

Haematology

Ulrike Köhl



Paediatric Haematology, Oncology and Haemostaseology
J.W. Goethe University Frankfurt, Germany



Haematology

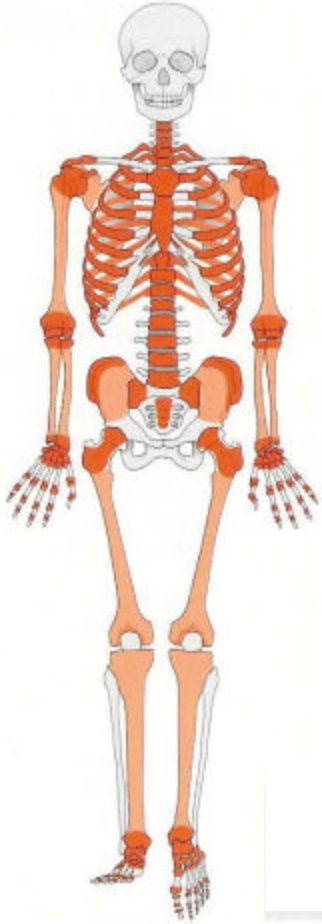
➤ Haematopoiesis – normal

➤ Diagnosis of blood diseases

- Red blood cells

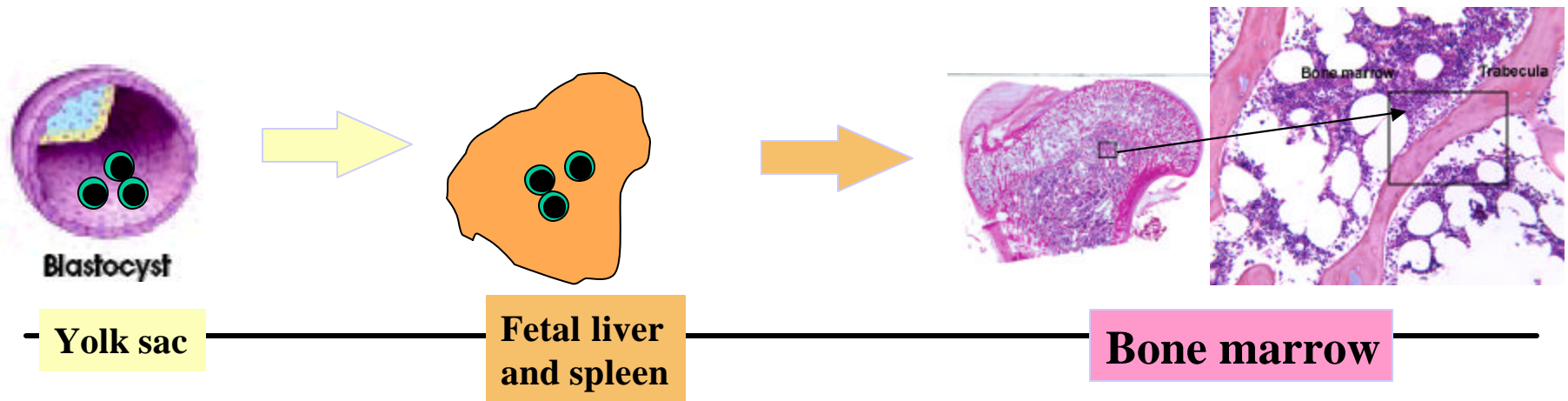
- White blood cells

Haematopoiesis



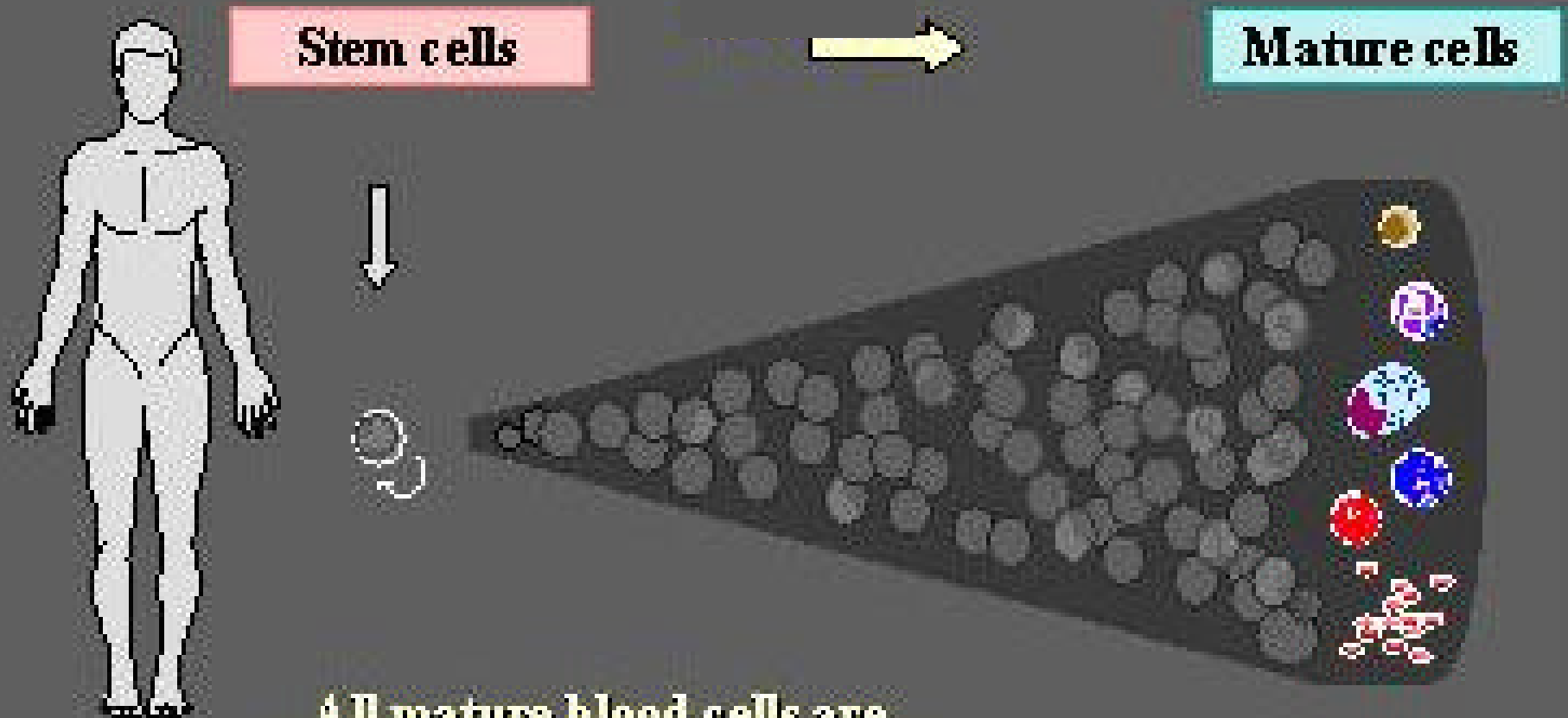
- **The production of blood cells is called haematopoiesis (haemopoiesis)**
- **Haematopoiesis starts 17 days after fertilisation**
- **Haematopoiesis continues throughout life**
- **Haematopoiesis is regulated**
- **Haematopoietic tissues can respond rapidly to increase cell production (blood loss, infection)**
- **Where does haematopoiesis occur?**

Sites of haematopoiesis during embryogenesis



- 17 days **Yolk sac** red cells and platelets
- 6 weeks **Liver** red cells, platelets, leukocytes
- 12 weeks **Liver and spleen** red cells, platelets, leukocytes
- 20 weeks **Bone marrow** red cells, platelets, leukocytes

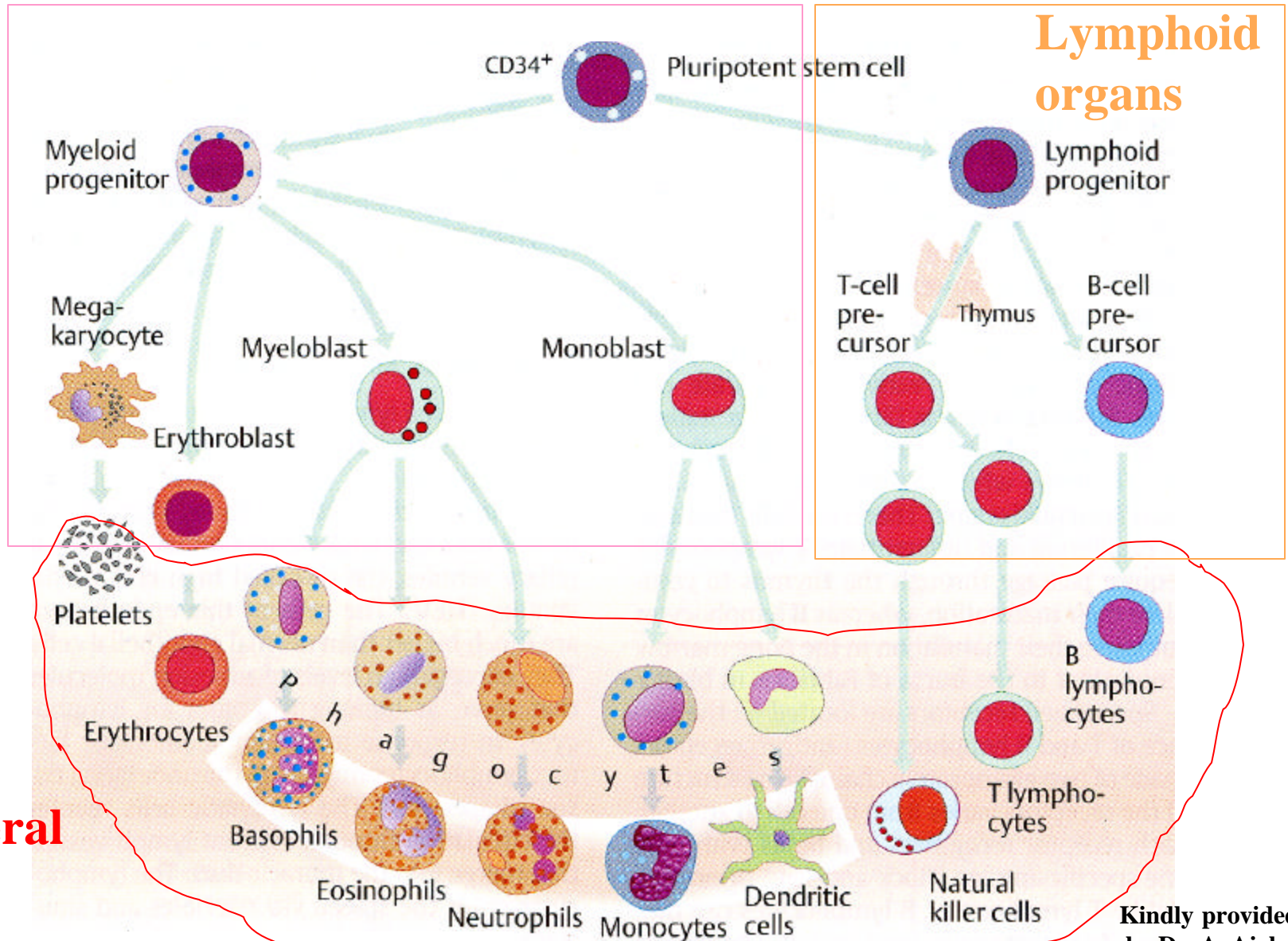
Haematopoietic stem cells



All mature blood cells are derived from a small population of "hematopoietic" stem cells

Model of haematopoiesis

Bone marrow

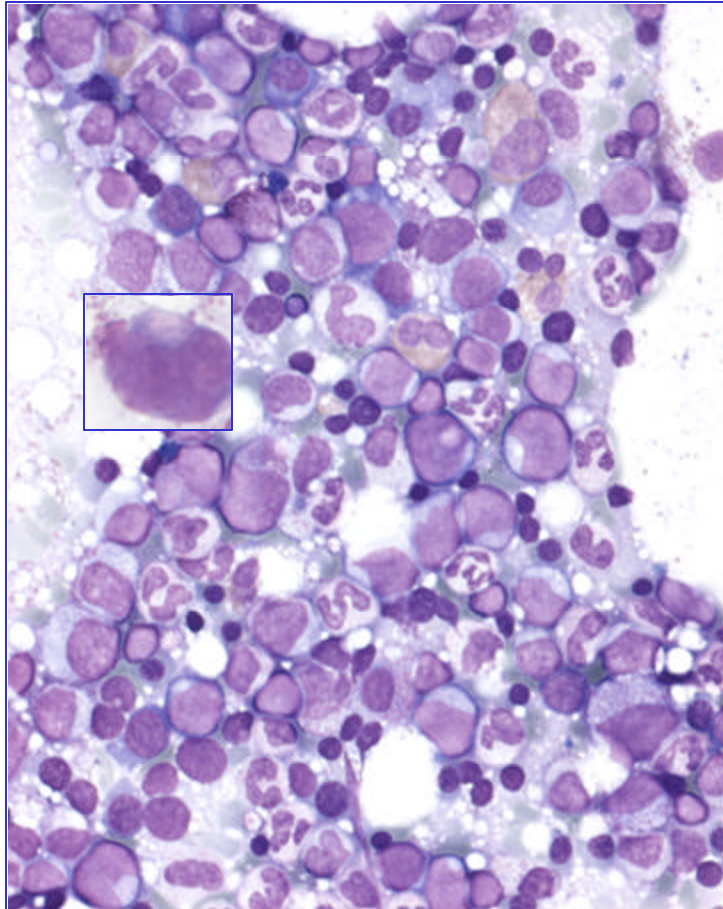


Lymphoid organs

Peripheral blood

Kindly provided by Dr. A. Aicher

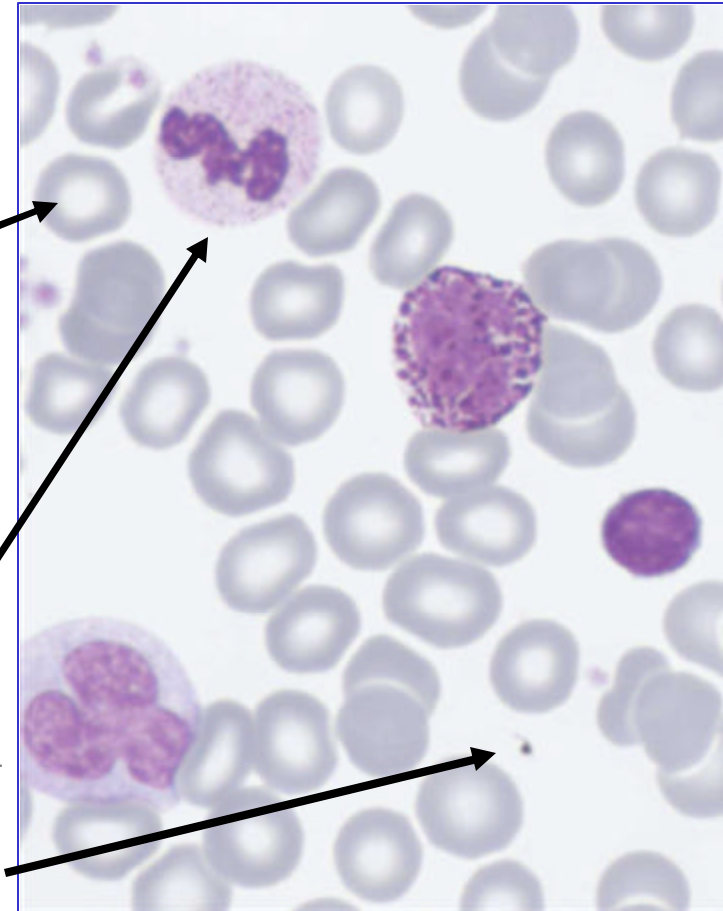
Production of each cell type per day



2×10^{11}
erythrocytes

1.2×10^{11}
granulocytes

1.5×10^{11}
platelets



Bone marrow



Peripheral blood

Content of blood

Blood volume

- ~ 8% of the body weight → 80 ml/kg
- Adults: ~ 5 liter
- New borns: ~ 300 ml

Blood contains

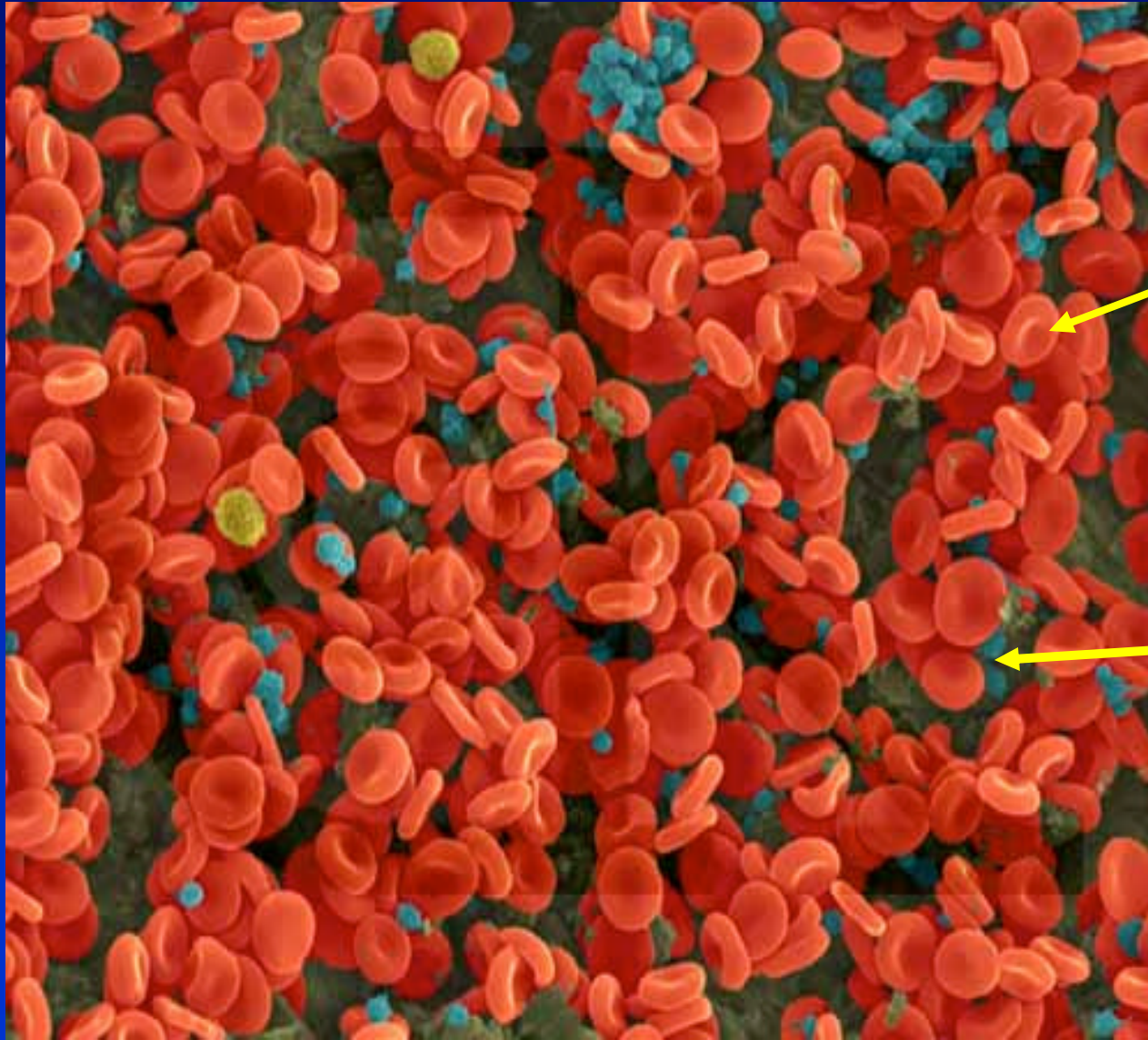
- ~ 45% cellular components
- ~ 55% plasma (water, proteins, clotting factors, elektrolytes...)

serum = plasma without factors for coagulation

Cellular components of the blood

Cells	Concentration	Duration of living	„juvenile form“
Erythrocytes	4 800 000/ μ l	100-120 days	Retikulozytes 1(-3) days
Platelets	200 000/ μ l	7-12 days	
Granulocytes	4 500/ μ l	2-3 days	„Stabkerniger“
Lymphozytes	3000/ μ l	up to many years	

Blood cells



Red
blood cells

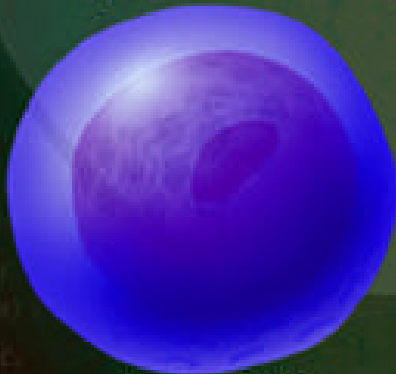
White
blood cells

Erythropoiesis

Function: O₂ transport



Proerythroblast



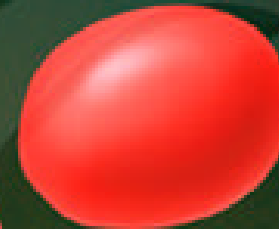
Basophilic erythroblast



Polychromatic erythroblast



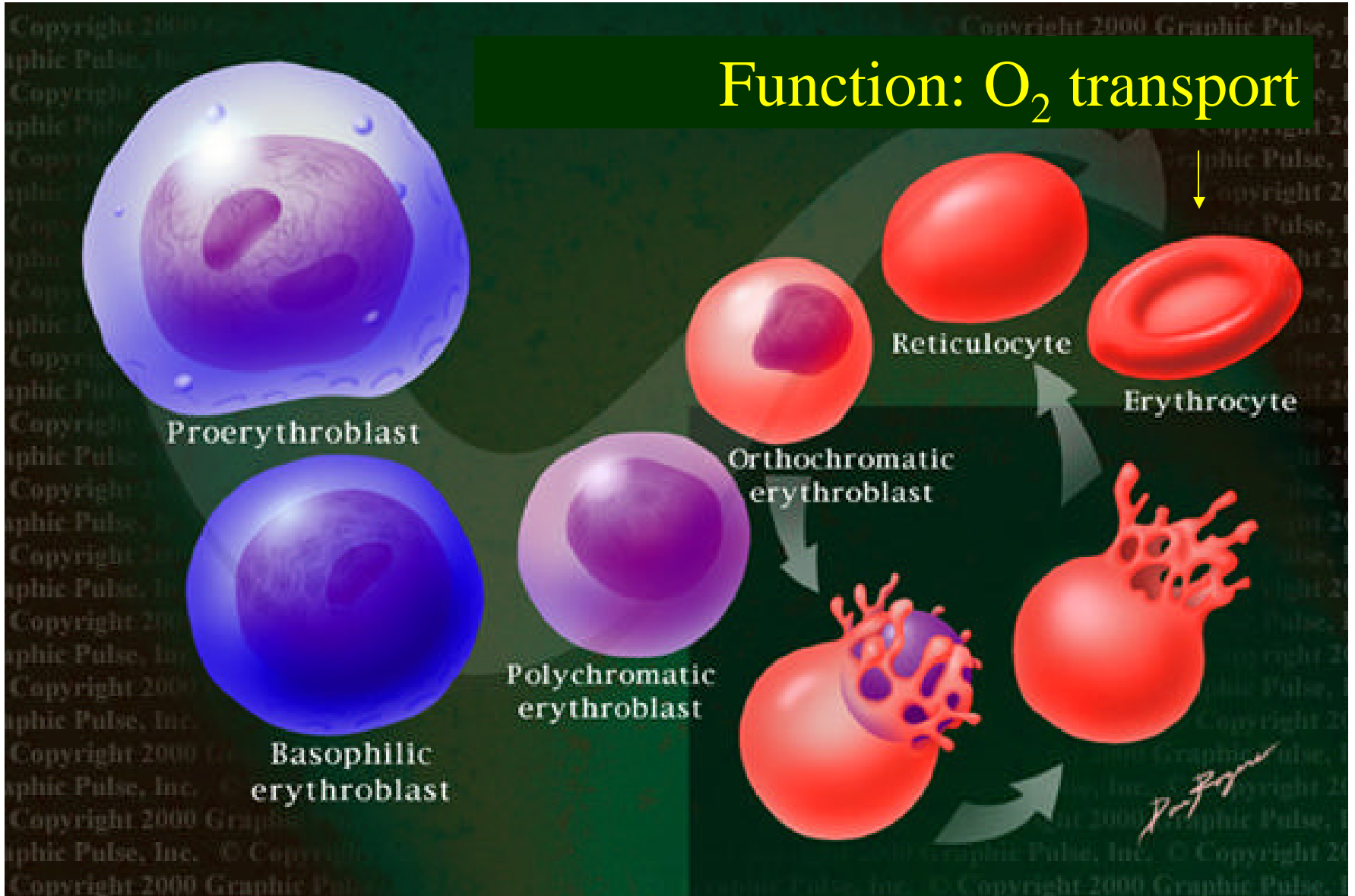
Orthochromatic erythroblast



Reticulocyte



Erythrocyte



Haematology

➤ Haematopoiesis – normal

➤ Diagnosis of blood diseases

- Red blood cells
- Thrombocytes

- White blood cells

Red blood cells

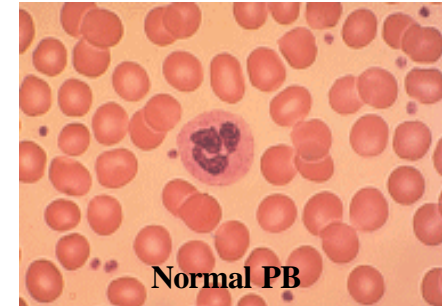
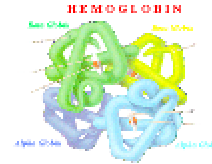
➤ **Erythrocytes:** 4 – 5.5/pl (4 - 5.5 Mio/ μ l)

➤ **Haemoglobin (Hb):** 12-17 g/dl

Hb increased → „Polyglobulia“

Hb decreased → „**Anaemia**“

Clinical symptoms: paleness, tired, exhausted



➤ **Mean corpuscular volume of erythrocytes (MCV):** 80-100fl

MCV increased → „macrocytosis“,,

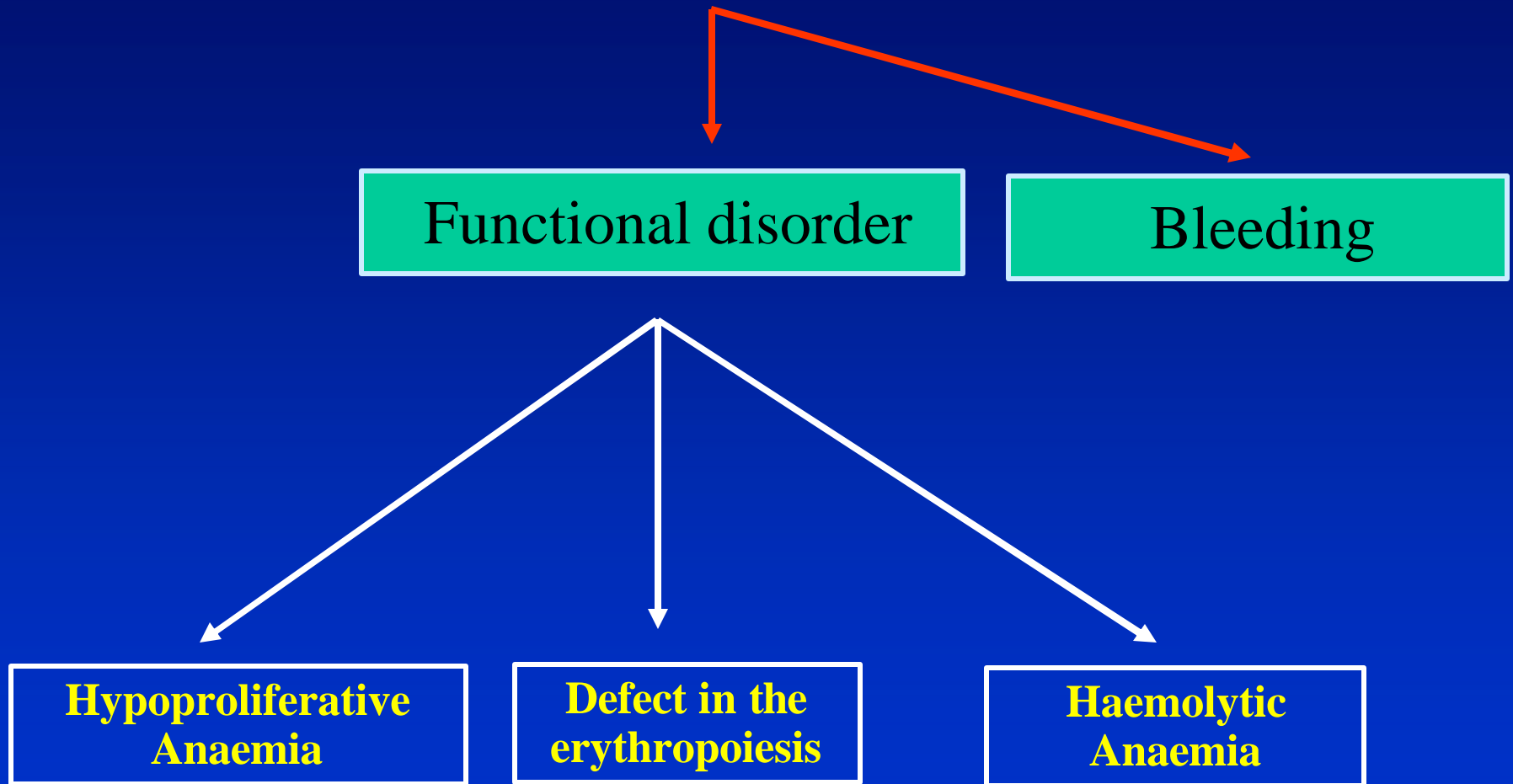
MCV decreased → „microcytosis“

➤ **Reticulocytes:** 0.5-1.5%

Reticulocytes decreased → „hypo- or aplastic“

Reticulocytes increased → exhausted use

Reason for Anaemia

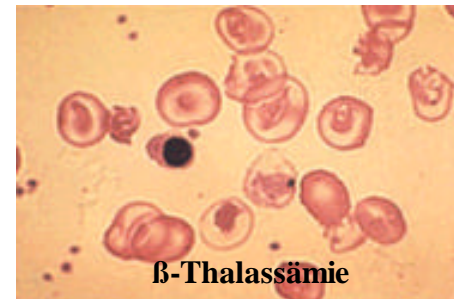


Kindly provided by Prof. Bergmann

Anaemia

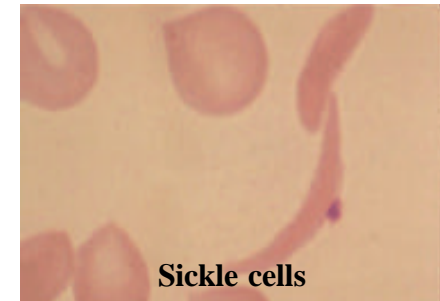
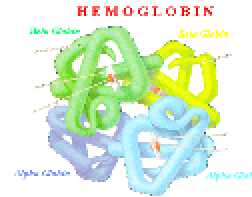
Mikrocytic:

- Iron decreased → Ferritin (marker for iron load) important during pregnancy
- Thalassaemia → LDH small increased, Reticulocytes very high; functional defect in the Haemoglobin synthesis ($\alpha_2\beta_2$)



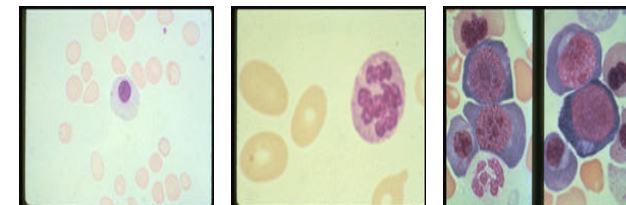
Normocytic:

- Bleeding, renal, aplastic
- Haemolytic → LDH increased, p.e. Haemolytic Uraemic Syndrom, Sickle Cell Disease

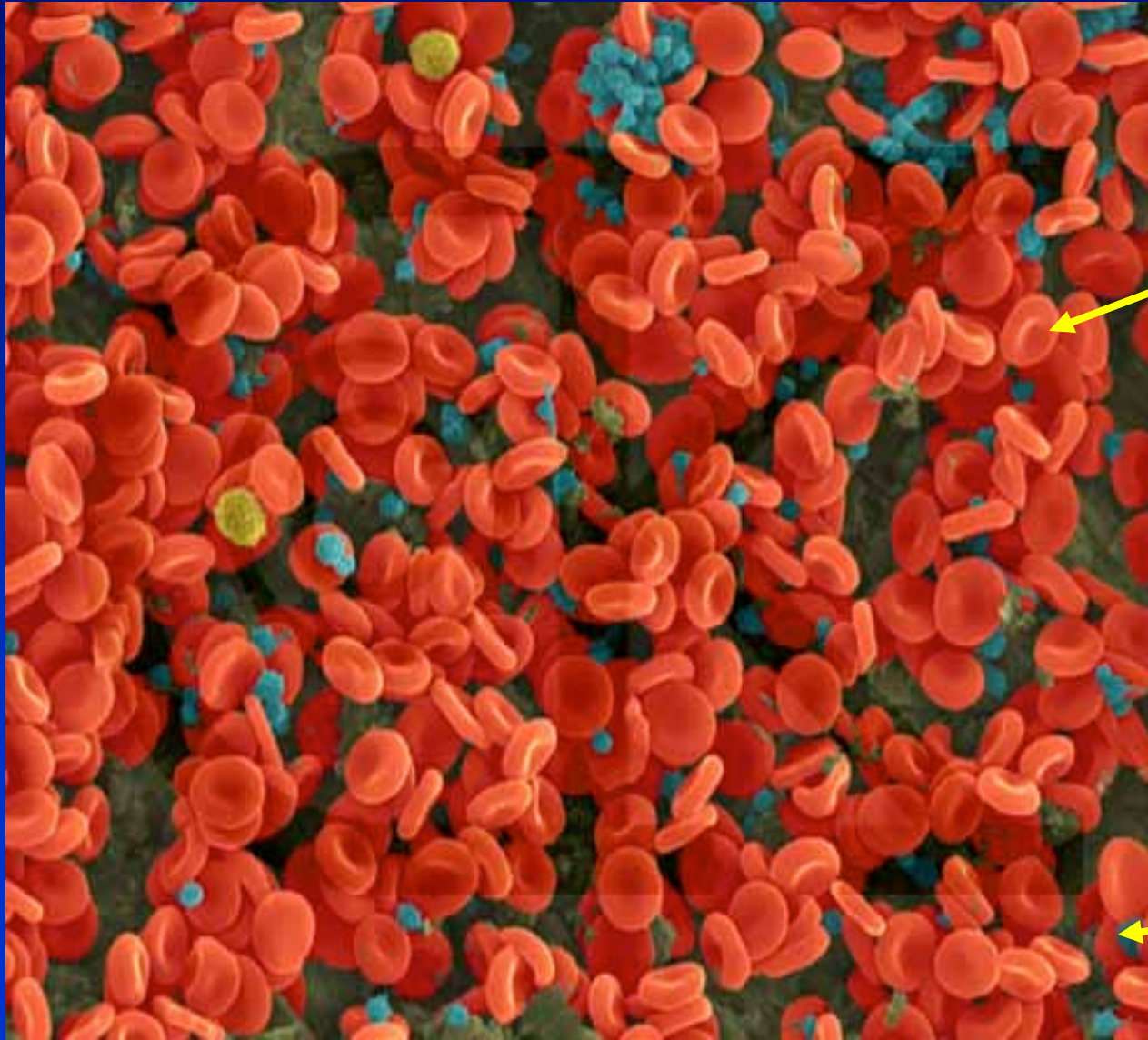


Makrocytic

- B12 or folic acid deficiency



Blood cells



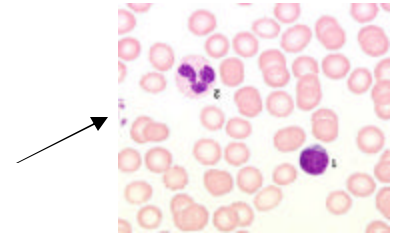
Red
blood cells

Platelets
where ?

White
blood cells

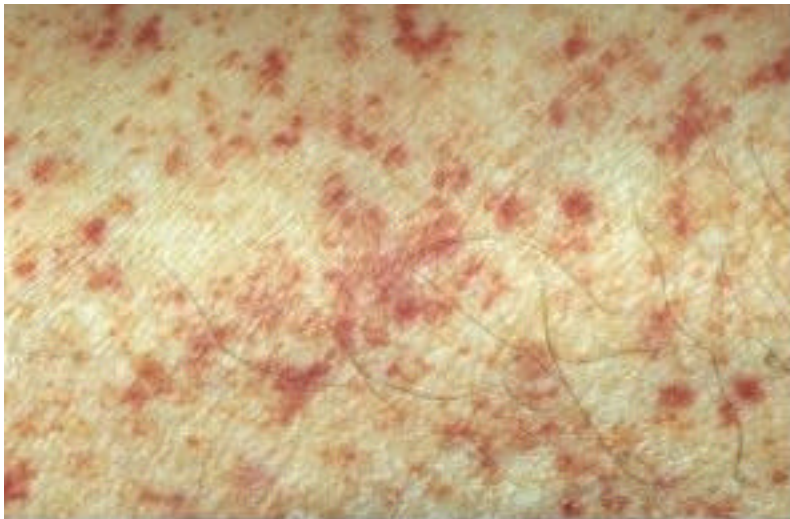
Platelets

Platelets: 150 - 400/nl (150 000 – 400 000/ μ l)



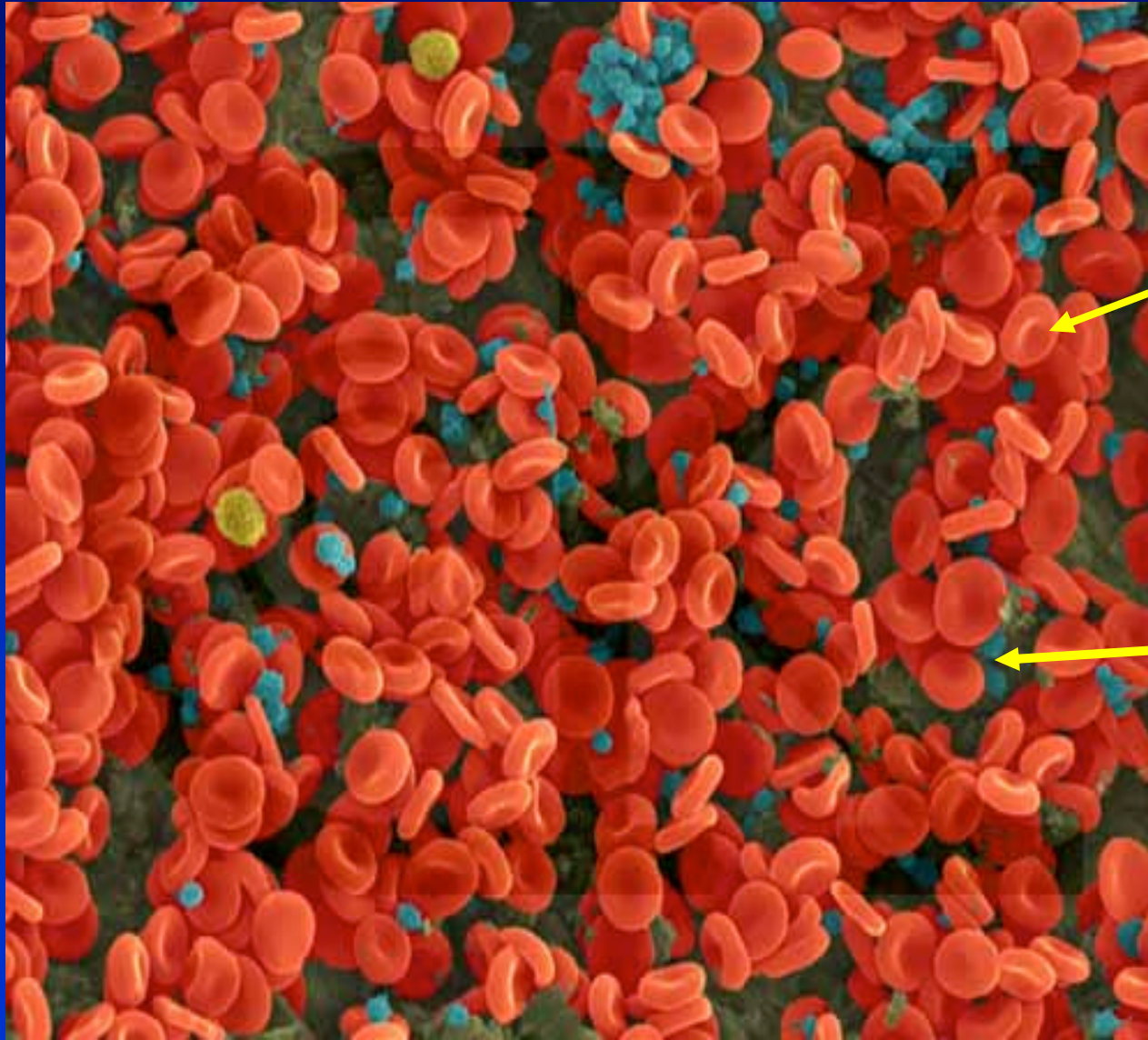
- Less Platelets: Thrombopenia, bleeding may start below 50 000/ μ l

Petechien



- Increased Platelets: Thrombozytosis, increased risk for thrombosis $> 800\ 000/\mu$ l

Blood cells



Red
blood cells

White
blood cells

Haematology

➤ Haematopoiesis – normal

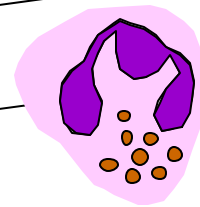
➤ Diagnosis of blood diseases

- Red blood cells

- White blood cells
normal versus illness

White blood cells

- **Basophil Granulocytes**
- **Eosinophil Granulocytes**
- **Neutrophil Granulocytes**

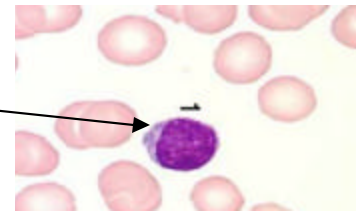
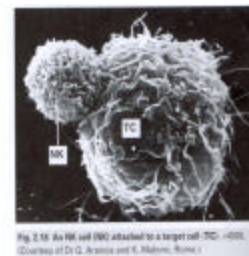
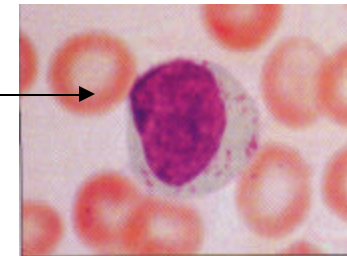
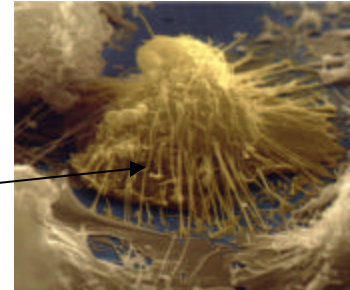
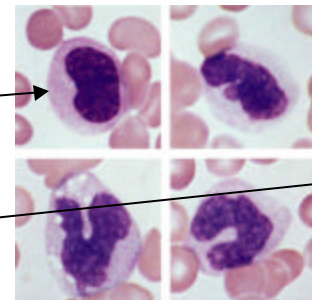
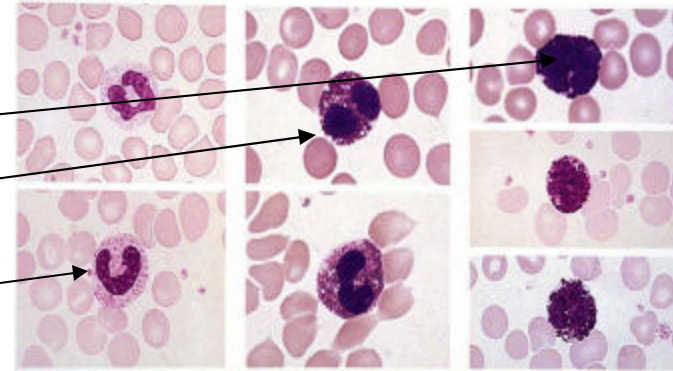


Neutrophil

- **Monocytes Macrophages**
- **Langerhans / Dendritic cell family**

- **Natural killer cells**

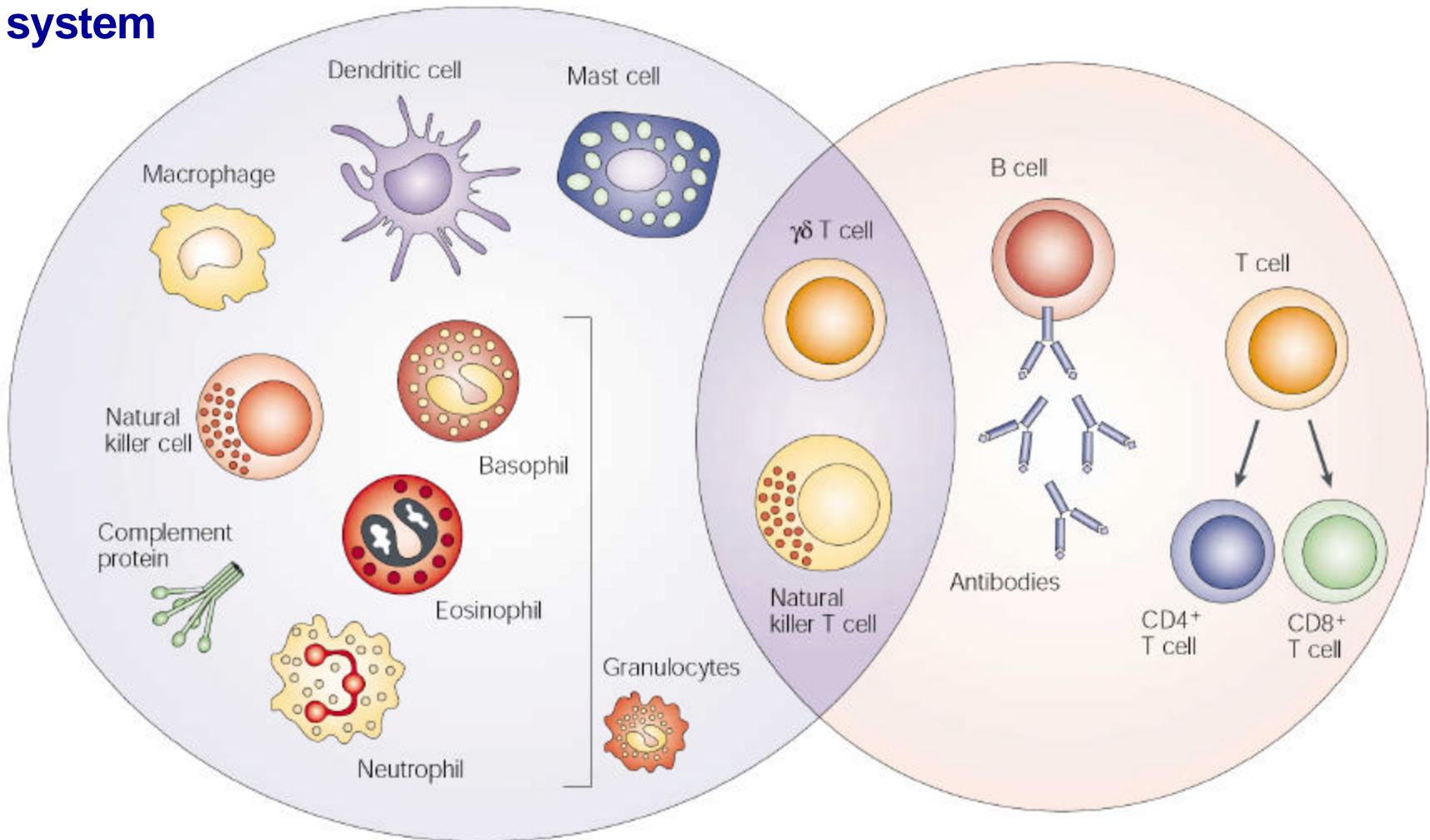
- **B lymphocytes**
- **T lymphocytes**



„Key players“ of the immune system

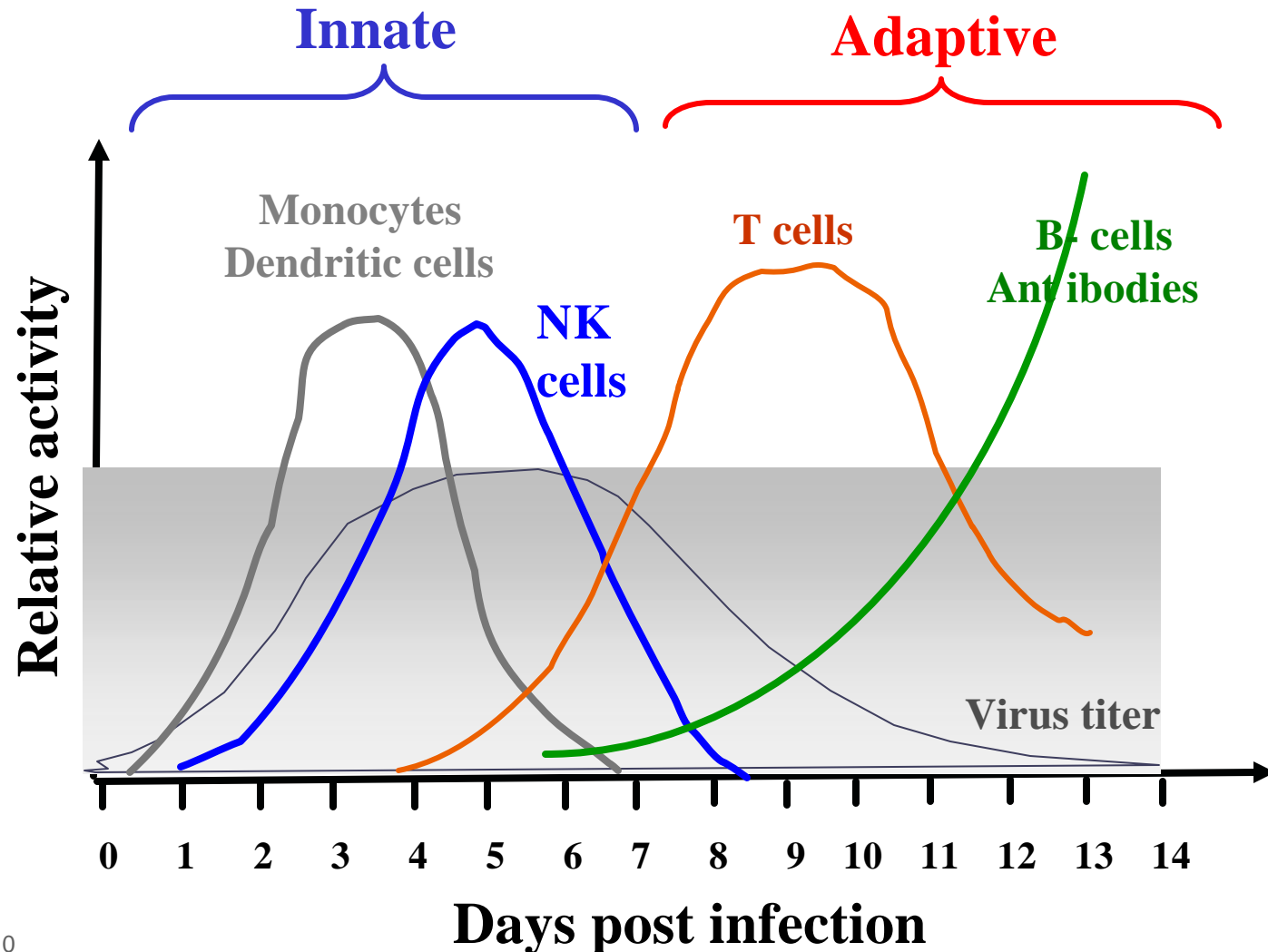
Innate immune system

Adaptive immune system



Dranoff D et al. Nat Rev 2004

The Innate Immune Response Slows Down A Pathogenic Viral Infection But The Adaptive Response Clears It

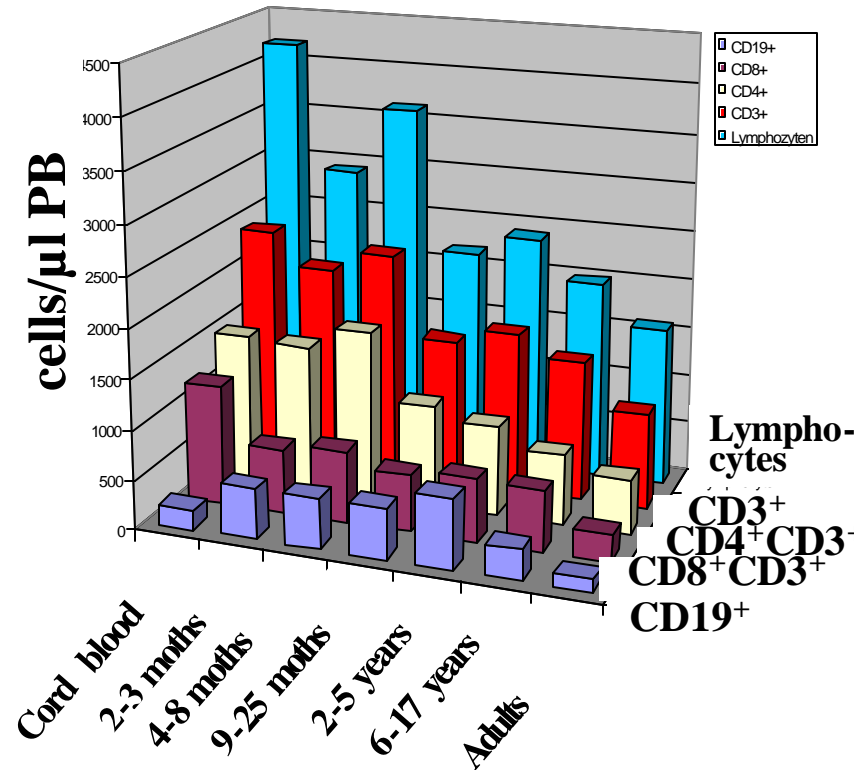


Lymphocytes

Lymphocytes: 2-5/nl 2000 – 5000/ μ l

- Less: Lymphopenia, p. e. HIV
- More: Lymphozytosis, p. e. various viral infections, Leukemia

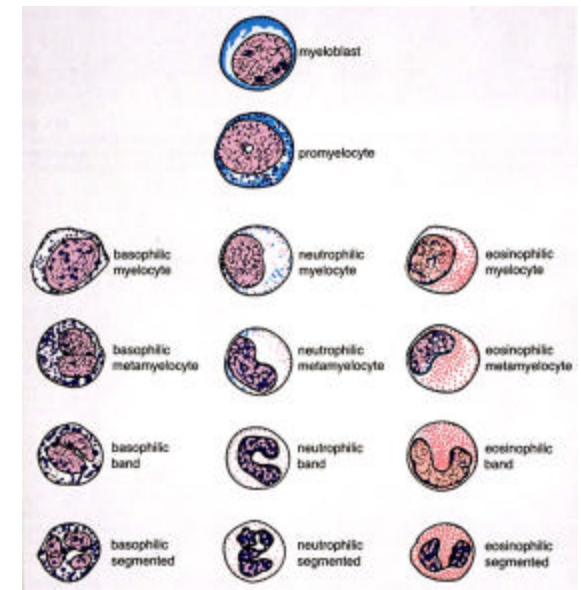
5. percentile of lymphocyte subsets



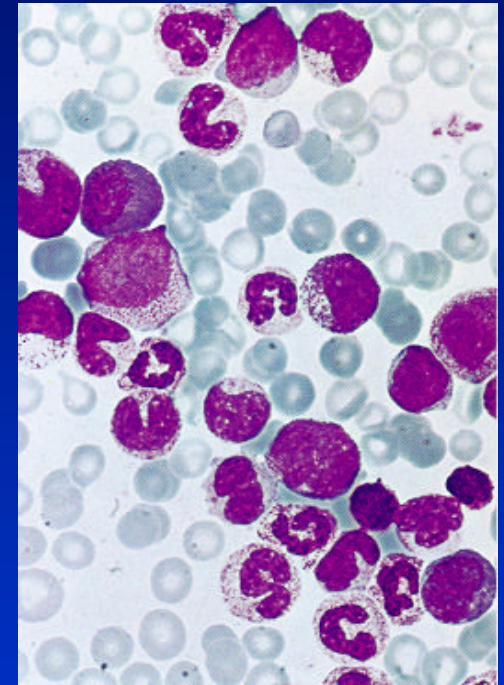
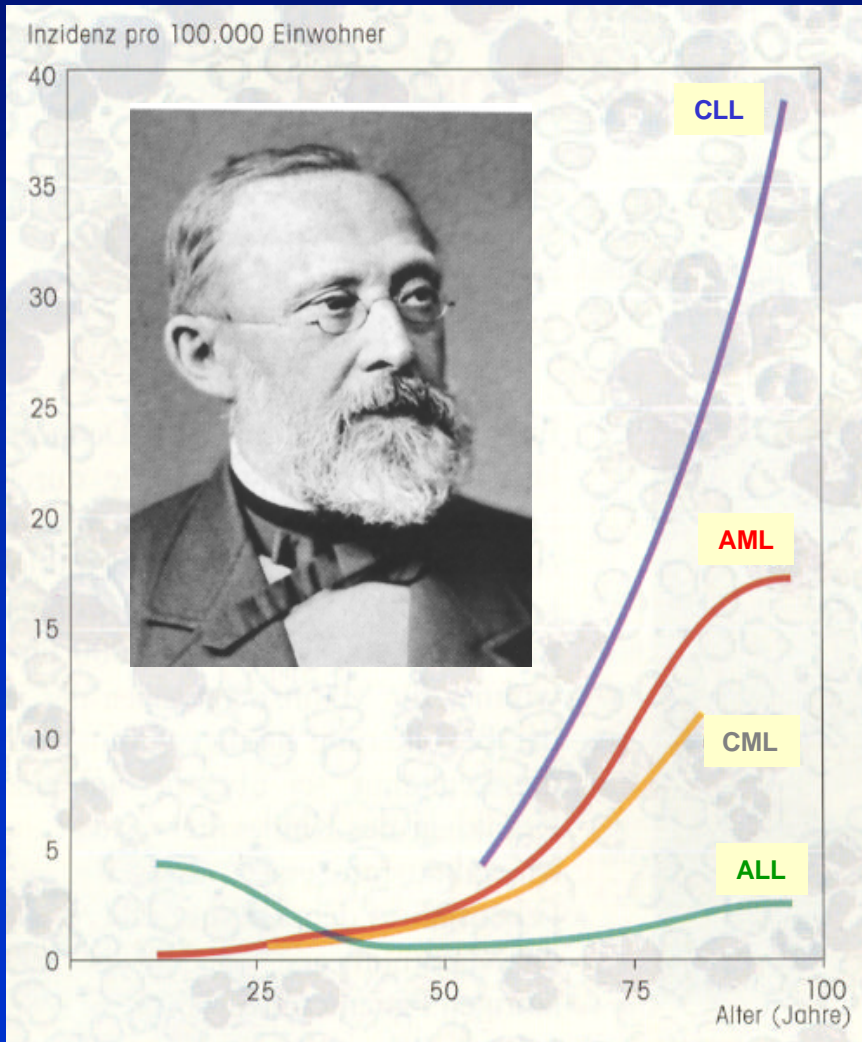
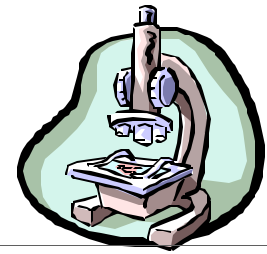
Neutrophil granulocytes

Neutrophils: 2-6/nl 2000 – 6000/ μ l

- Less: Neutropenia, Agranulozytosis, p.e. Bone marrow aplasia, Leukaemia, Chemotherapy, post-infection after viral diseases, Typhus
- More: Granulozytosis, p. e. acute (germs) Infection, Appendicitis, **Leukaemia**



Leukaemia



Leukaemia is a
clonal disorder

Kindly provided by PD Dr. H. Martin

Leukaemia, the current hypothesis

- Defect in maturation of white blood cells
- May involve a block in differentiation and/or a block in apoptosis
- Acquired genetic defect
- Initiating events unclear
- Transformation events involve acquired genetic changes
- Chromosomal translocation implicated in many forms of leukaemia
- Unspecific clinical signals like **pallor, tired, exhausted,**
- Sometimes **typical changes in the blood and bone marrow** (cell dissolution leads to increased LDH)

Leukaemia

➤ **Akute Leukaemia**

- Akute Lymphoblastic Leukaemia (ALL)
- Akute Myeloid Leukaemia (AML)

➤ **Chronic Leukaemia**

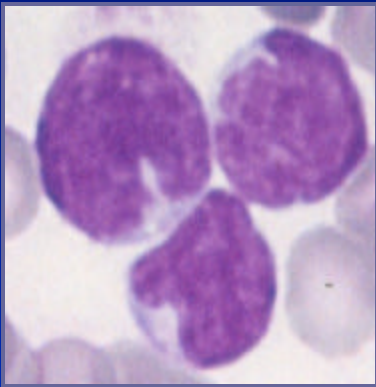
- Chronic Lymphoblastic Leukaemia (CLL)
- Chronic Myeloid Leukaemia (CML)

Symptoms

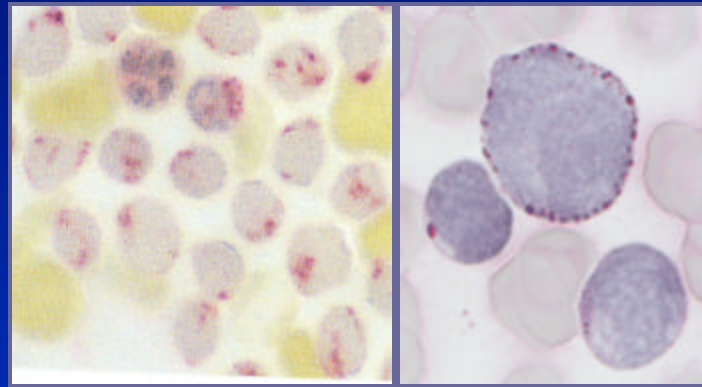
- Paleness
- Tired, exhausted
- Risk for bleeding
- Infectious disease (fever)
often CRP (C reactive proteine) increased
- Hepato-/Splenomegaly
- Lymph nodes (size enhanced)
- Bone pain
- Exanthema
- Changes in the blood and the marrow count

Diagnosis of Leukaemia

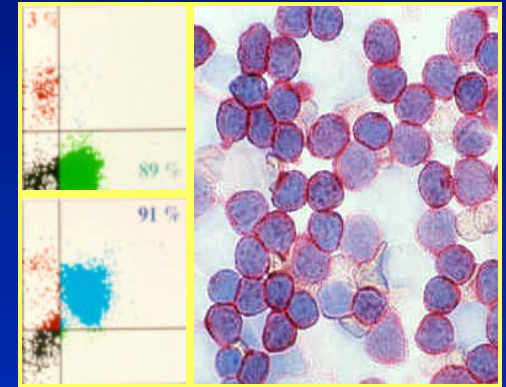
Morphology



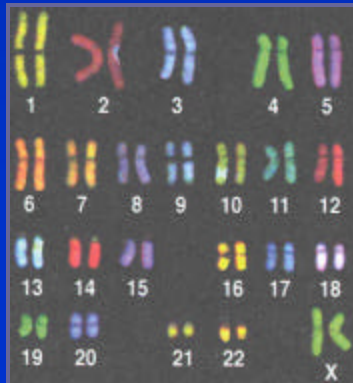
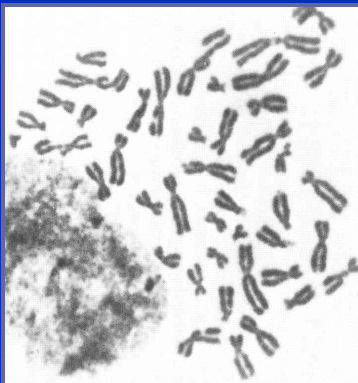
Cytochemistry



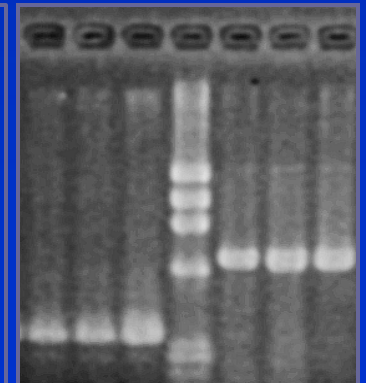
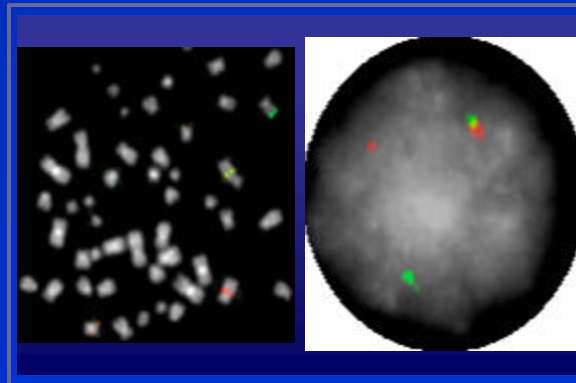
Immunology



Chromosome analysis

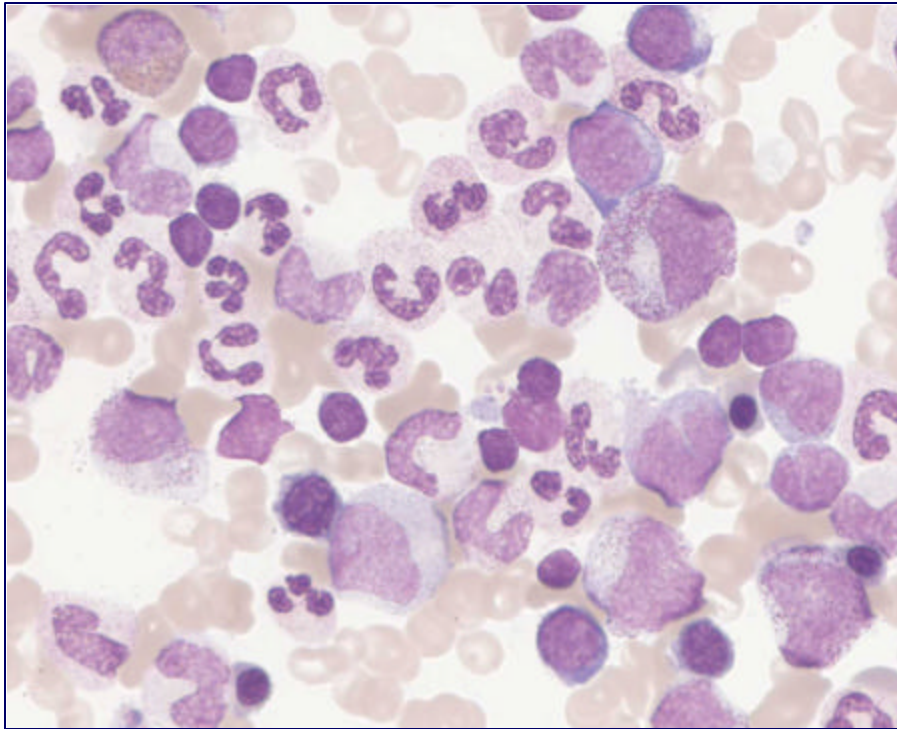


Cyto- / Molecular genetics



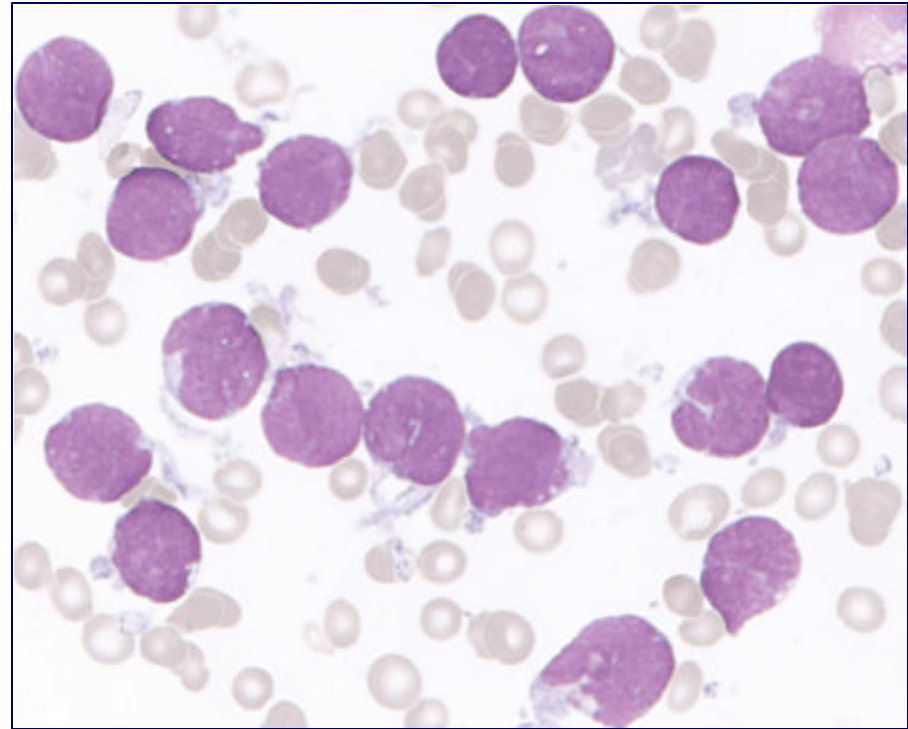
bone marrow: morphology

normal bone marrow



Different cells

Leukaemia

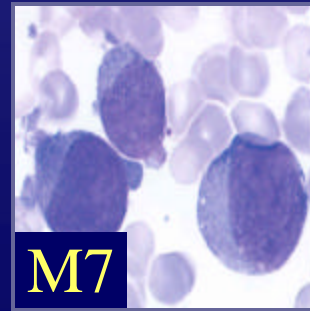
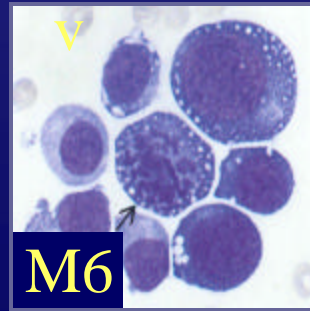
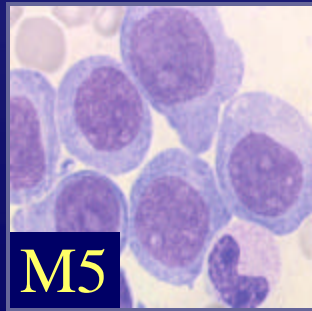
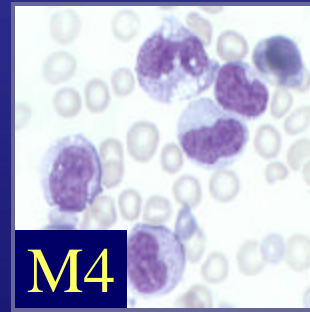
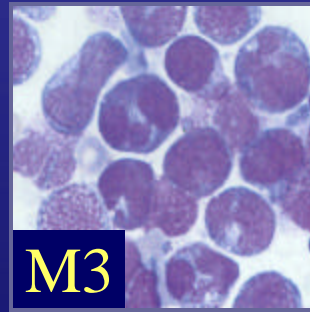
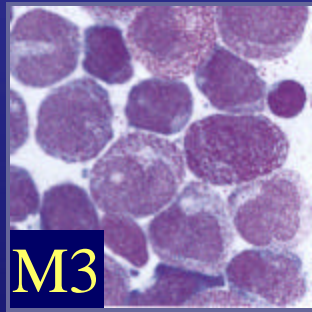
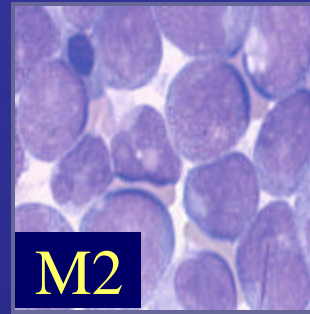
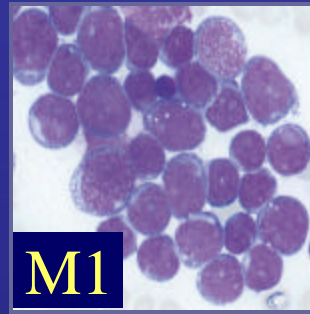
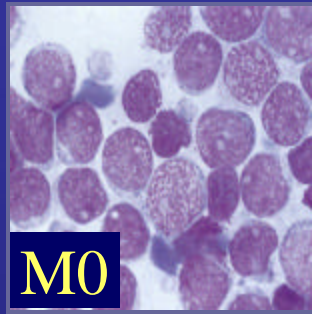


Cells uniform

Acute Lymphatic and Myeloid Leukaemia in Children

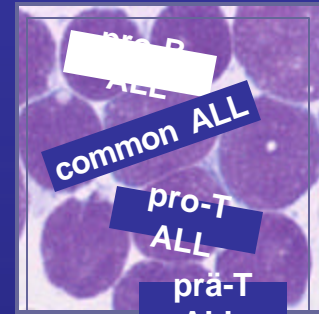
20%

AML

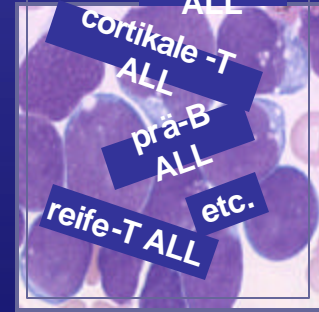


80%

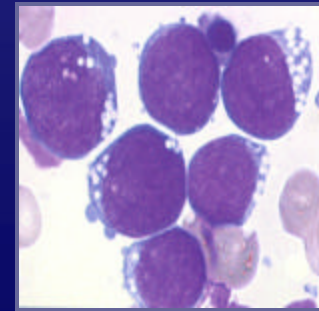
ALL



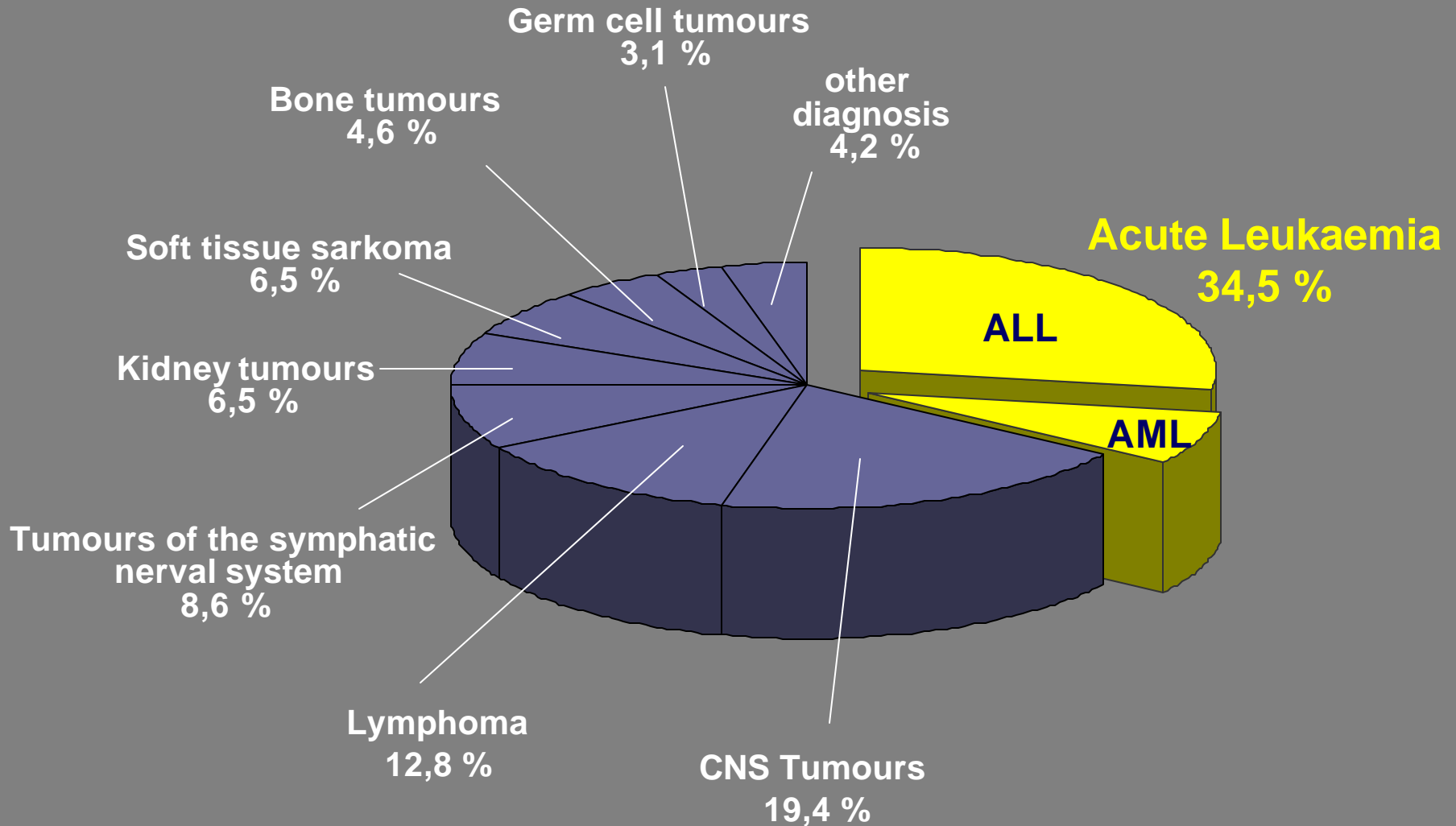
L1



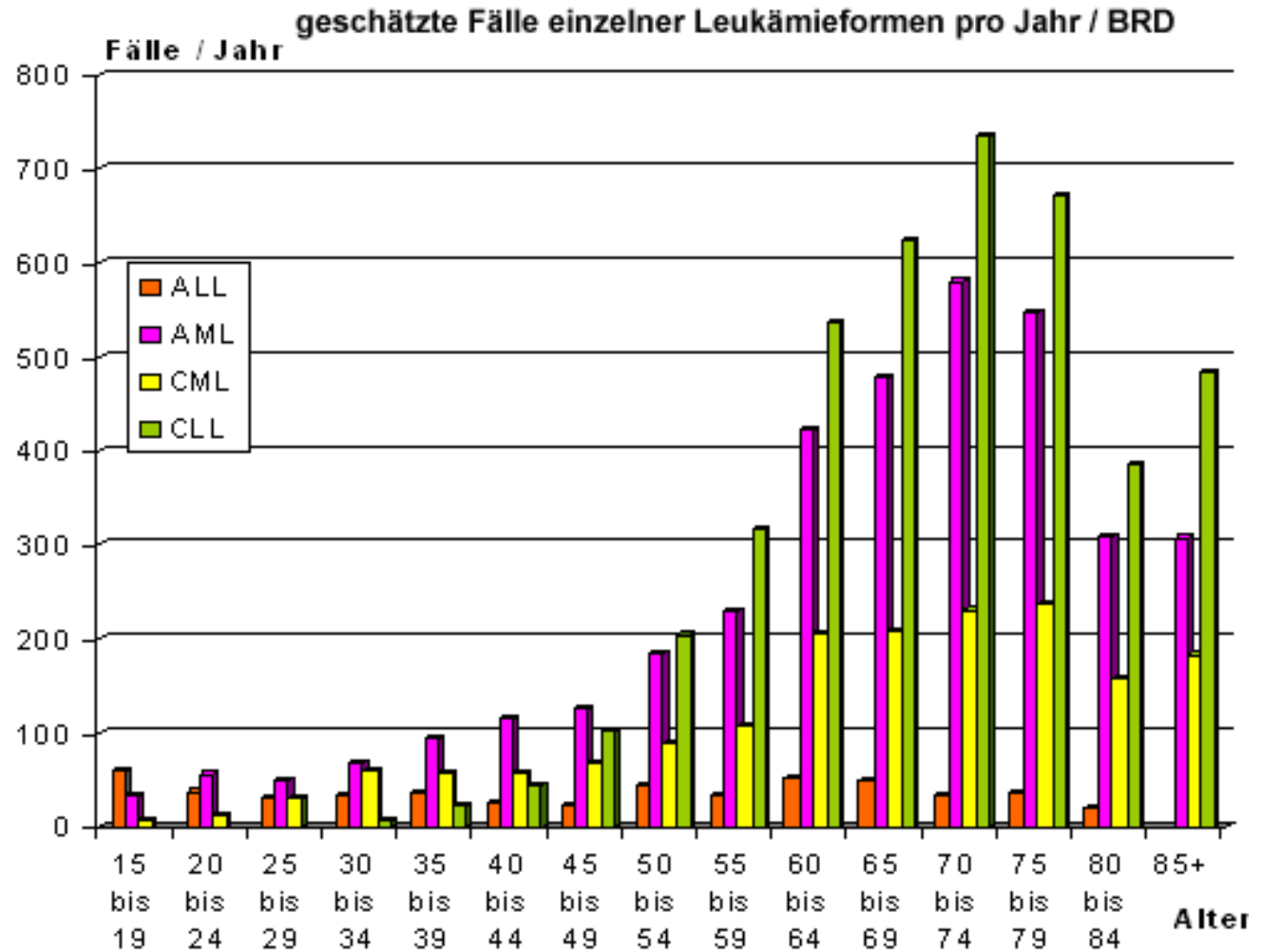
L2



Childhood Malignancies



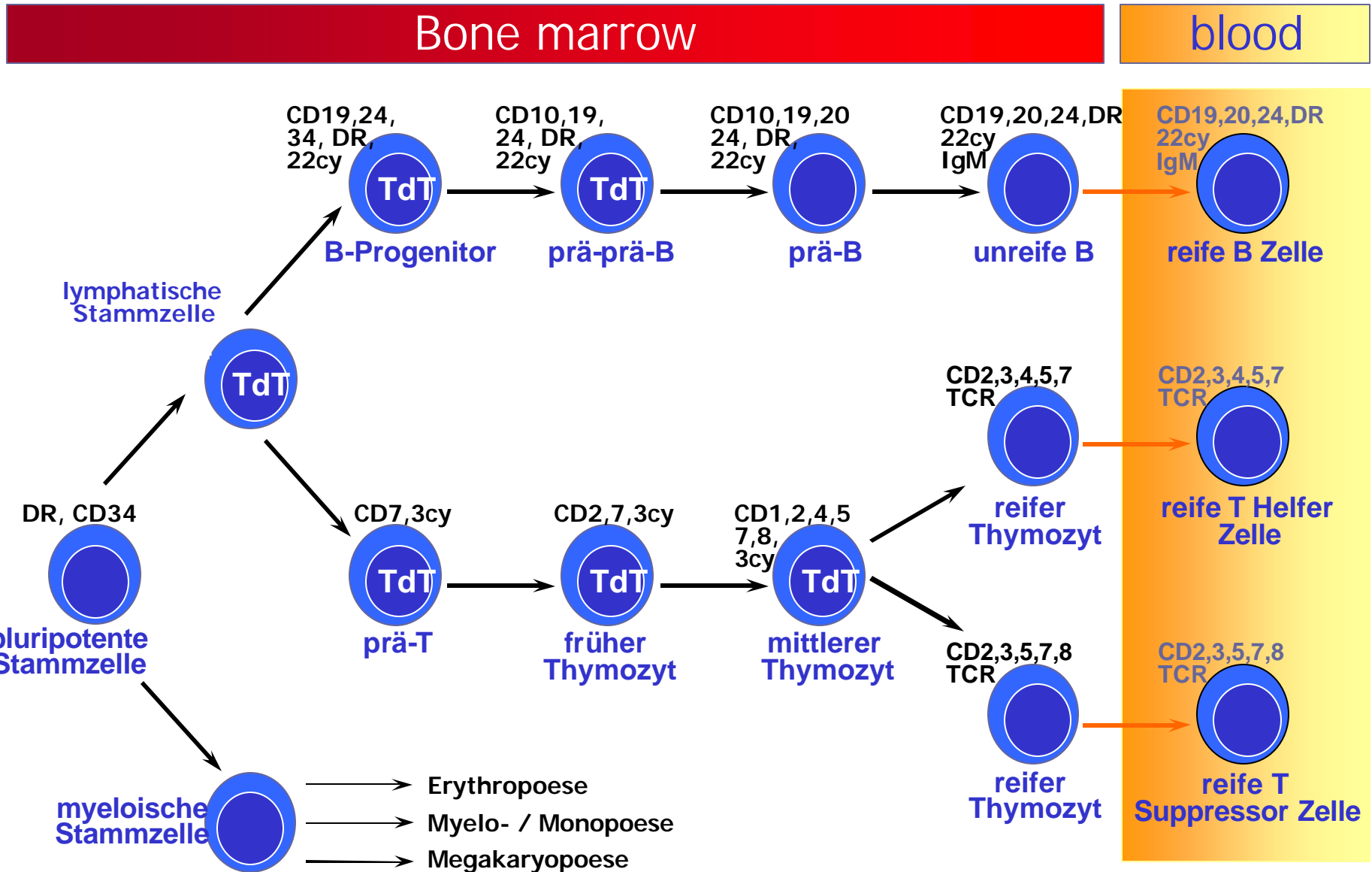
Incidence of Leukaemia in adults



ALL= Akute lymphatische Leukämie
 AML= Akute myeloische Leukämie
 CML= Chronisch myeloische Leukämie
 CLL= Chronisch lymphatische Leukämie

Häufigkeit von Leukämien bei Erwachsenen in Deutschland
 Autoren: A. Hellenbrecht, D. Messerer, N. Gökbüget Stand: 2003
 Aktualisierung der ALL-Inzidenzen 2007 (M. Hartog/TP2)

Possibility for clonal disorder of leukaemia

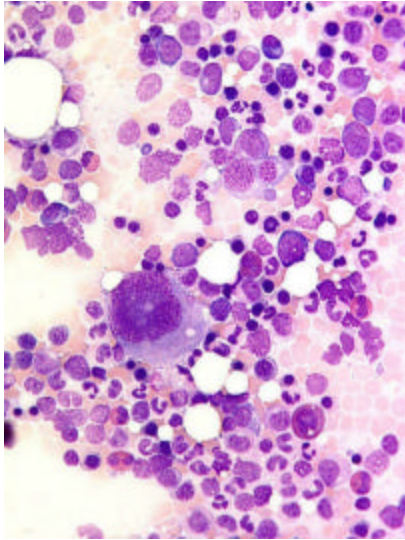


Immunophenotyping in AML

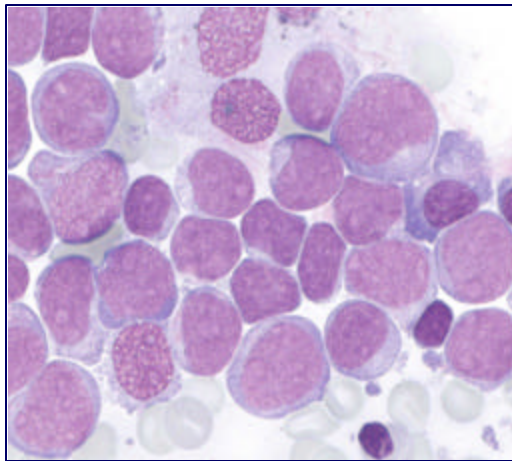
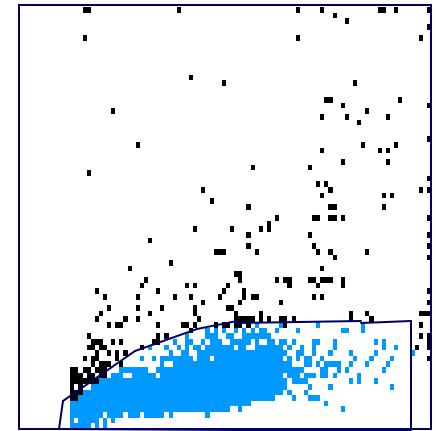
	FAB-Subtypen (AML)									
	M0	M1	M2	M3	M4	M5	M6	M7		
HLA-DR	+	+	+/-	-	+	+	+/-	+/-		
CD34	+	+	+/-	-	+/-	+/-	+/-	+/-		
CD13	+/-	+	+	+/-	+	+/-	+/-	+/-		
CD33	+/-	+	+	+	+	+	+	+/-		
CD65	+/-	+	+	+/-	+	+	+/-	+/-		
CD14	-	-	-	-	+	+/-	-	-		
CD15	-	-	+	-	+	+/-	+/-	-		
CD235	-	-	-	-	-	-	+	-		
CD41	-	-	-	-	-	-	-	+		
CD61	-	-	-	-	-	-	-	+		

Akute Myelogenous Leukaemia

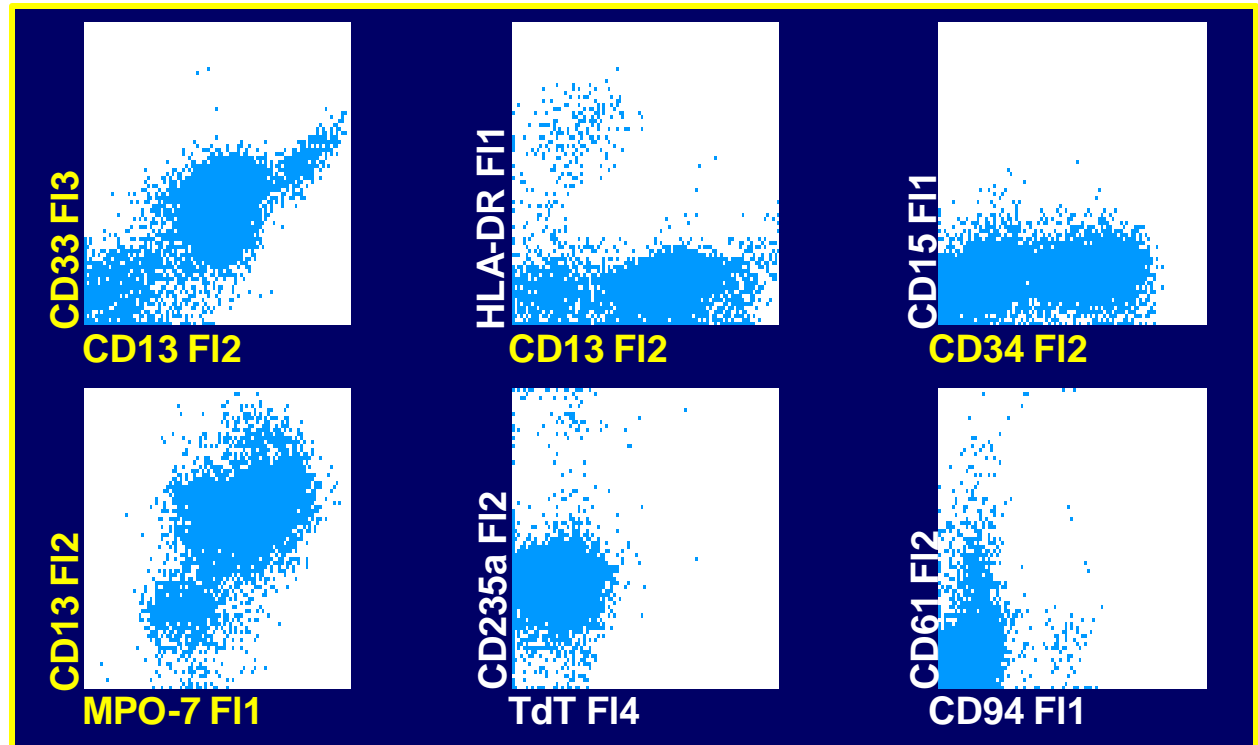
(FAB-AML-M1)



Normal
bone
marrow



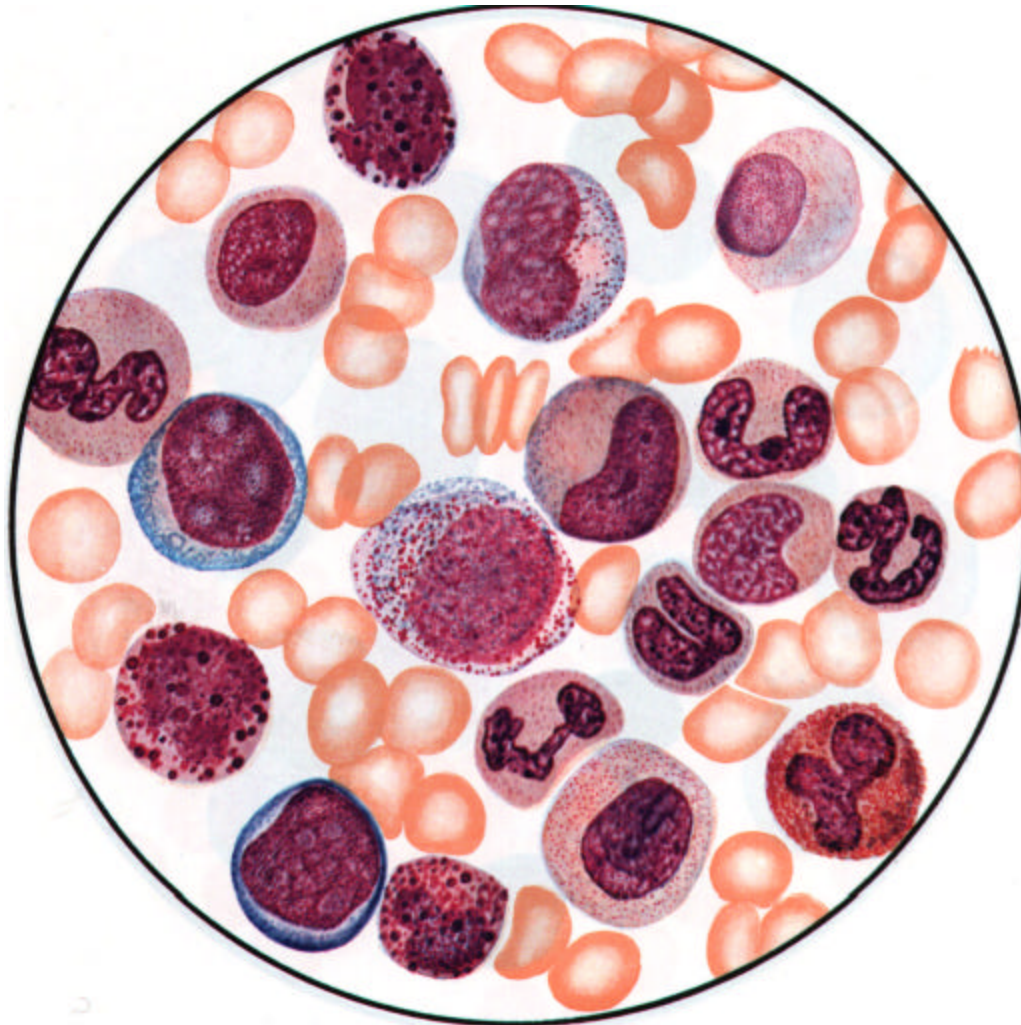
AML



Chronic Myeloid Leukaemia

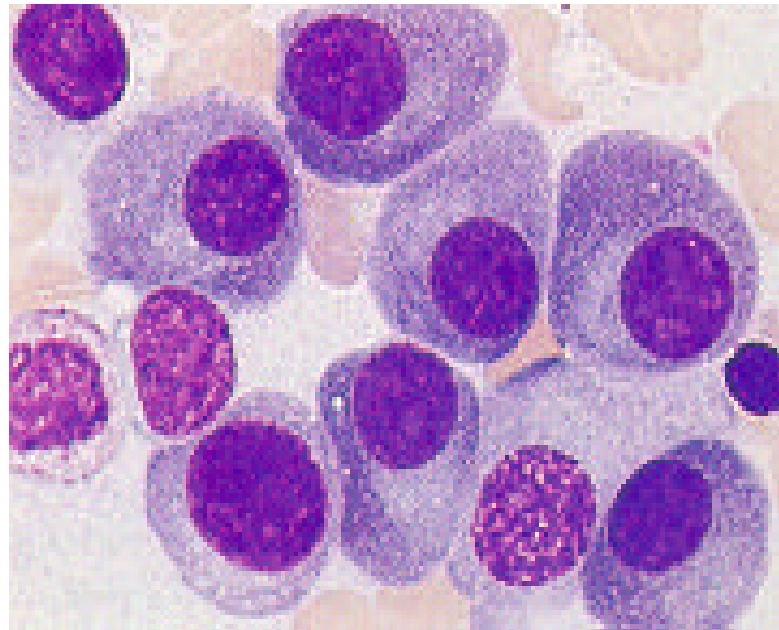
- Malignancy of the haemopoietic system
- Transformation of the pluripotent stem cell
- 9;22 translocation giving rise to the Philadelphia (Ph') chromosome
- Creation of a leukaemia specific mRNA (BCR-ABL)
- Resistance to apoptosis, abnormal signalling and adhesion

Chronic Myeloid Leukaemia



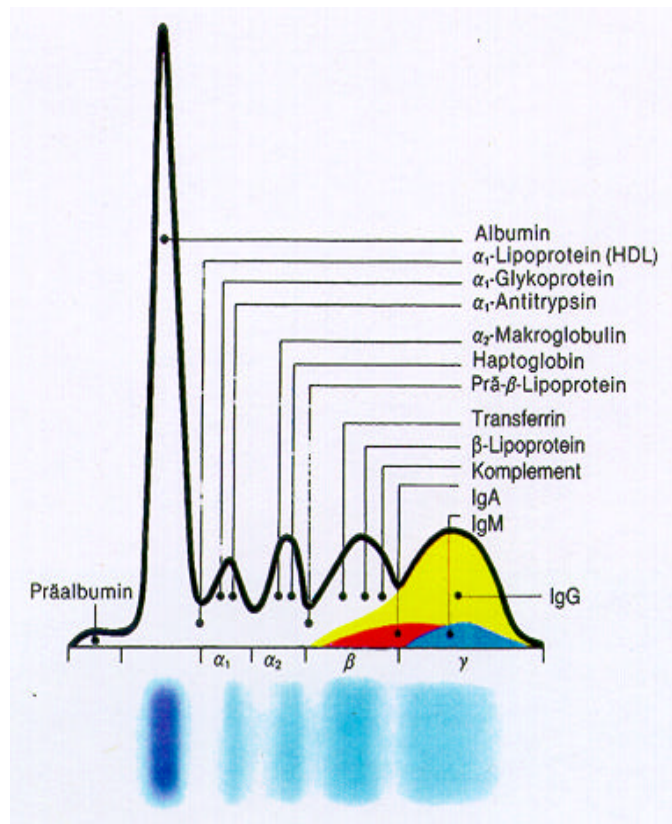
Multiple Myeloma

- **Maligne haematological disease**
- **Monoklonal proliferation of plasma cells**

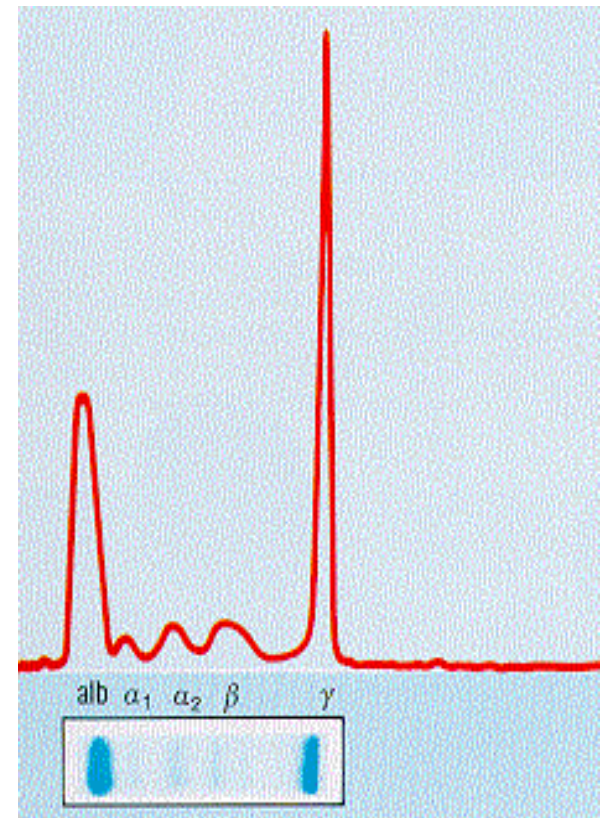


Multiple Myeloma

Serum electrophoresis



normal

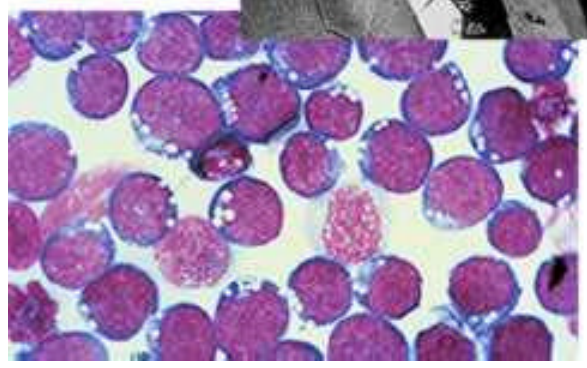


M protein

Kindly provided by PD Dr. H. Martin

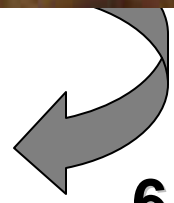
Burkitt's lymphoma: A highly malignant B cell tumour

Dennis Burkitt



BL cells in suspension

Can double in size by 24 hours



6 days treatment

Case

- 5 year old boy
- Good general condition
- Normal skin turgor
- Light red spotted exanthema
- T 38 ° C
- 14 days ago => no dejection
=> otitis
- Since 10 days increasing pains
right underbelly



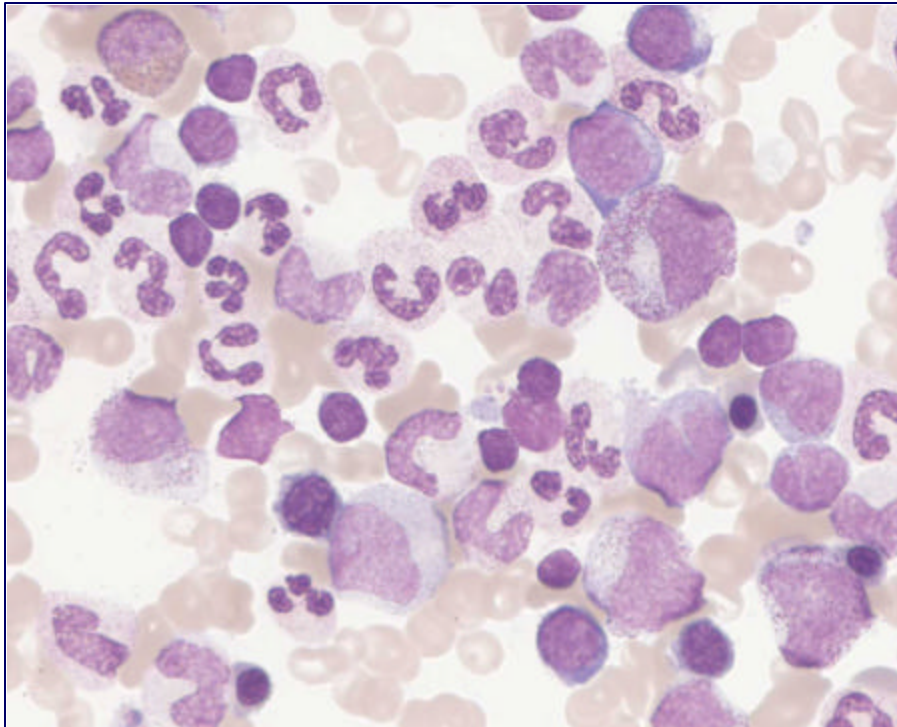
Case blood count

➤ Erythrocytes	3 310 000	/μl
➤ Hemoglobin	9.6	g/dl
➤ Leukocytes	50 200	/μl
➤ Granulocytes	1 300	/μl
➤ Platelets	15 200	/μl

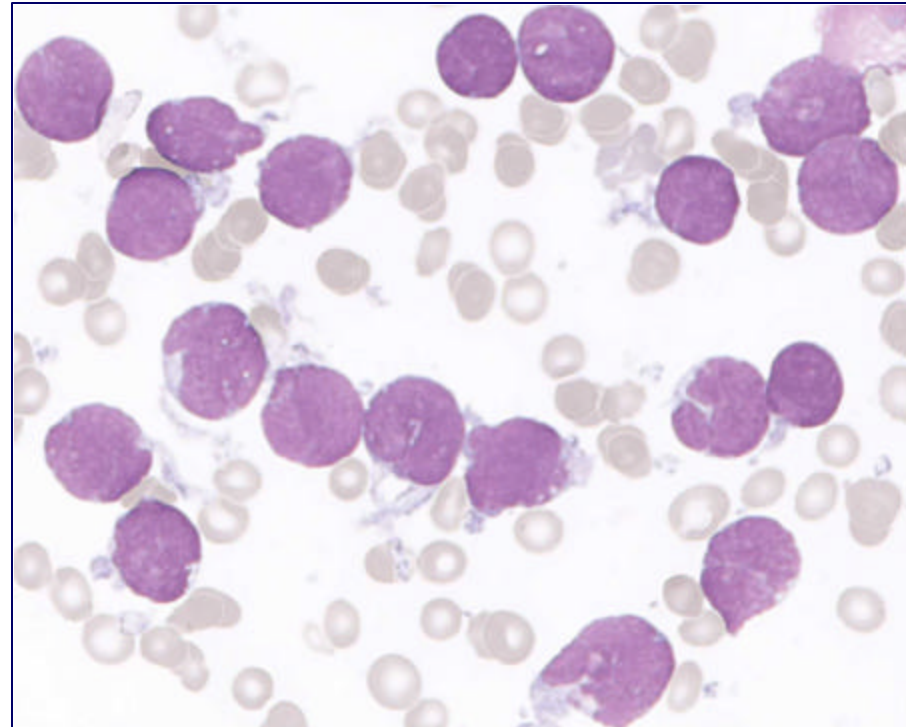
➤ CRP	6.12	mg/dl
➤ LDH	800	U/L
➤ uric acid	6.0	mg/dl

Case bone marrow morphology

normal bone marrow



patient



what is the difference ... ?

Case Immunophenotyping

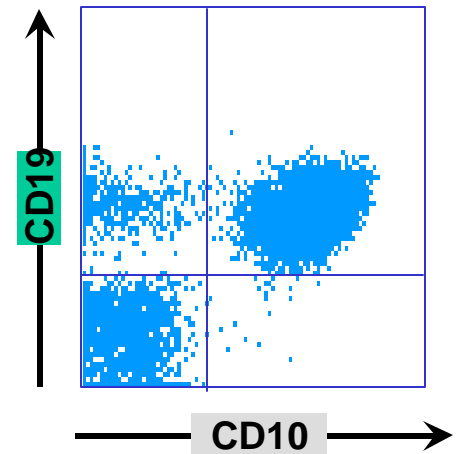
Antigen expression on the leukaemic cells:

Positive:

- CD19+ 89%
- CD19+CD10+: 88%
- CD10+CD79a: 85%
- CD10+cytCD22: 86%
- HLA-DR: 80%

Negative:

- CD1a, CD2, CD3, CD4, CD7
- CD34, CD117
- CD13, CD33, CD14, CD15, CD41, CD61



Diagnosis

1.) ALL (T cell)

2.) ALL (B cell)

3.) AML

4.) CML

5.) Severe infectious disease

Treatment of childhood ALL

AIEOP-Berlin Frankfurt Münster ALL 2009

➤ Drugs - Protocol IA

- Prednison
- Vincristine
- Daunorubicin
- PEG-L-Asparaginase
- Methotrexat i.th.

➤ Drugs - Protocol IB

- PEG-L-Asparaginase
- Cyclophosphamide
- Cytarabine (ARA-C)
- 6-Mercaptopurin (6-MP)
- Methotrexat i.th.

➤ Drugs - Protocol M

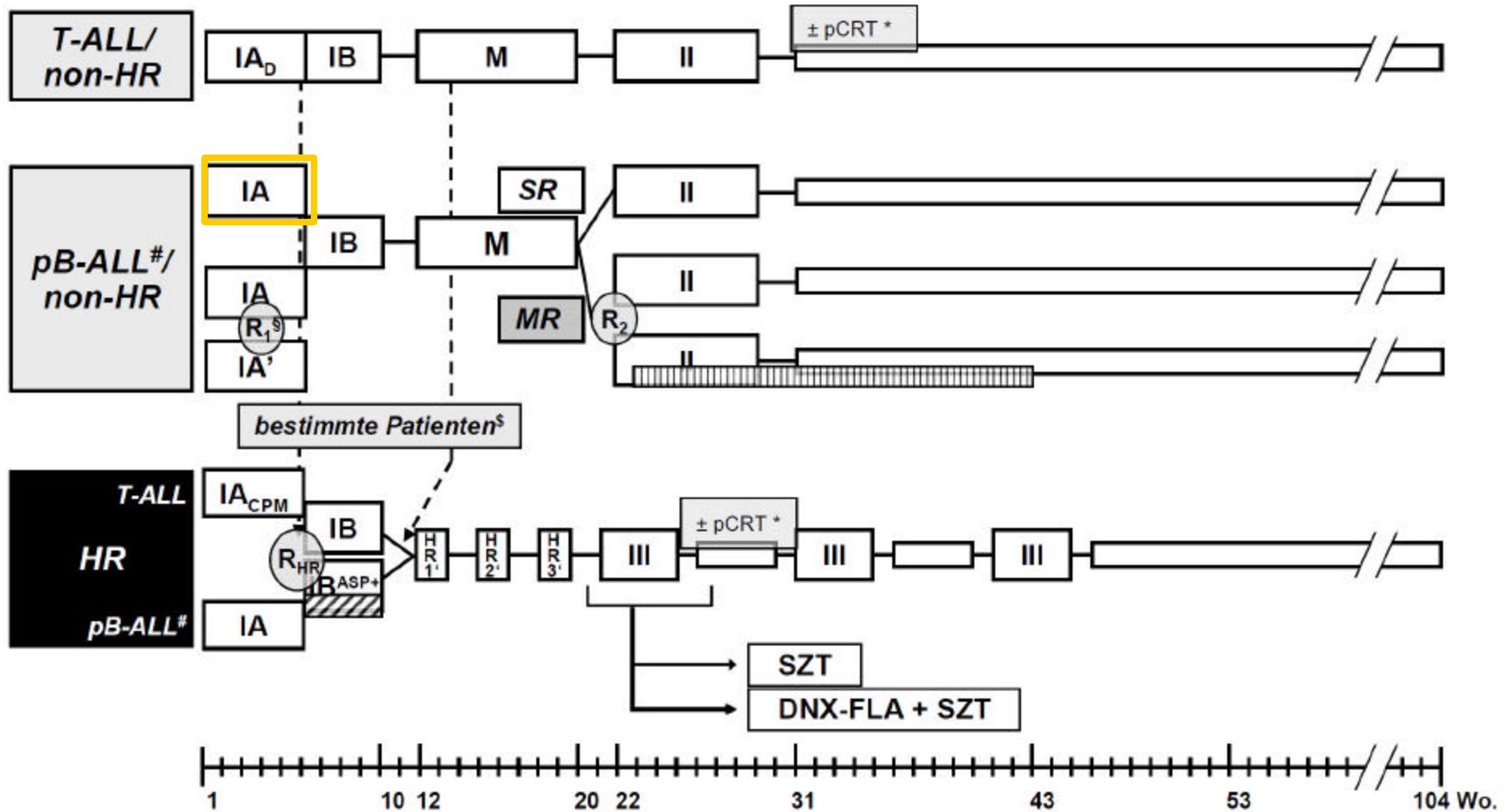
- 6-Mercaptopurin (6-MP)
- HD-MTX
- MTX i.th.

➤ Drugs - Protocol II

- Dexamethasone
- Vincristine
- Doxorubicin
- PEG-L-Asparaginase
- Cyclophosphamide
- Cytarabine (ARA-C)
- Thioguanine
- MTX i.th.

Treatment of childhood ALL

AIEOP-BFM ALL 2009

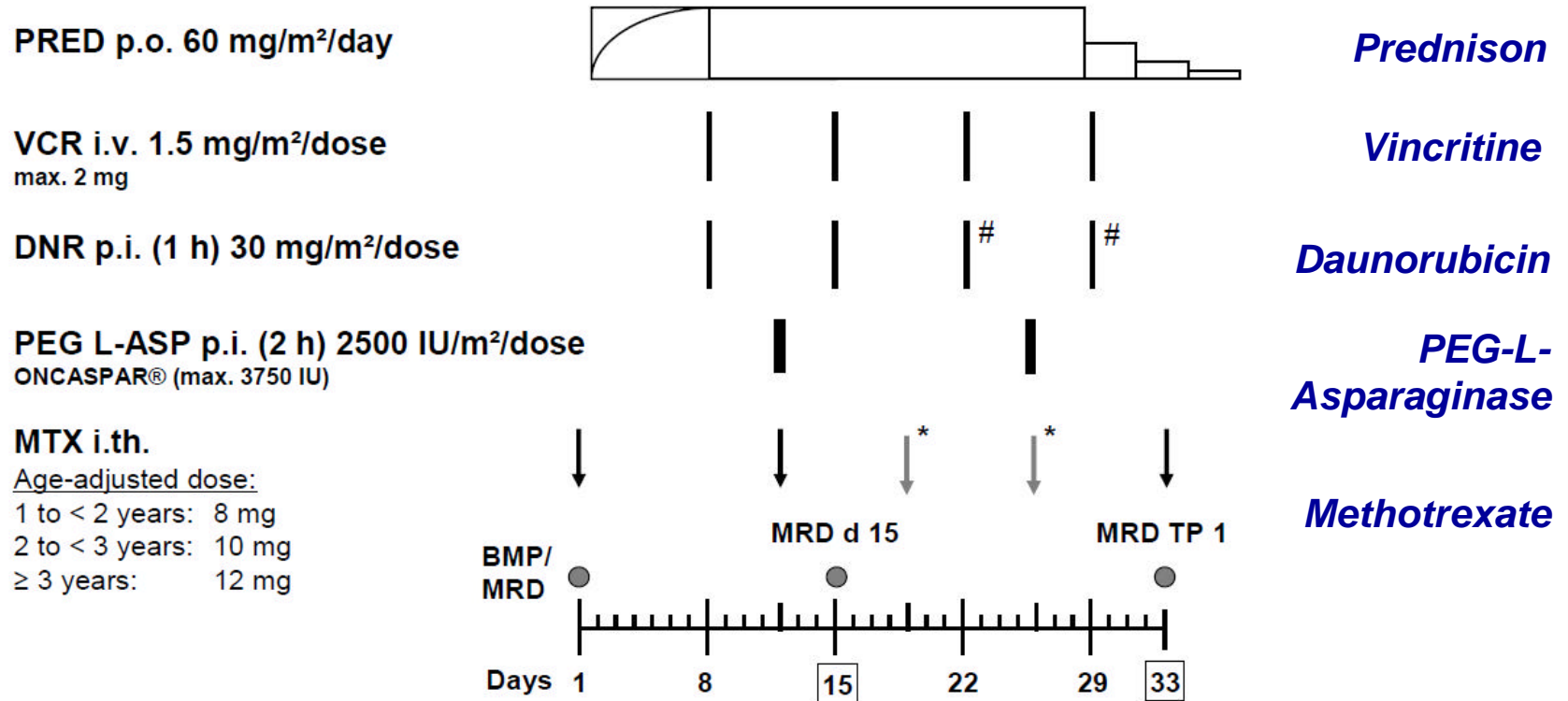


Treatment of childhood ALL

AIEOP-BFM ALL 2009

Protocol IA/IA' for pB-ALL

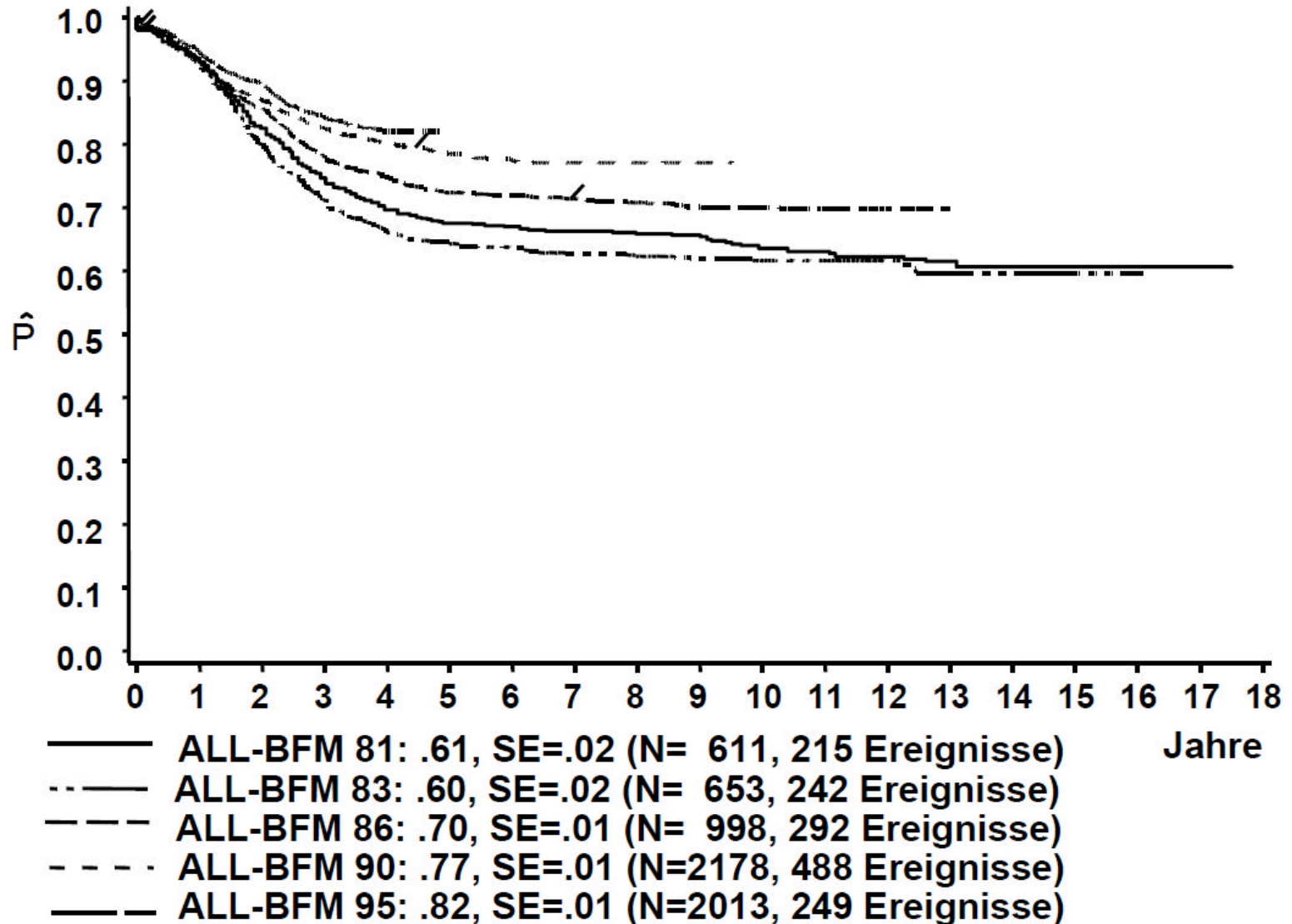
Protocol IA with prednisone and 4 (IA) or 2 (IA') doses daunorubicin



omitted in patients randomized (R₁) to the experimental arm

*additional i.th. MTX on days 19 and 26 if CNS-positive (CNS 3) or presence of blasts in initial cytospin (CNS 2)

Survival : ALL



Case 2 : Blood cell count

Hb: 13 g/dl

MCV: 90 fl

Thombo: 250 000/ μ l

Leuko: 8 000/ μ l

Granulo: 3 000/ μ l

Lympho: 4 000/ μ l

?

Normal

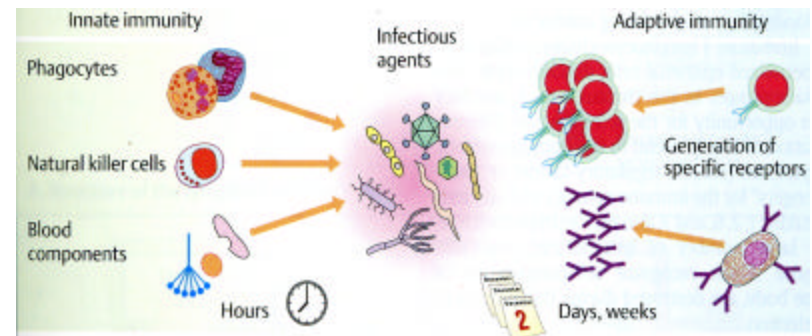
Cellular components of the blood

Cells	Concentration	Duration of living	„juvenile form“
Erythrocytes	4 800 000/ μ l	100-120 days	Retikulozytes 1(-3) days
Platelets	200 000/ μ l	7-12 days	
Granulocytes	4 500/ μ l	2-3 days	„Stabkerniger“
Lymphozytes	3000/ μ l	up to many years	

Take home message

- The production of cellular blood cells is called haematopoiesis (haemopoiesis)
- Differentiation of granulocytes in the bone marrow, of T- and B-cells in the lymphoid organs

- Innate immune system and adaptive immune system



- Living duration of blood cells: 100-120 days (Ery), 7-12 days (Thrombo), 2-3 days (Granulo) and years (Lymph)
- Haematopietic diseases can be related to the various cells types and lineage commitment (i.e. ALL, AML..)