ACUTE LEUKEMIAS

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ACUTE LEUKEMIAS

genetically heterogeneous group of malignant diseases of hematopoiesis

accumulation of acquired mutations in hematopoietic progenitor cells affects their ability to growth, proliferate and differentiate

leukemic clone:

- maturation arrest: blasts
- suppresses the normal hematopoiesis → cytopenias in the peripheral blood

leukemias are divided according to the affiliation of leukemic blasts with a particular developmental lineage:

- myeloid leukemias
- lymphoblastic leukemias
- mixed-phenotype leukemias

CLINICAL SYMPTOMS

non-specific symptoms resulting from impaired bone marrow function and eventual infiltration of other organs (last usually 1–3 months)

anemia: weakness, pale skin, fatigue, dyspnea, dizziness, palpitation, circulatory disturbances (angina pectoris, TIA)

thrombocytopenia: bleeding disorders (petechiae, suffusions, epistaxis, GIT bleeding)
often if thromocytes 10–20 x 10^9/l, almost always if <10 x 10^9/l

neutropenia: repeated infections (usually refractory to antibiotics) – ENT, skin, GIT, lungs, urogenital tract, often systemic, sepsis
etiology: bacteria (both G+ and G–), viruses, fungi
low number of neutrophillic segments (despite leukocytosis)

EXTRAMEDULLAR MANIFESTATION

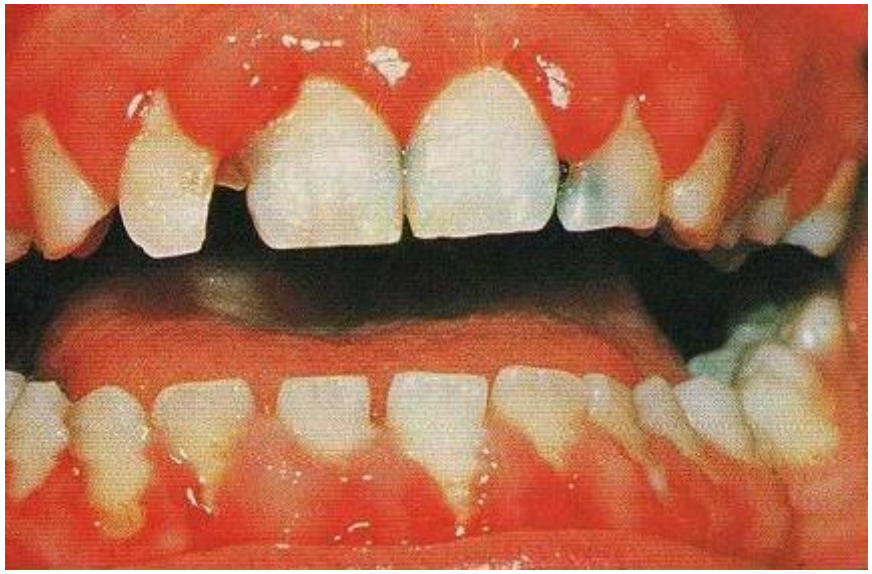
organomegaly (hepatomegaly, splenomegaly, adenomegaly): manifests with nausea, shortness of breath, early satiety, abdominal pressure or pain
may be present in ALL (rare in AML)

leukemic meningitis (infiltration of subarachnoideal space) or direct infiltration of CNS parenchyma: paresis, intracranial hypertension (headache, nausea, vomiting), cramps, cognitivie disturbances
common in ALL and AML with monocytic component

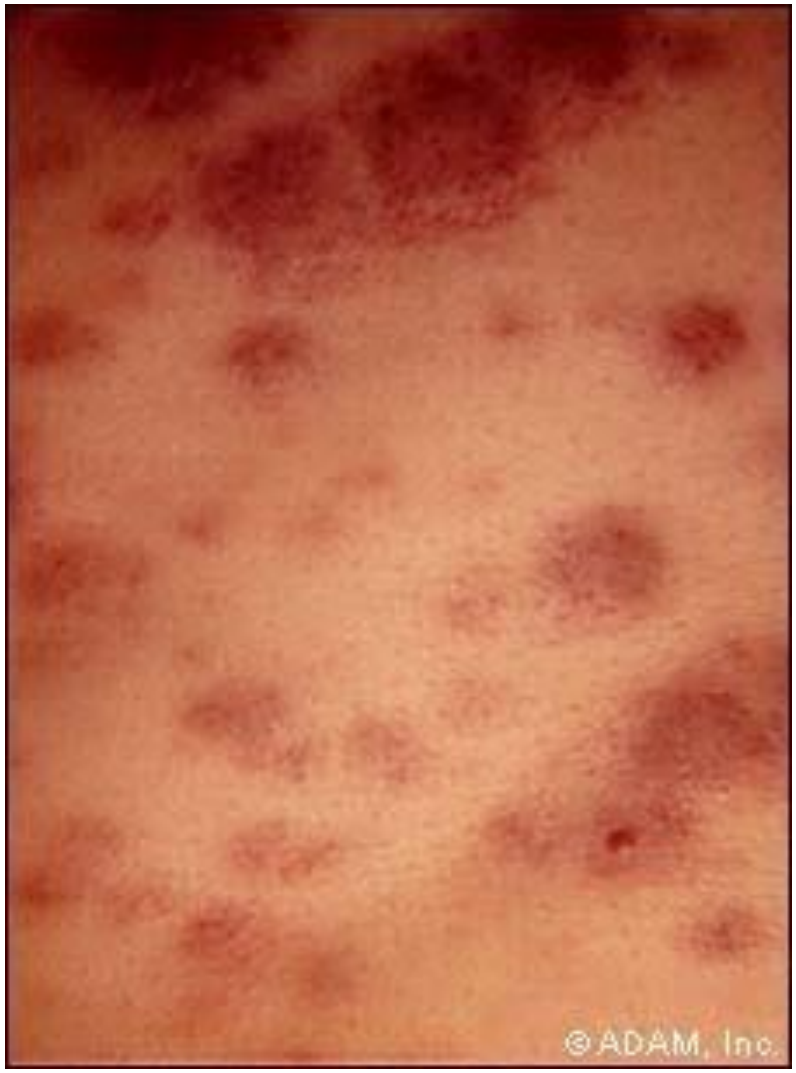
skin infiltrates and gingival hyperplasia
common in AML with monocytic component

chlorom: isolated extramedular leukemic mass

EXTRAMEDULLAR MANIFESTATION



↑ gingival hyperplasia



skin infiltrates →

HYPERVISCOSITY SYNDROME

= leukostasis syndrome

if WBC >100 x 10^9/l
in patients with AML (not in ALL)

clinical symptoms are resulting from hypoperfusion of lungs and CNS, bleeding and/or tissue ischemia:
dyspnea, headache, confusion, visual disturbances

immediate administration of chemotherapy may result in the manifestation of tumor lysis syndrome (TLS)

prevention of TLS: cytoreduction
mechanique (leukodepletion)
pharmacological (hydroxyurea)

DIAGNOSIS

peripheral blood count

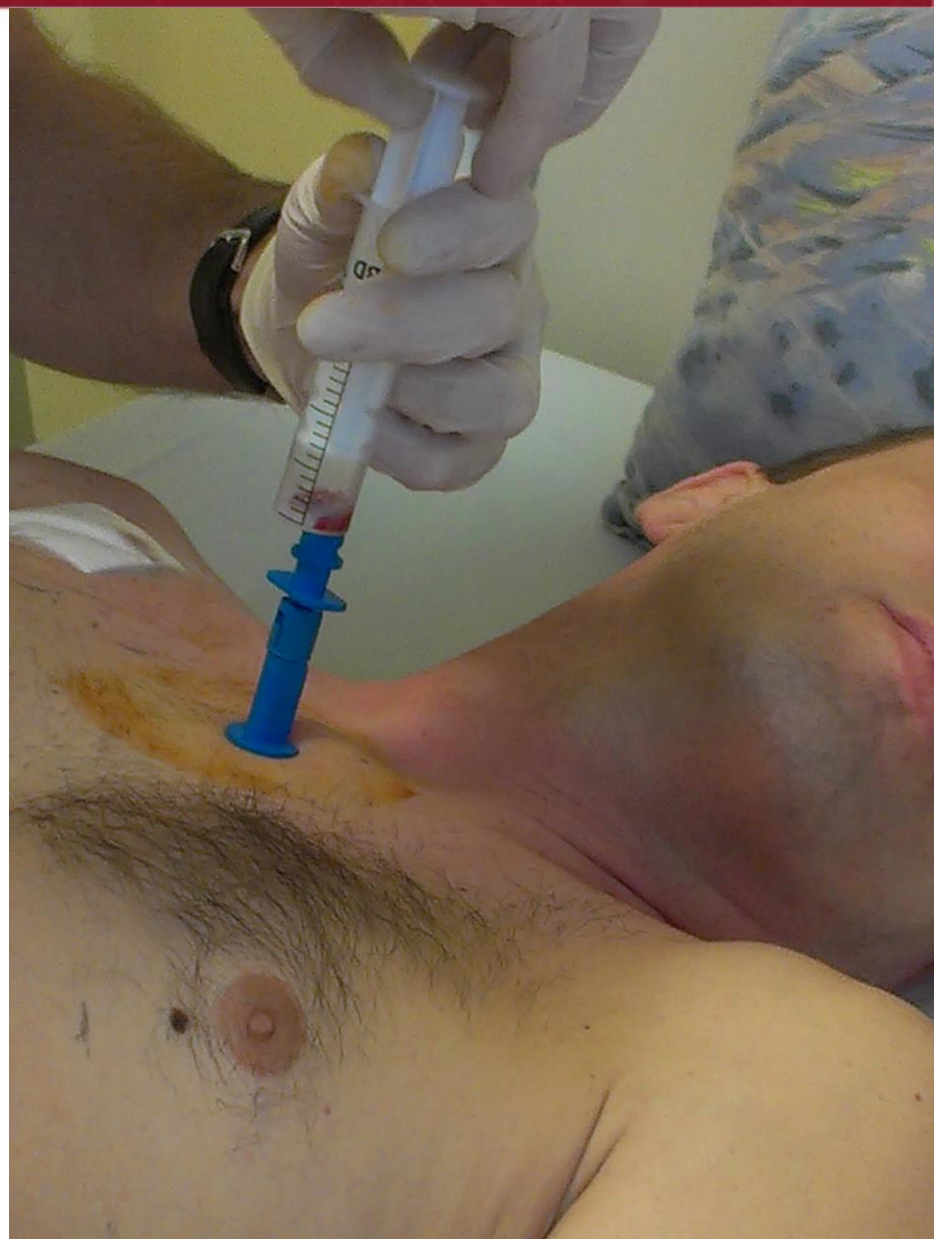
usually leukocytosis (less frequently normal WBC count or leukopenia)
anemia
thrombocytopenia

hiatus leucaemicus in differential count

bone marrow examination

aspiration (usually from sternum)

>20% blasts in bone marrow



HIATUS LEUCAEMICUS

WBC	**37.9**	x 10^9/l
RBC	2.15	x 10^{12}/l
Hgb	**68**	g/l
Hct	22.5	%
MCV	104.7	fl
MCH	31.6	pg
MCHC	302	g/l
Reti	0.033	x 10^{12}/l
PLT	**65**	x 10^9/l

<u>differential count (absolute):</u>

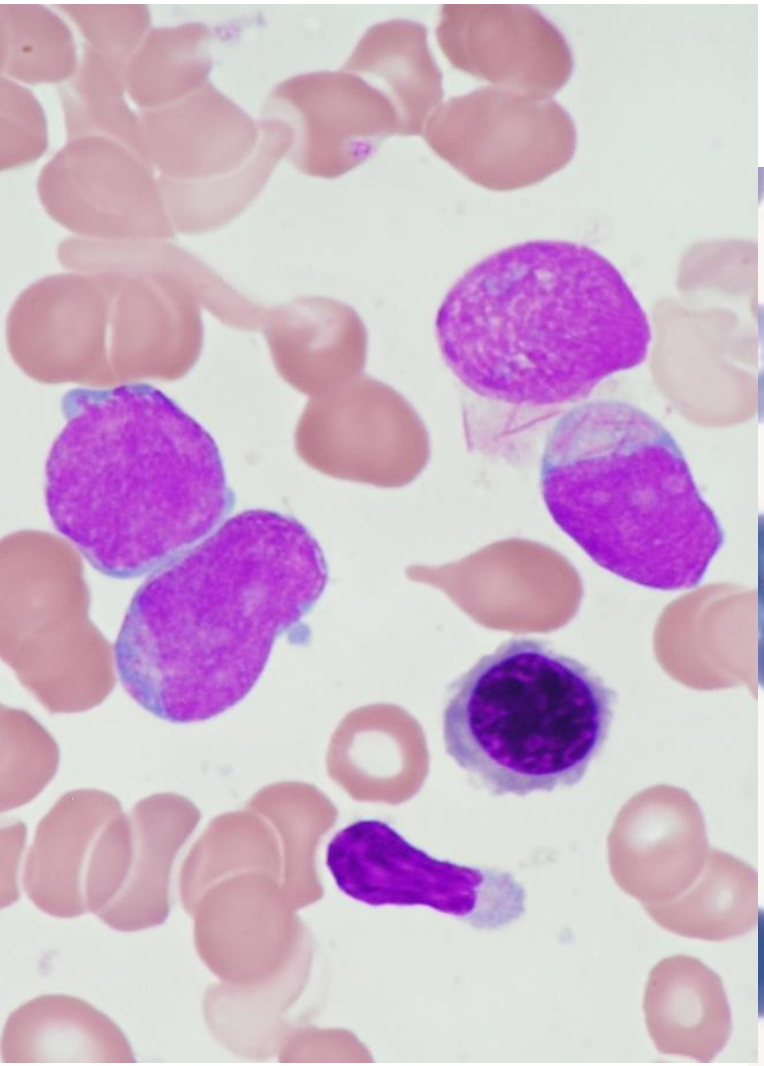
neutrophil	1.28	x 10^9/l
lymphfocyte	16.7	x 10^9/l
monocyte	19.9	x 10^9/l
eosinophil	0.01	x 10^9/l
basophil	0.02	x 10^9/l

<u>differential countt (relative):</u>

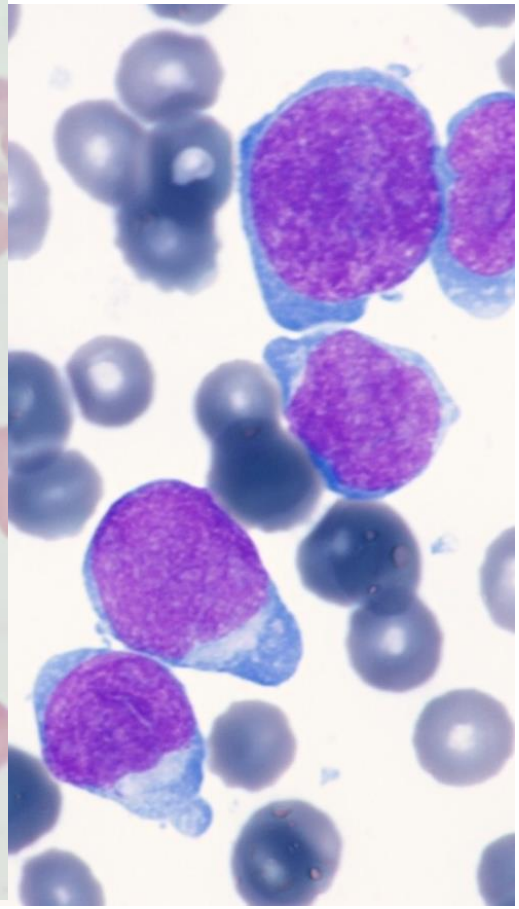
segment	4	%
band	-	
metamyelocyte	-	
myelocyte	-	
promyelocyte	-	
blast	82	%
basophil	1	%
monocyte	4	%
lymphocyte	9	%

← hiatus leucaemicus

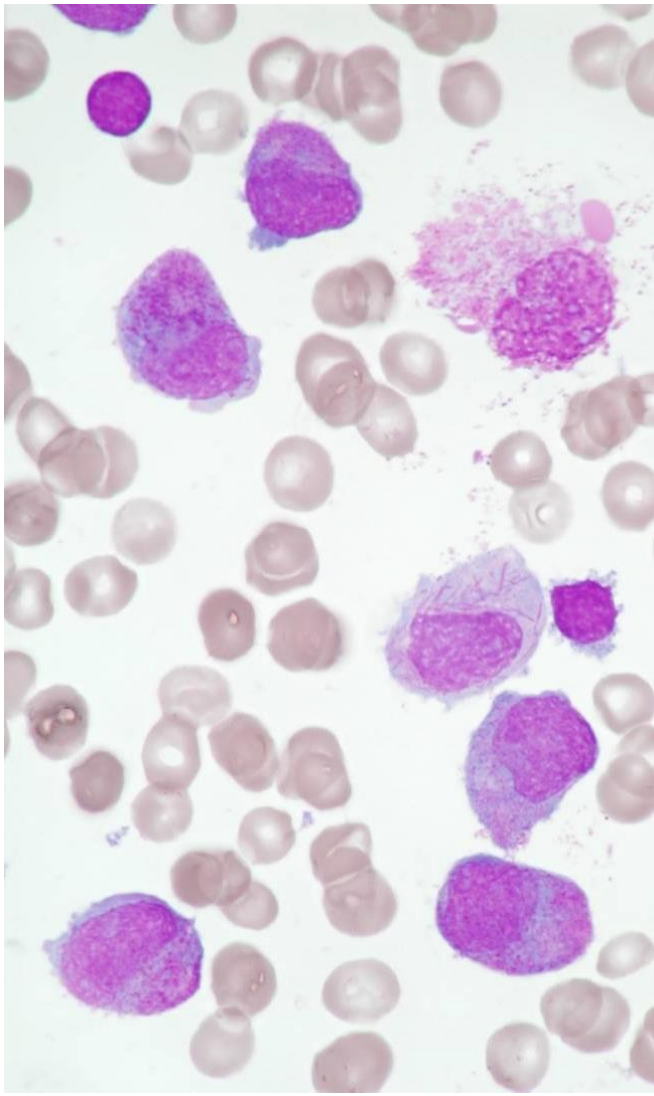
MORPHOLOGICAL EVALUATION OF BONE MARROW ASPIRATE



AML

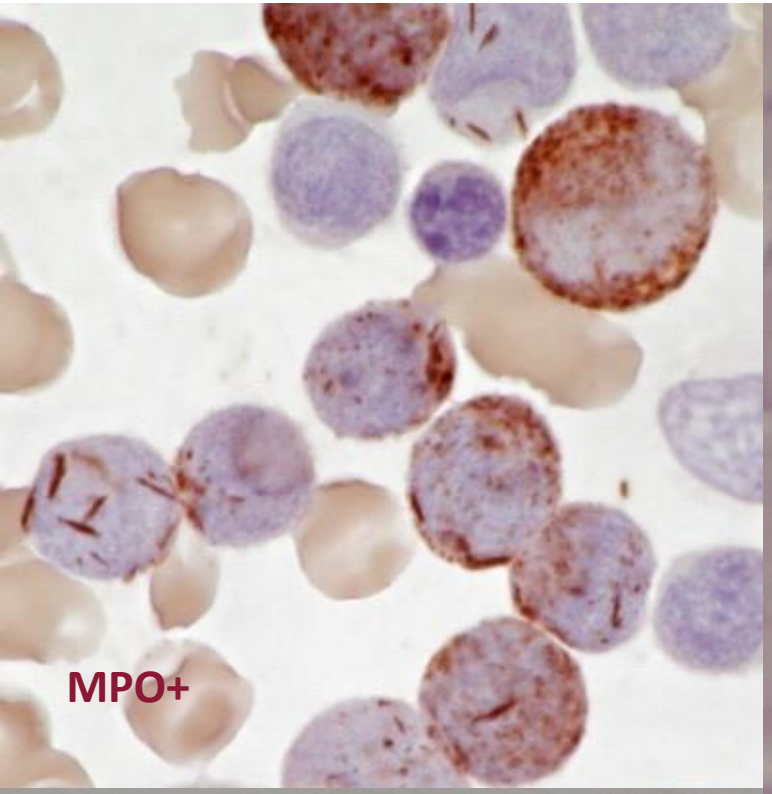


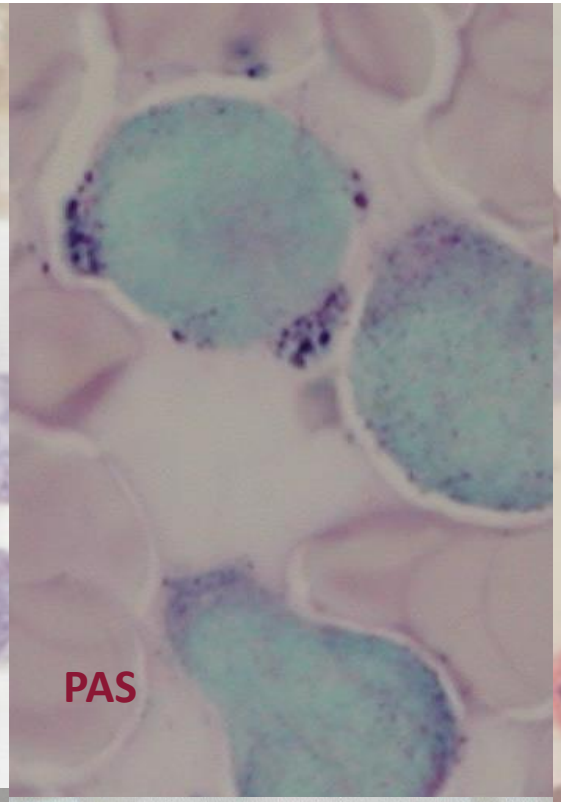
ALL

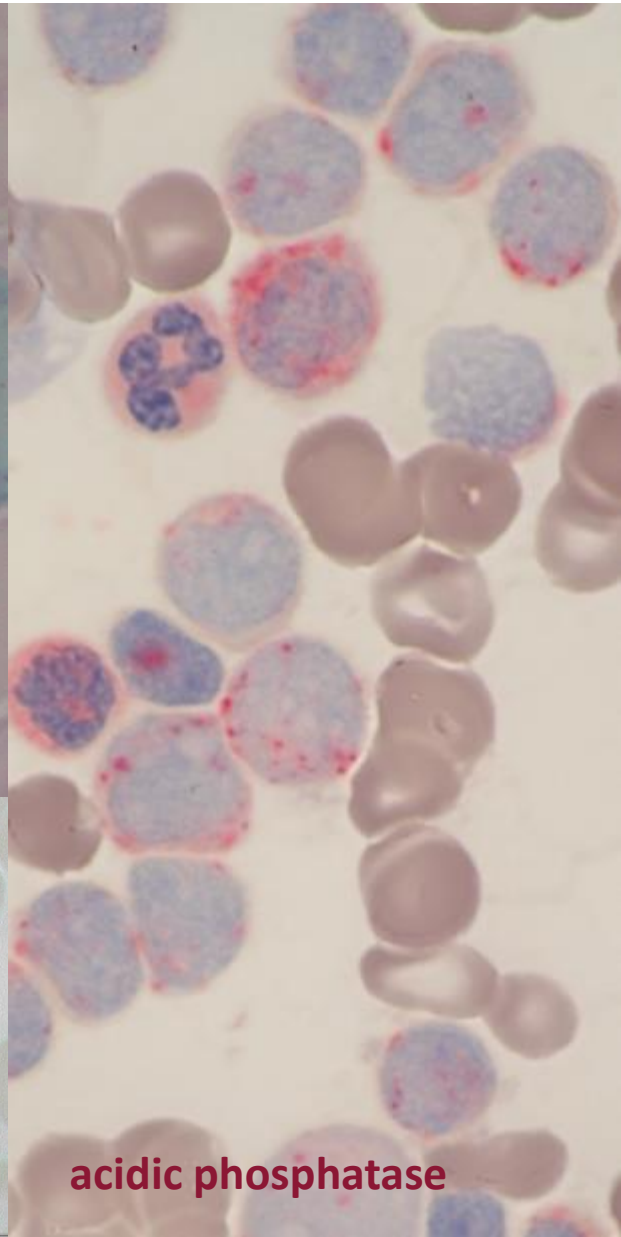


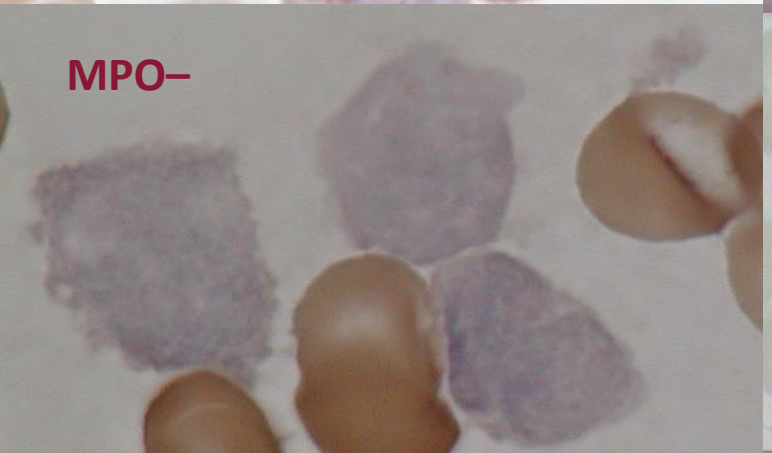
APL

CYTOCHEMISTRY

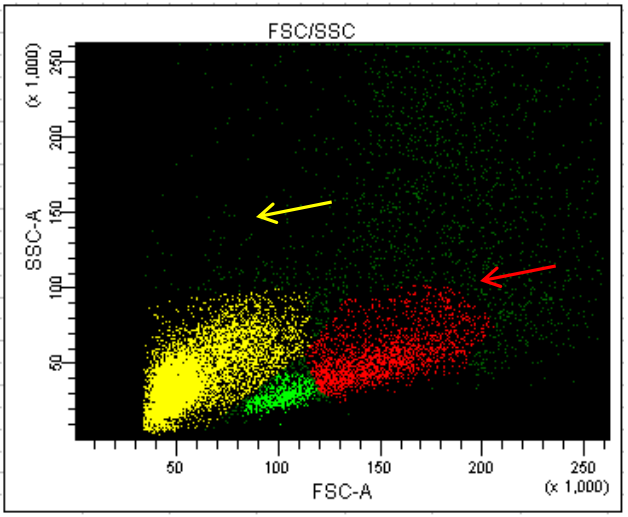


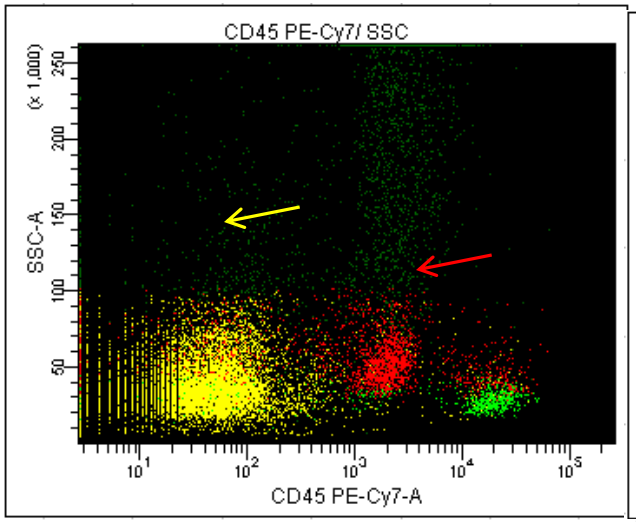


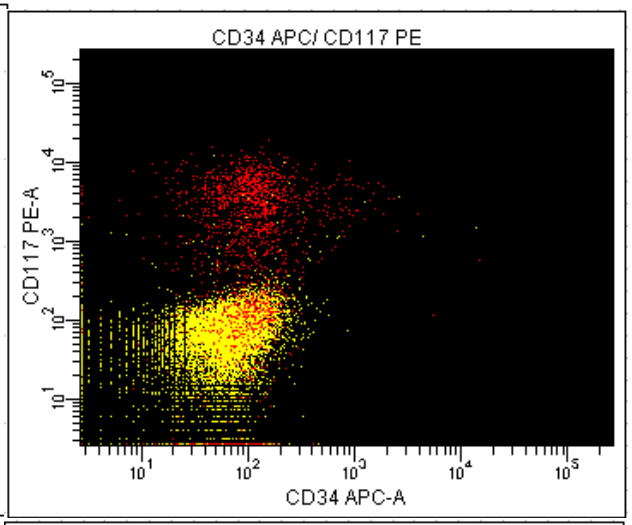


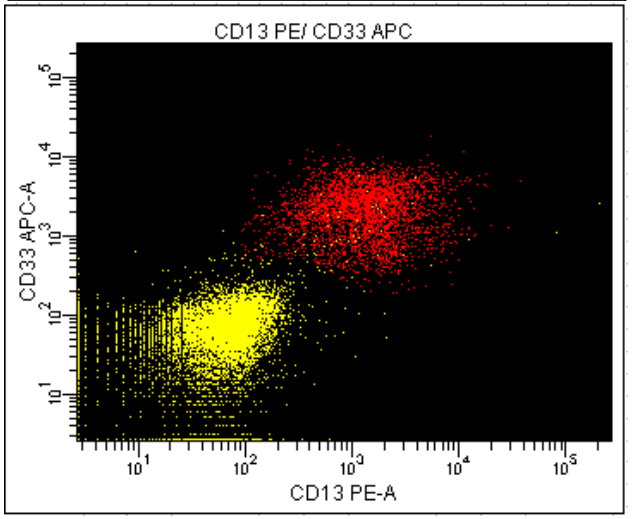


IMMUNOPHENOTYPIC EVALUATION BY FLOW CYTOMETRY (FACS)









Finding:

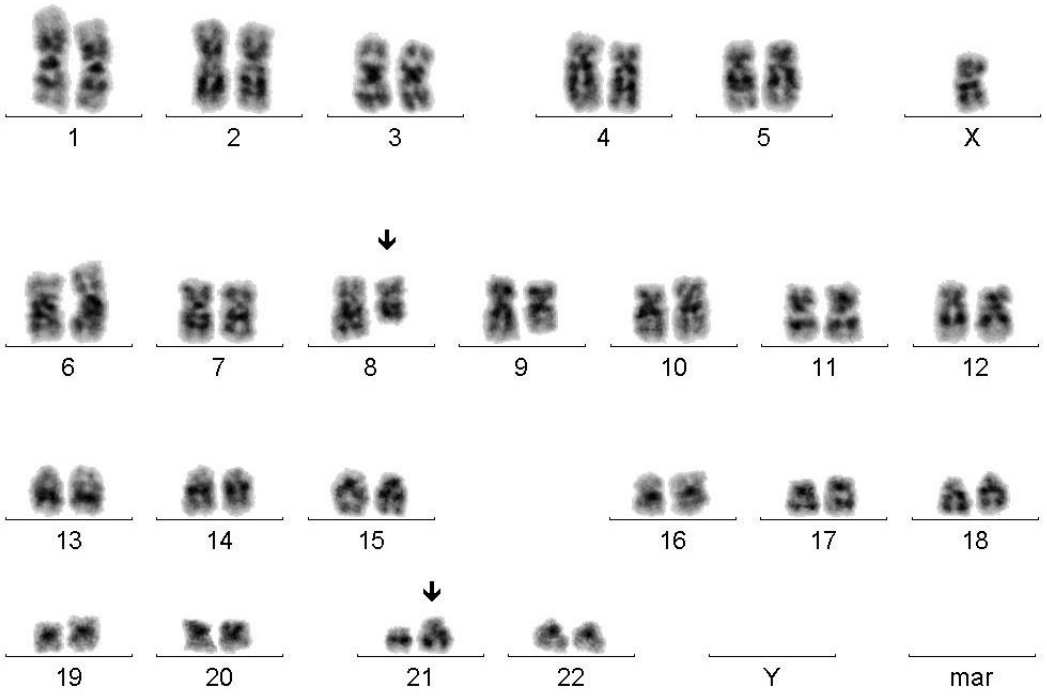
<u>pathologic/abnormal population:</u>

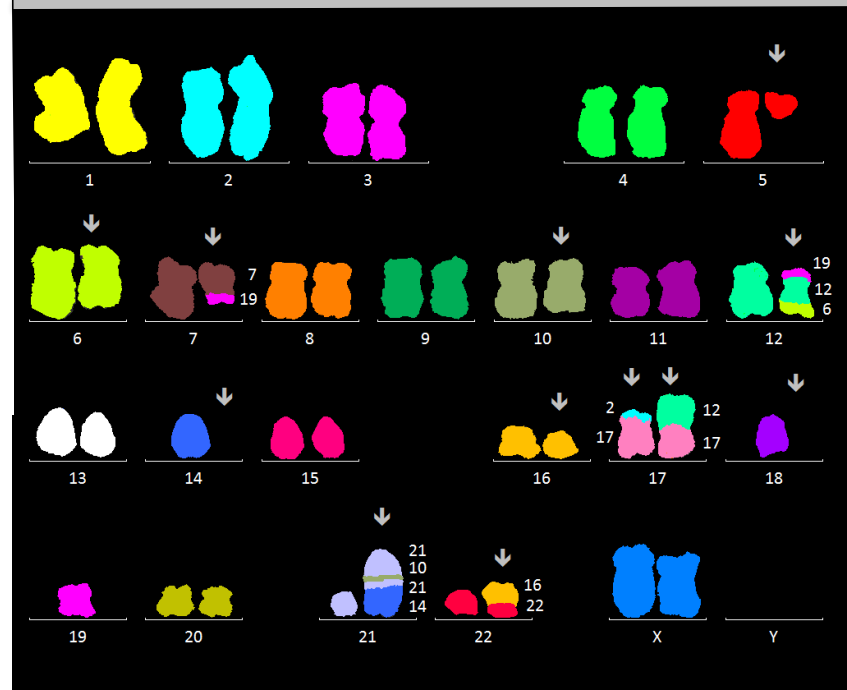
erythroid componen (yellow) + **population of myeloid blasts** (red): approx. 10–12% from nucleated cells / eg. **25% from NEC**

phenotype of myeloid blasts: CD45+(weak), CD117+, CD13+, CD33+, HLADR+ , CD34-

Conclusion: acute myeloid leukemia with erythroid hyperplasia, AML M6 (erythroid/myeloid) according to the FAB classification

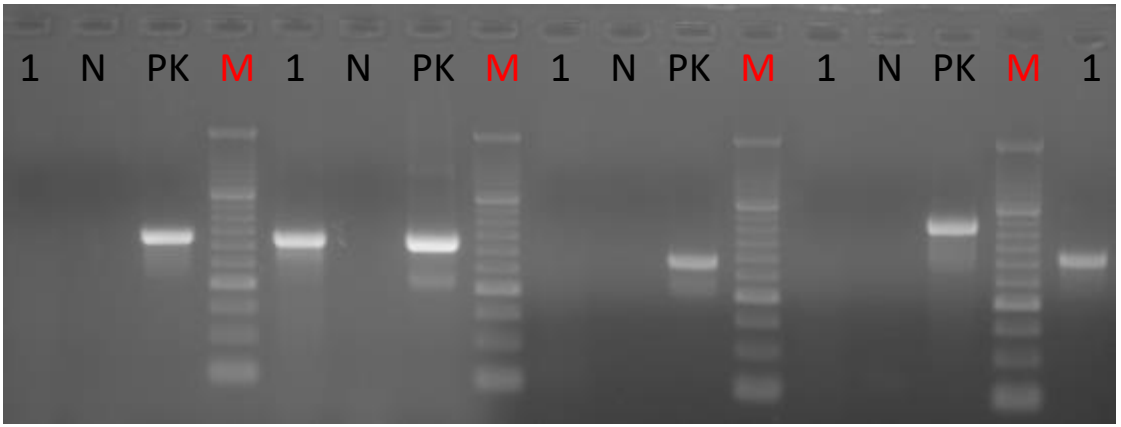
CYTOGENETICS





karyotype of a patient with AML M2:
45,X,-Y,t(8;21)(q22;q22)

MOLECULAR-GENETIC EVALUATION



← fusion genes:
qualitative assay at diagnosis

↙ fusion genes:
quantification

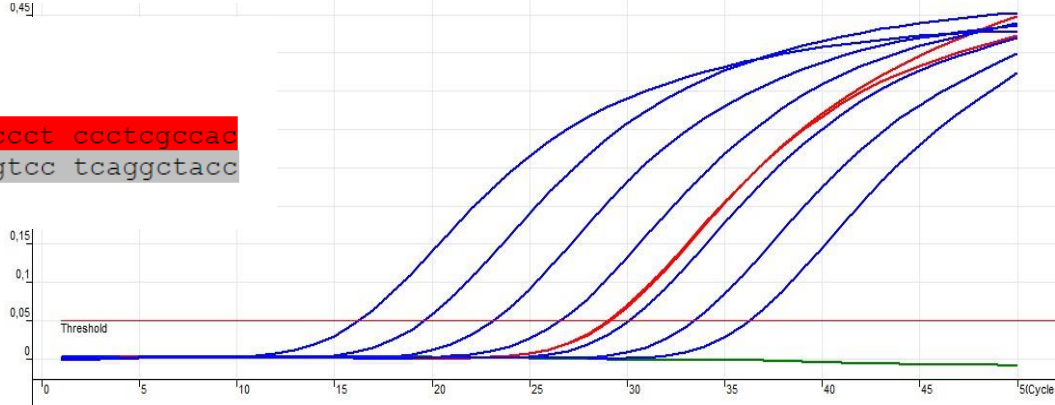
PML
chromosome: 15; **Location:** 15q22

agcgcgagct gctggaggct gtggacgcgc ggtaccagcg cgactacgag gagatggcca
gtcggctggg ccgcctggat gctgtgctgc agcgcatccg cacgggcagc gcgctggtgc
agaggatgaa gtgctacgcc tcggaccagg aggtgctgga catgcacggt ttcctgcgcc
aggcgctctg ccgcctgcgc caggaggagc cccagagcct gcaagctgcc gtgcgcaccg
atggcttcga cgagttcaag gtgcgcctgc aggacctcag ctcttgcatc acccagggga
aag*

RARα
chromosome: 17; **Location:** 17q21

*ccattga gacccagagc agcagttctg aagagatagt gcccagccct ccctcgccac
ccctctacc ccgcatctac aagccttgct ttgtctgtca ggacaagtcc tcaggctacc
actatggggt cagcgcctgt gagggctgca

forward primer for detection of bcr3
forward primer for quantification of bcr3
* breakpoint in PML for bcr3
reverse primer for detection of bcr3
probe for quantification
reverse primer for quantification
* breakpoint in RARα



THERAPEUTIC RESPONSE

complete remission
bone marrow blasts <5%
extramedullary disease not detectable
absolute count of neutrophilic granuloytes >1.0 x 10^9/l
thrombocytes >100 x 10^9/l
patient is independent of RBC transfusion

molecular complete remission
disease is not detectable even by molecular-genetic methods

MINIMAL RESIDUAL DISEASE

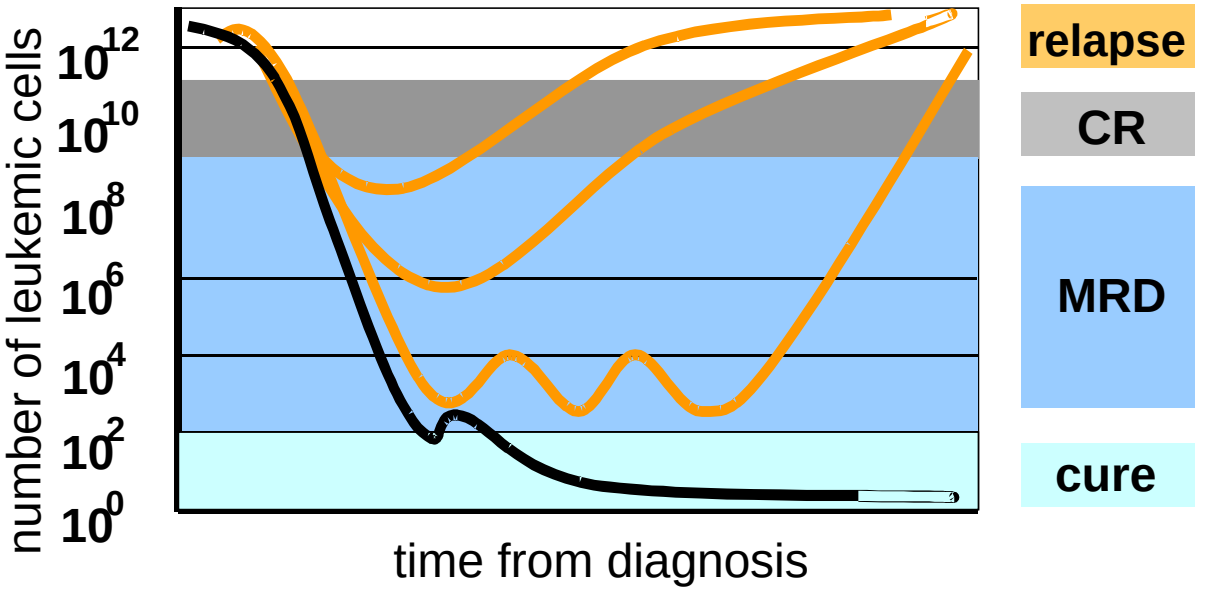
malignant cells persisting in the body after the remission is reached = minimal residual disease (MRD)

persistence of MRD causes relapse

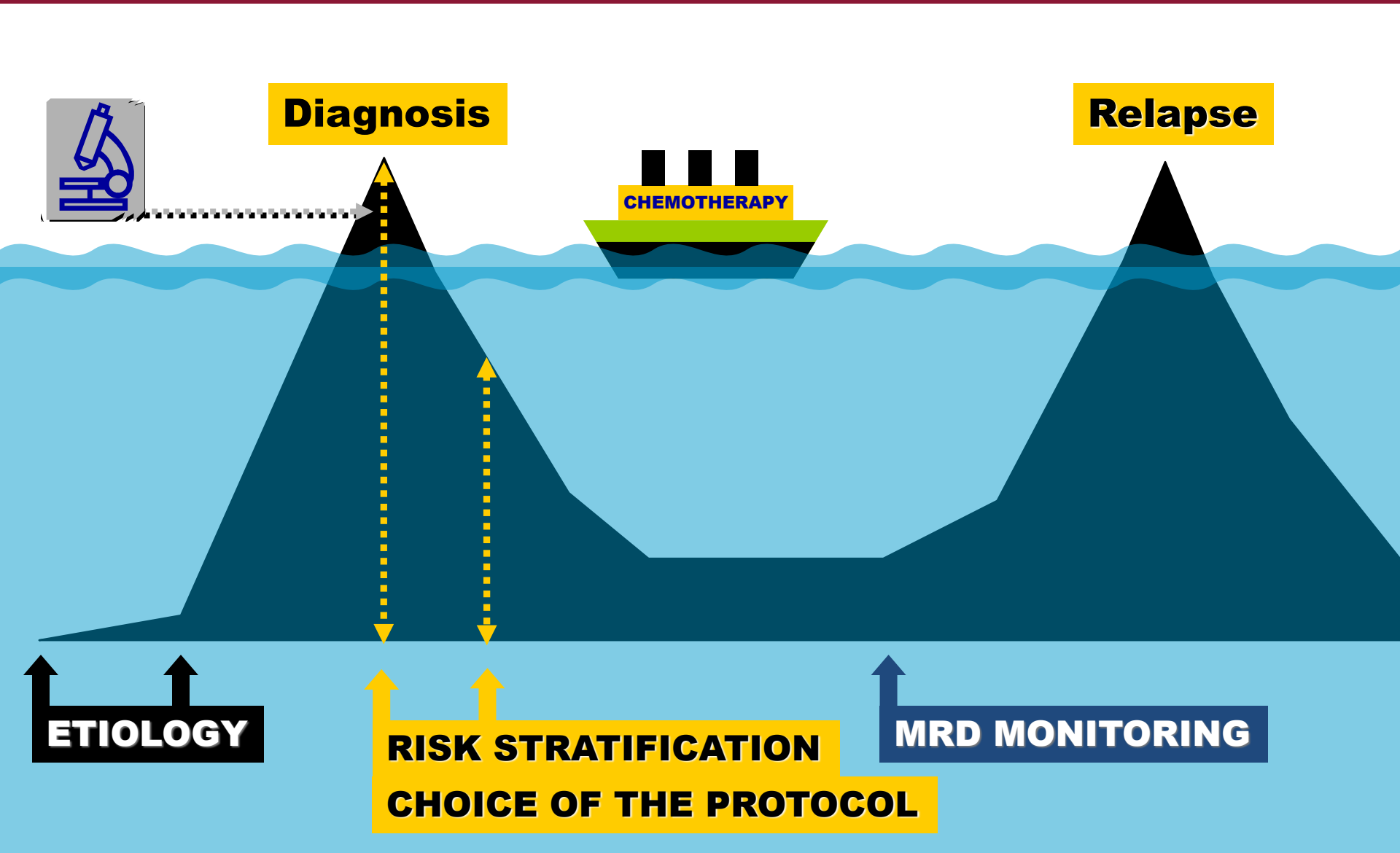
MRD evaluates the individual therapeutic response

- prognostic significance
- indicator of early relapse

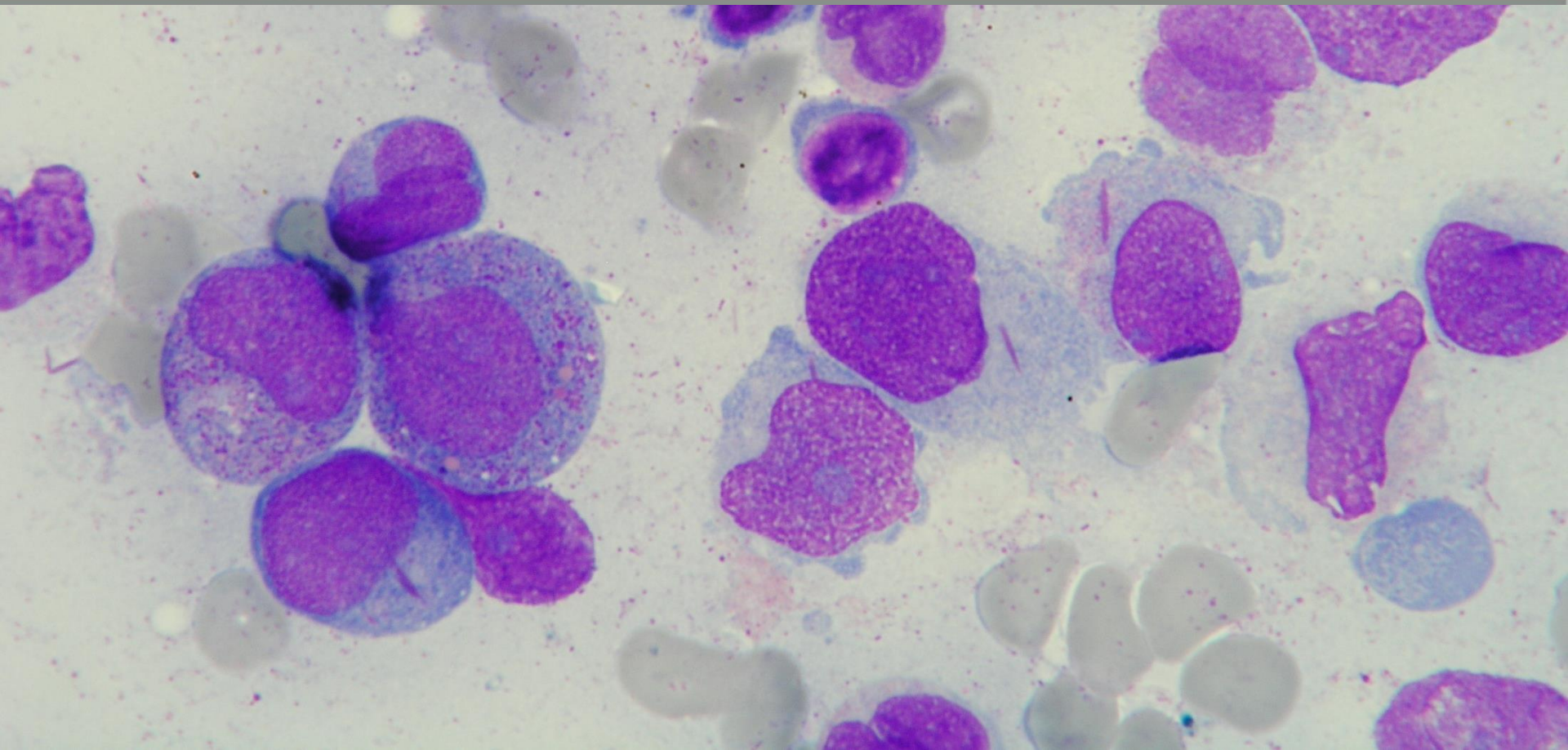
methods of detection:
flow cytometry
PCR



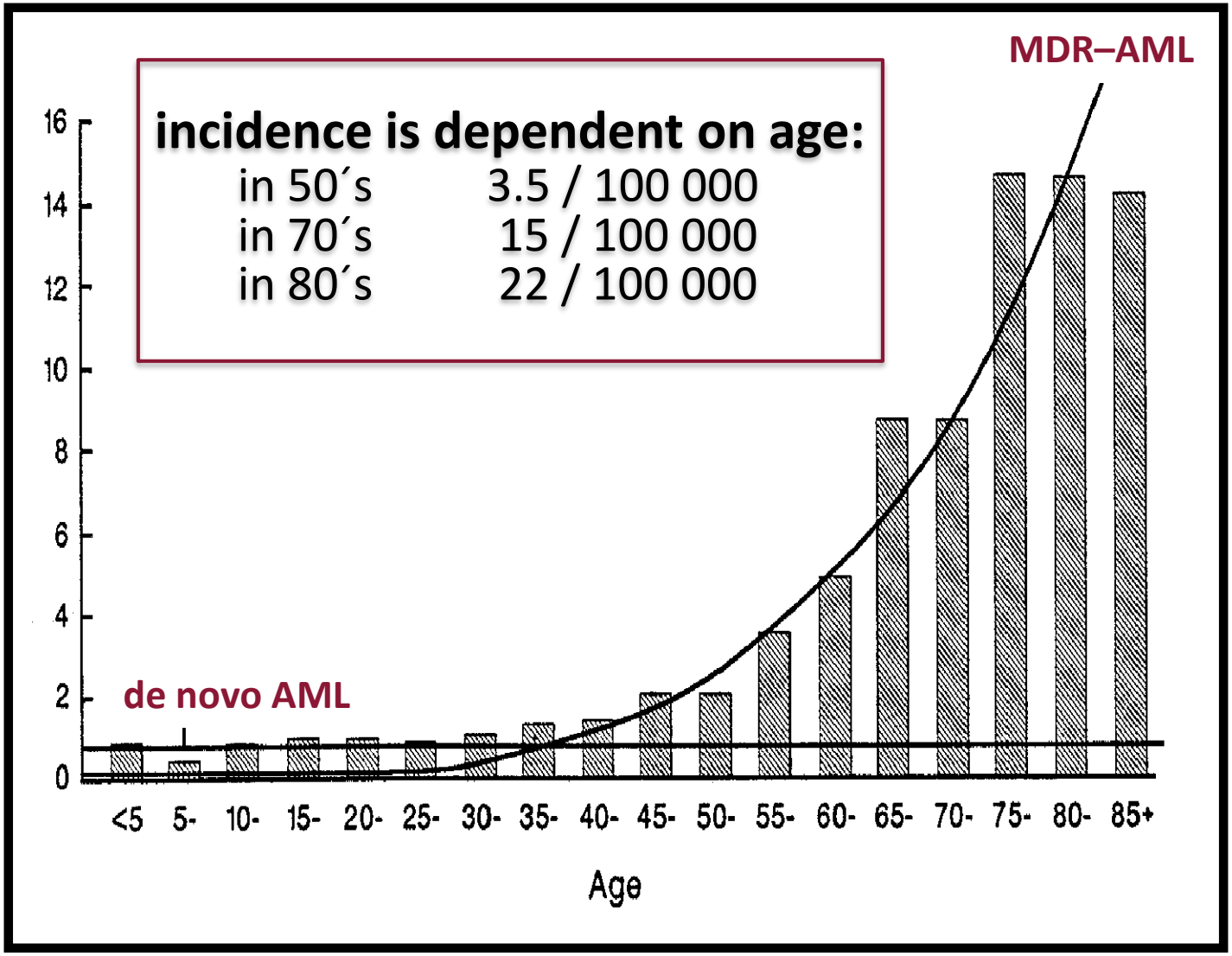
MRD IN CLINICAL PRAXIS AND RESEARCH OF ACUTE LEUKEMIAS



ACUTE MYELOID LEUKEMIA



INCIDENCE


MDR–AML
incidence is dependent on age:
in 50´s 3.5 / 100 000
in 70´s 15 / 100 000
in 80´s 22 / 100 000
de novo AML
16
14
12
10
8
6
4
2
0
<5 5- 10- 15- 20- 25- 30- 35- 40- 45- 50- 55- 60- 65- 70- 75- 80- 85+
Age

BASIC TYPES OF AML

primary disease

de novo AML – arises newly without known history of hematologic disease or genotoxic therapy

secondary disease

AML with myelodysplaia-related changes – arrises by transformation from MDS

cummulation of high-risk cytogenetic changes

therapy-related AML

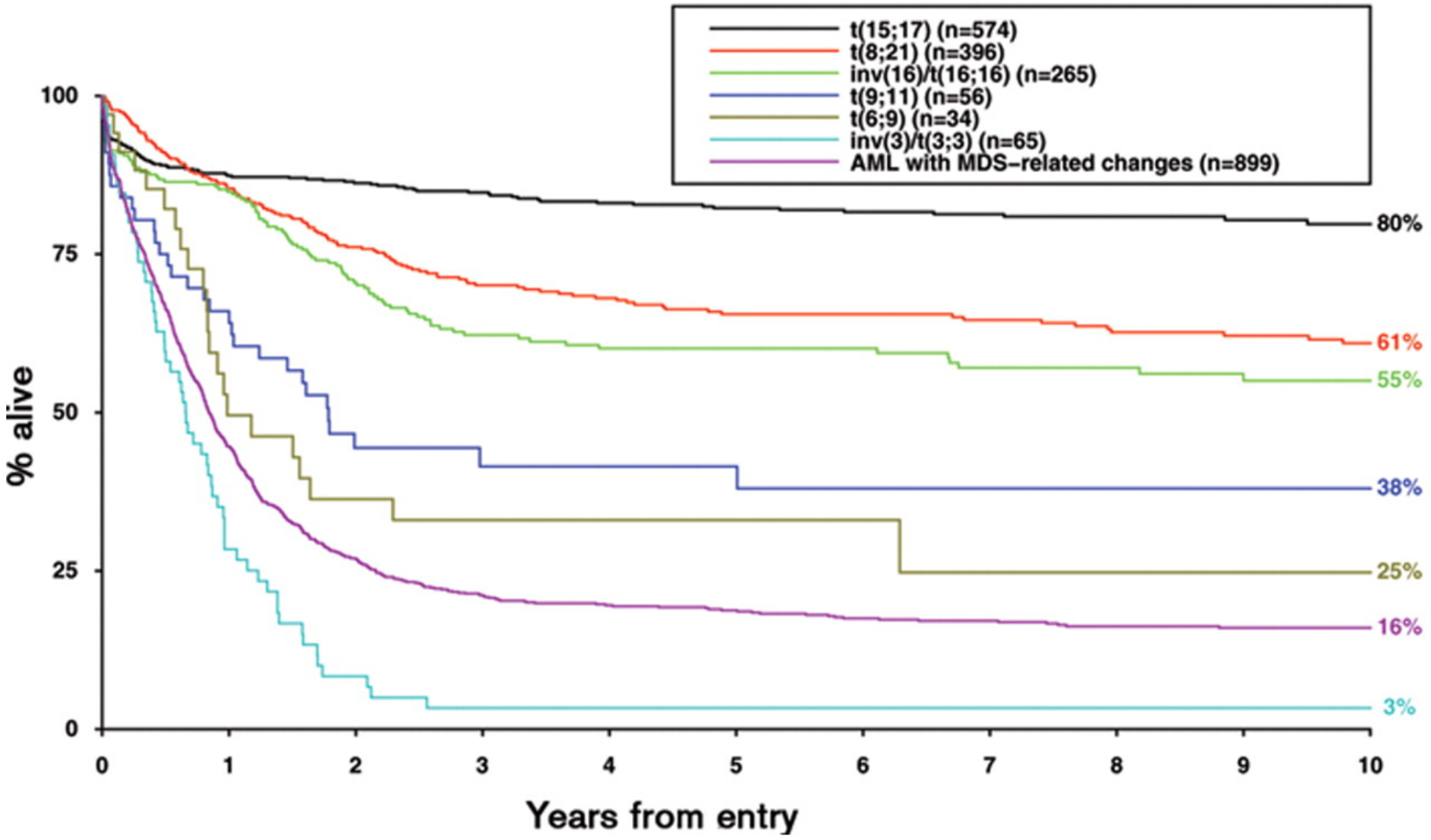
5–10 years after the Tx with alkylating agents (cyclophosphamide, busulfan, cisplatin) and radiotherapy

1–5 years after the Tx with topoisomerase II inhibitors (etoposide)

WHO CLASSIFICATION 2017

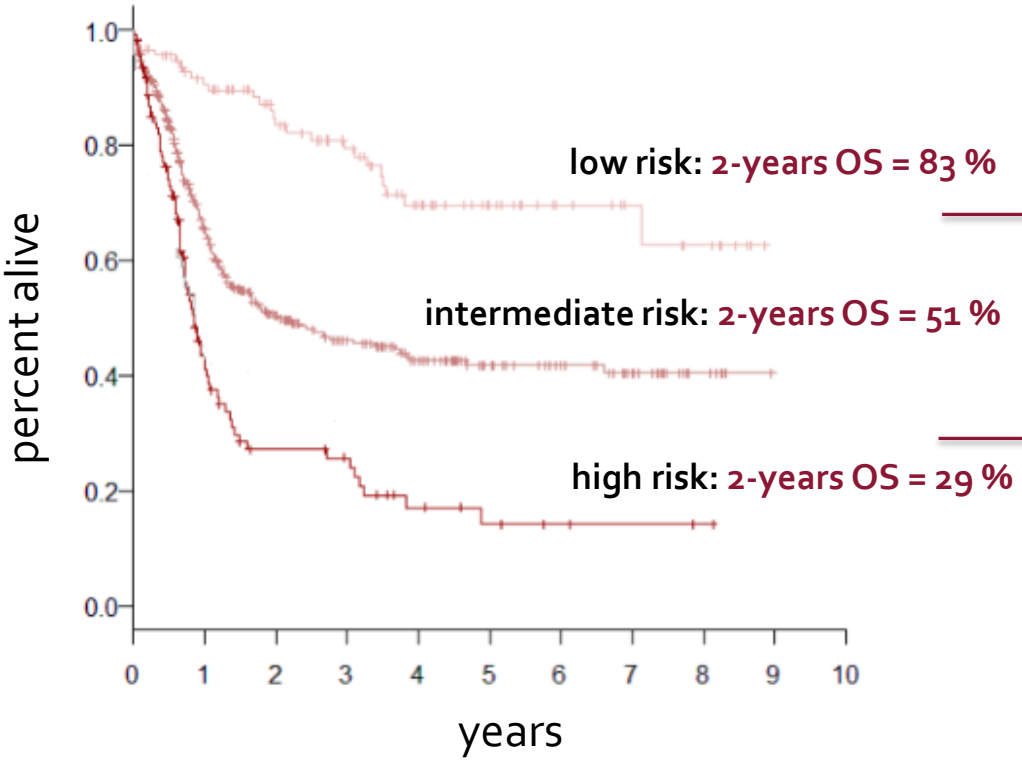
AML with recurrent genetic abnormalities
AML with t(8;21)(q22;q22), fusion gene *RUNX1-RUNX1T1*
AML with inv(16)(p13;q22) or t(16;16)(p13;q22), fusion gene *CBFB-MYH11*
acute promyelocytic leukemia with t(15;17)(q22;q12), fusion gene *PML-RARA*
AML with translocation t(9;11)(p22;q23), fusion gene *MLLT3-KMT2A*
provisional entity: AML with *BCR-ABL1*
AML with mutated *NPM1*
AML with biallelic mutations of *CEBPA*
AML with myelodysplastia-related changes
Therapy-related myeloid neoplasms
t-MDS
t-AML
AML not otherwise specified
AML with minimal differentiation
AML without maturation
AML with maturation
acute myelomonocytic leukemia
acute monoblastic and monocytic leukemia
acute erythroid leukemia
acute megakaryoblastic leukamia
acute basophilic leukemia
acute panmyelosis with myelofibrosis
Myeloid sarcoma
Myeloid proliferations related to Down syndrome
transient abnormal myelopoiesis (TAM)
myeloid leukemia associated with Down syndrome

PROGNOSTIC GROUPS: SURVIVAL



PROGNOSTIC GROUPS

overall survival according to the risk group (Czech data 2007-2017)



CBF mutations [t(8;21), inv(16)/t(16;16)]

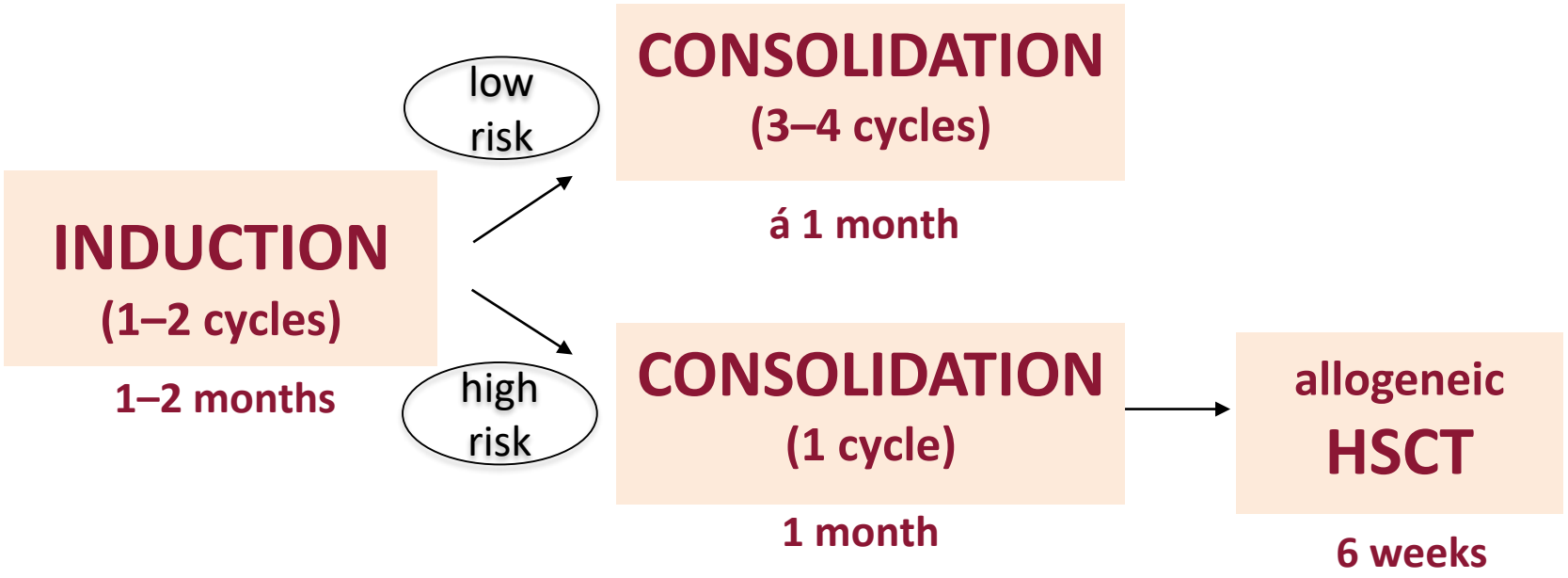
normal cytogenetics
risk can be further refined by
FLT3-ITD, *NPM1*, *CEBPA*
mutation status

numerical changes of chromosomes
(+8, +11, +13, +21, -5, -7)

complex karyotype
(tj. ≥3 chromosomal aberations)

cytogenetics has a key position in prognosic stratification of AML

THERAPY: GENERAL STRATEGY



in order to prevent tumor lysis syndrome chemotherapy is initiated after WBC drop to <50.000 /µl

cytoreduction: pharmacological (hydroxyurea)
instrumental (leukocytapheresis)

INDUCTION THERAPY

combination of anthracycline and cytarabine is the golden standard for >30 years: 3+7 regimen

- 3 days of daunorubicine 45–90 mg/m^2 or idarubicine 10 mg/m^2
- 7 days of cytarabine 100 mg/m^2 in continual infusion

identical for all AML subtypes except for M3

goal: to reach the 1st complete remission

- remission to be checked after 14–21 days

after the failure of the first induction therapy, the second induction cycle is to be administered:

- repeated administration of „3+7“ protocol
- more intensive protocol (e.g. Fla/Ida: idarubicine 10 mg/m^2 + fludarabine 30 mg/m^2 + cytarabine 2000 mg/m^2)

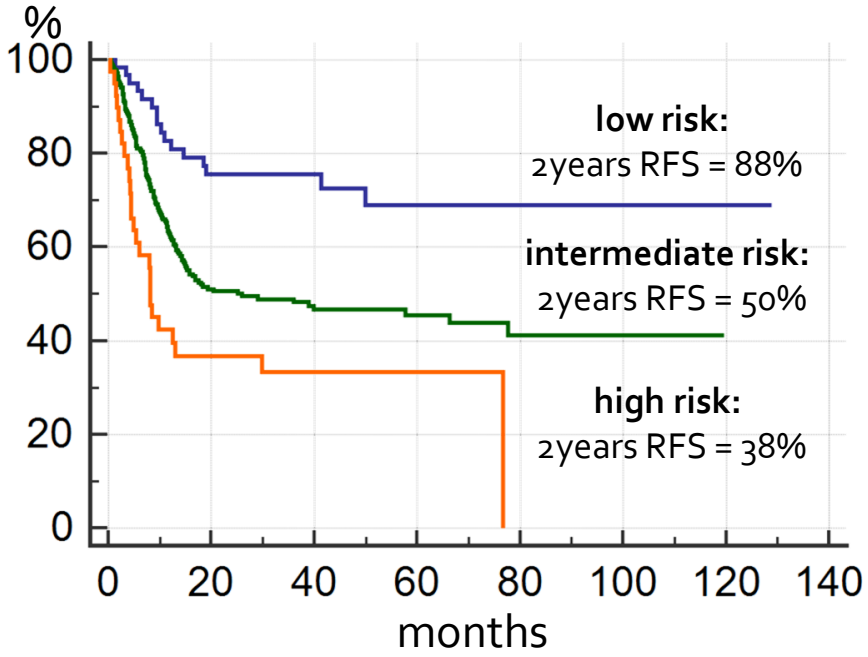
remission rate: 70%–80%

CONSOLIDATION THERAPY

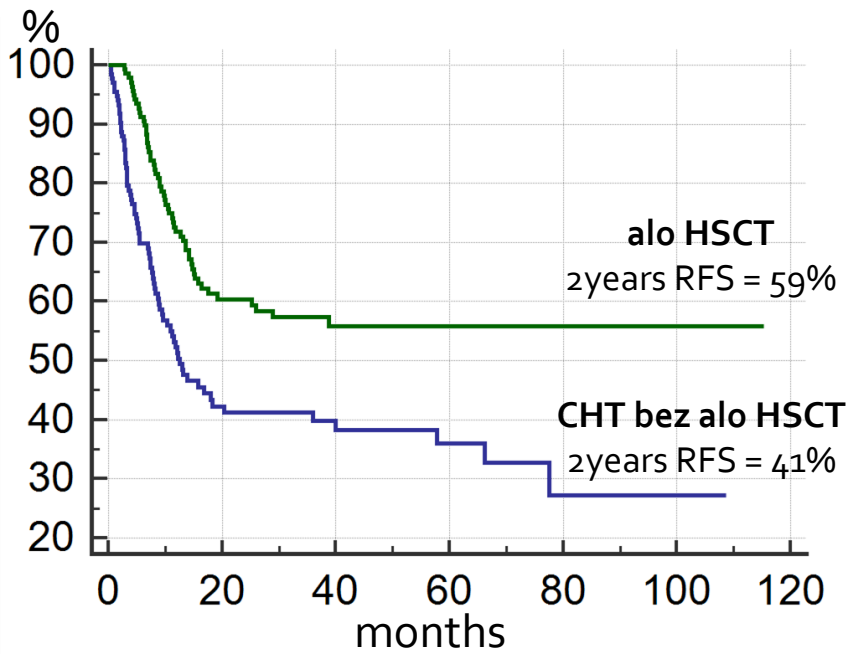
goal: **to sustain remission**

consolidation therapy is designed based on present prognostic factors

low risk:
3–4 cycles of high-dose cytarabine (3 g/m²)



high risk:
allogeneic hematopoietic stem cell transplantation



- risk of relapse after the induction chemotherapy is defined by cytogenetics
- risk of relapse in the intermediate group is lower in patients undergoing allo-HSCT

THERAPY OF ELDERLY PATIENTS

majority of AML cases

worse prognosis
more risky cytogenetic profile
higher percentage of secondary AML (history of MDS)
high chemoresistance (CR in only 50%–60% intensively treated pts.)
80%–90 % eventually relapse
high morbidity and mortality during the course of therapy

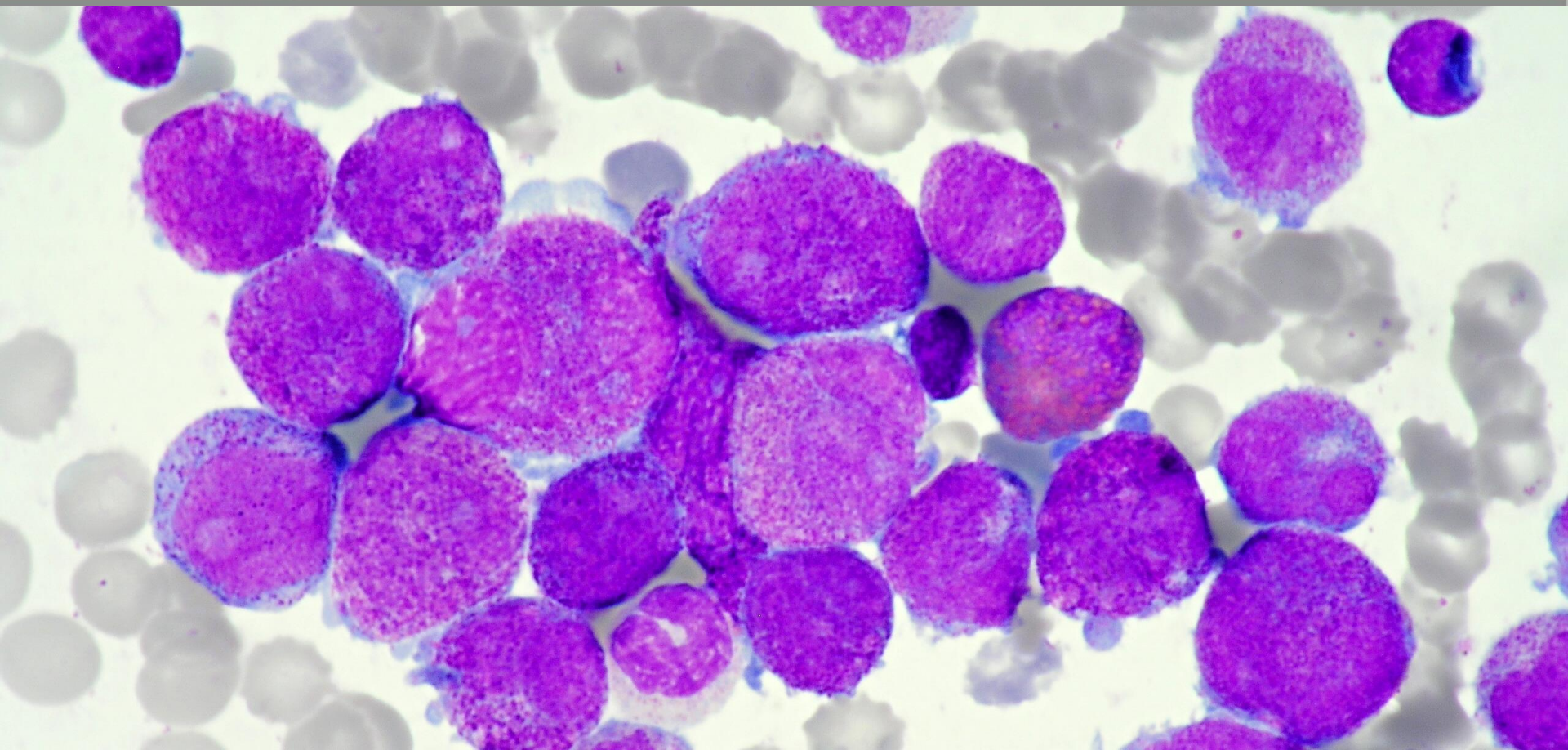
standard induction:
3+7 in the absence of serious comorbidities

consolidation therapy with a chance of CR induction:
low-doses of cytarabine s.c., hypomethylating agents (azacytidine)

palliative therapy: hydroxyurea orally + best supportive care

goal: to minimise hospitalisation, preserve quality of life
„less may be more"

ACUTE PROMYELOCYTIC LEUKEMIA



ACUTE PROMYELOCYTIC LEUKEMIA

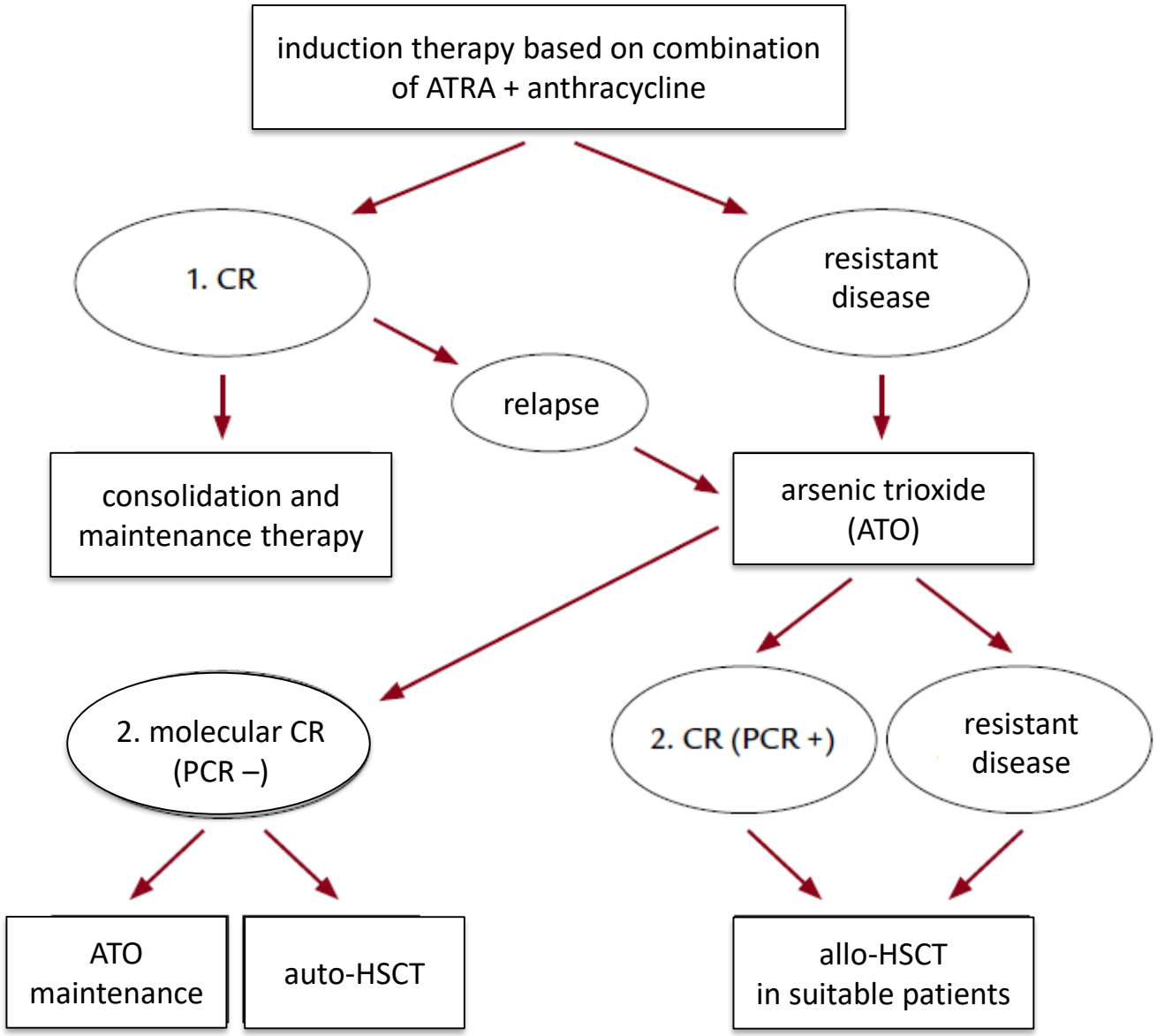
subtype of AML (M3)
accoutns for approx. 5%–8 % of AML cases (up to 15% in Italy, Spain and Latin America)
disease of **younger patients**, median age at diagnosis: 40 years

objective finding: bleeding
laboratory: pancytopenia, disseminated intravascular coagulation syndrome (DIC)
diagnosis: blood count+diff., FACS: HLA-DR neg., PCR: ***PML-RARA*** – results from chromosomal fusion t(15;17)

a leukemia with the best prognosis in the era of differentiating agents (ATRA, As_2O_3) – overall survival 80–90 %

life threatening bleeding is the major limiting factor

THERAPY: GENERAL STRATEGY



THERAPY

induction therapy: standard is idarubicine + ATRA
alternative scheme: combination of two differentiating agents (ATRA + As_2O_3)

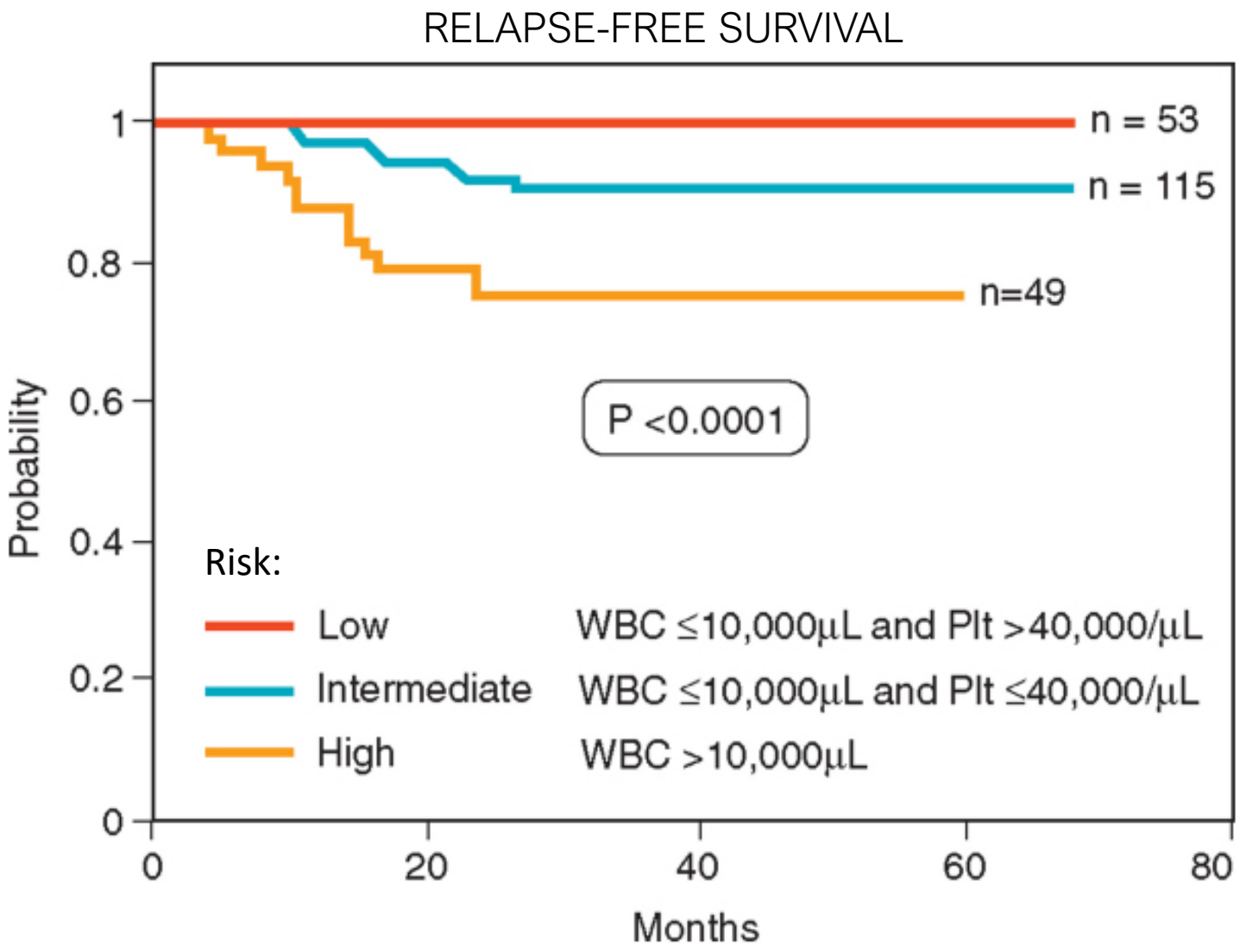
consolidation therapy: anthracycline + ATRA

therapy of DIC and prevention of bleeding (at the beginning of induction therapy)**:**
transfusion (thrombocytes, plasma), coagulation factors (fibrinogen, antithrombin), heparin

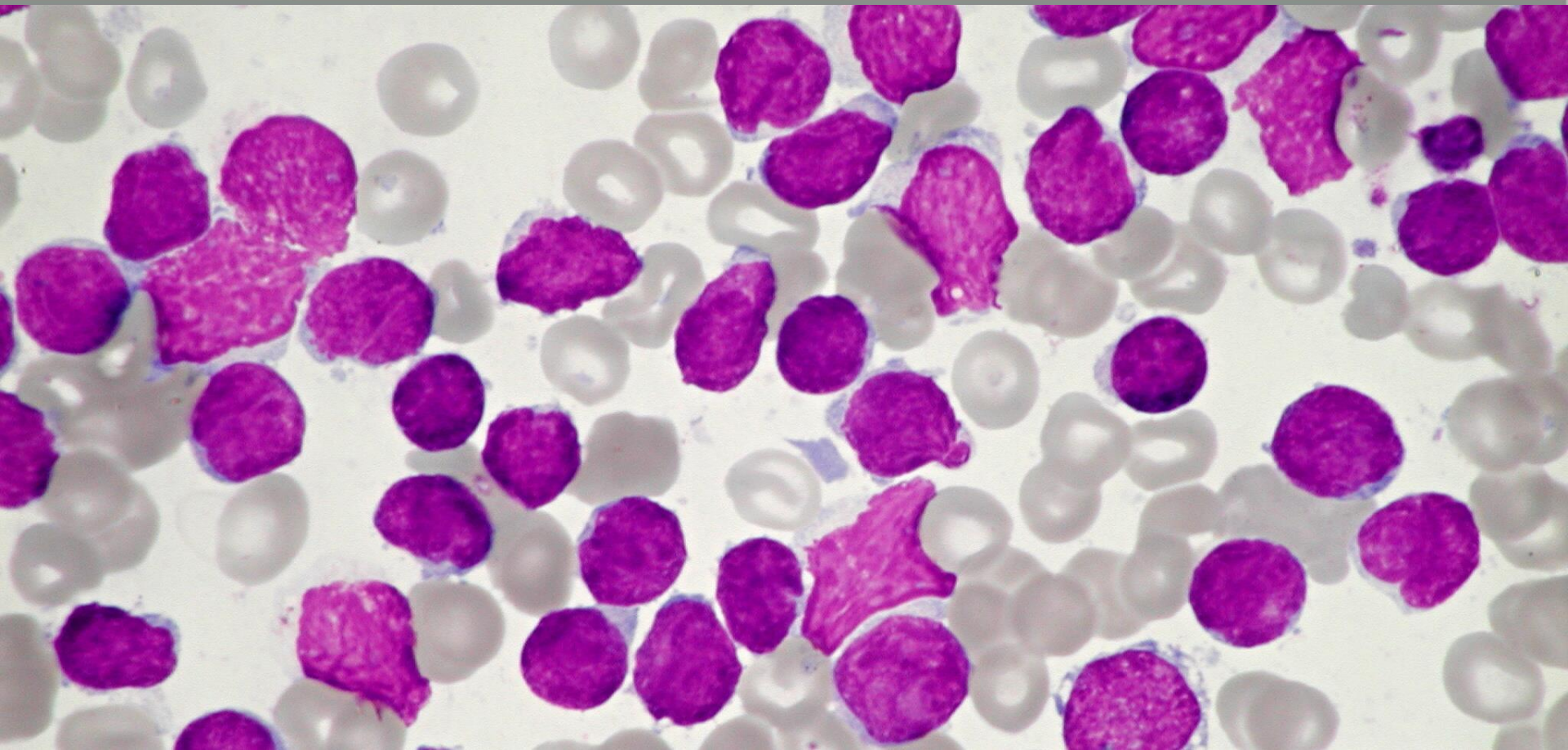
differentiation syndrome (= ATRA syndrome)
neutrophilia, fever, lung infiltrates, hypoxia, fluid retention
therapy: dexamethasone, hold on ATRA temporarily

dispensarisation: *PML-RARA* monitoring in peripheral blood and therapy of eventual molecular relapse

PROGNOSIS


RELAPSE-FREE SURVIVAL
Probability
1
0.8
0.6
0.4
0.2
0
0
20
40
60
80
Months
n = 53
n = 115
n=49
P <0.0001
Risk:
Low
WBC ≤10,000µL and Plt >40,000/µL
Intermediate
WBC ≤10,000µL and Plt ≤40,000/µL
High
WBC >10,000µL

ACUTE LYMPHOBLASTIC LEUKEMIA



ACUTE LYMPHOBLASTIC LEUKEMIA

the most common malignancy in childhood (peak between 2. a 5. year), in adults only 20% of AL

incidence: 1.6/100 000 people

maximum in children <5 yrs: 8.3/100 000

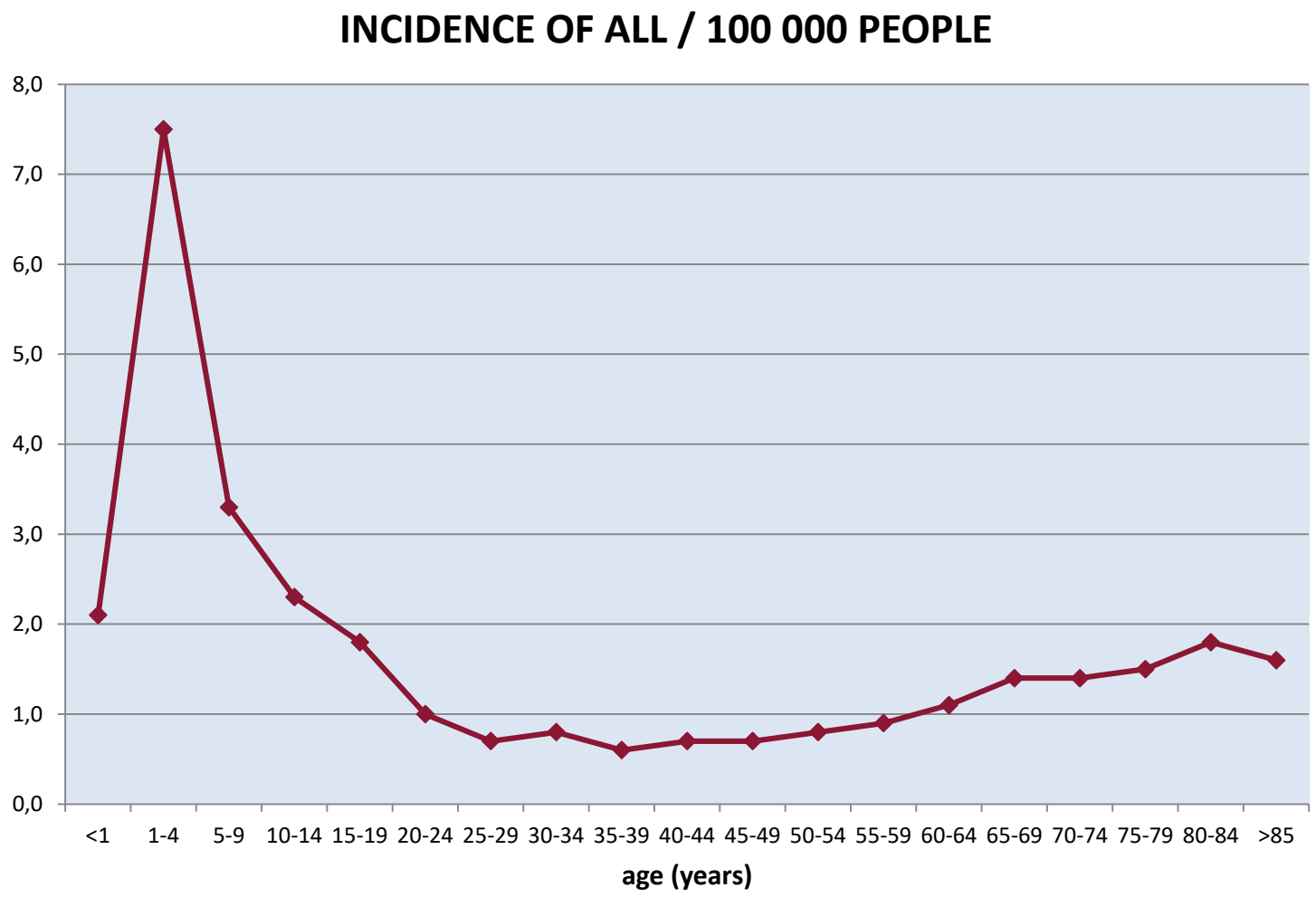
increment in adults >65 yrs, 2nd peak in >85 yrs: 2.0/100 000

different prognosis in children × adults:

children	remission: >95%	OS: 90%
adults	remission: 60%–80 %	OS: ~50%

higher incidence of high risk ALL in adults: mainly Ph+ (*BCR-ABL* positive) ALL (25% in adults × only 3% in children)

INCIDENCE


INCIDENCE OF ALL / 100 000 PEOPLE
8,0
7,0
6,0
5,0
4,0
3,0
2,0
1,0
0,0
<1
1-4
5-9
10-14
15-19
20-24
25-29
30-34
35-39
40-44
45-49
50-54
55-59
60-64
65-69
70-74
75-79
80-84
>85
age (years)

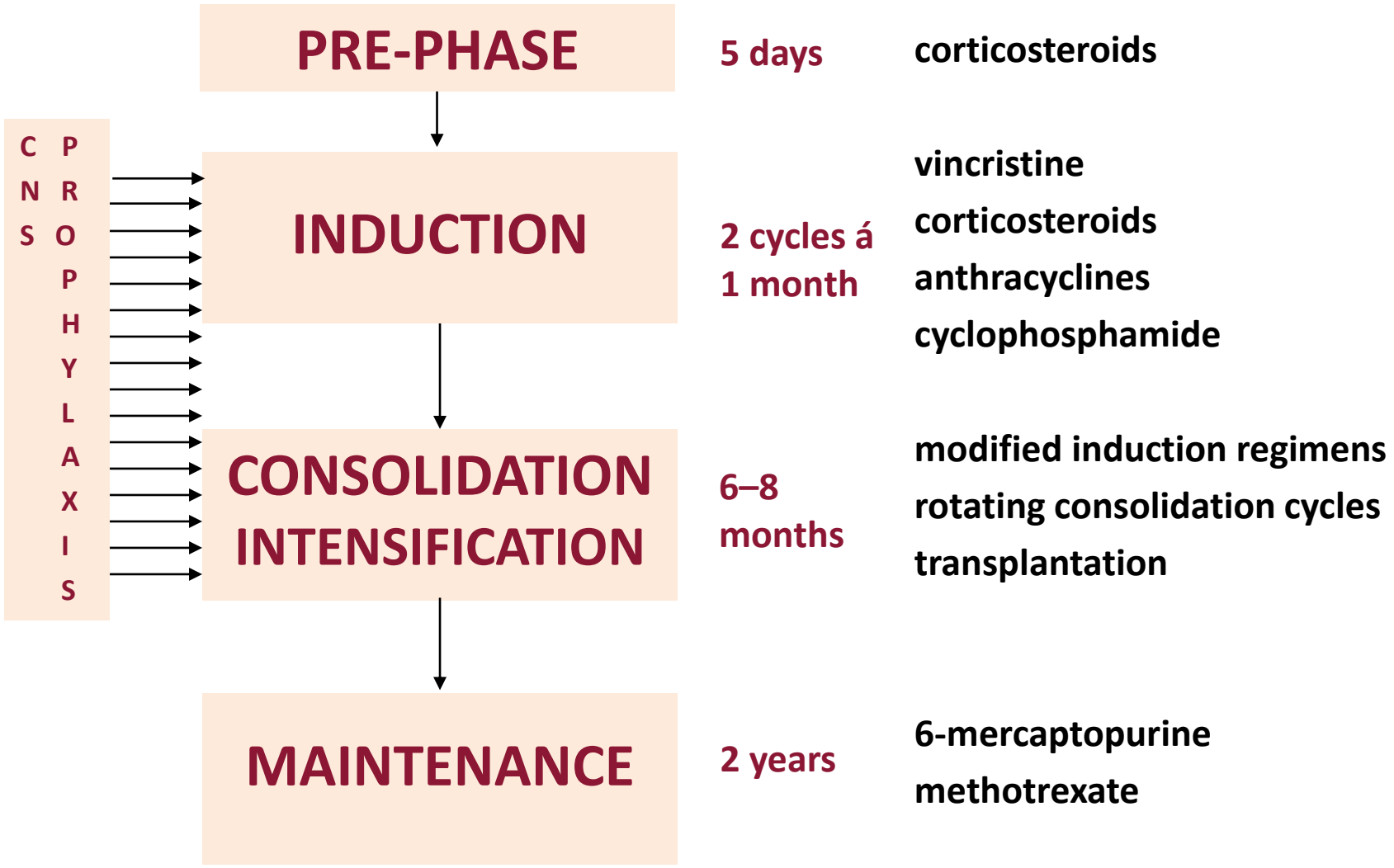
WHO CLASSIFICATION 2017

B-cell lymphoblastic leukemia / lymphoma
B-ALL / lymphoma, not otherwise specified
B-ALL / lymphoma with recurrent genetic abnormalities
- B-ALL / lymphoma with t(9;22), fusion gene *BCR-ABL1*
- B-ALL / lymphoma with *MLL* gene rearrangement
- B-ALL / lymphoma with t(12;21), fusion gene *ETV6-RUNX1*
- B-ALL / lymphoma with hyperdiploidy
- B-ALL / lymphoma with hypodiploidy
- B-ALL / lymphoma with t(5;14), fusion gene *IL3-IGH*
- B-ALL / lymphoma with t(1;19), fusion gene *E2A-PBX1*

Provisional entity: B-ALL / lymphoma *BCR-ABL*–like
Provisional entity: B-ALL / lymphoma with *iAMP21*

T-cell lymphoblastic leukemia / lymphoma
Provisional entity: Early T-cell precursor ALL / lymphoma

THERAPY: GENERAL STRATEGY



PRE-PHASE AND INDUCTION

pre-phase:
glucocorticoids (prednisone, dexamethasone)
used in majority of lymphoid malignancies for their antiproliferative and proapoptotic effect

induction therapy:
vincristine, glucocorticoids, daunorubicin

asparaginase – causes depletion of non-essential aminoacid asparagine which lymphoblasts are unable to synthetise itself

induction therapy in Ph+ ALL:
imatinib (tyrosine kinase inhibitor) parallel to chemotherapy
- higher number of remission
- lower intesity of chemotherapy → lower toxicity
- higher number of transplanted patients

CONSOLIDATION AND MAINTENANCE

consolidation:
the goal is to eliminate minimal residual disease and so lower risk of relapse
alternating cycles of chemotherapeutics used in induction + other agents: high dose **methotrexate**, high dose **cytarabine**, **etoposide**, others...

therapy/prophylaxis of CNS leukemia:
i.t. chemotherapy during induction and consolidation cycles
radiotherapy of the cranium (18–24 Gy)

CNS prophylaxis has lowered the risk of ALL relapse in CNS from 30% to <5%

maintenance therapy:
6-merkaptopurine p.o. daily + methotrexate p.o. once weekly during 2–3 years **in all patients not undergoing allo-HSCT**

TRANSPLANTATION

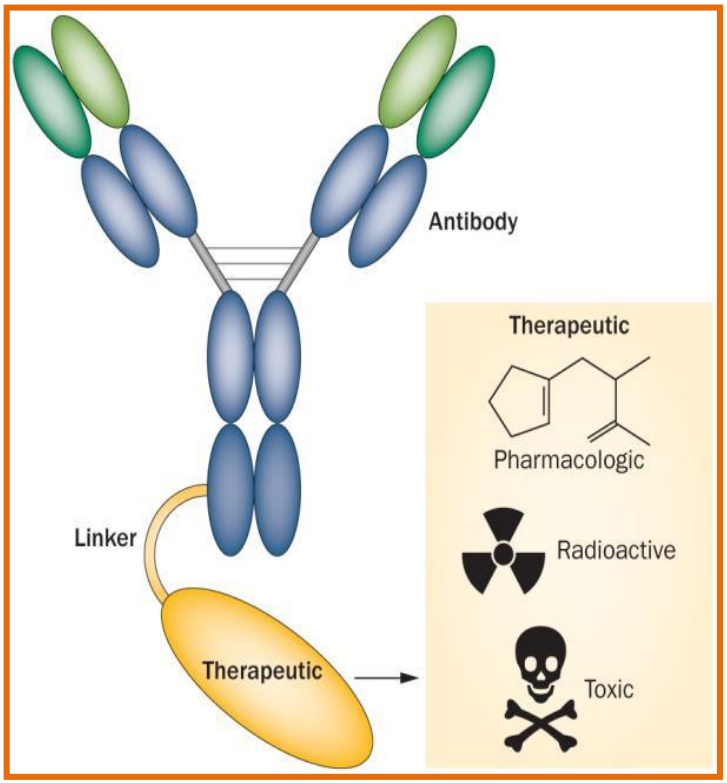
allogeneic hematopoietic stem cell transplantation is used as consolidation **in high risk ALL**

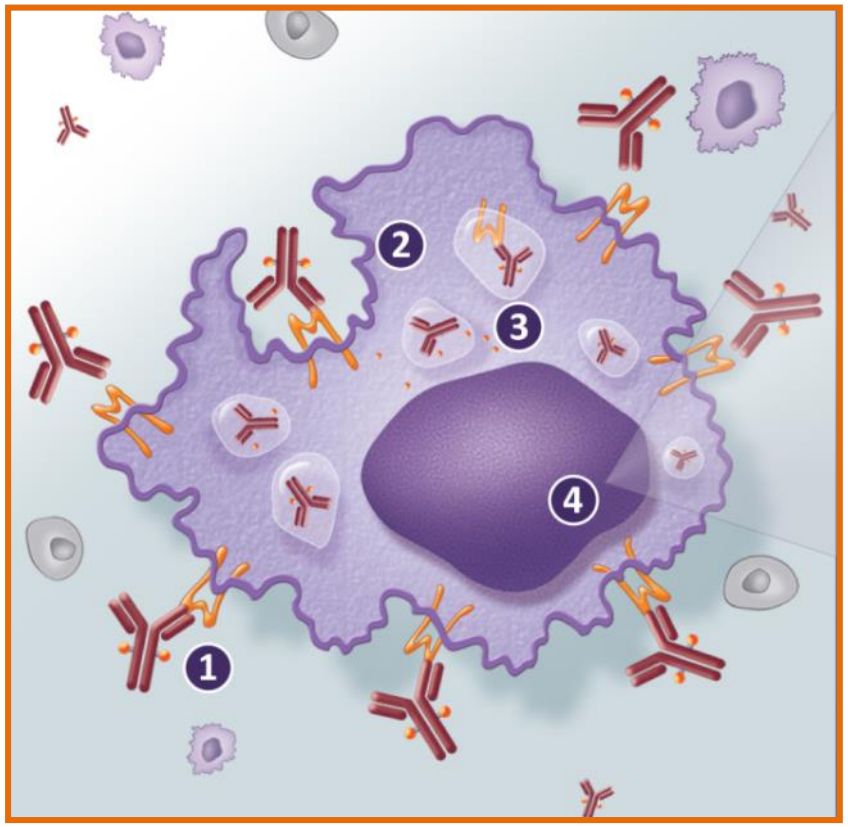
risk factors:
hyperleukocytosis (>30 x 10^9/l in B-ALL, >100 x 10^9/l in T-ALL)
imunophenotype (proB, earlyT, matureT)
cytogenetics: t(9;22) – i.e. **Ph+ ALL**, t(4;11)
slow hematological response (CR after >4 weeks)
MRD persistence

autologous transplanttion might be an alternative choice in patients who have reached MRD negativity
MRD meassured also in autologous graft

NOVEL MONOCLONAL ANTIBODIES

INOTUZUMAB OZOGAMICIN





immunoconjugate has 3 parts:

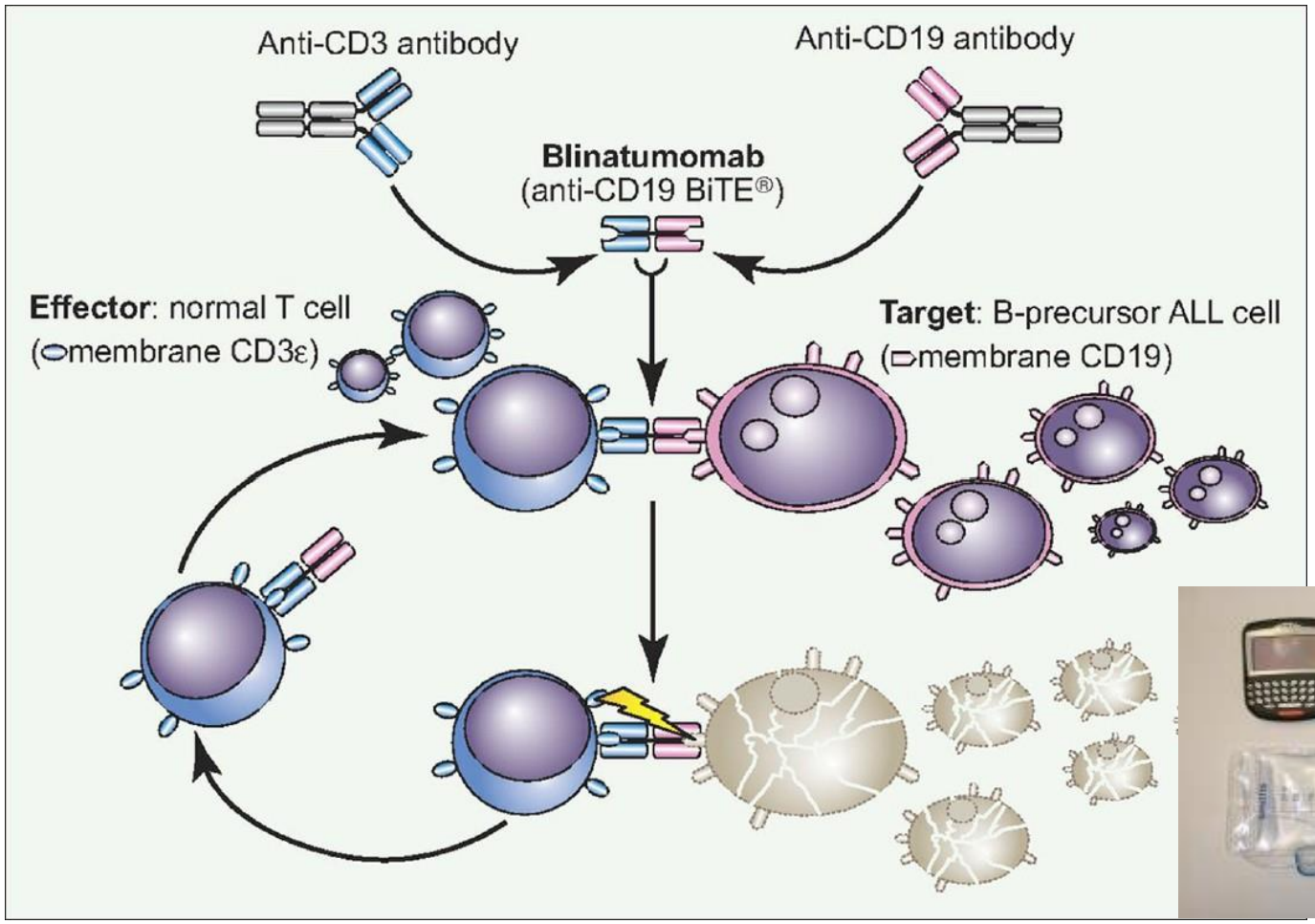
- antibody
- linker
- effector part

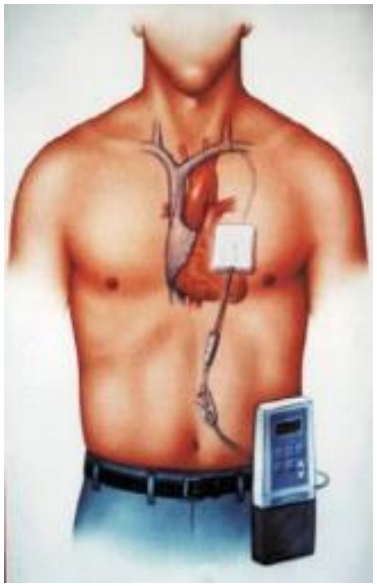
1. IO binds to CD22 antigen
2. the complex is internalized
3. toxin is detached from antibody
4. cytostatic effect in the nucleus

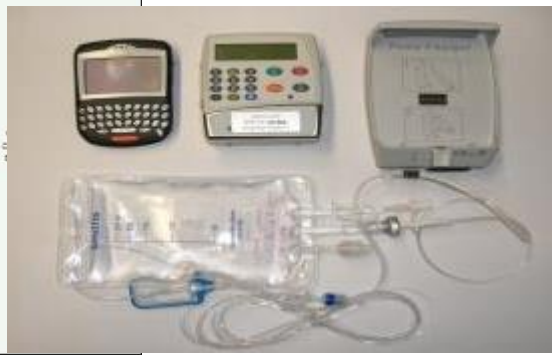
NOVEL MONOCLONAL ANTIBODIES

BLINATUMOMAB

continuous i.v. infusion
special pump
24 hrs/7 days for 4 weeks

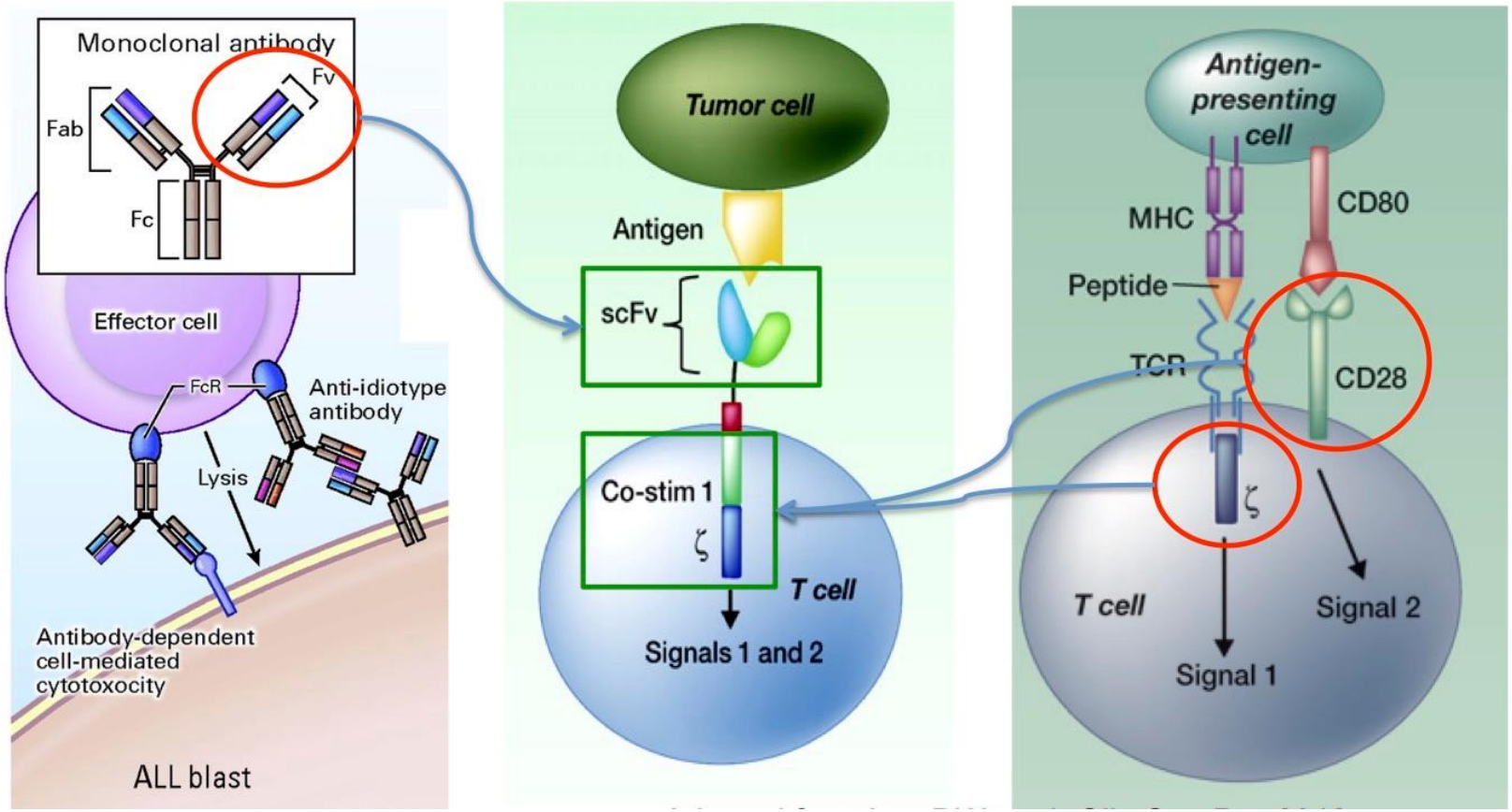






T-LYMPHOCYTES WITH A CHIMERIC ANTIGEN RECEPTOR (CAR)

targeted epitopes (dg. CD19) introduced via virus vector and attached to costimuatory membrane protein (eg. CD28, CD137), attached to the signalling domain of the ζ-chain

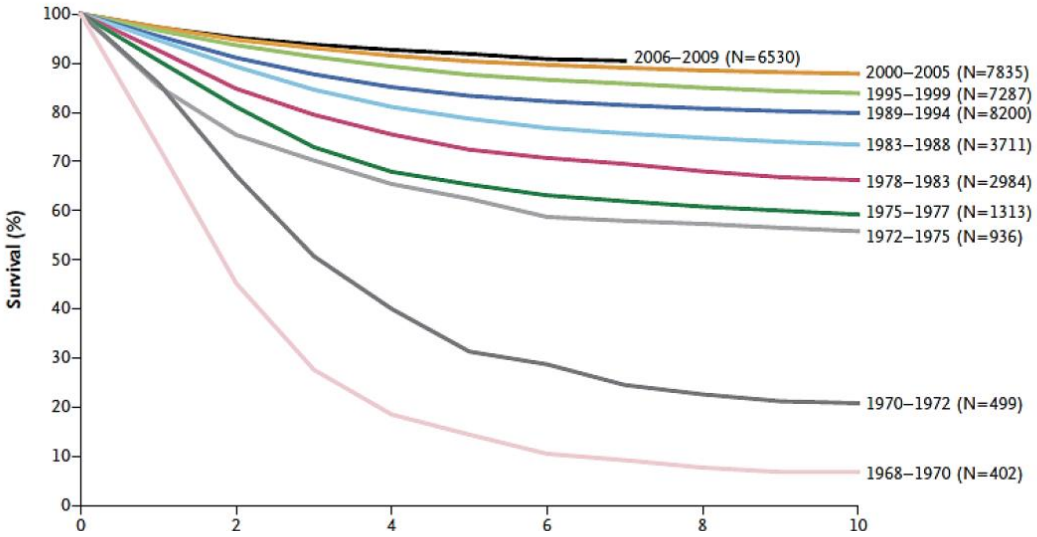


SURVIVAL ACCORDING TO AGE AND YEAR OF DIAGNOSIS

children

protokols of Children's Cancer Group

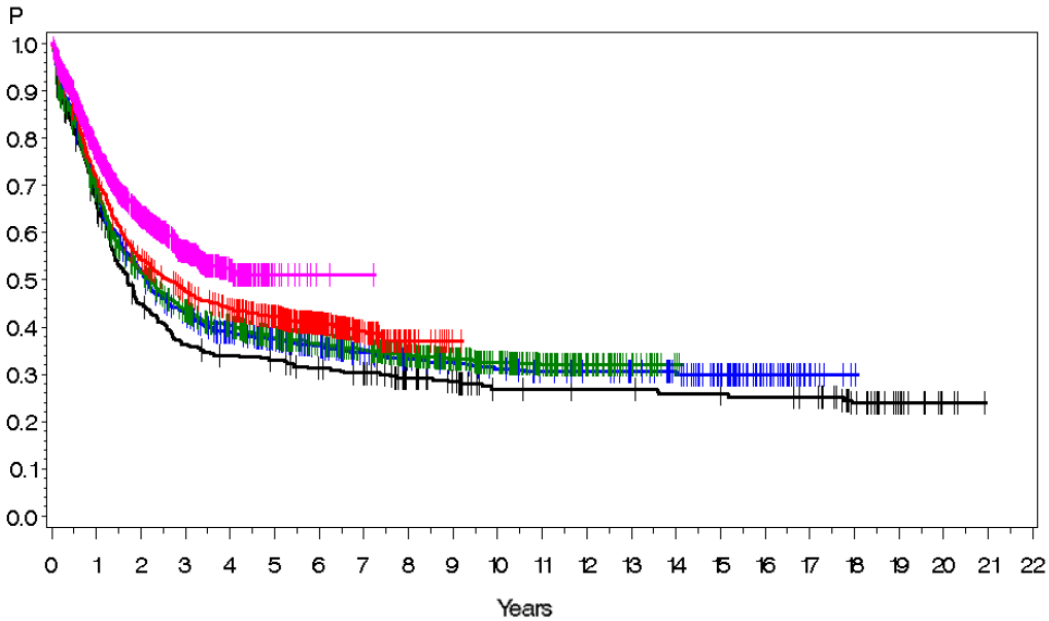
1968 - 2009



adults

protocols of GMALL group

1987 - 2015



CONCLUSION:
FOUR QUESTIONS

A finding of **whatever number of leukocytes in peripheral blood with the absense of middle developmental forms of granulocytes** is typical for:

1. chronic myeloid leukemia
2. leukemized non-Hodgkin lymphoma
3. acute leukemia
4. bone marrow failure

Vyberte odpověď

What finding is typical for **acute leukemia** in **bone m**

1. 5–10% of blasts
2. 10–20% of blasts
3. >20% of blasts
4. increased number of blasts, dysplastic changes in hematopoiesis must be present

Vyberte odpověď.

A finding of **pancytopenia in peripheral blood together with coagulopathy (↓ fibrinogen, ↑ D-dimers)** is suspicious of:

1. Ph-positive acute lymphoblastic leukemia
2. acute promyelocytic leukemia
3. essential thrombocytemia with a thromboembolic complication
4. myelodysplastic syndrome

Vyberte odpověď

What is the major **risk factor** predicting prognosis of **acute myeloid leukemia**:

1. age
2. number of WBC at diagnosis
3. immunophenotype
4. karyotype

TAKE HOME MESSAGES

any abnormality of the WBC count on routine peripheral blood examination warrants a differential WBC count

diagnosis of acute leukemia is made by bone marrow examination demonstrating >20 % of blasts

acute leukemia does not necessarily mean a high WBC count

a pacient with pancytopenia and bleeding manifestation is to be urgently examined by a hematologist (suspected APL)