Pediatric Hematology

Anemia in children and adolescents

Age (y)		*Hb (g/dl)*
0.5 – 2		< 10.5
2 – 14		< 11.5
15 – 18	girls	< 12.0
	boys	< 13.0

Classification of anemias according to the size of red cells

- **Microcytic anemia**
- **Macrocytic anemia**
- **Normocytic anemia**

Red Cells Indexes

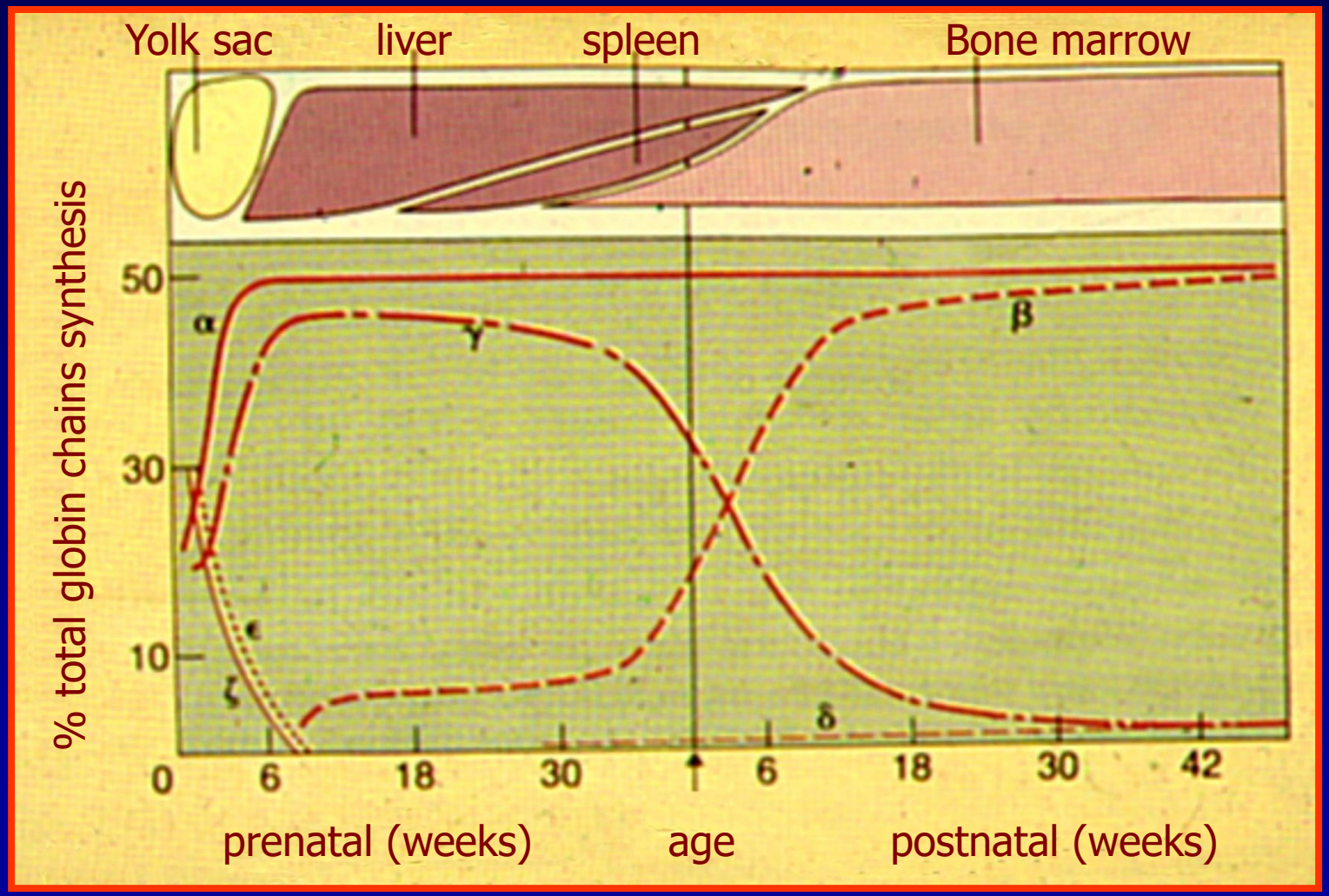
- **MCV (mean corpuscular volume):**
- Lower normal limit in children < 10 y: 70 fl + age in years
- Upper normal limit since 6th mo: 84 + 0.6fl/y till achievement a limit for adults 95 fl
- **MCH (mean corpuscular Hb):** usually follows changes in MCV
- **MCHC (mean corpuscular Hb concentration):**
- value > 35g/dl is typical for HS, decreased values for iron deficiency, thalassemia
- **RDW (red cell distribution width):** range of variation in red cell size (anisocytosis)

Development of hemoglobin and erythrocyte volume values since birth into adulthood.

(adapted from: Dallman PR, J Pediatr 1979, 94: 26; Stockman III JA, Pochedly C, Developmental and Neonatal Hematology, Raven Press New York 1988)

	Hemoglobin (g/dl)		MCV (fl)	
Age	Mean	Lower limit	Mean	Lower limit
1. day	19.0	17.0	119	101
1 m	14.0	11.0	105	90
2 m	11.0	9.3	100	83
3 m	11.0	9.5	88	78
6m-2y	12.5	11.0	77	70
2-5y	12.5	11.0	79	73
5-9	13.0	11.5	81	75
9-12	13.5	12.0	83	76
12-14 years				
females	13.5	12.0	85	77
males	14.0	12.5	84	76
14-18 years				
females	14.0	12.0	87	78
males	15.0	13.0	86	77
18-49 years				
females	14.0	12.0	90	80
males	16.0	14.0	90	80

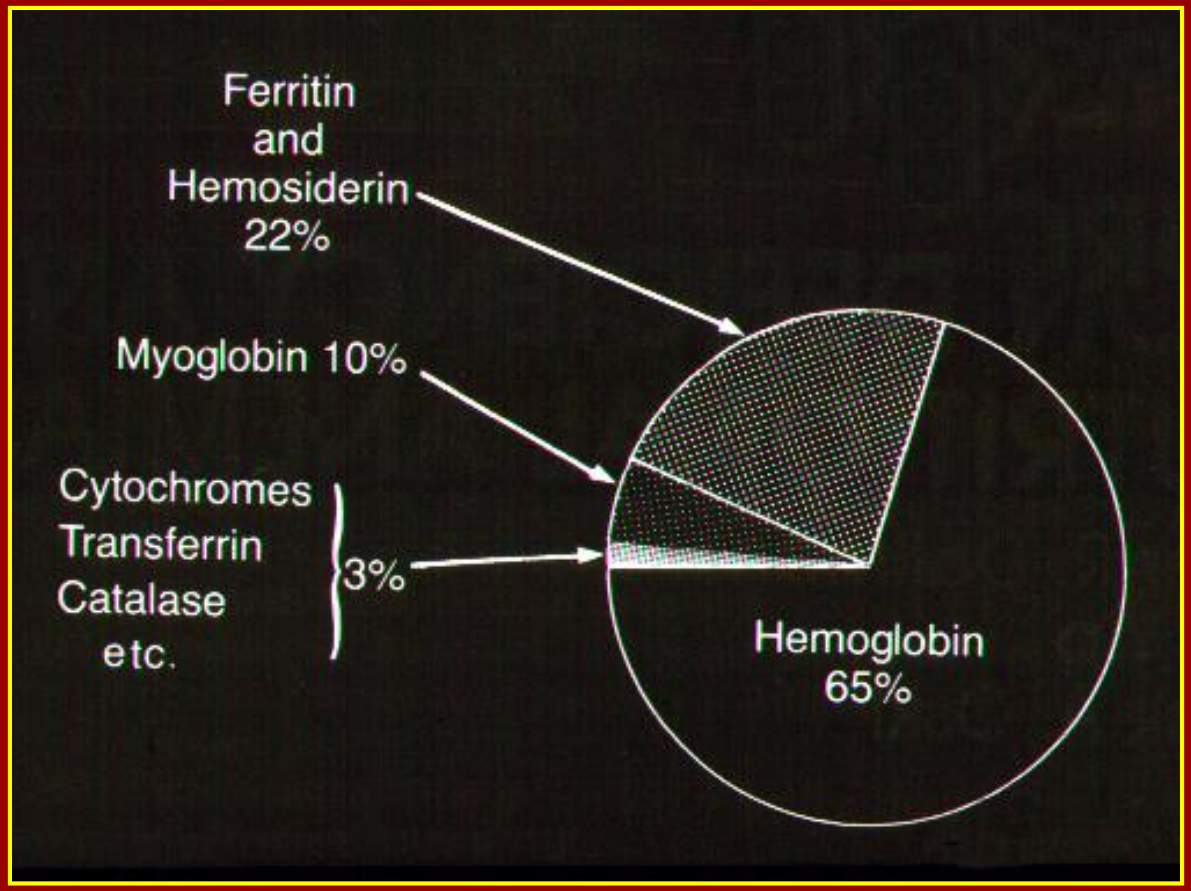
Hemoglobin development – fetal and neonatal period



Microcytic anemias

- **Iron deficiency**
- **Chronic inflammation/malignancy**
- **Thalassemias**
- **Sideroblastic anemia**
- **Congenital hemolytic anemias with unstable hemoglobin**
- **Chronic lead poisoning**

DISTRIBUTION OF IRON IN MAN (adult male: ≈3,5 gr)



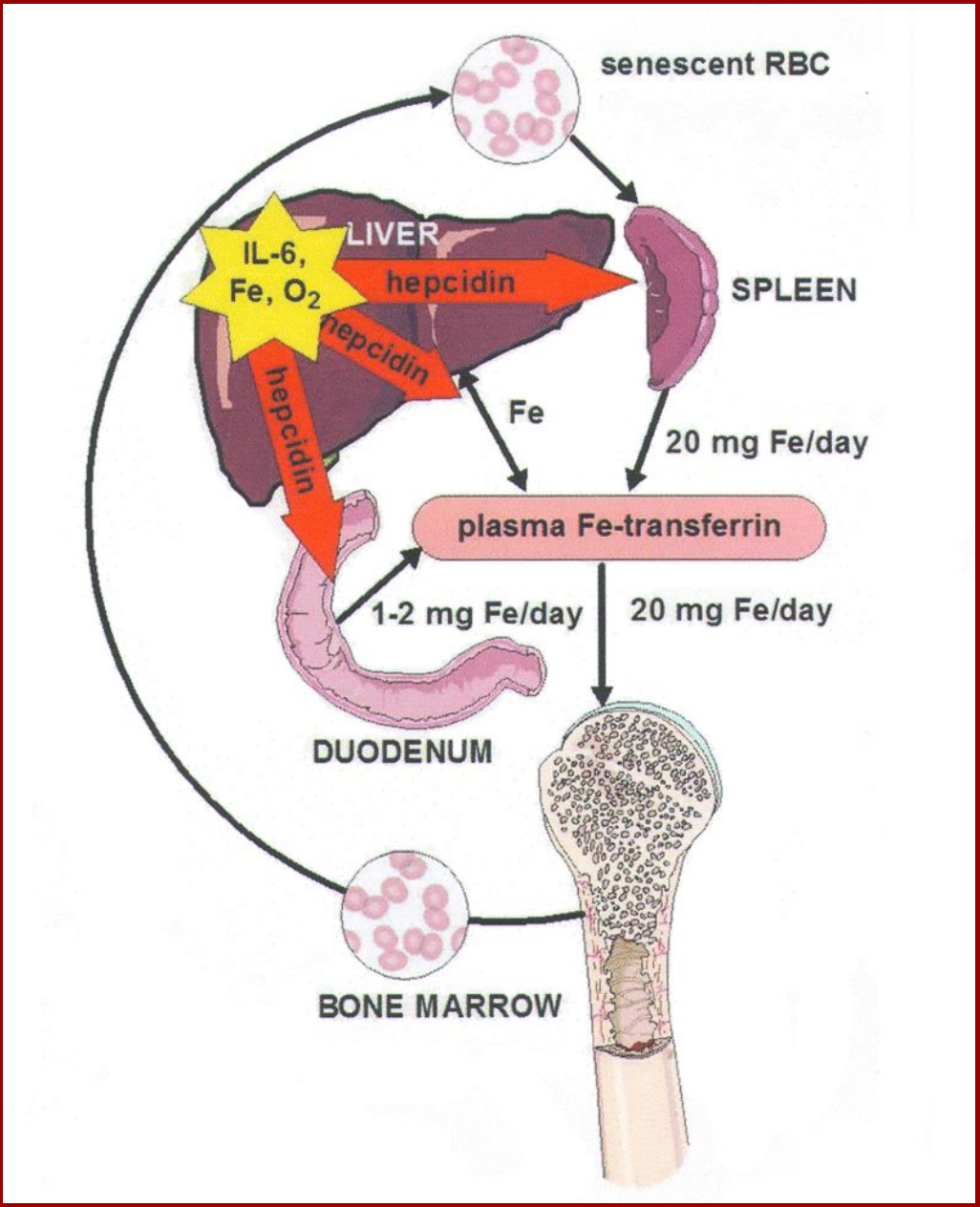
Iron absorption

- Duodenum – acid production in stomach decreases pH in duodenum, Fe^{3+} (insoluble)→Fe^{2+} (valid for inorganic iron)
- Only 10% inorganic Fe absorbed
- Hem (meat) is absorbed independently by pH, easily, mechanism poorly understood
- Iron deficiency increases absorption, overload decreases
- The exception is ineffective erythropoiesis (thalassemia, PK deficiency, CDA, MDS) and hereditary hemochromatosis

Hepcidin

- Peptid, synthesis is induced by iron and inflammation, suppressed by tissue hypoxia, anemia
- Regulates iron metabolism
- Deficiency causes hereditary hemochromatosis
- Binds to ferroportin (export canal Fe) and blocks iron intake from macrophages, liver, duodenal enterocytes, placent. syncyciotrofoblast into blood
- Consequence is iron deficiency, failure of hemoglobin production and microcytic anemia
- Later on a real iron deficit may develop

Hepcidin and iron metabolism



Intercellular Iron Transport

- 60-80% body iron is in erythropoiesis
- 4 mg Fe circulates in plasma (ery-RES), bound in Trf
- Trf is synthetised in liver, increases in iron deficiency (TBC), decreases in inflammation and in iron overload
- Normally cca 30% binding capacity of Trf is saturated by iron, one molecule Trf binds 2 atoms Fe
- Transport into erytropoiesis, RES, liver

Iron deficiency anemia causes

- Increased requirements for iron intake in the periods of fast growth
- Inadequate iron absorption (food deficient in iron, antacids, H2 blockers, tea, coffee, malabsorption, gut resection, inflammation, Helicobacter pylori)
- Inadequate/non-available iron stores – GIT bleeding (parasits, Rendu-Osler), meno/metrorrhagia, hematuria, pulmonary hemosiderosis
- Atransferrinemia (Fe bound to albumin, anemia, liver iron overload)
- Abnormal intracellular iron transport/utilization

Iron deficiency anemia
Laboratory diagnostics

- **Screening:**
- ✓ **Hb**
- ✓ **Transferrin saturation**
- ✓ **MCV**
- ✓ **MCH, MCHC**
- ✓ **RDW**
- **Definitive diagnosis:**
- ✓ **ferritin in serum**
- ✓ **hemosiderin in bone marrow**
- ✓ **sTfR (sTfR/ferritin)**

Iron deficiency anemia
Developmental stages

- **Prelatent iron deficiency:** iron stores deficit (decreased ferritin)
- **Latent iron deficiency:** iron deficit in stores and erythrocytes (decreased iron in serum, ferritin, increased TBC, sTfR)
- **Iron deficiency anemia**: iron deficit in stores and erythrocytes**,** anemia (decreased Hb, Ht, MCV, MCH, MCHC, ferritin, increased sTfR, TBC)
- **Dx criteria: Low Hb, serum ferritin < 30 µg/l, norm. CRP, increased sTfR**

Iron deficiency anemia

- Two peaks in the incidence of anemia in childhood: toddlers 1-3 y and adolescents (highest iron consuption)
- In first 4 m of life in full-term infants sufficient storage iron (from mother, decrease of Hb from 180 to 140g/l)
- Amount of Hb doubles in full-term infant at the age 1 year (180 → 340mg), in a child weighing 1 kg increases 6x (2 kg 3x)
- Adolescent boys increase muscle mass (myoglobin), girls increase iron consumption after menarche, (diets, sport)

Iron deficiency anemia
Prophylaxis

- Absorption of iron from cow´s milk (10%) lower in comparison with mother´s milk (25-50%)
- Amount of iron in mother´s milk decreases to 0.3 mg/ml after 5 mo of breastfeading
- Since 6th mo there is necessary to introduce fortified cereals, vegetable, meat
- In pre-term babies supplementation of iron as drops from 2nd mo (2-3 mg element. Fe/kg/day)

Iron deficiency anemia Treatment

- 3-5 mg element. Fe/kg/day, infants drops 3x daily, toddlers sirup 1x daily, between meals, evening (better tolerance)
- Parenteral iron: 1.5 mg/kg/dose, 3x weekly
- GIT difficulties in 10-40% pts – induced by release ionic iron, irritating mucous membrane (retarded tbl lower local iron concentration)
- Reticulocyte incease after 1 week, Hb increases in about 20g/l after 1 mo, stores replenished after 3-5 mo

Macrocytic anemia

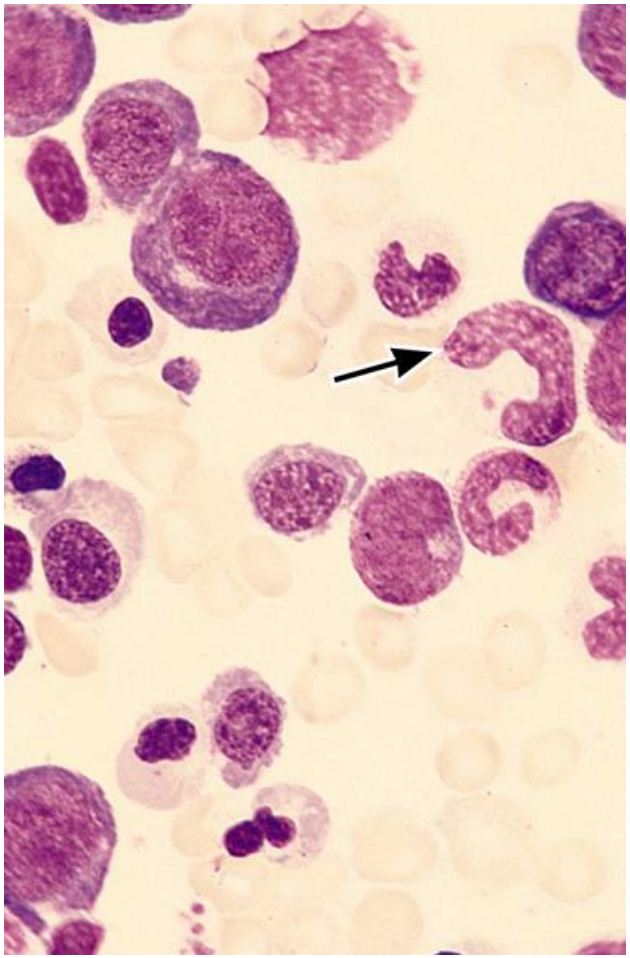
1. With megaloblastic bone marrow:

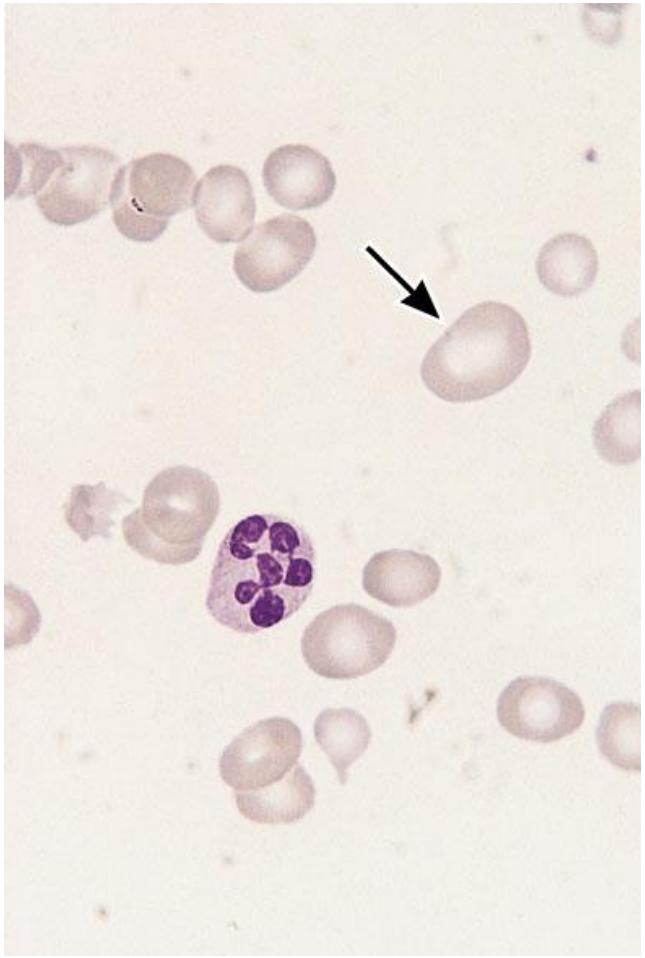
- **Deficiency vitamin B12**
- **Deficiency folic acid**
- **Hereditary orotic aciduria**

2. Without megaloblastic bone marrow:

- **Aplastic anemia**
- **Diamond-Blackfan anemia**
- **Hypothyreoidism**
- **Liver disorders**
- **BM infiltration**
- **Dyserythropoetic anemias**

Megaloblastic anemia
BM + PB





Causes of megaloblastic anemia (makrocytic anemia with megaloblastic erythropoiesis).

Vitamin B12 deficiency:
1. Inadequate nutrition
2. Inadequate gastric intrinsic factor
Pernicióus anemia
Chronic atrophic gastritis
Congenital intrinsic factor deficiency
3. Disease of small intestine (terminal ileum)
Crohn´s disease
Surgical resection
Imerslund-Gräsbeck syndrome
4. Inherited defects of metaboiism
Transcobalamin II deficiency
Cobalamine A-G
Folates deficiency:
1. Inadequate nutrition
2. Defects in absorption (jejunum)
Celiak disease
Surgical resection
3. Folate inhibitos (dihydrofolát reductase) – MTX, pyrimethamine, thrimethoprim, sulfones
4. Increased requirements
Prematurity
Chronic hemolytic anemias
Chronic inflammatory disorders
5. Inherited defects
Hereditary folate malabsorption
Other causes of megaloblastic anemia:
1. Defects in purine and pyrimidine synthesis due to treatment by purine analogs (azathioprine, 6-merkaptopurien, 6-thioguanine, acyklovir), pyrimidine (cytarabin), cyklophosfamid, procarbazin
2. Inborn error of neucleic acid synthesis
Orotic aciduria
3. Other – antiepileptics (valproic acid, carbamazepine, phenytoin), CDA type I and III.

Normocytic anemia

1. Congenital hemolytic anemias:

- **Defective red cell membrane**
- **Red cell enzymes deficiencies**
- **Hemoglobinopathies**

2. Acquired hemolytic anemias:

- **Autoantibodies (AIHA)**
- **Microangiopathic hemolytic anemia**
- **Secondary caused by infection**

3. Acute blood loss

4. Hypersplenism

5. Chronic renal failure

Hereditary spherocytosis

- **Most common hemolytic anemia due to red cell membrane defect**
- **Clinically, biochemically and genetically heterogenous**
- **Osmotically fragile spherocytes are selectively trapped in the spleen and destroyed**
- **Clinical signs: anemia, jaundice, splenomegaly**
- **Autosomal dominant inheritance in 75% of cases, autosomal recessive form, *de novo* mutations**

Hereditary spherocytosis
Treatment

- **Splenectomy is indicated for HS pts with anemia or significant hemolysis (reticulocytes > 5%) or a family history of gallbladder disease (prevention of gallstones)**
- **Mild hemolysis + gallstones (cholecystectomy and splenectomy)**
- **Splenectomy at the school-age, total *vs.* partial, laparotomy *vs.* laparoscopy**
- **Risk od sepsis (Pneumococcus, H.influenzae, meningococcus) – 4%, mortality 2%**
- **Vaccination at least 2 weeks before SE, revaccination after 5 years**
- **ATB prophylaxis – PNC 250mg x2, amoxicillin 250 mg x 1 for at least 2 years after SE, at the first sign of febrile infection life-long**
- **Education of the patients**
- **Folate supplementation in severe/moderate form**
- **Follow-up: US for gallstones from the age of 5 years every 3 years, hematological supervision during viral infections-risk of decompensation, TAC**

Glucose-6-phosphate dehydrogenase deficiency
Clinical manifestation

- **Most individuals are asymptomatic and develop symptoms only in response to oxidant stress**
- **Chronic-non-spherocytic hemolytic anemia – class I variant rare mutations, low enzyme activity,non-endemic, males with history of neonatal jaundice and exchange transfusion, chronic anemia worsened by infection or drugs, gallstones, splenomegaly**
- **Neonatal jaundice: kernicterus, hyperbilirubinemia is largely result of G6PD deficiency in the liver**

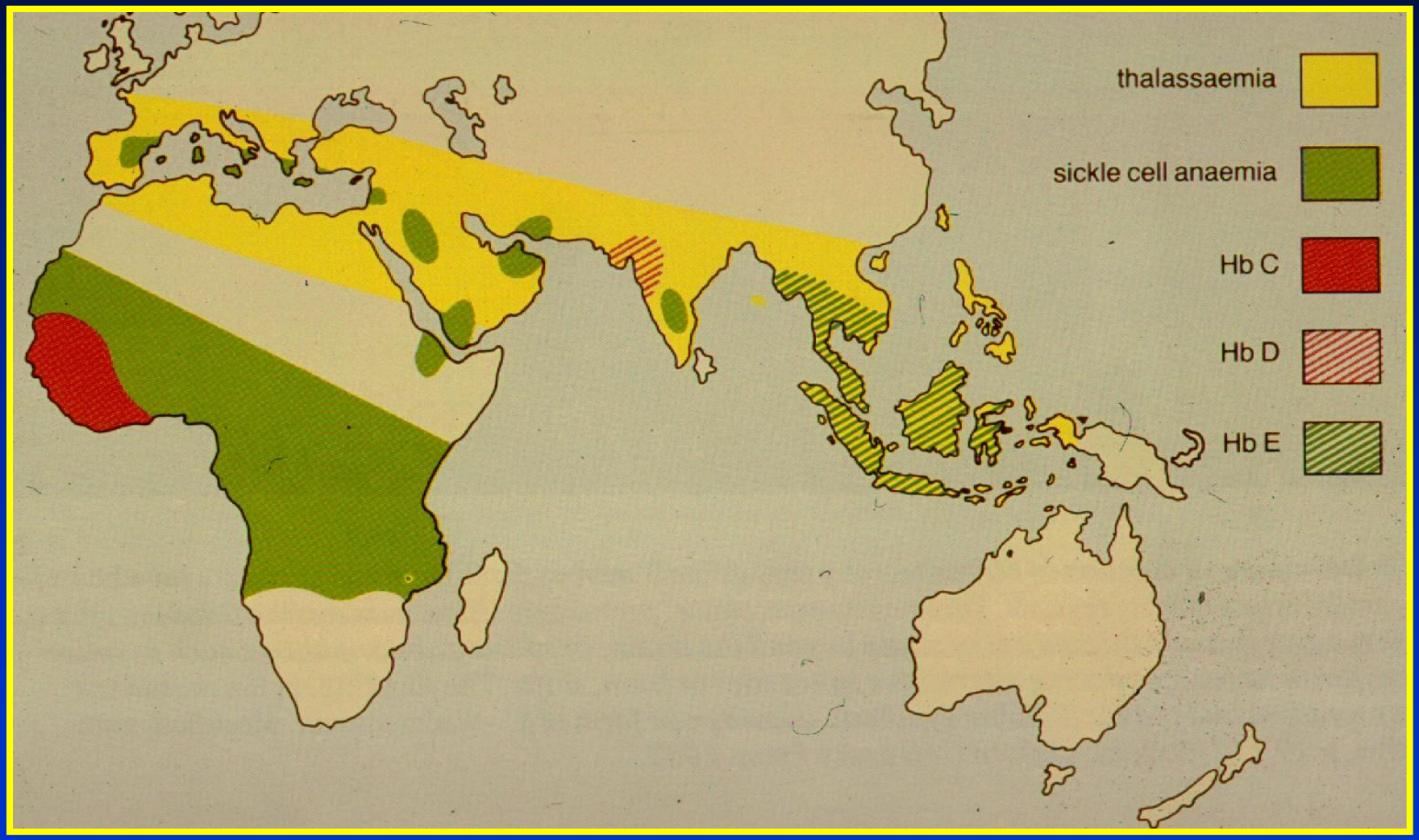
Glucose-6-phosphate dehydrogenase deficiency
Clinical manifestation

- **Infection-induced hemolysis: viral (hepatitis A), bacterial, rickettsial, hemolysis acute and intravascular (renal failure), relate to release of oxidants by leukocytes during phagocytosis**
- **Favism: acute hemolysis within 24-48 hours of ingestion of fava beans, boys aged 2-6 years, „bite“red cells in the smear (Heinz bodies)**
- **Drug-induced hemolysis: antimalarials, sulphonamides, sulphones, nitrofurantoin, urate oxidase, within 2-3 days, intravascular, hemoglobinuria**

Glucose-6-phosphate dehydrogenase deficiency Prevention and treatment

- **Avoidance the precipating causes of hemolysis**
- **Neonatal screening, health education**
- **CNSHA – splenectomy beneficial in transfusion dependent individuals, folate supplementation**

Geographic distribution of hemoglobinopathies



THALASSEMIAS (definition)

- reduced rate of synthesis of one or more globin chains (α, β, δ, γ, $\delta\beta$, HPFH)
- leading to: imbalanced globin chain synthesis, defective hemoglobin synthesis, and damage of red cells or their precursors
- many varieties are currently recognized
- Heterozygous carrier confers a selective advantage against malaria

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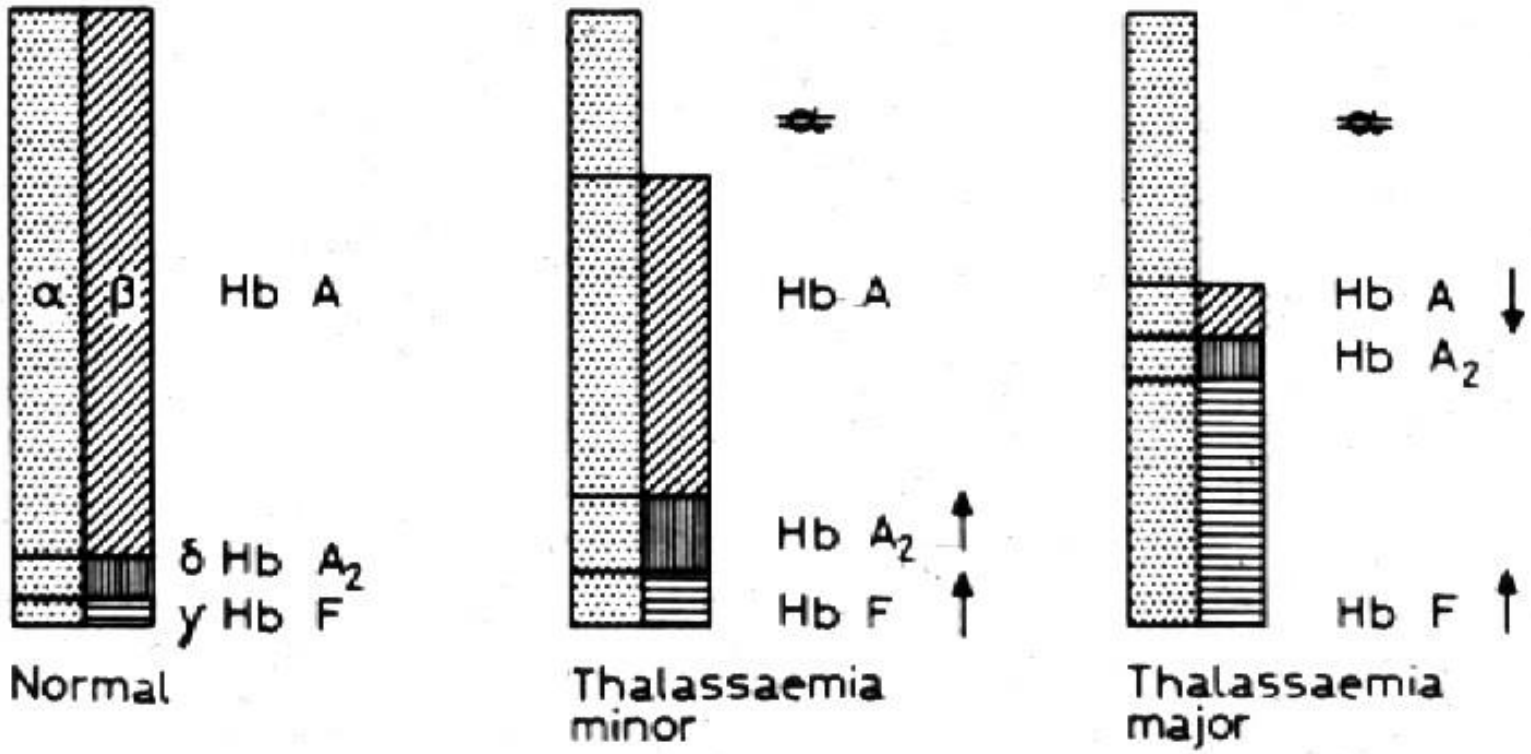
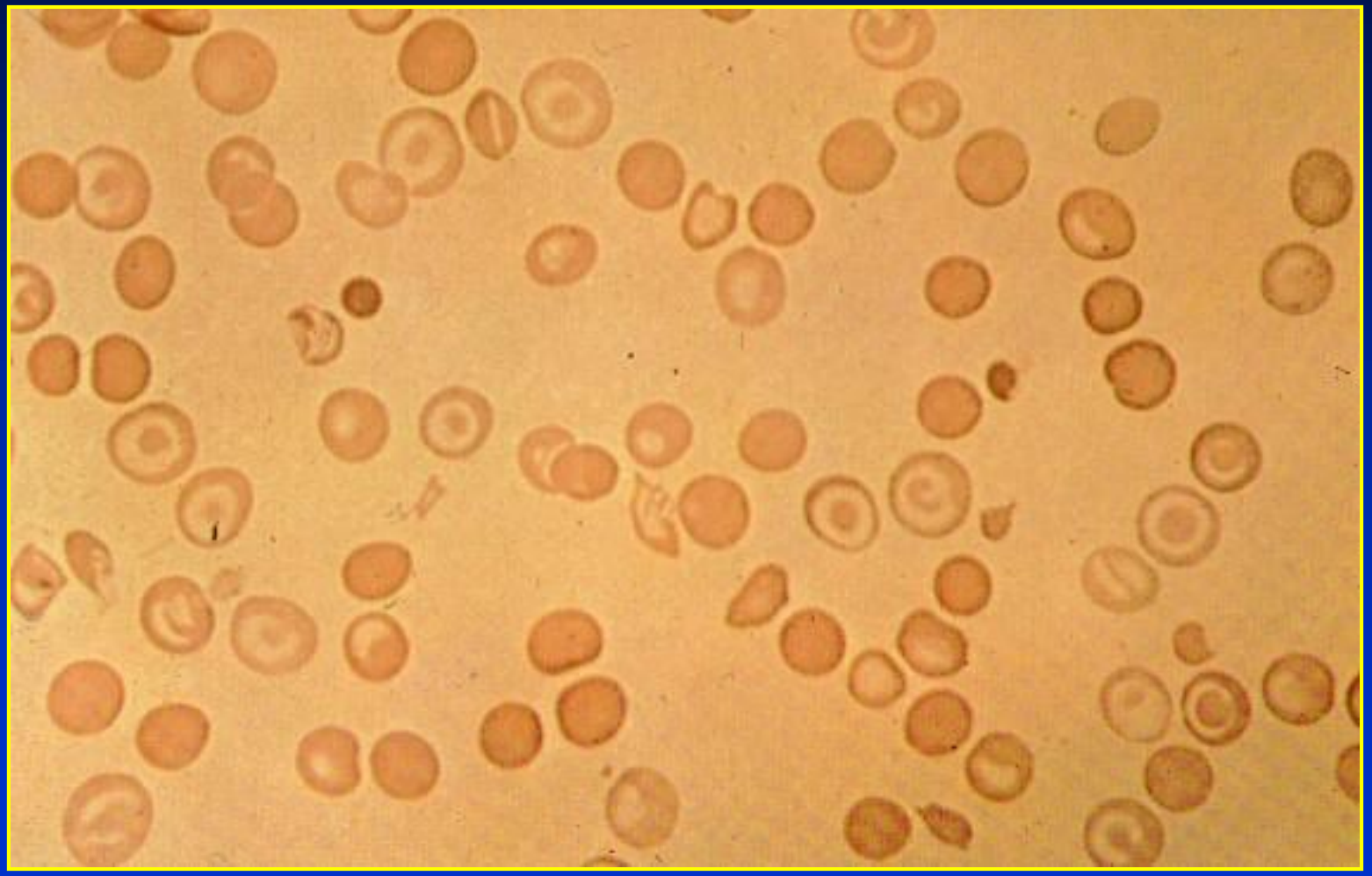
HPFH* = Hereditary persistence of fetal Hb

β-THALASSEMIA - GLOBIN CHAINS



β-THAL. - HYPOCHROMIC AND TARGET CELLS



β-THALASSEMIA (clinical picture)

1. Thalassemia major = the homozygous state for Th.
   = heterozygotes for two different Th. mutations
2. Thalassemia intermedia: some patients with β-Th. genes from both parents

   - a milder clinical picture
   - later onset
   - few or no transfusions requirements
3. Thalassemia minor: heterozygous carrier state

THALASSEMIA MAJOR (course)

- depends entirely on the maintenance of adequate transfusion programe (to maintain relatively normal Hb levels by regular blood transfusion)
- inadequately transfused child develops typical features: stunted growth, bossing of the skull, overgrowth of maxillary region („mongoloid" appearence of the face)
- adequately transfused child develops normally, has no abnormal physical signs

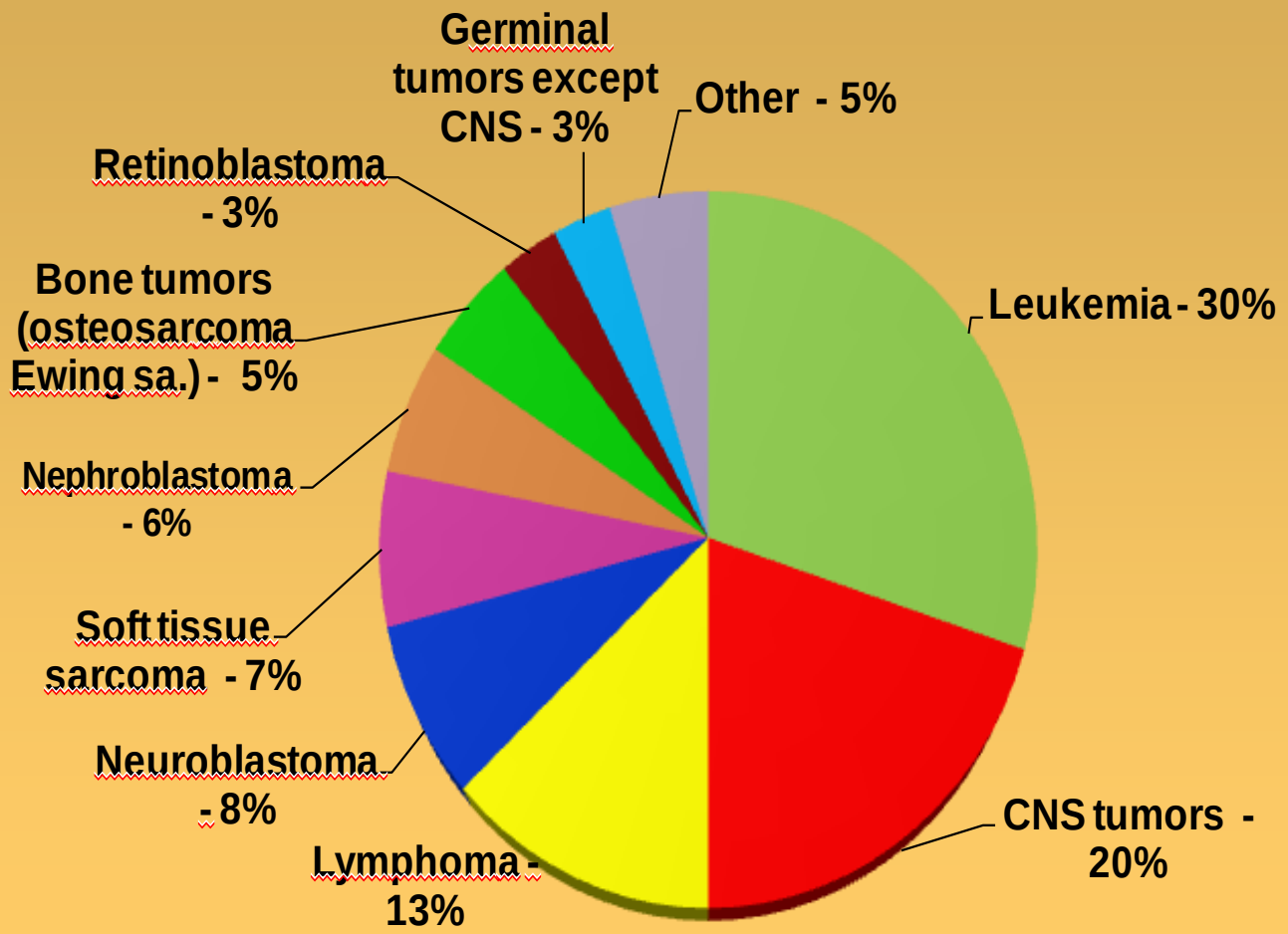
THALASSEMIA MAJOR (Iron overloading)

- First symptoms:
  - absence of the pubertal growth spurt
  - failure of the menarche
- Endocrine disturbances:
  - diabetes mellitus
  - adrenal insufficiency
- Cardiac complications:
  - arrhytmia
  - cardiac failure

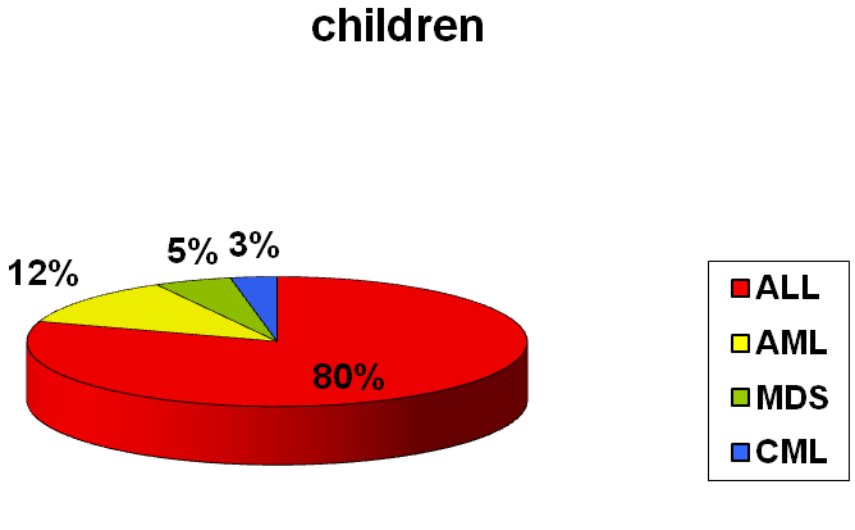
ThThalassemia major-Treatment

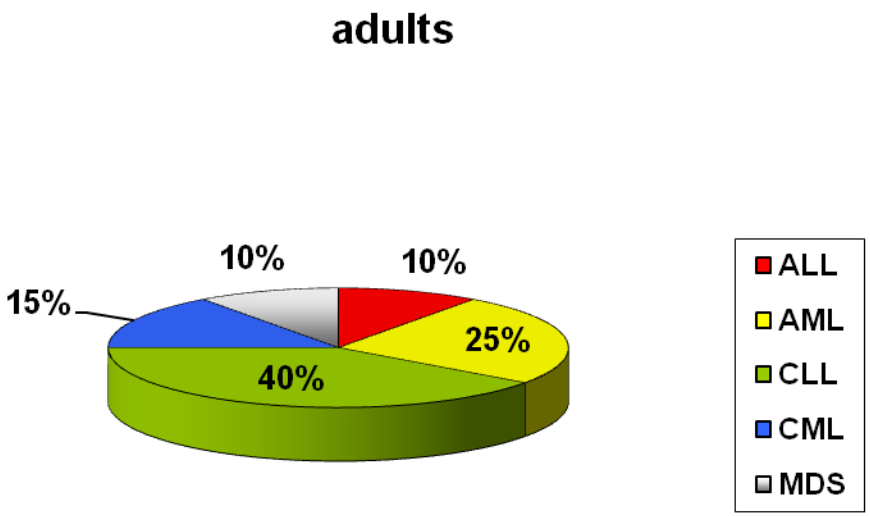
- Transfusion policy to maintain Hb above 9 g/dl
- Chelators
- Bone marrow transplantation

Epidemiology of cancer in children



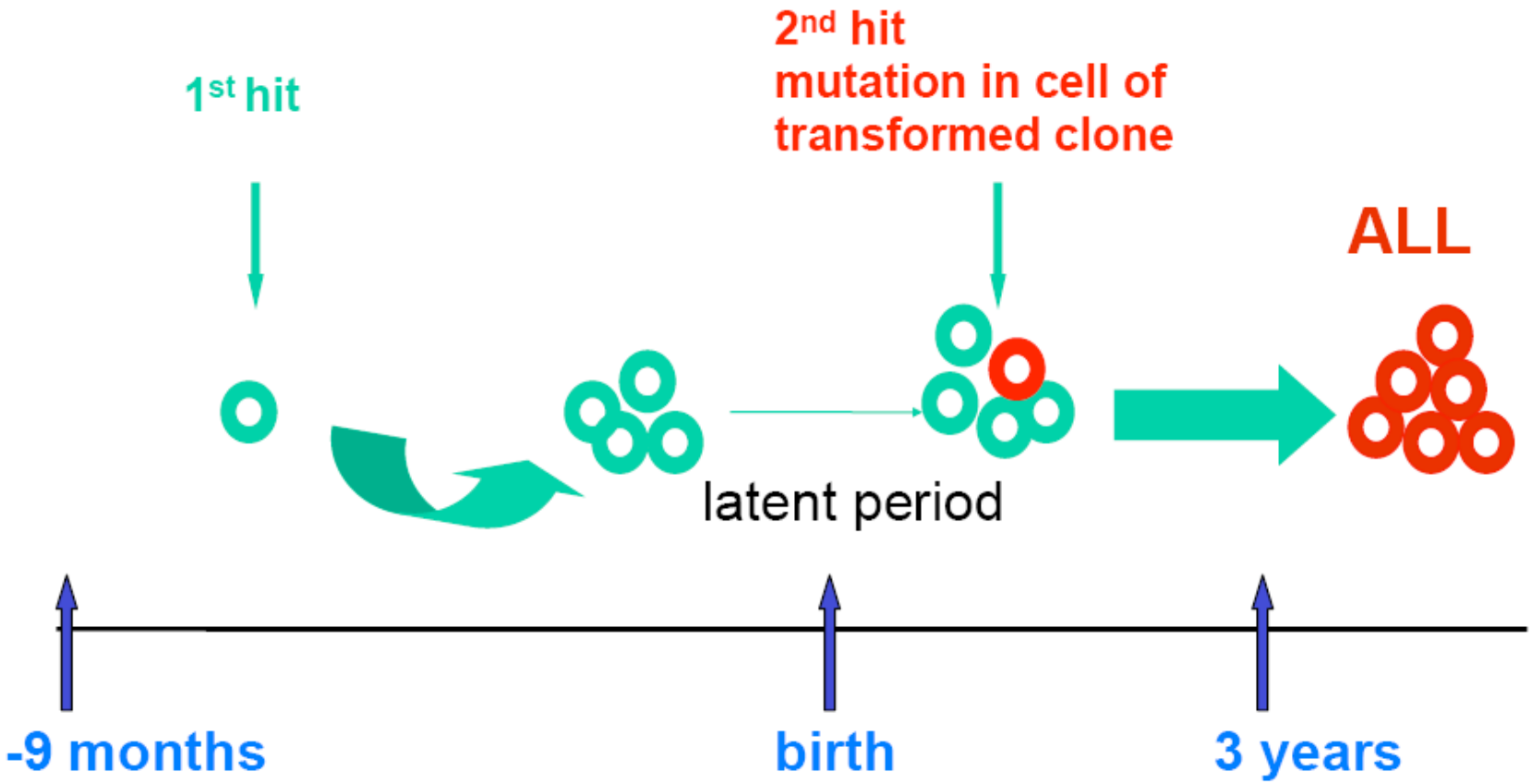
Leukemia in children and adults



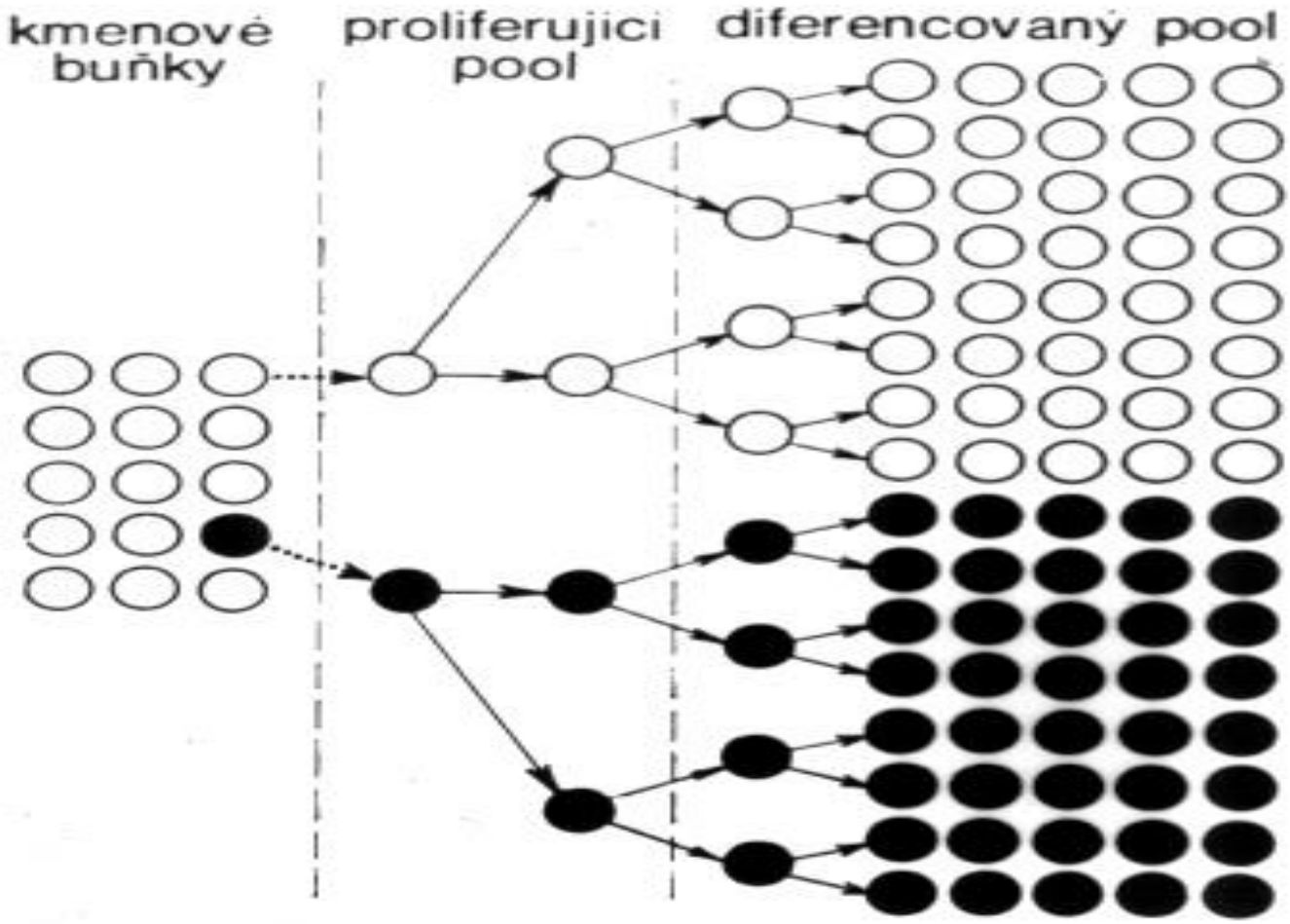


Model for the etiology of childhood common ALL

Mel Greaves, 1988



Acute leukemia-etiopathogenesis



leukemia- symptoms

• typical manifestation: fatigue, pallor, haemorrhagic diathesis, lymphadenopathy, hepatosplenomegaly, fever, infection

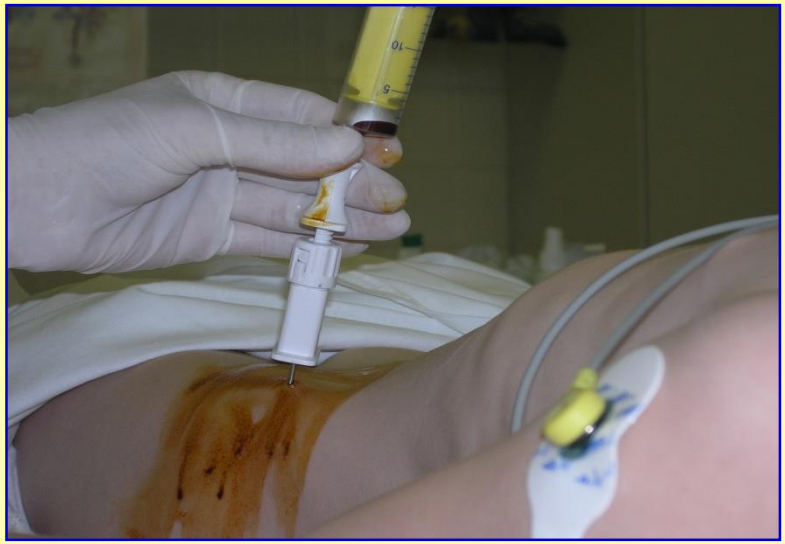
• bone and joint pain in 20-50% ALL cases, exceptionaly in AML, CML
  • back pain, limbs
  • often the only symptom, smoldering leukemia

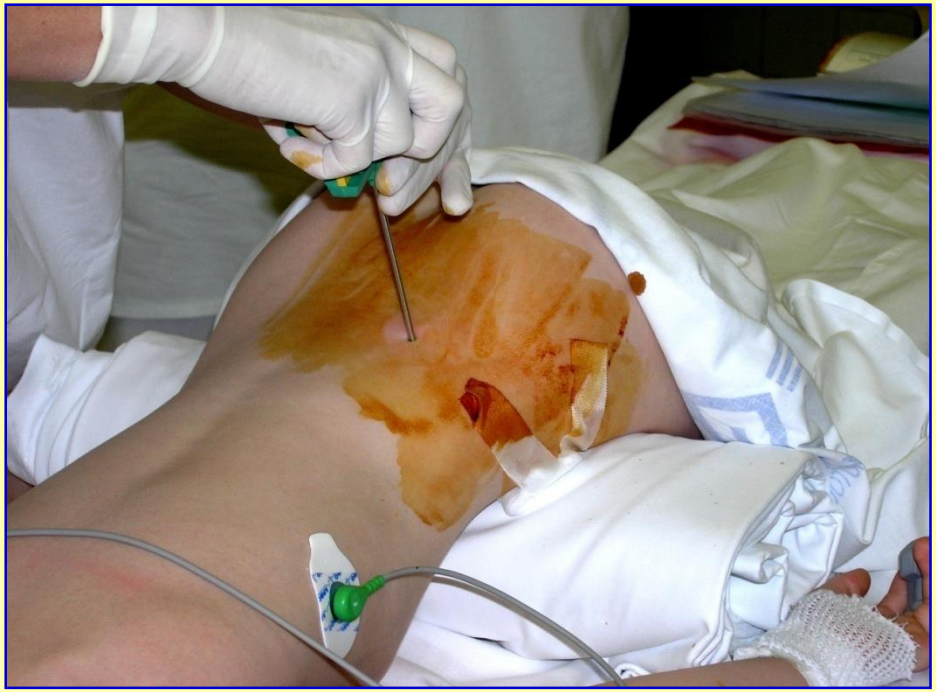
• dyspnoe, vena cava superior syndrome, leukemia cutis, gingival hyperplasia, orbital chloroma, testicular leukemia, priapism, intracranial hypertension, seazures, paresis …

leukemia– laboratory changes

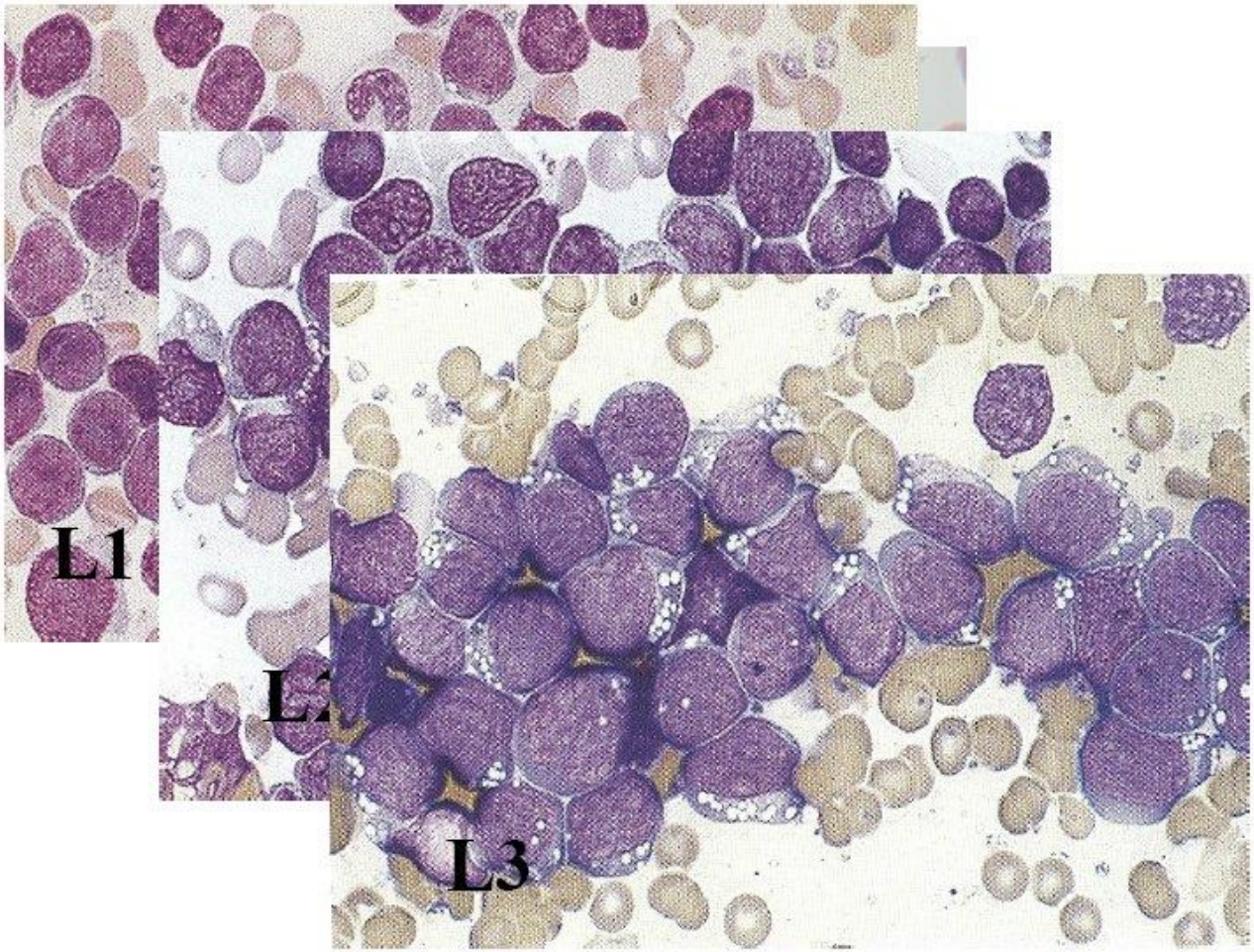
• CBC: typicaly leukocytosis, anemia (normocytic or macrocytic), trombocytopenia, hiatus leucemicus, lymfocytosis, neutropenia

- !! But also normal CBC or leukopenia, pancytopenia, lymphocytosis
- rarely polycytemia
- hyperurikemia, renal insufficiency, increased LDH, increased transaminases
- exceptionaly hypercalcemia

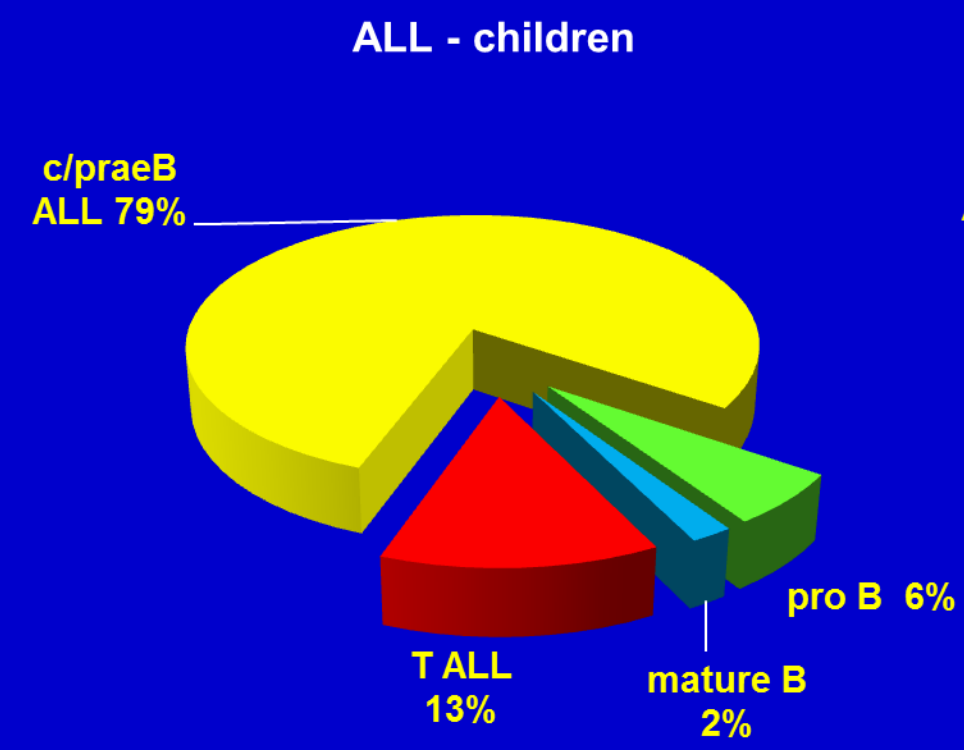


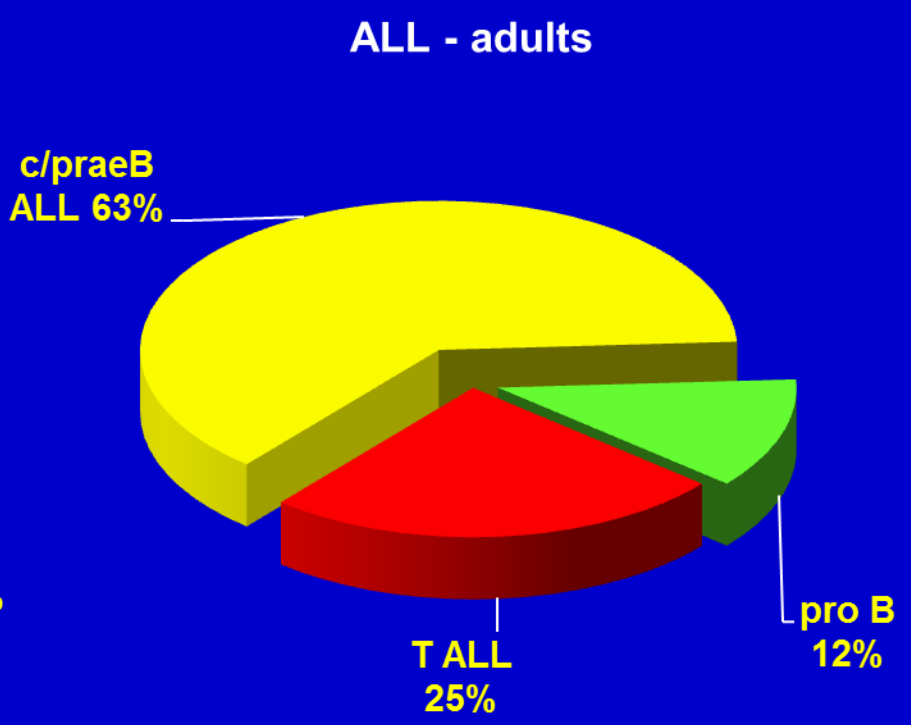


Molecular aberrations in childhood ALL



ALL - immunophenotypes

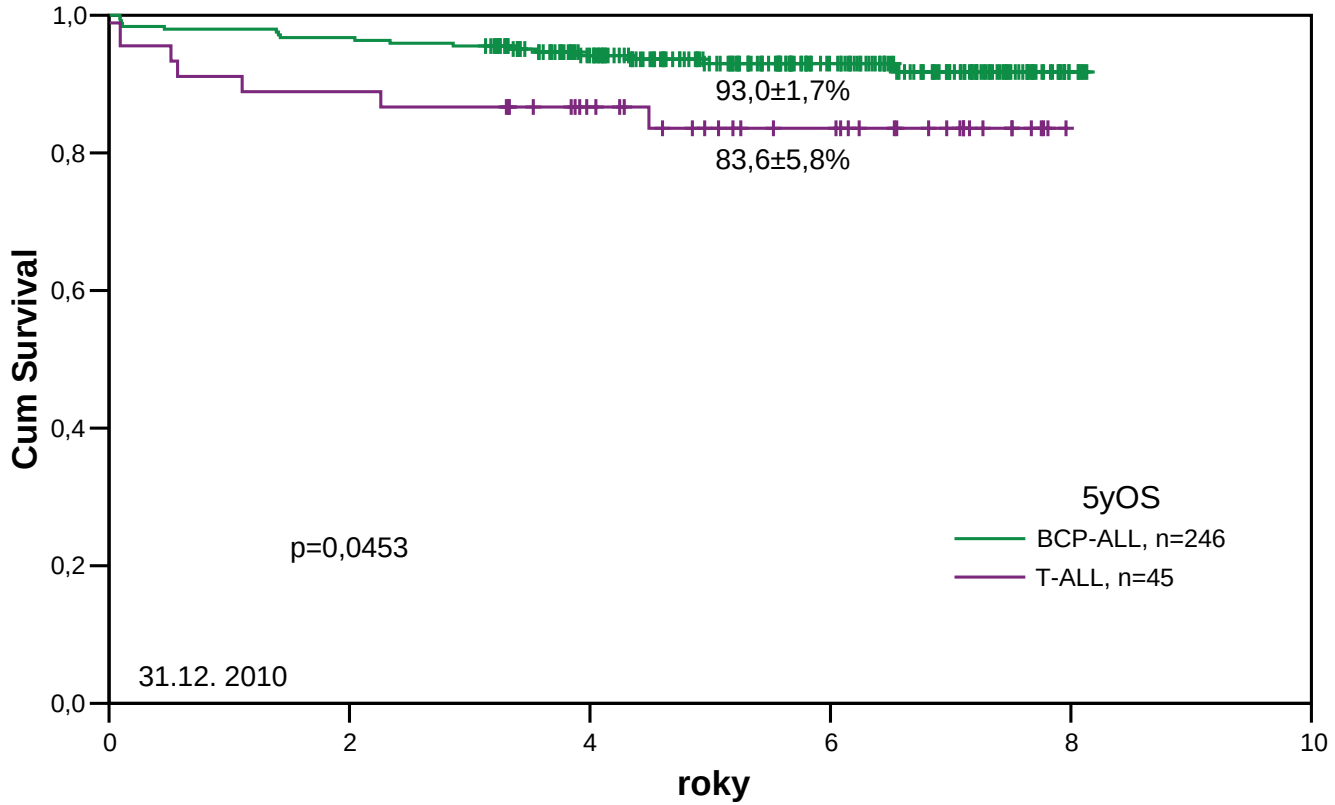




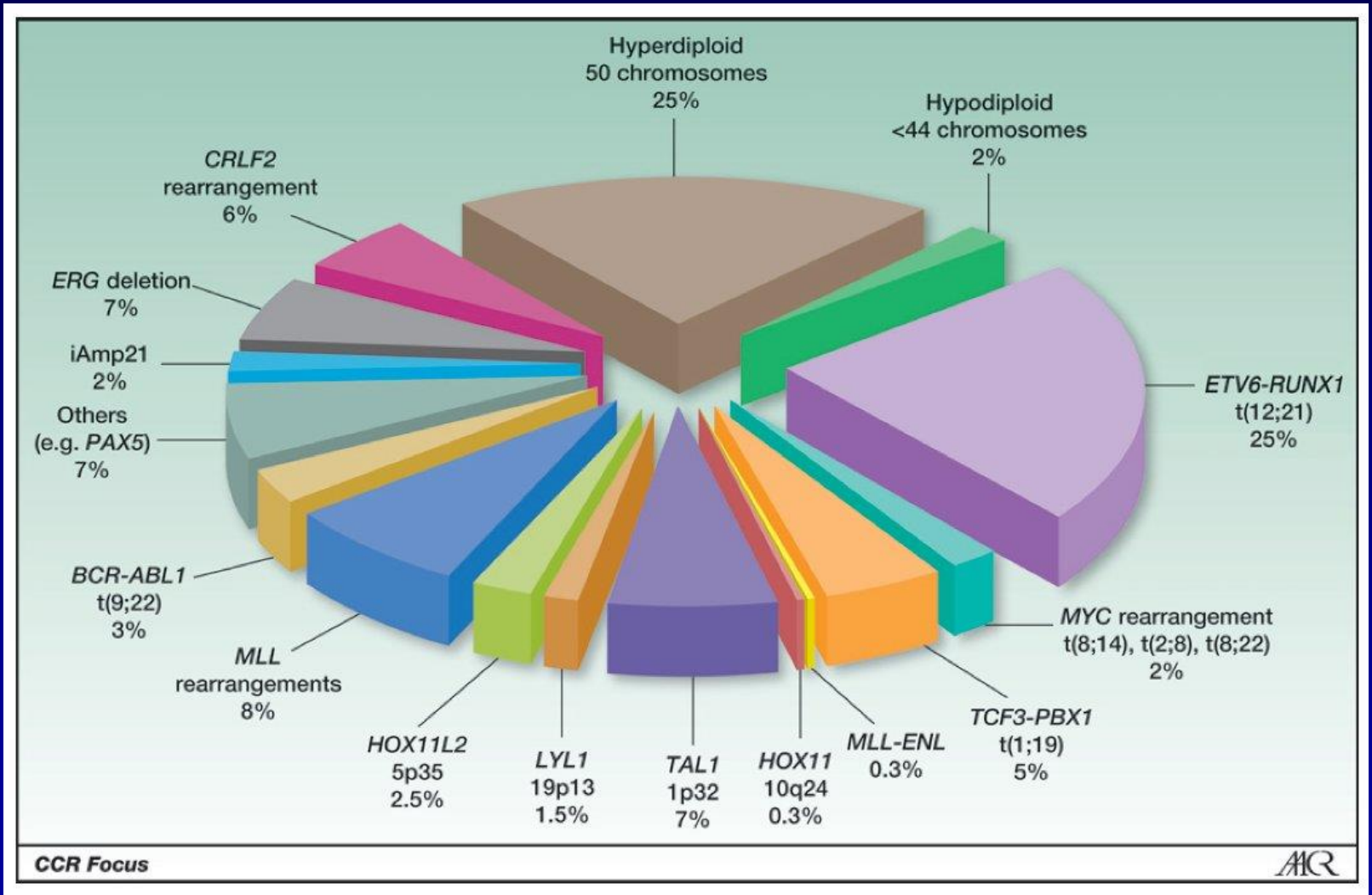
CLIP

PSDH ČR

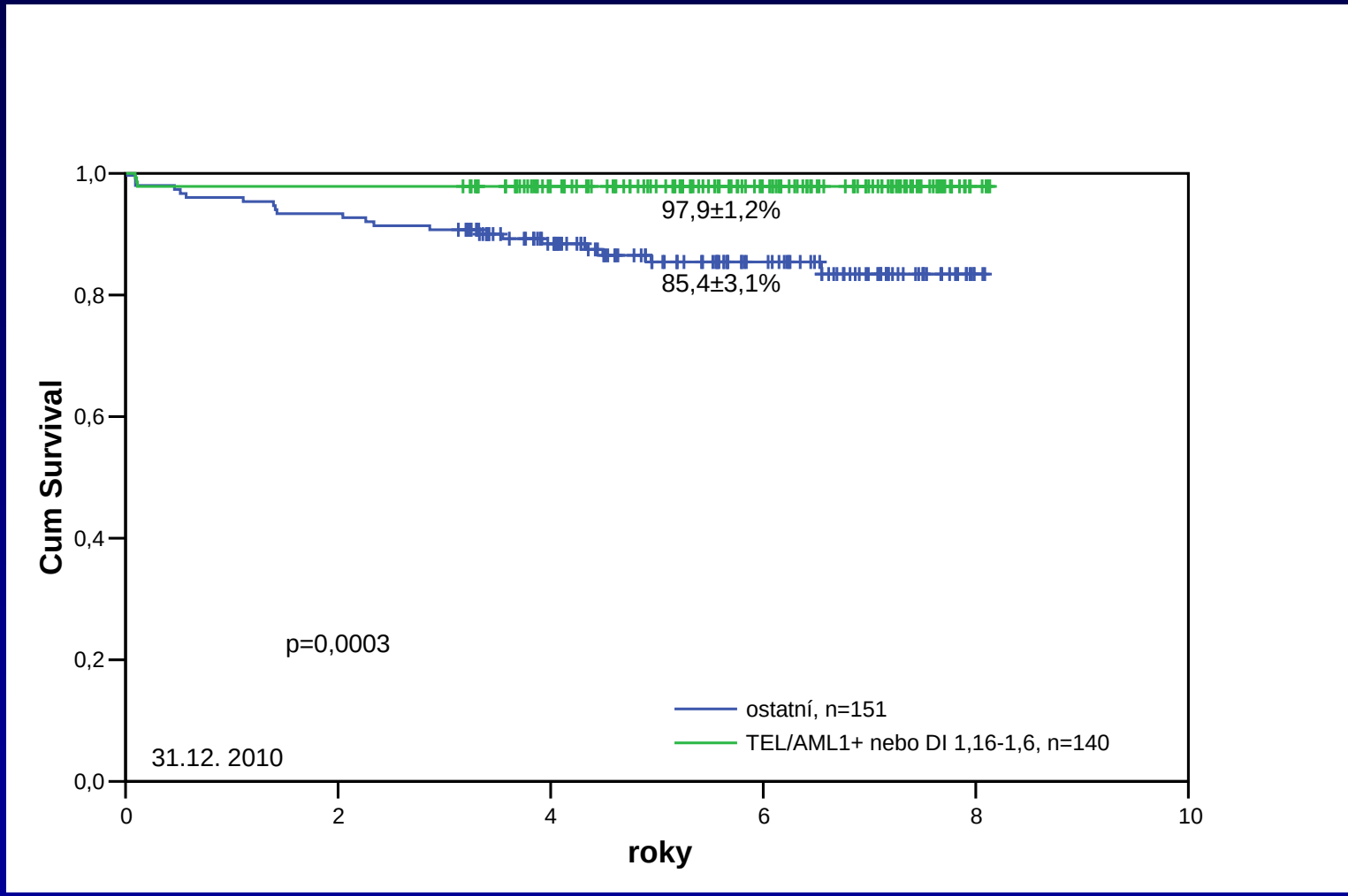
ALLIC (N=291)
5-y OS BCP-ALL vs. T-ALL



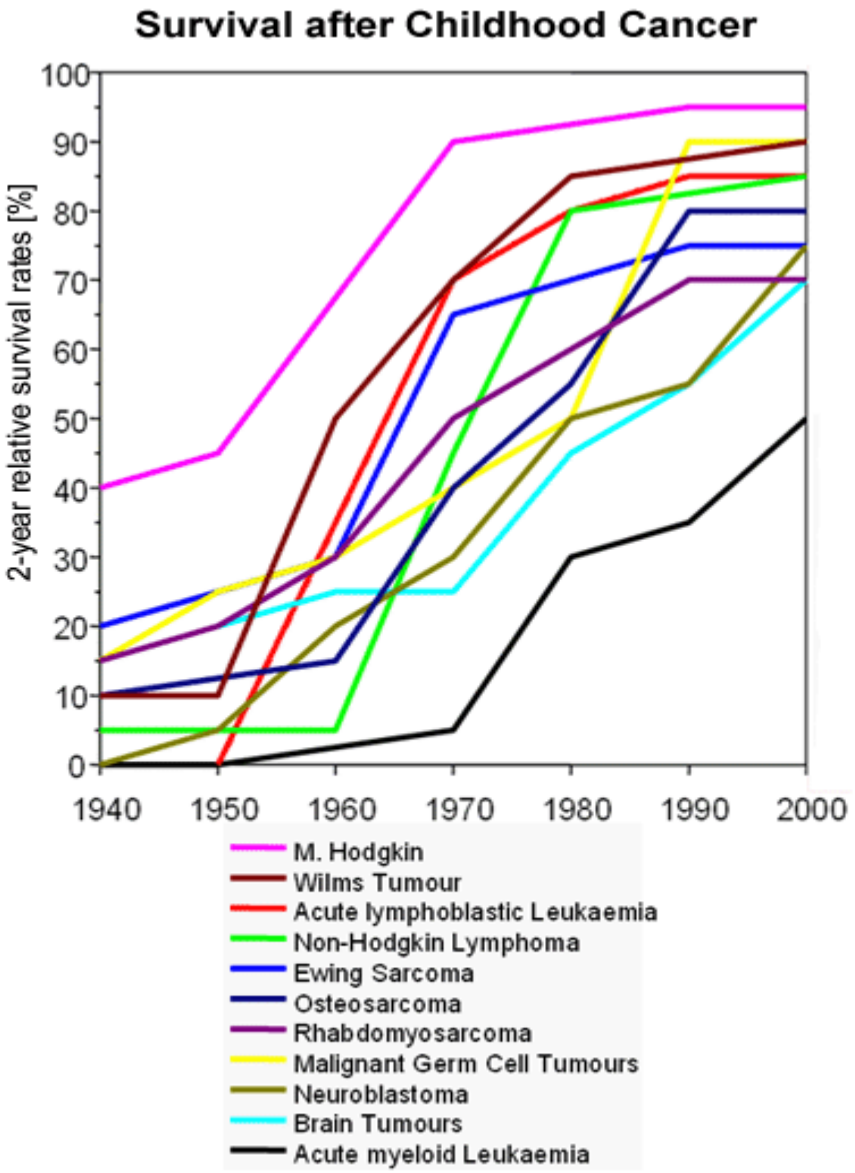
ALL in children - genotype

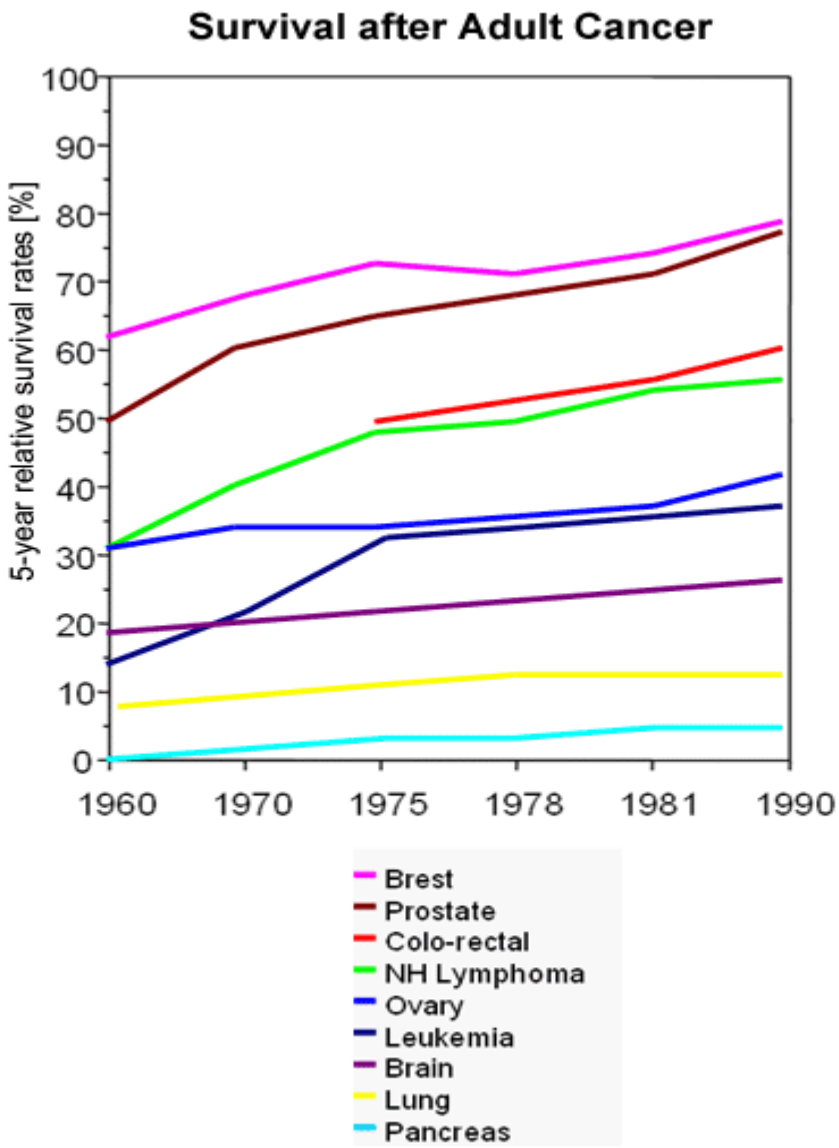


ALLIC (N=291)
5-y OS according to genotype



Survival of children and adults with cancer





ALL treatment

Induction

4-5 weeks, at the end 98% pts in remission (despite up to 10^{10} malignant cells remains)

Consolidation

few months

Late intensification

re- induction 6 months since diagnosis

Maintenance treatment

gradual „comeback to life“, peroral cytostatics until total length of treatment 2 years

+ since the beginning prophylaxis of CNS leukemia

Cytostatics

Antimetabolites (Methotrexate, Cytosin arabinosid, 6-mercaptopurine, 6-thioguanine)

Alkylating agens (Cyclophosfamide, Ifosfamide)

Antibiotics (Doxorubicine, Daunorubicine, Idarubicine; Mitoxantron)

Alkaloids

Vinca alkaloids

(Vincristin, Vinblastin, Vindesin)

Podophyllotoxins

(Etoposide)

Various (L-asparaginase)

BFM studies – professor H. Riehm

BFM 2003 MS

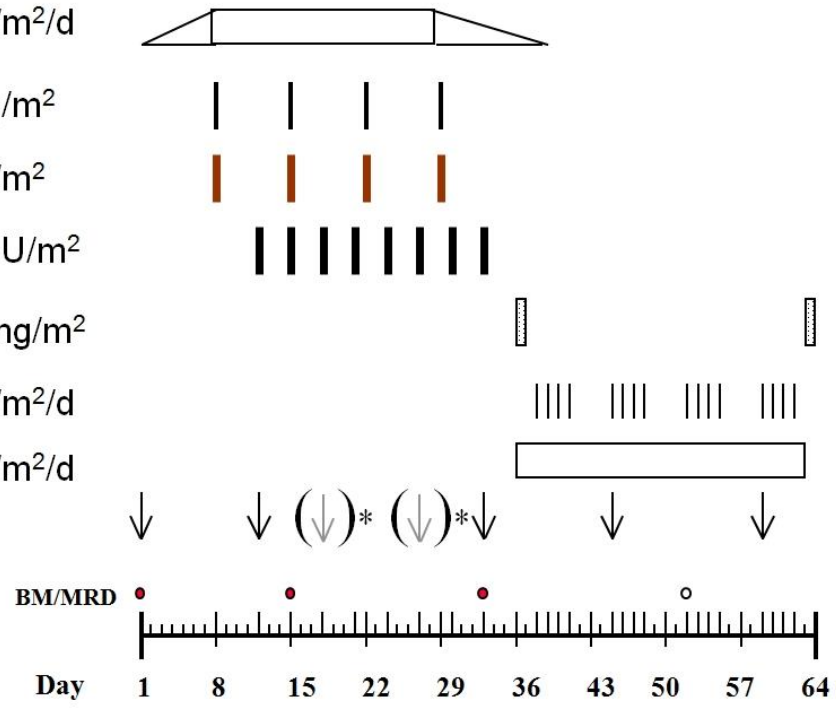
Protocol I

PRED	**p.o.**	60mg/m²/d
VCR	**i.v.**	1.5mg/m²
DNR	**p.i.** (1h)	30mg/m²
L-ASP (E.coli- MEDAC/KYOWA)	**p.i.** (1h)	5,000 U/m²
CPM	**p.i.** (1h)	1000mg/m²
ARA-C	**i.v.**	75mg/m²/d
6-MP	**p.o.** (28 d)	60mg/m²/d
MTX	***IT***	

Dose age-adapted:

MTX IT	<1J	1J	2J	≥3J
	6mg	8mg	10mg	12mg

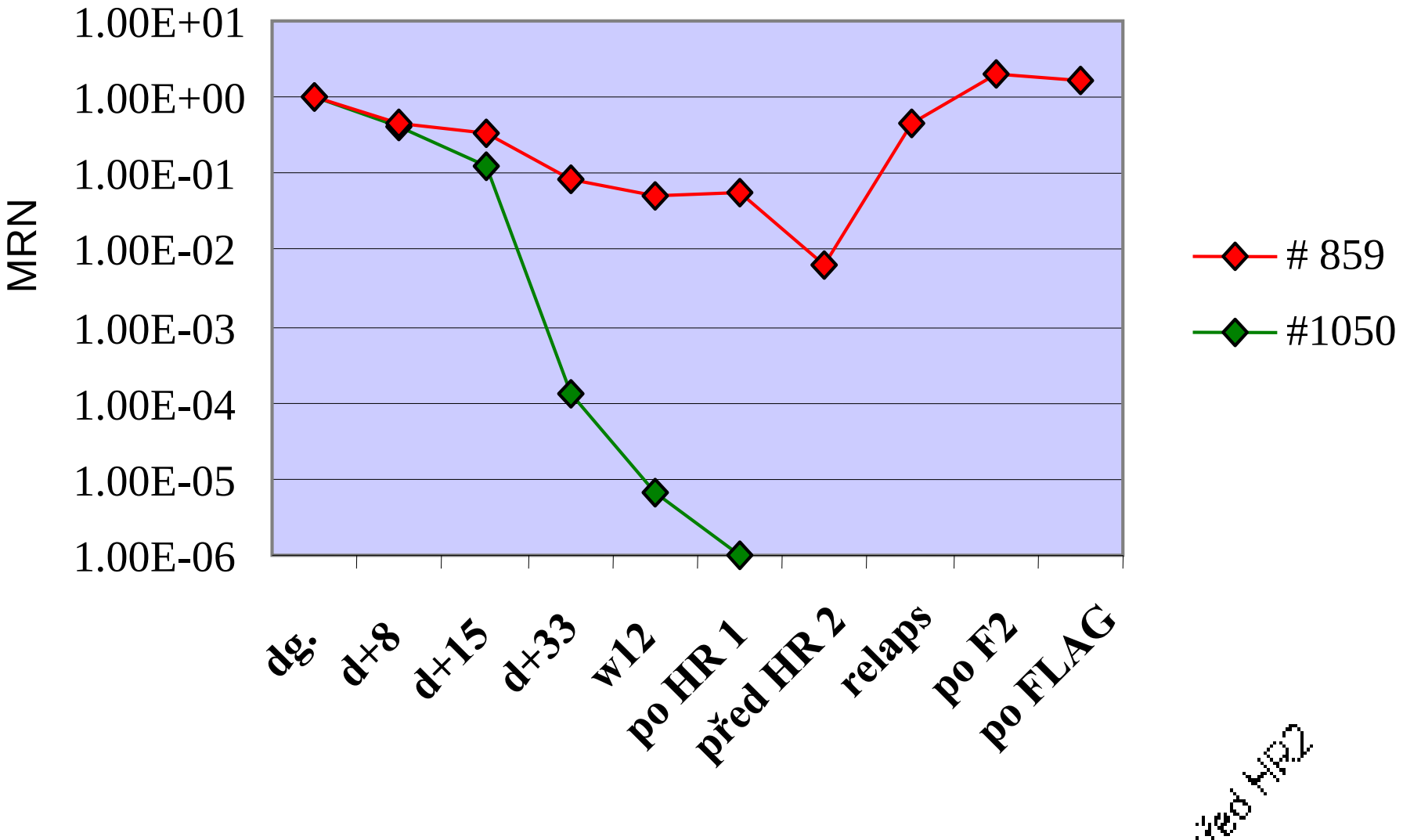
* if CNS positive, or CNS neg. but blasts in CSF, or traumatic puncture: additional MTX *IT:* d 18 and 29



MRD in ALL treatment

- MRD as a research method since 1988
- Procedure:
  quantification of fusion transcripts by the help of qPCR
  quantification of clonal specific rearrangements by Ig/TCR genes by the help of qPCR
  polychromatic flow cytometry

T-ALL+ PPR+ BMd15 M3+ BMd33 CR
Comparison according to MRD



Risk stratification AIEOP-BFM 2009

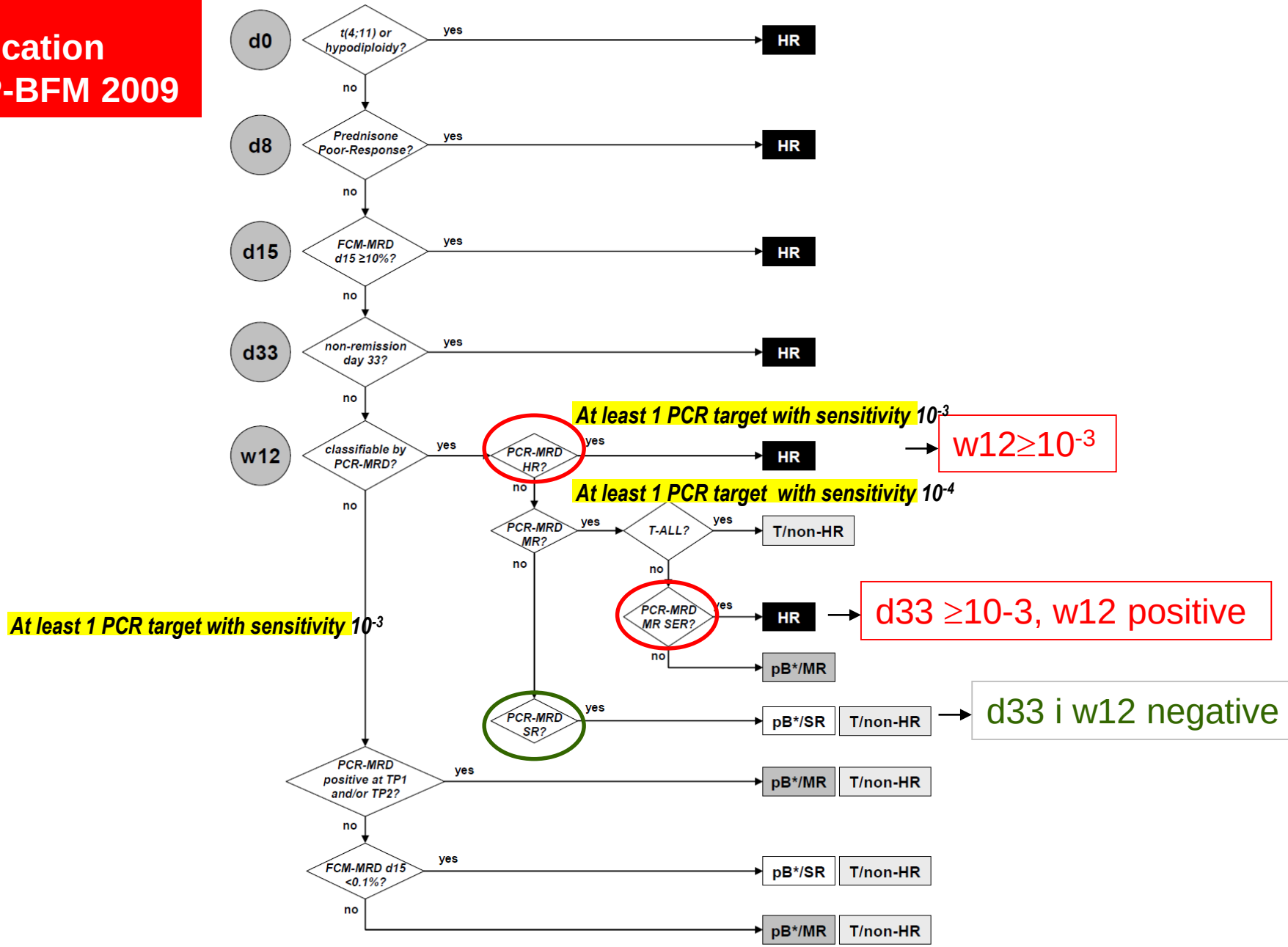
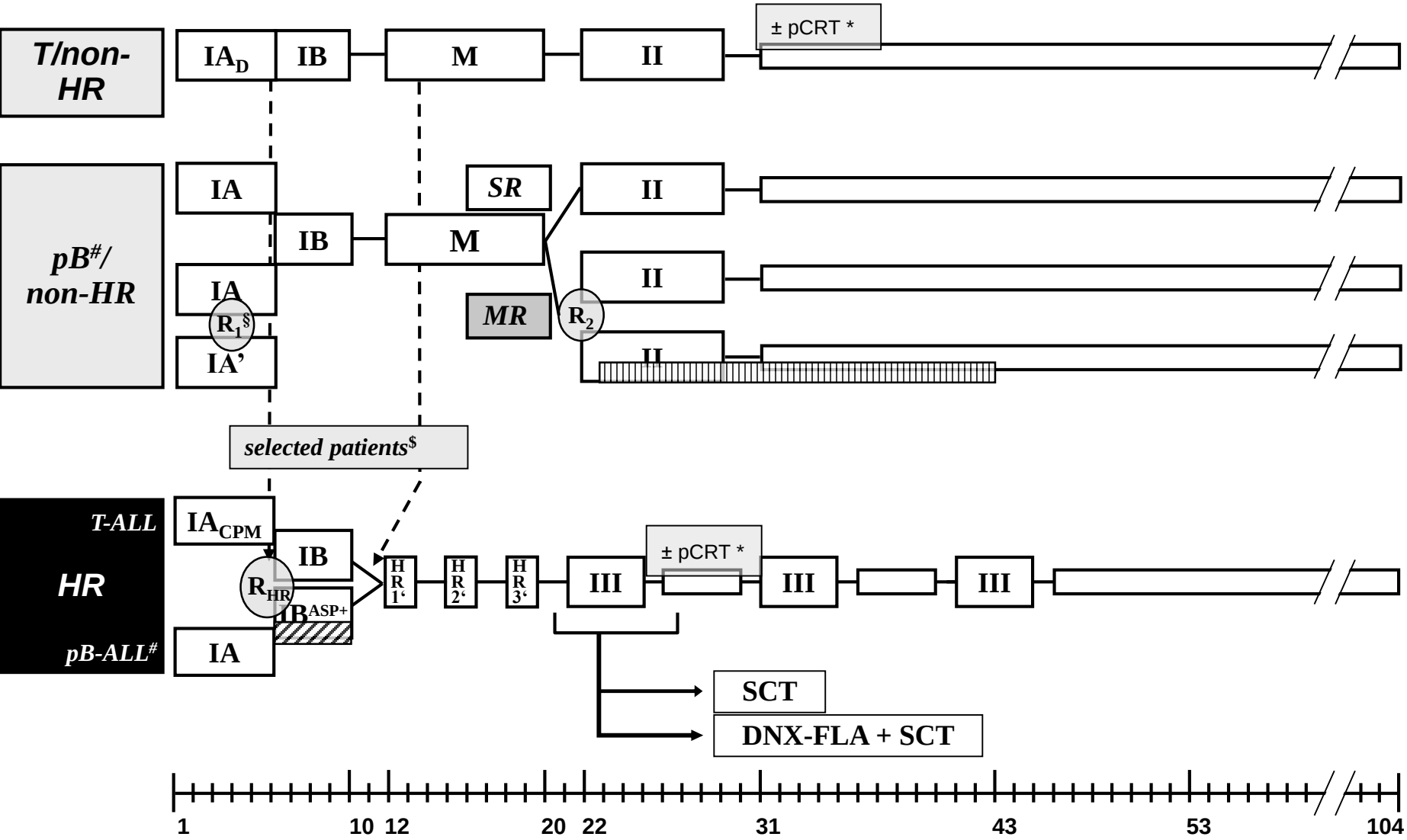


Figure 1 Flow-chart for identification of the treatment group
(*or immunophenotype unknown)

AIEOP-BFM ALL 2009



IA	Prot. IA (with Pred and 4 DNR doses on day 8, 15, 22 and 29)
IA'	Prot. IA' (with Pred and 2 DNR doses on day 8 and 15)
IA$_{CPM}$	Prot. IA$_{CPM}$ (with Pred, 4 DNR doses and 1 dose CPM on day 10)
IA$_D$	Prot. IA$_D$ (with Dexa and 4 DNR doses on day 8, 15, 22 and 29)
IB^{ASP+}	Prot. IB-ASP+ (with 4 x 2500 E PEG-L-ASP)
	PEG-L-ASP given for 20 weeks

\# or immunophenotype unknown

* pCRT 12 Gy if age ≥ 2 yrs / in selected subgroups no pCRT + 6x i.th. MTX / in patients with CNS disease (CNS 3) tCRT with 12 Gy or 18 Gy (dose age-adapted)

§ for eligibility for randomization see protocol

$ see protocol

ALL in Czech Republic 1985-2007
5-y OS