MUNI
MED

Pathophysiology of hematopoietic system I– hematological malignancies

Assoc. Prof. RNDr. Sabina Ševčíková, PhD.
Babak Myeloma Group
Department of Pathophysiology

MUNI
MED

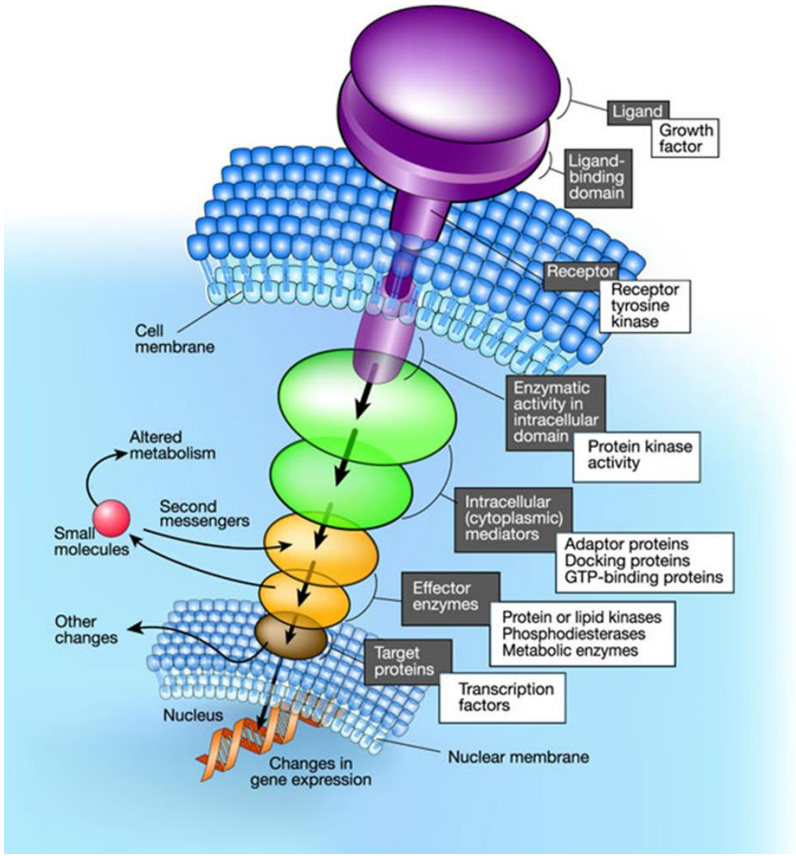
I. Hematological malignancies

Targeted therapy

- Treatment aiming at specific properties of cancer cells (growth, survival…)
- Need to know specific changes in cancer cells
- Trastuzumab – targeting HER2 positive breast cancer

MUNI
MED

Signaling pathway



MUNI MED

End of 1 disease = 1 treatment

- Time of precision and translational medicine
- Precision medicine
  - Takes into account genetics, environment and lifestyle of patient
  - Patients with same diagnosis react vs do not react to treatment…why?
- Translational medicine – bench to bedside
- Genetic dispositions influence almost all diseases
- There are different molecular subtypes in one diagnosis
  - 5 subtypes of breast cancer

MUNI
MED

Agnostic approach to cancer treatment

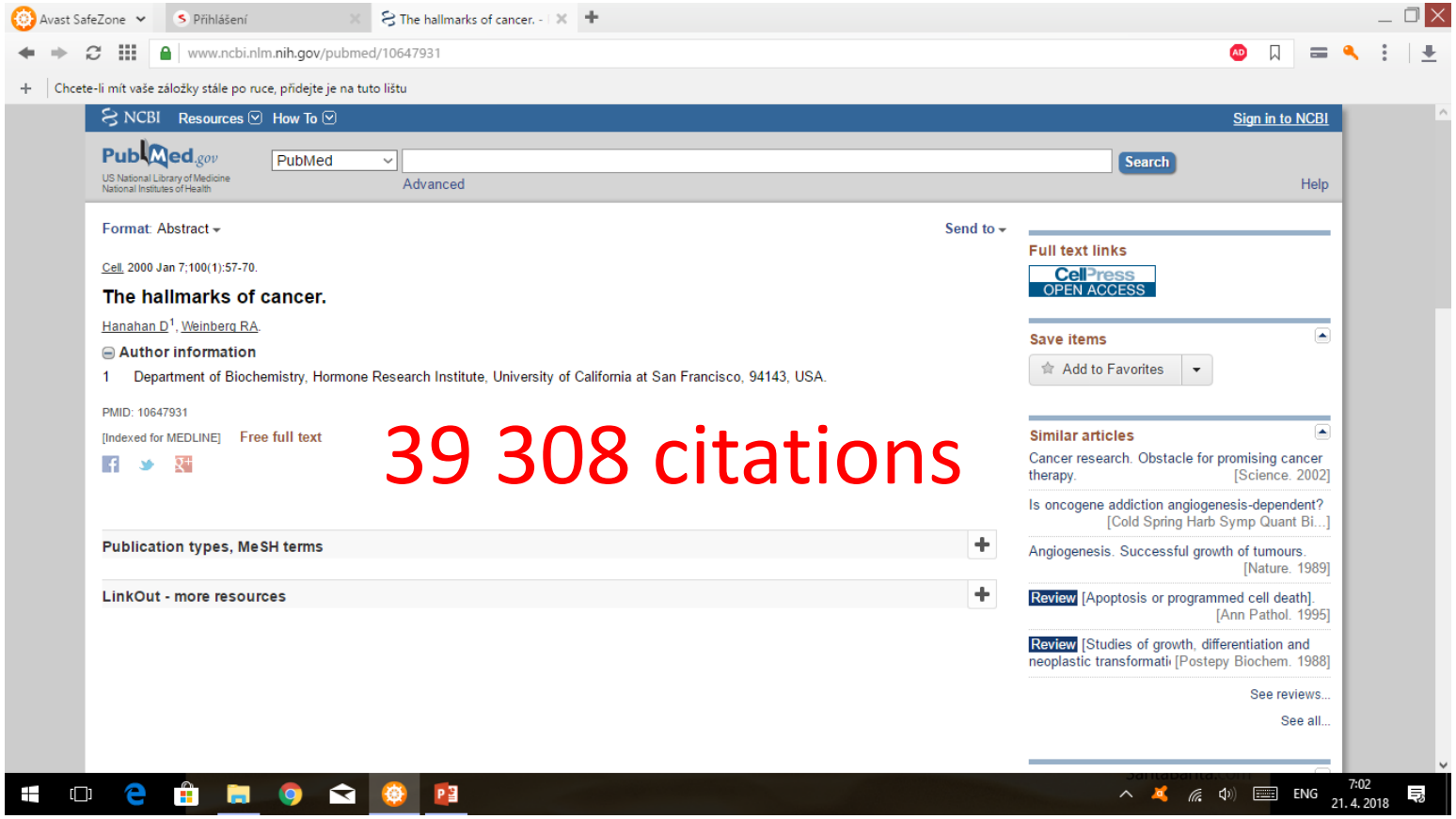
- Treatment based on mutation, not based on tumor
- Mutation BRAF*V600E* – melanoma, non small cell lung carcinoma, colorectal cancer
- New tyrosine kinase inhibitor vemurafenib targets BRAF*V600E*
- The BRAF*V600E* – oncogenic „driver“ mutation associated with agresive phenotype, shorter survival than wt BRAF tumors
- FDA approved treatment of other tumors

MUNI
MED

I. Hallmarks of cancer

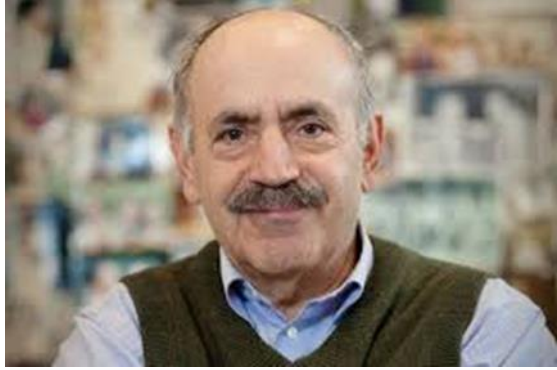
MUNI
MED

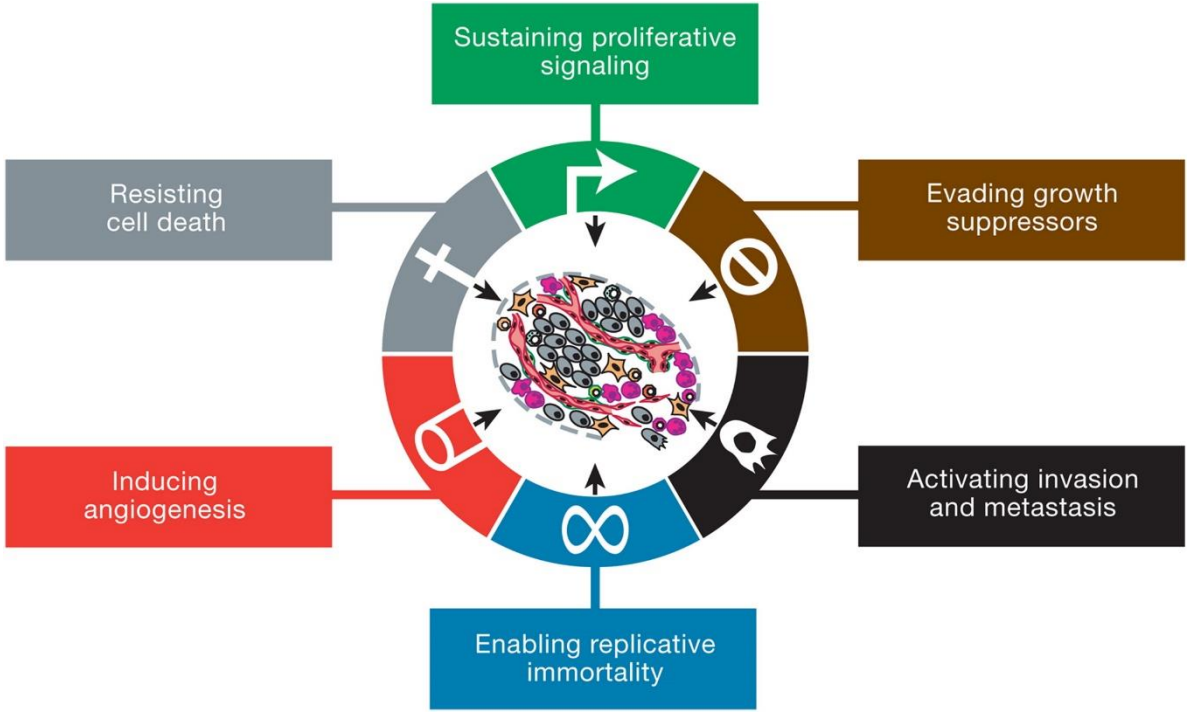
Hallmarks of cancer



MUNI
MED

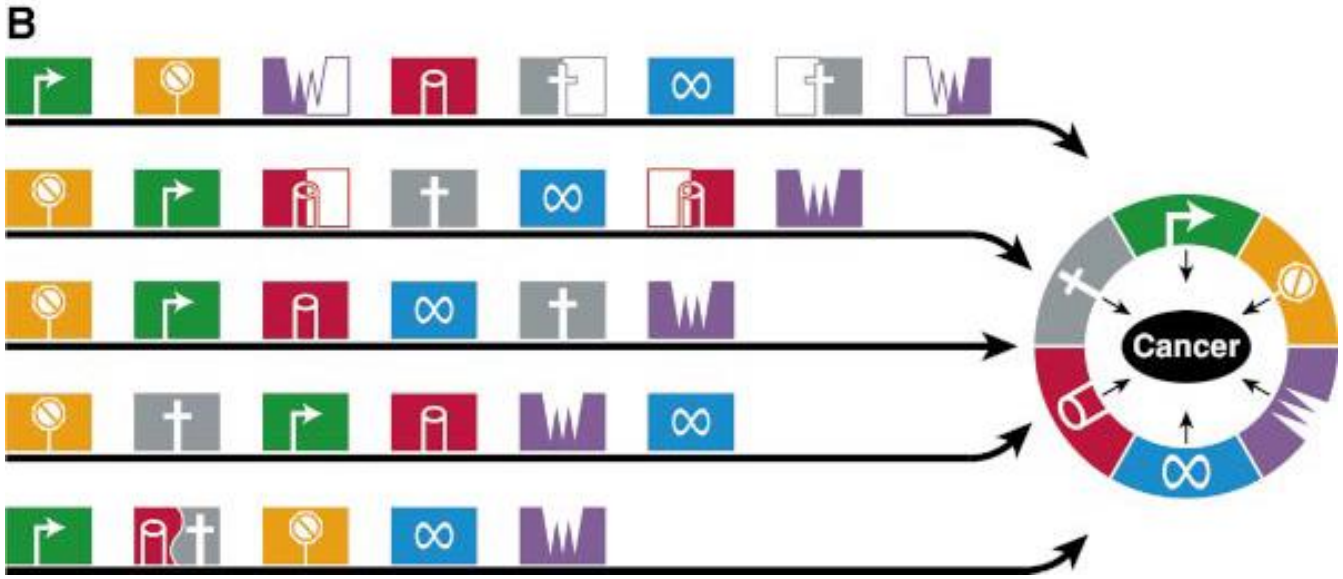
Hallmarks of cancer





MUNI MED

Carcinogenesis is individual



Order, number of hits and specific genes are individual

@Jana Šmardová

Six hallmarks of cancer


Sustaining proliferative signaling


Evading growth suppressors


Resisting cell death


Enabling replicative immortality


Inducing angiogenesis


Activating metastasis

Tumor microenvironment

@Jana Šmardová

Six hallmarks of cancer


Sustaining proliferative signaling


Evading growth suppressors


Resisting cell death


Enabling replicative immortality


Inducing angiogenesis

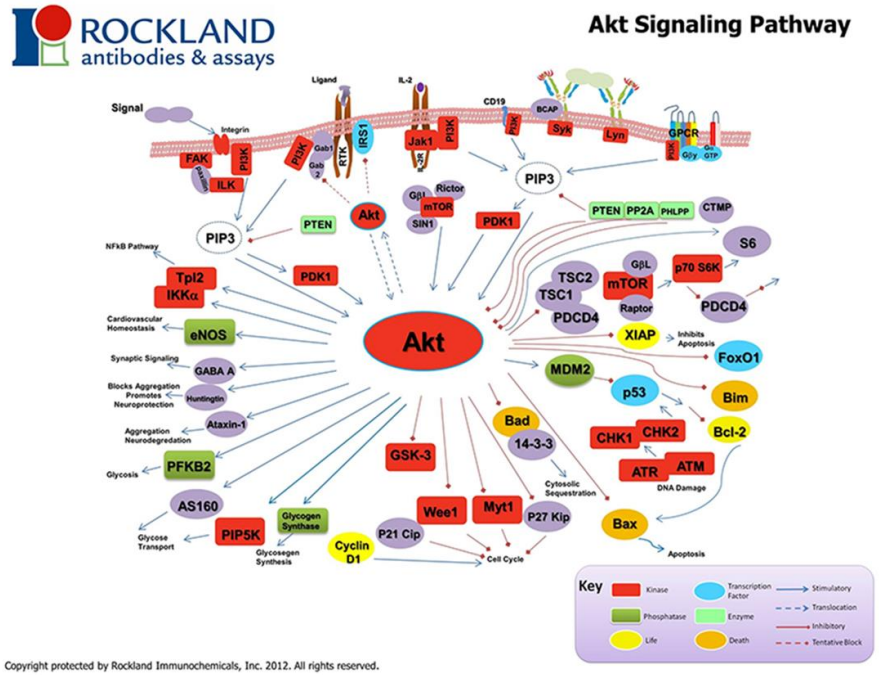

Activating metastasis

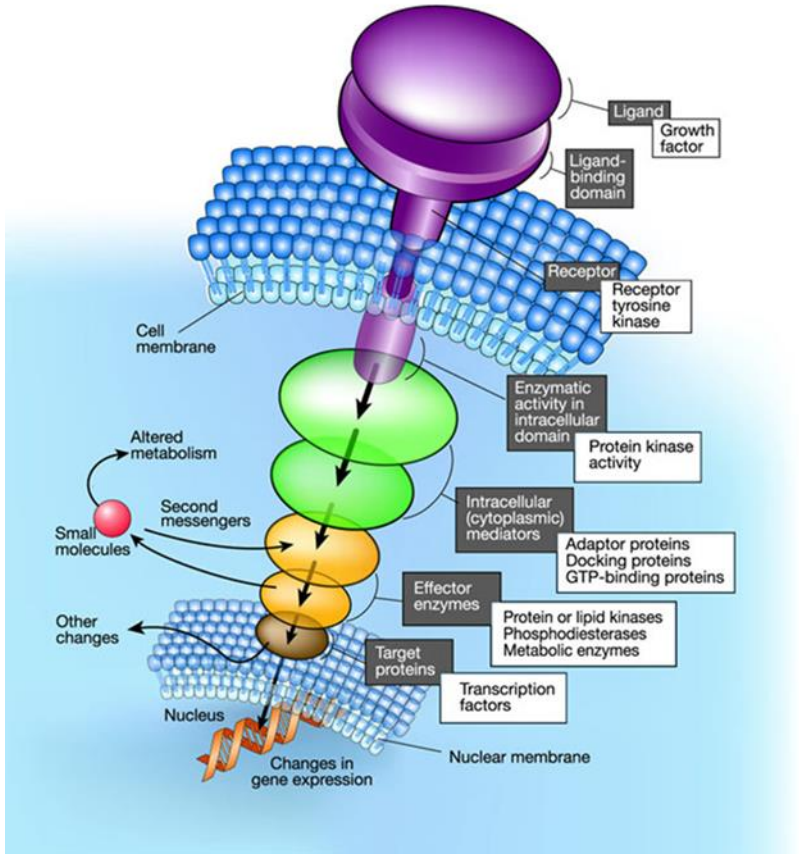
Tumor microenvironment

@Jana Šmardová

Sustaining proliferative signaling

- Homeostasis – cells are dependent on growth signals





MUNI MED

Sustaining proliferative signaling

- Tumor cells – dysregulation of critical pathways for survival
- Tumor cells
  - Uncontrolled proliferation
  - Invasivity
  - Resistance to death signals
  - Angiogeneiss
  - Metastasis
  - Resistance to apoptosis

Six hallmarks of cancer


Sustaining proliferative signaling


Evading growth suppressors


Resisting cell death


Enabling replicative immortality


Inducing angiogenesis

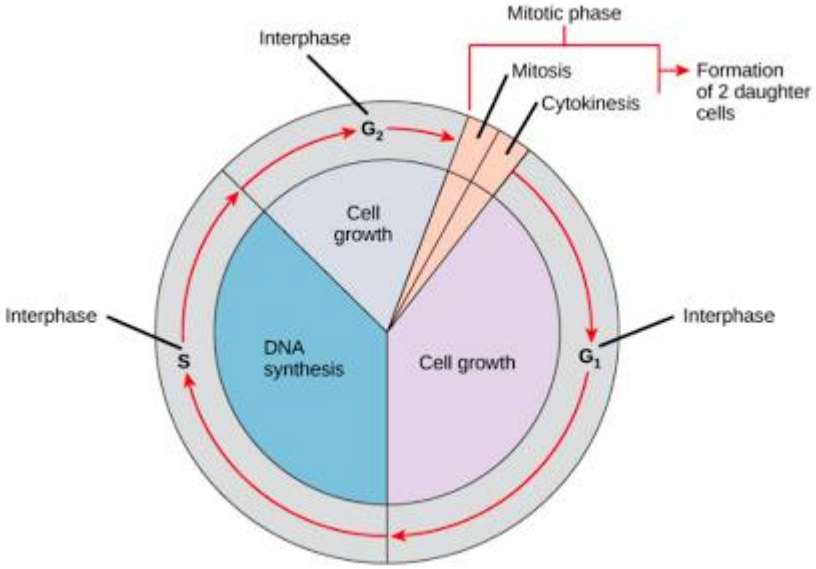

Activating metastasis

Tumor microenvironment

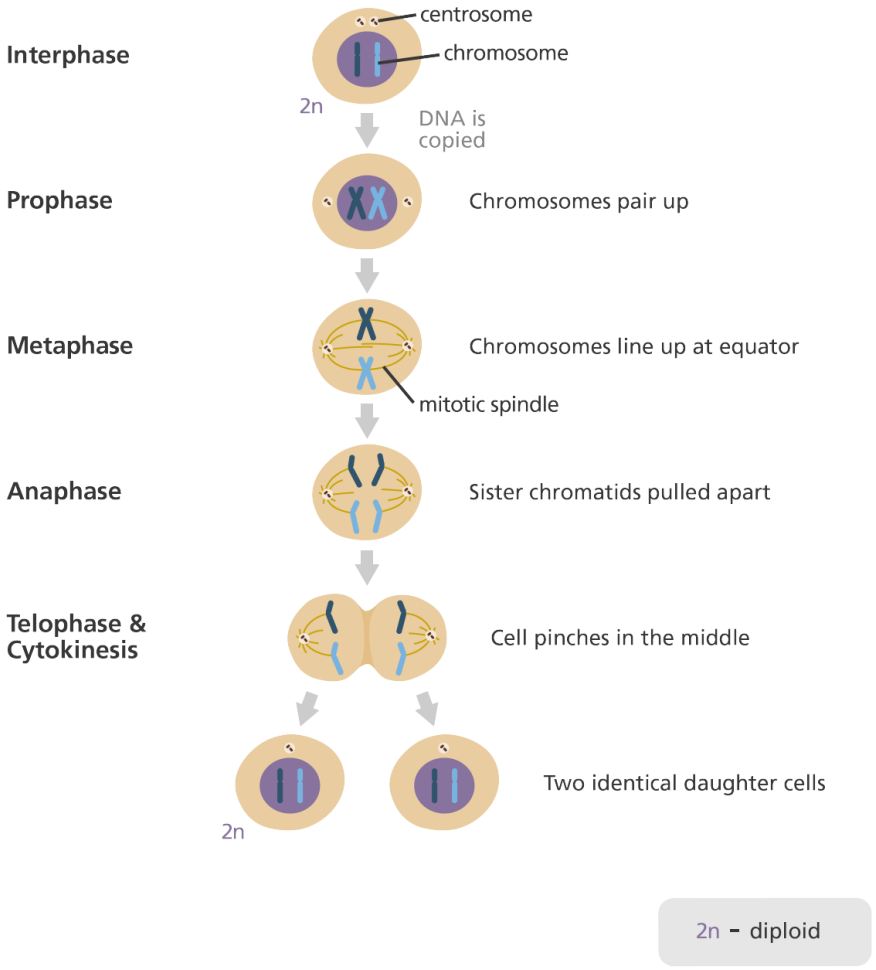
@Jana Šmardová

Cell cycle

- Basic principle for growth and reproduction – formation of new cells
- End product: 2 daughter cells from 1 maternal cell
- From mitosis to mitosis
- Nuclear division followed by cellular division
    - **G1 phase:** cell is small and young, creating organelles
    - **S phase:** DNA synthesis, replication of DNA
    - **G2 phase:** cytoskeleton and protein development
    - **M phase:** mitosis, 2 new cells



Mitosis



MUNI MED

Tumor as a cell cycle disease?

- Loss of CC regulation critical for transformation
- Loss of CC regulation is not the only part of carcinogenesis
- Does not lead to full transformation

MUNI
MED

Six hallmarks of cancer


Sustaining proliferative signaling


Evading growth suppressors


Resisting cell death


Enabling replicative immortality


Inducing angiogenesis


Activating metastasis

Tumor microenvironment

@Jana Šmardová

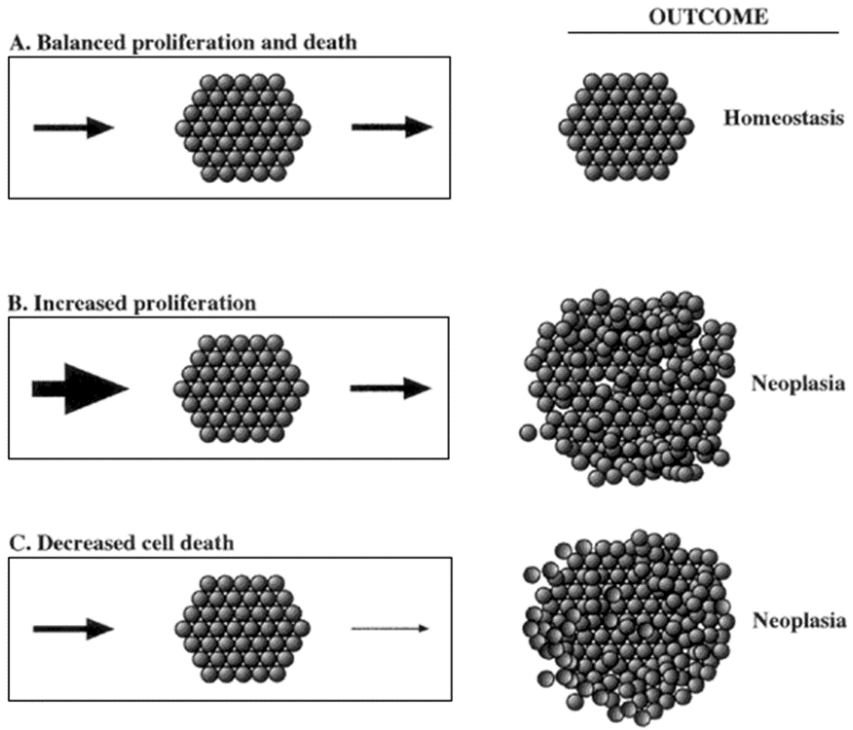
Apoptosis

- Programmed cell death
- Cell survive for a limited time – then apoptosis
- Tumor cells are resistant to proapoptotic signaling

MUNI
MED

Dysregulation of apoptosis

- Growth of tissues – balance between proliferation and cell death (homeostasis)
- In tumors, balance dysregulated



MUNI MED

Six hallmarks of cancer


Sustaining proliferative signaling


Evading growth suppressors


Resisting cell death


Enabling replicative immortality


Inducing angiogenesis


Activating metastasis

Tumor microenvironment

@Jana Šmardová

Replicative potential

- Mammal cells have replicative potential of about 60-70 divisions (Hayflick limit)
- Then senescence – change of morphology, metabolically active, do not divide
- 10^{-7} – immortal cell
- Most tumor cells – immortal – unlimited replicative potential

MUNI
MED

Six hallmarks of cancer


Sustaining proliferative signaling


Evading growth suppressors


Resisting cell death


Enabling replicative immortality


Inducing angiogenesis


Activating metastasis

Tumor microenvironment

@Jana Šmardová

Angiogenesis

- Growth of blood vessels
- Tumor – population of quickly and uncontrollably growing cells
- Tumors cannot grow more than 1-2 mm^3 - several million cells (not enough nutrients and oxygen)
- Need angiogenesis – until then, tumor growth slowly and linearly, then exponentially

MUNI
MED

Increasing angiogenesis

- Growth of new vessels is key for metastasis and for nutrients and oxygen
- In tumors, new vessels are not regular, blood flow irregularly,
- Different cells in the tumor microenvironment

MUNI
MED

Tumor vasculature is abnormal

- Highly disorganized
- Blood flow chaotic
- Places of hypoxia in certain parts of tumors
- Influences therapy effect
- Selection and clonal expansion of tumor cells

Six hallmarks of cancer


Sustaining proliferative signaling


Evading growth suppressors


Resisting cell death


Enabling replicative immortality


Inducing angiogenesis

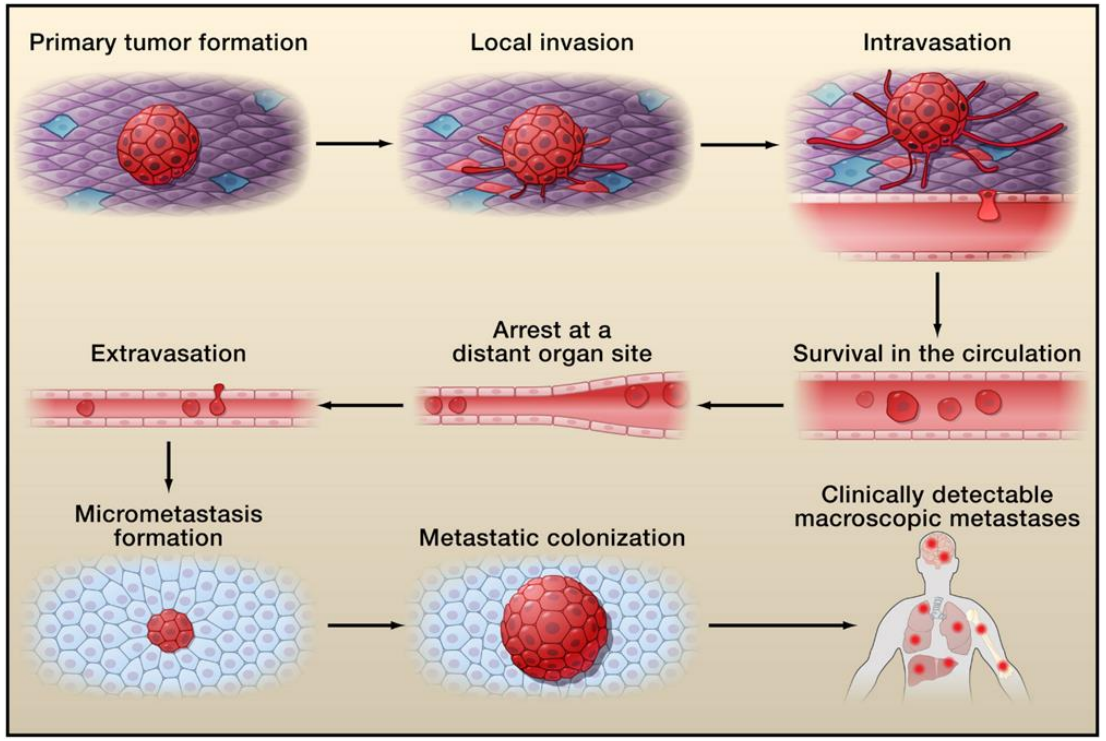

Activating metastasis

Tumor microenvironment

@Jana Šmardová

Formation of metastasis

- Tumor cells invade surrounding tissue
- Migrate from primary tumor to other tissues forming secondary tumors



MUNI MED

Metastases – most common cause of death

- Cause of about 90% of death of tumor patients
- Less common – effect of primary tumors
  - Brain tumors
  - Leukemia, lymphoma

MUNI
MED

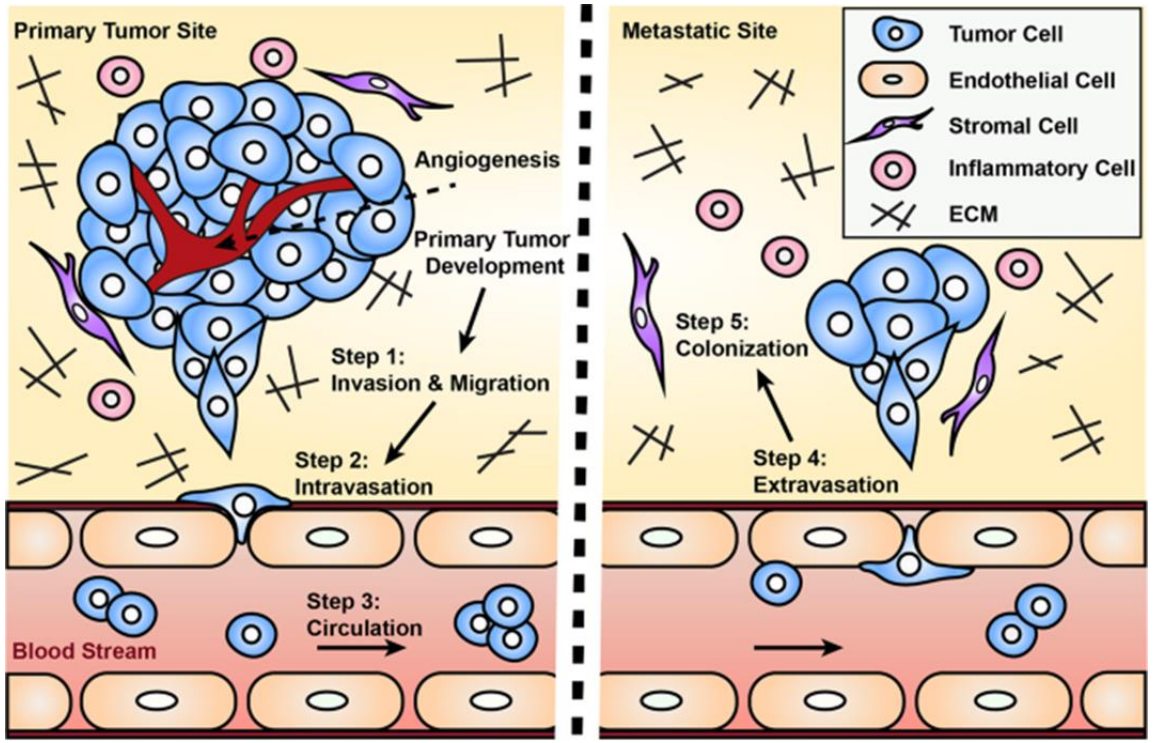
Bad news about metastases

- More than 70% of patients with invasive tumors have metastases at the time of diagnosis
- Invasive character – early in tumor progression
- Millions of tumor cells get into blood every day
- Angiogenesis influences metastasis formation

Good news about metastases

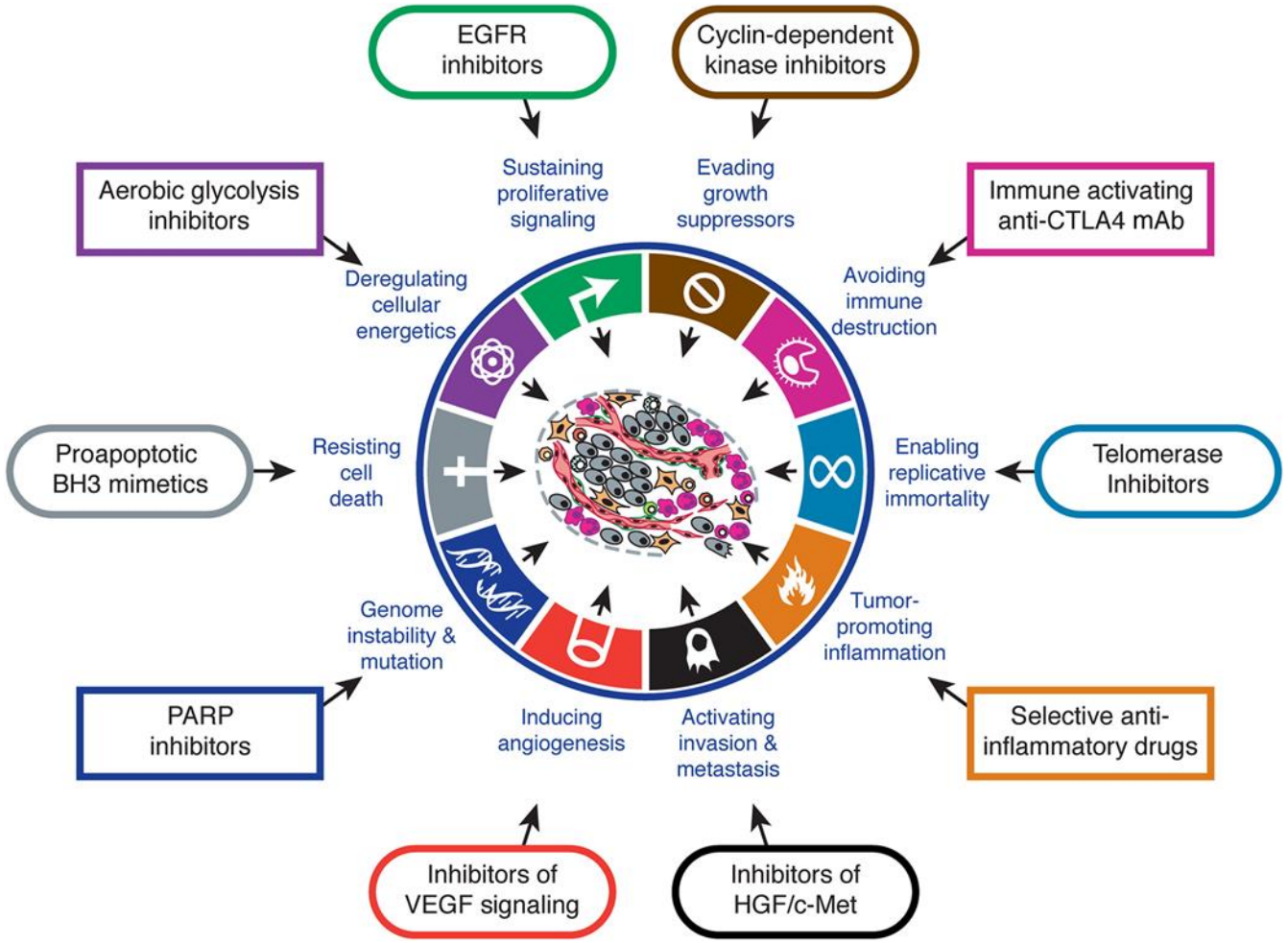
- Not very effective: less than 0.01% of circulating tumor cells is able to form metastasis
- We can detect circulating tumor cells early
- Leading to early therapy

Tumor – complex tissue



Tumor microenvironment supports growth of tumor, other cell types

@Jana Šmardová


EGFR inhibitors
Cyclin-dependent kinase inhibitors
Aerobic glycolysis inhibitors
Immune activating anti-CTLA4 mAb
Sustaining proliferative signaling
Evading growth suppressors
Deregulating cellular energetics
Avoiding immune destruction
Proapoptotic BH3 mimetics
Telomerase Inhibitors
Resisting cell death
Enabling replicative immortality
Genome instability & mutation
Tumor-promoting inflammation
PARP inhibitors
Selective anti-inflammatory drugs
Inducing angiogenesis
Activating invasion & metastasis
Inhibitors of VEGF signaling
Inhibitors of HGF/c-Met

MUNI MED

MUNI
MED

II. Hematological malignancies

Important definitions

- Incidence - number of new cases of a disease diagnosed each year
- Prevalence - total number of people living with a certain disease during a given period of time
- Overall survival - length of time from either the date of diagnosis or the start of treatment

MUNI
MED

Important definitions

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

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

- Partial remission - decrease of leukemic cells by at least 50% (observing total number of leukemic cells)

- Relapse - return 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

MUNI MED

Minimal residual disease - MRD

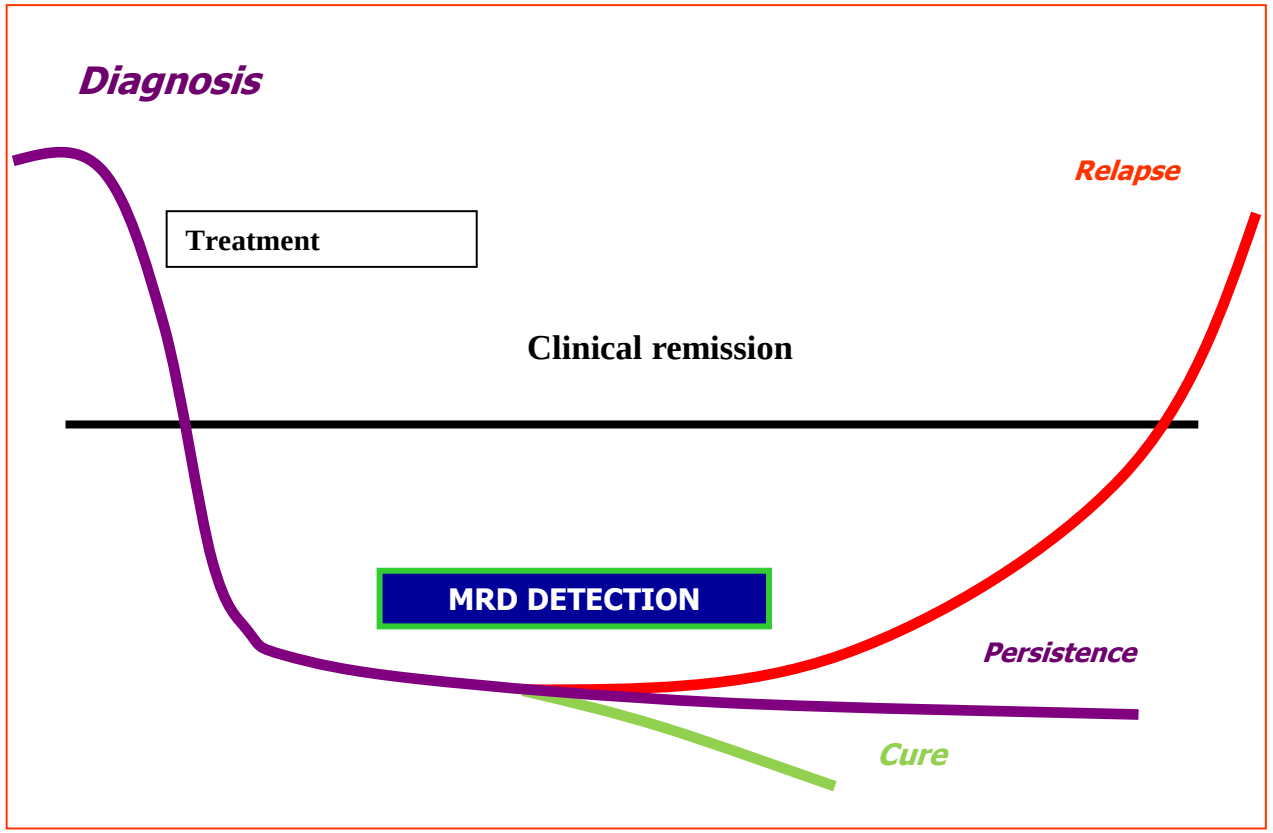
- Tumor cells not eradicated by the treatment
- Usually results in growth of these cells – resistance to treatment
- Emerging component of CR assessment in MM patients

- MRD negativity - associated with significantly longer OS

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

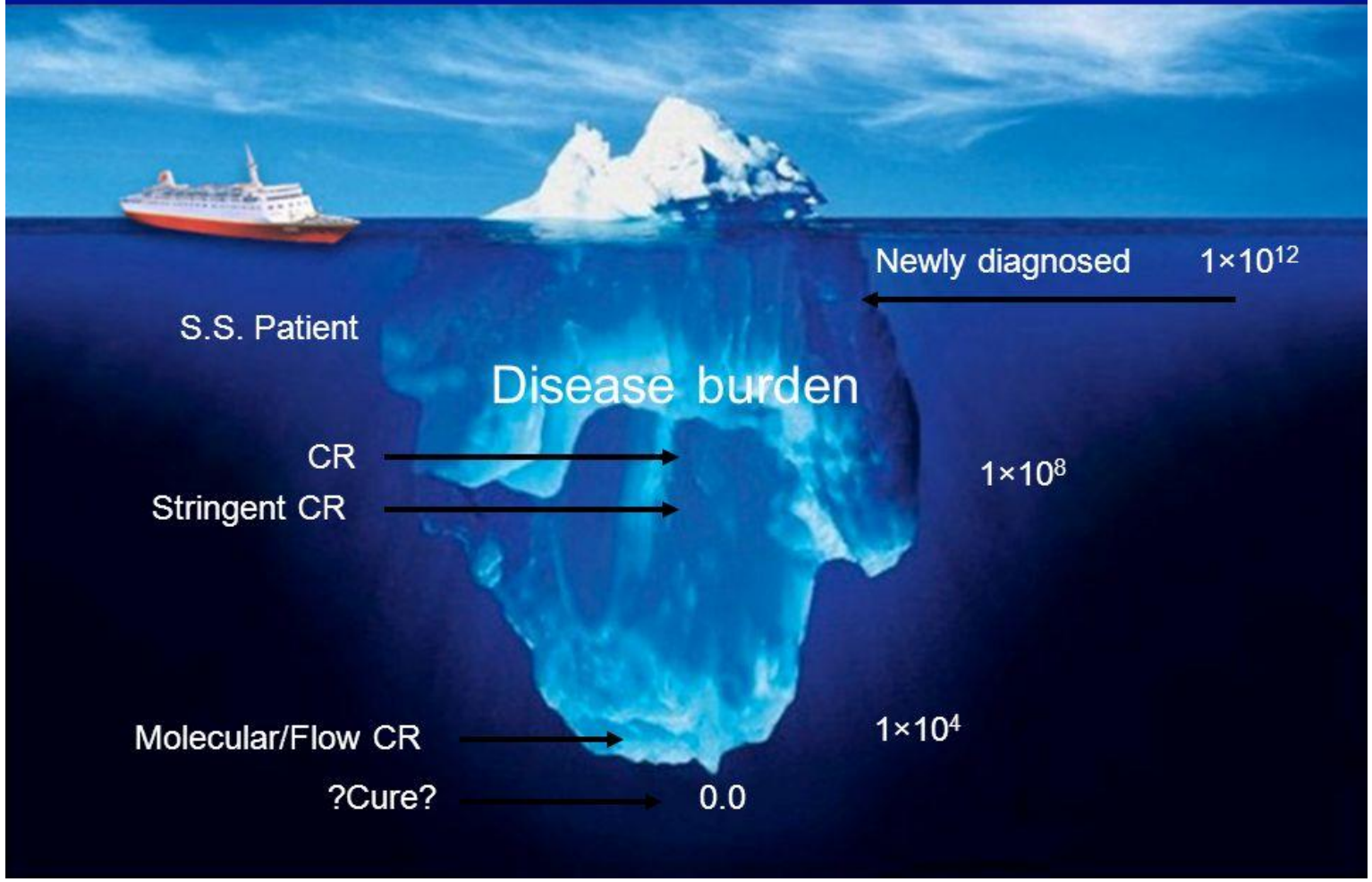
MUNI
MED

Minimal residual disease



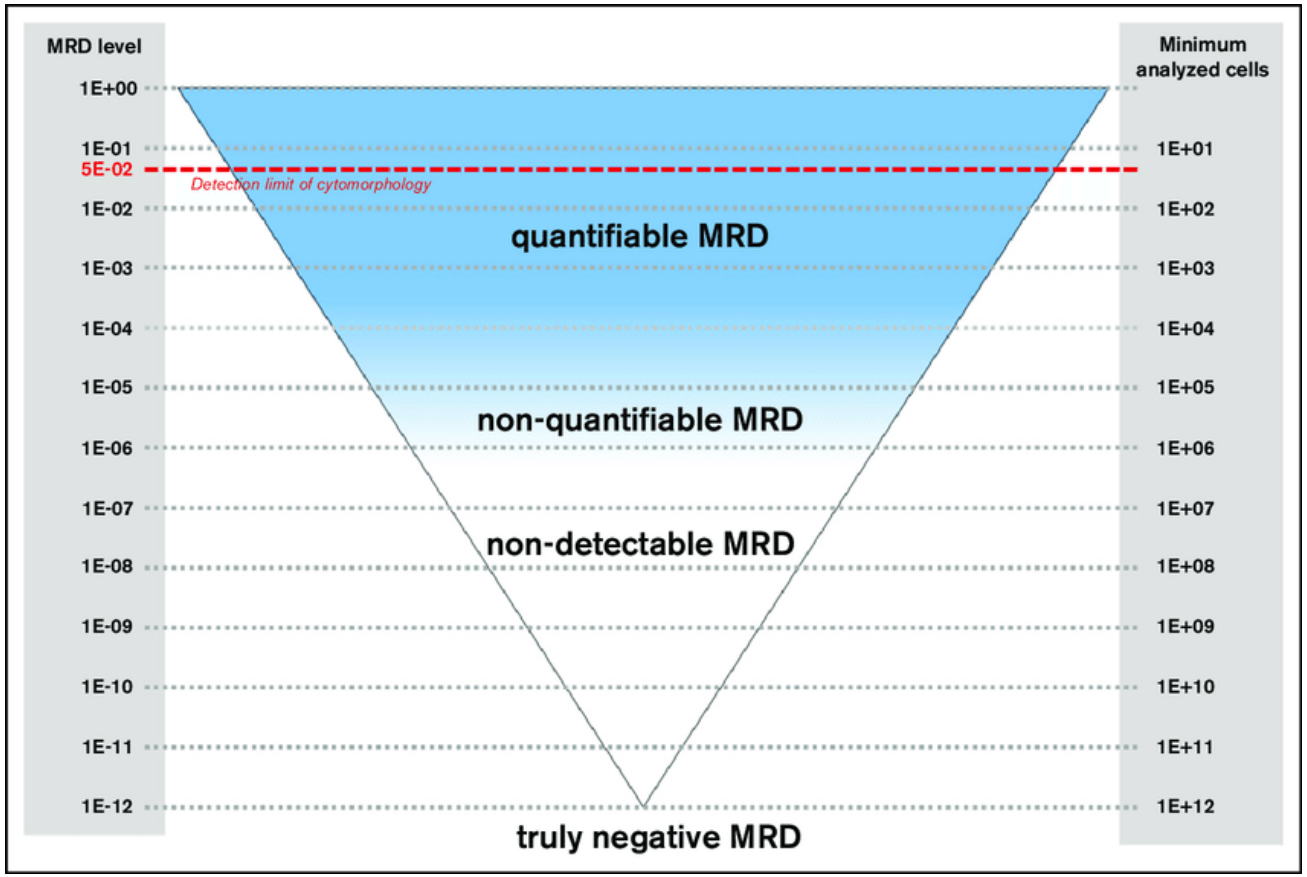
MUNI
MED

Getting to Minimal Residual Disease (MRD)



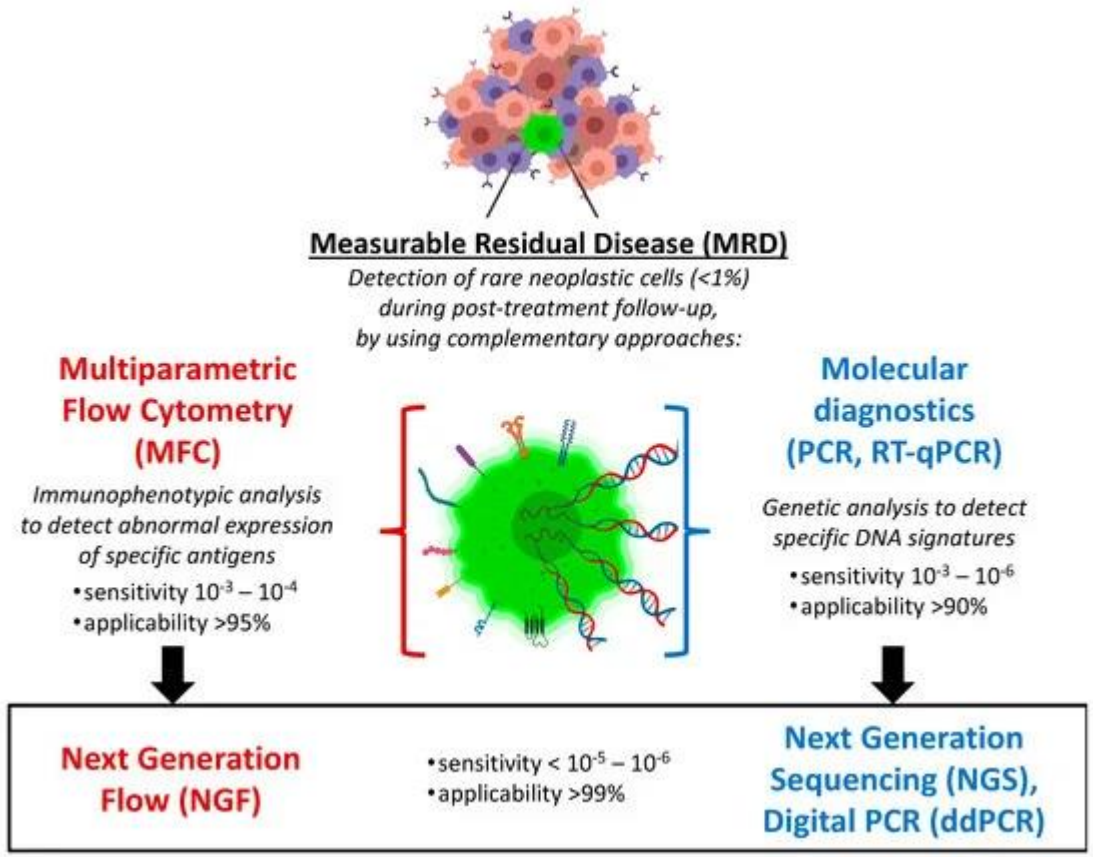
MUNI
MED

MRD


MRD level
1E+00
1E-01
5E-02
Detection limit of cytomorphology
1E-02
1E-03
1E-04
1E-05
1E-06
1E-07
1E-08
1E-09
1E-10
1E-11
1E-12
Minimum analyzed cells
1E+01
1E+02
1E+03
1E+04
1E+05
1E+06
1E+07
1E+08
1E+09
1E+10
1E+11
1E+12
quantifiable MRD
non-quantifiable MRD
non-detectable MRD
truly negative MRD

MUNI MED

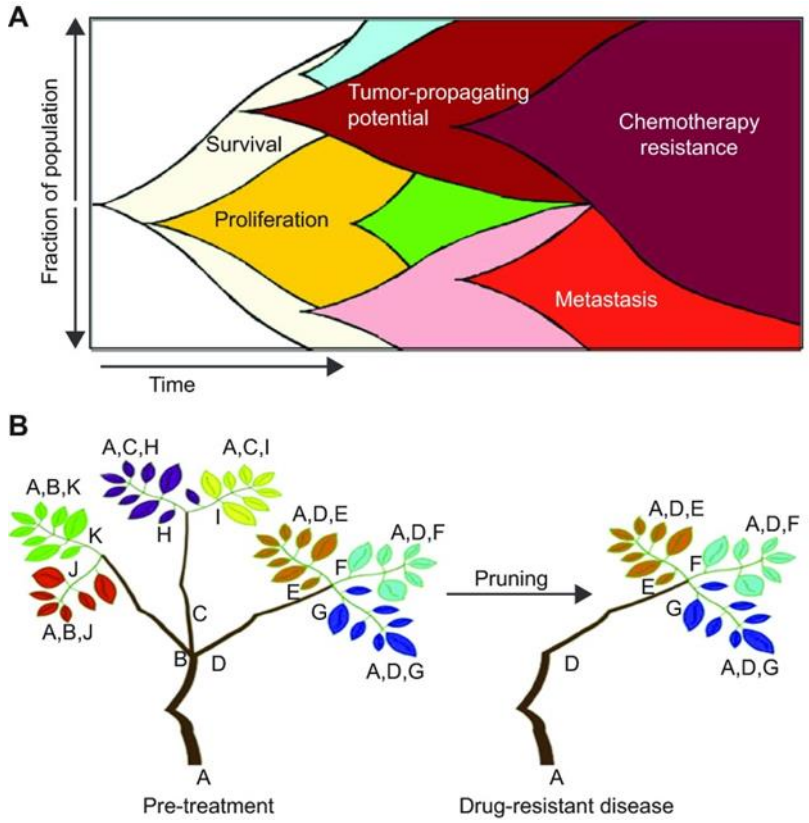
MRD


Measurable Residual Disease (MRD)
Detection of rare neoplastic cells (<1%)
during post-treatment follow-up,
by using complementary approaches:
Multiparametric Flow Cytometry (MFC)
Immunophenotypic analysis to detect abnormal expression of specific antigens
•sensitivity 10^{-3} – 10^{-4}
•applicability >95%
Molecular diagnostics (PCR, RT-qPCR)
Genetic analysis to detect specific DNA signatures
•sensitivity 10^{-3} – 10^{-6}
•applicability >90%
Next Generation Flow (NGF)
•sensitivity < 10^{-5} – 10^{-6}
•applicability >99%
Next Generation Sequencing (NGS), Digital PCR (ddPCR)

MUNI MED

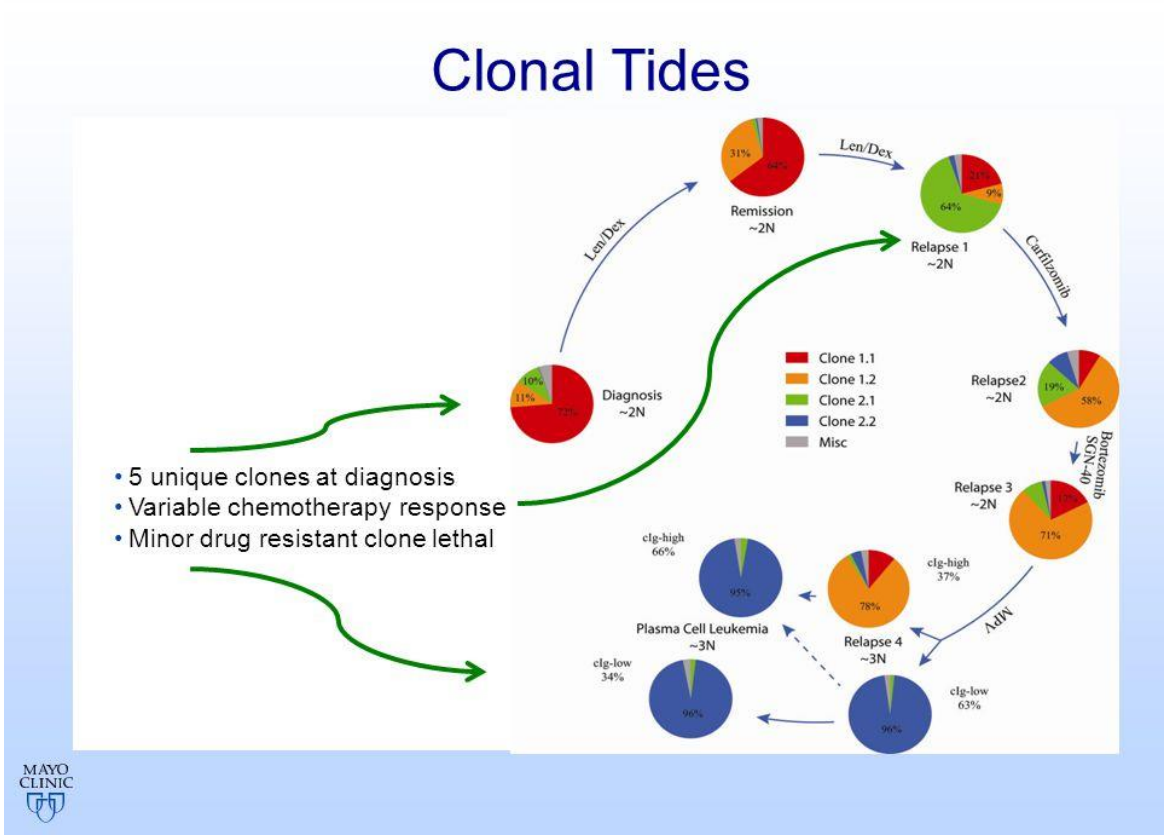
Clonal evolution of tumors

- Clonal expansion and selection
- Presence of many subclones, sometimes undetectable at diagnosis
- Different response to treatment
- Change of treatment



MUNI MED

Clonal tides



MUNI MED

Hematological malignancies

Leukemia

Lymphoma

Multiple myeloma

MUNI
MED

Hematological malignancies

Leukemia

Lymphoma

Multiple myeloma

MUNI
MED

Leukemia

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

MUNI
MED

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

MUNI
MED

Clinical features

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

MUNI
MED

Prognosis of leukemia

Morphology

Chromosomal aberrations

Age – worse prognosis

B cells - worse prognosis

MUNI
MED

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)

MUNI
MED

After chemotherapy

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

MUNI
MED

Leukemia

Acute

Chronic

MUNI
MED

Leukemia

Acute

Chronic

Myeloid

Lymphoid

MUNI
MED

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

MUNI
MED

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

MUNI
MED

ALL – more common in children

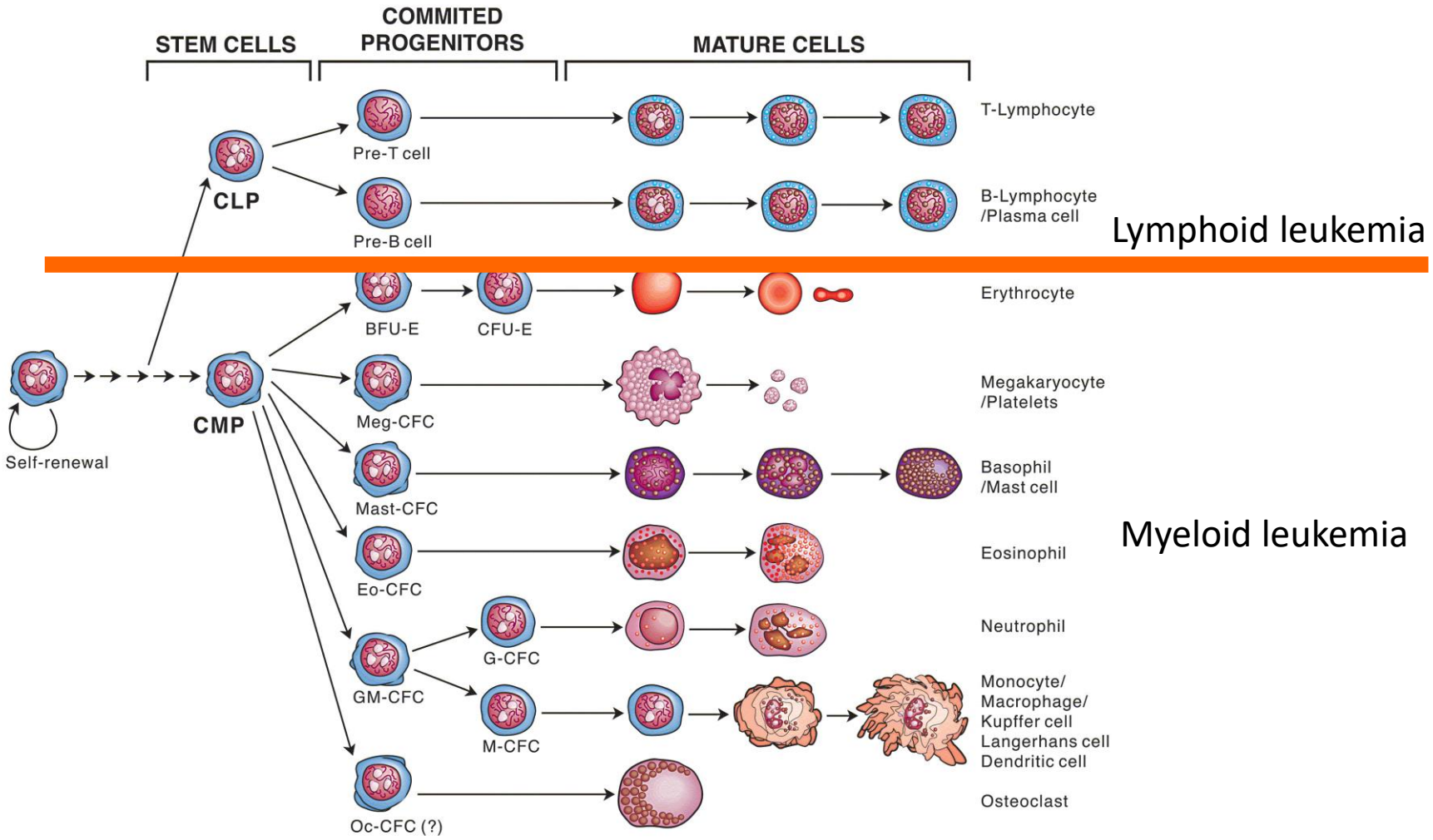
AML – more common in elderly

CLL – most common in adults

CML – mostly in adults

MUNI MED

Hematopoesis



MUNI MED

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

MUNI
MED

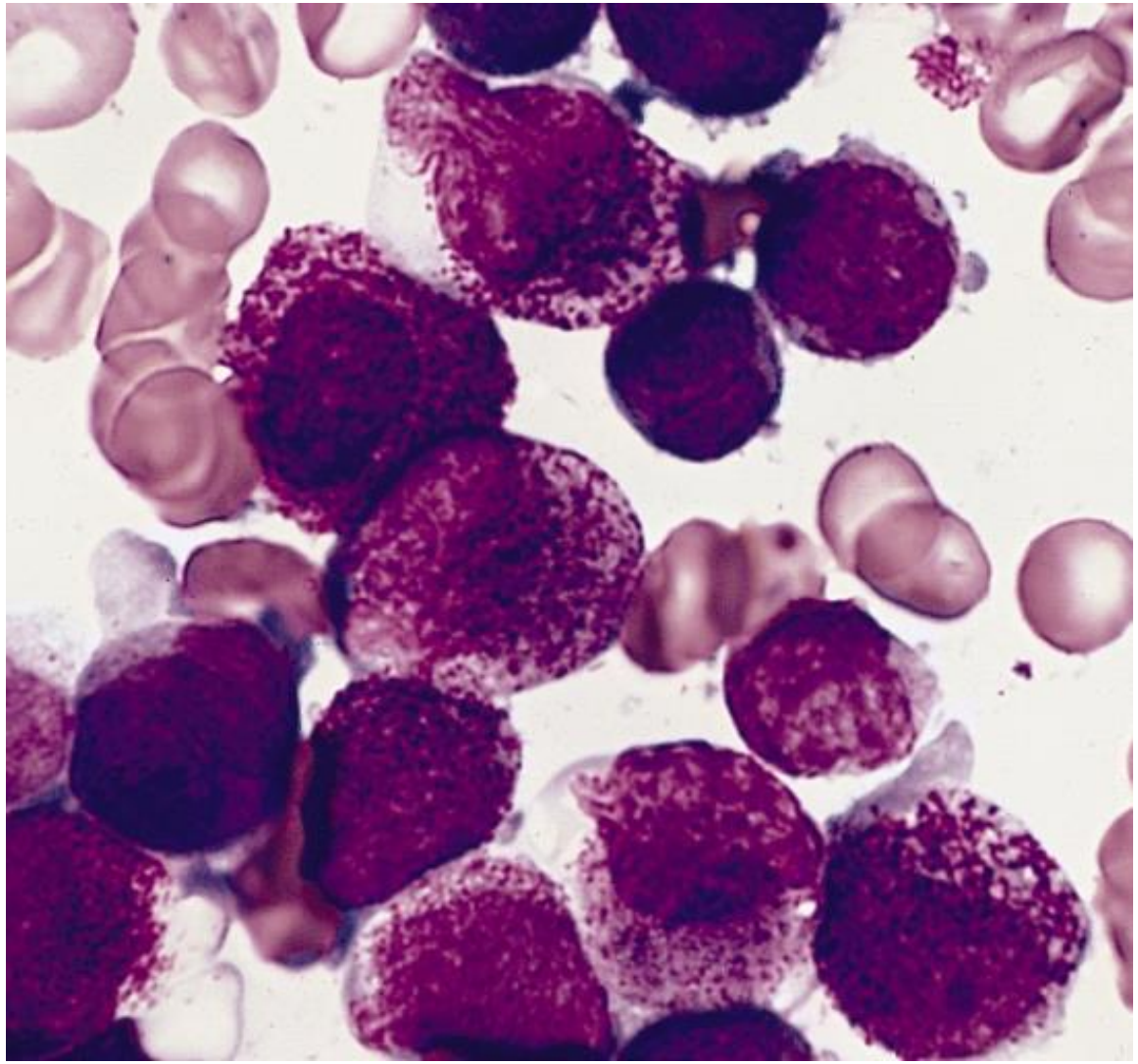
Acute myeloid leukemia - AML

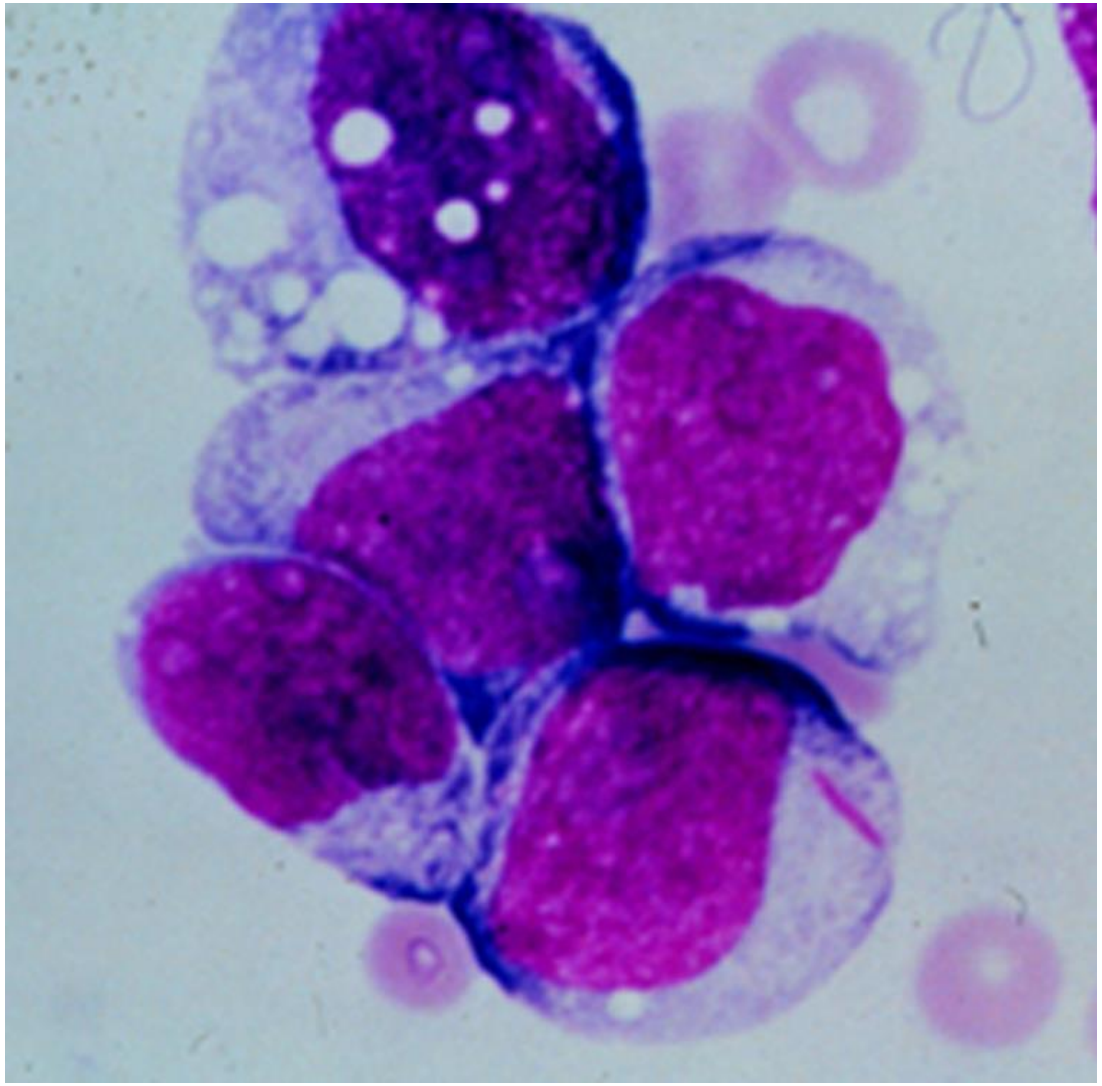
MUNI
MED

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

MUNI
MED

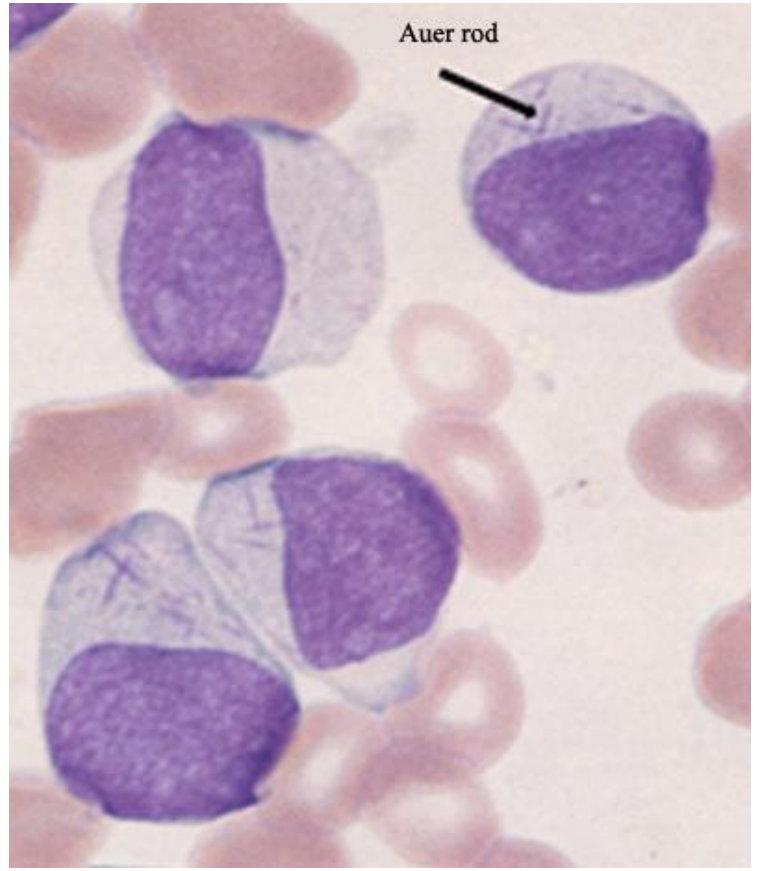




MUNI
MED

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

MUNI
MED

Classification of AML

FAB -

French American British classification

- 8 subtypes
- based on morphology and cytochemistry

WHO classification

- based on molecules, morphology and clinics

MUNI MED

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

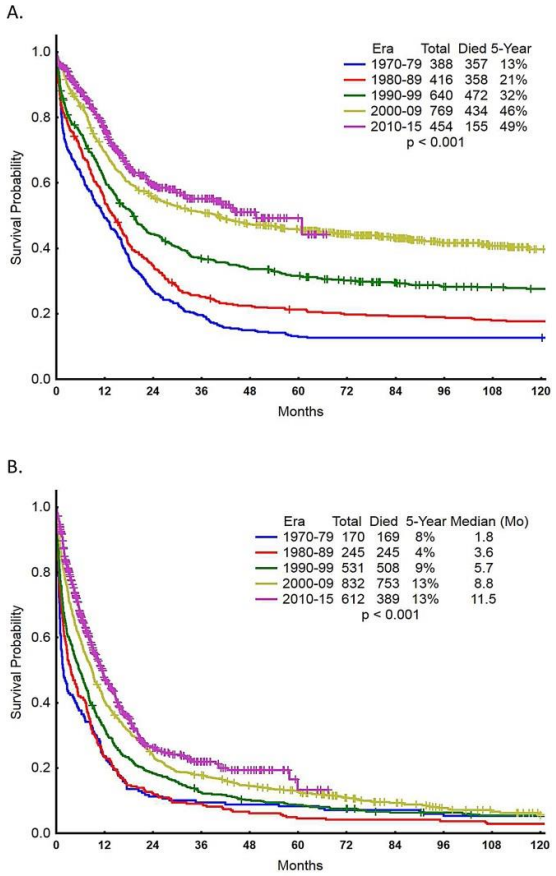
Mature B-cell neoplasms
Chronic lymphocytic leukemia/small lymphocytic lymphoma
Monoclonal B-cell lymphocytosis*
B-cell prolymphocytic leukemia
Splenic marginal zone lymphoma
Hairy cell leukemia
Splenic B-cell lymphoma/leukemia, unclassifiable
Splenic diffuse red pulp small B-cell lymphoma
Hairy cell 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

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

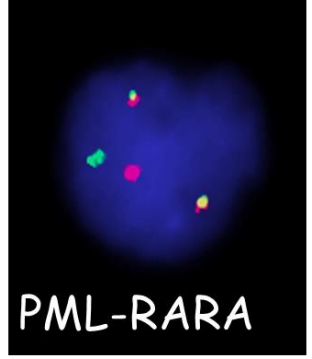
MUNI MED

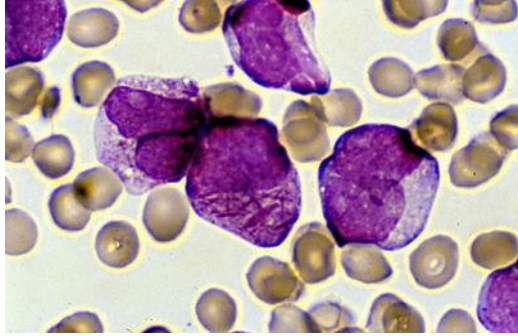
Acute promyelocytic leukemia - APL

the most malignant human leukemia

MUNI
MED

APL

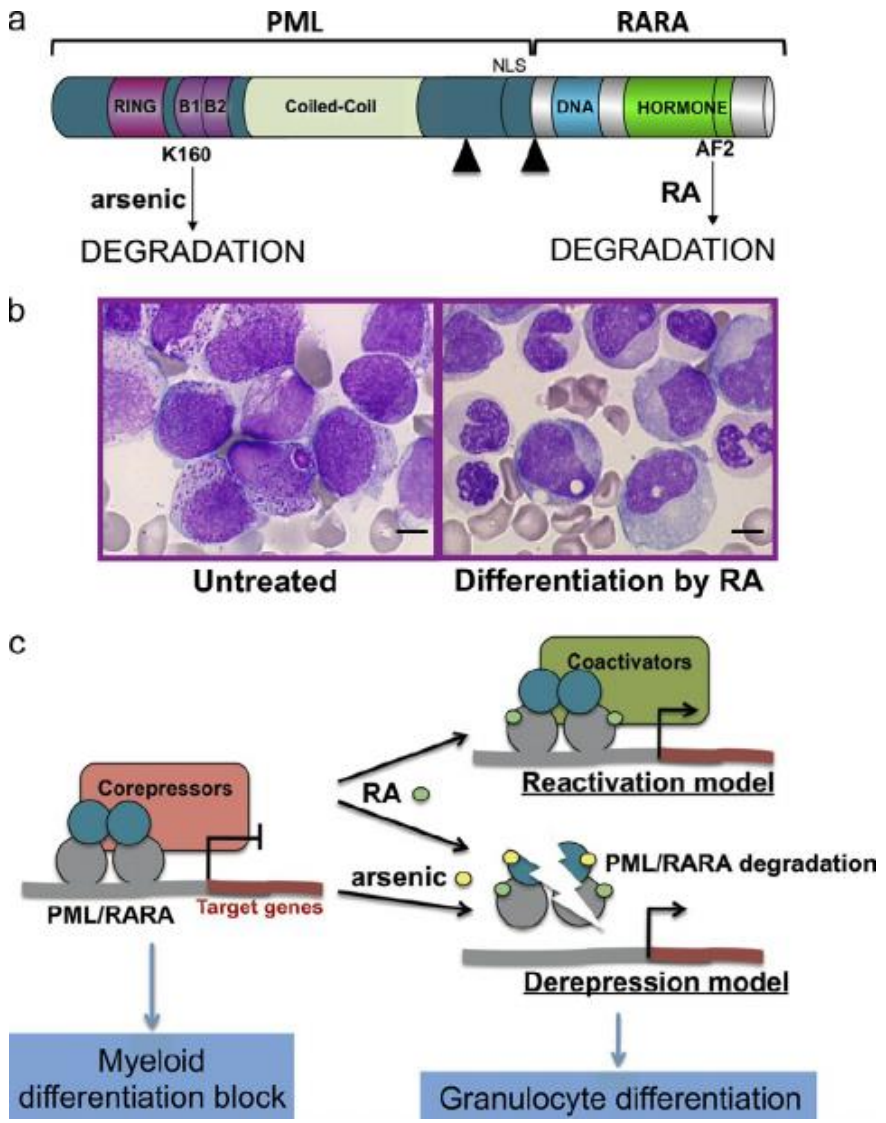




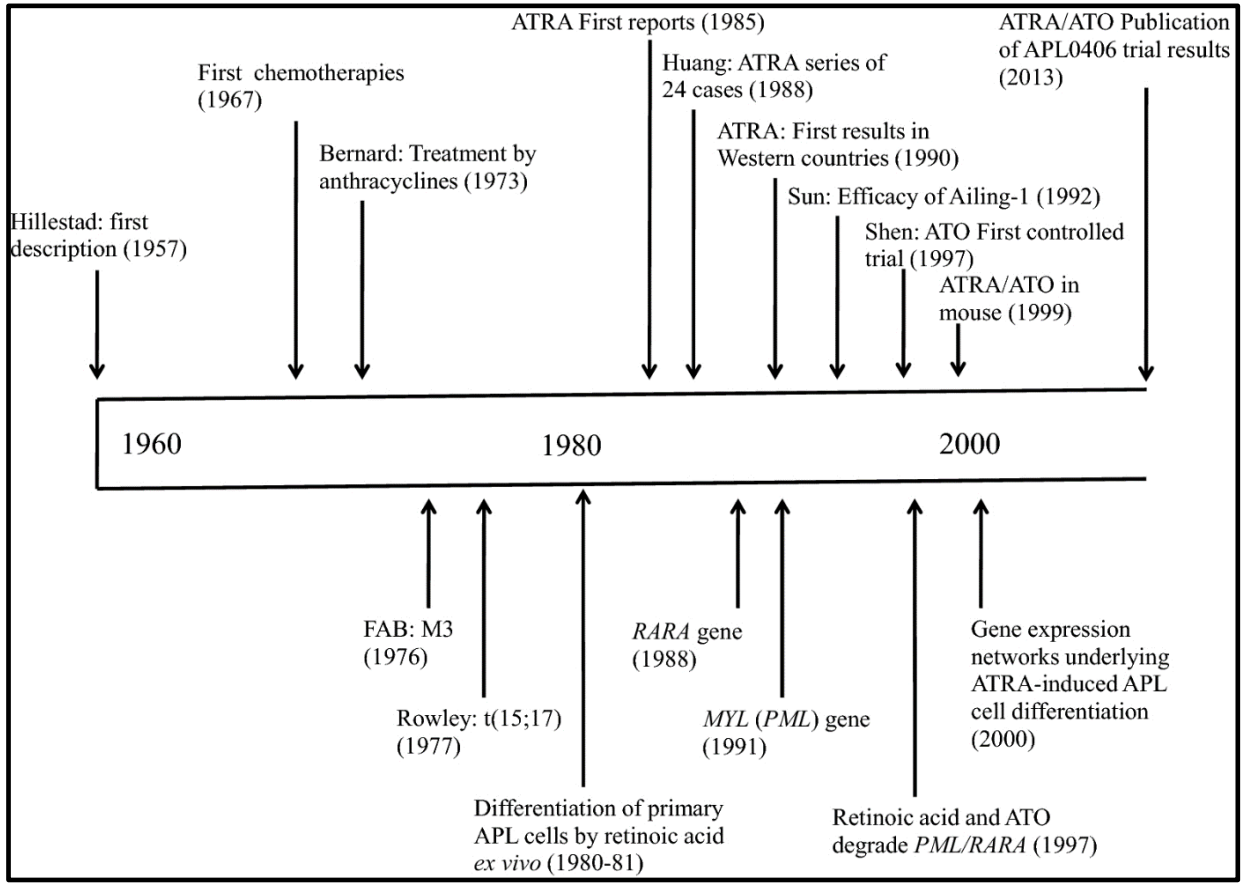
- accumulation of promyelocytes (differentiation stage of granulocytes)
- M3 classification based on FAB
- treatment commenced immediately – medical emergency
- for a diagnosis - detection of translocation necessary
- median age at diagnosis 40 - same risk throughout lifetime
- 1957 - subtype of leukemia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

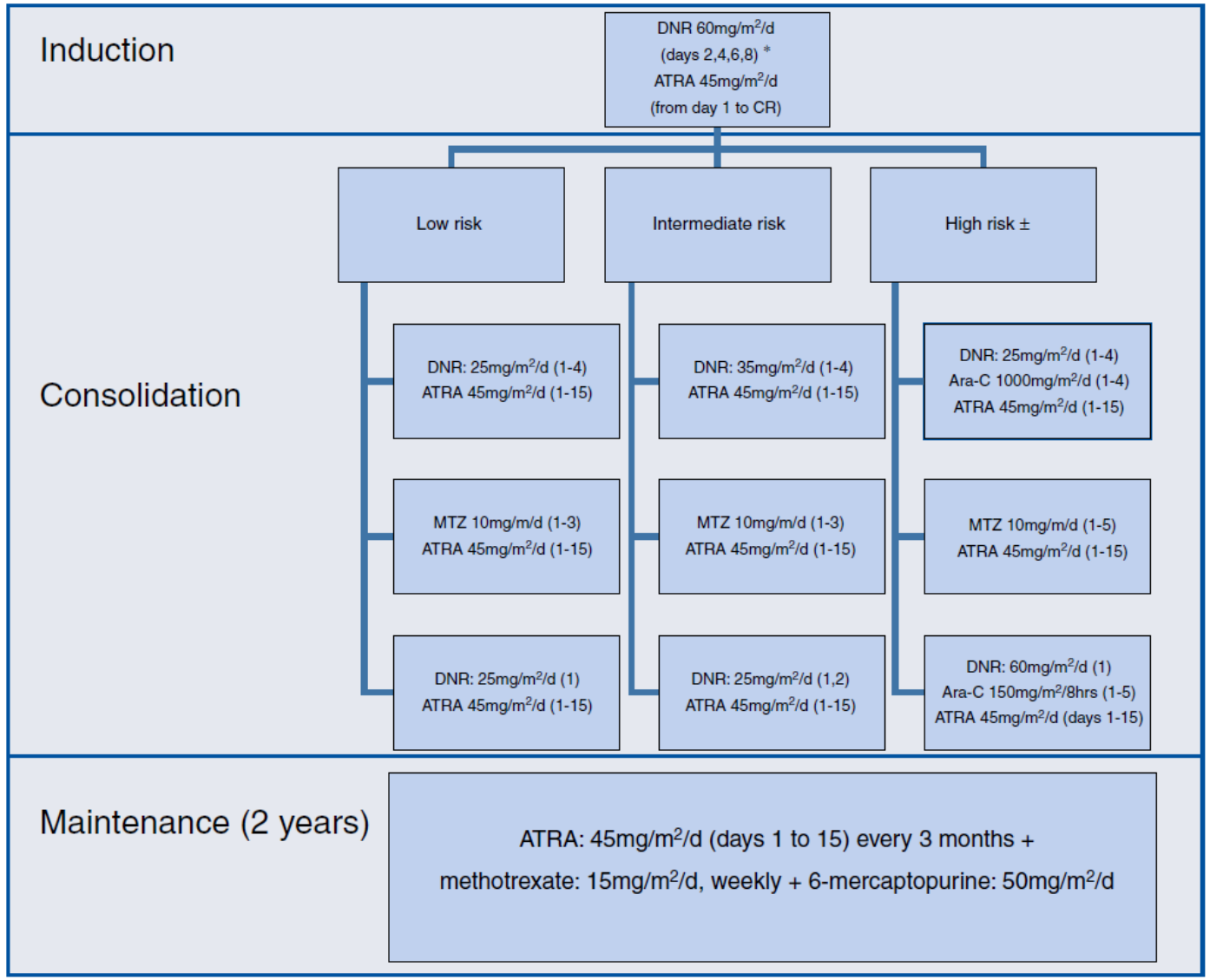


APL treatment



MUNI MED

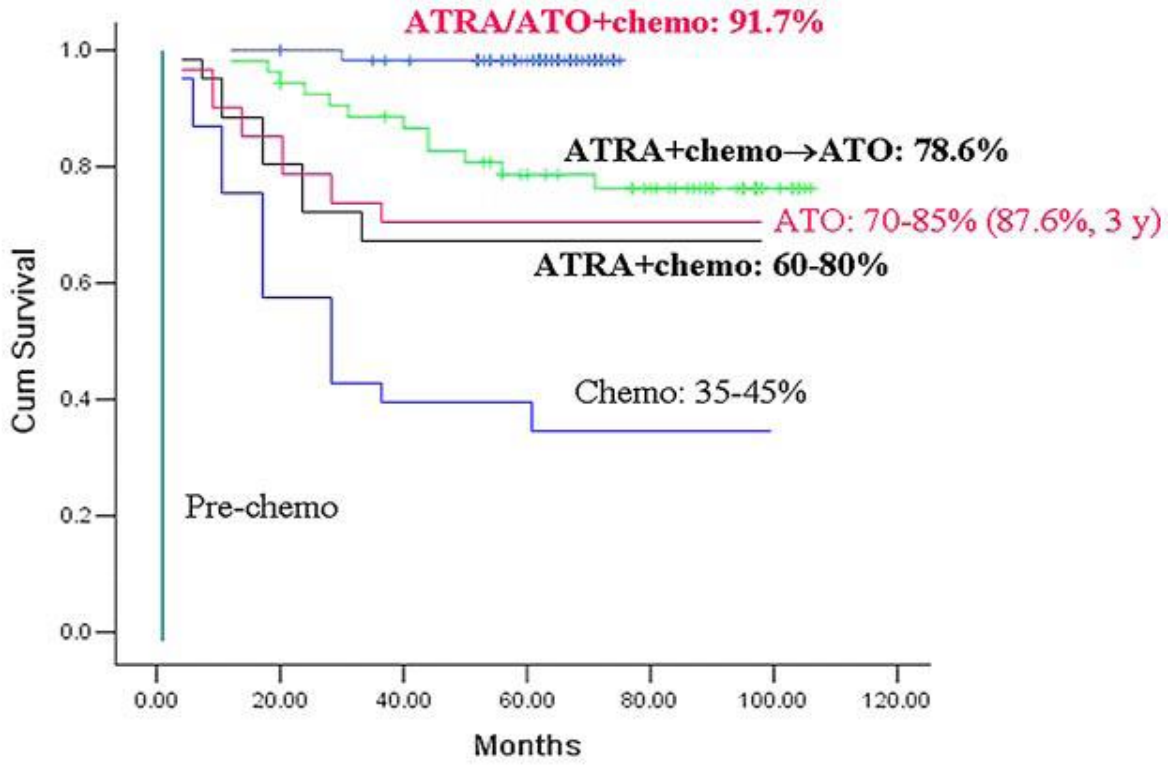
APL treatment



Crespo-Solis 2016

MUNI MED

APL survival



MUNI MED

Acute lymphoid leukemia - ALL

MUNI
MED

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

MUNI
MED

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

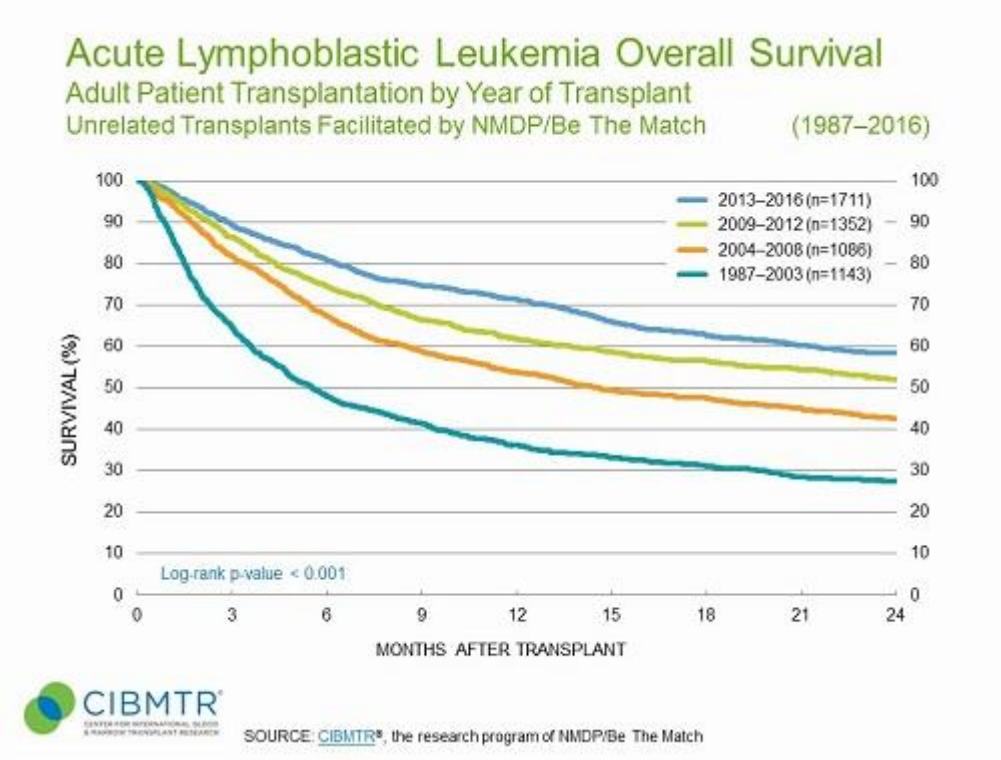
MUNI
MED

ALL treatment

- Induction (vincristin, corticosteroids, anthracyclins)
- Transplantation of bone marrow

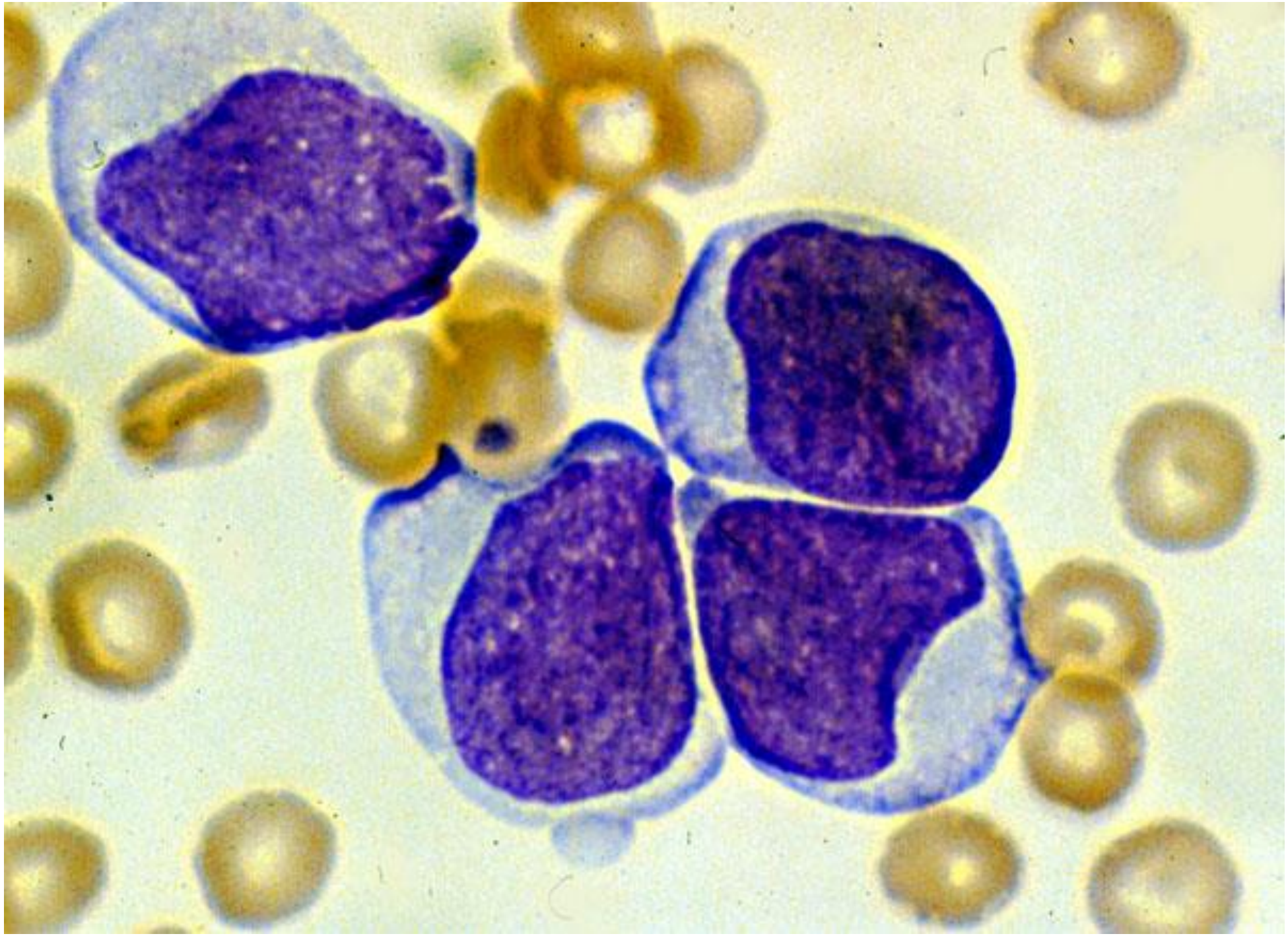
Or

- Consolidation
- Maintenance 2-3 years



Terwilliger 2017

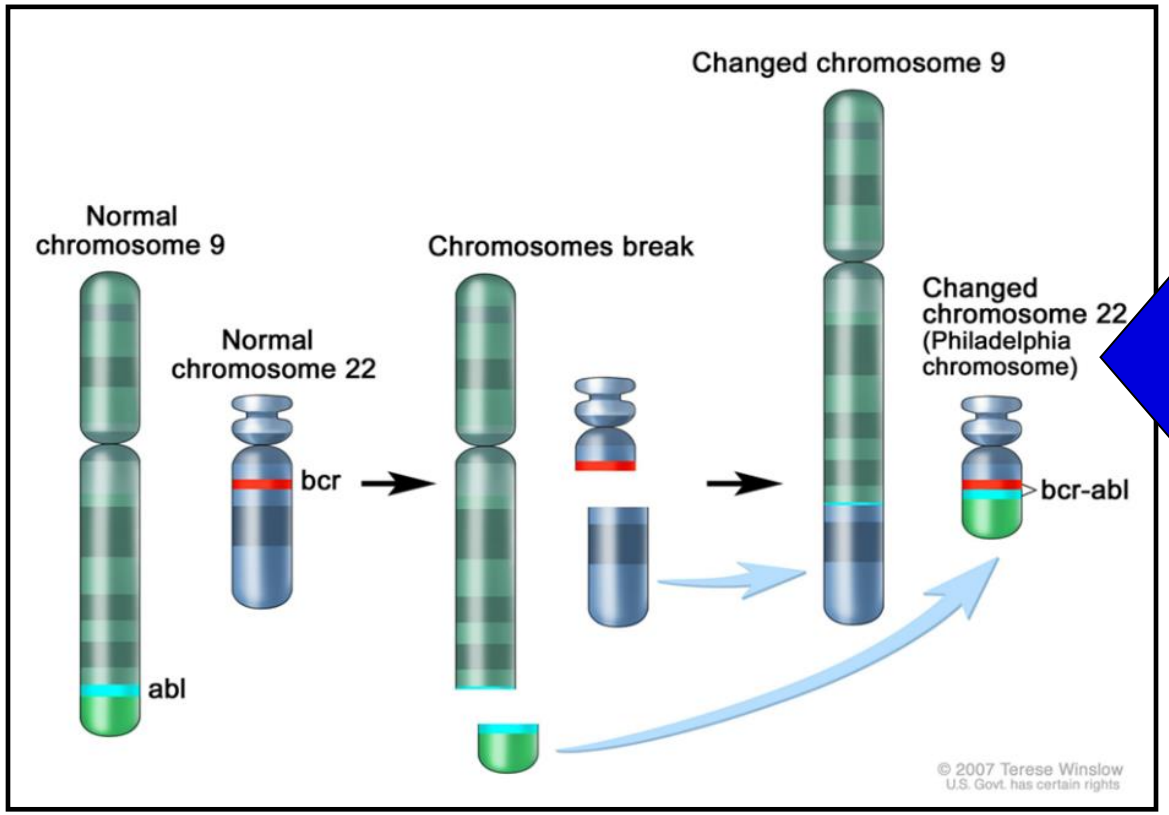
MUNI MED



Chronic myeloid leukemia - CML

MUNI
MED

Chronic myeloid leukemia - CML



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

MUNI MED

Philadelphia chromosome

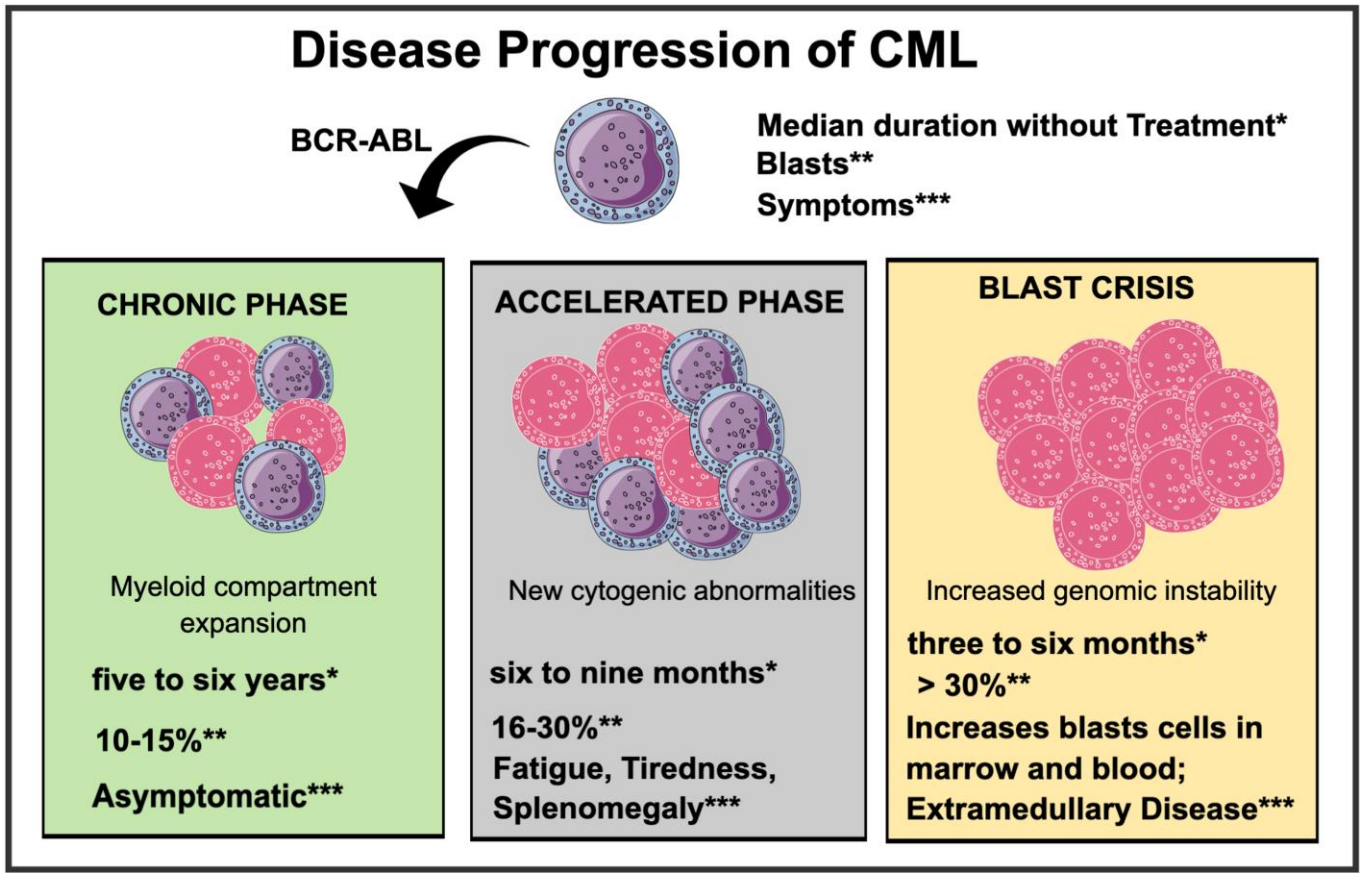
- 1960 – Peter Nowell and David Hungerford described an abnormal chromosome in CML
- First genetic cause of tumors
- 1972 – reason or consequence? Janet Rowley – t(9,22)

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)

MUNI
MED

CML progression



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)

MUNI
MED

CML treatment

- Until 2000 – hydroxyurea, IFNα
- Transplantation of bone marrow curative but high mortality

MUNI
MED

Gleevec (1993) Novartis

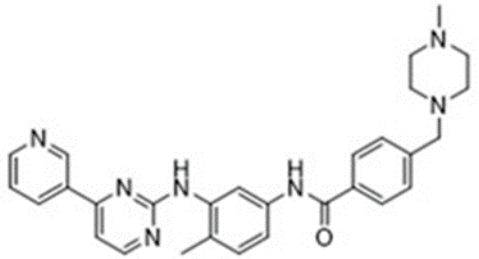
- Imatinib mesylate
- Active against CML colonies (Druker 1996)
- 2 years later – clinical study: 31 patients, 98% response rate
- Clinical study phase III: 16 countries, 177 centers, 1000 patients – study stopped, all patients on Gleevec
- Survival 95%, survival 65% in blast crisis (8 years)
- Molecular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

MUNI
MED

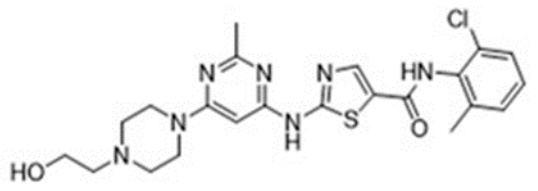
Current treatment of CML

- **Imatinib (Gleevec)**
- **Dasatinib (Sprycel)**
- **Nilotinib (Tasigna)**
- **Bosutinib (Bosulif)**
- Ponatinib (Iclusig)
- Asciminib (Scemblix)

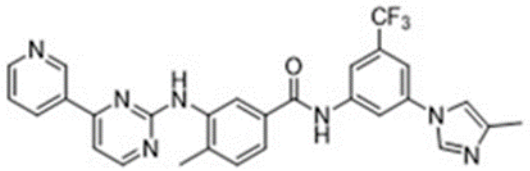
FDA approved



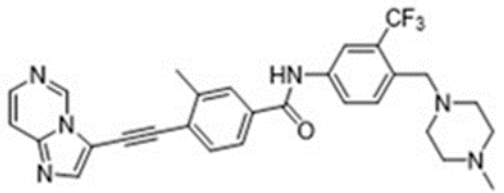
A, Imatinib



B, Dasatinib



C, Nilotinib

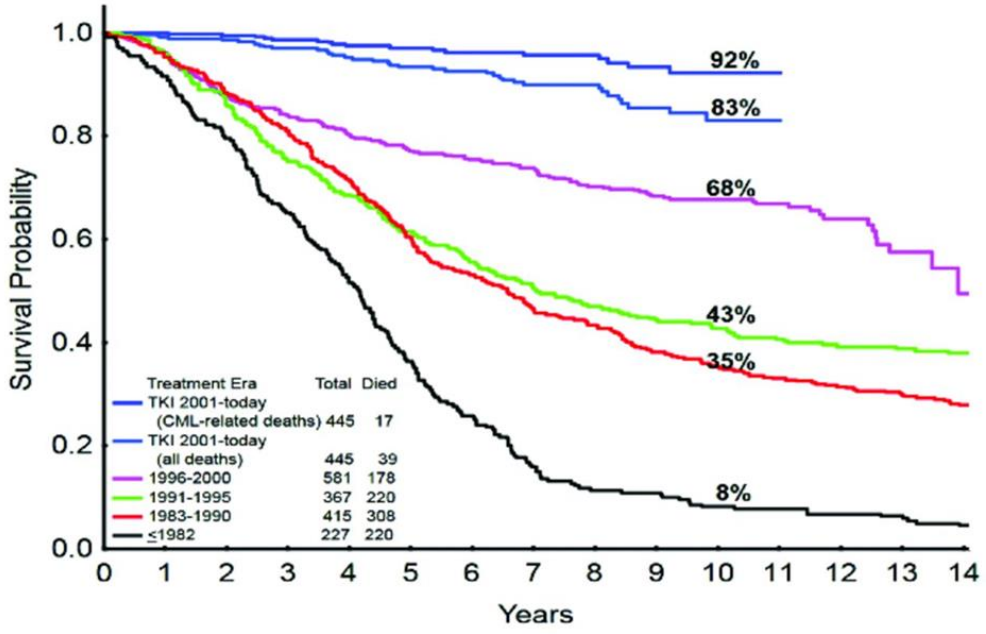


D, Ponatinib

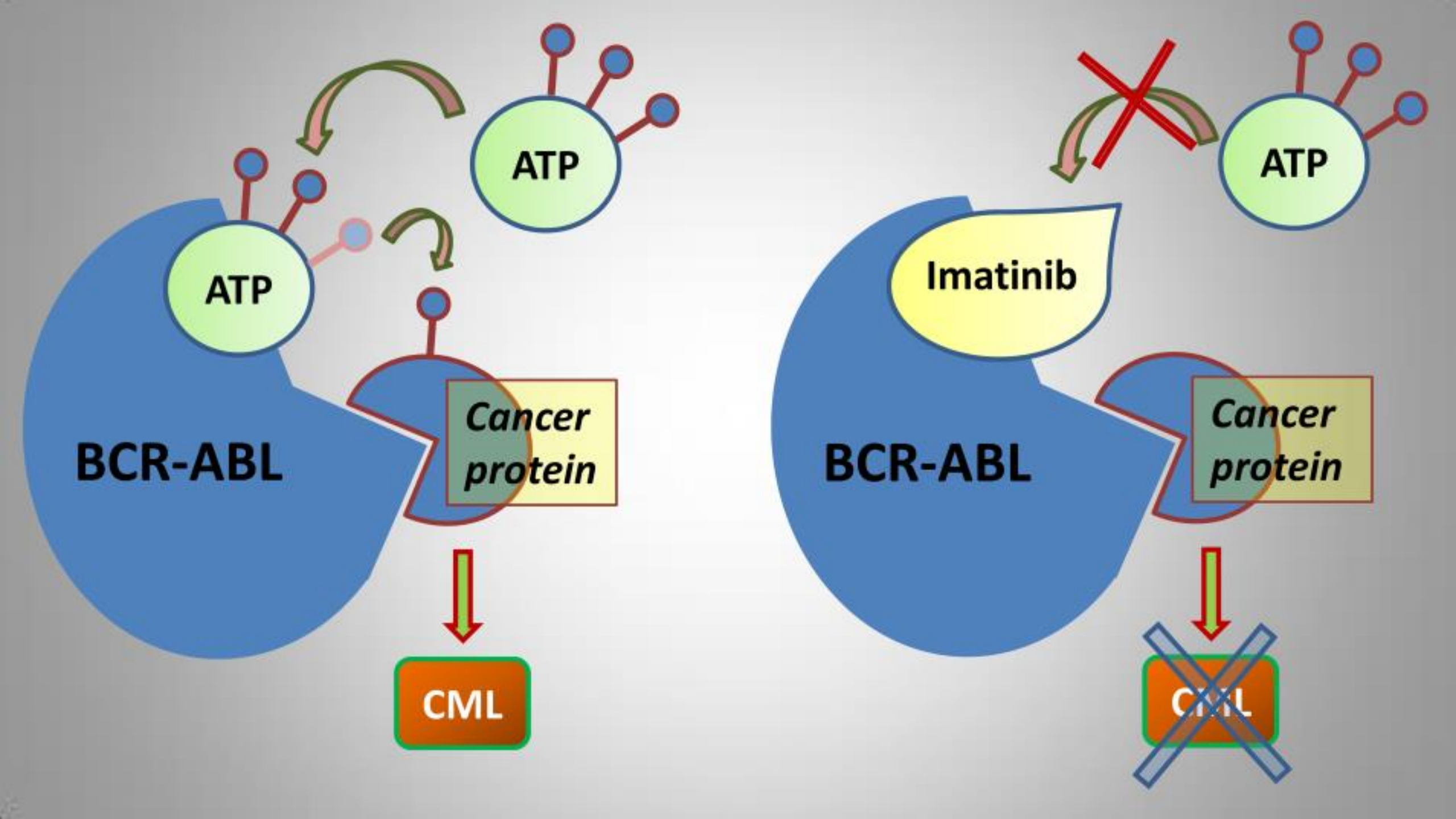
MUNI MED

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



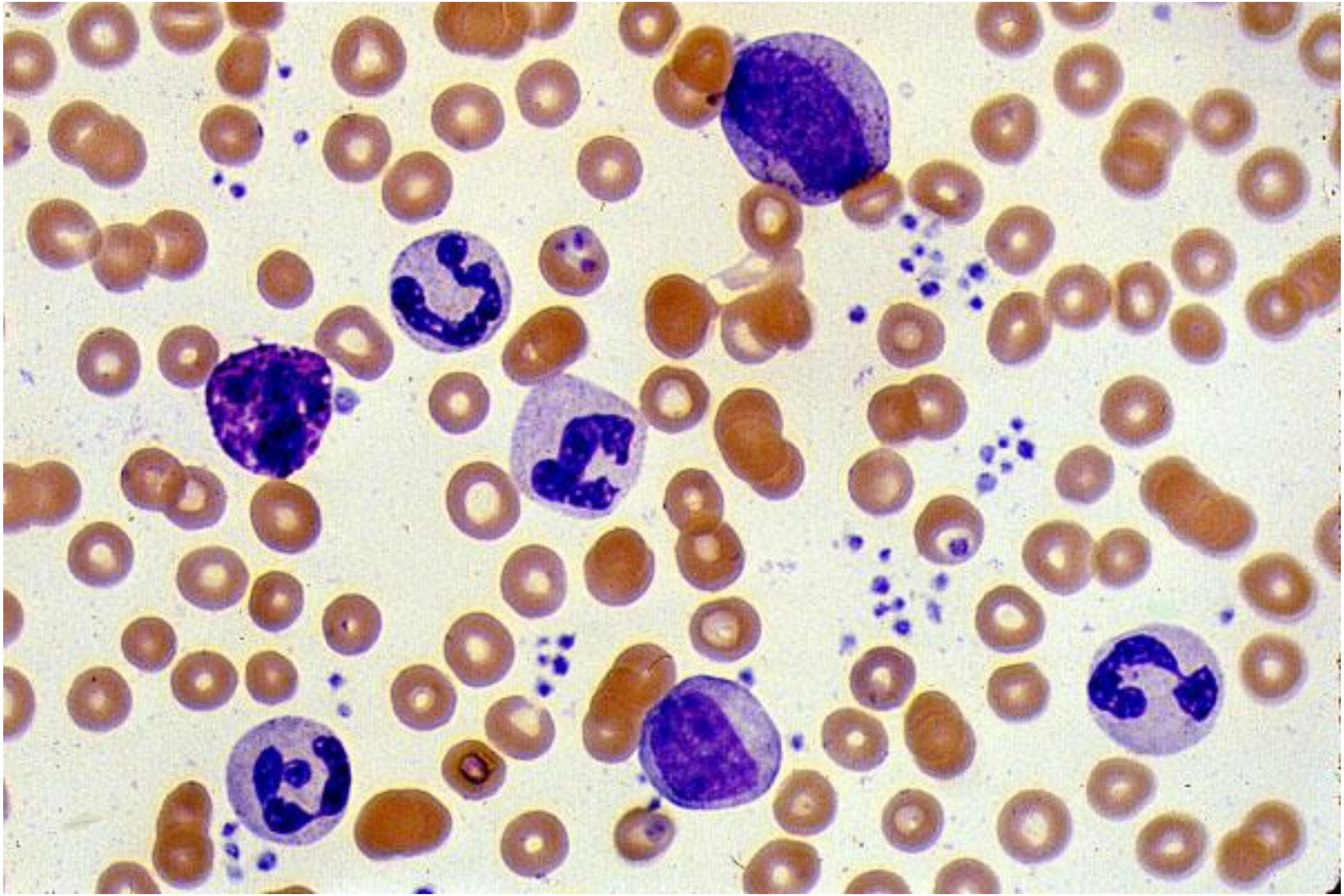
MUNI MED


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

MUNI
MED



Chronic lymphocytic leukemia - CLL

MUNI
MED

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

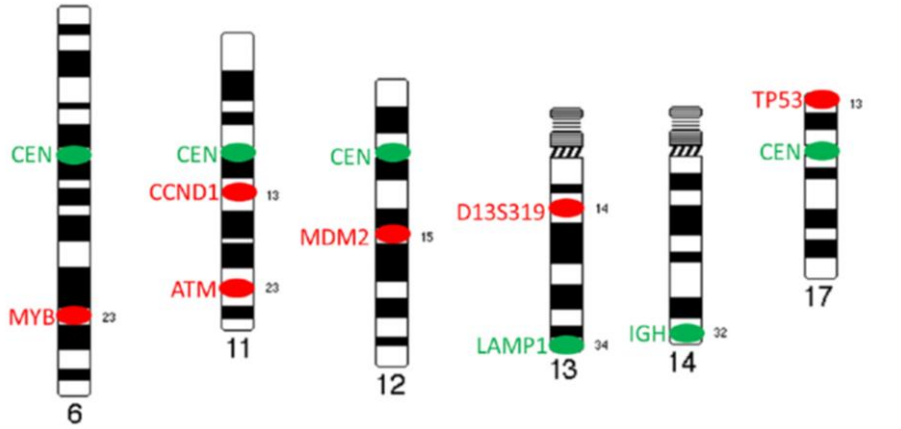
MUNI MED

CLL etiology

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

MUNI
MED

CLL genetic changes

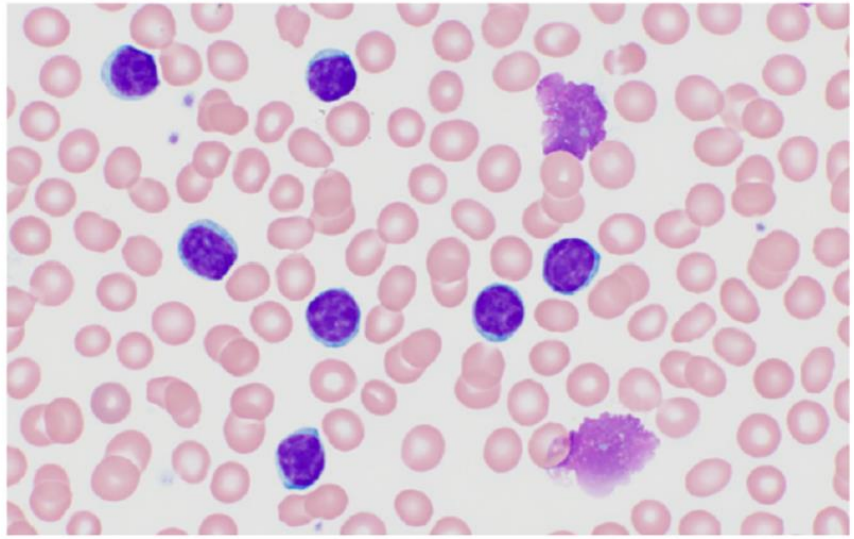


- primary changes in multipotent hematopoietic stem cell
- Deletion 13q, deletion 11q, trisomy of chromosome 12
- Del(13q14) primary change - 55% of cases
- Del(11q) - 25% of patients – deletion 11q23- gene *ATM* – decreased OS
- Trisomy 12- 10-20% of patients
- Del(17q) – 5-8% of patients – resistence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

MUNI MED

CLL diagnosis

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



MUNI
MED

CLL risk factors

- deletion or mutation of *TP53*
- *IGHV* mutation
- Serum β2 macroglobulin
- Age over 65

The chronic lymphocytic leukemia – international prognostic index (CLL-IPI).

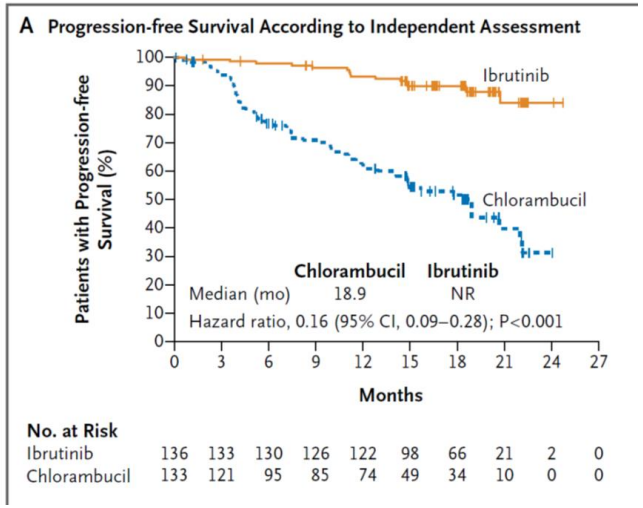
Characteristic	Points
Age > 65 years	1
Rai Stage I-IV	1
Unmutated *IGHV* genes	2
Serum β2 microglobulin >3.5 g/dL	2
Del17p13 by FISH or *TP53* mutation	4
Total score	0–10
Total Score	CLL-IPI Risk Group
0–1	Low
2–3	Intermediate
4–6	High
7–10	Very high

MUNI MED

CLL treatment

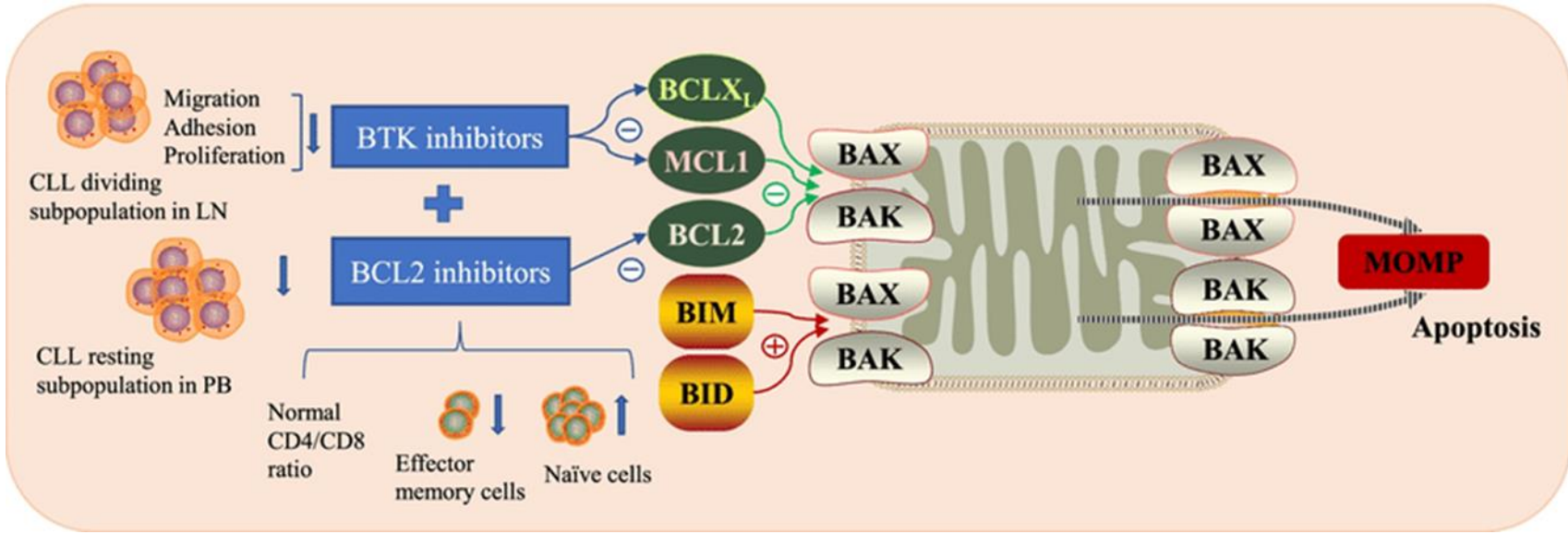
- Chlorambucil – alkylator
- Purine analogues - fludarabin, pentostatin, cladribin
- Monoclonal antibodies – antiCD20 (rituximab)

- Ibrutinib – inhibitor of bruton tyrosine kinase, FDA approved
- Venetoclax – inhibitor of BCL2, FDA approved



MUNI MED

CLL treatment



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 targeted agents or treatment in clinical trials

MUNI MED

Hematological malignancies

Leukemia

Lymphoma

Multiple myeloma

MUNI
MED

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)

MUNI
MED

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

MUNI MED

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

MUNI
MED

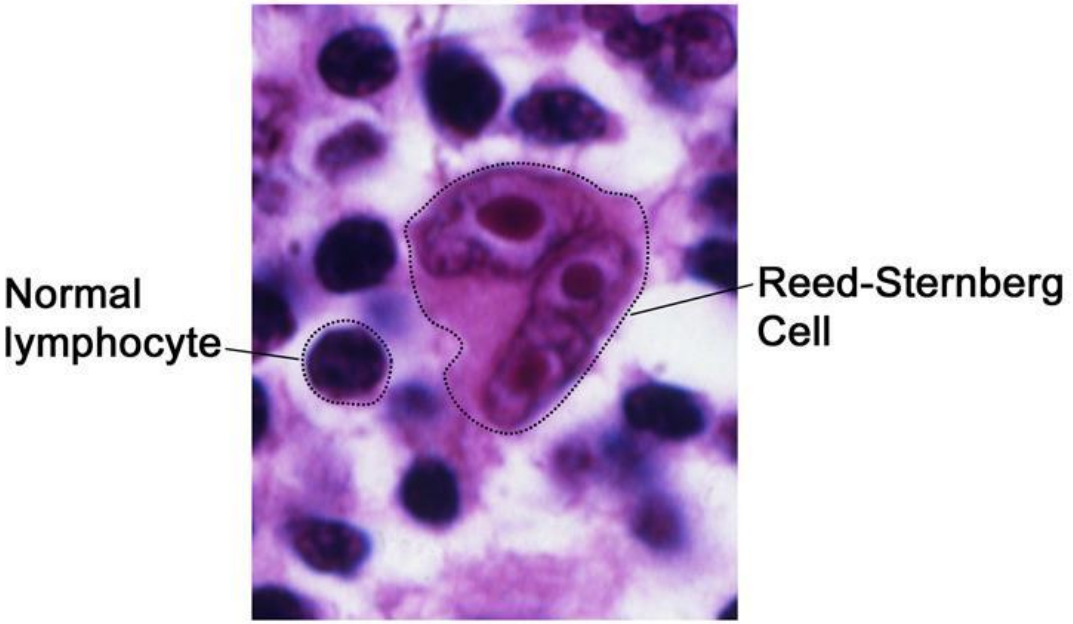
Hodgkin lymphoma

- **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%)

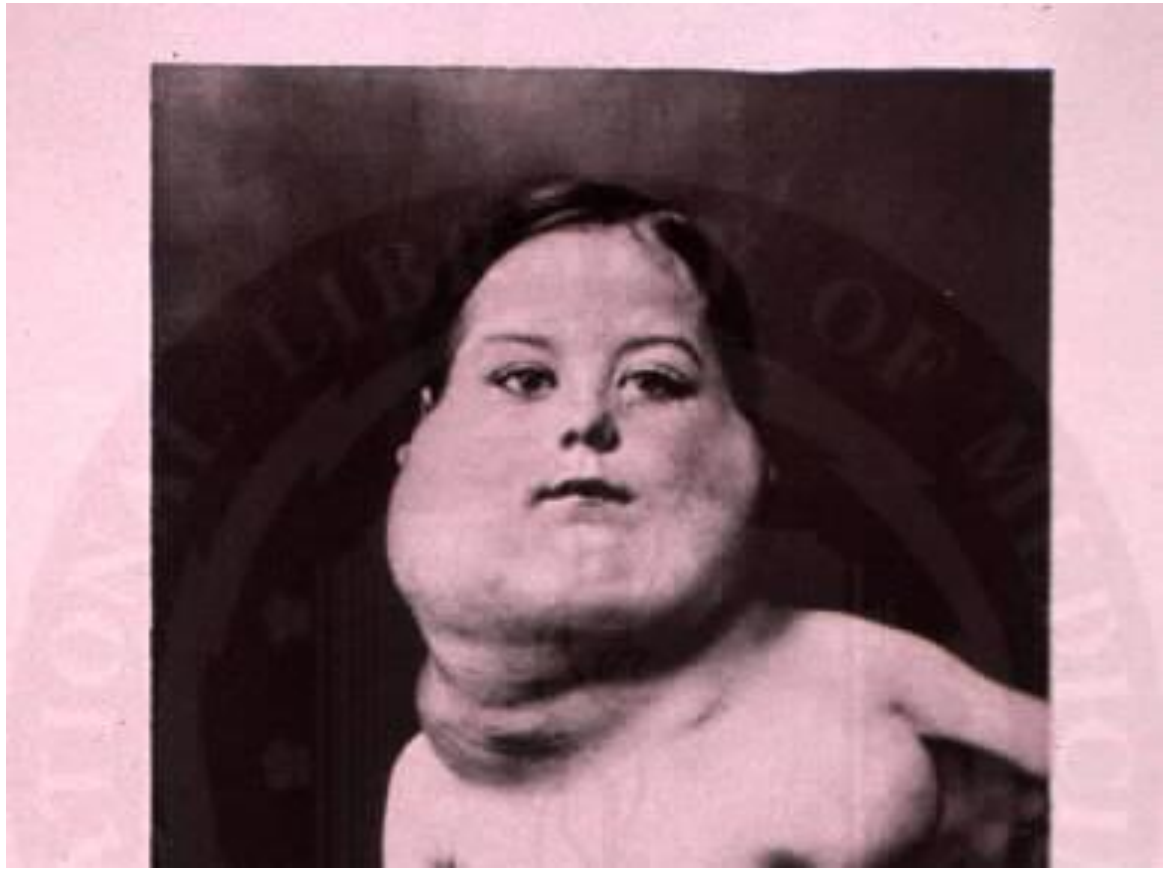
- **type III -** mixed cellularity (20–25%)

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



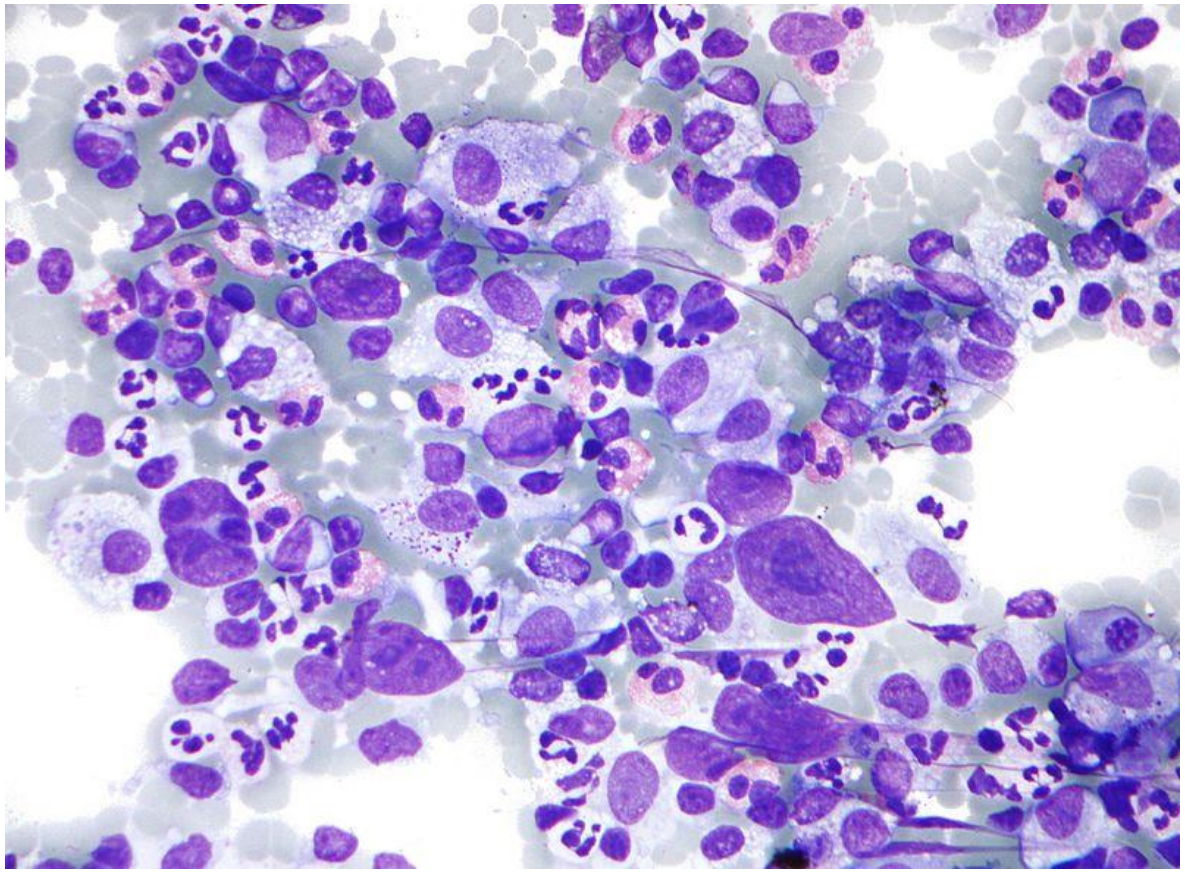
Reed-Sternberg cells – abnormal lymphocytes, characteristic for lymphomas, multinucleated cells

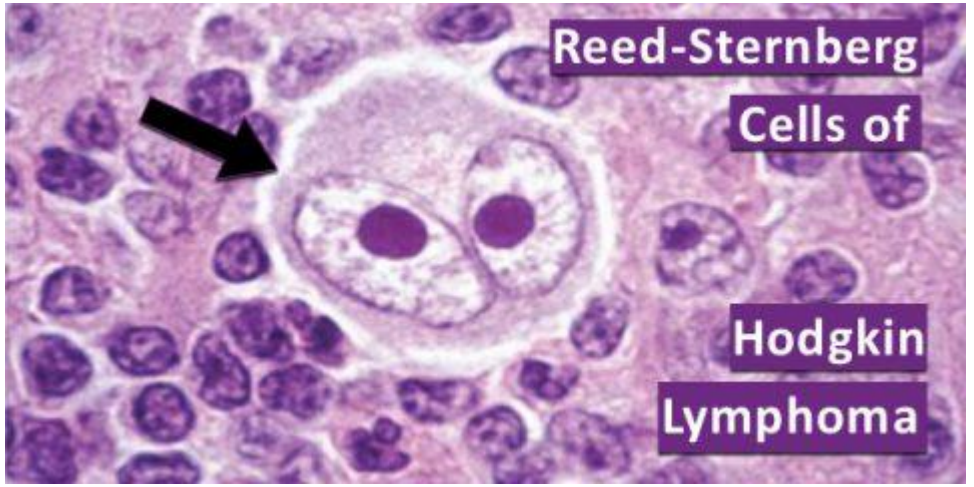
Hodgkin lymphoma



MUNI
MED

Hodgkin lymphoma





MUNI
MED

Non-Hodgkin lymphoma

- Heterogeneous group of tumors (cca 40 types)
- Arising from lymph nodes – fast migration into 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 - intense chemo treatment - successful in 80% of cases
- In adults – less malignant

MUNI
MED

Myelodysplastic syndromes - MDS

MUNI
MED

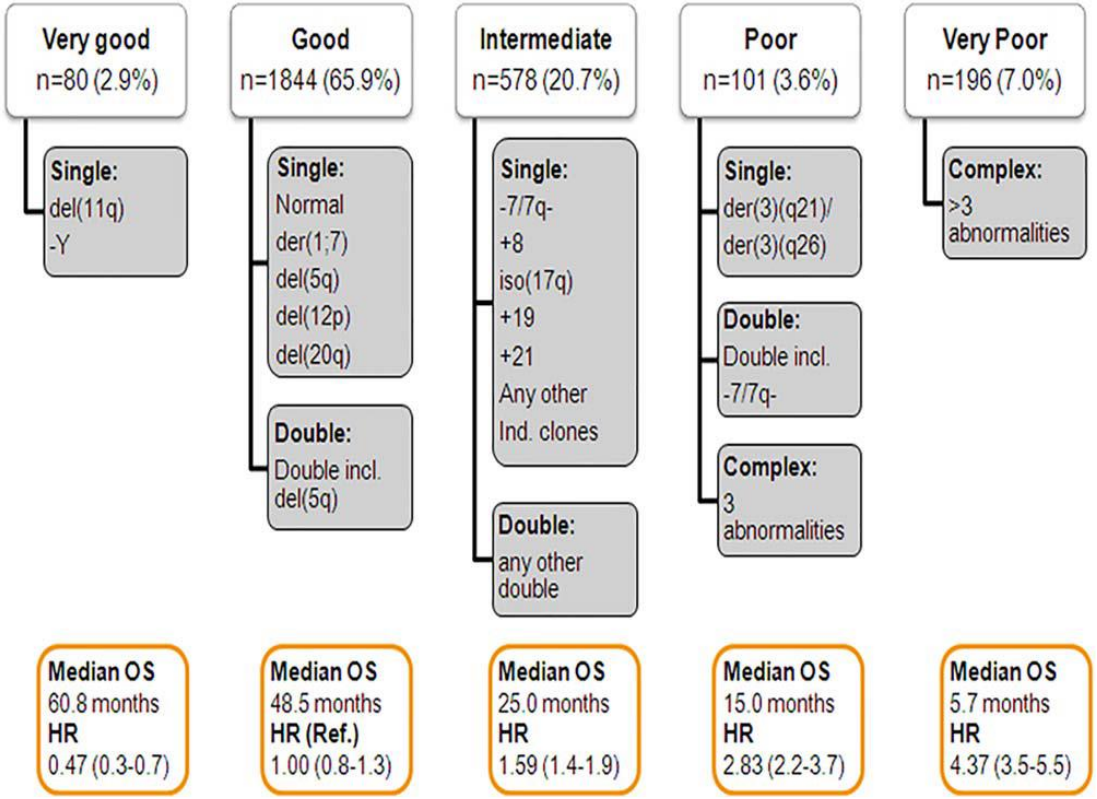
Myelodysplastic syndromes - MDS

- 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

MUNI MED

Cytogenetic classification of MDS

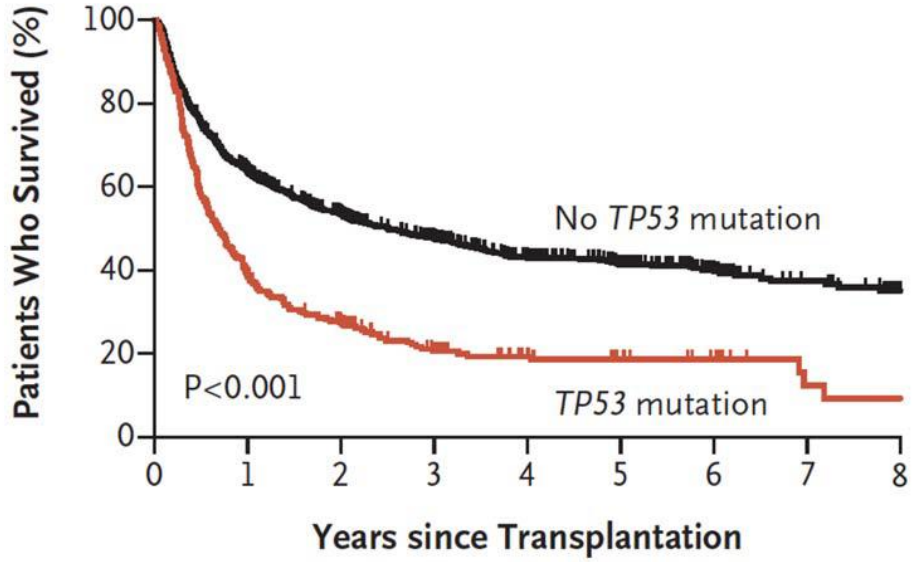


Montelban-Bravo, 2018

MUNI MED

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

MUNI MED

Hematological malignancies

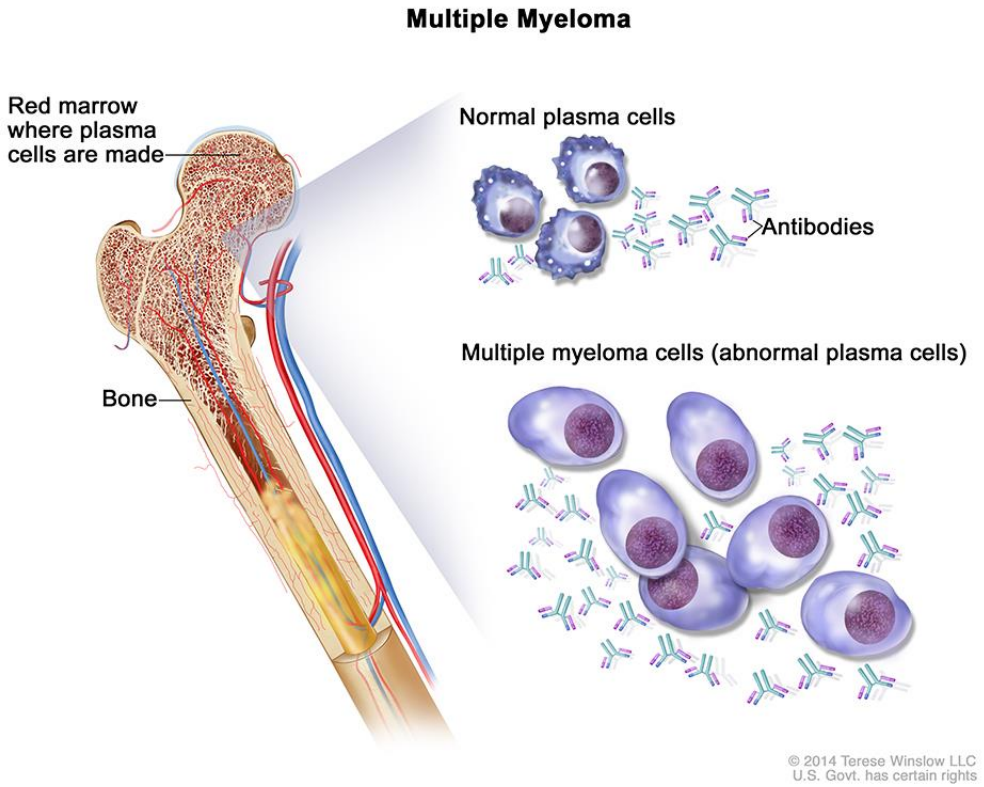
Leukemia

Lymphoma

Multiple myeloma

MUNI
MED

Multiple myeloma MM



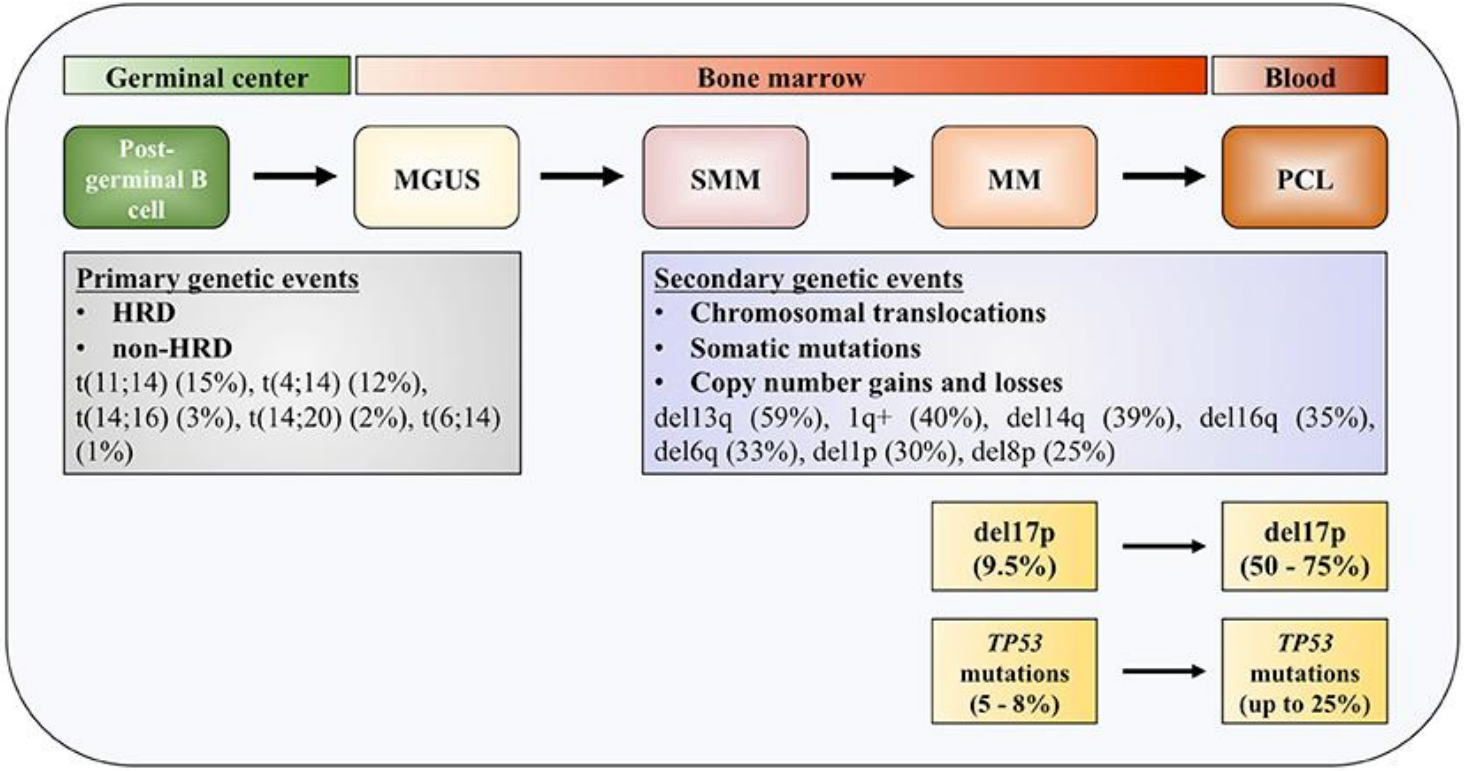
- second most common hematological malignancy
- 10% of hematological malignancies
- median age at diagnosis - 65
- Incidence 4/100 000
- more common in men
- multistep pathogenesis

Hájek, 2012

Anderson, 2011

MUNI MED

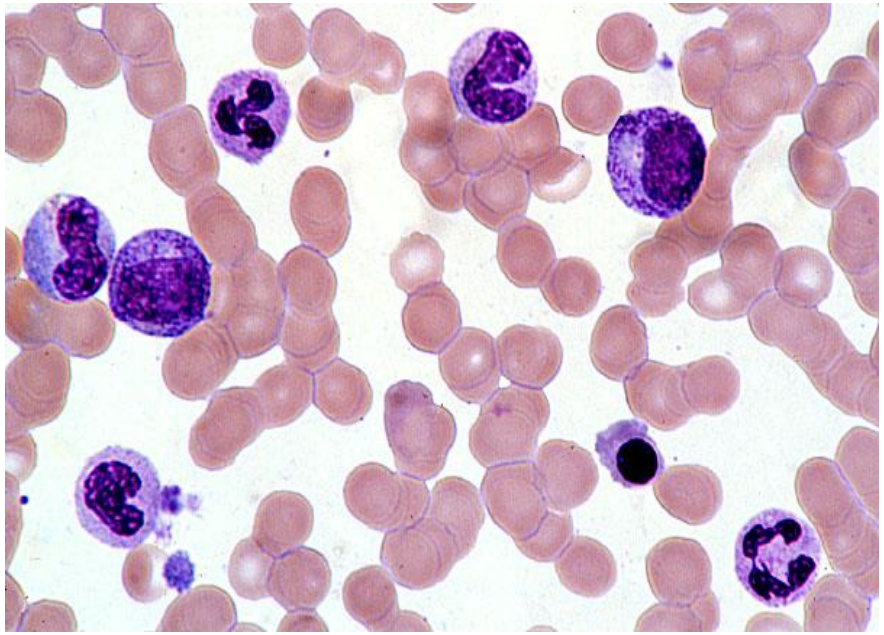
Pathogenesis of MM - multistep process



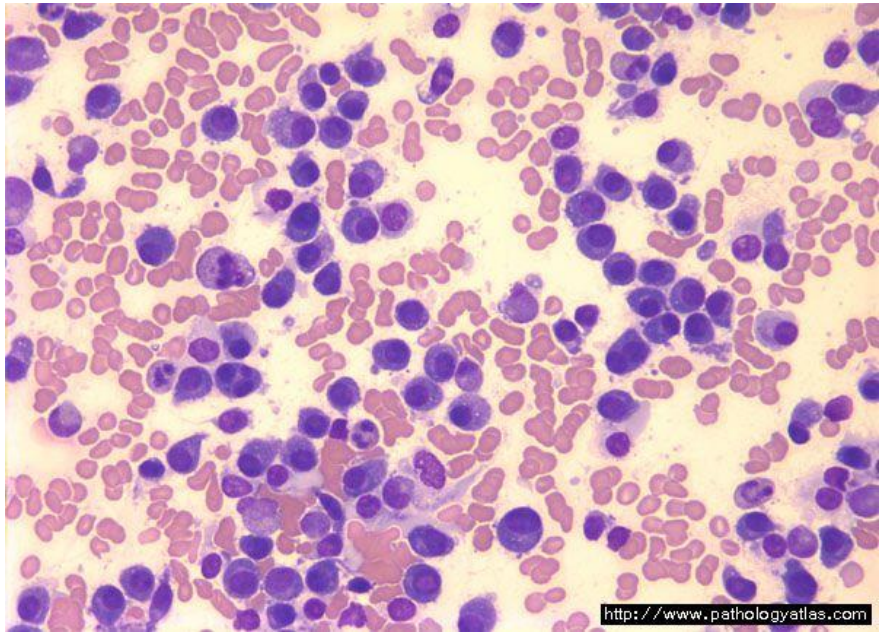
Jovanovic 2019

MUNI MED

healthy bone marrow



MM bone marrow



www.pathologyatlas.com

MGUS

- Monoclonal gammopathy of undetermined significance
- Benign, asymptomatic
- accumulation of genetic changes in plasma cells leading to malignant transformation
- bone marrow infiltrated by <10 % of malignant plasma cells
- 15 % people with MGUS progress into MM
- 1 % risk of progression to MM every year
- Incidence 3 % of population over 50 (increases with age)

MUNI
MED

MM

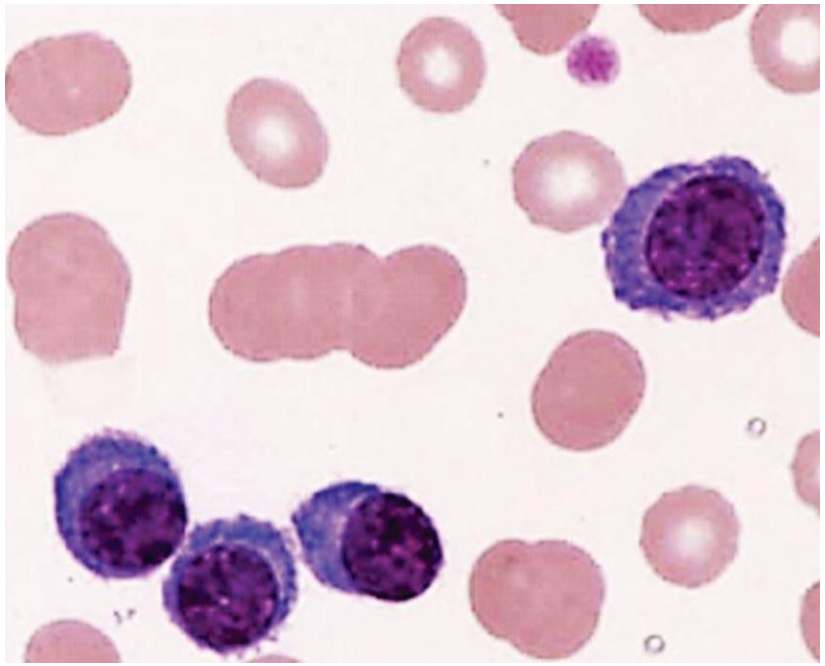
- infiltration of bone marrow by malignant plasma cells
- bone lesions
- presence of monoclonal immunoglobulin (M-Ig) in serum and/or urine

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

MUNI
MED

Plasma cell leukemia

- loss of dependence of plasma cells on bone marrow microenvironment
- > 20 % circulating plasma cells in peripheral blood
- 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

MM symptoms

- Effect on bone marrow: anemia, decrease of immune reactions, bleeding
- Osteolytic lesion: pain, fractures
- Presence of defective immunoglobulins: hyperviscosity, decrease of immunity

MUNI
MED

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

MUNI
MED

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
- monoclonal antibodies

Hájek, 2012
Anderson, 2011

MUNI
MED

Prognosis of MM

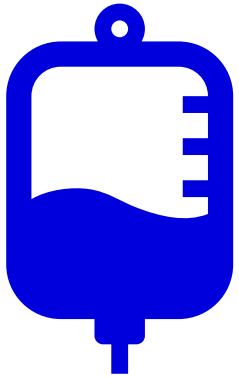
- untreated patients survive 14 months
- standard therapy 3 - 4 years
- Transplantation 6 – 7 years
- New drugs increase five-year survival for about 80% of patients

Hájek, 2012

MUNI
MED

Treatment possibilities for MM

IMIDs (immunomodulatory drugs)



Proteasome inhibitors

MUNI
MED

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)

MUNI
MED

Thalidomide children… today

MUNI
MED

Thalidomide – continuation

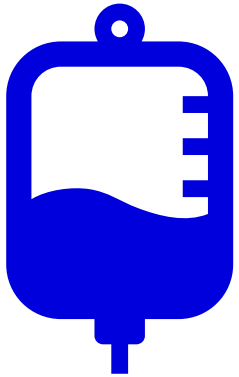
- 1964 – Jason Sheskin – patient with leprosy and complications
- 1993- Judah Folkman – angiogenesis important not only for solid tumors but also hematological
- 1994 – refractory MM patient – thalidomide – clinical study 1/3 of patients responded
- 2006 – FDA – treatment of MM approved
- unpleasant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

Sedlaříková, 2012

MUNI MED

Treatment possibilities for MM

IMIDs (immunomodulatory drugs)



Proteasome inhibitors

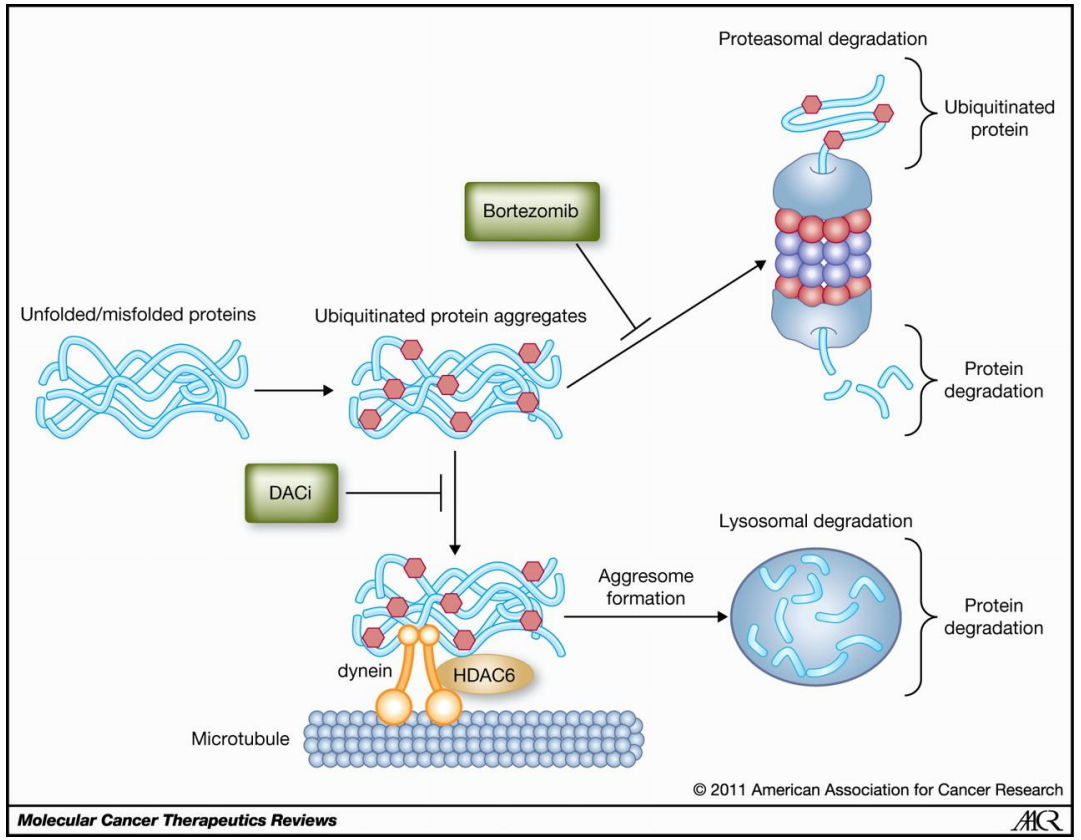
MUNI
MED

Proteasome inhibitors

- Proteasome – a proteolytic complex for degradation of ubiquitinated proteins

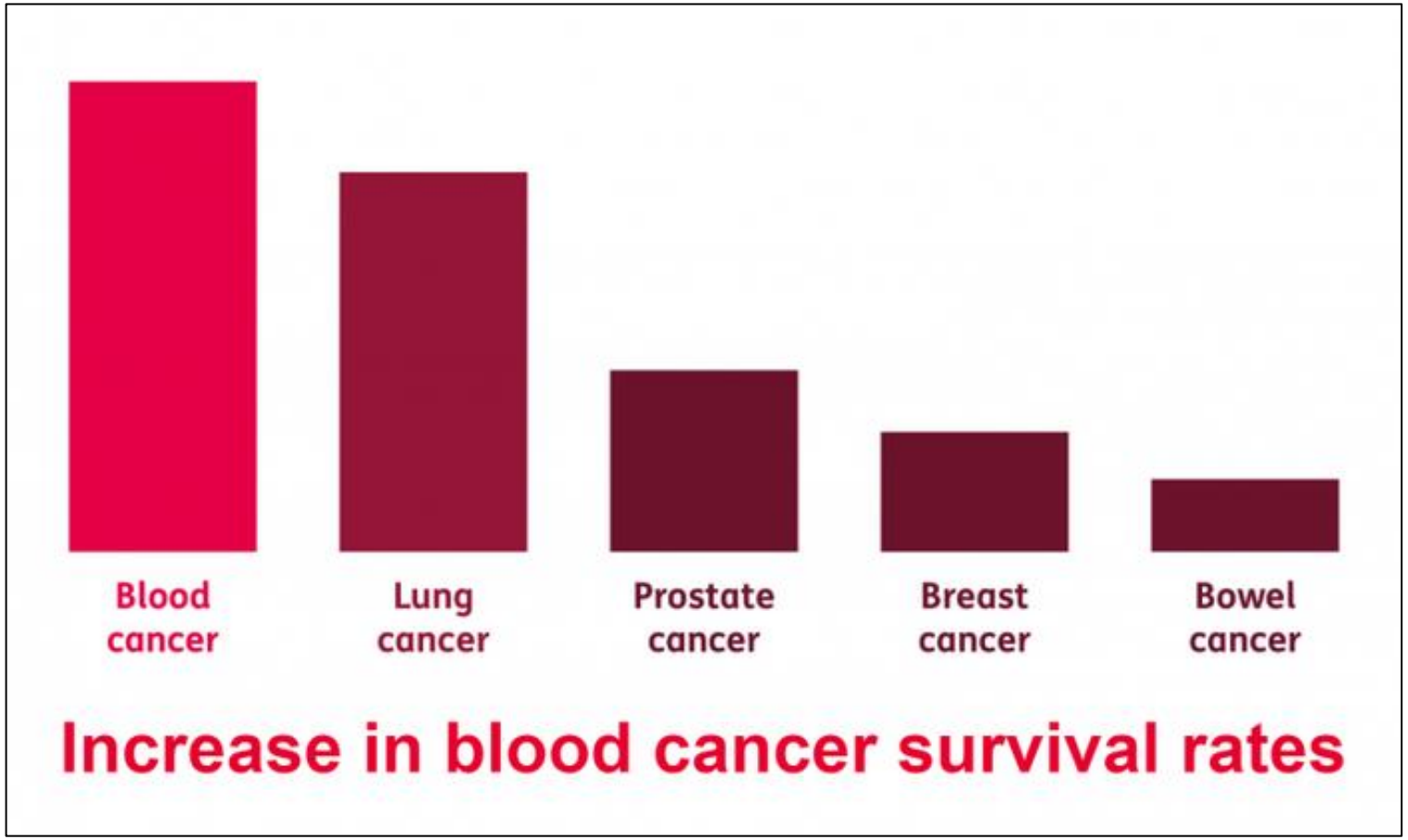
- MM cells produce large amount of proteins - inhibition of proteasome leads to accumulation of proteins in the cells and apoptosis

- Bortezomib – first proteasome inhibitor approved for treatment of MM



MUNI
MED

III. Survival of patients with hematological malignancies


Blood cancer
Lung cancer
Prostate cancer
Breast cancer
Bowel cancer
Increase in blood cancer survival rates

MUNI MED

Incidence and survival

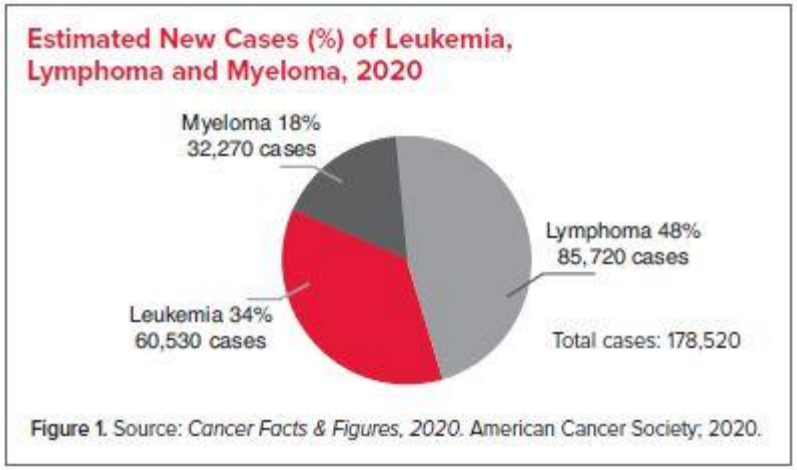


Figure 1. Source: *Cancer Facts & Figures, 2020.* American Cancer Society; 2020.

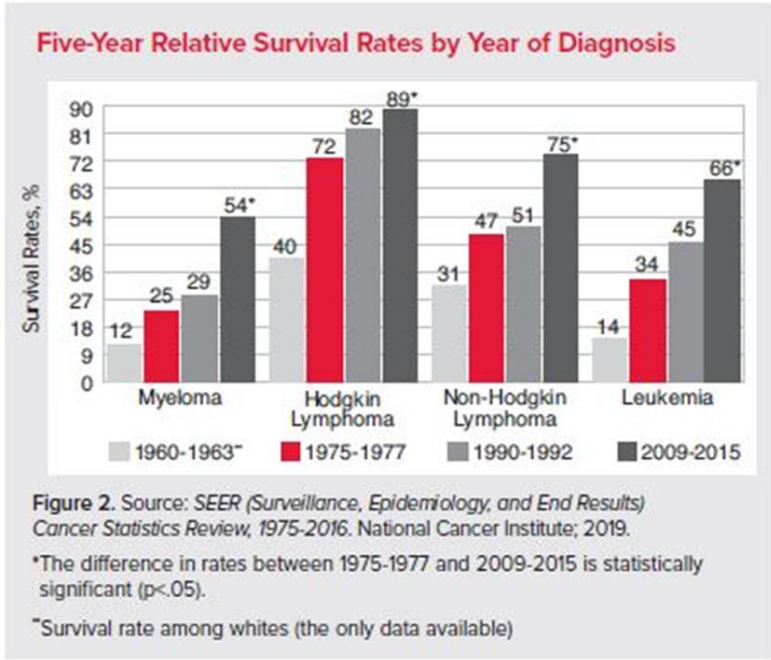


Figure 2. Source: *SEER (Surveillance, Epidemiology, and End Results) Cancer Statistics Review, 1975-2016.* National Cancer Institute; 2019.

*The difference in rates between 1975-1977 and 2009-2015 is statistically significant (p<.05).

"Survival rate among whites (the only data available)

MUNI MED

MUNI
MED

and that is all ….

MUNI
MED

There are papers for your further studies in IS

MUNI
MED

Thank you for your attention