IMPORTANCE OF CYTOGENETICS IN HEMATOLOGIC MALIGNANCIES

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What is cytogenetics?

- Study of number and structure of chromosomes, their properties, behavior during cell division, influence on phenotype
- Any changes in number or structure may lead to disease
- Changes lead to disrupted genes leading to proteins that do not function correctly
- Depending on size, location, and timing, structural changes in chromosomes can lead to birth defects, syndromes or even cancer

Cytogenetics

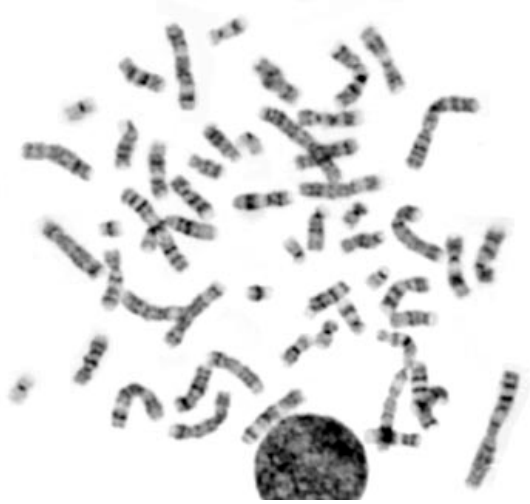
- Clinical cytogenetics (germline alterations)
  - All cells are effected
  - Changes are stable
- Tumor cytogenetics (acquired alterations)
  - Only a portion of cells is effected
  - Clonal evolution or regression
  - Role of sensitivity of any method

Tumor genetics

- A tumor is a genetic disease that arises as a consequence of accumulation of various genetic changes
- Every 3rd person will get a tumor disease
- In the Czech Republic, more than 70 thousand people are diagnosed with a tumor every year
- Tumor cells are characterized by chromosomal changes: numerical or structural changes of chromosomes

Tumor cytogenetics

- Studies of acquired chromosomal changes of tumor cells
- Analyses of numerical and structural changes of chromosomes
- Basic method – G- banding
- One analysis checks the entire genome

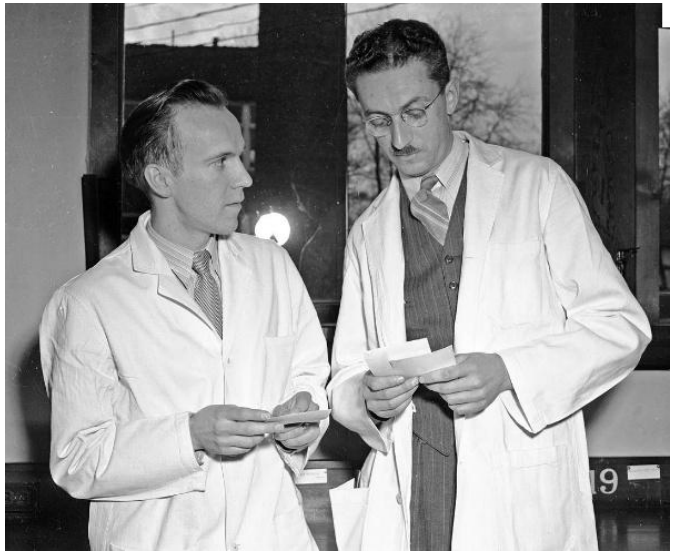


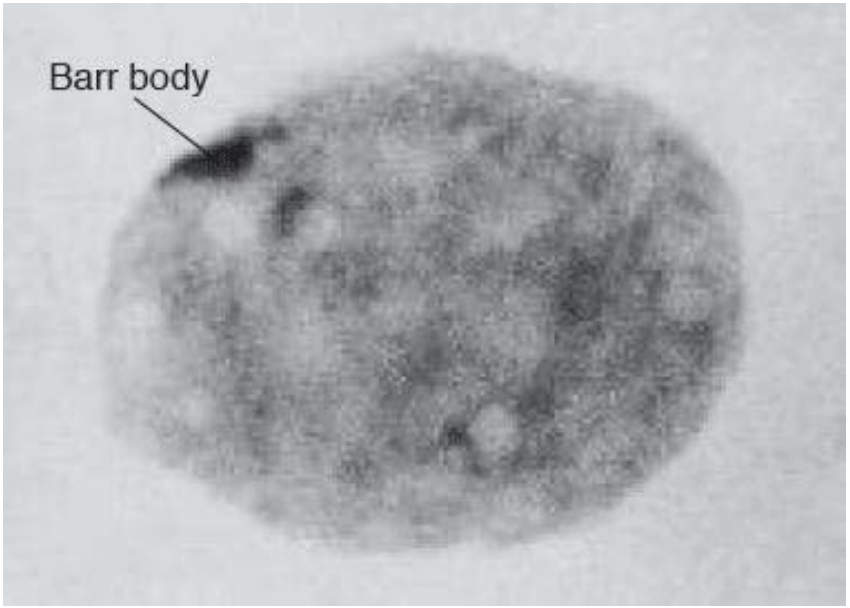
History of cytogenetics

Early cytogenetics

- 1842 – Carl Nageli- cell division (anomaly), botanist
- 1879 – Walther Flemming – chromosomal movement during mitosis
- 1882 – published his work
- 1888 – Waldeyer - chromosome

1940s – the sex revolution

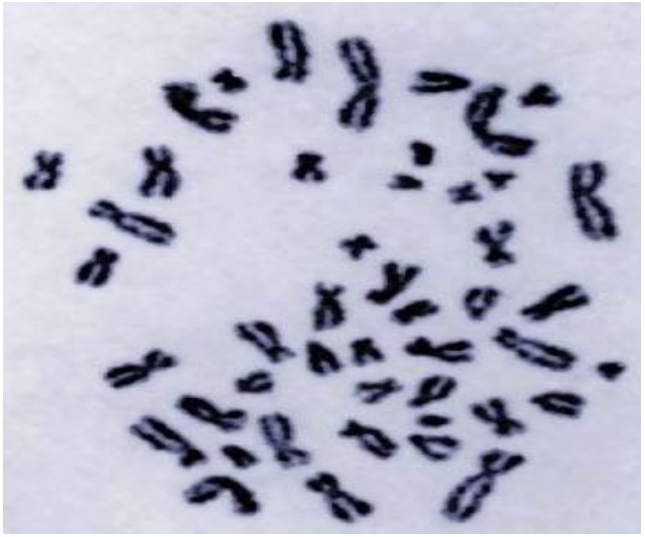




Dr. Barr and Dr. Bertram discovered sex-chromatin, now known as the Barr body, while working at The University of Western Ontario, in 1949.

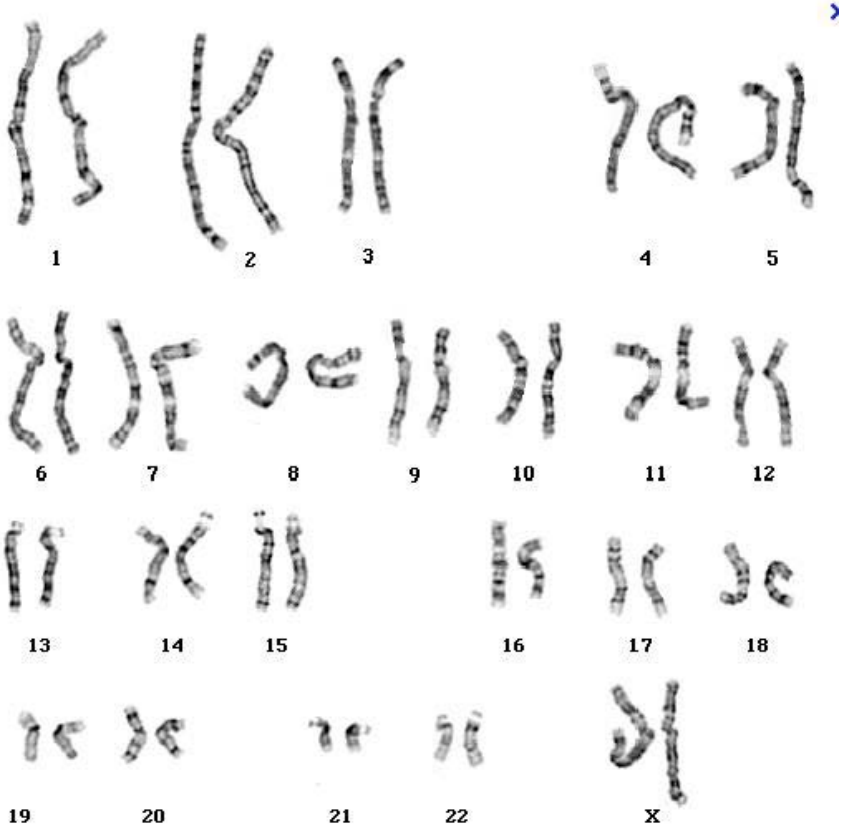
1950s – the hypotonic revolution

- T. C. Hsu discovered the utility of hypotonic solution in 1952
- Tijo and Levan reported the correct chromosome number in humans *in vitro*.
- This was confirmed within a year, *in vivo* by Ford and Hamerton (1956)

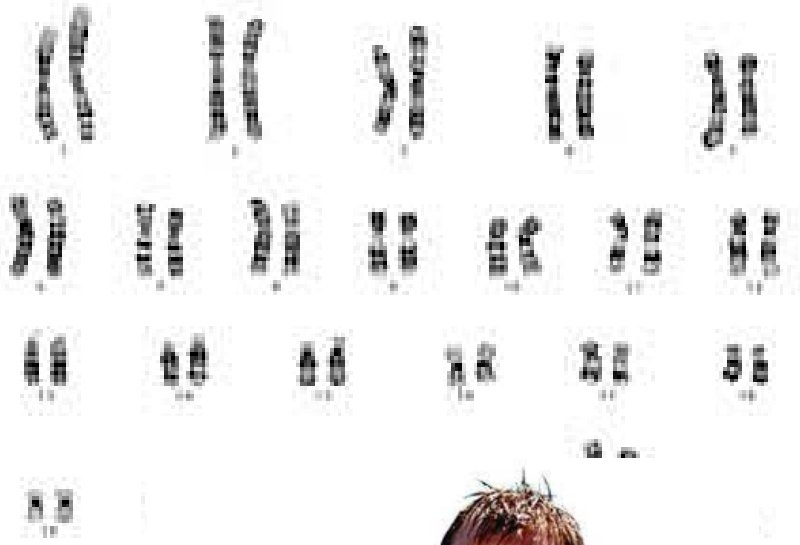


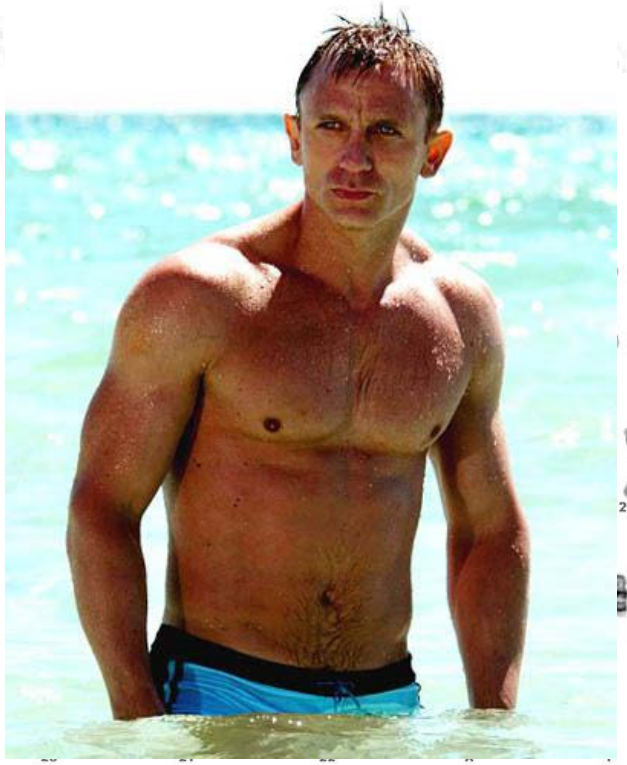
They could count the chromosomes but classification was rough and approximate.

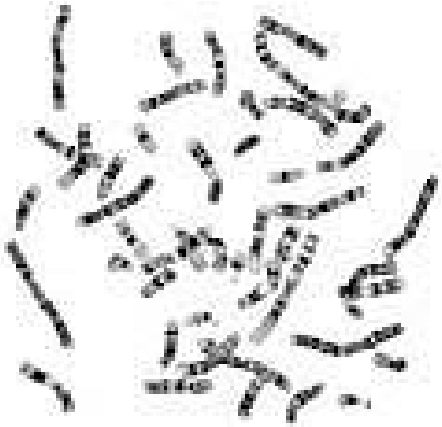
1970s – the banding revolution

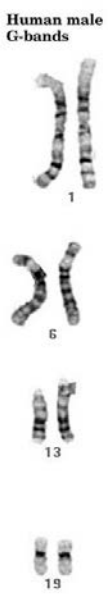


High Resolution G-banding, Yunis, 1975








Human male
G-bands
1
6
13
19

♂

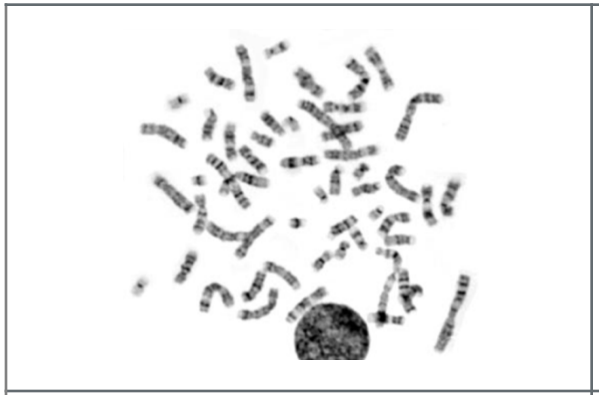
80s, 90s, 00s

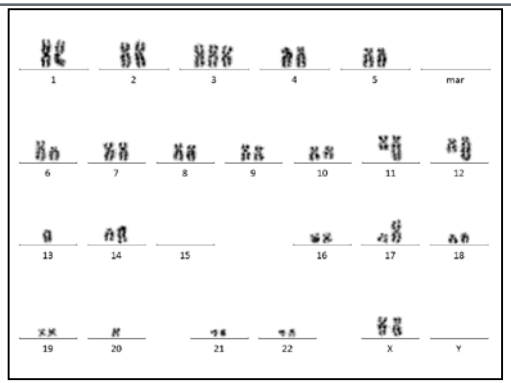
- 80s – imaging – automated imaging
- 90s – colors – unique locus probes
- 00s – genomics – SNP arrays, next gen sequencing

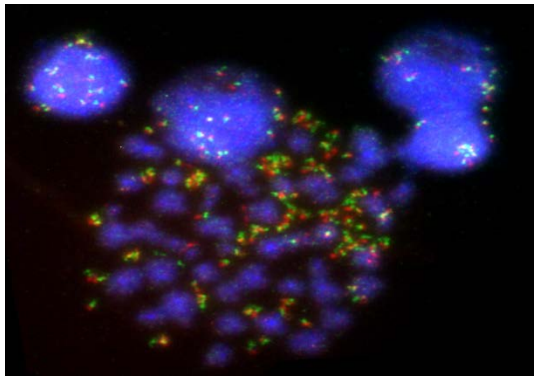
Cytogenetic methods

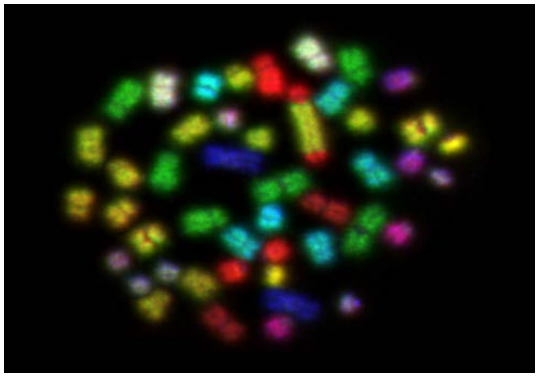
Cytogenetics

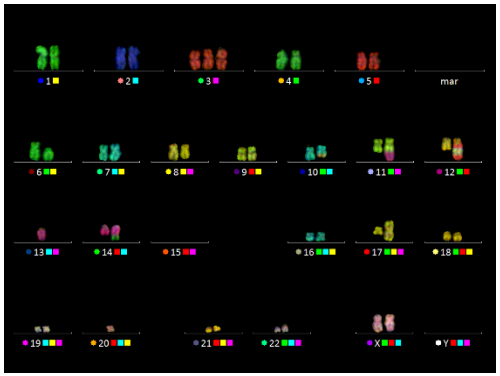
FISH

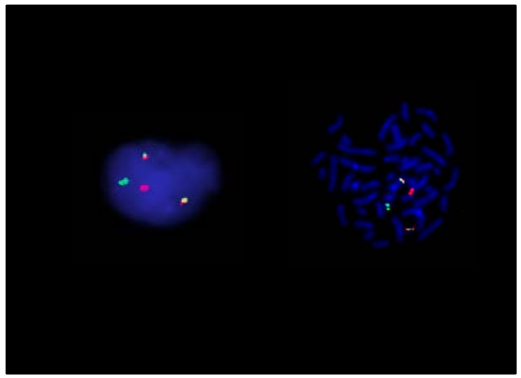


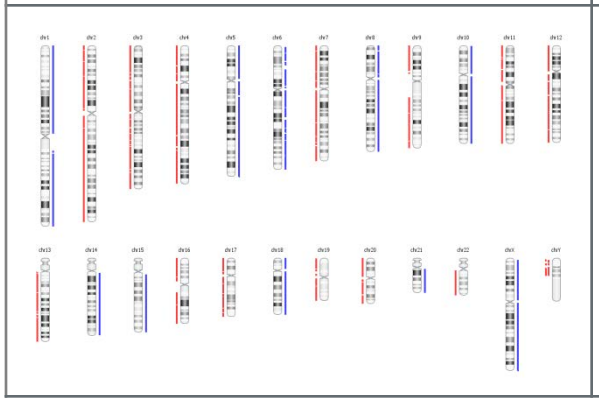


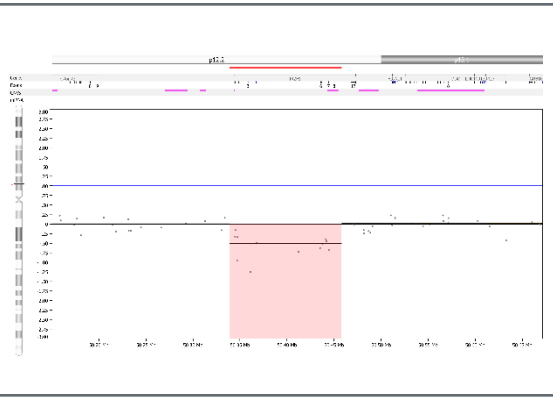


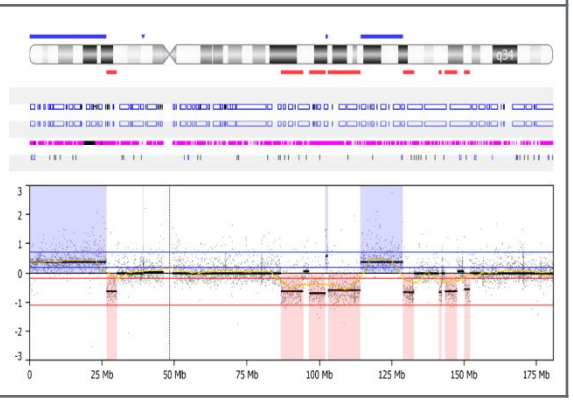






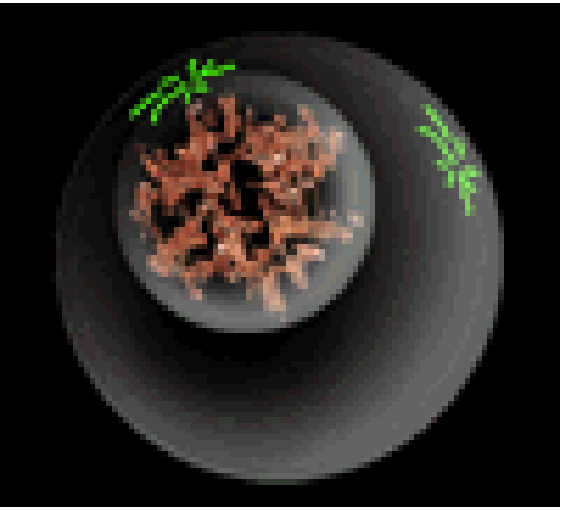


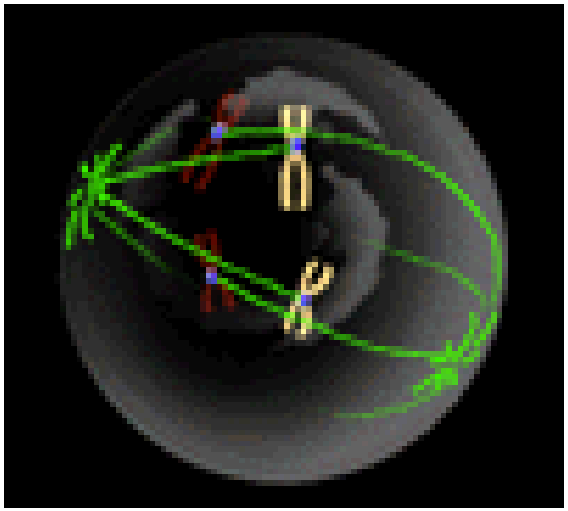


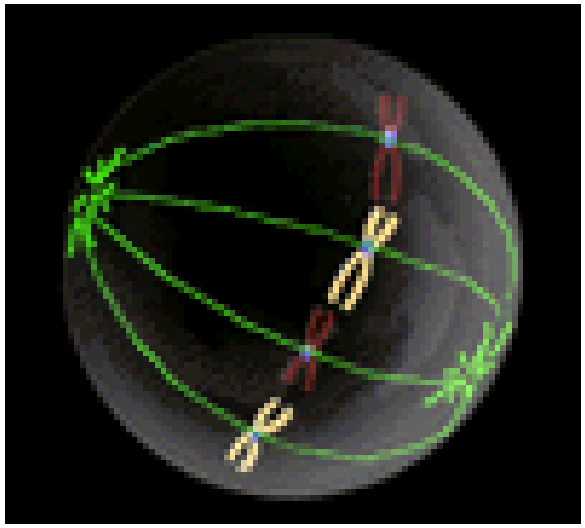


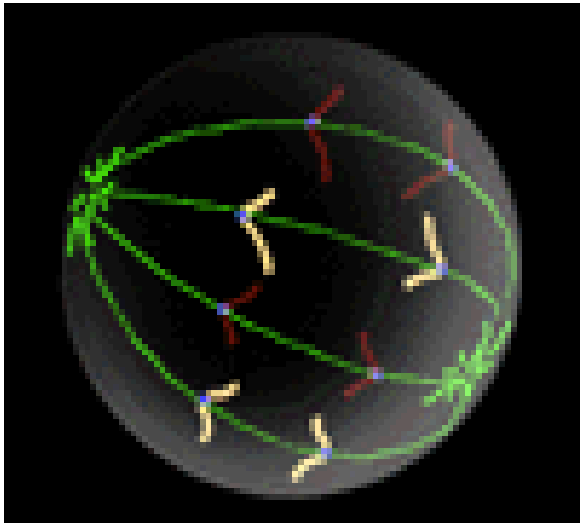
arrayCGH/SNP array

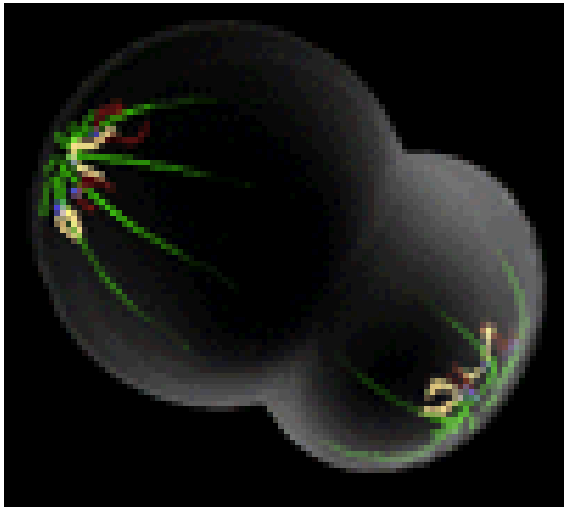
Metaphase

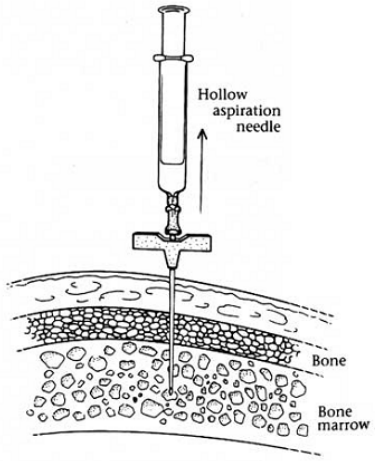












1-2ml

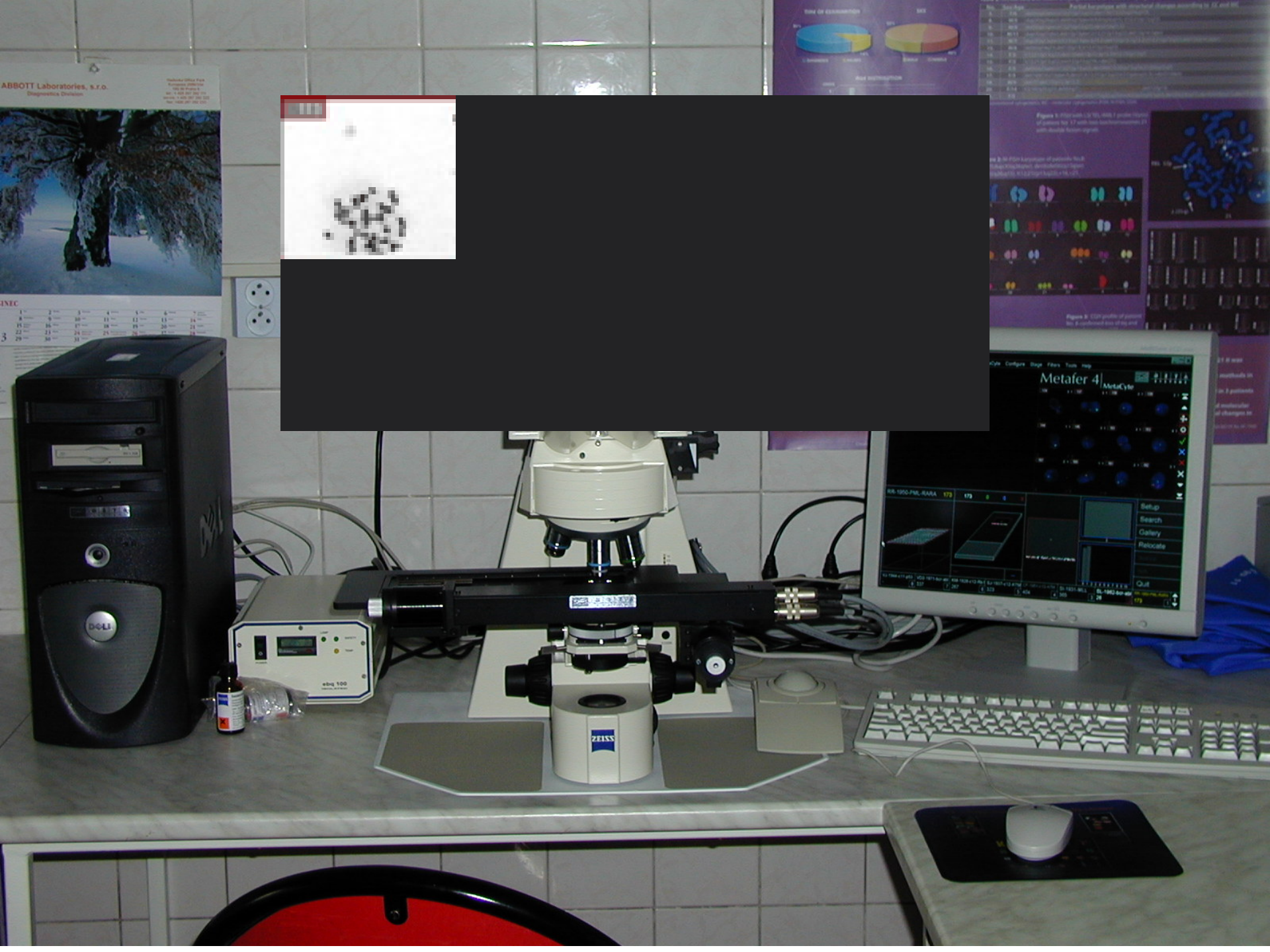
+

colcemide

- ✓bone marrow
- ✓peripheral blood
- ✓lymph node

cultivation
2/24/72 hrs

COLCEMIDE BLOCKS MITOSIS IN METAPHASE


ABBOTT Laboratories, s.r.o.
Diagnostics Division
Metafer 4
MetaCyte
Setup
Search
Gallery
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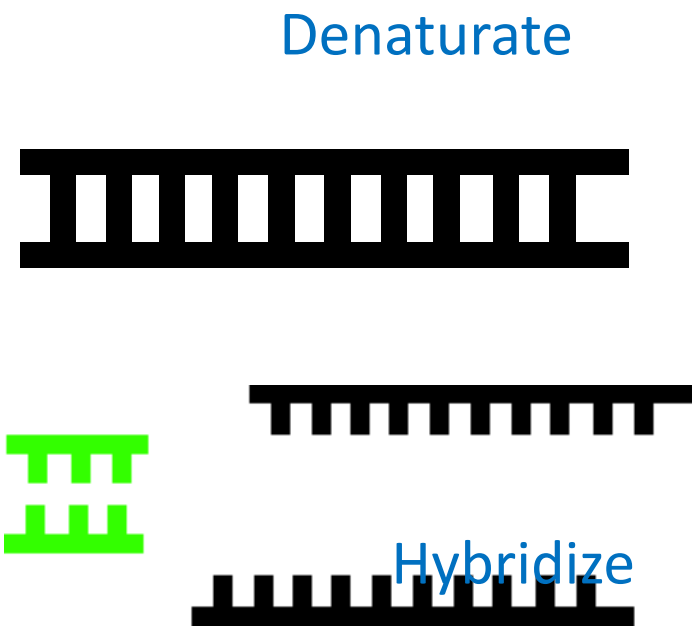
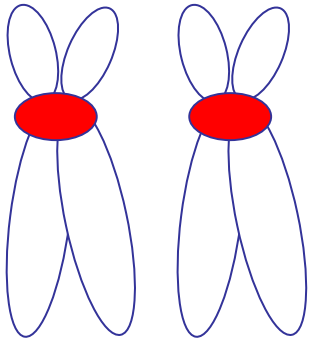
ZEISS

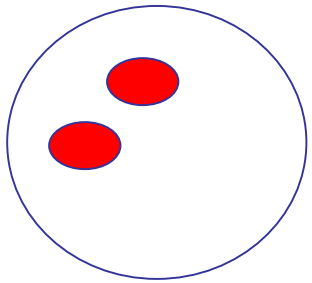
Molecular cytogenetics

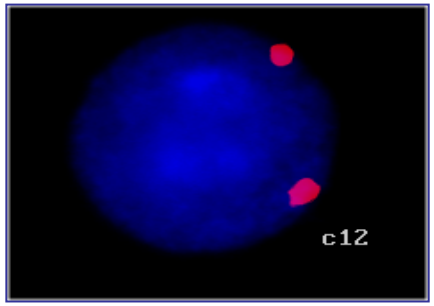


- Methods based on fluorescence in situ hybridization (FISH) – based on molecular as well as classical cytogenetics
- Methods use the basic property of single stranded DNA to bind together based on complementarity

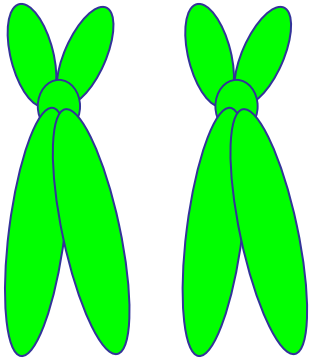
Types of probes

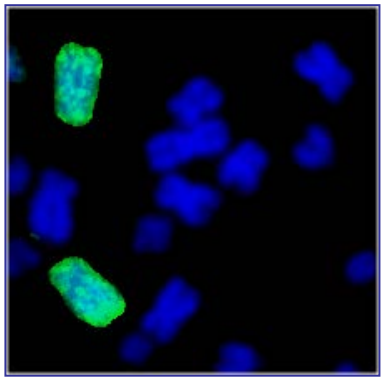




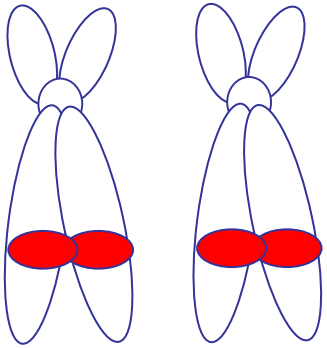


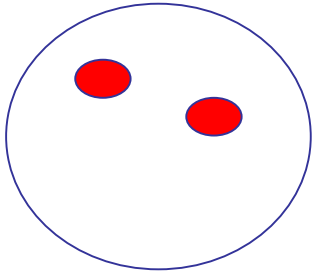
centromeric

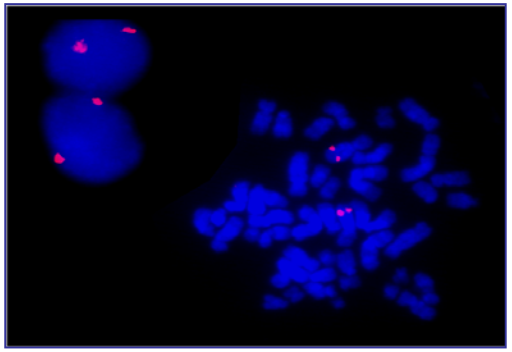




Whole chromosome







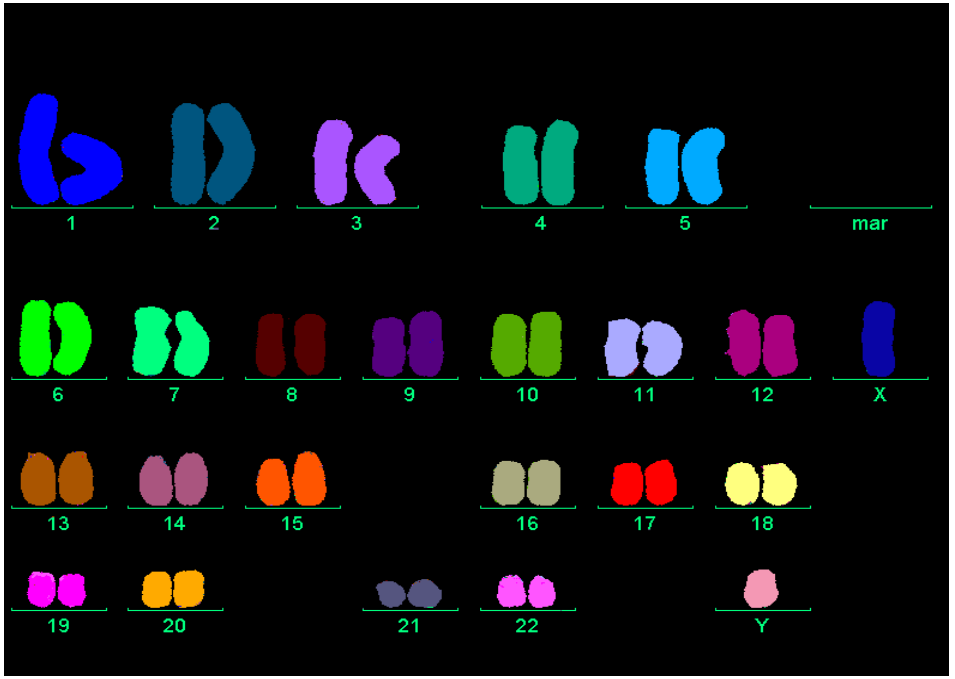
gene

Multicolor fluorescence in situ hybridization (M-FISH)

M-FISH is based on hybridization of 24 fluorescently labeled whole chromosome probes that allow the staining of all chromosomal pairs by different colors

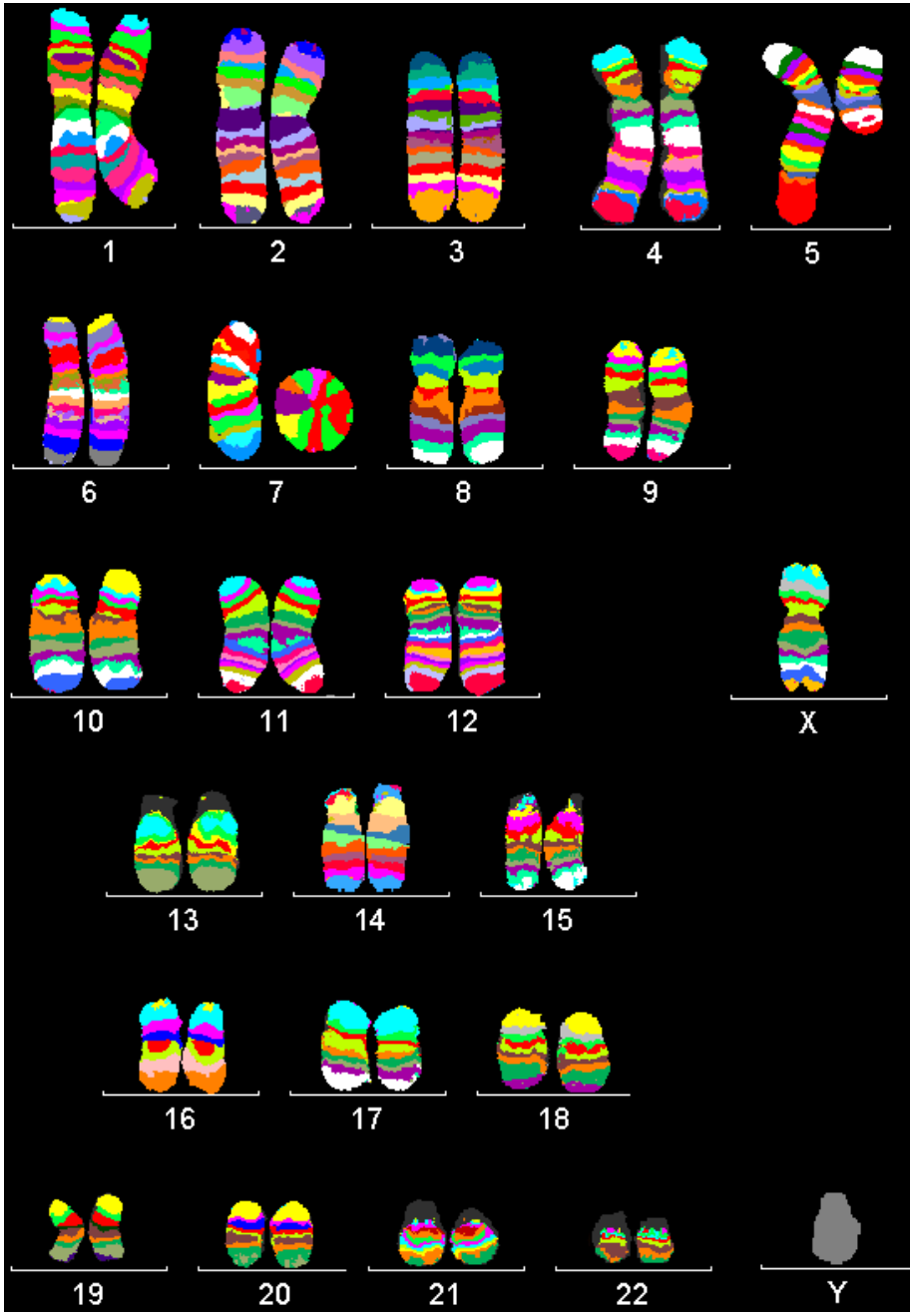
24 color karyotyping *hybridization and detection kit*

Chr.	FITC	Spectrum Orange	Texas Red	Cy5	DEAC
1					
2					
3					
4					
5					
6					
7					
8					
9					
10					
11					
12					
13					
14					
15					
16					
17					
18					
19					
20					
21					
22					
X					
Y					



Mband FISH

- Combines paint probes specific for a region of a chromosome
- Banding covers the whole chromosome



Převzato I.Chudoba

Array CGH: The Complete Process

Steps 1-3 Patient and control DNA are labeled with fluorescent dyes and applied to the microarray.

Step 4 Patient and control DNA compete to attach, or hybridize, to the microarray.

Step 5 The microarray scanner measures the fluorescent signals.

Step 6 Computer software analyzes the data and generates a plot.

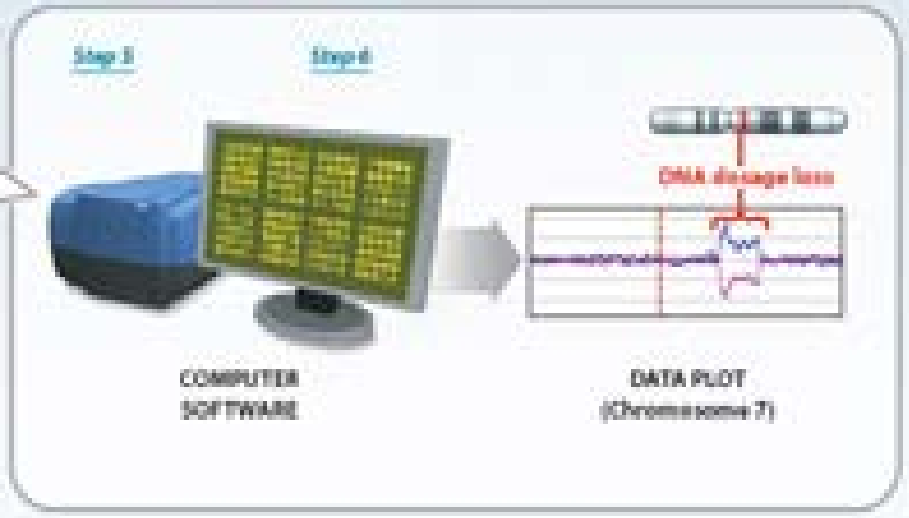
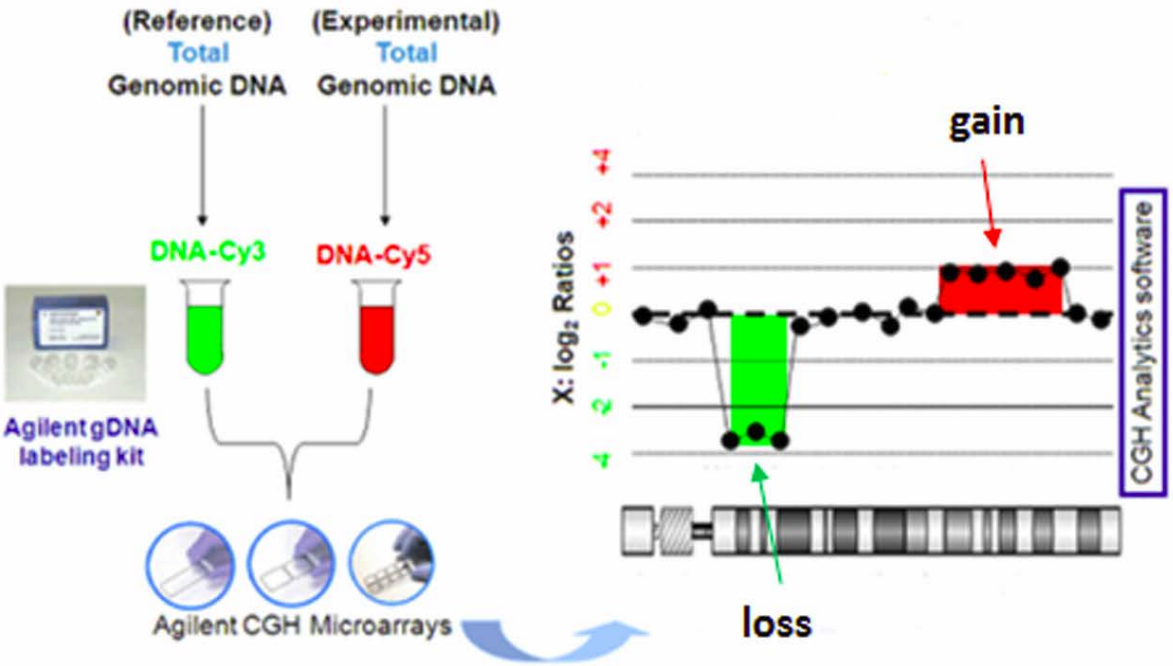
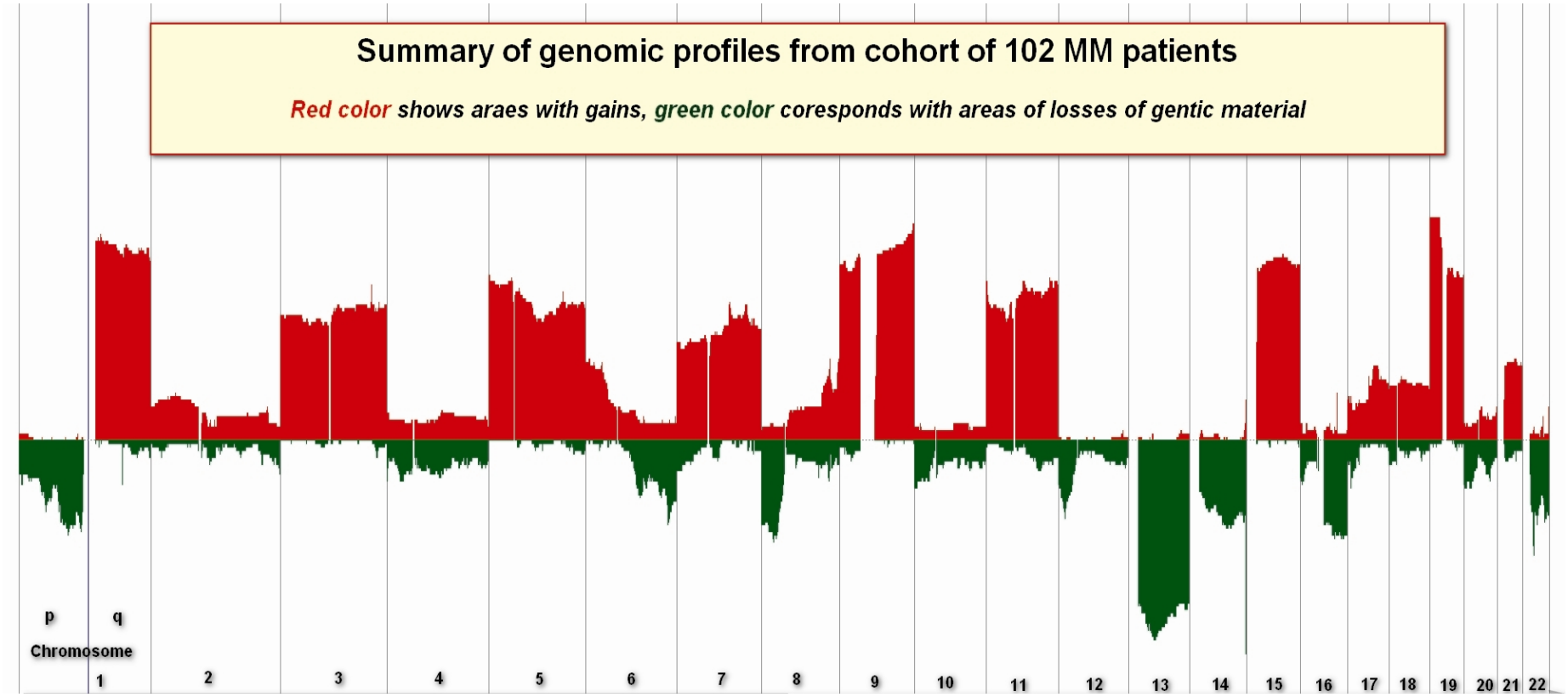


Figure 3

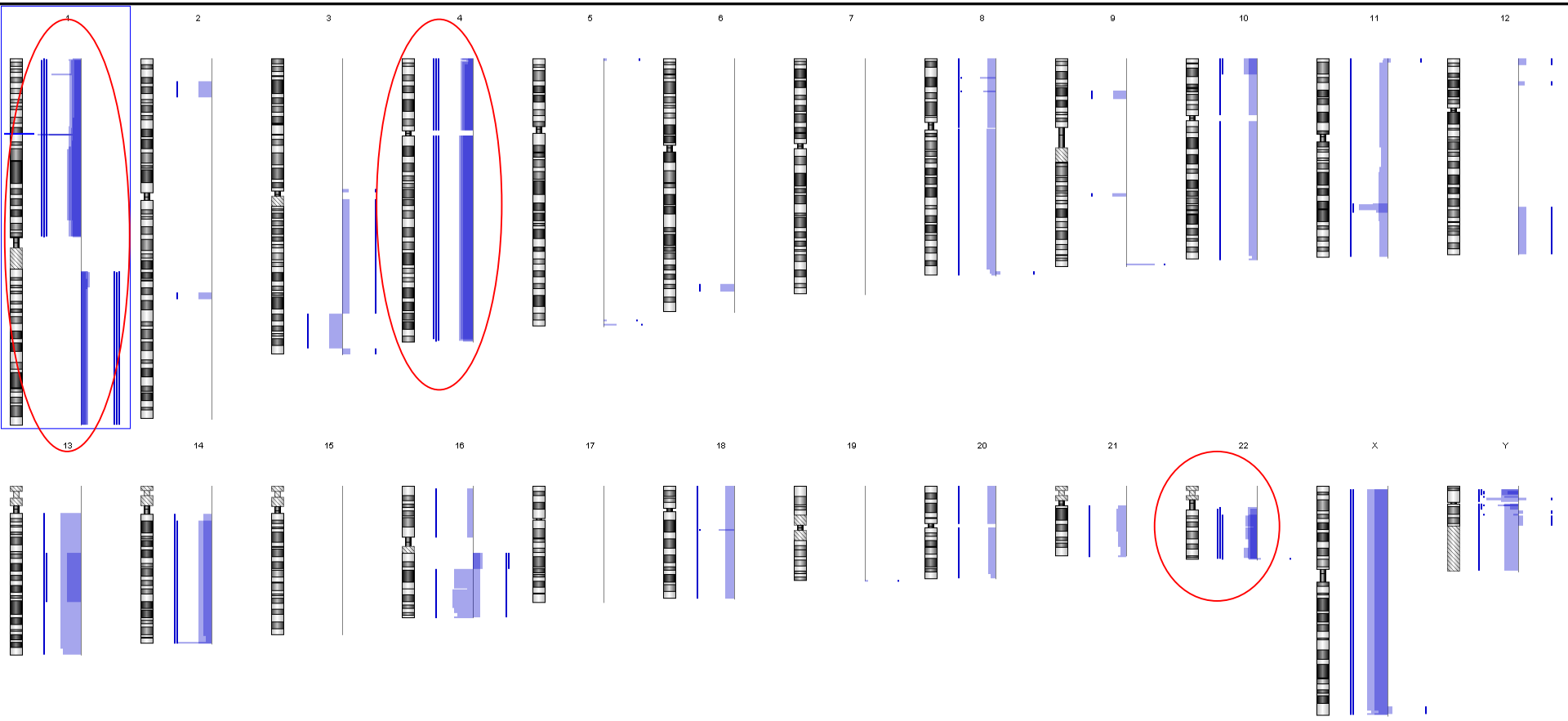

(Reference)
Total
Genomic DNA
(Experimental)
Total
Genomic DNA
DNA-Cy3
DNA-Cy5
Agilent gDNA
labeling kit
Agilent CGH Microarrays
gain
loss
X: log2 Ratios
+4
+2
+1
0
-1
-2
-4
CGH Analytics software

aCGH in MM



J. Smetana

Analysis



J. Smetana

Cytogenetics in hematology

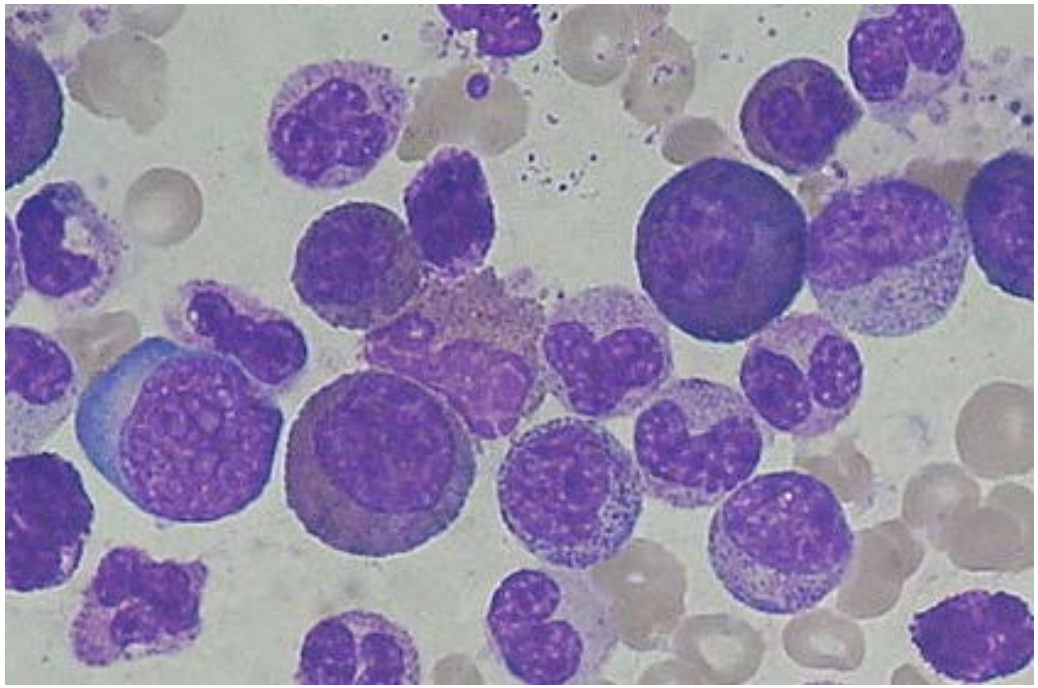
- Diagnosis
- Prognosis
- Treatment decisions

Genetic changes in hematological malignancies

- 90-95% of CML
- 60-80% of AML
- 60% of MDS
- 50-80% of CLL
- 60-90% of NHL
- 70-90% of MM
- 70-90% of ALL

Philadelphia chromosome (Ph)

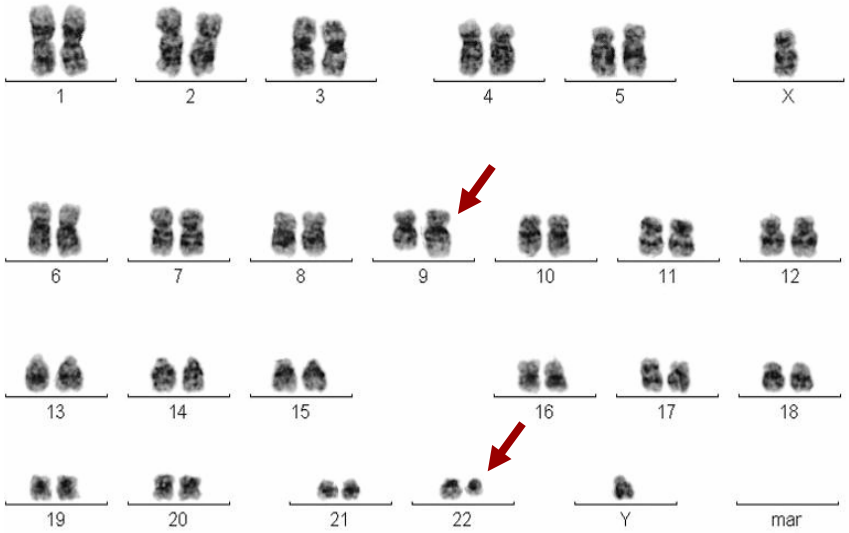
First specific chromosomal aberration linked to a tumor



Cytogenetics of CML

Diagnosis

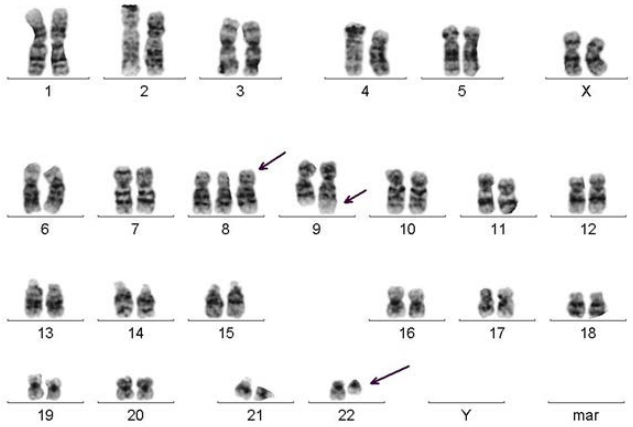
90-95% Ph1 result of translocation
t(9;22)(q34;q21)



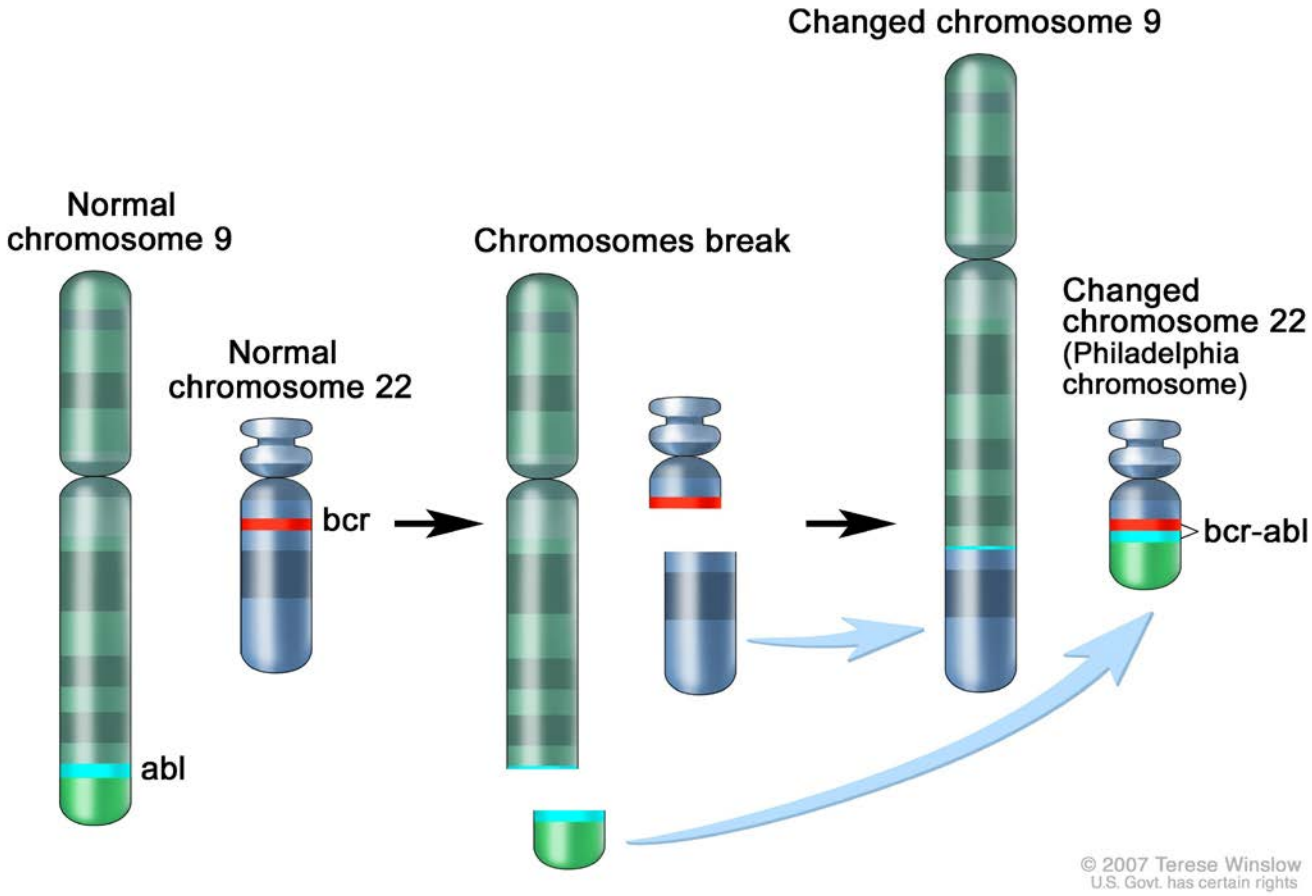
Prognosis

Additional chromosomal changes

Diagnosis: ~12%
Accelerated phase: ~30%
Blast crisis : ~70%

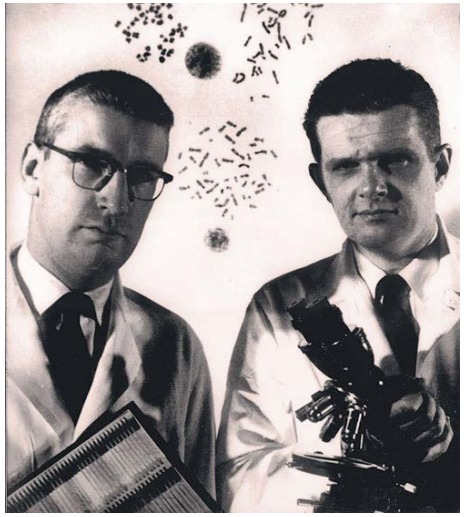


Philadelphia chromosome



Philadelphia chromosome (Ph)

- 1960 – Peter Nowell and David Hungerford described abnormal chromosome in CML
- First genetic signature of cancer – growth advantage for abnormal cells?
- Cause or consequence?
- Janet Rowley in the 1972 – t(9,22)



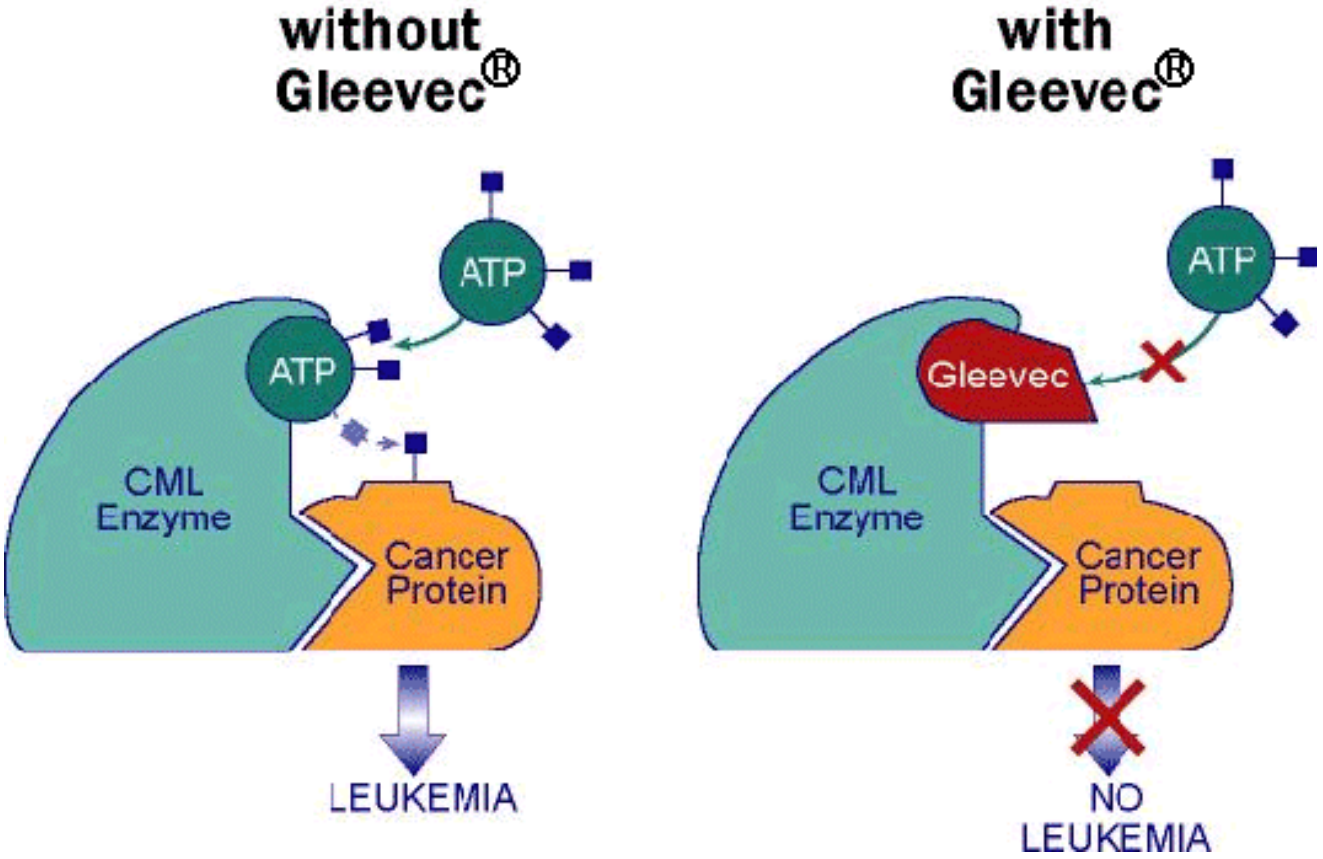
Philadelphia chromosome (Ph)

- 1983 abl (Heisterkamp)
- 1984 bcr (Groffen)
- 1990 bcr-abl cause of CML (Daley)
- 1990 - bcr-abl- abnormal tyrosine kinase activity (Lugo)
- Chronic phase, accelerated phase, blast crisis
- Bad prognosis

Gleevec - Imatinib mesylate (1993)

- Activity against CML colonies (Druker 1996)
- 2 years later – clinical study, 31 patients, 98% response
- Clinical trial phase III – 16 countries, 177 centers, 1100 patients – closed early
- All patients moved to Gleevec arm
- Survival 95%, 65% in blast crisis
- Molecular positivity still a problem
- Dasatinib, nilotinib….

Gleevec: HOW IT WORKS

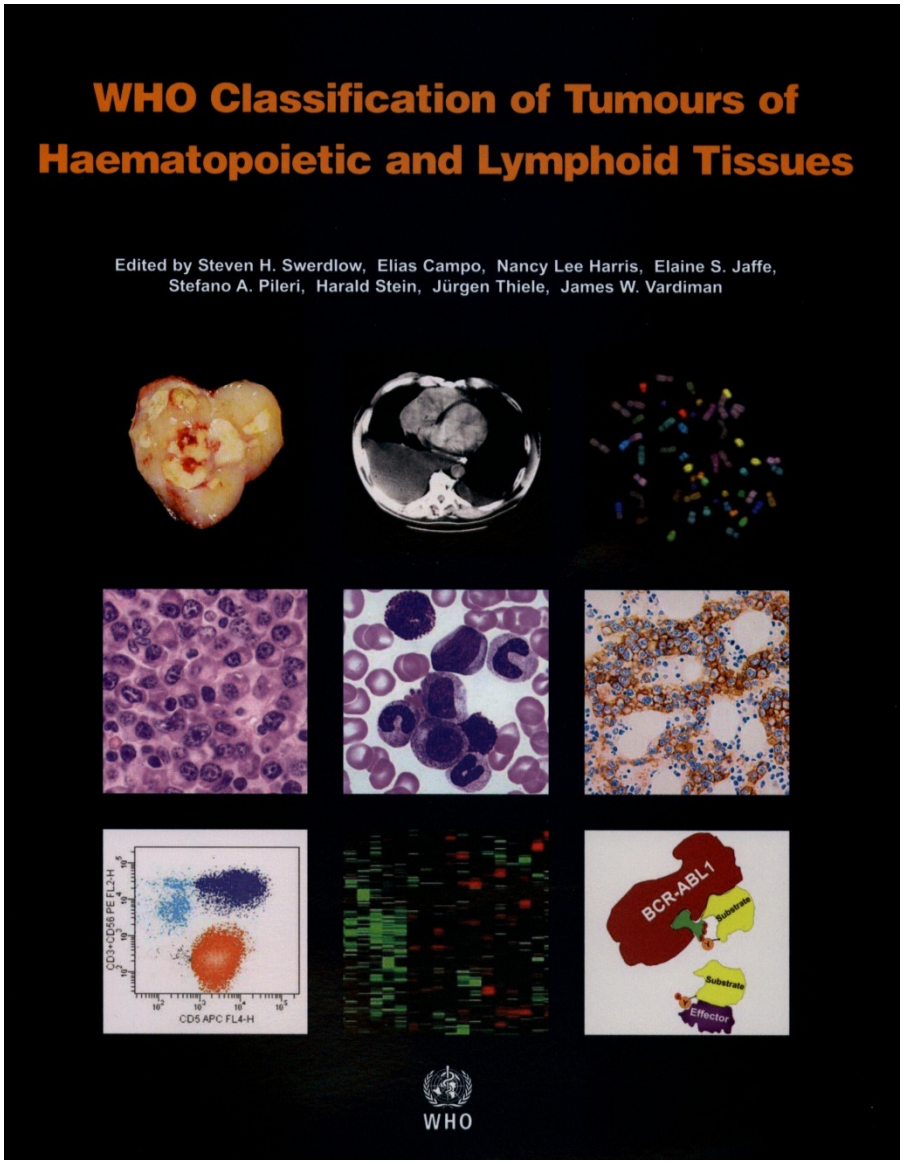


https://www.google.cz/search?q=gleevec+cml&dcr=0&source=lnms&tbm=isch&sa=X&ved=0ahUKEwjokaX99NnWAhXMDMAKHUfAAisQ_AUICigB&biw=1366&bih=604#imgrc=TNlp3Ot1Yx6sbM:

MRD in CML

	Type of Response	Definition
CHR	Complete Hematologic Response	Normal differential, WBC & platelets ≤ ULN
MCyR	Major cytogenetic Response	0–35% Ph+marrow metaphases
CCyR	Complete Cytogenetic Response	0% Ph+marrow metaphases
MMR	Major Molecular Response	BCR-ABL/ABL ≤ 0.1% (International Scale)
$MR^{4.0}$		BCR-ABL/ABL ≤ 0.001% (IS) “4-log reduction”
$MR^{4.5}$		BCR-ABL/ABL ≤ 0.003% (IS) “4.5-log reduction”
CMR	Complete Molecular Response	Undetectable BCR-ABL (test of sensitivity ≥4.5 logs)

WHO Classification 2008



WHO Classification

- Since 2008, cytogenetics is part of diagnosis and classification for many hematological malignancies
  - Cytogenetics is a part of WHO classification of AML
  - Together with cytomophology stratifies MDS patients
  - Classification of lymphomas – histology, cytogenetics and FISH confirm classification
  - Is part of prognostic stratification of MM

WHO classification of AML

The 2016 revision to the World Health Organization classification of myeloid neoplasms and acute leukemia

Daniel A. Arber,[1] Attilio Orazi,[2] Robert Hasserjian,[3] Jürgen Thiele,[4] Michael J. Borowitz,[5] Michelle M. Le Beau,[6] Clara D. Bloomfield,[7] Mario Cazzola,[8] and James W. Vardiman[9]

Acute myeloid leukemia (AML) and related neoplasms

- AML with recurrent genetic abnormalities
  - AML with t(8;21)(q22;q22.1);*RUNX1-RUNX1T1*
  - AML with inv(16)(p13.1q22) or t(16;16)(p13.1;q22);*CBFB-MYH11*
  - APL with *PML-RARA*
  - AML with t(9;11)(p21.3;q23.3);*MLLT3-KMT2A*
  - AML with t(6;9)(p23;q34.1);*DEK-NUP214*
  - AML with inv(3)(q21.3q26.2) or t(3;3)(q21.3;q26.2); *GATA2, MECOM*
  - AML (megakaryoblastic) with t(1;22)(p13.3;q13.3);*RBM15-MKL1*
  - *Provisional entity: AML with BCR-ABL1*
  - AML with mutated *NPM1*
  - AML with biallelic mutations of *CEBPA*
  - *Provisional entity: AML with mutated RUNX1*
- AML with myelodysplasia-related changes
- Therapy-related myeloid neoplasms
- AML, NOS
  - AML with minimal differentiation
  - AML without maturation
  - AML with maturation
  - Acute myelomonocytic leukemia
  - Acute monoblastic/monocytic leukemia
  - Pure erythroid leukemia
  - Acute megakaryoblastic leukemia
  - 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

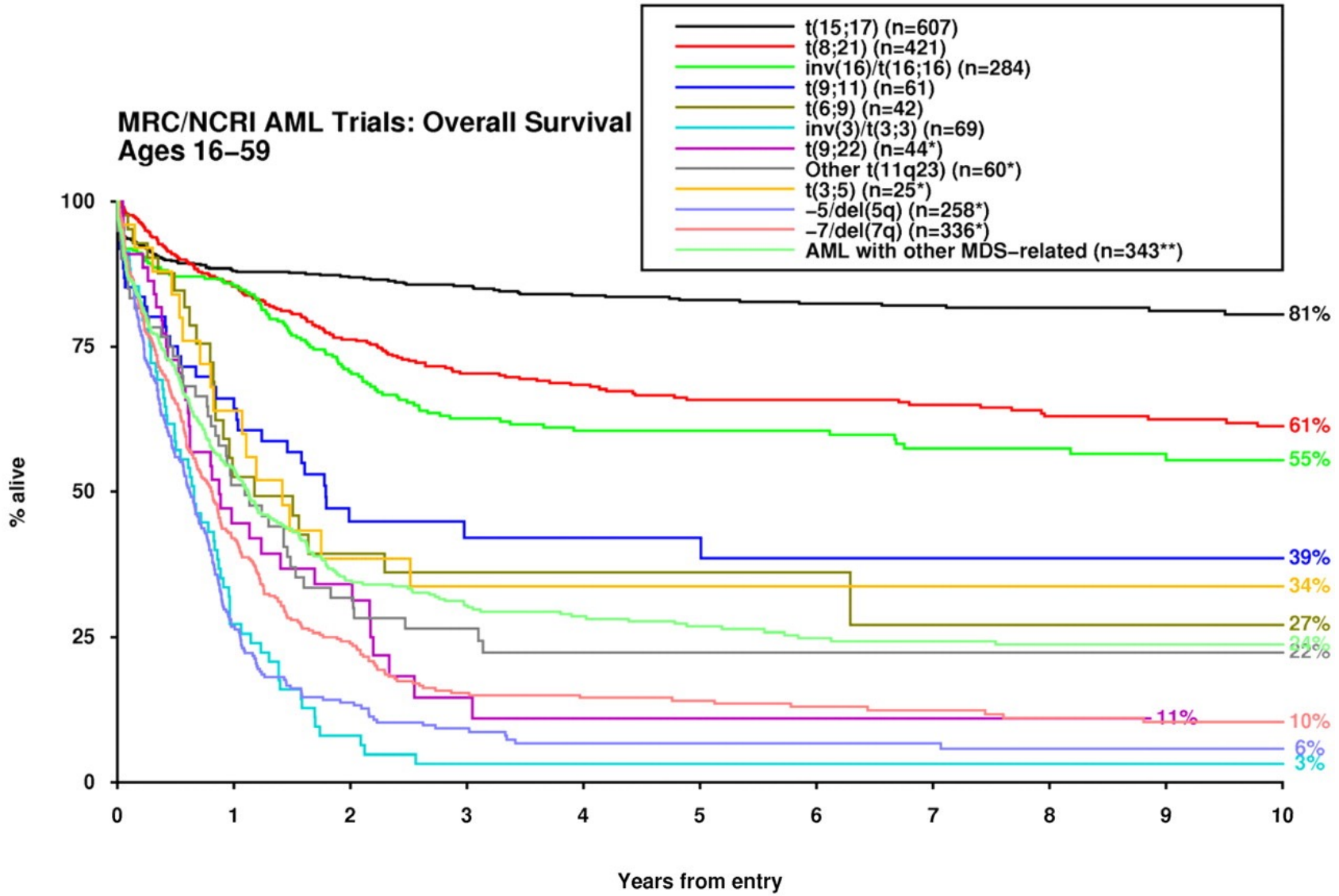
WHO myeloid neoplasm and acute leukemia classification
Blastic plasmacytoid dendritic cell neoplasm
Acute leukemias of ambiguous lineage
Acute undifferentiated leukemia
Mixed phenotype acute leukemia (MPAL) with t(9;22)(q34.1;q11.2); *BCR-ABL1*
MPAL with t(v;11q23.3); *KMT2A* rearranged
MPAL, B/myeloid, NOS
MPAL, T/myeloid, NOS
B-lymphoblastic leukemia/lymphoma
B-lymphoblastic leukemia/lymphoma, NOS
B-lymphoblastic leukemia/lymphoma with recurrent genetic abnormalities
B-lymphoblastic leukemia/lymphoma with t(9;22)(q34.1;q11.2);*BCR-ABL1*
B-lymphoblastic leukemia/lymphoma with t(v;11q23.3);*KMT2A* rearranged
B-lymphoblastic leukemia/lymphoma with t(12;21)(p13.2;q22.1); *ETV6-RUNX1*
B-lymphoblastic leukemia/lymphoma with hyperdiploidy
B-lymphoblastic leukemia/lymphoma with hypodiploidy
B-lymphoblastic leukemia/lymphoma with t(5;14)(q31.1;q32.3) *IL3-IGH*
B-lymphoblastic leukemia/lymphoma with t(1;19)(q23;p13.3);*TCF3-PBX1*
Provisional entity: B-lymphoblastic leukemia/lymphoma, BCR-ABL1–like
Provisional entity: B-lymphoblastic leukemia/lymphoma with iAMP21
T-lymphoblastic leukemia/lymphoma
Provisional entity: Early T-cell precursor lymphoblastic leukemia
Provisional entity: Natural killer (NK) cell lymphoblastic leukemia/lymphoma

WHO prognostic stratification of AML

Genetic group	Subsets
Favorable	t(8;21)(q22;q22); *RUNX1-RUNX1T1*
	inv(16)(p13.1q22) or t(16;16)(p13.1;q22); *CBFB-MYH11*
	Mutated *NPM1* without *FLT3*-ITD (normal karyotype)
	Mutated *CEBPA* (normal karyotype)
Intermediate-I*	Mutated *NPM1* and *FLT3*-ITD (normal karyotype)
	Wild-type *NPM1* and *FLT3*-ITD (normal karyotype)
	Wild-type *NPM1* without *FLT3*-ITD (normal karyotype)
Intermediate-II	t(9;11)(p22;q23); *MLLT3-MLL*
	Cytogenetic abnormalities not classified as favorable or adverse†
Adverse	inv(3)(q21q26.2) or t(3;3)(q21;q26.2); *RPN1-EVI1*
	t(6;9)(p23;q34); *DEK-NUP214*
	t(v;11)(v;q23); *MLL* rearranged
	−5 or del(5q); −7; abnl(17p); complex karyotype‡

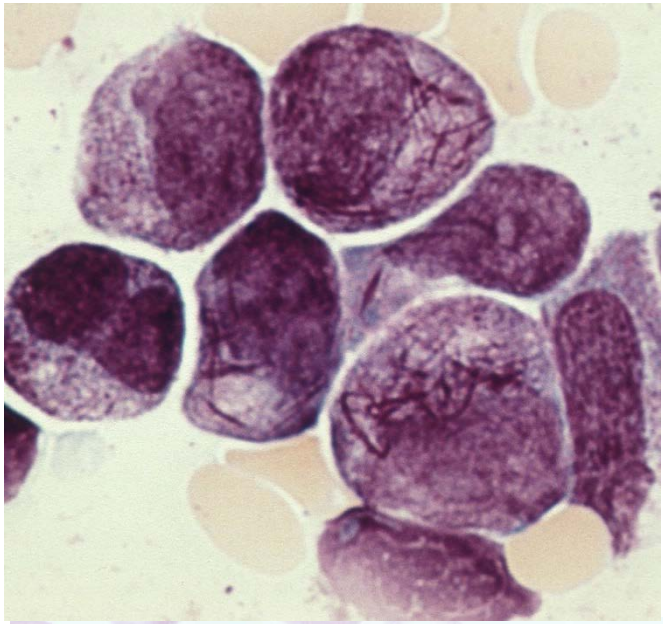
Döhner H.et al. for the ELN, Blood 2010

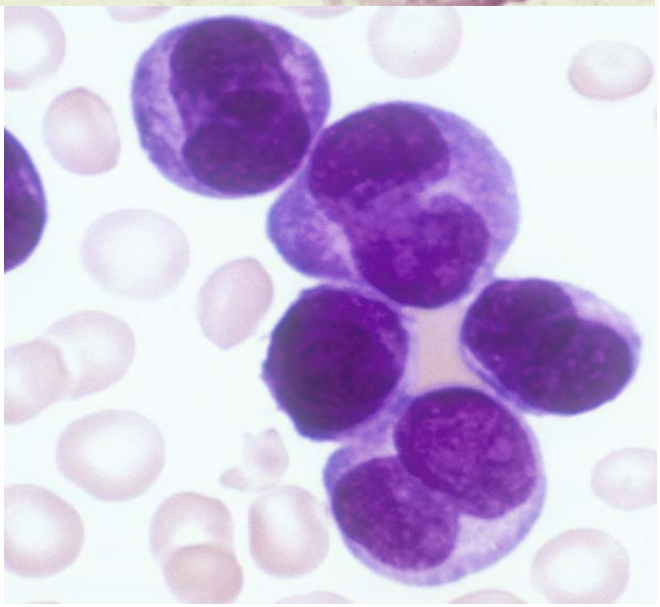
Stratification based on cytogenetics



Grimwade D et al. Blood 2010;116:354-365

APL t(15;17)(q22;q12) / *PML-RARA*





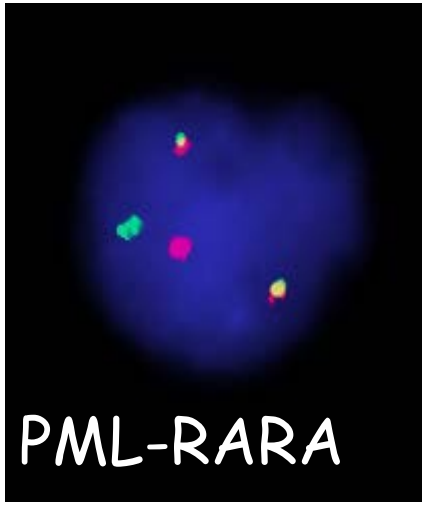
15/17 TRANSLOCATION, A CONSISTENT CHROMOSOMAL CHANGE IN ACUTE PROMYELOCYTIC LEUKAEMIA

SIR,—We have described a similar chromosomal abnormality in two patients with acute promyelocytic leukæmia

Department of Medicine,
Franklin McLean Memorial
Research Institute,
University of Chicago,
Chicago, Illinois 60637, U.S.A.

JANET D. ROWLEY
HARVEY M. GOLOMB
CHARLOTTE DOUGHERTY

t(15;17)(q22;q12)



Janet D Rowley et al. Lancet 1977

Targeted treatment of APL

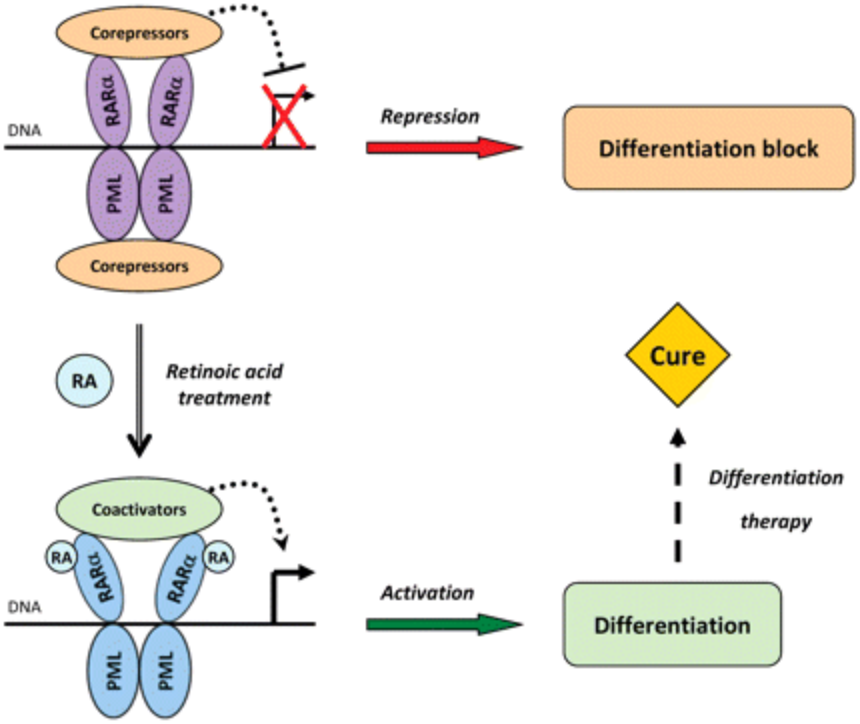
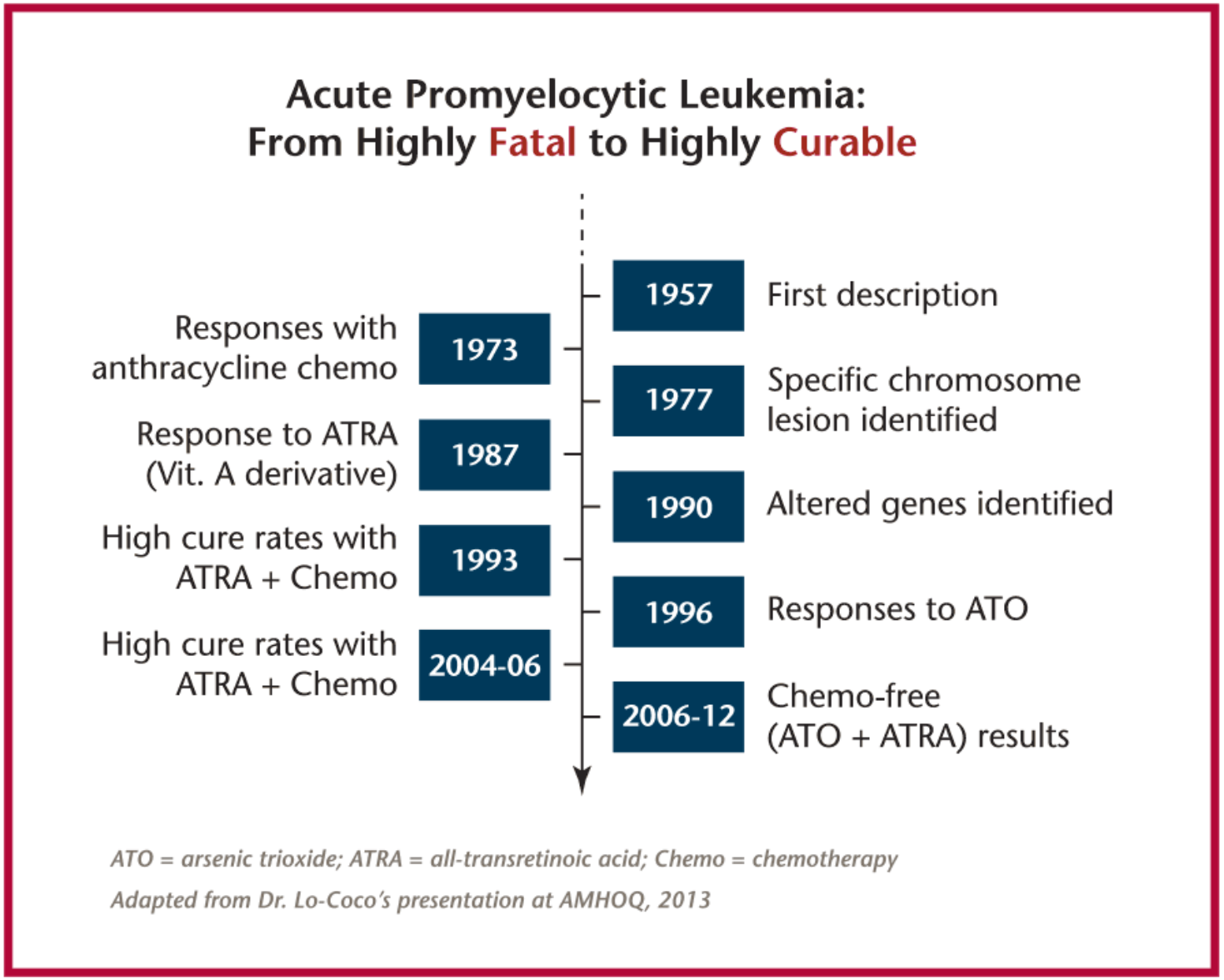


Figure 1. History of APL



ATO = arsenic trioxide; ATRA = all-transretinoic acid; Chemo = chemotherapy

Adapted from Dr. Lo-Coco's presentation at AMHOQ, 2013

ALL

- heterogeneous disease with monoclonal proliferation and expansion of lymphoid cells in BM, PB and other organs
- Cytogenetics- prognostic significance
- Imunophenotyping – diagnostic significance

TABLE 2: WHO 2008 classification of acute lymphoblastic leukemia (ALL)

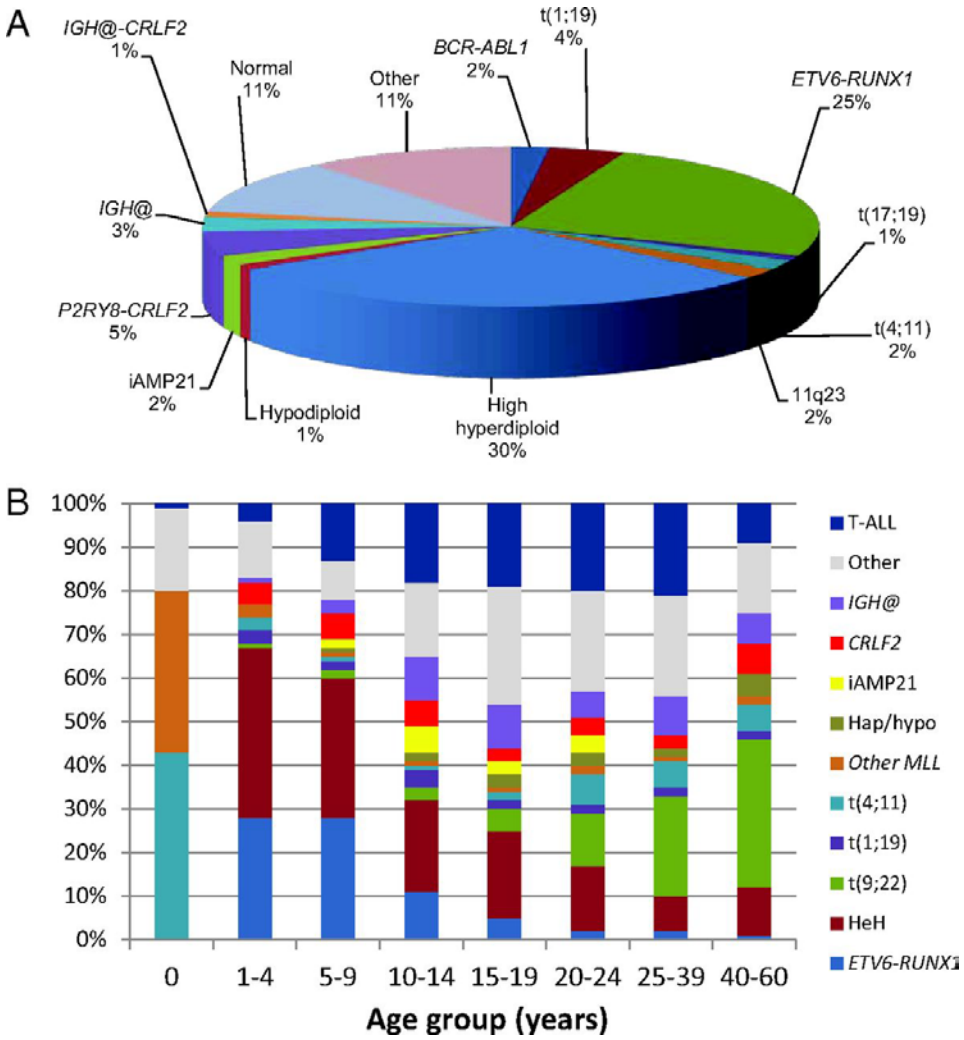
Precursor lymphoid neoplasms
B-cell lymphoblastic leukemia/lymphoma, not otherwise specified
B-cell lymphoblastic leukemia/lymphoma with recurrent genetic abnormalities
B-cell lymphoblastic leukemia/lymphoma with t(9;22)(q34;q11.2); *BCR-ABL1*
B-cell lymphoblastic leukemia/lymphoma with t(v;11q23); *MLL* rearranged
B-cell lymphoblastic leukemia/lymphoma with t(12;21)(p13;q22); *TEL-AML1 (ETV6-RUNX1)*
B-cell lymphoblastic leukemia/lymphoma with hyperploidy
B-cell lymphoblastic leukemia/lymphoma with hypoploidy (hypodiploid ALL)
B-cell lymphoblastic leukemia/lymphoma with t(5;14)(q31;q32); *IL3-IGH*
B-cell lymphoblastic leukemia/lymphoma with t(1;19)(q23;p13.3); *E2A-PBX1 (TCF3-PBX1)*
T-cell lymphoblastic leukemia/lymphoma

WHO = World Health Organization

Swerdlow SH, Campo E, Harris NL, et al (eds): WHO classification of tumours of haematopoietic and lymphoid tissues. Lyon, France: IARC Press; 109–138, 2009.

Pediatric ALL

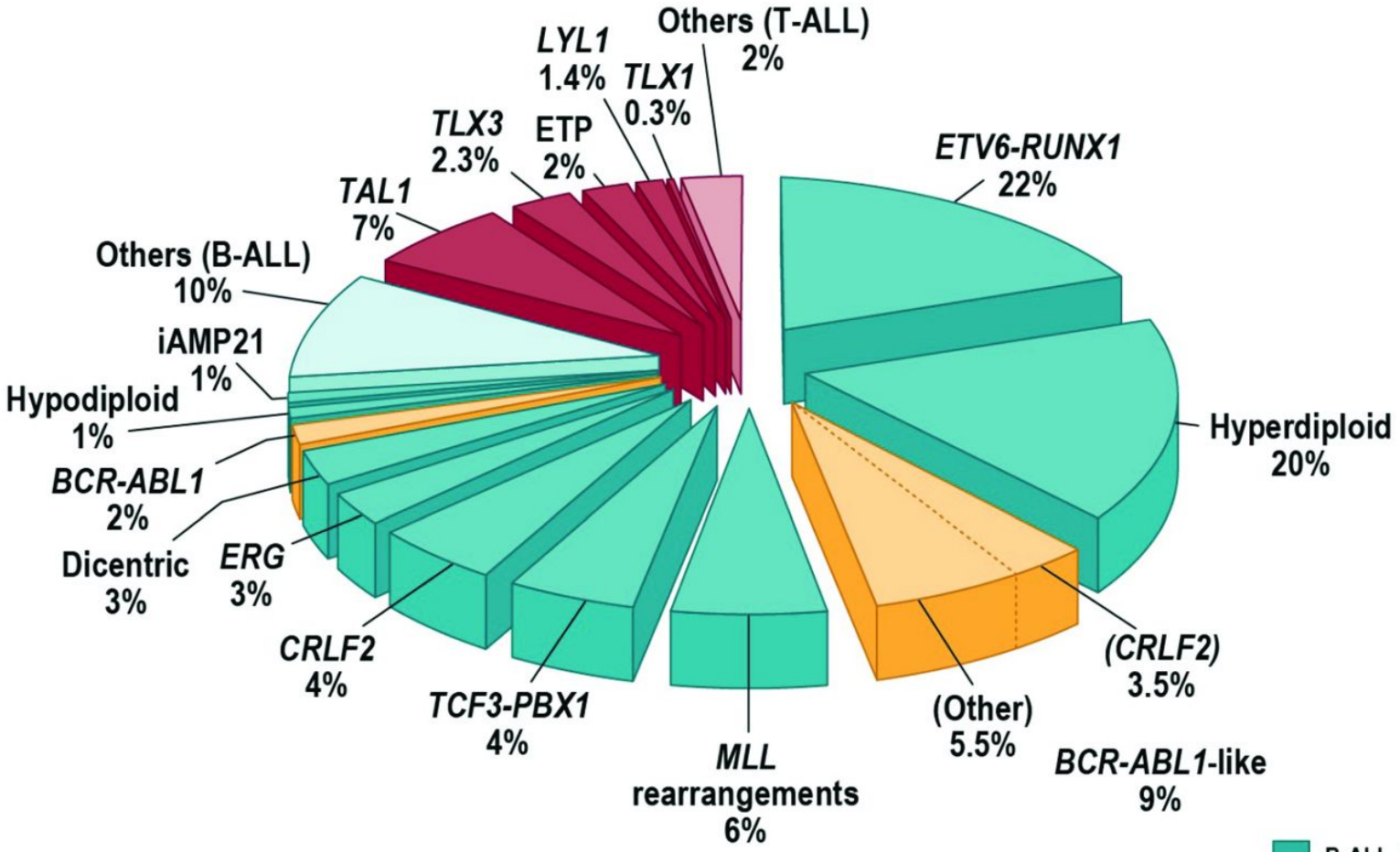
cca 30% of all pediatric tumors



Christine J. Harrison Hematology 2013;2013:118-125

Distribution of cytogenetic abnormalities from data collected from UK childhood ALL treatment trials.

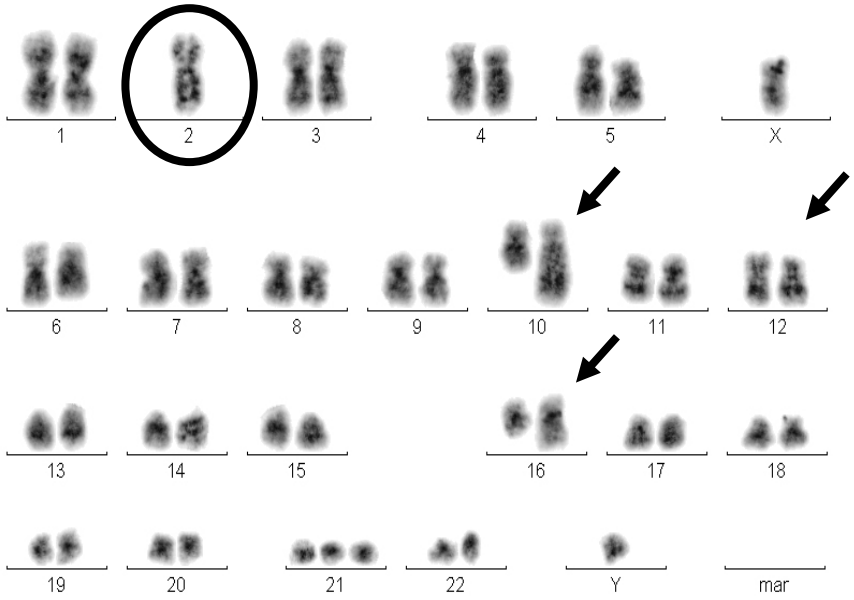
Frequency of cytogenetic subtypes of pediatric ALL

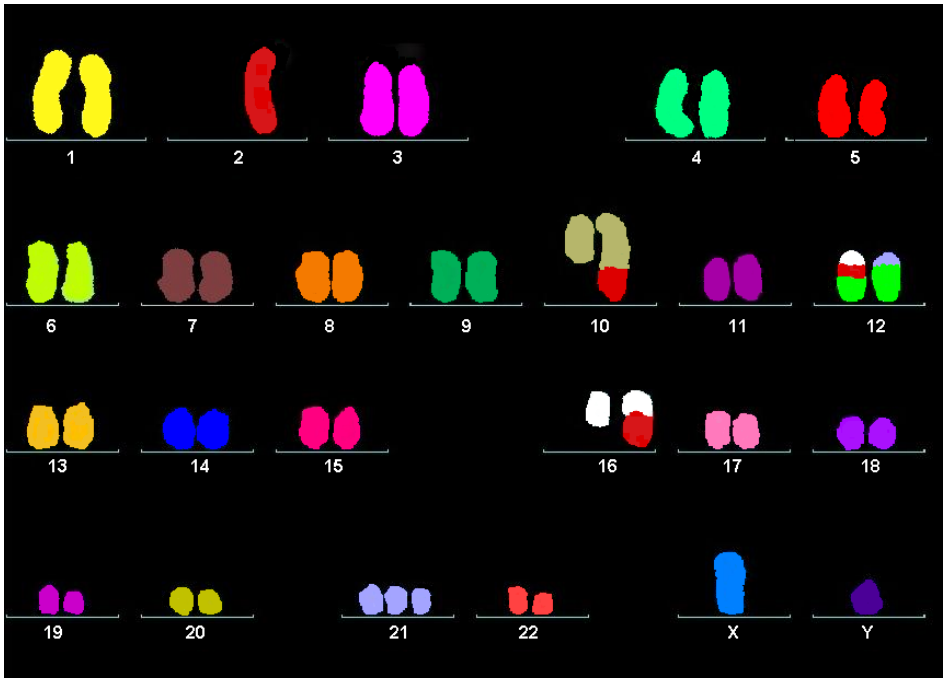


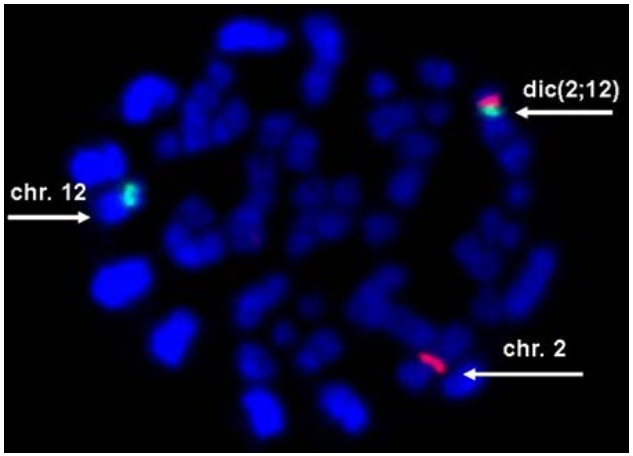
Charles G. Mullighan Hematology 2012;2012:389-396

Boy b 1998, dg. BCP-ALL 2003

46,XY,dic(2;12)(?;p?12)t(2;16)(?;q?),der(10)t(2;10)(q?12;q?12),t(12;21)(p13;q22),+21







MDS

WHO classification

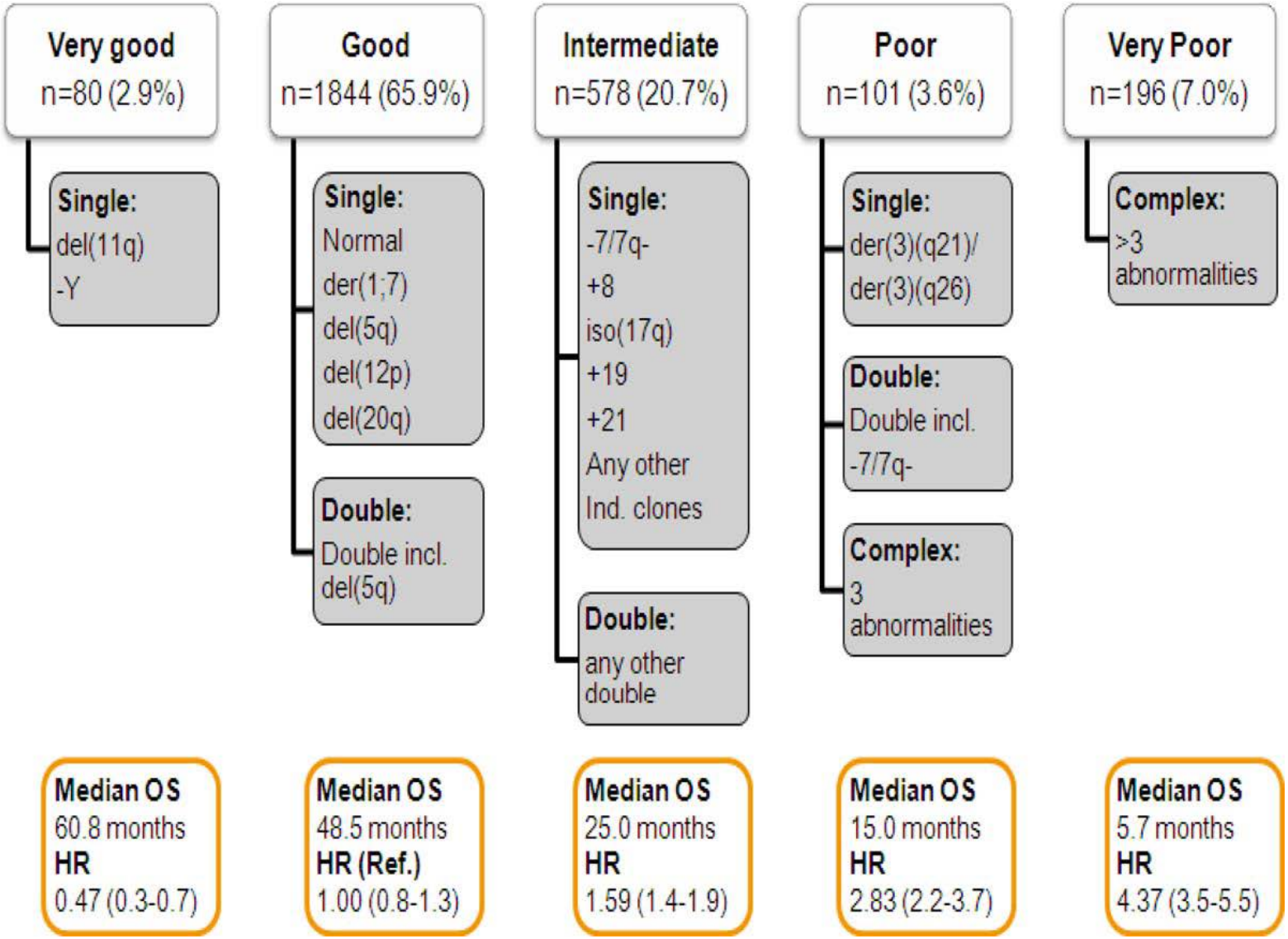
- Refractory cytopenia with unilineage dysplasia (RCUD)
- Refractory anemia with ringed sideroblasts (RARS)
- Refractory cytopenia with multilineage dysplasia (RCMD)
- Refractory anemia with excess blasts-1 (RAEB-1)
- Refractory anemia with excess blasts-2 (RAEB-2)
- Myelodysplastic syndrome, unclassified (MDS-U)
- Myelodysplastic syndrome associated with isolated del(5q)

Clinical heterogeneity is mirrored in heterogeneity of acquired genetic changes

Chromosomal changes in MDS

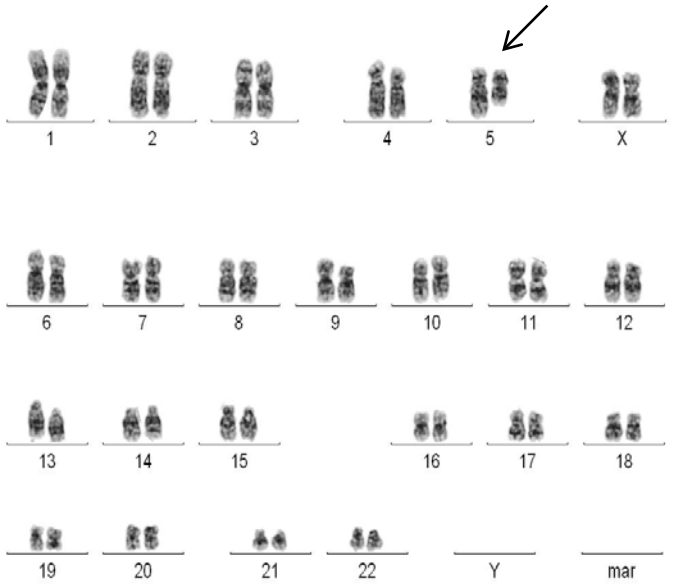
- de novo MDS 40-60%
- t-MDS or secondary MDS 90%
- SNPs+arrayCGH 70%

Prognostic stratification of MDS



5q- SYNDROME

46,XX,del(5)(q31)

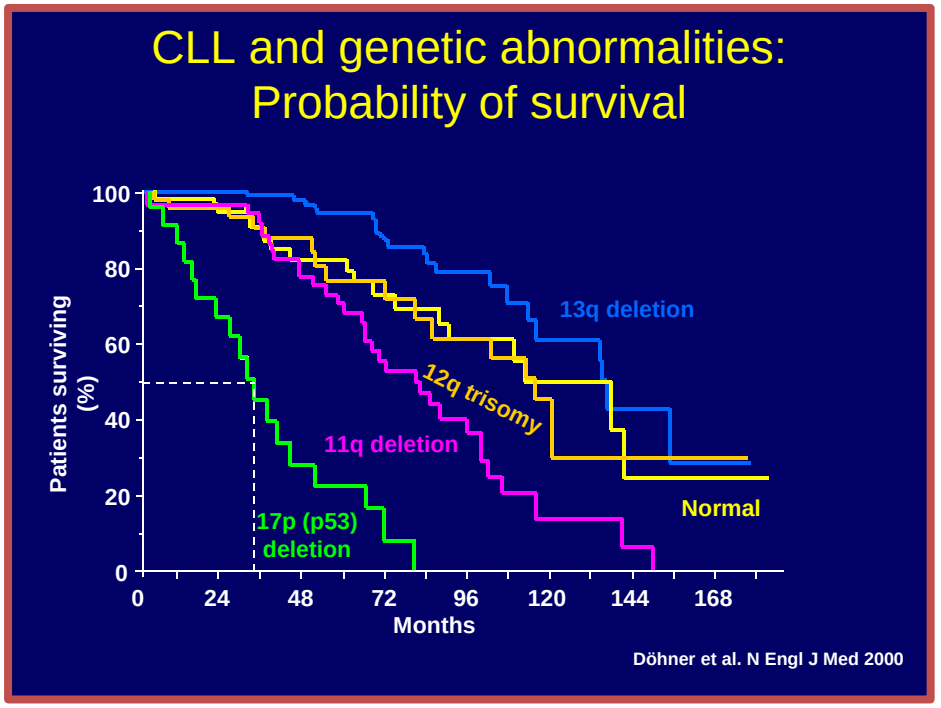


- 10% of patients
- good prognosis
- 5-16 % progress into AML

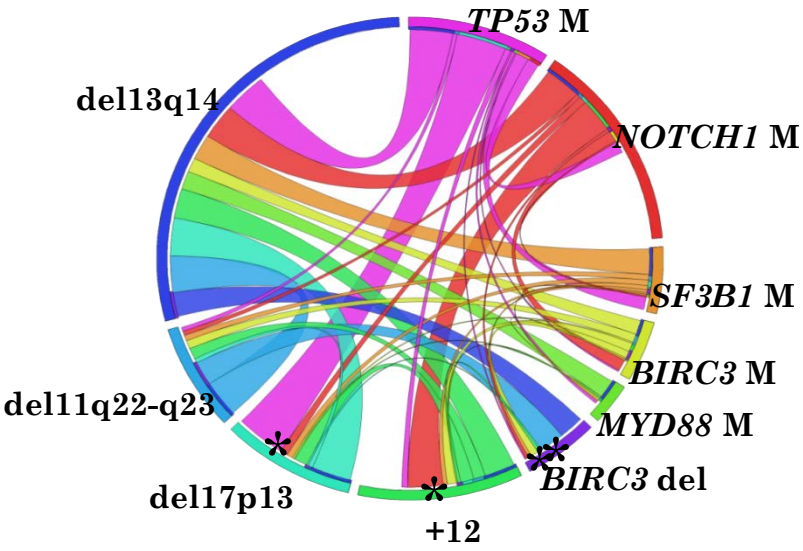
Prognostic significance of cytogenetics in CLL

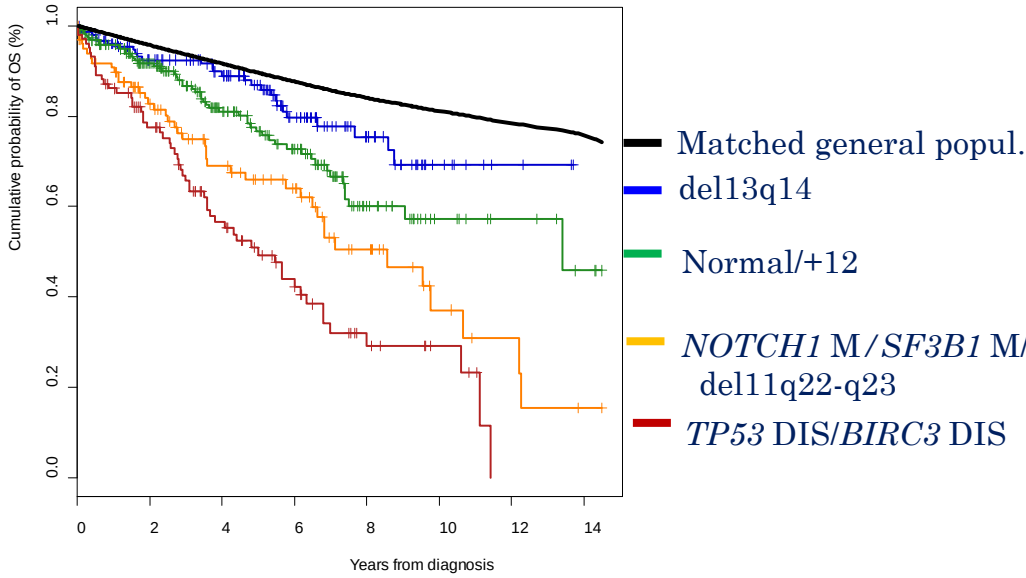
Döhner H, Stilgenbauer S, Benner A, Leupolt E, Krober A, Bullinger L, Dohner K, Bentz M, Lichter P: Genomic aberrations and survival in chronic lymphocytic leukemia.

N Engl J Med 2000; 343:1910-1916.



Mutational and cytogenetic model of CLL





Rossi et al. Blood 2013

CLL – prognostic and treatment stratification

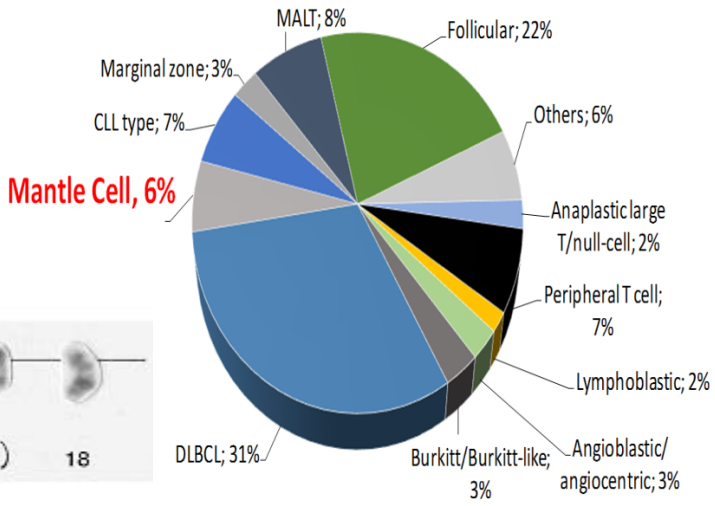
Category	Associated genetic factors	Therapeutic strategies
Very high risk	del(17p)* /*TP53* mutation and/or *BIRC3* mutation	p53-independent drugs, BTK inhibitors, allogeneic stem cell transplantation
High risk	del(11q)* /*ATM* mutation and/or *NOTCH1* mutation and/or *SF3B1* mutation	FCR
Intermediate risk	Trisomy 12 Normal karyotype and FISH	Not recommended
Low risk	Isolated del(13q)*	Not recommended

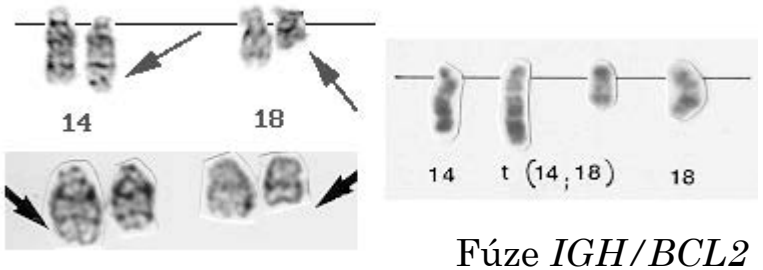
Puiggros et al, BMRI 2014

NON HODGKIN LYMPHOMA

- Heterogenous group of tumors of the lymphatic tissues
- Arise from genetic changes in originally normal cells
- Classification – histopathology WHO 2008
- Cytogenetics and FISH used for classification

Folicular lymphoma (FL)
indolent B cell lymphoma
~20% of all lymphomas
Clinically heterogenous, OS up to 20 years
90% of patients t(14;18)(q32;q21)

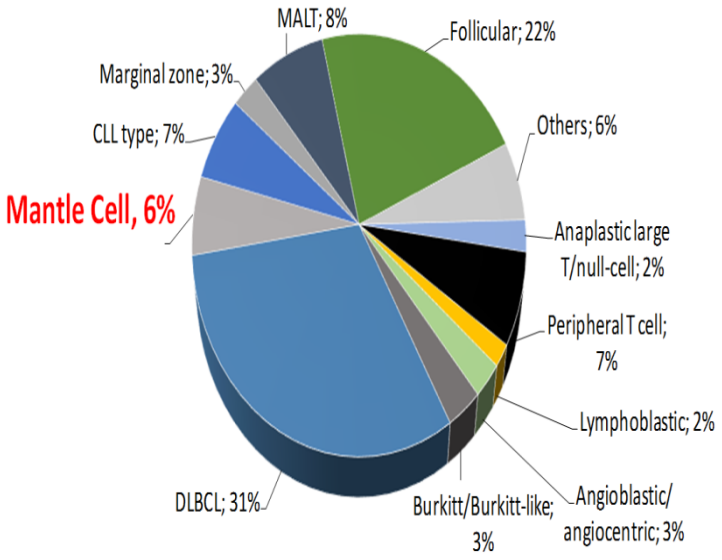




Fúze *IGH/BCL2*

MANTLE CELL LYMPHOMA

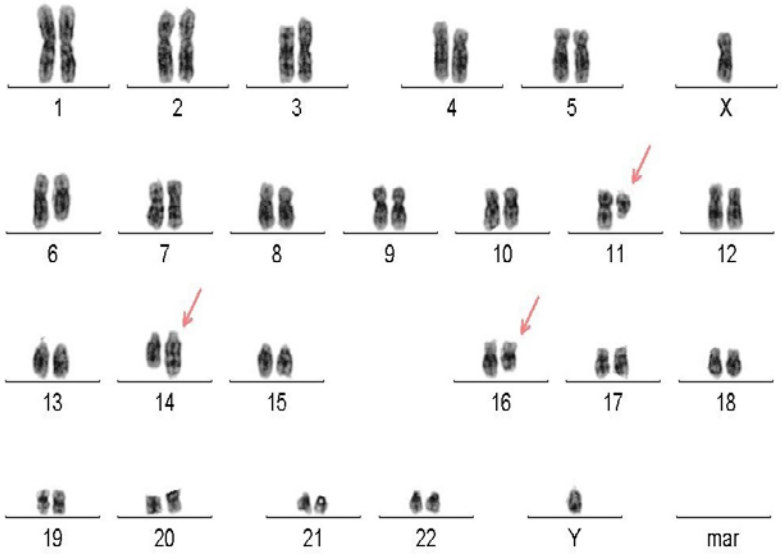
- Aggressive disease (OS 3-5 years)
- ~ 6 % všech NHL
- Diagnostics:
  - Morphology
  - Imunohistochemistry
  - Imunophenotyping
  - Genetics:
    - cytogenetics
    - FISH
    - Molecular genetics - PCR



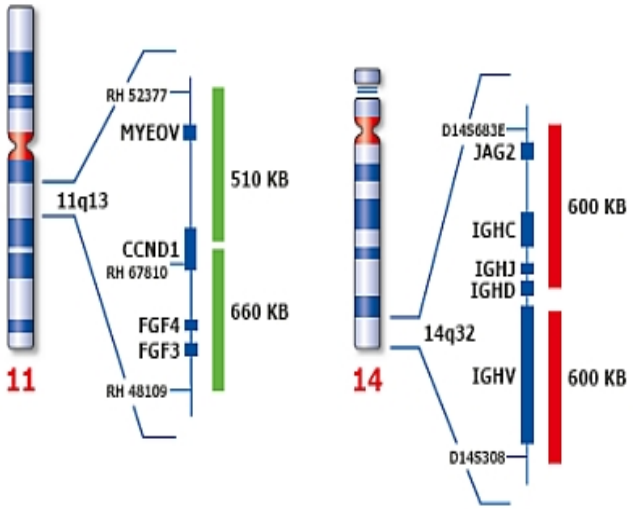
MANTLE CELL LYMPHOMA

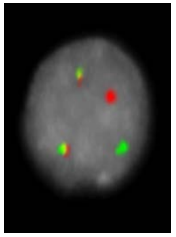
- Conventional cytogenetics

t(11;14)



- FISH
  dual color dual fusion probes

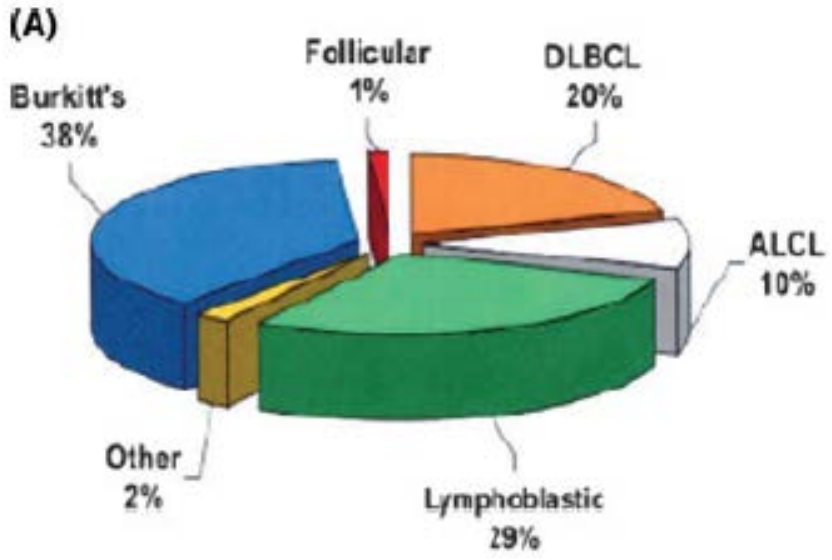




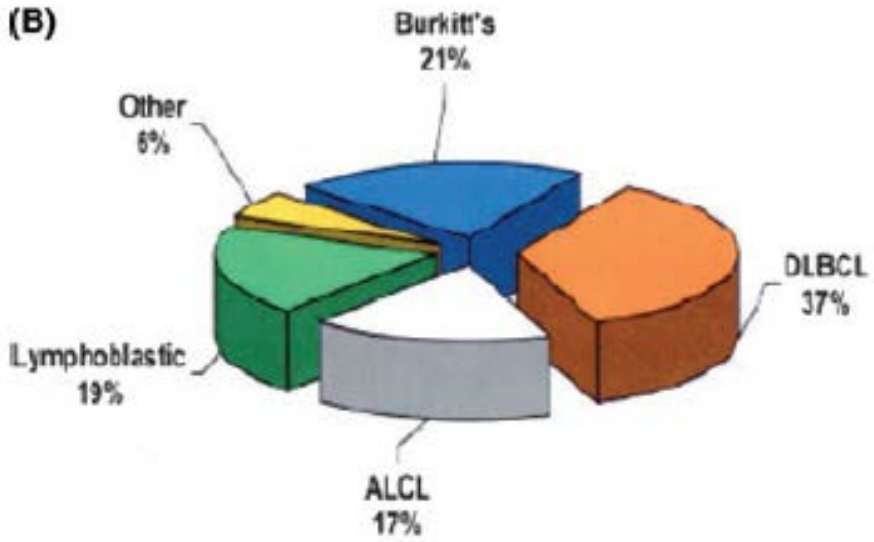
IgH/CCND1 DC
DF Kreatech

NON-HODGKIN LYMPHOMAS IN CHILDREN

- 4-7% of tumors in children and young adults
- Incidence increases with changes
- WHO classification 2008
- frequency of histological subtypes different from adults



0–14 year

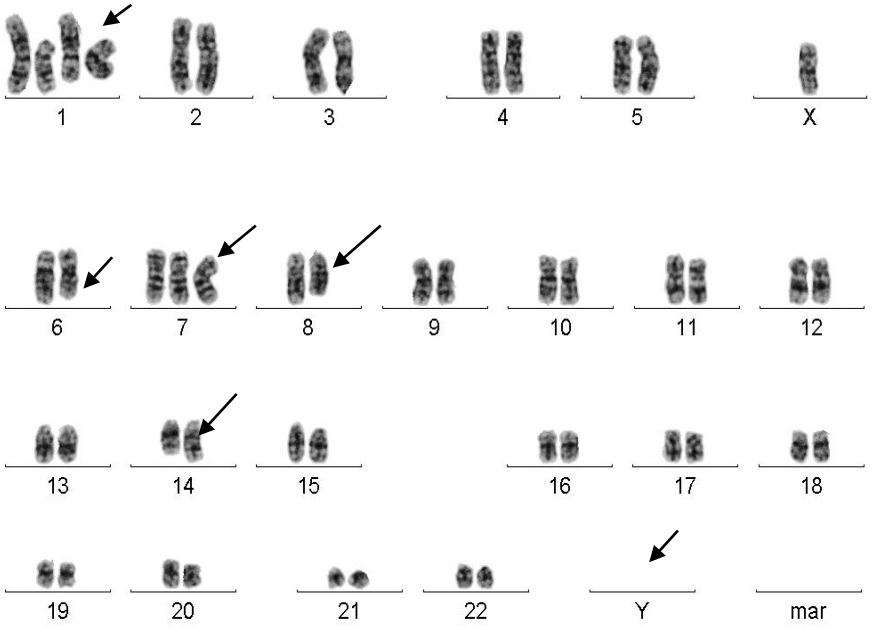


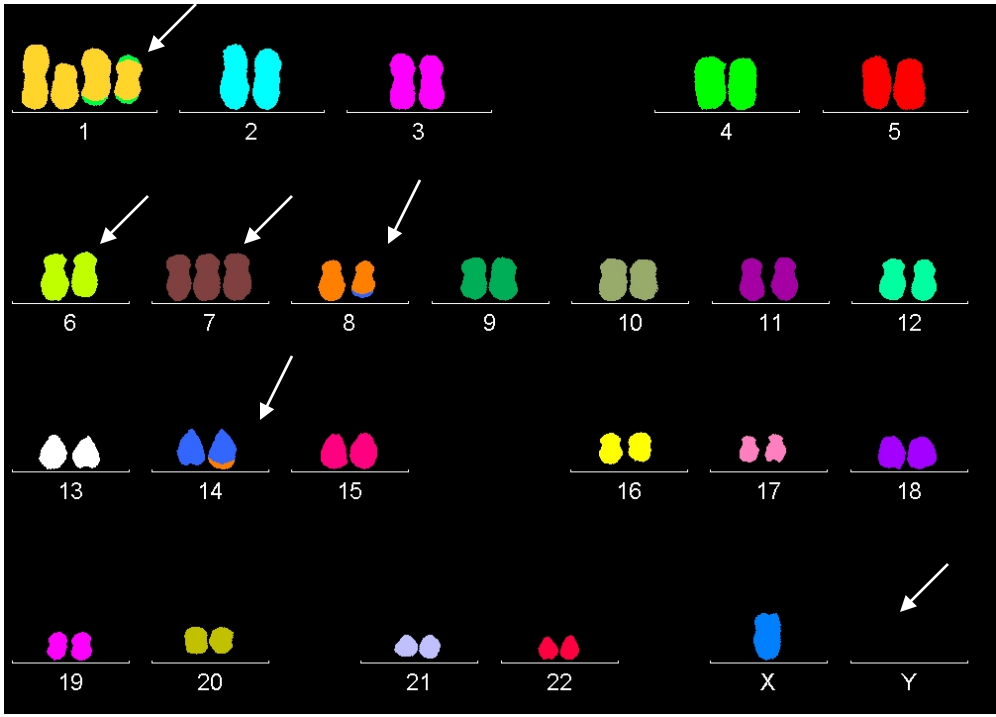
15–19 year age group

Převzato: Hochberg et al, BJH 2008

BURKITT LYMPHOMA

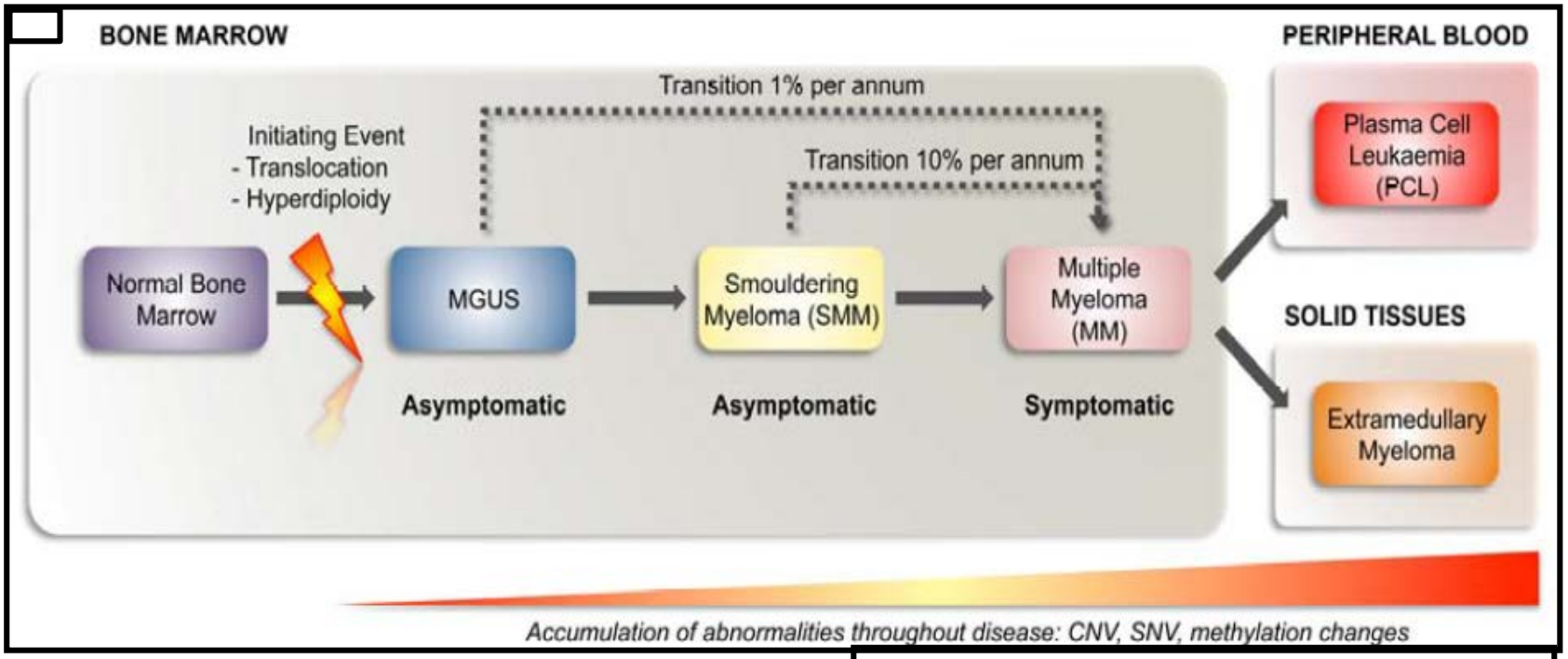
48,X,-Y,del(1)(p13pter),+der(1)del(1)(q?24q?ter)t(1;4)(q23;?q?),
+ider(1)(q11)del(1)(q?24q?ter)t(1;4)(q23;?q?),del(6)(q?15),+7,t(8;14)(q24;q32)(1.klon-56%)





11/2011

MULTIPLE MYELOMA



Walker B, Wardell CP, Melchor L et al., *Submitted*

SUMMARY - CYTOGENETICS

- Diagnostics and prognostic stratifications of hematologic malignancies
- Can analyze the entire genome in one run
- Allows for clarification of diagnosis by specific chromosomal aberration
- Recurrent nonrandom changes determine prognosis of disease
- Aberration classification allows monitoring of treatment efficacy

Thanks for your attention