colonies (Druker 1996)
- 2 years later – clinical study: 31 patients, 98% response rate
- Clinical study phase III: 16 countries, 177 centers, 1000 patients – study stopped, all patients on Gleevec
- Survival 95%, survival 65% in blast crisis (8 years)
- Molecular positivity of bcr-abl a problem - leukemic cells survive - danger of relapse?

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Current treatment of CML

- Imatinib – in recent years even generics
- Dasatinib
  - 350 More potent than imatinib
  - inhibition of Src pathway
  - five years survival similar to imatinib
- Nilotinib
  - structural analogue of imatinib but binds better
  - Five-year survival better than imatinib
- Bosutinib - Src/Abl inhibitor
  - for patients resistant to previous lines of therapy

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Cost of treatment

- Imatinib – 30 000 USD/year – at the beginning
- Today 132 000 USD/year
- Generics – 8000 USD/year (2016) – but are they as efficient?

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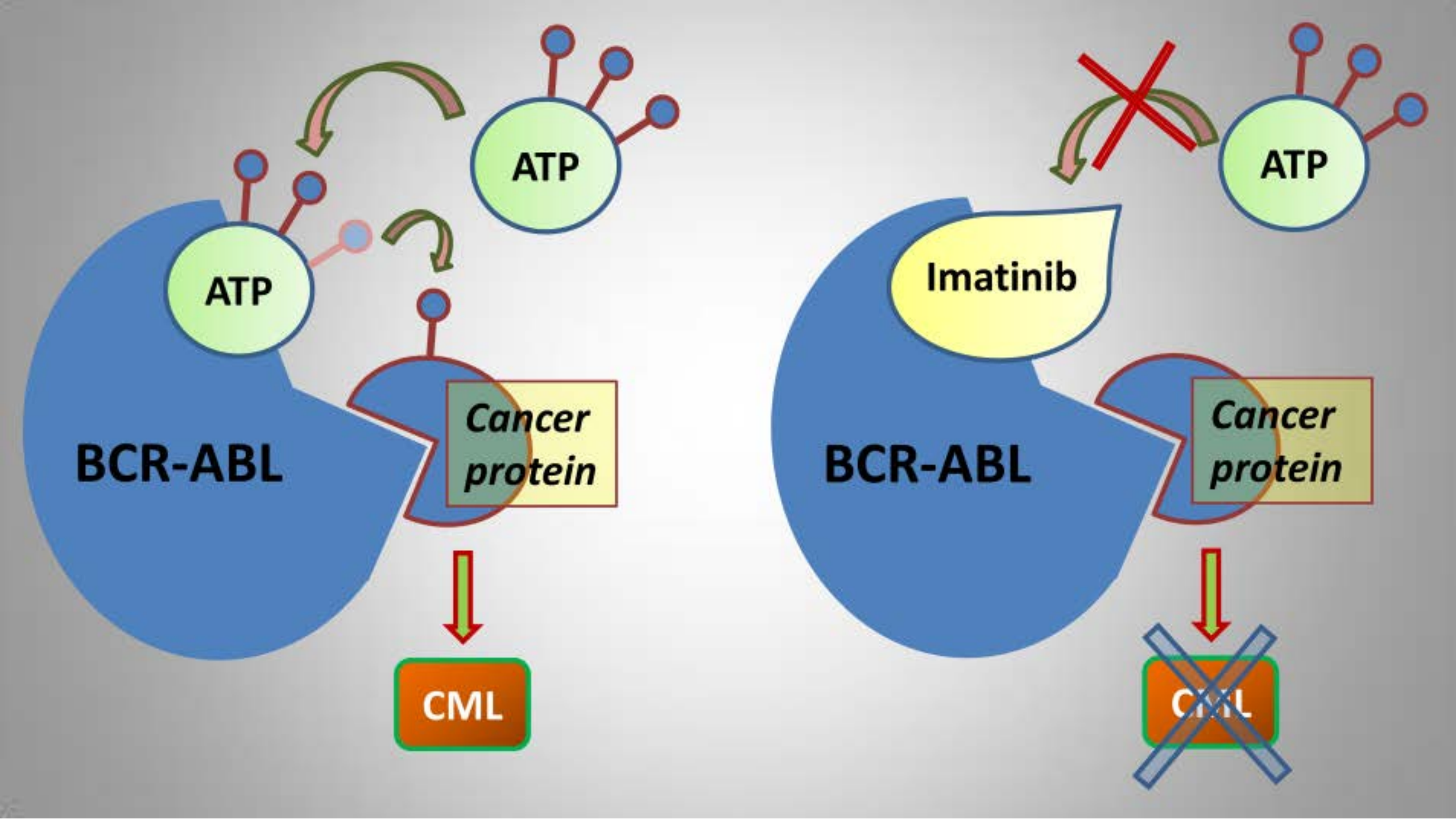
ATP
ATP
BCR-ABL
Cancer protein
CML
ATP
Imatinib
BCR-ABL
Cancer protein

CML diagnosis

- 50% patients asymptomatic
- Anemia, splenomegaly, fatigue, weight decrease
- Cytogenetics for diagnosis
- 100% of patients - bcr-abl, but also other aberrations (trisomy 8, …)
- bone marrow biopsy

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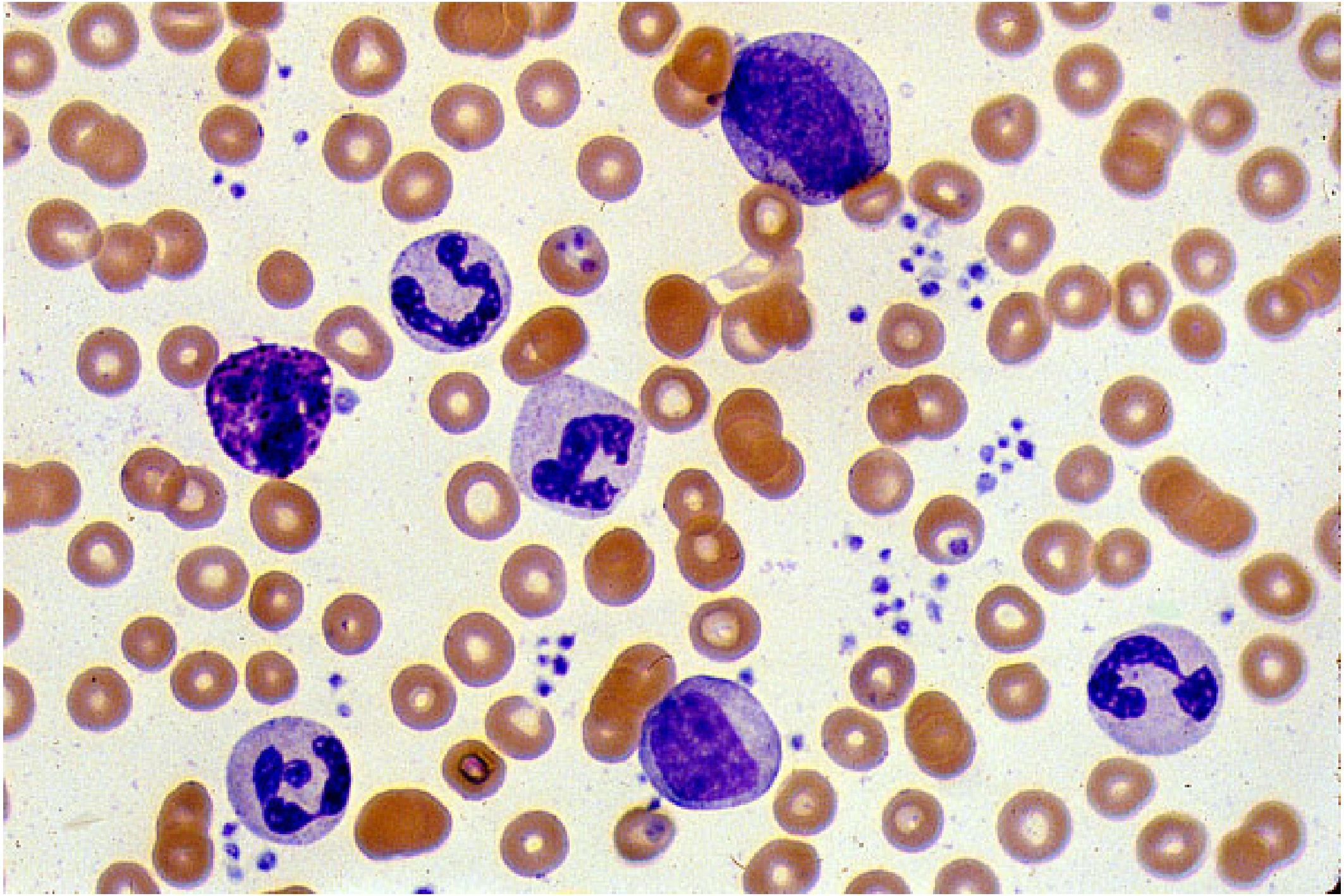

Chronic lymphocytic leukemia - CLL

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Chronic lymphocytic leukemia - CLL

- 30% of all leukemias
- the most common type of leukemia in Western countries
- clonal expansion of B cells - CD5 positive in peripheral blood, bone marrow, lymph nodes and spleen
- more common in men (1.7:1)
- Incidence 4.1/100 000
- Median age at diagnosis 67

Hallek 2019

MUNI MED

CLL etiology

- Genetics
- Viruses (EBV, HIV)
- Radiation
- Chemicals
- Smoking

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CLL genetic changes

- primary changes in multipotent hematopoietic stem cells
- Deletion 13q, deletion 11q, trisomy of chromosome 12
- Del(13q14) primary change - 55% of cases
- Del(11q) - 25% of patients – deletion 11q23- gene *ATM* – decreased OS
- Trisomy 12- 10-20% of patients
- Del(17q) – 5-8% of patients – resistence to chemotherapy

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CLL diagnosis

- Blood smear, immunophenotyping
- More than 5000 B cells/1 µl of peripheral blood
- Clonality based on flow cytometry

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CLL risk factors

- deletion or mutation of *TP53*
- *IGHV* mutation (gene for heavy chain of immunoglobulin)
- Serum β2 macroglobulin
- Age over 65

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CLL treatment

- Chlorambucil – alkylator
- Purine analogues - fludarabin, pentostatin, cladribin
- Monoclonal antibodies – antiCD20 (rituximab)

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CLL

CLL-IPI category	OS at 5 years (%)	Potential clinical consequence
Low risk	93.2	Do not treat
Intermediate risk	79.3	Do not treat except if the disease is really symptomatic
High risk	63.3	Treatment indicated except if the disease is asymptomatic
Very high risk	23.3	If you need to treat, do not use chemotherapy but rather novel agents or treatment in clinical trials.

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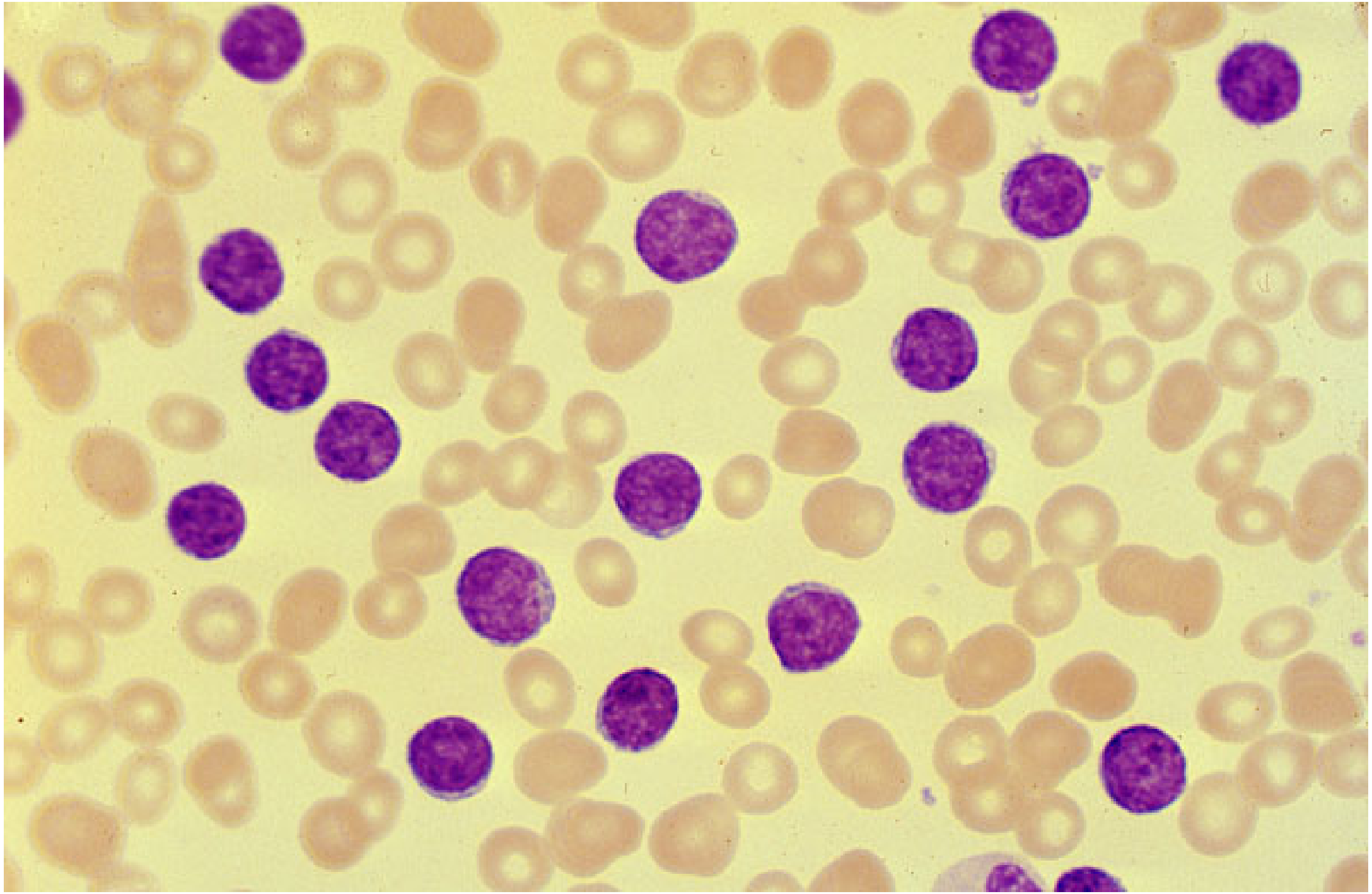

Hematological malignancies

Leukemia

Lymphoma

Multiple myeloma

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Lymphoma

- malignant proliferation of lymphatic tissue – B, T cells
- Solid tumor of blood cells
- 1832 described by Dr. Hodgkin
- most common hematological malignancy
- 5.3 % of all tumors
- Diffusing into other lymph nodes and tissues
- Histology:
  - Hodgkin (more common in men)
  - Non-Hodgkin (B,T, NK cells)

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Lymphoma

Most common lymphoma:

- Diffuse large B cell lymphoma (30 %)
- follicular lymphoma (22 %)
- MALT-lymphoma (8 %)
- chronic B lymphocytic leukemia (7 %)
- mantle cell lymphoma (6 %)

All malignant lymphoma may present as B-symptoms:

- Weight loss (10 % / 6 months)
- Fever, night sweats

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Hodgkin lymphoma

- Painless enlargement of nodes (neck, axillary)
- Fever, sweating, fatigue, weight loss
- splenomegaly
- Cough, emphysema
- Infiltration of parenchymous organs

- Etiology unknown – genetics, HIV, EBV
- Common in adults between 20-30 and over 50

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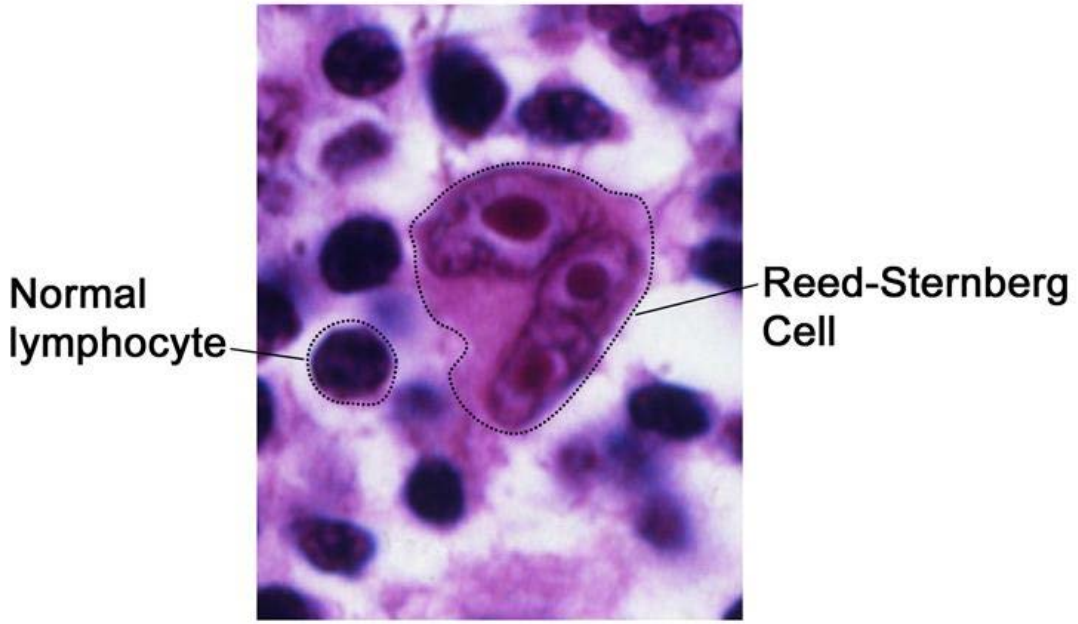
Hodgkin lymphoma

- **type I** – lymphocyte –rich - majority of lymphocytes (few Reed-Sternberg cells, best prognosis) (5% of cases)

- **type II** nodular-sclerosis (nodular deposits, cells – reticular, lymphocytes, histiocytes) in collagen fibres (70%)

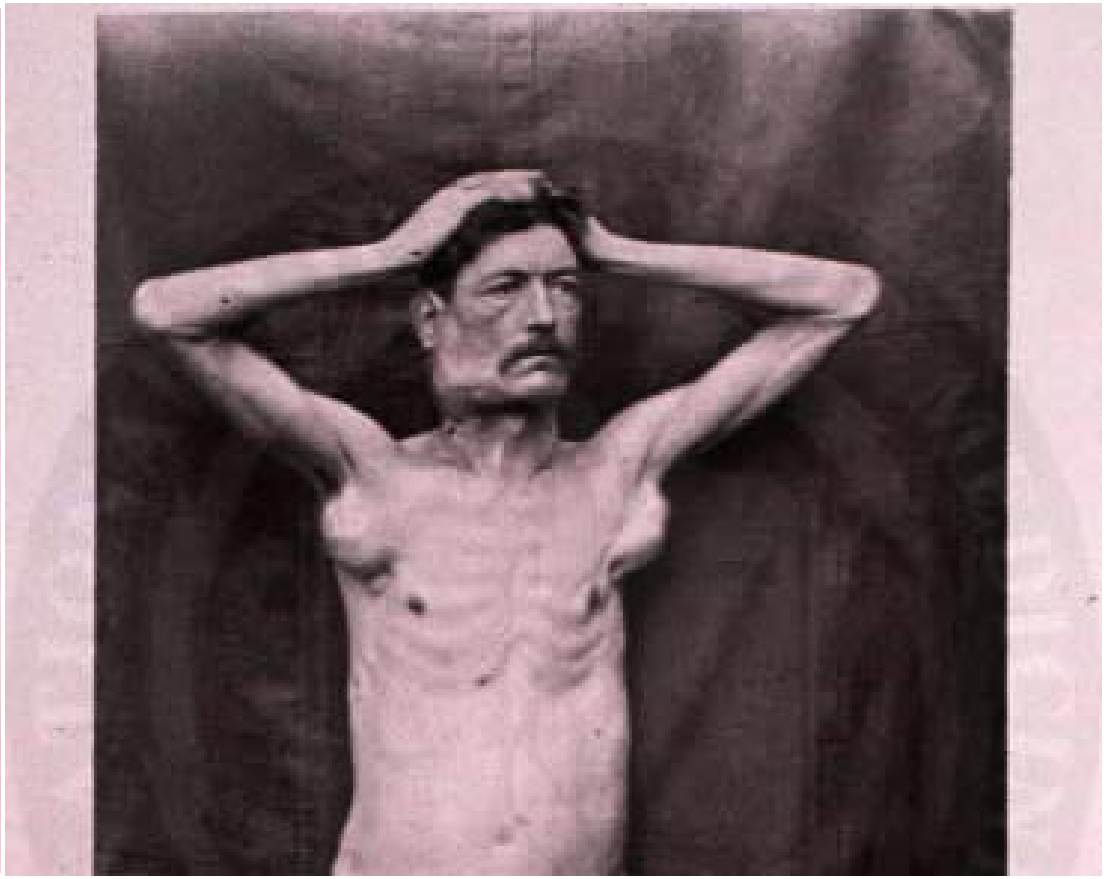
- **type III** mixed cellularity (20–25%)

- **type IV** lymphocyte-depleted (Reed-Sternberg cells increased, worst prognosis) (1%)



Reed-Sternberg buňky – abnormal lymphocytes, characteristic for lymphomas, multinucleated cells

Hodgkin lymphoma



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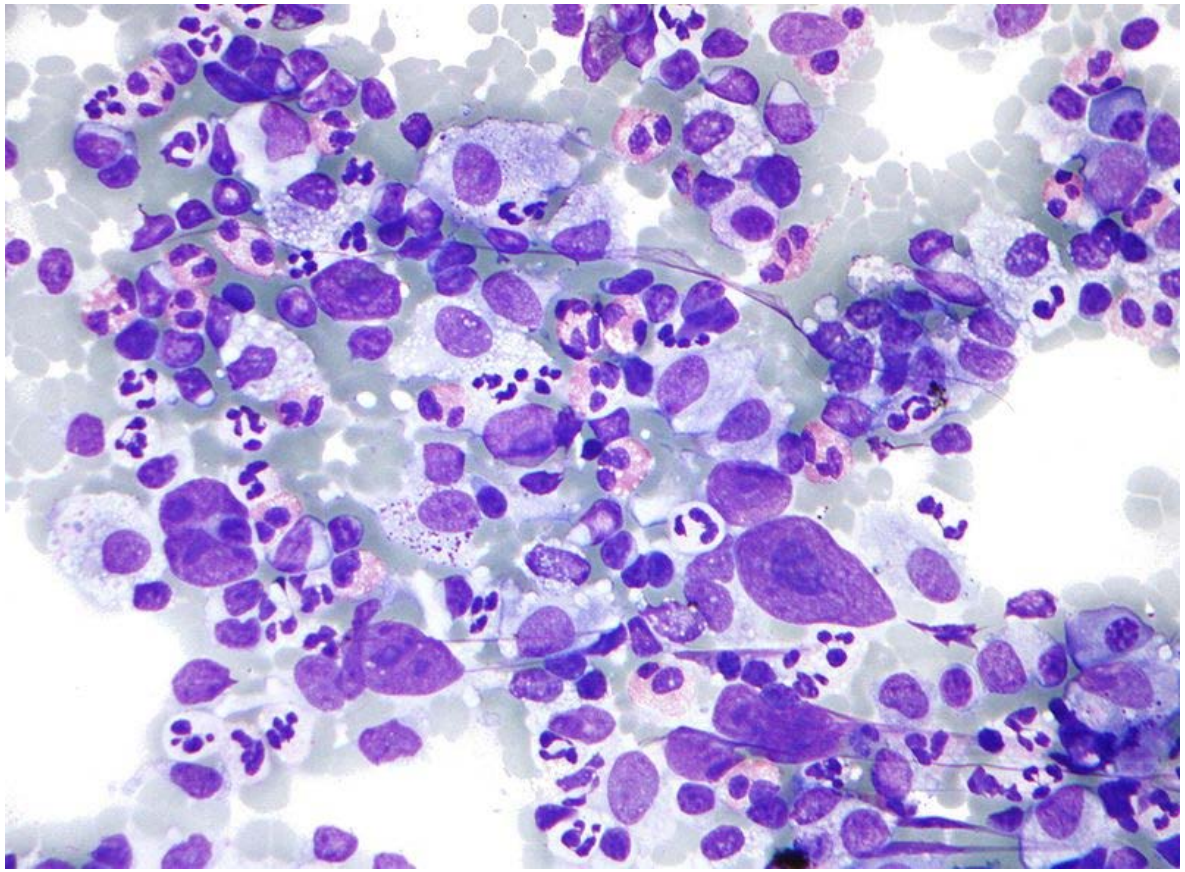
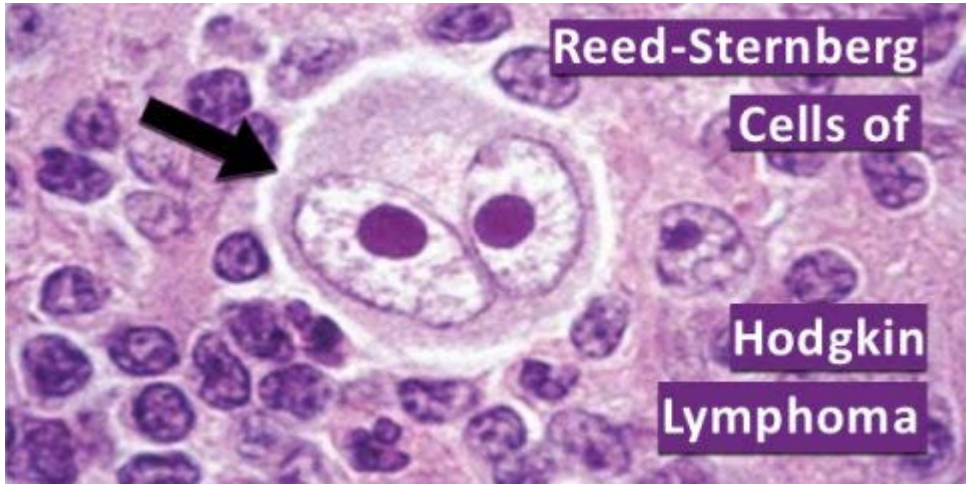
Hodgkin lymphoma





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Non-Hodgkin lymphoma

- Heterogenous group of tumors (cca 40 types)
- Arising from lymph nodes – fast migration into surrounding tissues and metastases in children
- At the time of diagnosis – 2/3 of patients have advanced stage of the disease
- in children highly malignant tumors - very intense chemo treatment - successful in 80% of cases
- In adults – less malignant

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Myelodysplastic syndromes - MDS

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Myelodysplastic syndromes - MDS

- Heterogenous group of myeloid disorders characterized by cytopenia in peripheral blood and increased risk of progression into secondary AML
- Incidence 3-4/100 000 (USA)
- Prevalence increases with age
- Diagnosis: bone marrow biopsy
- Stratification: analysis of peripheral cytopenia, percentage of blasts in the bone marrow, cytogenetic analysis

Montelban-Bravo, 2018

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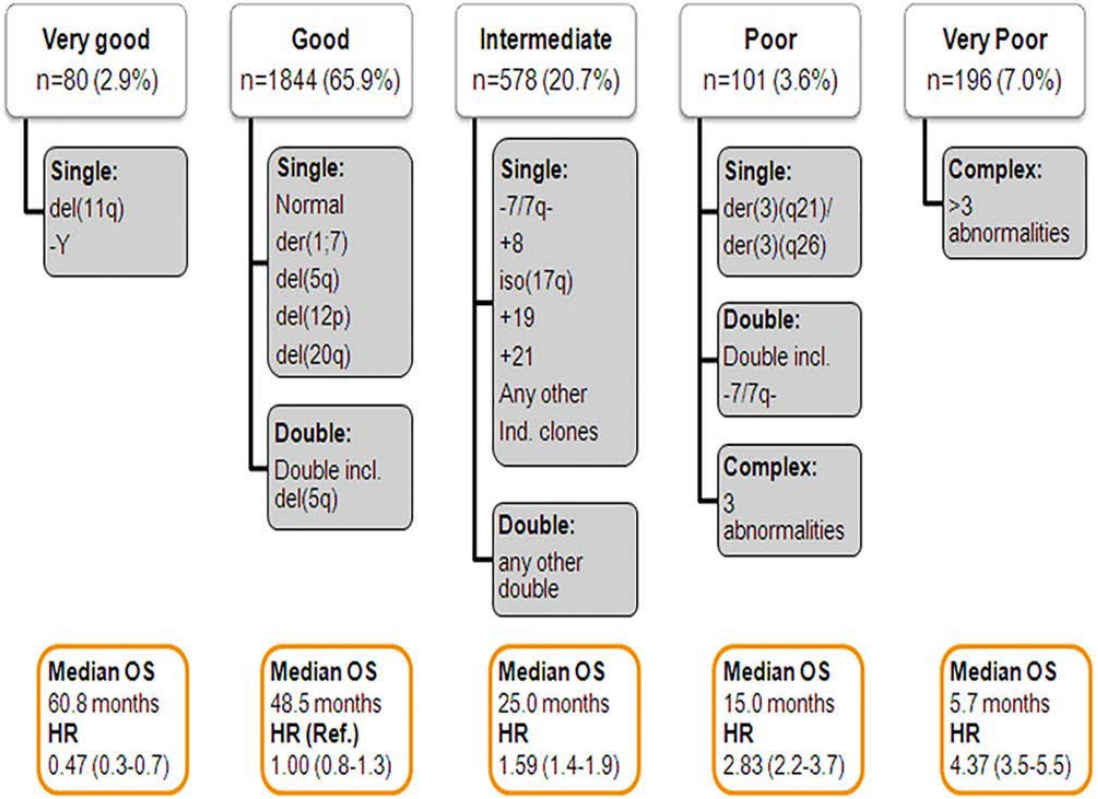
Cytogenetic classification of MDS



Montelban-Bravo, 2018

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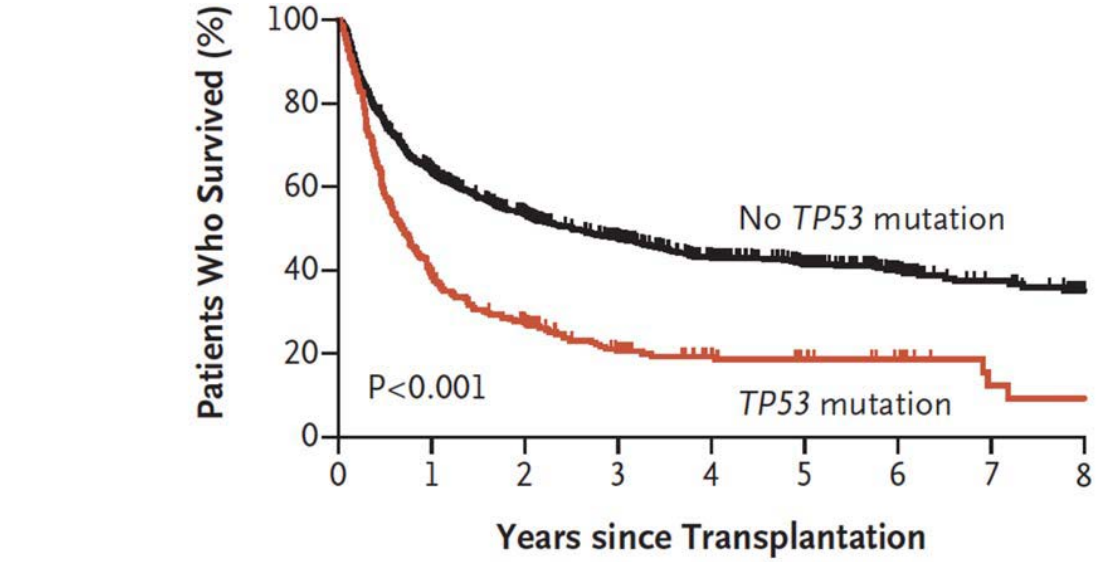
Survival of MDS patients depends on *TP53* mutation

- Mutations in *TP53*, *RUNX1*, *ASXL1*, *JAK2* and *RAS* genes is connected to significantly shorter OS after allotransplantation of the bone marrow
- *TP53* mutations have a strong negative effect



No. at Risk

No *TP53* mutation	1224	757	529	370	261	183	109	53	32
TP53 mutation	289	109	66	39	26	20	14	6	5

Montelban-Bravo, 2018

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Hematological malignancies

Leukemia

Lymphoma

Multiple myeloma

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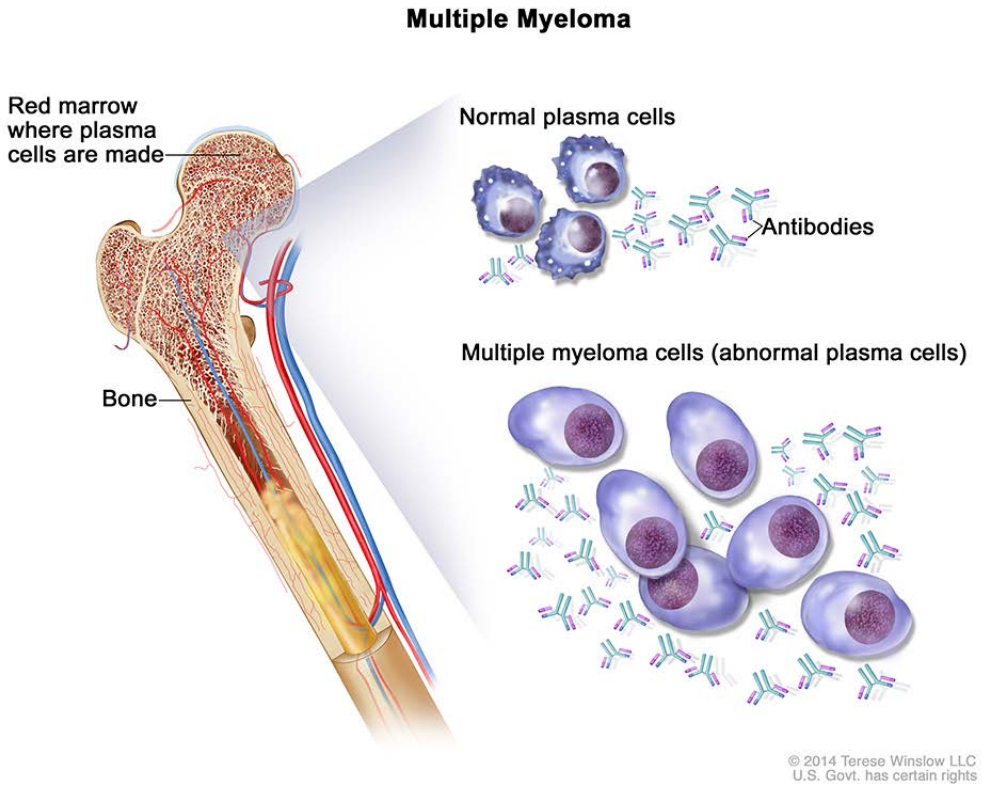
Multiple myeloma MM



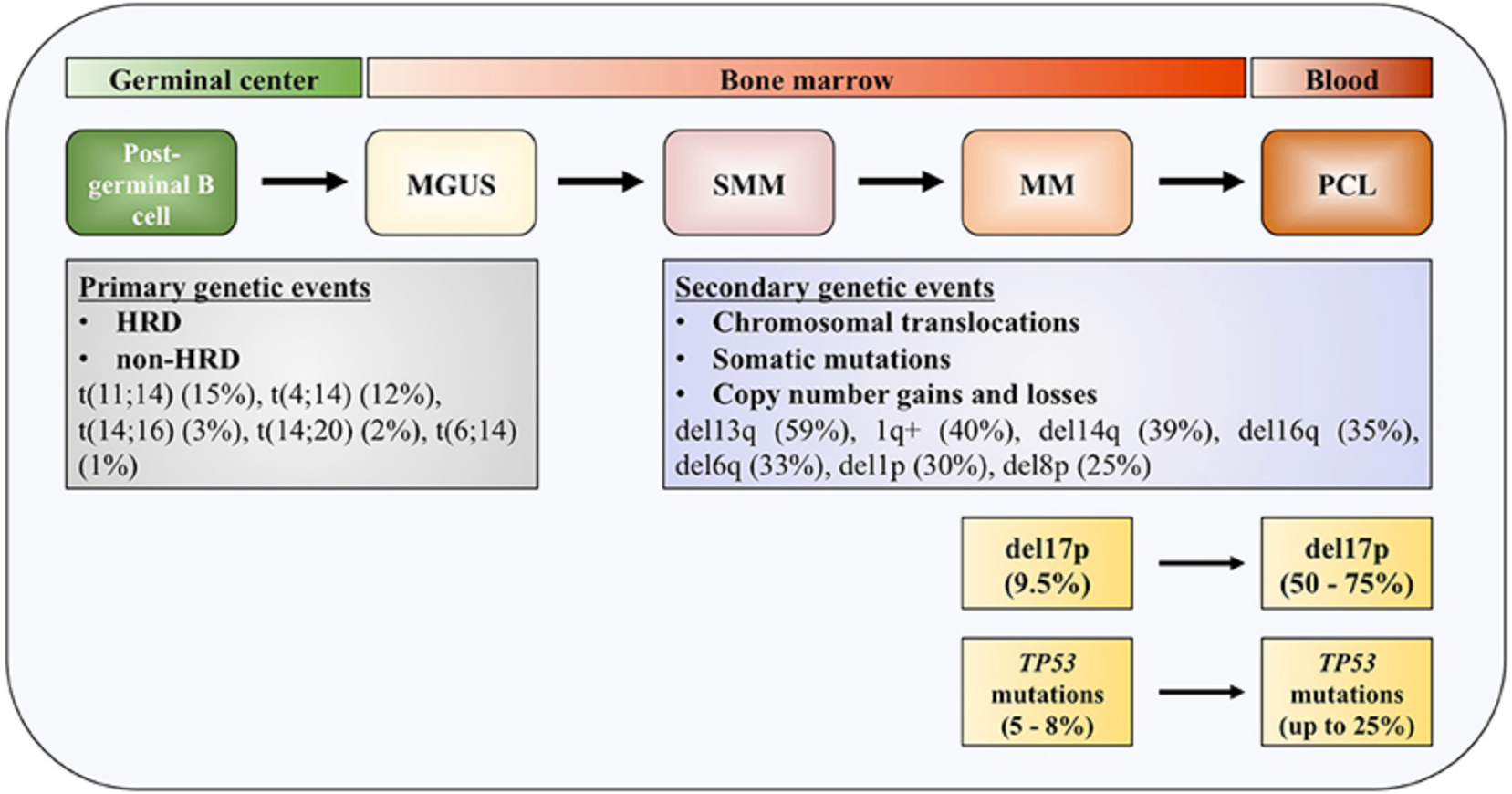
- second most common hematological malignancy
- 10% of hematological malignancies
- median age at diagnosis - 65
- Incidence 4/100 000
- more common in men
- multistep pathogenesis

Hajek, 2012

Anderson, 2011

MUNI MED

Pathogenesis of MM - multistep process



Jovanovic 2019

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MGUS monoclonal gammopathy of unknown significance

- accumulation of genetic changes in plasma cells leading to malignant transformation
- In MGUS - bone marrow infiltrated by <10 % of malignant plasma cells
- Asymptomatic – not found by routine tests
- 15 % people with MGUS progress into MM
- 1 % risk of progression to MM every year
- Incidence 3 % of population over 50 (increases with age)

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MM

- infiltration of bone marrow by malignant plasma cells
- bone lesions
- presence of monoclonal immunoglobulin (M-Ig) in serum and/or urine

- Bone marrow niche supports proliferation and survival of malignant myeloma cells

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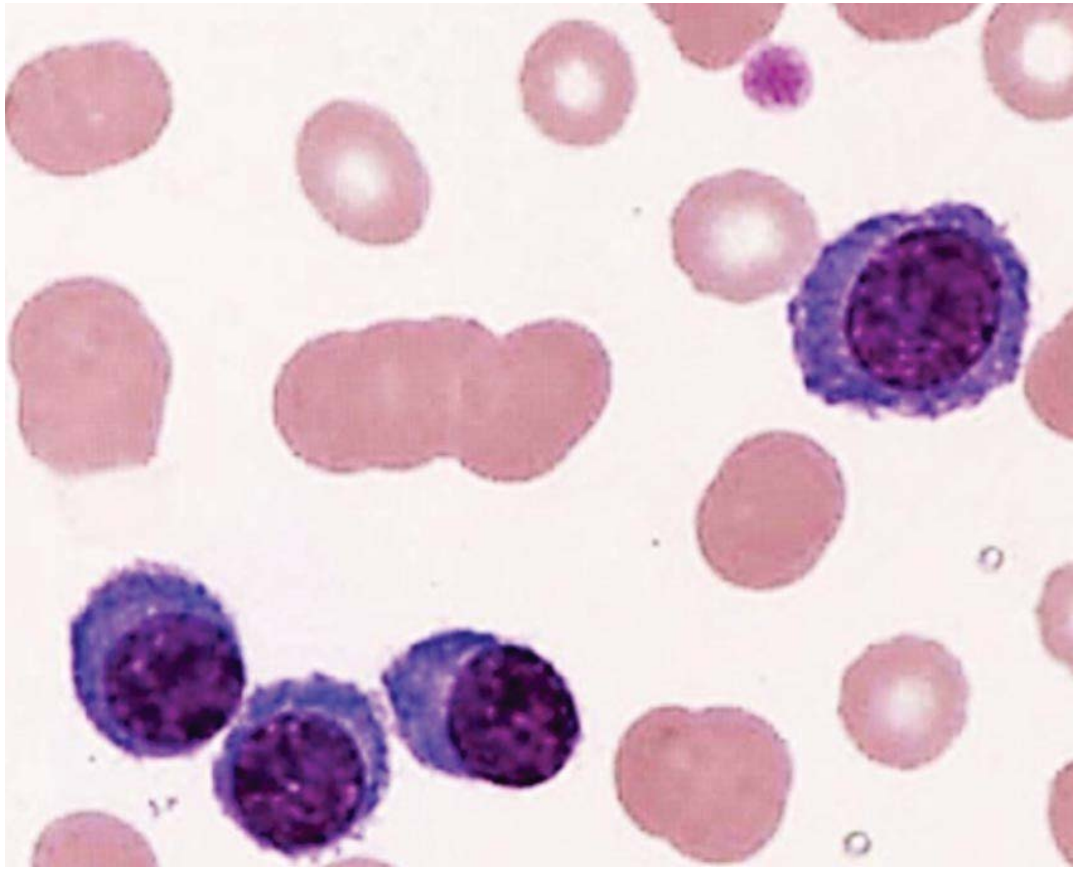
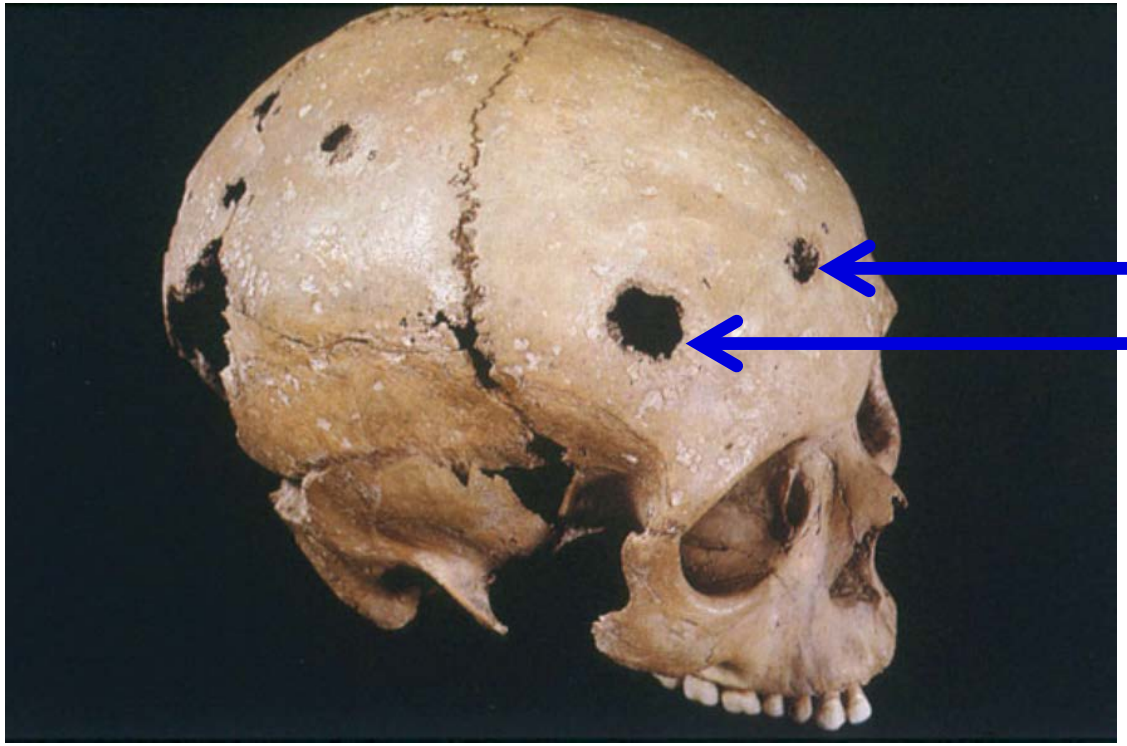
Plasma cell leukemia

- loss of dependency of plasma cells on bone marrow microenvironment, migration into peripheral blood
- > 20 % circulating plasma cells in periphery
- Incidence 4/ 10 000 000
- transformation from MM - 21 months
- Very bad prognosis - 2 - 3 months



https://www.labmedica.com/hematology/articles/294776427/new-definition-for-plasma-cell-leukemia-proposed.html

History of MM
Male skull from the bronze age



Osteolytic lesions – typical feature of MM

Capasso, 2005

MUNI MED

History of MM

- 1844 - First documented case – Sarah Newbury (Dr. Solly)

distraction of sternum broken bones distraction of femur

Kyle et Rajkumar, 2008

MUNI MED

History of MM

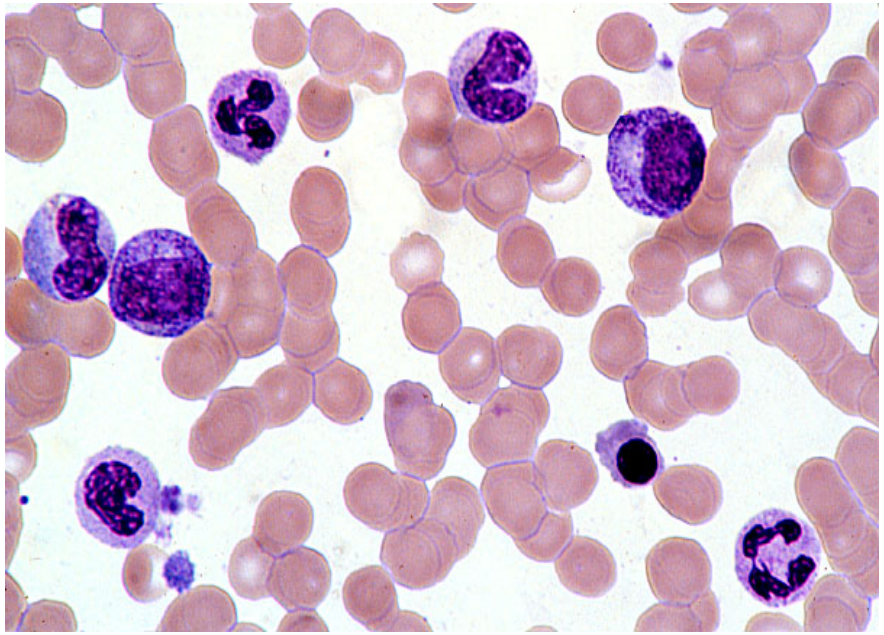
- 1845 – presence of protein in urine of a patient (Dr. Bence Jones – Bence Jones protein)
- MM=Kahler disease – Prague MD Dr. Otto Kahler described MM

Otto Kahler (1849-1893)

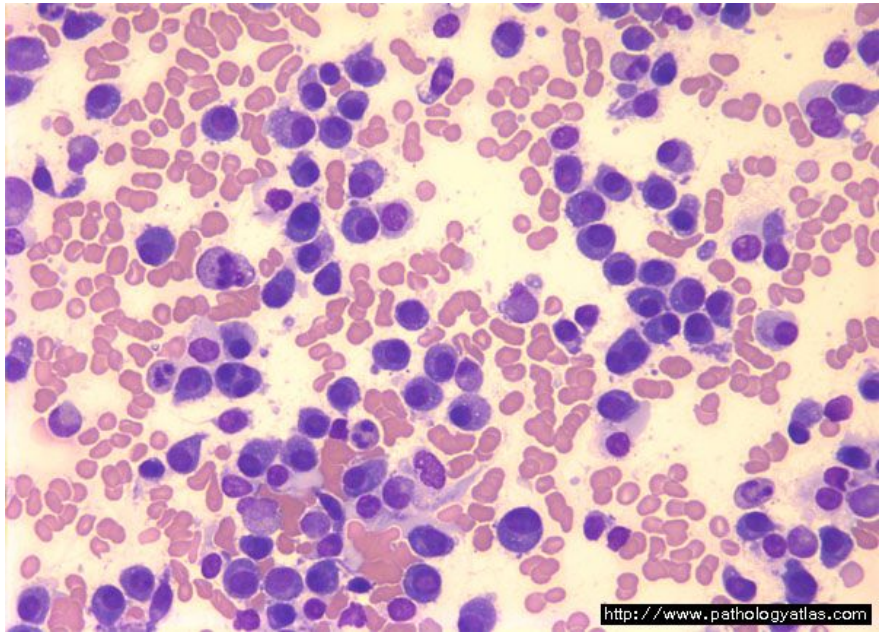
Kyle et Rajkumar, 2008

MUNI MED

healthy bone marrow



MM bone marrow



www.pathologyatlas.com

MM symptoms

1) effect on bone marrow:

↓ erythrocytes → Anemia
↓ white blood cells → decrease of immune reactions
↓ thrombocytes k → bleeding

2) Osteolytic lesions:

- pain
- fragile bones
- fractures
- calcium increase in serum

3) presence of defective immunoglobulins

- hyperviscosity
- accumulation of these proteins in small veins
- decrease of immunity - decreased number of regular immunoglobulins

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MM diagnosis

quite difficult – pain, fatigue, repeated infections common for other diseases

1) number of myeloma cells in the bone marrow
2) presence of abnormal protein in blood or urine
3) typical changes on the bones

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Treatment of MM

….this is what we tried

Hájek, 2012
Anderson, 2011

MUNI
MED

Treatment of MM

…and this is what we're currently using

- chemotherapy
- transplantation of bone marrow
- immunomodulatory drugs
- proteasome inhibitors

Hájek, 2012
Anderson, 2011

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Prognosis of MM

- untreated patients survive 14 months
- standard therapy 3 - 4 years
- Transplantation 6 – 7 years
- New drugs increase five-year survival for about 80% of patients

Hájek, 2012

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Chemotherapy and transplantation

- used even nowadays
- treatment program junior vs senior (intensive versus less intensive)
- Melphalan (alkylator)
- Prednisone (Glukokortikoid – induces apoptosis of hematological cells)

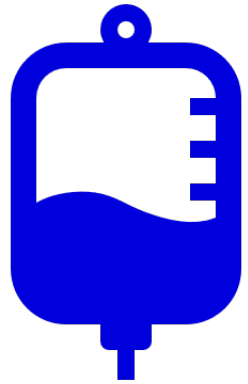
- Transplantation used since 1957
  - Autologous – generally until 65 years of age of patient
  - Allogenous – rare, only in clinical trials

Hájek, 2012
Anderson, 2011

MUNI MED

Treatment possibilities for MM

IMIDs (immunomodulatory drugs)



Proteasome inhibitors

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Thalidomide – first IMID

- 1953- created by Chemie Grünenthal
- 1957- distribution (without prescription)
- Sedative
- Relieves morning sickness
- Heavy teratogen
- Insufficient testing in animals
- About 10 000 children effected – around 40 % survived

- FDA - Dr. Francis Kelsey – did not allow usage of thalidomide in the United States

White House Archive

MUNI MED

Dr. Francis Kelsey (1914-2015)

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MED

Thalidomide children… today

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MED

Thalidomide – continuation

- 1964 – Jason Sheskin – patient with leprosy and complications
- 1993- Judah Folkman – angiogenesis important not only for solid tumors but also hematological
- 1994 – refractory MM patient – thalidomide – clinical study 1/3 of patients responded
- 2006 – FDA – treatment of MM approved
- unpleasant side effects - neuropathy

Sedlaříková, 2012

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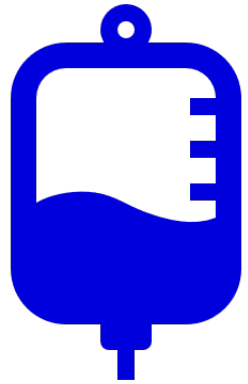
Treatment possibilities for MM

IMIDs (immunomodulatory drugs)



Proteasome inhibitors

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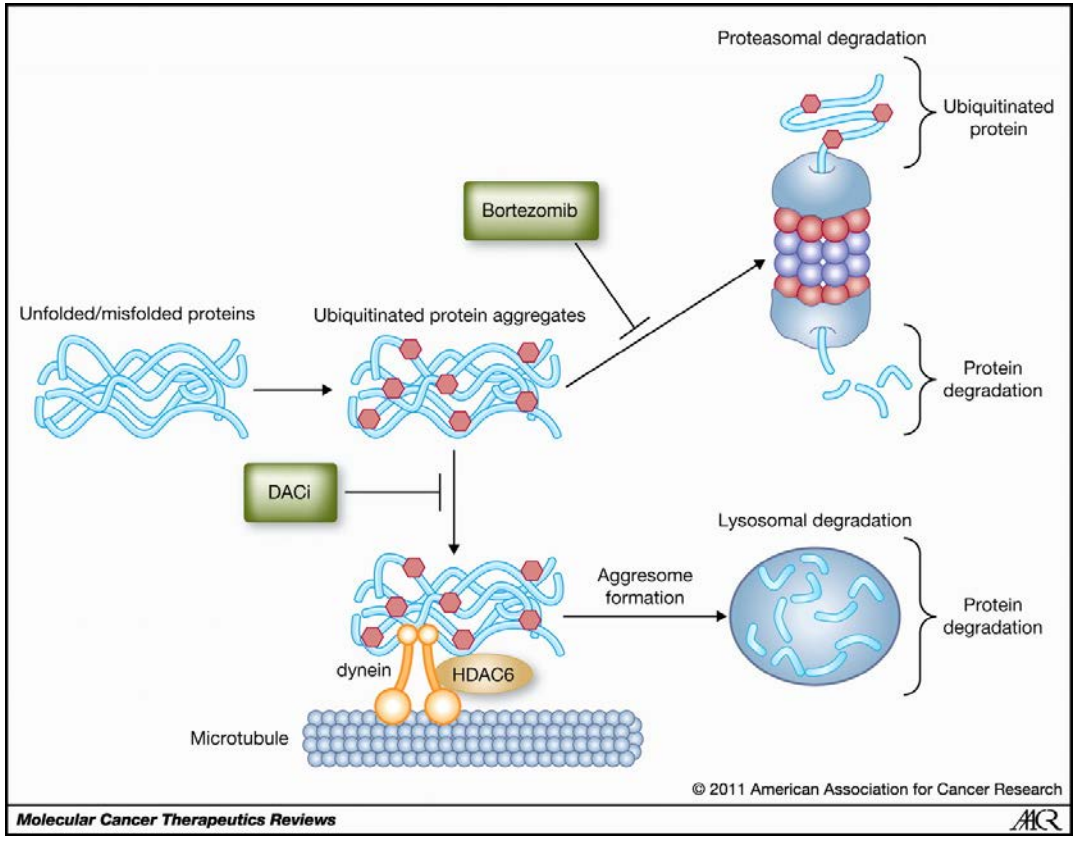
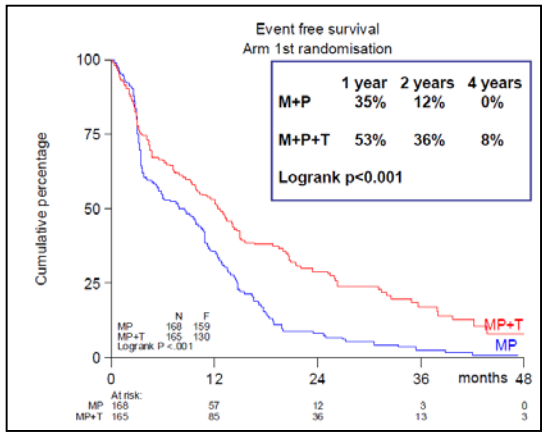
Proteasome inhibitors

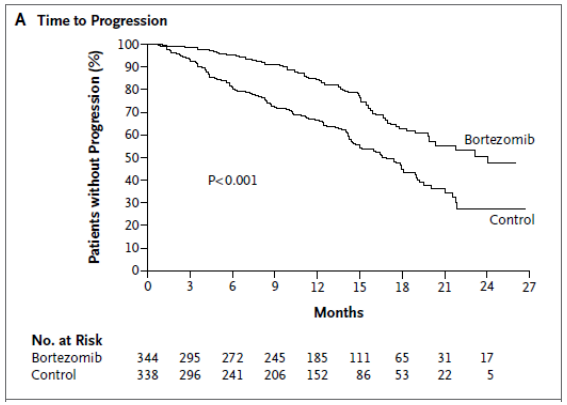
- Proteasome – a proteolytic complex for degradation of ubiquitinated proteins
- MM cells produce large amount of proteins - inhibition of proteasome leads to accumulation of proteins in the cells and apoptosis
- Bortezomib – first proteasome inhibitor approved for treatment of MM



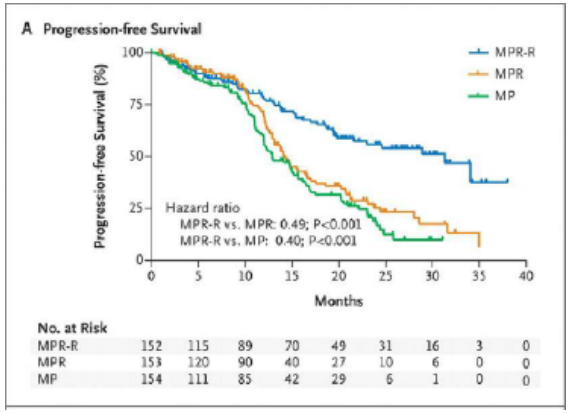
New drugs increase survival but do not cure ….not yet



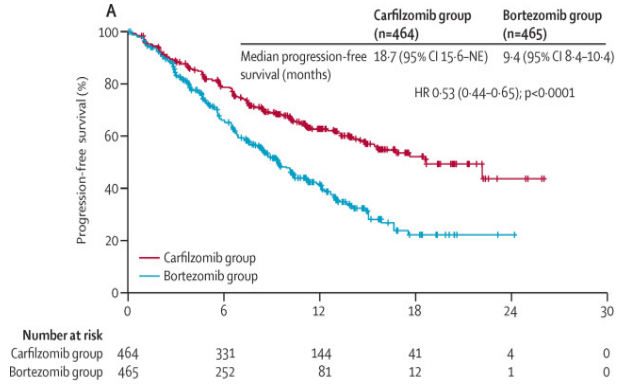
Thalidomid (Myrin)



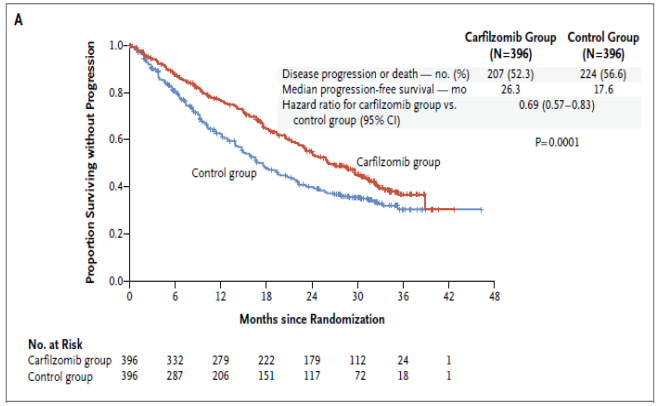
Bortezomib (Velcade)



Lenalidomid (Revlimid)



Carfilzomib > Bortezomib



Carfilzomib+Revlimid> Revlimid

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IV. Survival of patients with hematological malignancies

Estimated New Cases (%) of Leukemia, Lymphoma and Myeloma, 2019

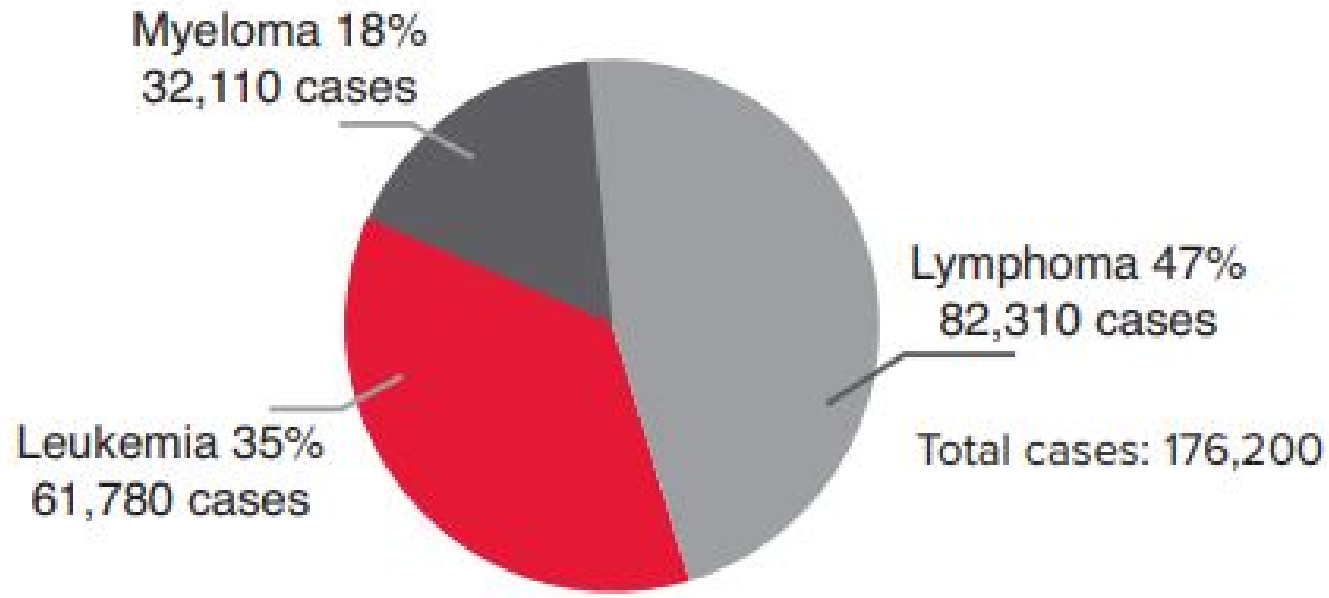

Figure 1. Source: *Cancer Facts & Figures, 2019*. American Cancer Society; 2019.

Five-Year Relative Survival Rates by Year of Diagnosis

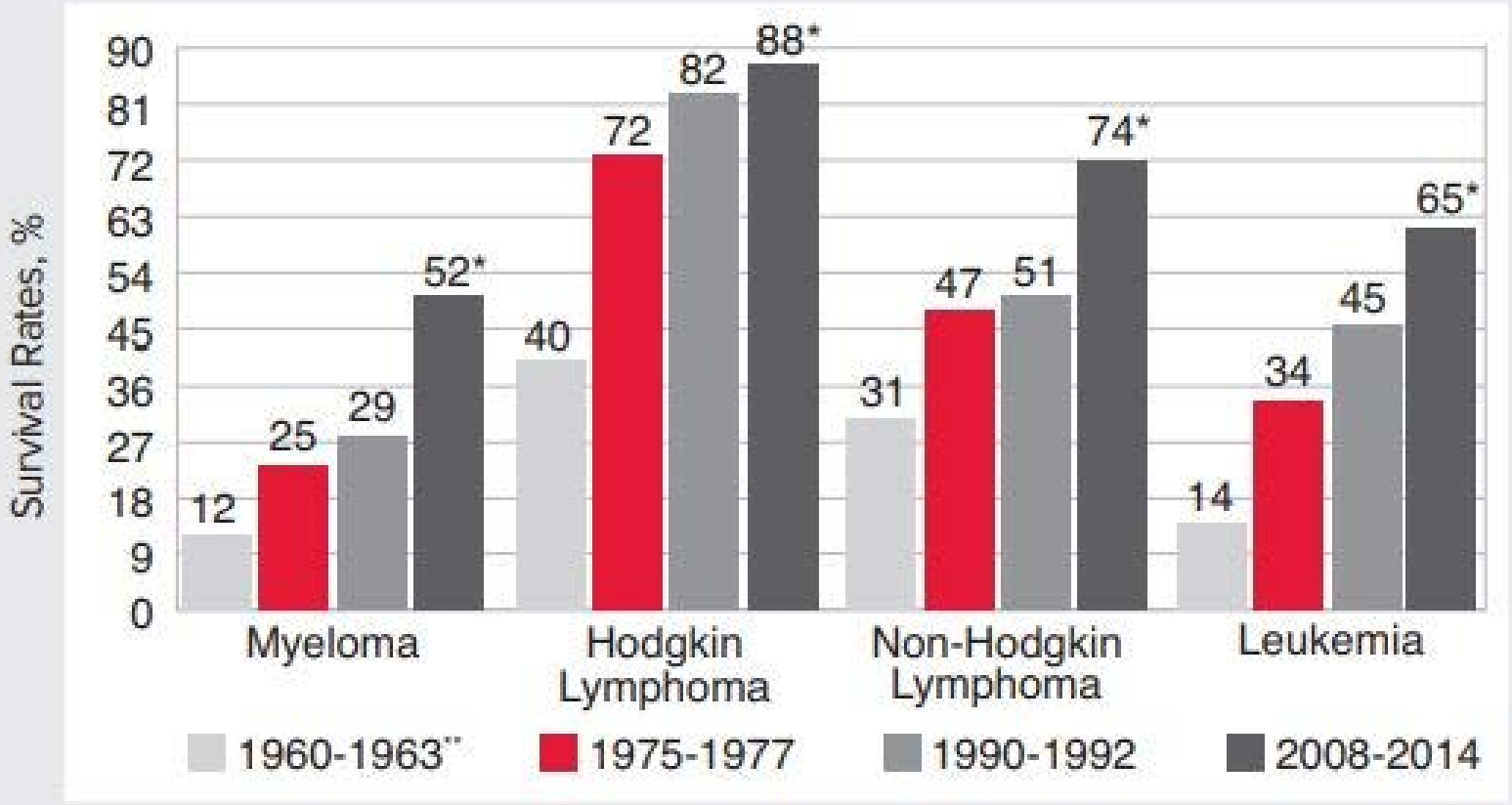

Figure 2. Source: *SEER (Surveillance, Epidemiology, and End Results) Cancer Statistics Review, 1975-2015*. National Cancer Institute; 2018.

*The difference in rates between 1975-1977 and 2008-2014 is statistically significant ($p<.05$).

**Survival rate among whites.

https://www.lls.org/facts-and-statistics/facts-and-statistics-overview/facts-and-statistics

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and that is all ….

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There are papers for your further studies in IS

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Thank you for your attention