CHRONIC LEUKEMIAS

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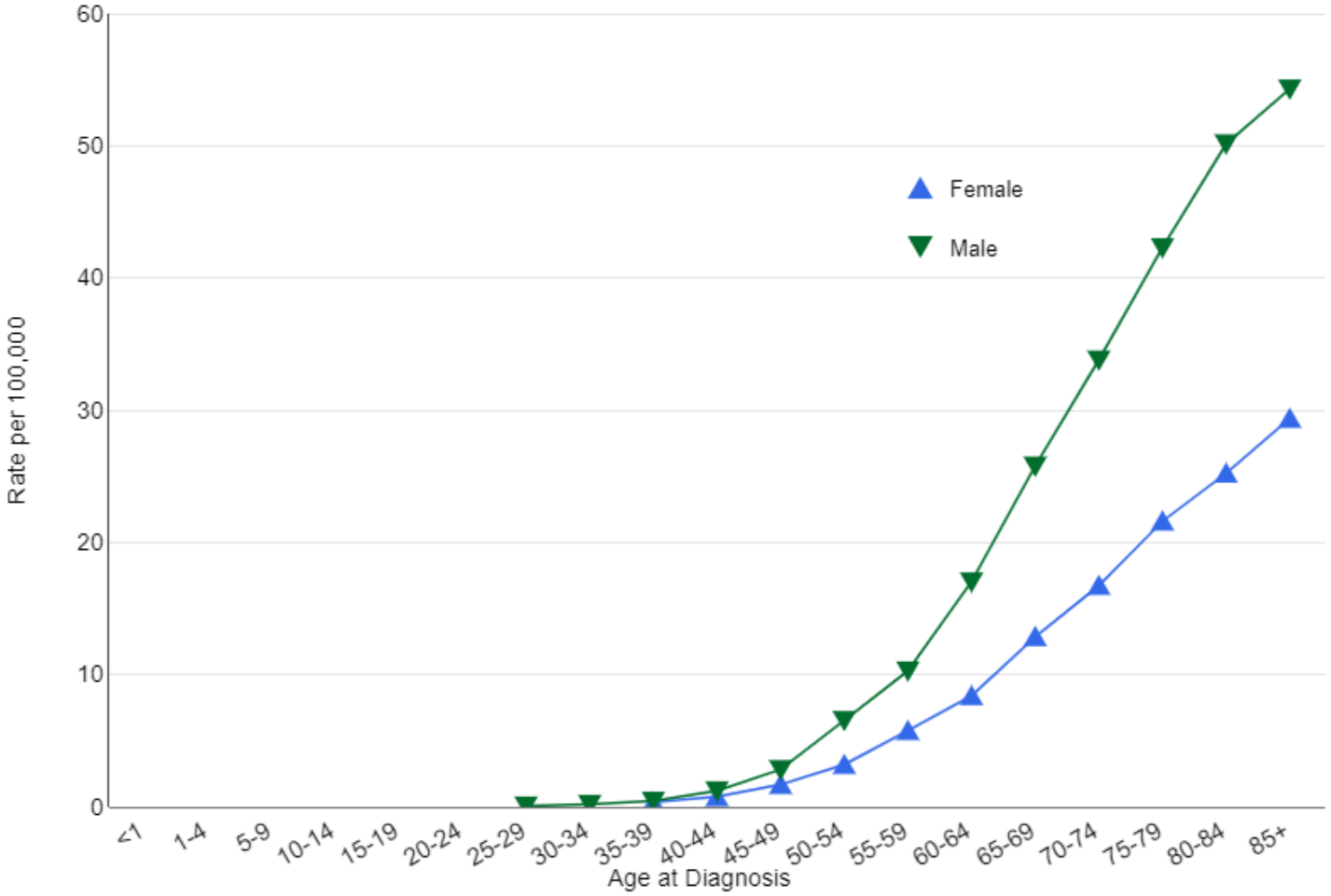
CHRONIC LYMPHOCYTIC LEUKEMIA

Introduction

- B- lymphoproliferative disease
- most common leukemia in our country
- incidence ~ 50: 100 000 (over 70 years)
- mostly elderly patients (median 65 - 68) x about 1/3 <60 years at the time of dg.
- men 2 times more often than women (2: 1)
- heterogeneous clinical course (survival ranges from 5 years to more than 25 years)

Age distribution of patients with CLL



data seer.cancer.gov 2012 - 2016

Pathophysiology

- etiology unclear
- clonal proliferation of the lymphocyte at some level of its normal maturation
- apoptosis disorder (extended lifetime of tumor lymphocytes)
- accumulation of tumor B-lymphocytes and peripheral blood lymphocytosis
- bone marrow failure
- lymphadenopathy, splenomegaly

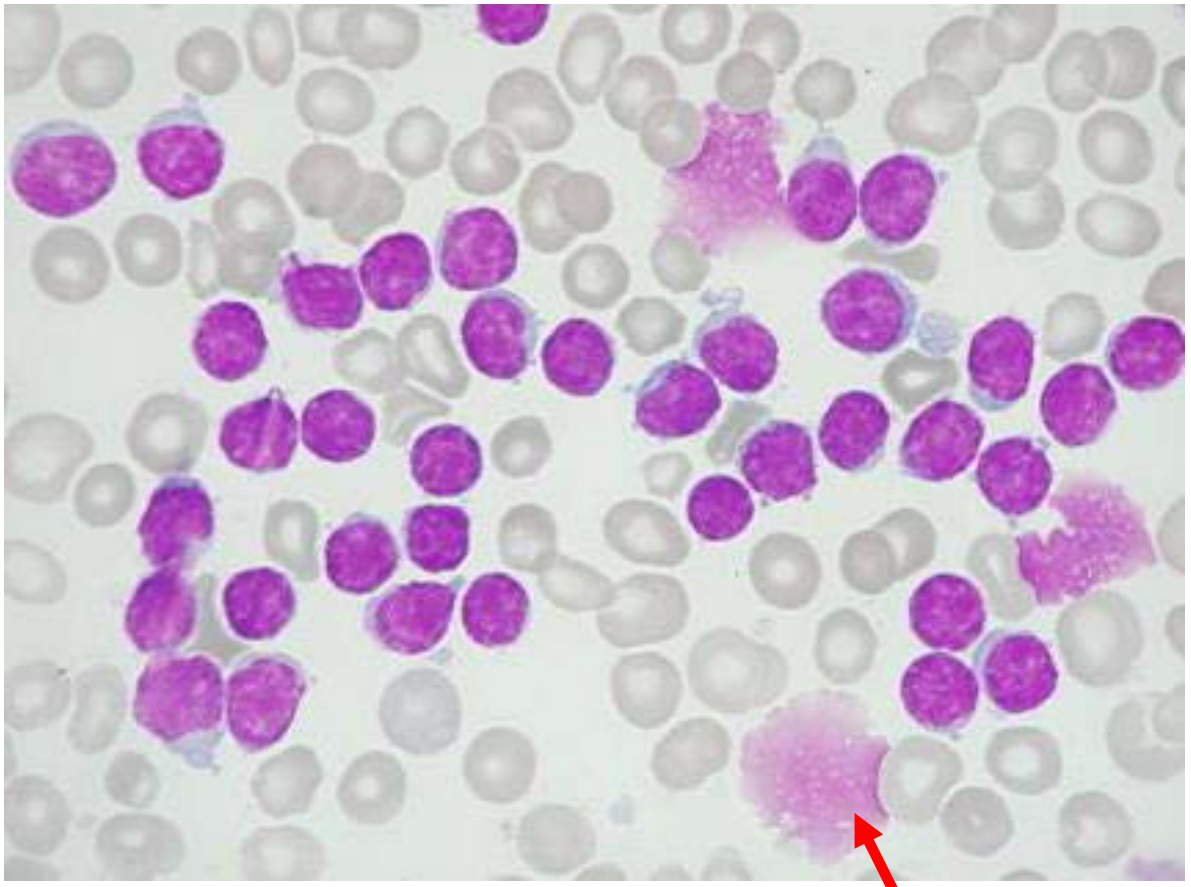
Clinical feature

- may initially be asymptomatic (accidental laboratory finding)
- asymptomatic is up to 40% of patients, they have only changes in blood count
- later show:
  - anemic symptoms (bone marrow failure)
  - general symptoms: weight loss, night sweats, subfebrile
  - enlargement of lymph nodes
  - with or without splenomegaly, hepatomegaly
  - frequent infections

Peripheral blood smear

B--Le	188,30
B--Ery	3,98
B--Hb	123
B--HTK	0,362
B--Obj ery.	91
B--Hb ery	31,0
B--Hb konc	340
B--Erytr.křivka	15,6
B--Trombo	110
Dif mikr.	
B--Seg	0,05
B--Ly	0,89
B--Mo	0,03
B--Prolymfocyt	0,03
B--Jader. stíny	+++

B--Le	540,40
B--Ery	2,76
B--Hb	88
B--HTK	0,264
B--Obj ery.	96
B--Hb ery	31,9
B--Hb konc	333
B--Erytr.křivka	16,4
B--Trombo	92
Dif mikr.	
B--Seg	0,01
B--Ly	0,88
B--Mo	0,01
B--Prolymfocyt	0,10
B--Jader. stíny	++



Gumprechtovy stíny

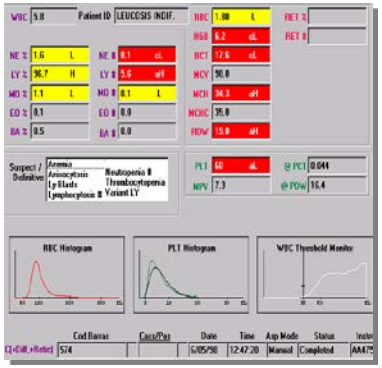
- leukocytosis with a predominance of lymphocytes
- Gumprecht's shadows
- anemia, thrombocytopenia

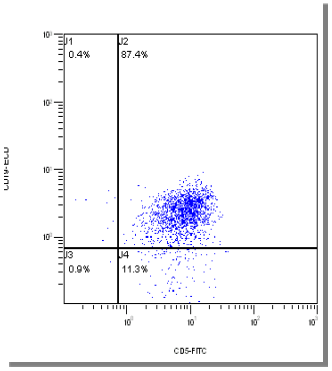
Diagnosis

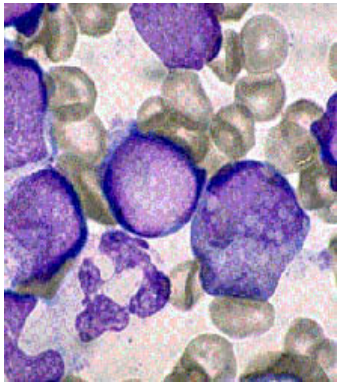
lymfocytosis: > 5 x 10^9/l
≥ 1 B lymfocytární znak (CD19, CD 20)

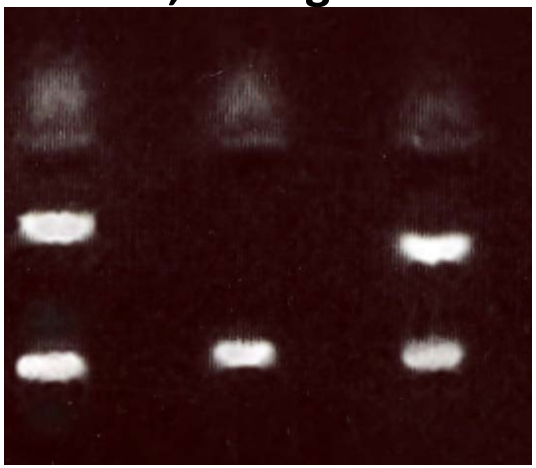
bone marrow infiltration: ≥ 30 % lymfocytů

KO | ***flow cytometry*** | **histology** | **FISH, mol. genetics**



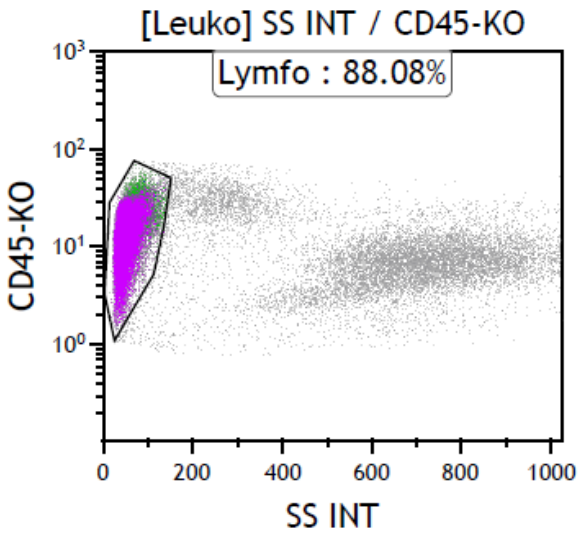


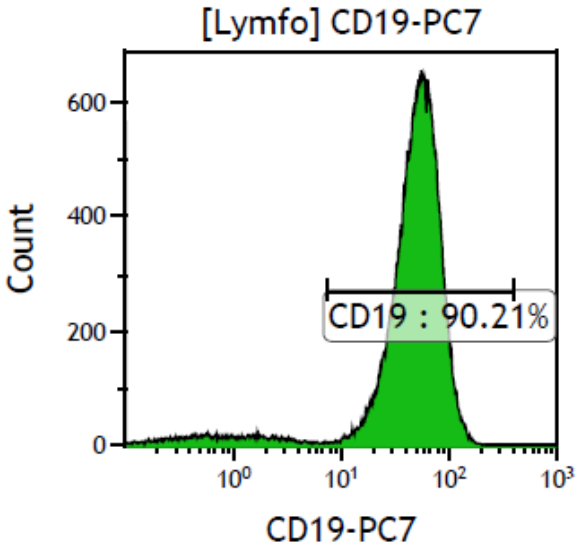




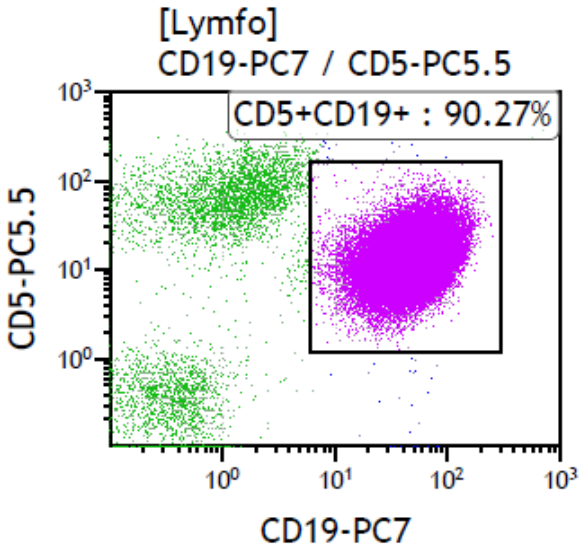
lymfocytosis | **imunophenotyping** | **BM infiltration** | **prognostic factors**

Laboratory findings - cytometry





Gate	Number	%Gated
All	100,000	100.00
Leuko	97,042	98.40
Lymfo	85,473	88.08
CD19	77,107	90.21
CD20	13	0.02
CD200	79,517	93.03
CD23	70,556	82.55
CD5+CD19+	77,157	90.27



Clinical stages of CLL

- the disease can be divided into several clinical stages
- various systems, most commonly used by Raie

➡ 5 stages:

stage	findings	median survival (years)
0.	lymphocytes ≥ 15 x 10^9/l + BM infiltration	> 12
I.	st. 0 + lymphadenomegaly	9
II.	st. 0 + splenomegaly or hepatomegaly	5
III.	st. 0 + Hb < 110 g/l	1,5
IV.	st. 0 + trombo < 100 x 10^9/l	1,5

Course of CLL

part of pat. - in the long term (years) without signs of progression, without therapy

part of pat. - early failure of marrow hematopoiesis, ⬇KO
- the need to start therapy early
- Transformation into more aggressive NHL

we can estimate by stage:

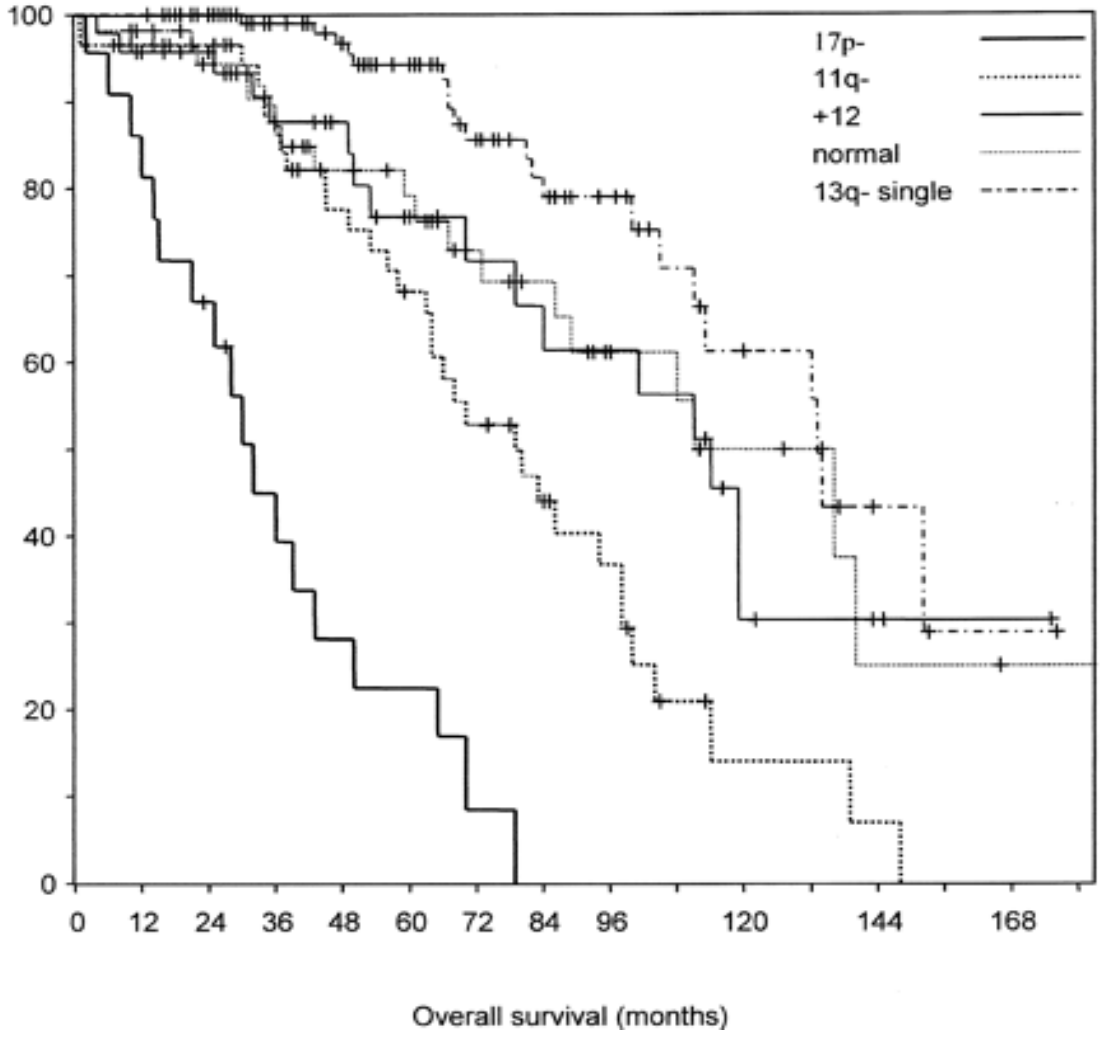
Rai:	0	> 10 years
	I - II	5 - 7 years
	III - IV	1 - 3 years

- unfortunately, limited information value for a **particular patient**:
  - the course of the disease in patients of the same stage differs
  - approximately 80% of patients diagnosed as early stage (0 - I)

Prognostic factors

prognostic factor:		**median survival (years):**
BM infiltration	difuse no x yes	10 x 3 - 5
doubling time lymfo	> 12 months x < 12 months	10 x 5
genetic abnormality (FISH):		
• normal karyotyp		~ 10
• del 14q		3 - 4
• del 17q		1.5 - 2
• multiple aberations		5 – 6
mutate status IgV_H	mutate x unmutate	7 - 8 x 10 - 20

Influence of cytogenetics on survival



median survival (months):

17p-	**30**
11q-	**79**
+12	**114**
normal	**111**
13q-	**133**

Byrd JS, Hematology, 2004

CLL therapy

- patients with low stage are not treated, they only follow up
- lymphocytosis alone in the blood count is not a reason to initiate therapy

- **indications for initiation of treatment:**
  - general symptoms
  - bone marrow failure (anemia, thrombocytopenia)
  - massive adenomegaly, splenomegaly
  - short lymphocyte doubling time (LDT <6 months)

LDT = lymphocyte doubling time

CLL modern therapy

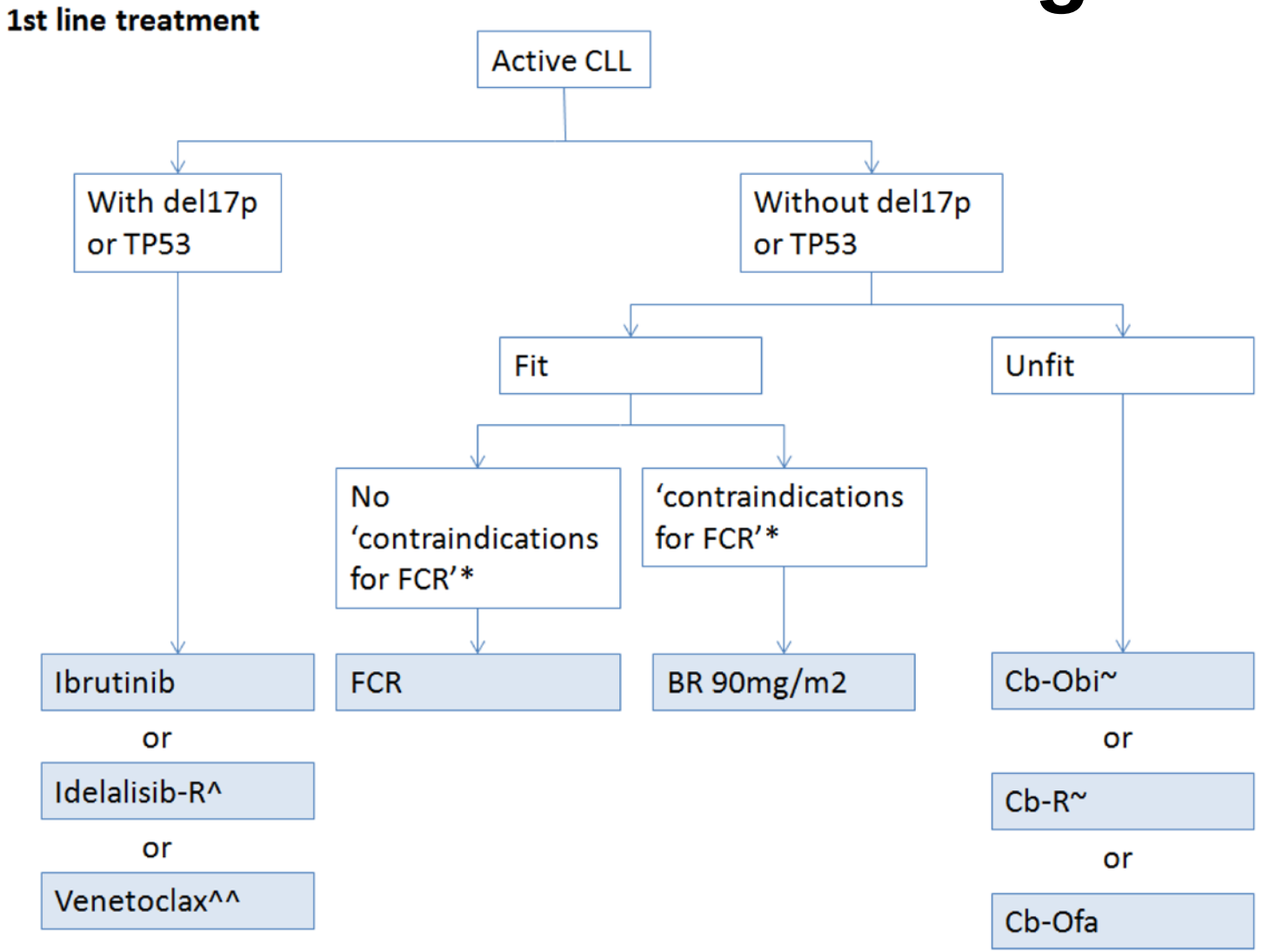
- **monoclonal antibodies:** alemtuzumab (anti-CD52) and rituximab (anti-CD20), ofatumumab, obinutuzumab (anti-CD20)

- **chemo- immuno therapy:**
  - FCR protocol: fludarabine + cyclophosphamide and rituximab
  - BR protocol: bendamustine + rituximab
  - chlorambucil + anti-CD20 antibody

- **new molecules / inhibitors:**
  - BCR signaling inhibitors (ibrutinib, idelalisib)
  - inhibitor of anti-apoptotic protein bcl-2 (venetoclax)

BCR = B cell receptor

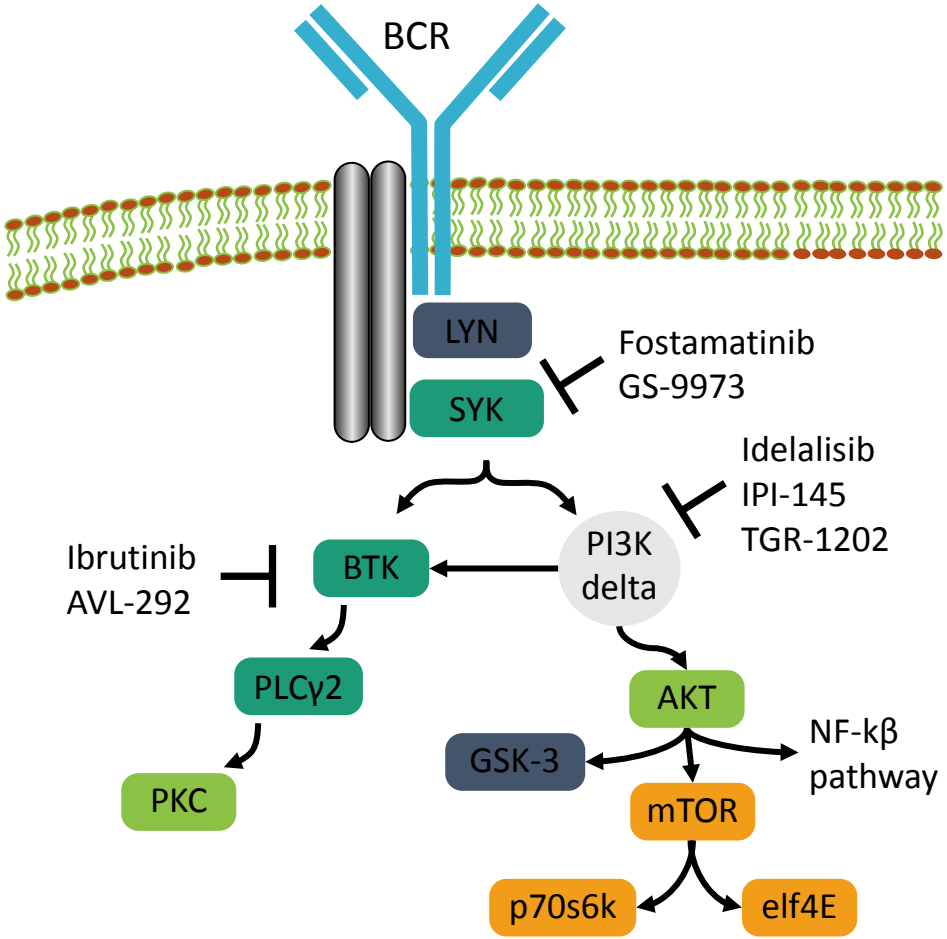
CLL treatment algorithm



Cb = chlorambucil, Obi = obinutuzumab,
Ofa = ofatumumab, R = rituximab

Kersting S, Clin Lymphoma Myeloima Leuk 2017

Interference with BCR signaling in CLL



- BCR-associated kinases are targets of novel inhibitors that are used in therapy
  - BTK inhibitors (Bruton kinase): ibrutinib PI3δ kinase inhibitors: idelalisib
  - Syk inhibitors: under development

BCR, B-cell antigen receptor; BTK, Bruton's tyrosine kinase; GSK-3, glycogen synthase kinase 3; mTOR, mammalian target of rapamycin; NF-kβ, nuclear factor kappa-light-chain-enhancer of activated B cells; PI3K, phosphatidylinositide 3-kinases; PKC, protein kinase C; PLC, phospholipase C; Syk, spleen tyrosine kinase.

Evaluation of response to treatment

classical:

- disappearance of clinical signs, adenomegaly
- normal blood count
- bone marrow histology

} **CR, PR**

today extra:

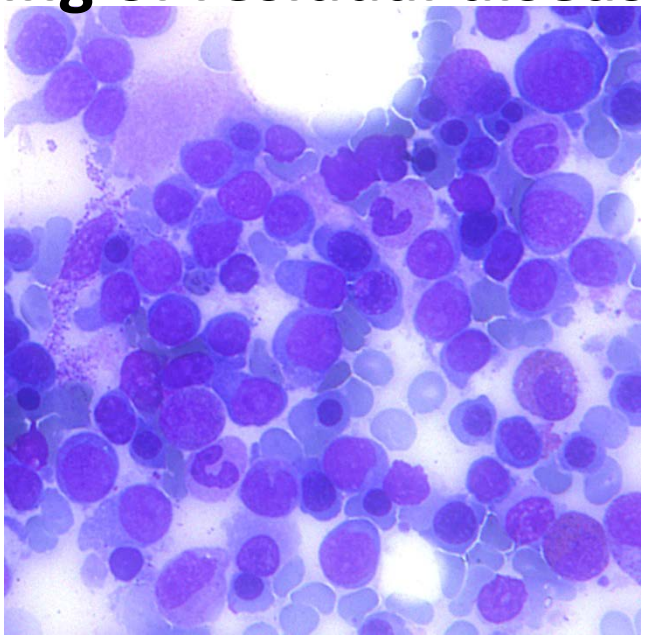
- flow cytometry 10^{-3} - 10^{-4}
- molecular genetics 10^{-4} - 10^{-5}

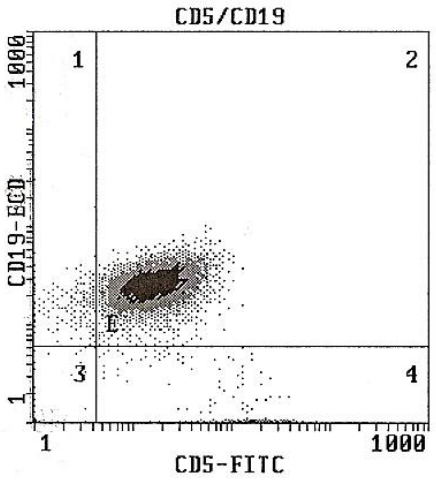
CR = complete remission, PR = partial remission etc..

Monitoring of residual disease - cytometry

2/2009: 3.progression

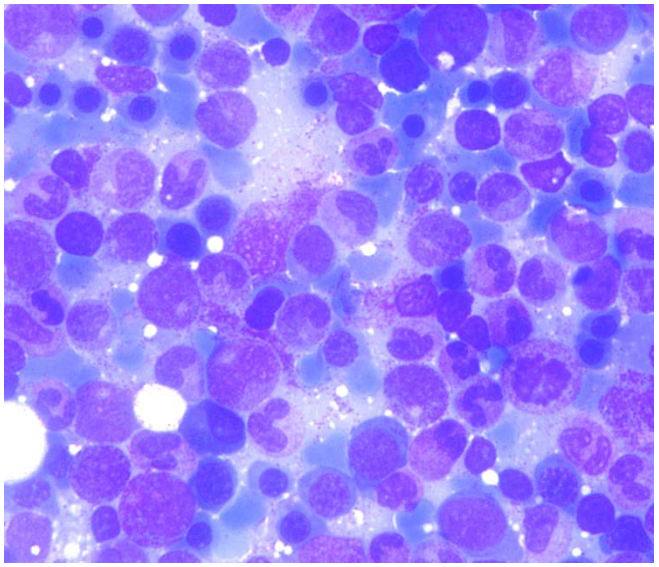
23,50
4,62
140
0,412
89
30,4
341
16,6
153

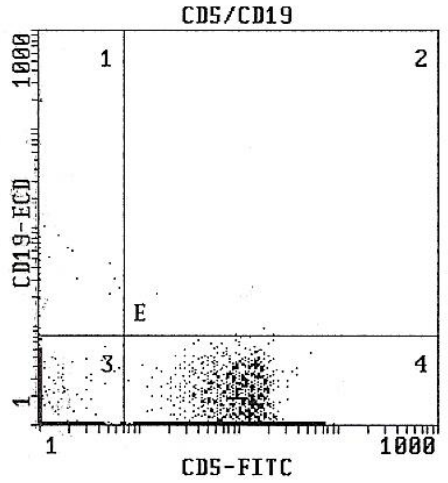




2/2010: remission after alogeneic Tx

7,30
4,36
137
0,410
94
31,4
334
17,9
165





YES! immunophenotypic remission was achieved

Complications of CLL

- **Richter transformation:**
  - transition to another type of lymphoma - most commonly diffuse large cell lymphoma, rarely Hodgkin's lymphoma
  - it occurs in about 2 - 10% of patients
- **autoimmune cytopenia**:
  - autoimmune hemolytic anemia (AIHA)
  - immune thrombocytopenia (ITP)
  - pure red cell aplasia (PRCA)
- **secondary tumors**:
  - a higher risk of developing tumors is due to abnormalities of immune function in CLL but also to treatment and immunosuppression
- **defect of cellular and humoral immunity**:
  - recurrent infectious complications
  - bacterial, viral (herpes), fungi
  - immunosuppression caused by treatment

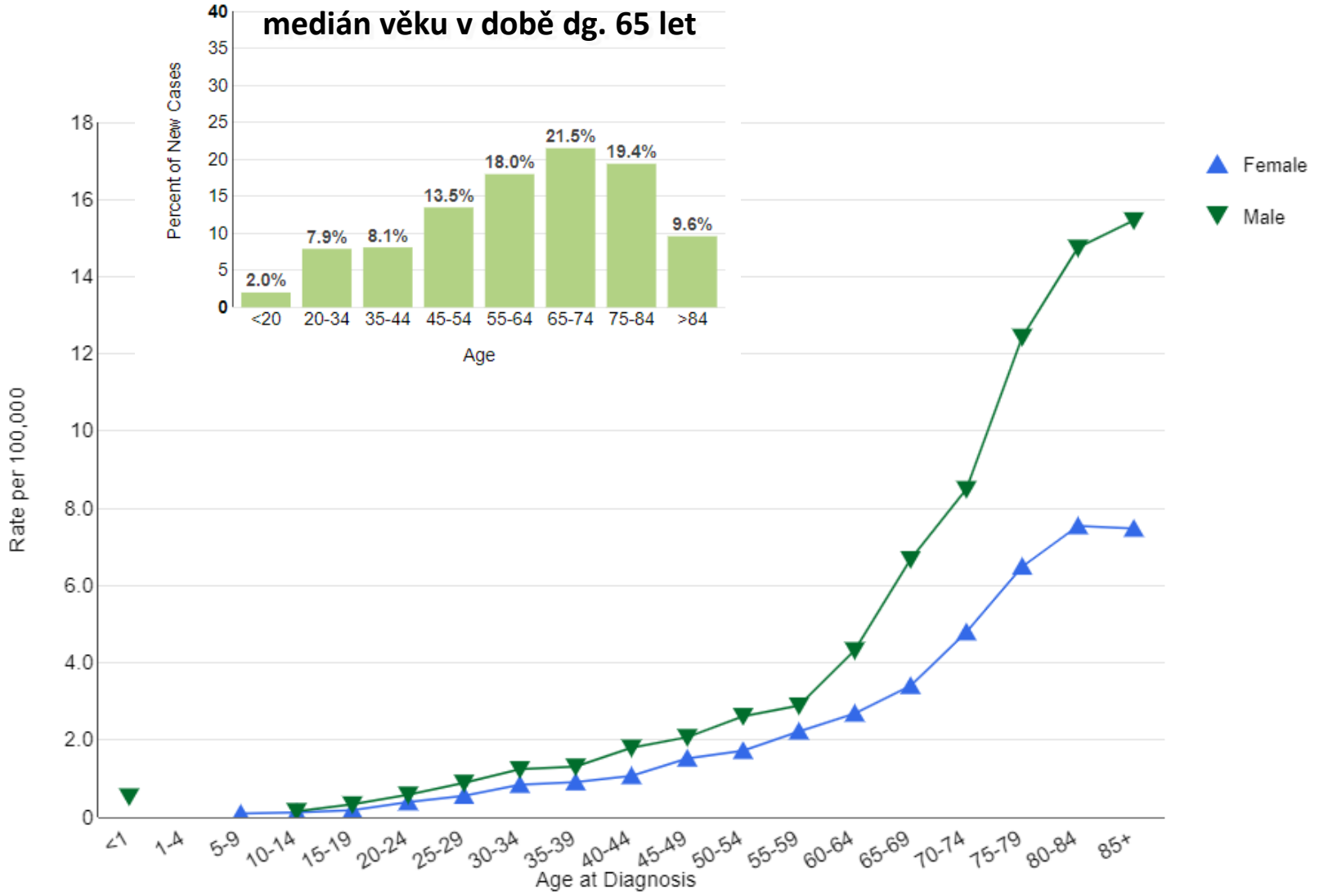
CHRONIC MYELOID LEUKEMIA

Introduction

- incidence of 1 - 2/100 000 people
- typically 3 phases:
  - chronic (4 - 6 years)
  - accelerated (less than 1 year)
  - blastic (several months… inevitable death)

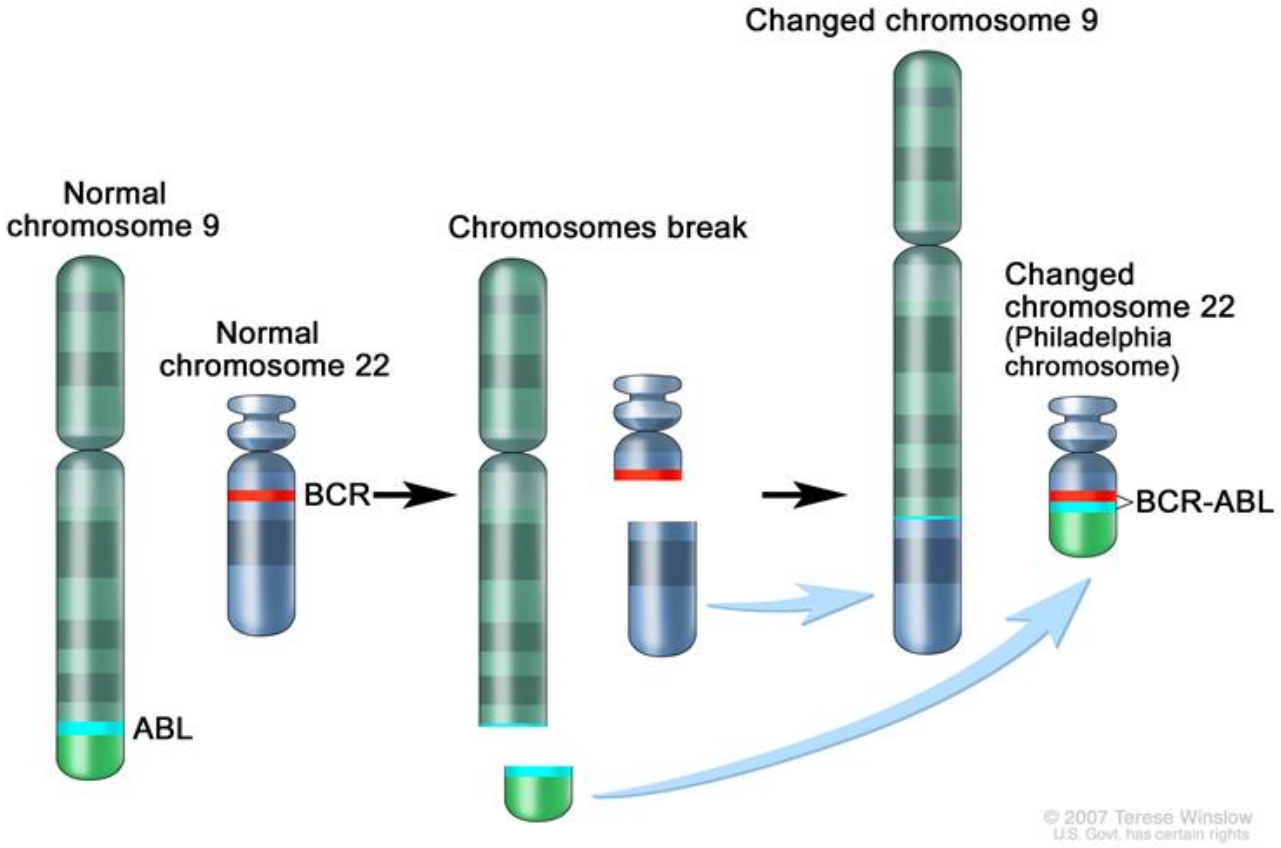
- a clonal disease affecting a pluripotent stem cell
- malignant clone is characterized by the presence of Philadelphia chromosome (Ph-chromosome)
- Ph-chromosome is present not only in the myeloid series, but also erythro, mono, lympho

Age distribution of patients with CML



data seer.cancer.gov 2012 - 2016

Philadelphia chromosome



- arises from reciprocal translocation of chromosomes 9 and 22
- translocation ➡ fusion of breakpoint cluster region (BCR) gene to chr. 22 with the ABL gene located on chr. 9

  ➡ results in a **bcr-abl fusion gene** and protein with tyrosine kinase activity

BCR-ABL tyrosine kinase

- **Abl tyrosine kinase**
  - acts as a catalyst for the transfer of phosphate groups from ATP to the tyrosine series of proteins
  - catalyzing the transfer of energy to the protein
  - regulates cell proliferation and differentiation

- **Bcr - Abl kinase**
  - significantly activated Abl gene
  - strong energy transfer from ATP to other proteins
  - growth advantage of leukemia clone

Clinical feature - chronic phase

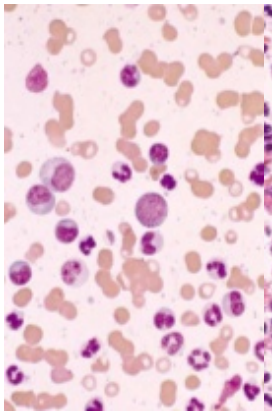
- weight loss, night sweats
- splenomegaly (pressure in the left lower ribs)
- Hepatomegaly
- often a random finding (blood donors, preoperative exam.)
- rarely leukostasis or thrombosis:
  - cerebrovascular accidents
  - priapism
  - venous thrombosis
  - heart-attack ….

Diagnosis of CML

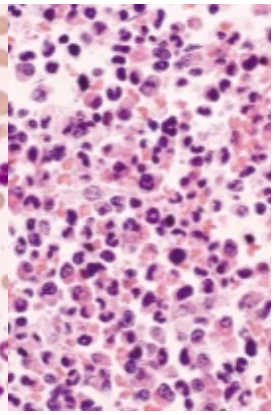
Sensitivity

Hematology | **Cytogenetics** | **Mol. gen.**

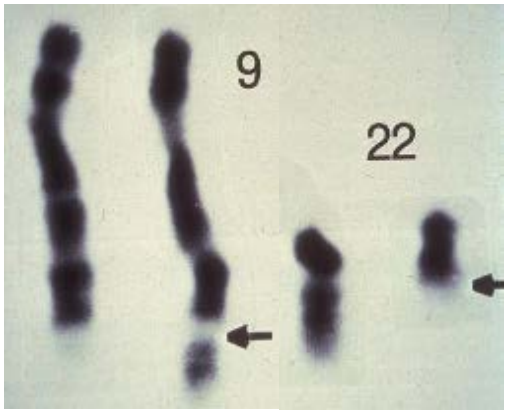
Karyotype (Ph chromosome) | FISH | PCR (BCR-ABL fusion)



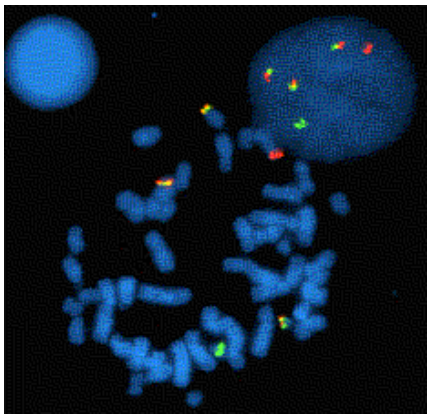
Peripheral blood (with myeloid cells)



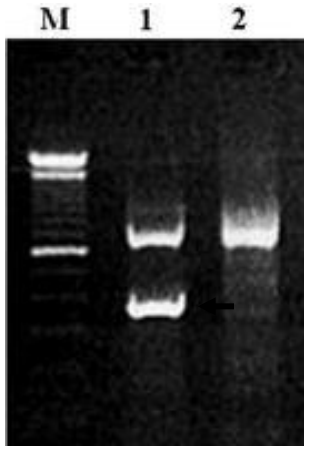
Bone marrow (with myeloid hyperplasia)



Chromosomal translocation t(9;22)(q34;q11)



Abnormal BCR-ABL
Red: BCR
Green: ABL
Yellow: fusion



Abnormal BCR-ABL
Lane 1: BCR-ABL+
Lane 2: BCR-ABL-

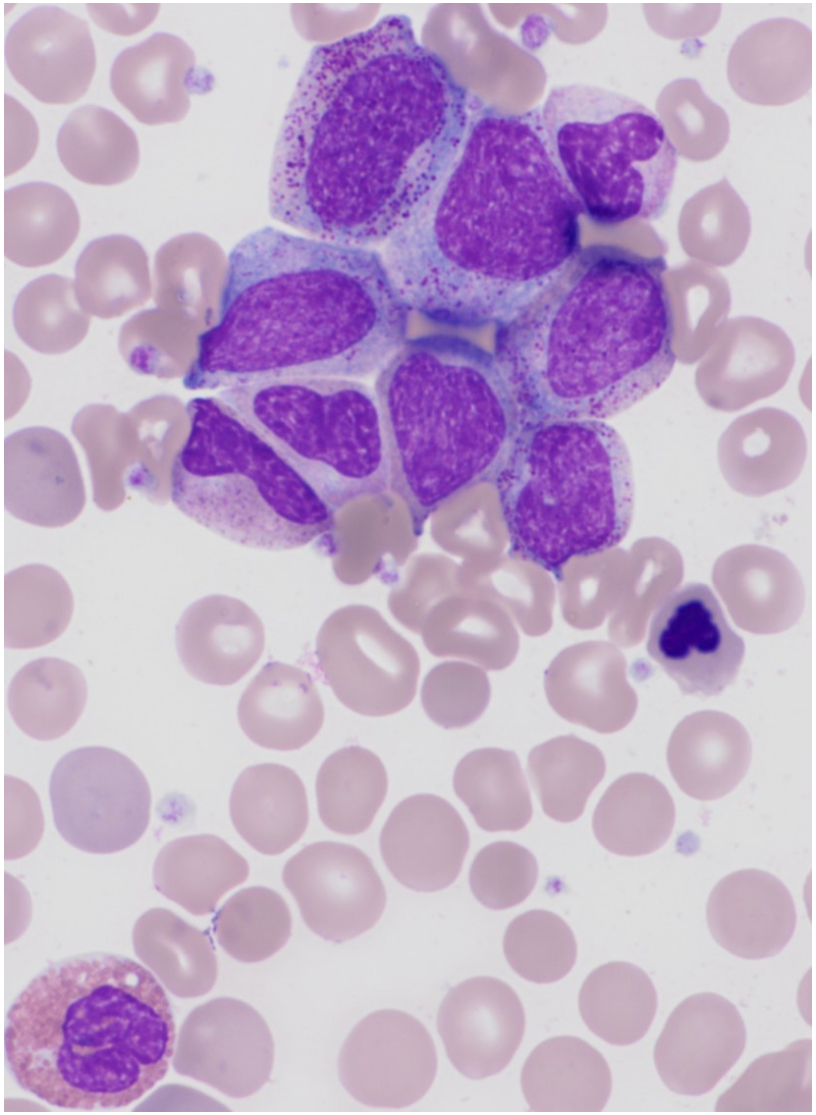
Blood count

Krevní obraz			
B--Le	320,30	4 - 10	10^9/l
B--Hb	106	130 - 170	g/l
B--HTK	0,313	0,32 - 0,53	1
B--Obj ery.	91	80 - 98	fl
B--Hb ery	31,0	26 - 32	pg
B--Hb konc	339	320 - 360	g/l
B--Trombo	272	150 - 400	10^9/l
Dif mikr.			
B--Seg	0,34	0,5 - 0,75	1
B--Tyc	0,28	0,01 - 0,031	1
B--Ly	0,06	0,25 - 0,4	1
B--Mo	0,02	0,03 - 0,08	1
B--Eo	0,04	0,01 - 0,03	1
B--Ba	0,04	0 - 0,01	1
B--MMc	0,06	0 - 0	1
B--Mc	0,13	0 - 0	1
B--PMc	0,01	0 - 0	1
B--Nbl	2/100	0 - 0	1

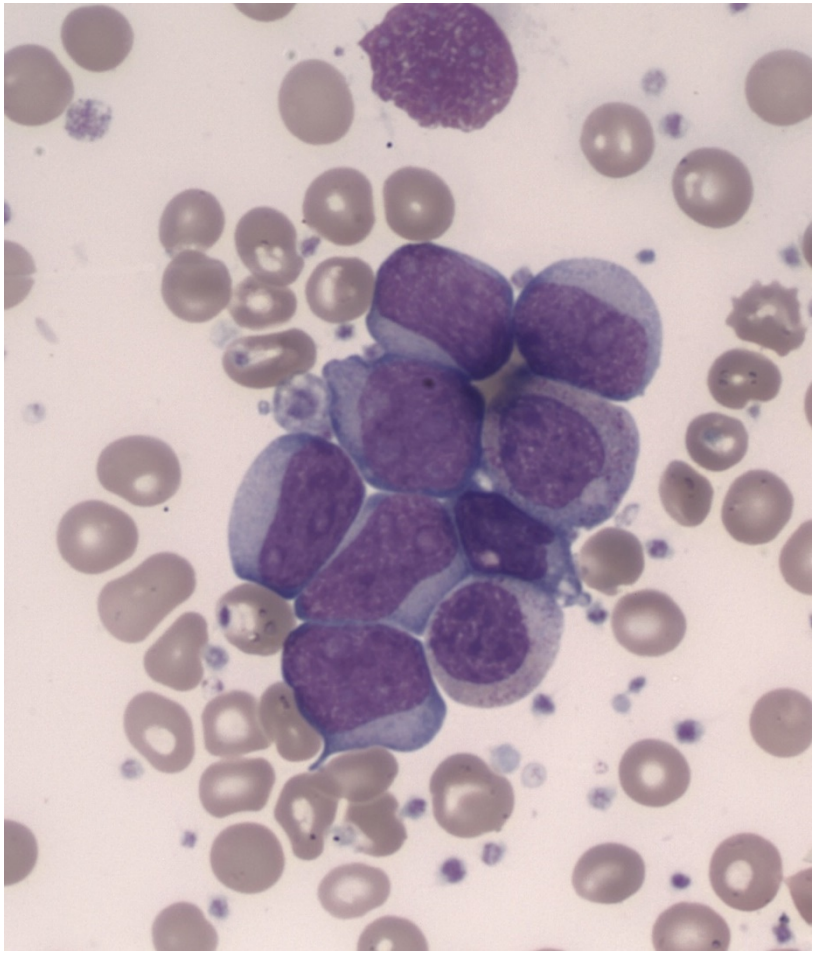
Krevní obraz			
B--Le	45,40	4 - 10	10^9/l
B--Ery	4,21	3,5 - 5,6	10^12/l
B--Hb	125	130 - 173	g/l
B--HTK	0,379	0,42 - 0,53	1
B--Obj ery.	90	80 - 98	fl
B--Hb ery	29,8	26 - 35	pg
B--Hb konc	331	310 - 370	g/l
B--Erytr.křivka	15,8	11,6 - 15,2	%
B--Trombo	727	150 - 400	10^9/l
Dif mikr.			
B--Seg	0,56	0,47 - 0,7	1
B--Tyc	0,12	0 - 0,04	1
B--Ly	0,05	0,2 - 0,45	1
B--Mo	0,01	0,02 - 0,1	1
B--Eo	0,03	0 - 0,07	1
B--Ba	0,06	0 - 0,02	1
B--MMc	0,03	0 - 0	1
B--Mc	0,11	0 - 0	1
B--PMc	0,01	0 - 0	1
B--Blasty	0,02	0 - 0	1
B--Nbl	1/100	0 - 0	1

- leukocytosis, in budget all developmental stages of granulocytes
- increased platelet count
- often higher basophils, sometimes mild anemia

Peripheral blood smear

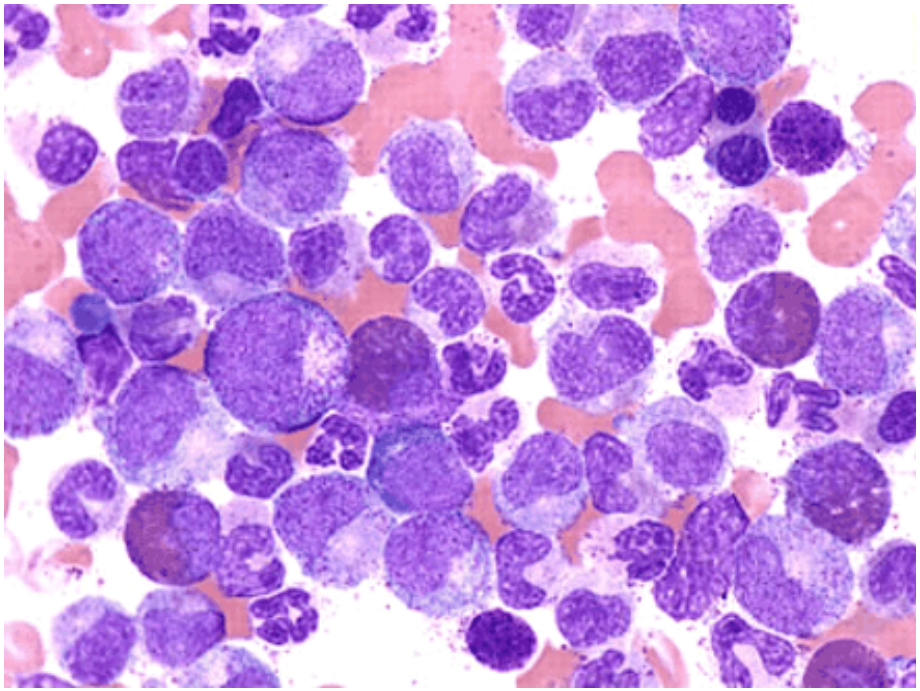


chronic phase

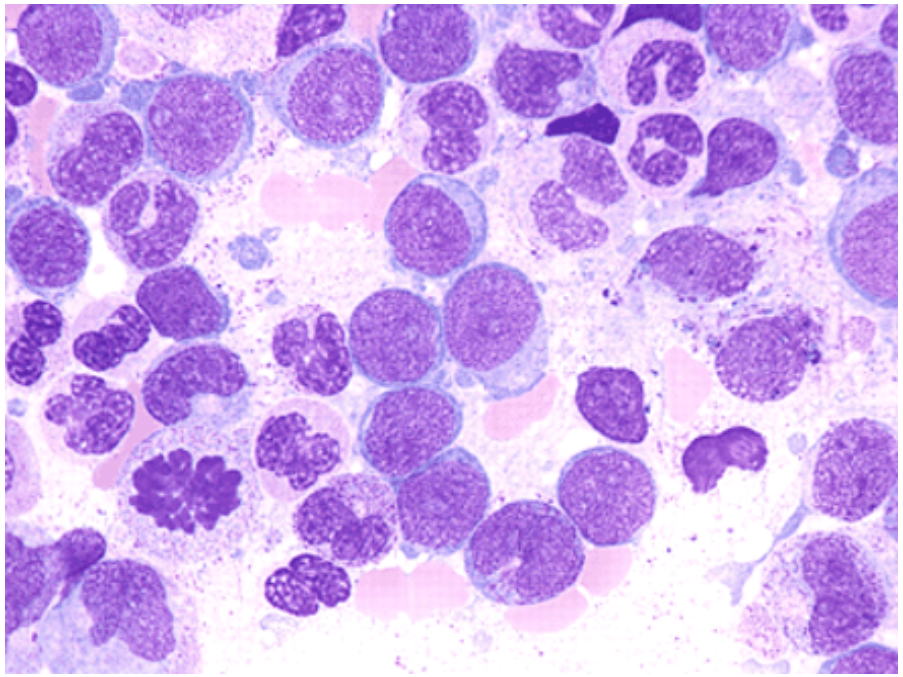


blastic phase

Bone marrow smear

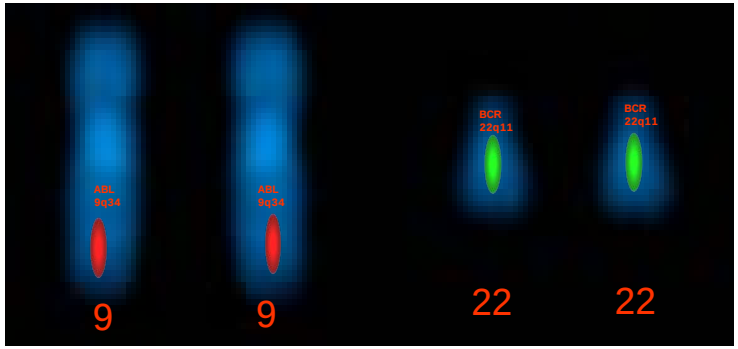


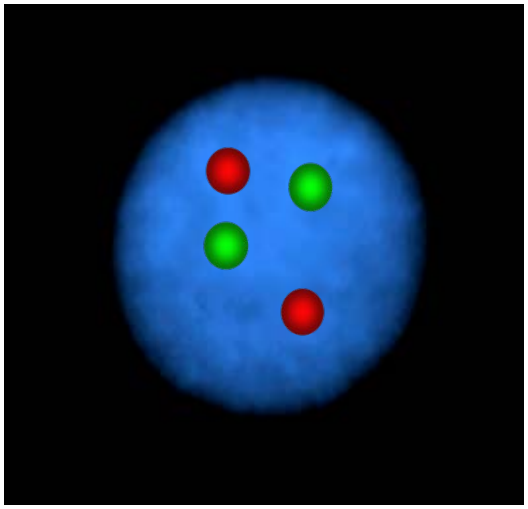
Chronic phase



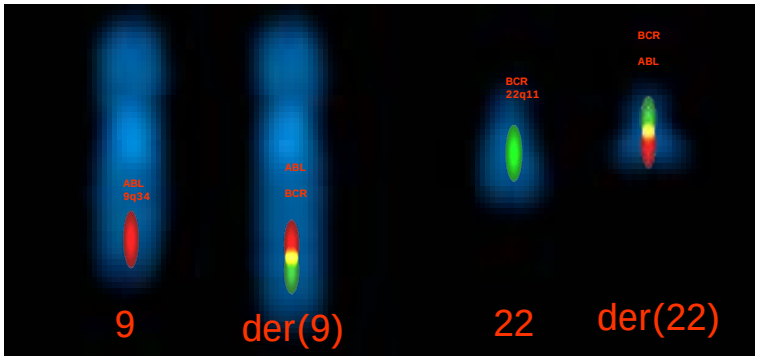
Blastic phase

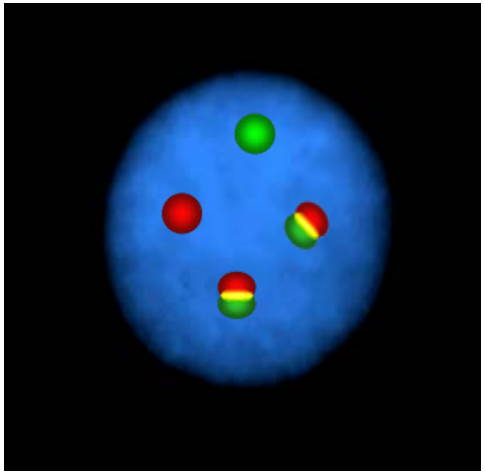
FISH
Fluorescent *In Situ* Hybridisation





Normal signal





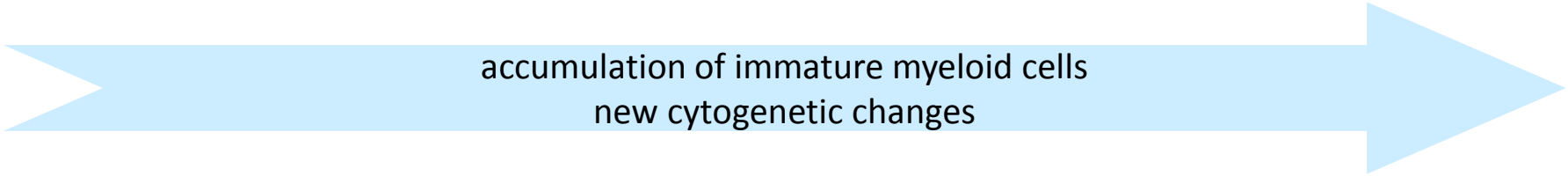
BCR/ABL

dual fusion

t(9;22)(q34;q11)

Abnormal signal

Natural development of CML



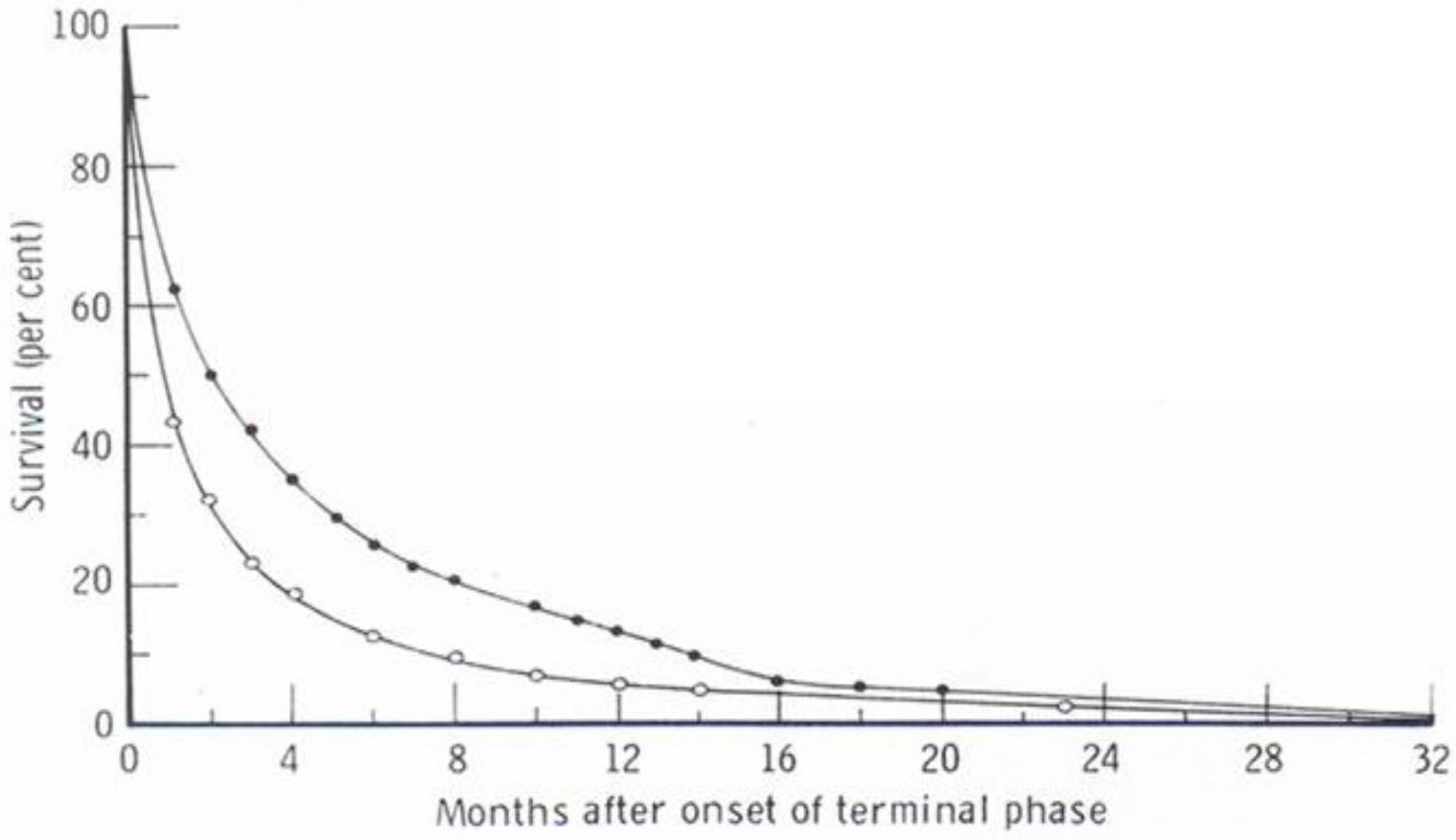
	Chronic phase	**Akcelerated phase**	**Blastic phase**
Duration	Not treated, 3-5 years	different	median survival in months
Prognosis	responds to treatment	decreased response to treatment	Resistant to treatment
Symptoms	asymptomatic **OR** fatigue, weight loss abdominal pain or discomfort night sweats	progressive splenomegaly myelofibrosis	haemorrhagic complications infectious complications

Akcelerated and blastic phase

- inevitable (no treatment), appears with a median of about 3 years after diagnosis (in untreated patients)

- the following symptoms may indicate progress:
  - progression of leukocytosis, ↑ blast count, cytogenetic clonal evolution
  - thrombocytosis or thrombocytopenia, anemia increasing painful splenomegaly and hepatomegaly
  - bone and joint pain
  - thrombotic or bleeding complications
  - increasing dose of drugs used to control the disease

Blastic phase survival



Karanas A, Silver RT. Blood. 1968

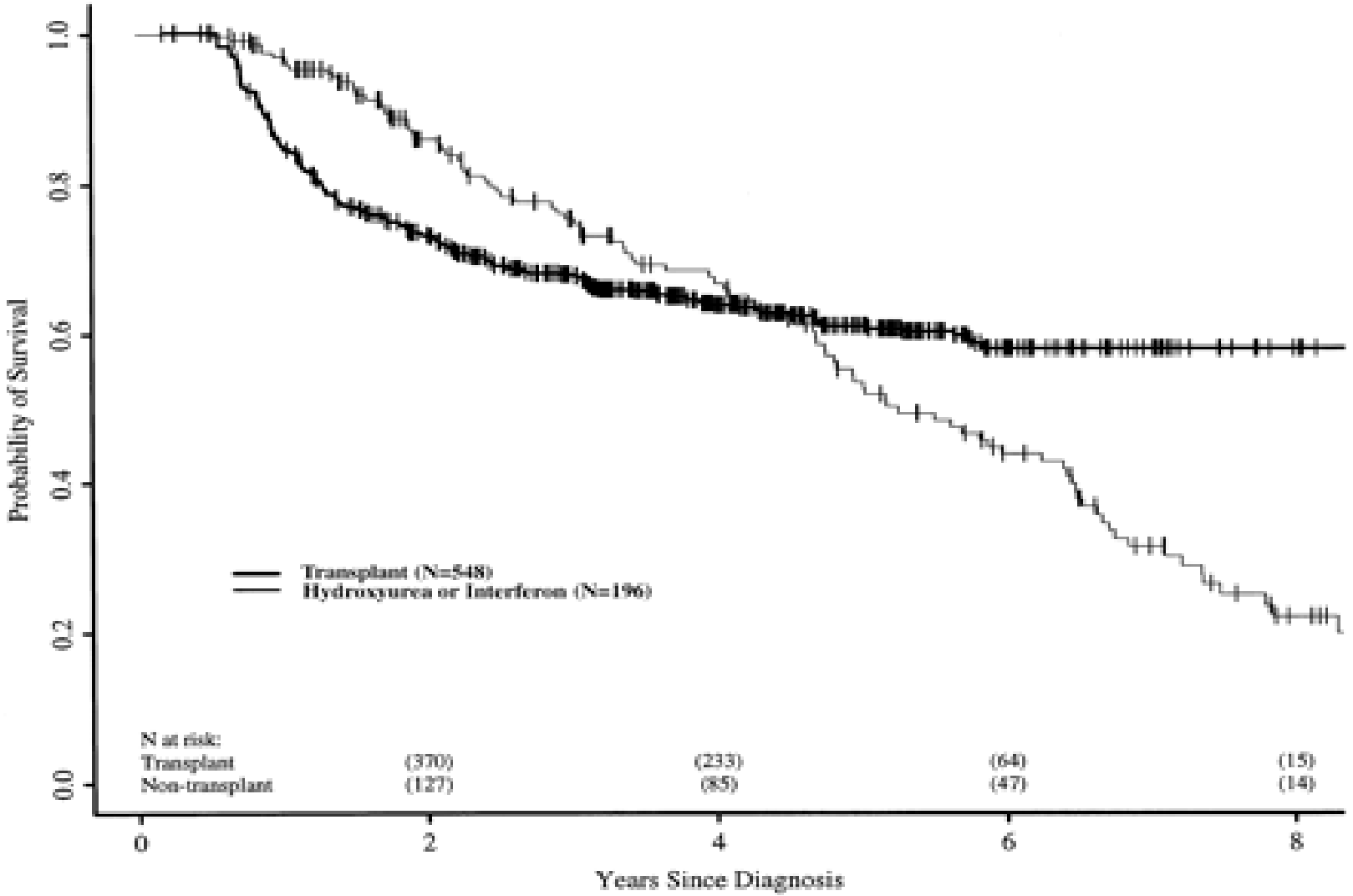
Treatment

- until 2000, the treatment options were as follows:

- **non-curative:**
  - busulfan, hydroxyurea, interferon

- **curative:**
- allogeneic bone marrow transplantation
- 30 - 60% transplant mortality

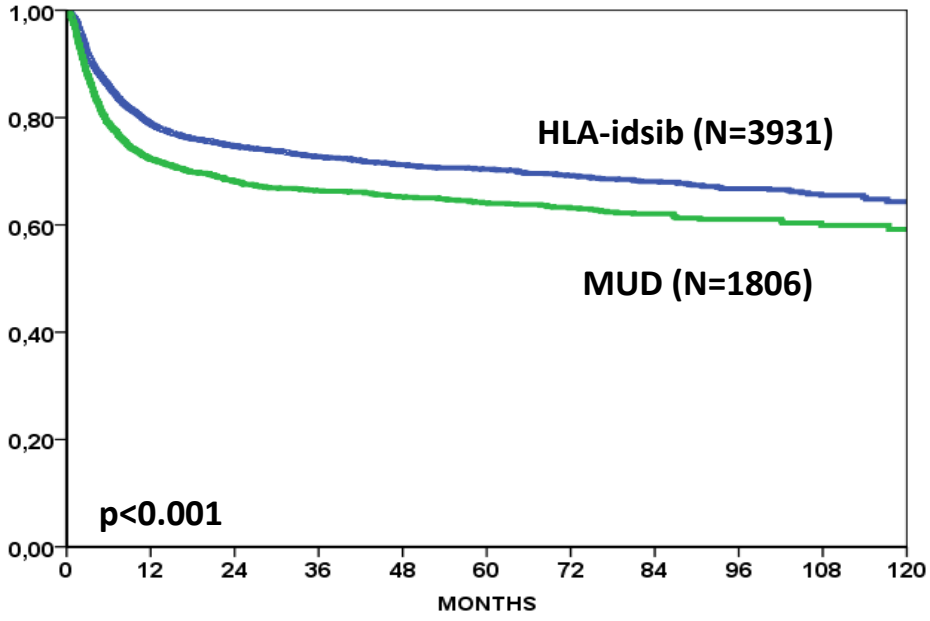
Survival of patients treated with transplantation or hydroxyurea / interferon



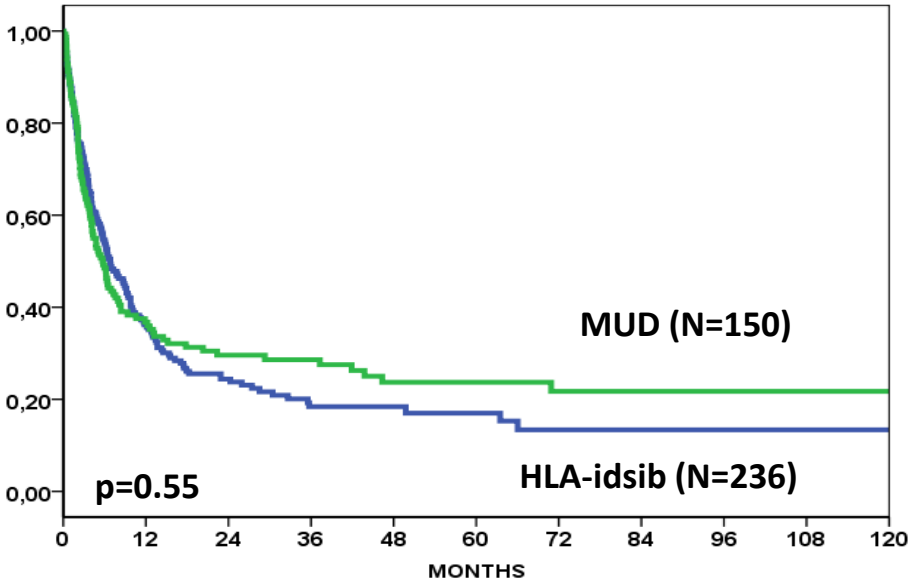
RP Gale, Blood 1998

Survival of patients after alloTx (1997-2008)

chronic phase



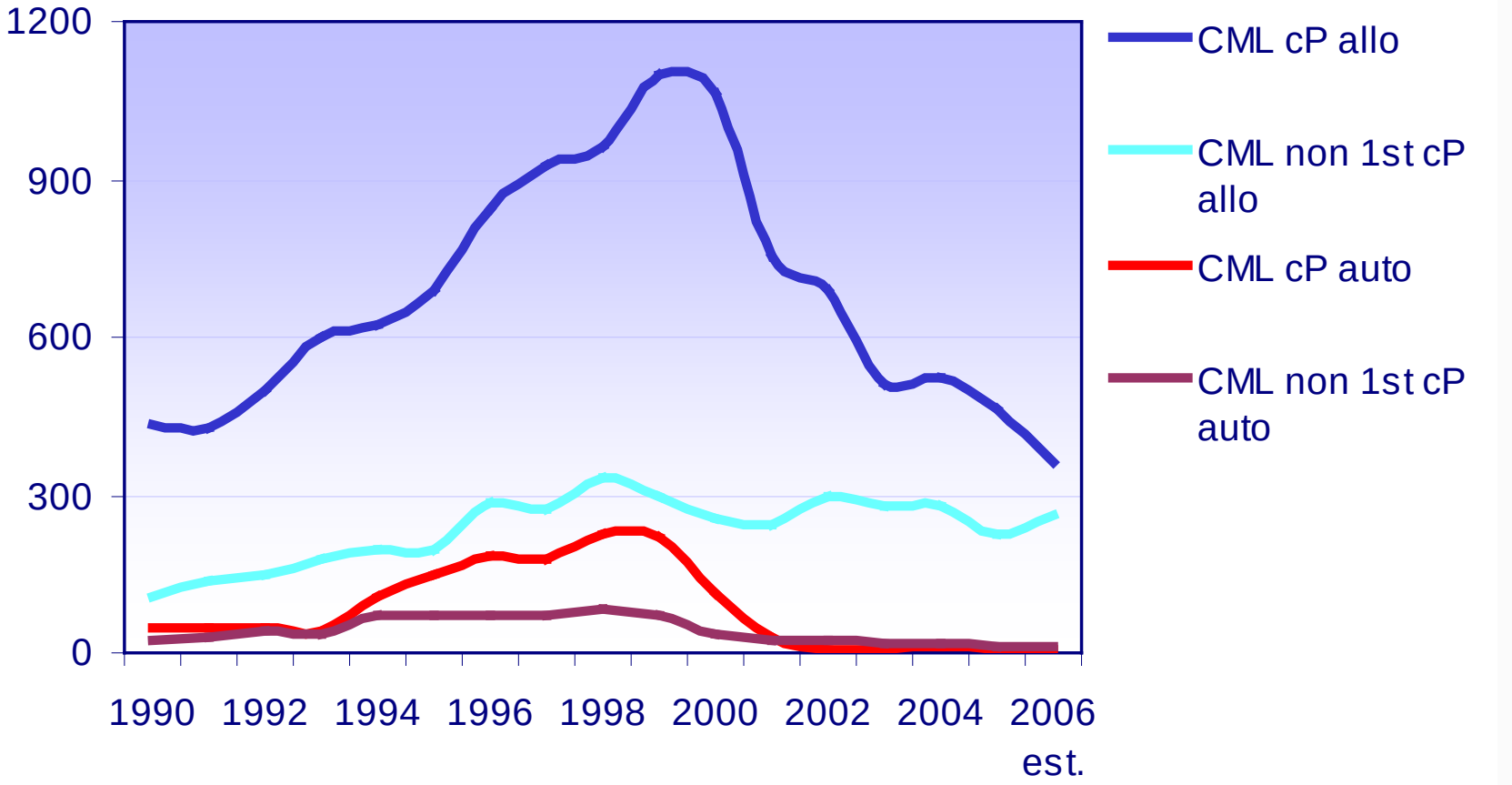
blastic phase



MUD = matched unrelated donor
HLA-idsib = HLA identical sibling

data www.EBMT.org

Development of CML transplantation treatment



before the Imatinib CML era the most common indication for allogeneic transplantation

Gratwohl A, Bone Marrow Transplant , 2006

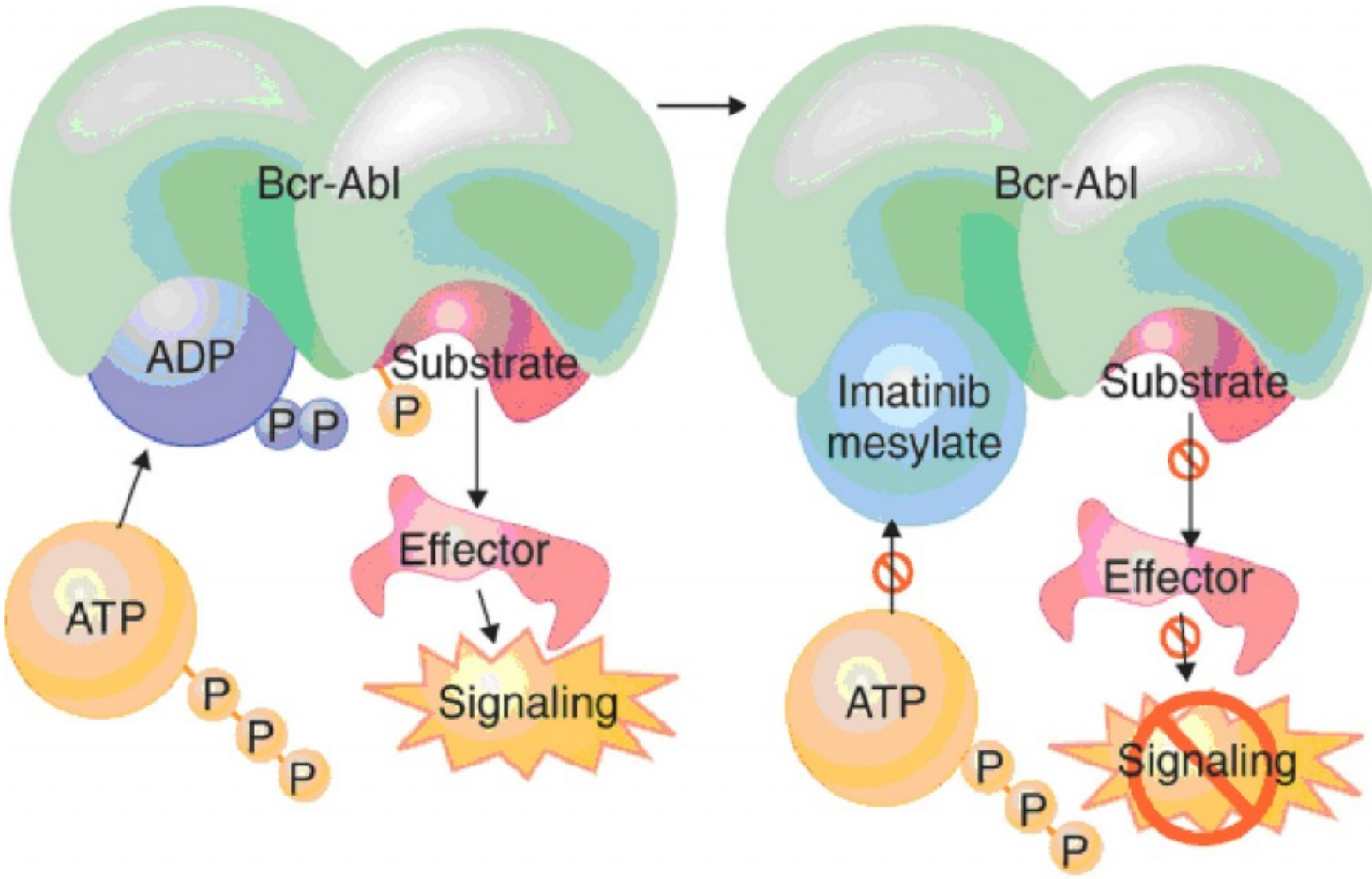
Inhibitors in the treatment of CML

tyrosine kinase inhibitors - first drug: imatinib (Gleevec)

- a tyrosine kinase antagonist with specific and potent inhibition of Abl tyrosine kinase
- specifically blocks its ATP binding site on the Abl kinase
- Thus, by releasing phosphate groups from ATP, Abl kinase cannot release energy and activate other proteins
- is a first-line drug for newly diagnosed CML

other inhibitors available today: nilotinib, dasatinib, ponatinib etc.

Imatinib inhibits ATP binding to abl tyrosine kinase



Biochemical pathways, Kohn Pharmaceuticals; https://kohnpharmaceuticals.weebly.com/

Monitoring of treatment

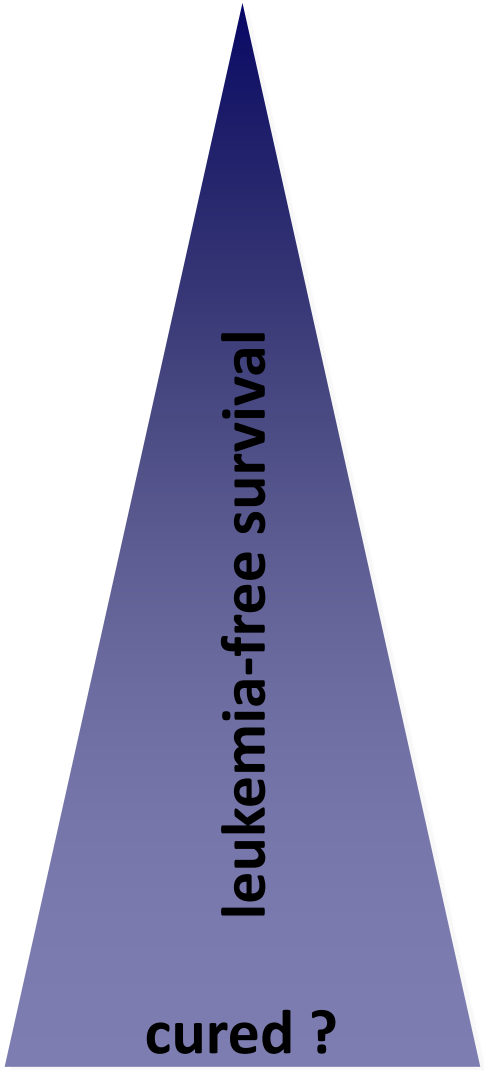
- **hematological parameters**:
  - leukocyte and platelet count, differential budget
  - BM aspiration - no signs of acceleration (blasts)
  - spleen size

- **cytogenetics:**
  - percentage of Ph-positive mitoses in bone marrow
  - FISH (sensitivity 1 in approx. 100 cores, 10-2)

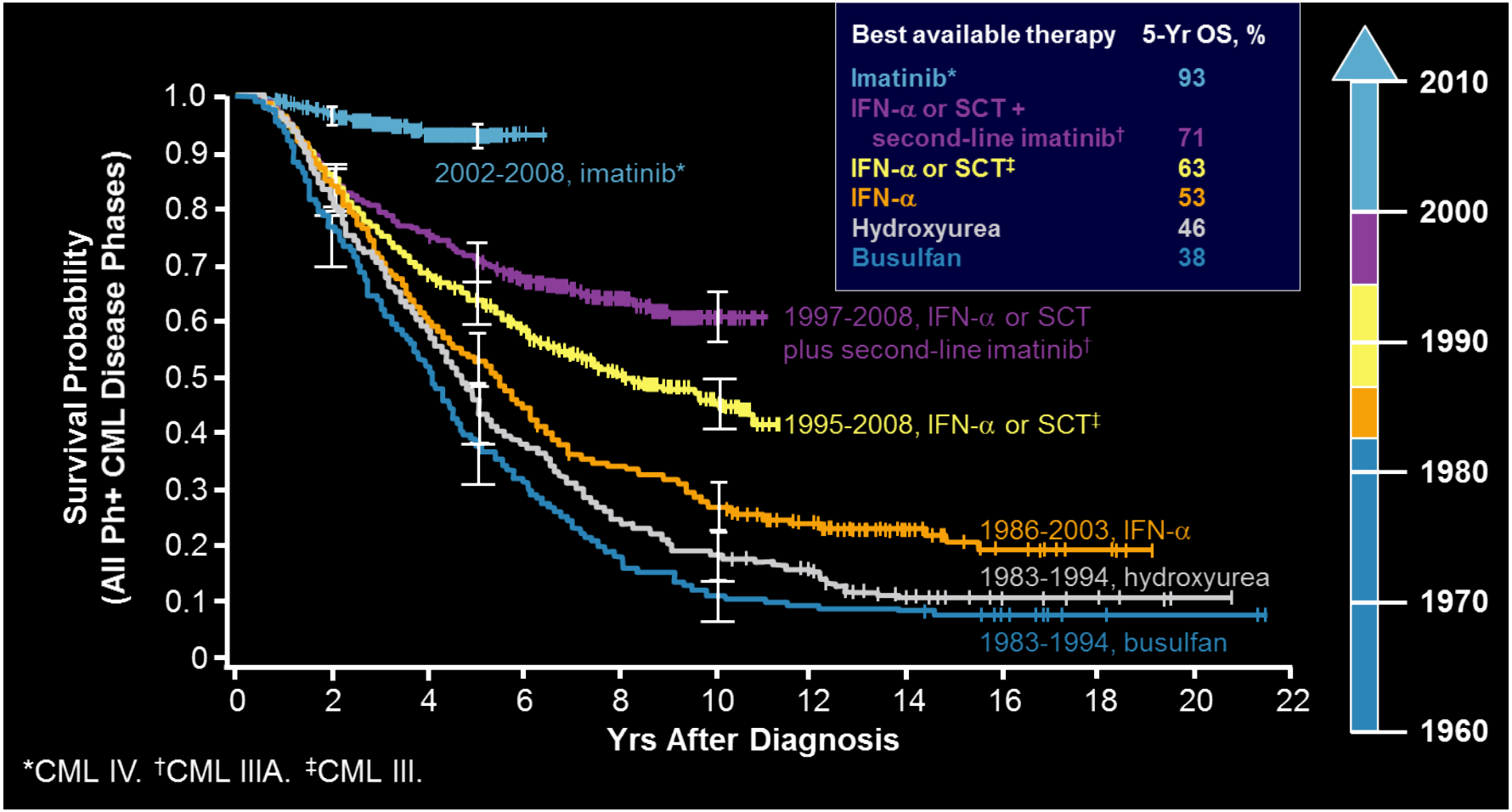
- **molecular genetics (quantitative-PCR)**:
  - number of BCR-ABL transcripts
  - sensitivity 10-6

Course and objectives of treatment

- **haematological remission**
  - normalization of blood count
- **cytogenetic remission**
  - Ph-chromosome disappearance
- **molecular genetic remission**
  - not detectable by BCR-ABL



Imatinib fundamentally altered CML therapy and its results



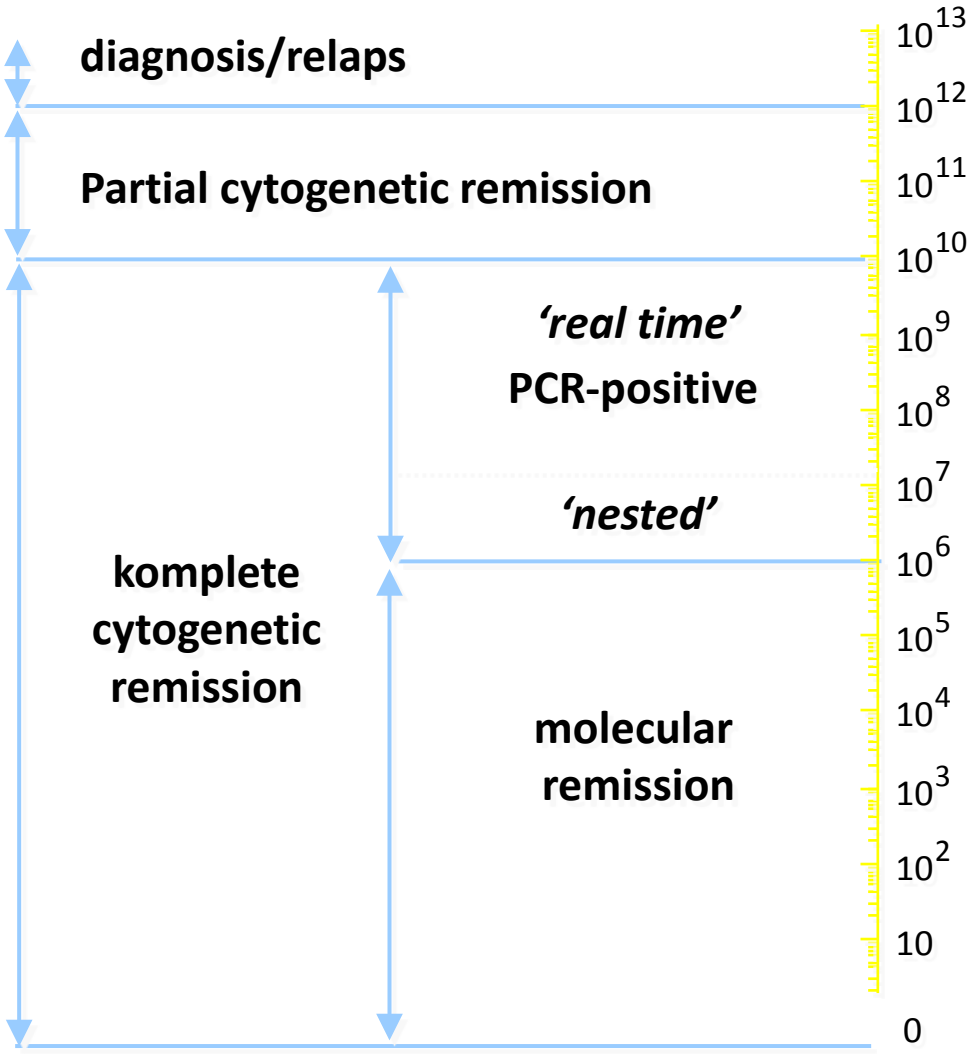
IFN-α, interferon-alpha; OS, overall survival; Ph, Philadelphia chromosome; SCT, stem cell transplantation

Survival by type of treatment

treatment	5-years survival
busulfan	40%
hydroxyurea	50%
interferon	60%
alogeneic transplant	> 60%
imatinib	90%

A 55-year-old patient diagnosed in 1980 had a probability of survival of about 4 years, with a diagnosis in 2010 of 30 years

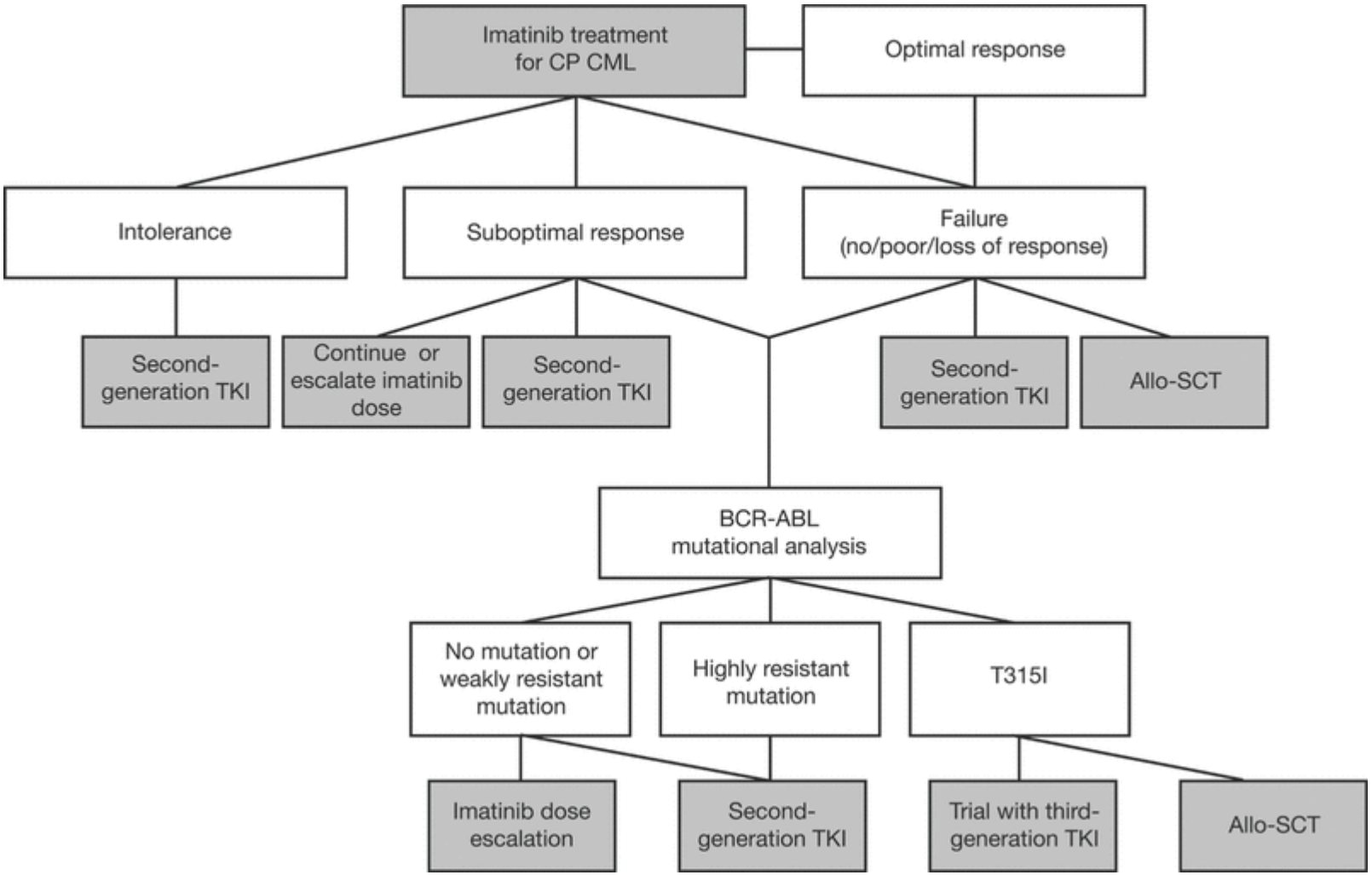
Development of tumor mass during treatment



total leukemia count cells

the goal of treatment is to achieve eradication of all BCR / ABL containing transcript = **molecular remission**

Treatment algoritm *(example)*



Assouline S, Current Oncology 2011

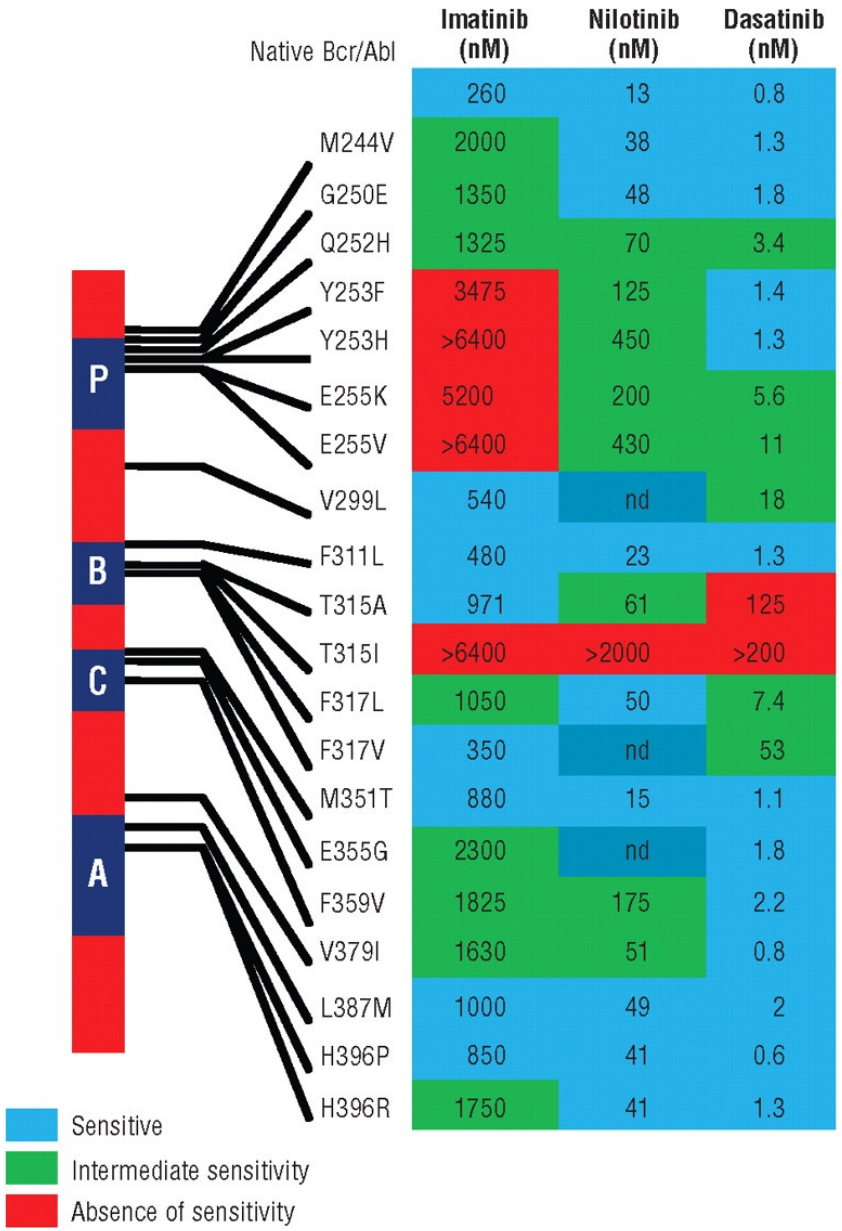
Definition of optimal treatment response

Time	Optimal response	Warning	Failure
Baseline		High risk Major route CCA/Ph+	
3 mos.	BCR-ABL^{IS} ≤10%* Ph+ ≤35% (PCyR)	BCR-ABL^{IS} >10%* Ph+ 36-95%	No CHR* Ph+ >95%
6 mos.	BCR-ABL^{IS} <1%* Ph+ 0% (CCyR)	BCR-ABL^{IS} 1-10%* Ph+ 1-35%	BCR-ABL^{IS} >10%* Ph+ >35%
12 mos.	BCR-ABL^{IS} ≤0.1%* (MMR)	BCR-ABL^{IS} 0.1-1%*	BCR-ABL^{IS} >1%* Ph+ >0%
Then, and at any time	MMR or better	CCA/Ph- (-7, or 7q-)	Loss of CHR Loss of CCyR Loss of MMR, confirmed** Mutations CCA/Ph+

- newly diagnosed CML - imatinib 400 mg / day
- if the optimal response is not achieved after 6 to 12 months - dose increase, second generation TKI or consideration of allogeneic transplantation

ELN: European Leukemia Net

IS = international scale



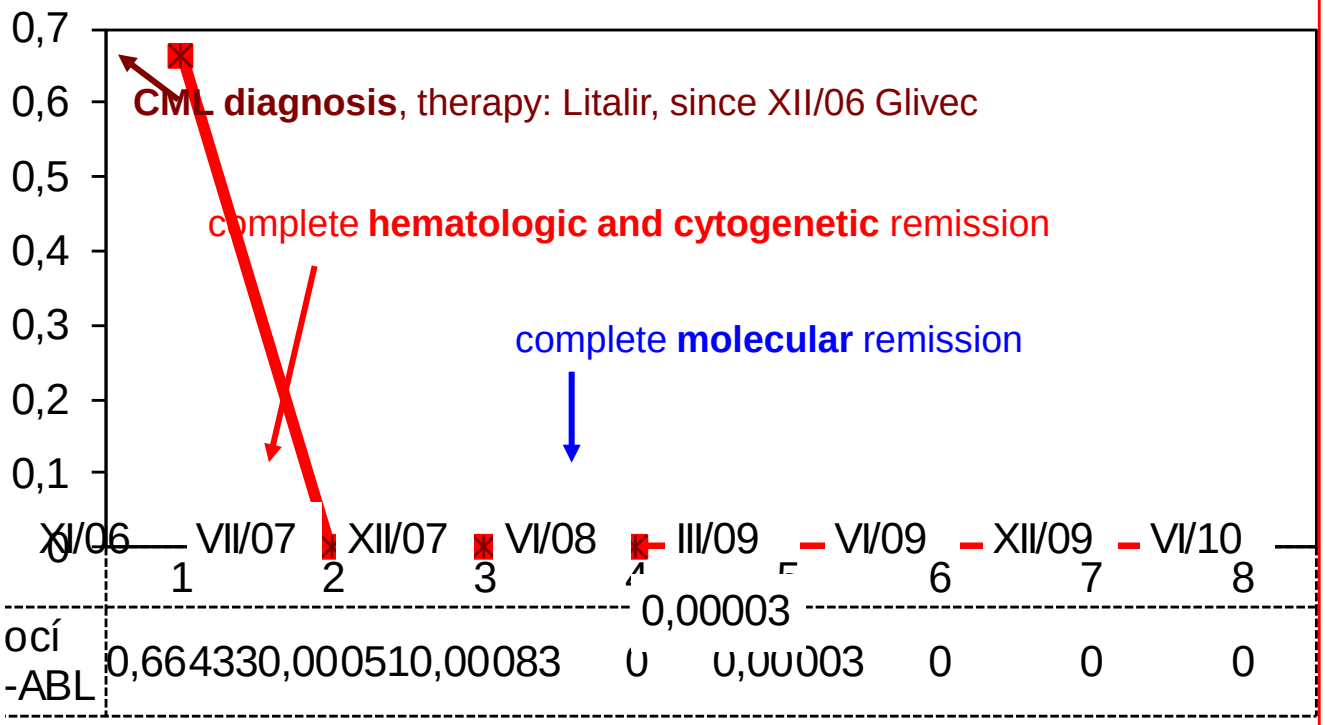
Imatinib Resistance

- some patients do not respond to treatment
- in others, long-term treatment leads to the emergence of resistance
- mutations of the BCR / ABL protein kinase domain responsible for resistance to inhibitors

Baccarani M. et al, Hematologica 2008

Case report I. - long-term response to treatment

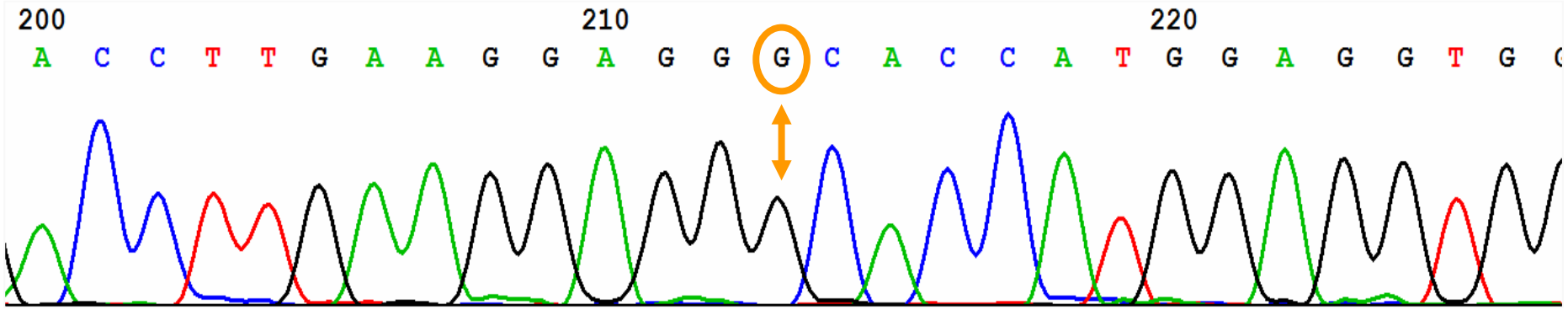
Relative quantification by RQ-PCR of the BCR-ABL fusion gene



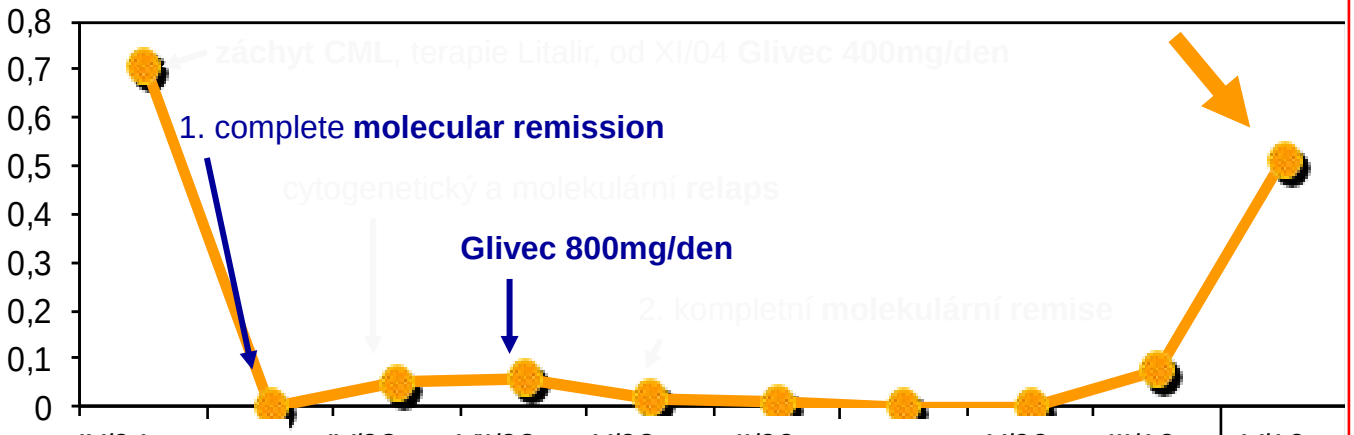
kit Ipsogen

ocí -ABL	0,66433	0,00051	0,00083	0	0,00003	0	0	0

Case report II. - relapse, imatinib resistance



Relative quantification by RQ-PCR of the BCR-ABL fusion gene

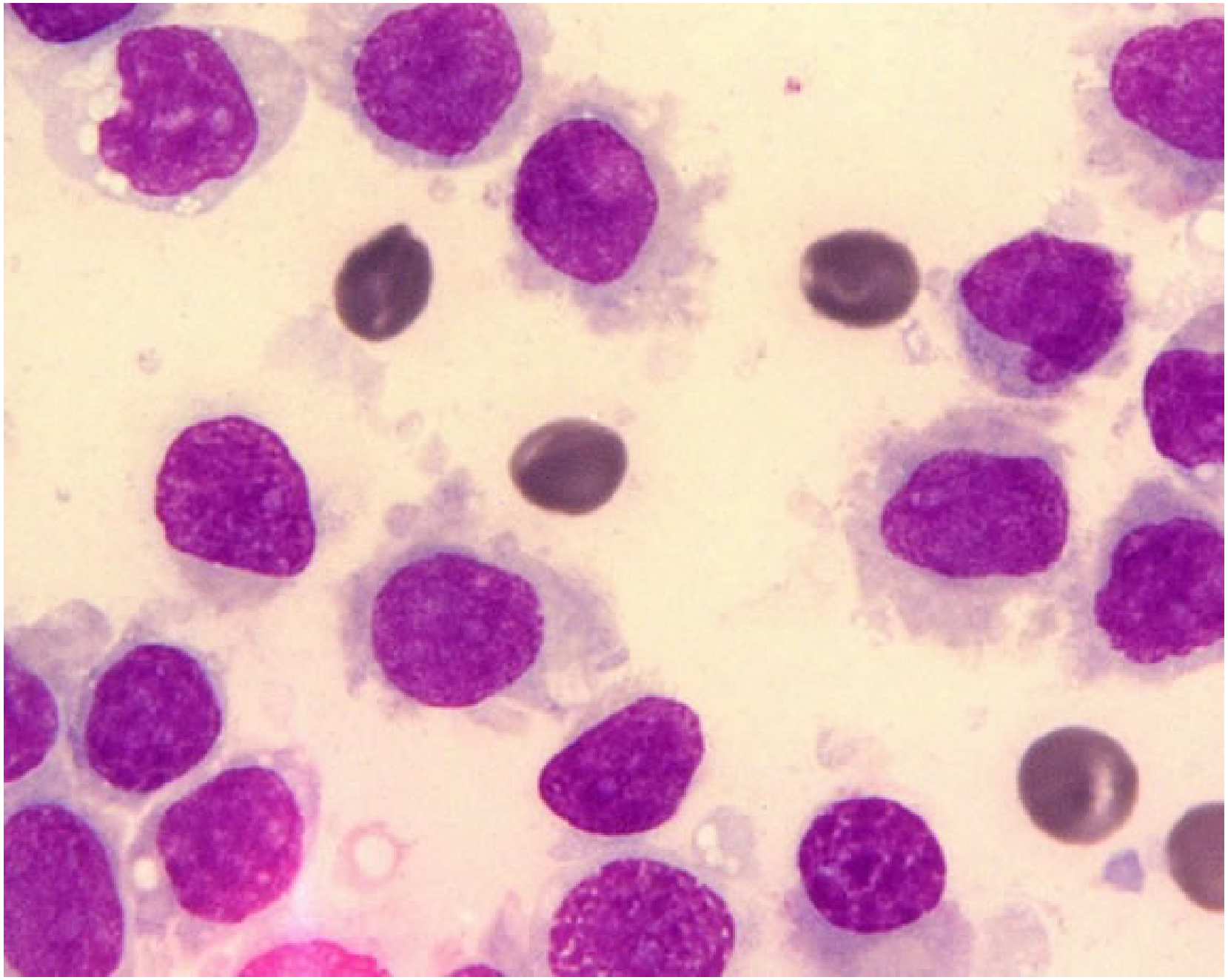


Relativní kvantifikace pomocí RQ-PCR kit Ipsogen	IX/04	X/05	IV/08	VII/08	X/08	II/09	VII/09	X/09	III/10	V/10
	0,70892	0,00028	0,0498	0,05945	0,0193	0,00775	0	0	0,077	0,5135

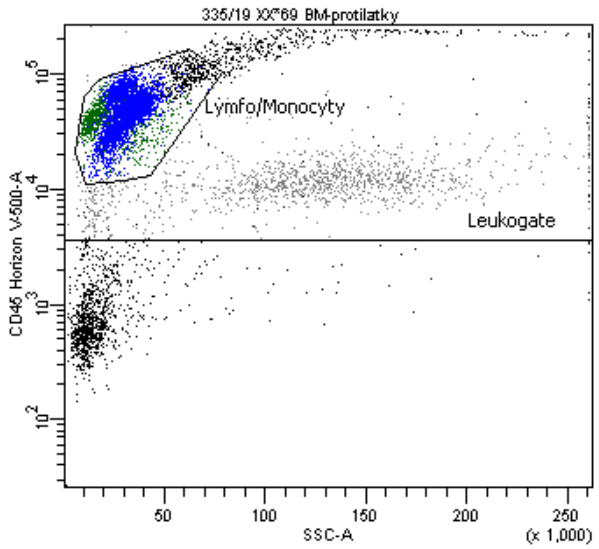
HAIRY CELL LEUKEMIA

Introduction

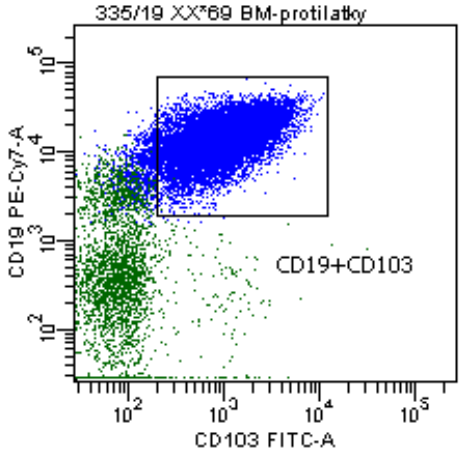
- mature B-cell neoplasia
- proliferation of pathological lymphoid cells with multiple cytoplasmic processes ➡ **hairy cell leukemia**
- tumor lymphocytes infiltrate the bone marrow and circulate in peripheral blood
- produce cytokines (TNF, ..), which contribute to pancytopenia synonyms: trichocellular leukemia, hairy cell leukemia
- median age of patients 50-55 years, more frequently affected men
- prognostically very favorable leukemia

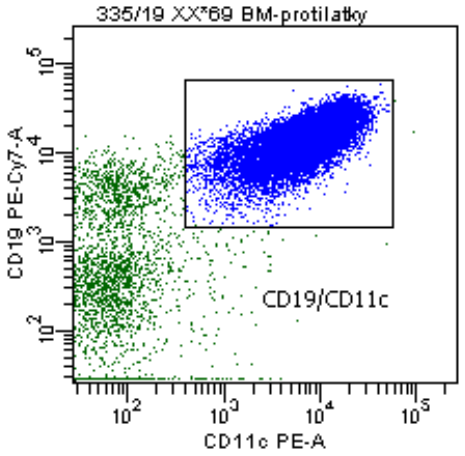


Laboratory findings



Krevní obraz				
B--Le	11,10	-	4 - 10	10^9/l
B--Ery	2,84	-	4 - 5,8	10^12/l
B--Hb	106	-	135 - 175	g/l
B--HTK	0,310	>	0,4 - 0,5	1
B--Obj ery.	109	-	82 - 98	fl
B--Hb ery	37,4	-	28 - 34	pg
B--Hb konc	343	-	320 - 360	g/l
B--Erytr.křivka	16,6	-	10 - 15,2	%
B--Trombo	88	-	150 - 400	10^9/l
B--shluky trombo	nejsou			
Dif mikr.				
B--Seg	0,02		0,47 - 0,7	1
B--Tyc	0,01		0 - 0,04	1
B--Ly	0,06		0,2 - 0,45	1
B--Eo	0,01		0 - 0,05	1
B--Ostatní buňky	0,90		0 - 0	1
B--Nbl	5/100		0 - 0	1





CD19+, CD103+, CD25+, … → B- NHL malignant lymphocytes are bigger than normal

Clinical feature

- symptomatology resulting from pancytopenia (anemic syndrome, bleeding symptoms)
- splenomegaly (pressure in the left lower ribs)
- less often hepatomegaly, adenomegaly
- general symptoms (temperature, sweating, weight loss)
- immunity disorder - febrile, infection

laboratory diagnostics:

- in the blood count pancytopenia
- bone marrow morphology (cytology and histology)
- immunophenotyping of tumor lymphocytes

Treatment

- formerly interferon alfa (long-term treatment)
- splenectomy rarely (resistant cases)
- now the drug of choice 2-CDA (2-chlorodeoxyadenosine, belongs to adenosine analogues)
- weekly treatment induces complete remission lasting years (median duration of response ~ 7 years)
- in relapse, treatment can be successfully repeated or combined with an anti-CD20 antibody

SÉZARY SYNDROME

Introduction

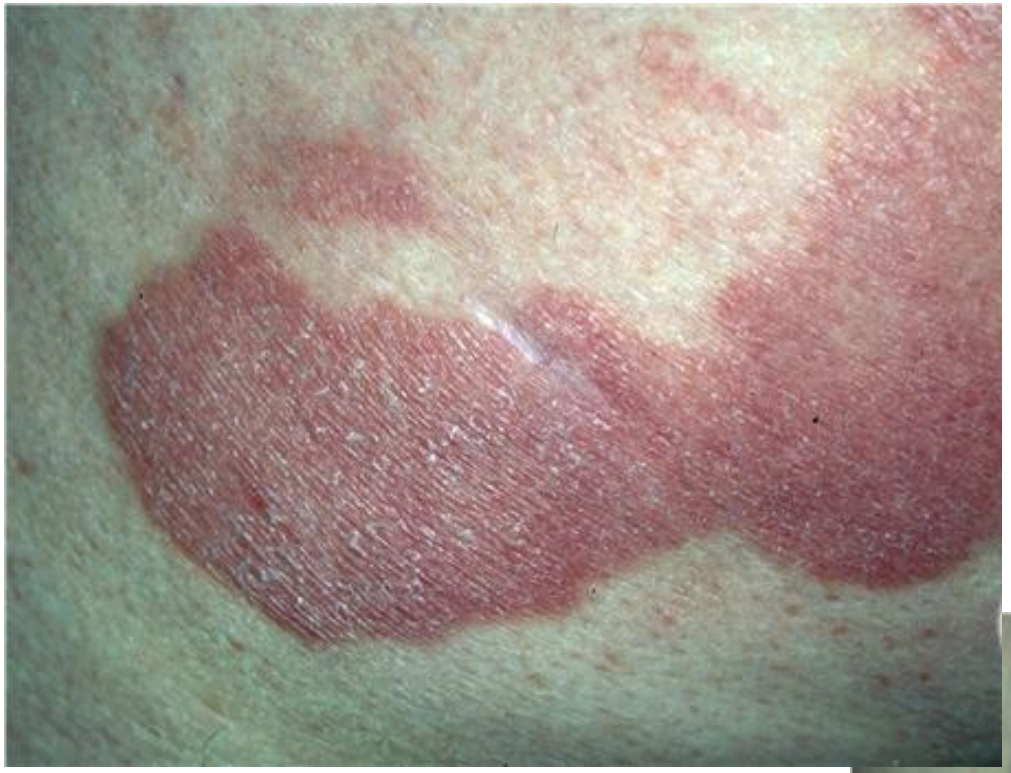
- **mycosis fungoides** is a low aggressive cutaneous T-lymphoma
- **Sezary syndrome** is a leukemic variant of the disease

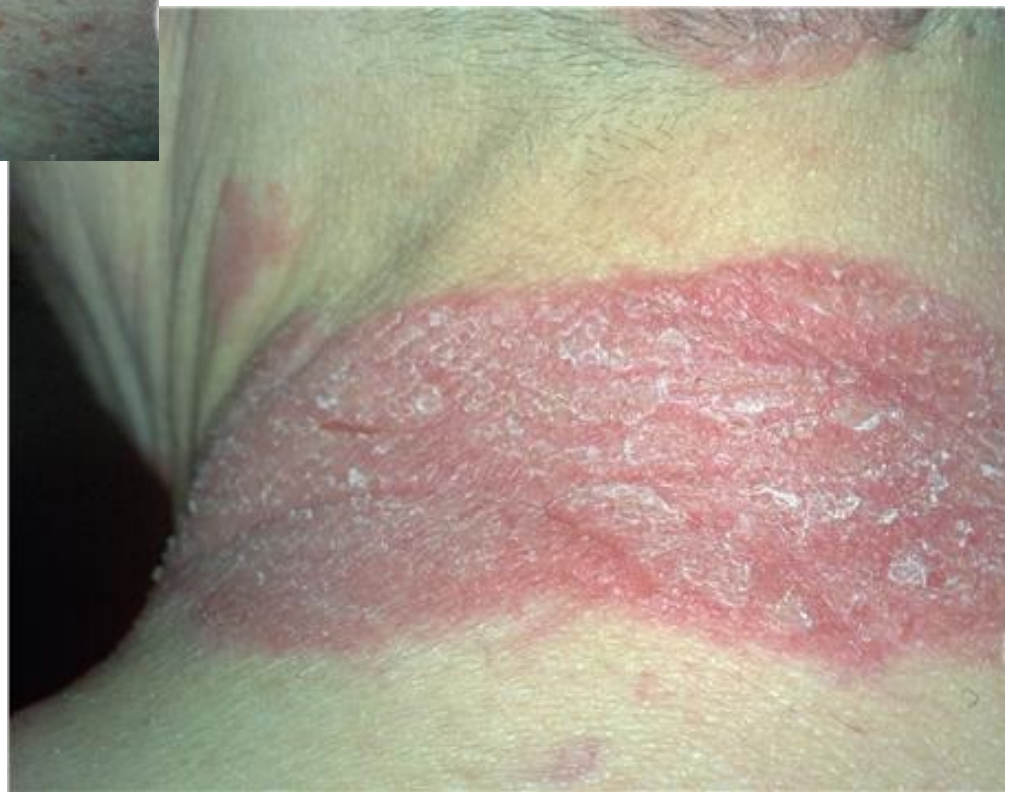
- relatively rare disease (incidence 0.4 / 100 000)
- more often, dermatologists encounter the disease
- typical age of patients 45 - 65 years
- the predisposing factor is atopic eczema

Clinical feature

- first symptoms very unspecific (itching)
- the first manifestations on the skin transient, after some time disappear
- later (months to years) typical morphs appear
- infiltrates are gradually expanding - even generalized erythroderma
- after years, malignant lymphocytes can infiltrate nodules, internal organs

- in Sezary's sy, pathological T-lymphocytes are also present in peripheral blood
- prognosis worse and treatment more difficult than Mycosis fungoides





typical morphs:

- creeping, sharply bounded edge
- sometimes a central gaps

Treatment

- topical therapy: corticosteroids
- radiotherapy: whole body irradiation with low energy electrons
- photochemotherapy:
  - PUVA: psoralen + UVA
  - ECP (extracorporeal photopheresis): irradiation of lymphoid outside the body
- interferon alpha, retinoids
- systemic chemotherapy:
  - advanced stages with nodule and organ infiltration
  - adenosine analogs

methoxypsoralen is irreversibly
binds after photoactivation to DNA and
causes damage to T-lymphocytes

Thanks for your attention