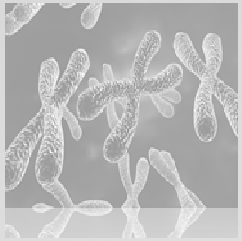


Nádorová cytogenomika
(ONKOCYTOGENETIKA)

Metody v onkocytogenetice

Marie Jarošová

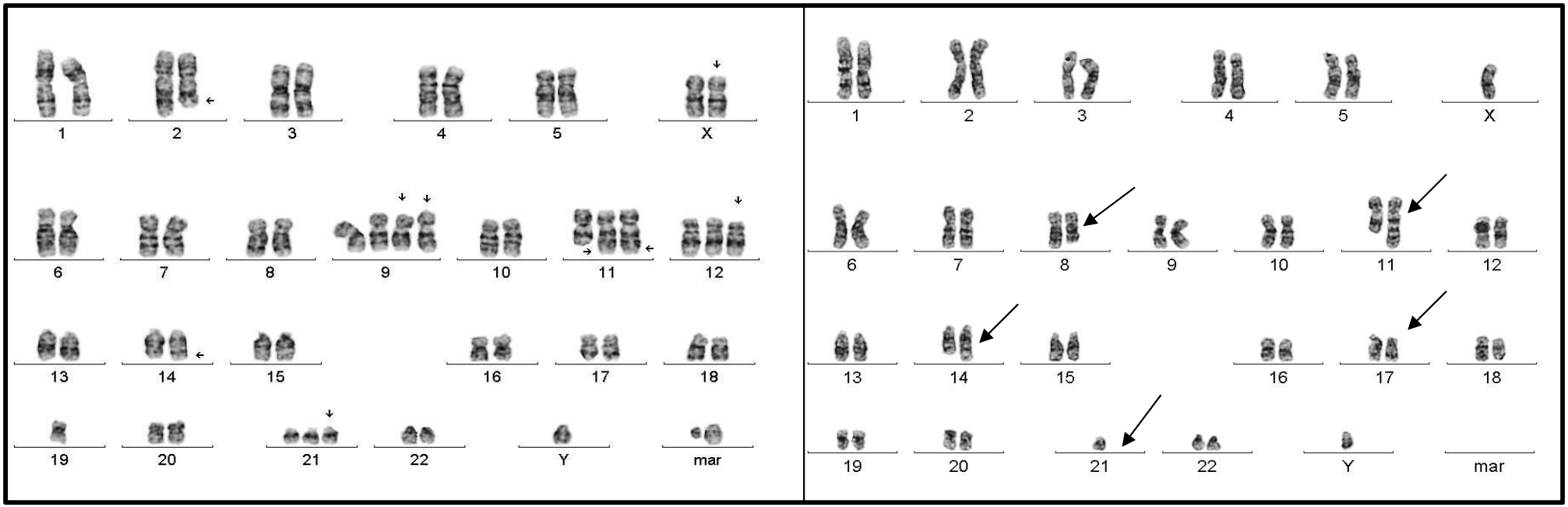
Centrum molekulární biologie a genové terapie
Interní hematoonkologická klinika FN a LF MU Brno
Ústav lékařské genetiky a genomiky MU Brno

Nádorová cytogenomika

- *Historie*
- *Metody cytogenetiky*
- *Klasické cytogenetické vyšetřené*
- *Metody molekulární cytogenetiky*
  - *FISH*
  - *mFISH*
  - *Mband*
  - *arrayCGH/SNP array*
- *Význam a využití metod v hematologii a onkologii*

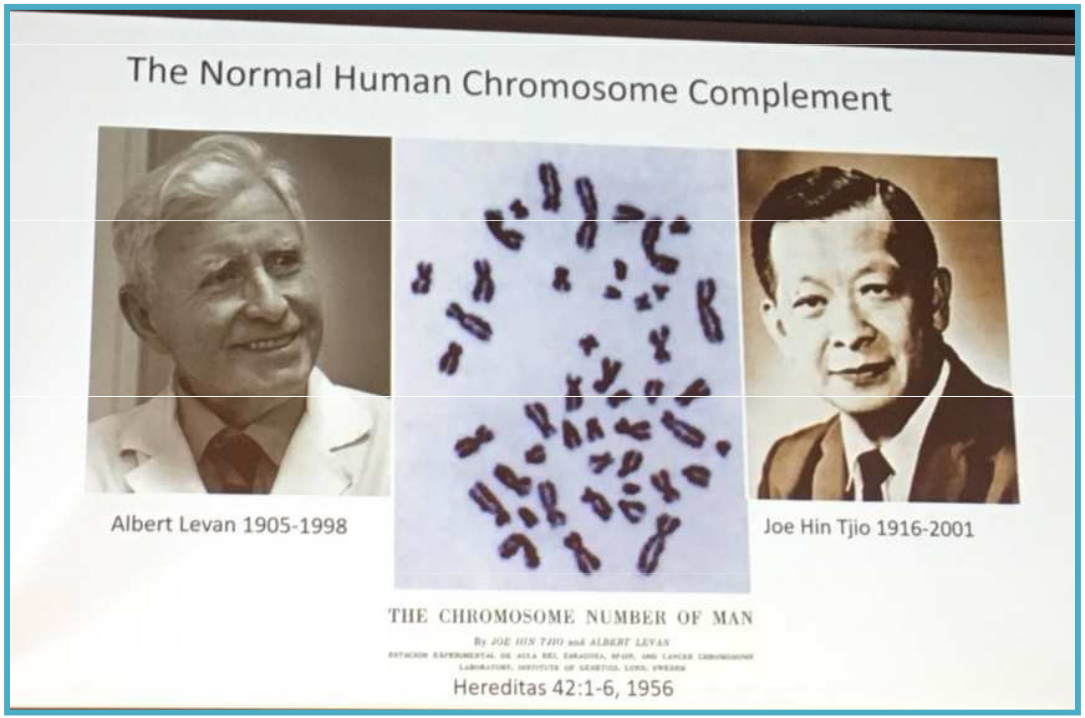
Nádorová cytogenomika

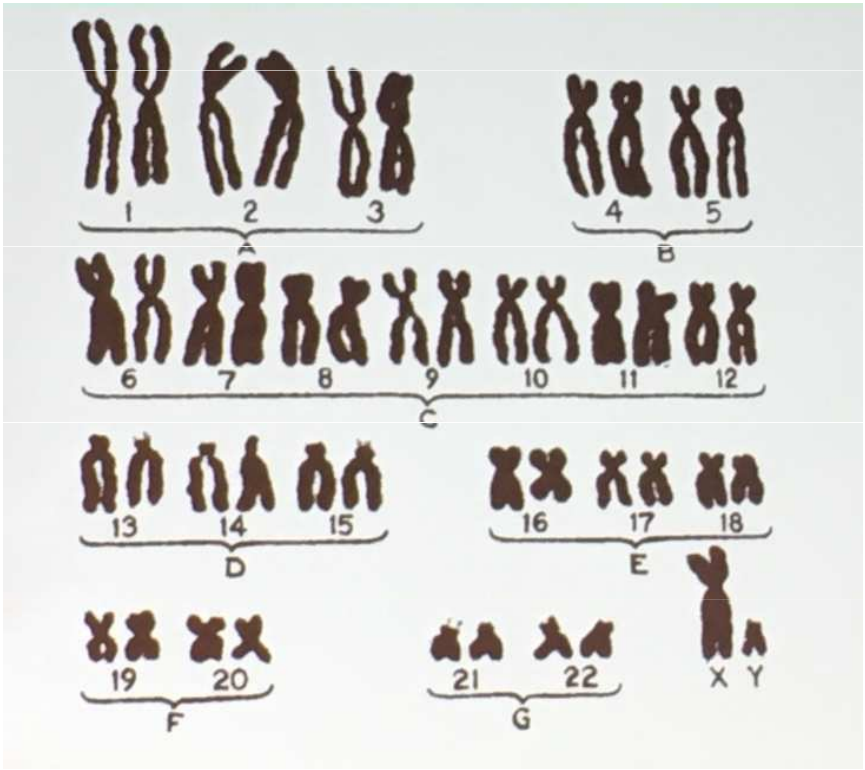
- Charakteristickou vlastností nádorových buněk jsou chromosomové změny : početní změny chromosomů
  strukturní změny chromosomů



Historie cytogenetiky

Cytogenetics is the study of the structure and properties of chromosomes, their behaviour during somatic cell division during growth and development (mitosis), and germ cell division during reproduction (meiosis), as well as their influence on phenotype. Cytogenetics also includes the study of factors that cause chromosomal changes.
Hare &Singh1979



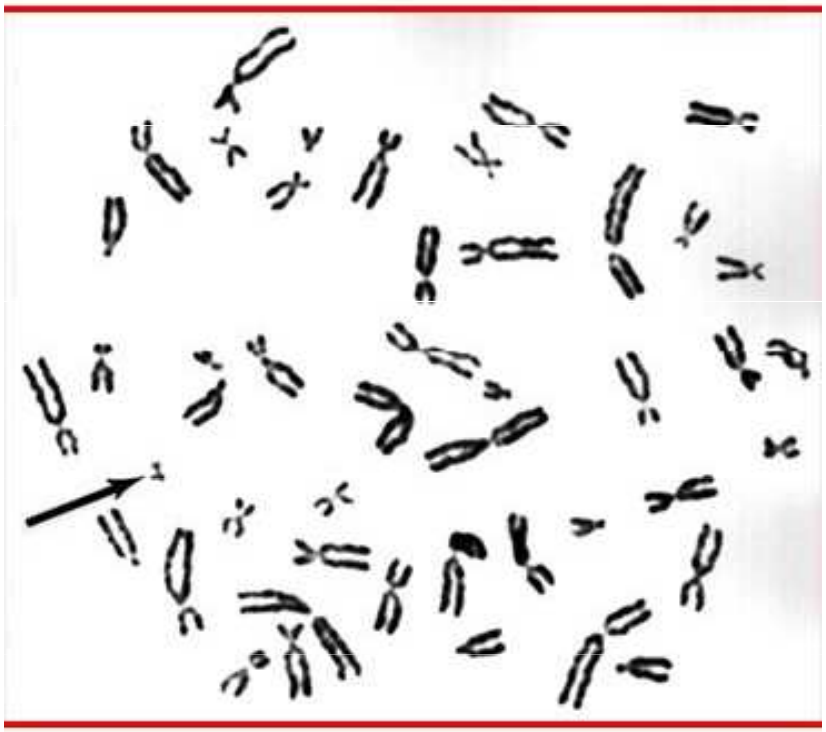


1956 - určen přesný počet 46 lidských chromosomů

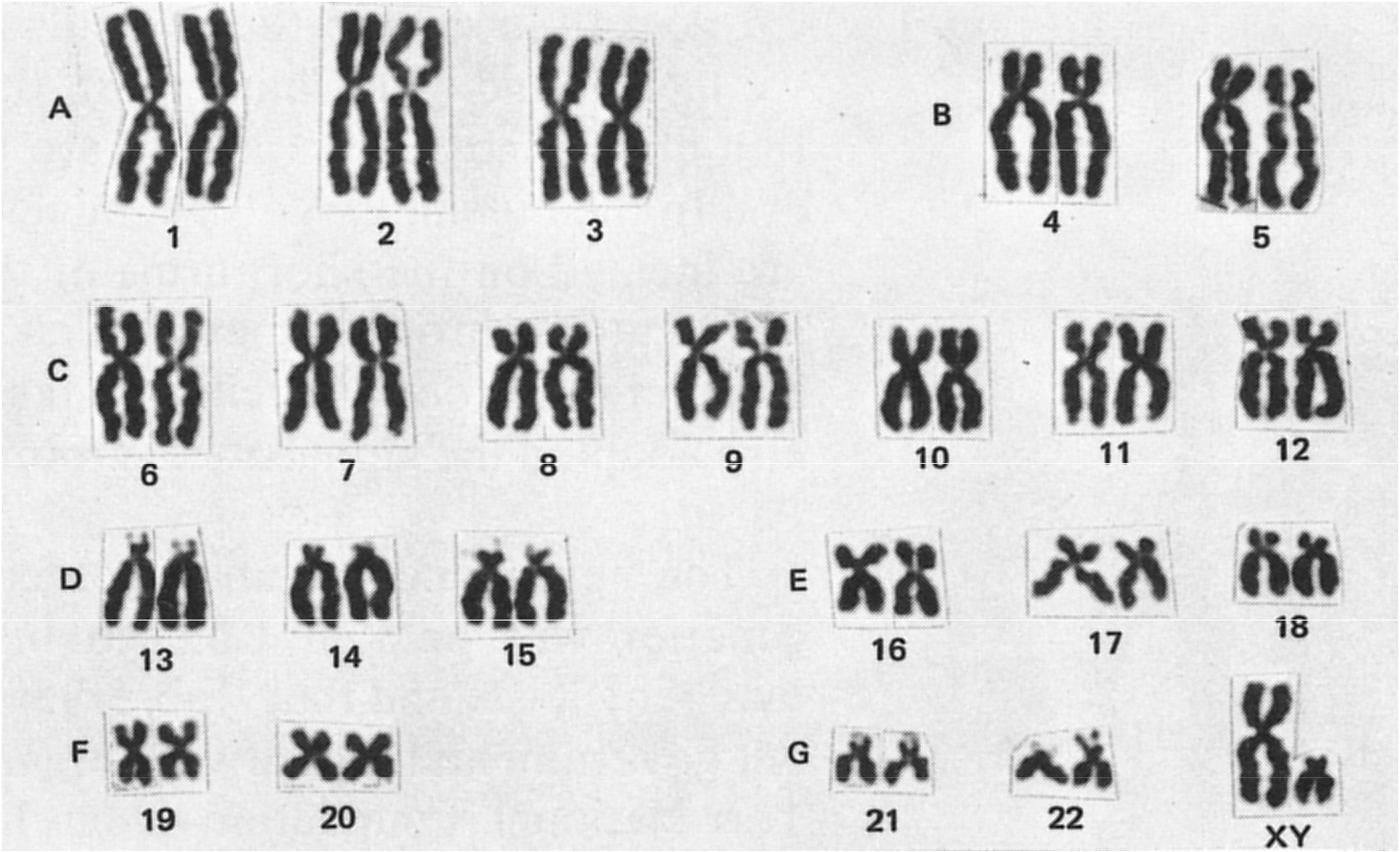
Historie nádorové cytogenetiky

Philadelphia chromosome (Ph_1)

Peter Nowell & David Hungerford
Science 1960,132:1497



Historie nádorové cytogenetiky



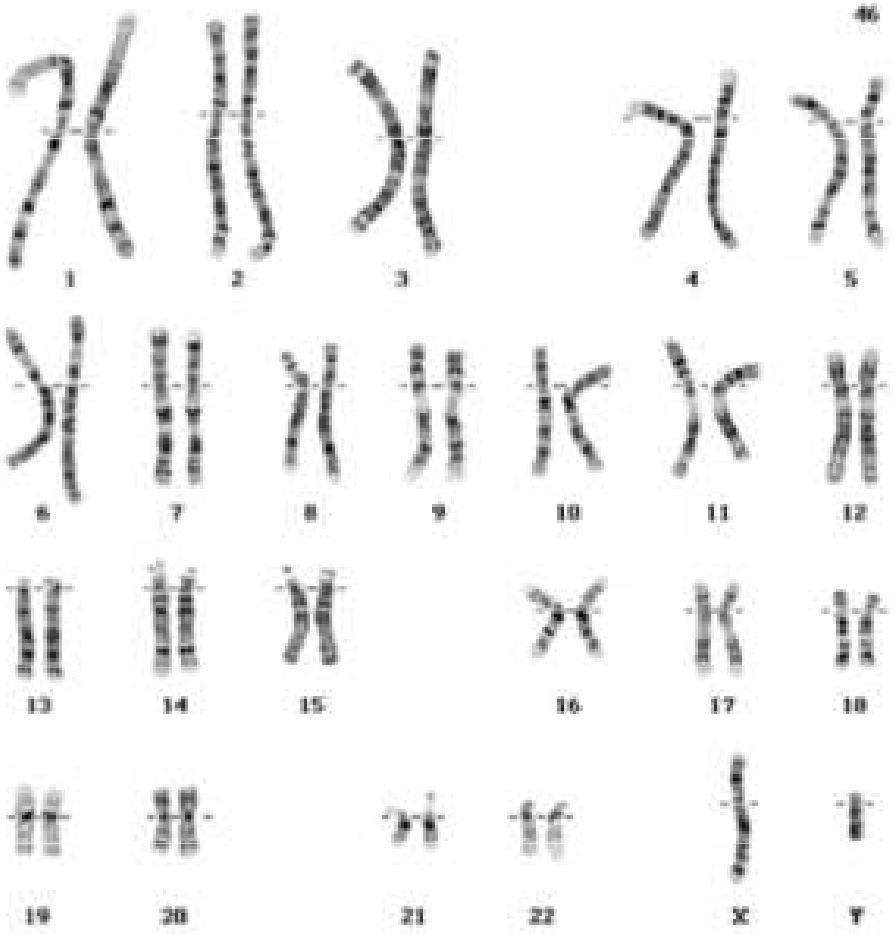
Classification of chromosomes into seven groups by size and relative centromere position established the so-called "Denver System" (right) in 1960. Chromosomes within groups B - G were not readily distinguishable from each other. The X chromosome is in the C group, and the Y is in the G group: males are recognizable by five small G-type chromosomes.

Historie nádorové cytogenetiky

Pruhování chromosomů

G-banded Ideogram

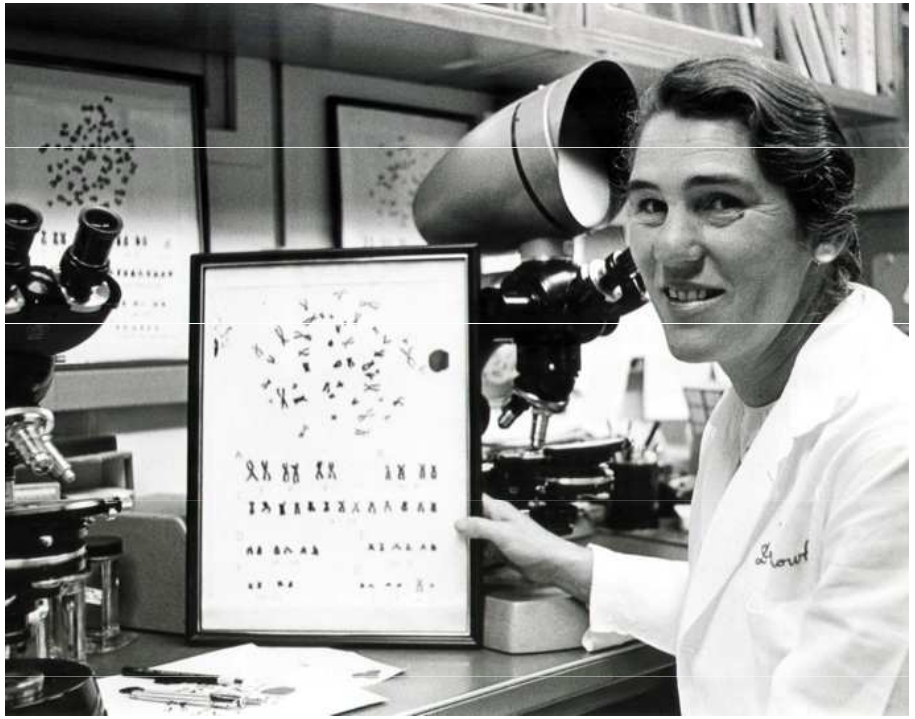
- Controlled digestion of chromosomes with trypsin
- Stain with Giemsa, a DNA binding chemical dye
- Produces alternating light (GC rich) and dark (AT rich) bands
- Banding pattern allows chromosome identification



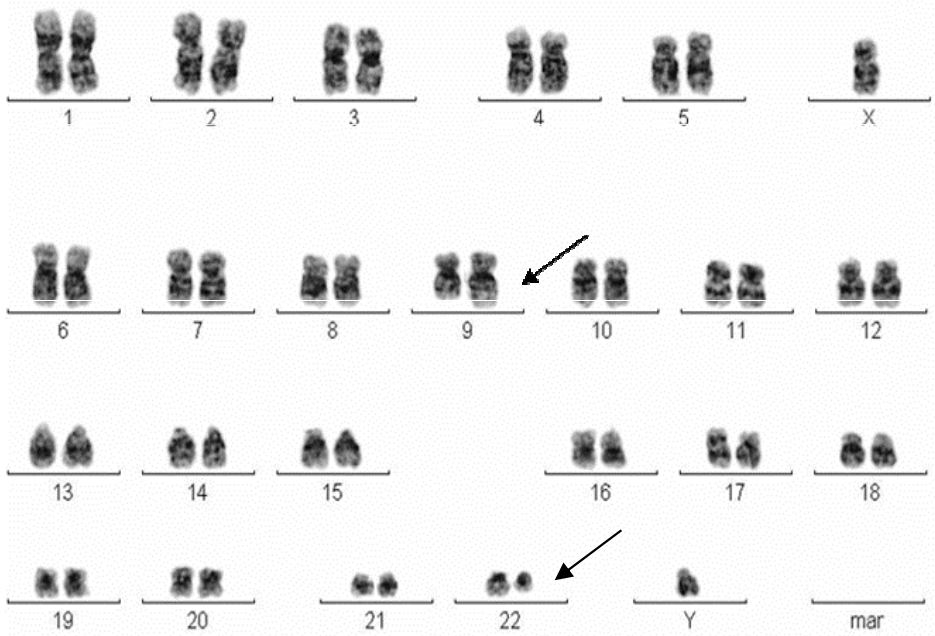
Banding techniques: Casperson & Zech,1970

Historie nádorové cytogenetiky

Philadelphia chromosome (Ph1)
(1973)



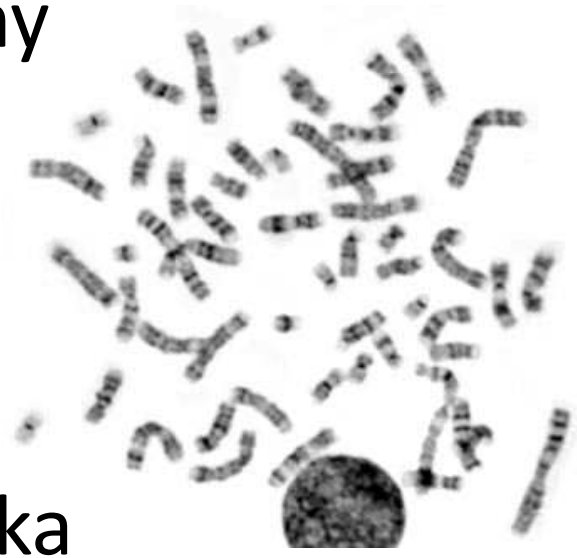
t(9;22)(q34;q11)



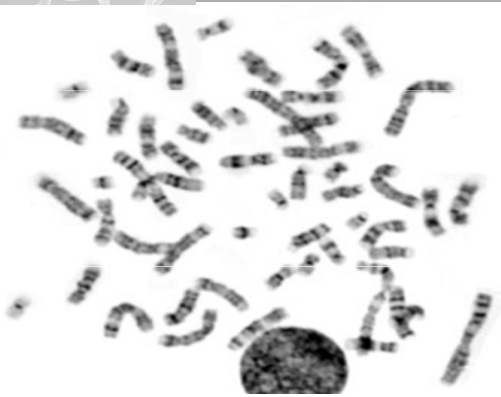
Dr. Rowley received the Lasker Award, given for distinguished contributions to medical science; the National Medal of Science from President Bill Clinton; and the Presidential Medal of Freedom from President Obama, among many other honors (1925-2013)

Nádorová cytogenetika (onkocytogenetika)

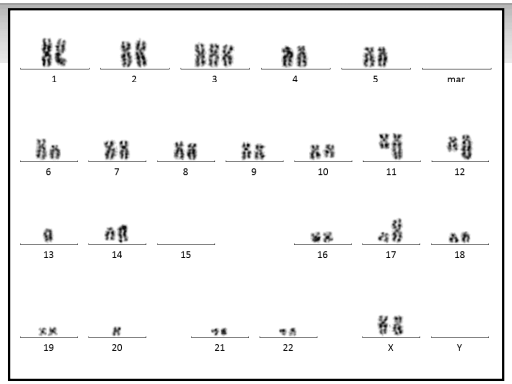
- Zkoumá získané chromosomové změny nádorových buněk
- Hodnotí početní a strukturní změny chromosomů
- Základní metoda – G-pruhovací technika (rozlišení kolem 3-5Mb)
- <u>V jednom vyšetření analyzuje celý genom</u>

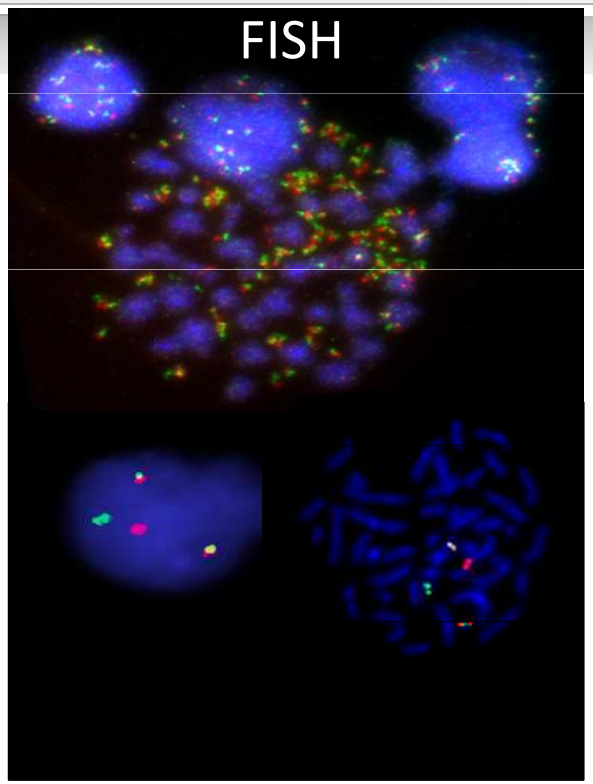


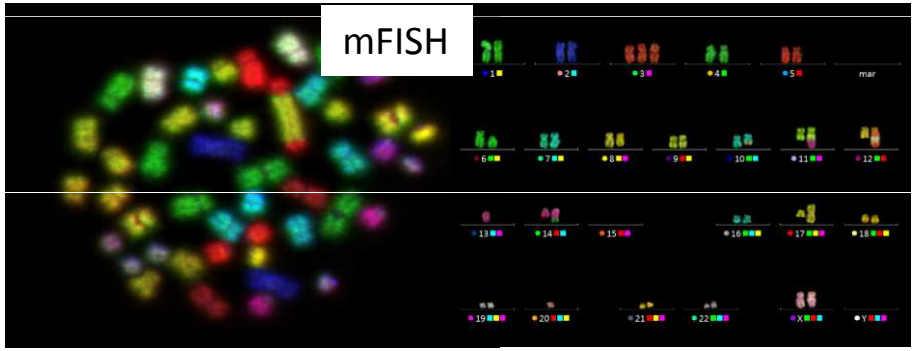
Nádorová cytogenomika - metody

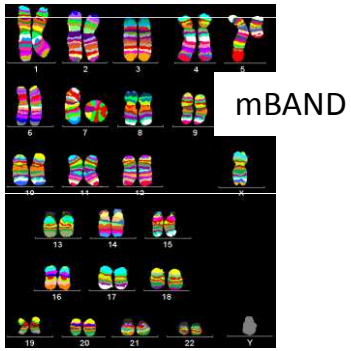


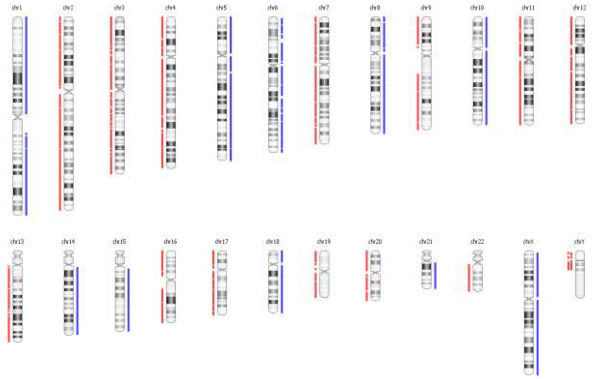
Konvenční cytogenetická analýza

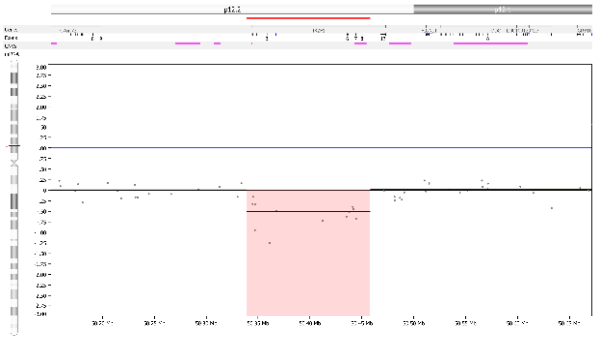


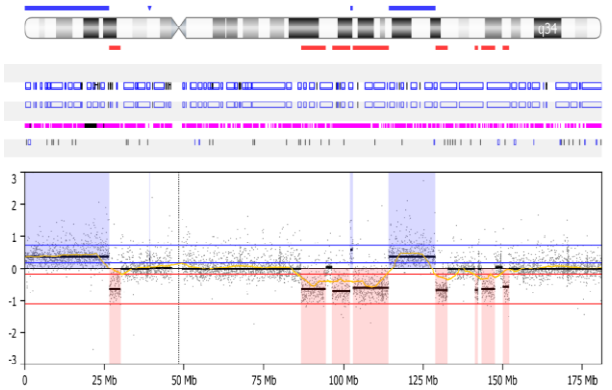






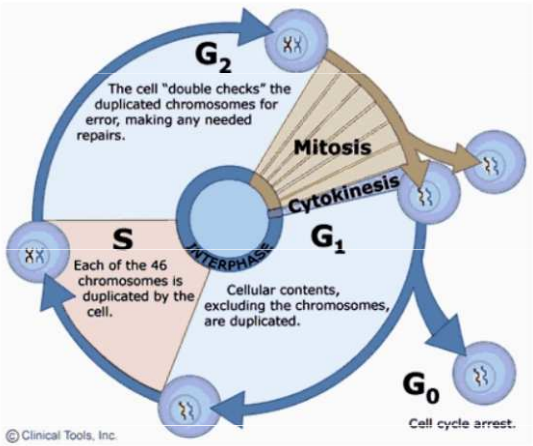


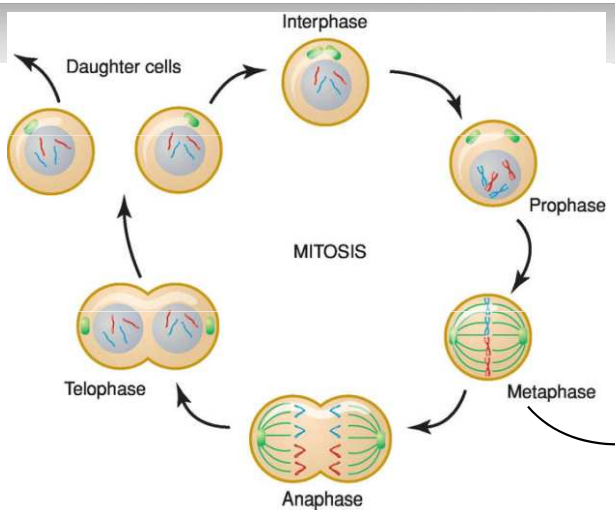


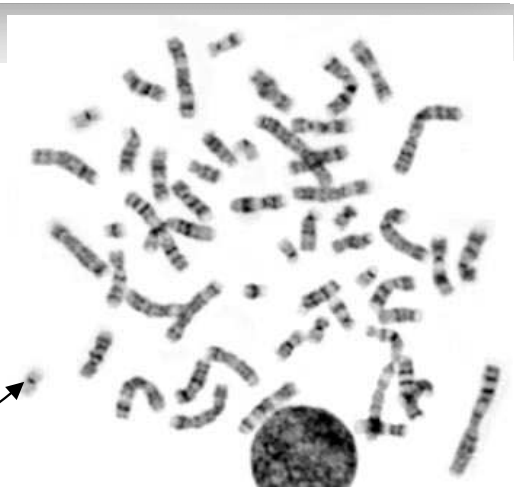


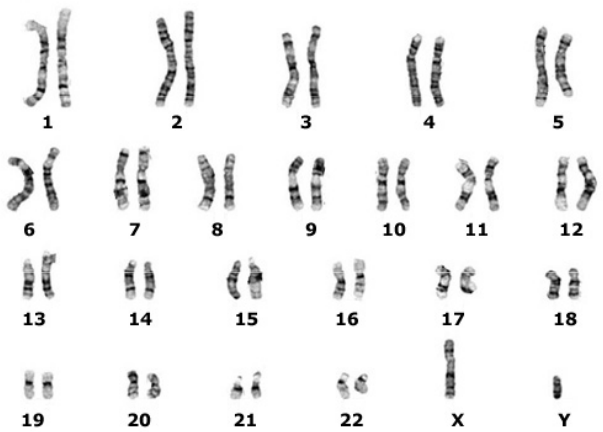
arrayCGH/SNP array

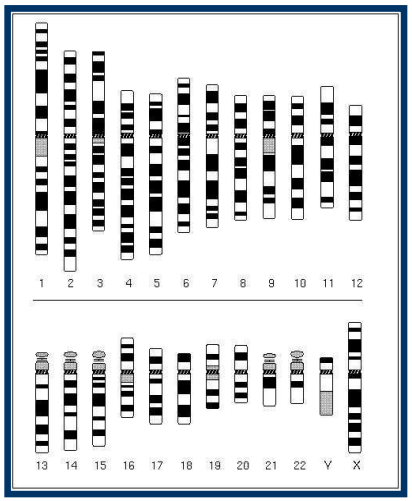
Konvenční cytogenetika

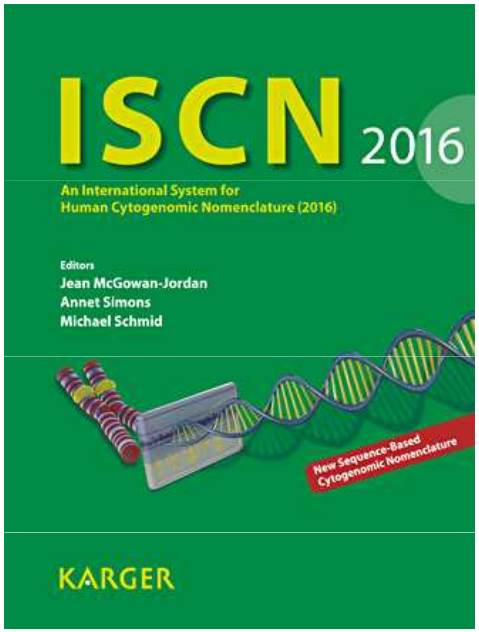




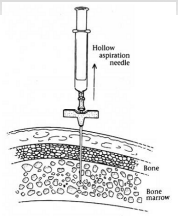






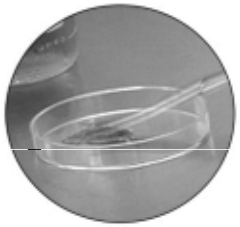


Postup kultivace buněk nádorů



1-2ml

- ✓ kostní dřeň
- ✓ periferní krev
- ✓ uzlina
- ✓ nádorová tkáň



37°C/ 5%CO2

Kultivace
2/24/72hod/týdny

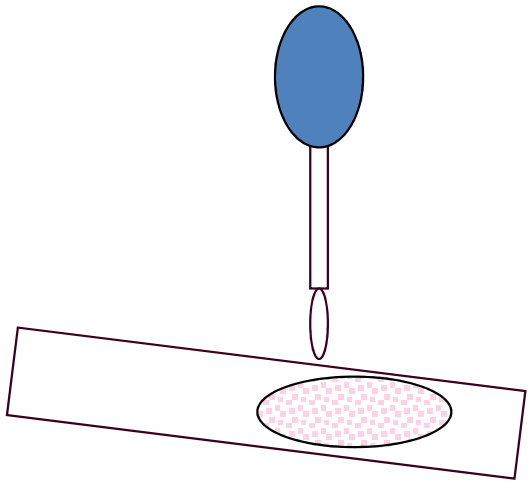
ZPRACOVÁNÍ BUNĚČNÉ KULTURY

HYPOTONIZACE
0,075M KCl

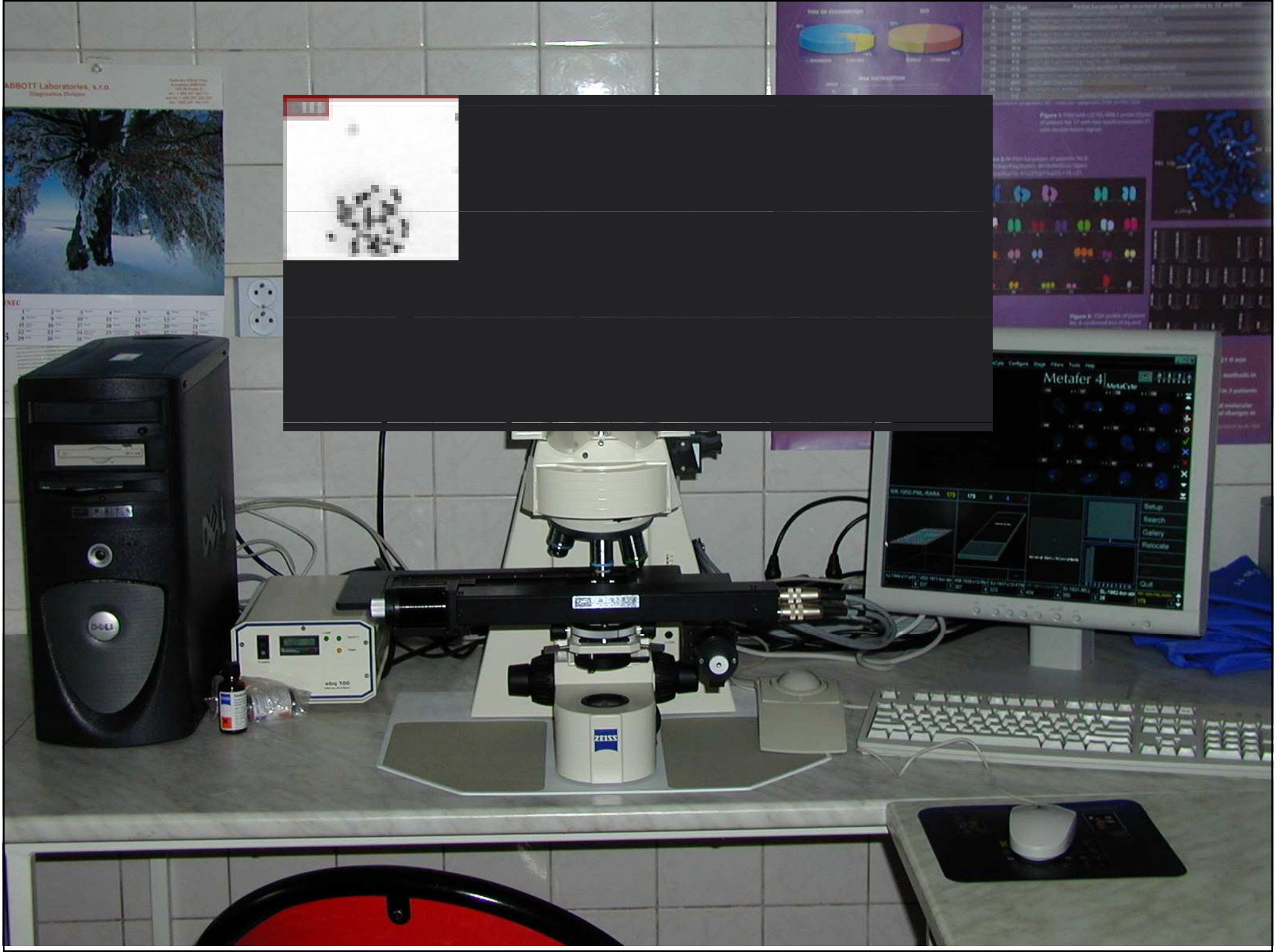
COLCEMIDE BLOKUJE MITÓZY V METAFÁZI

FIXACE ROZTOKEM
KYS.OCTOVÉ A METANOLU v poměru
1:3

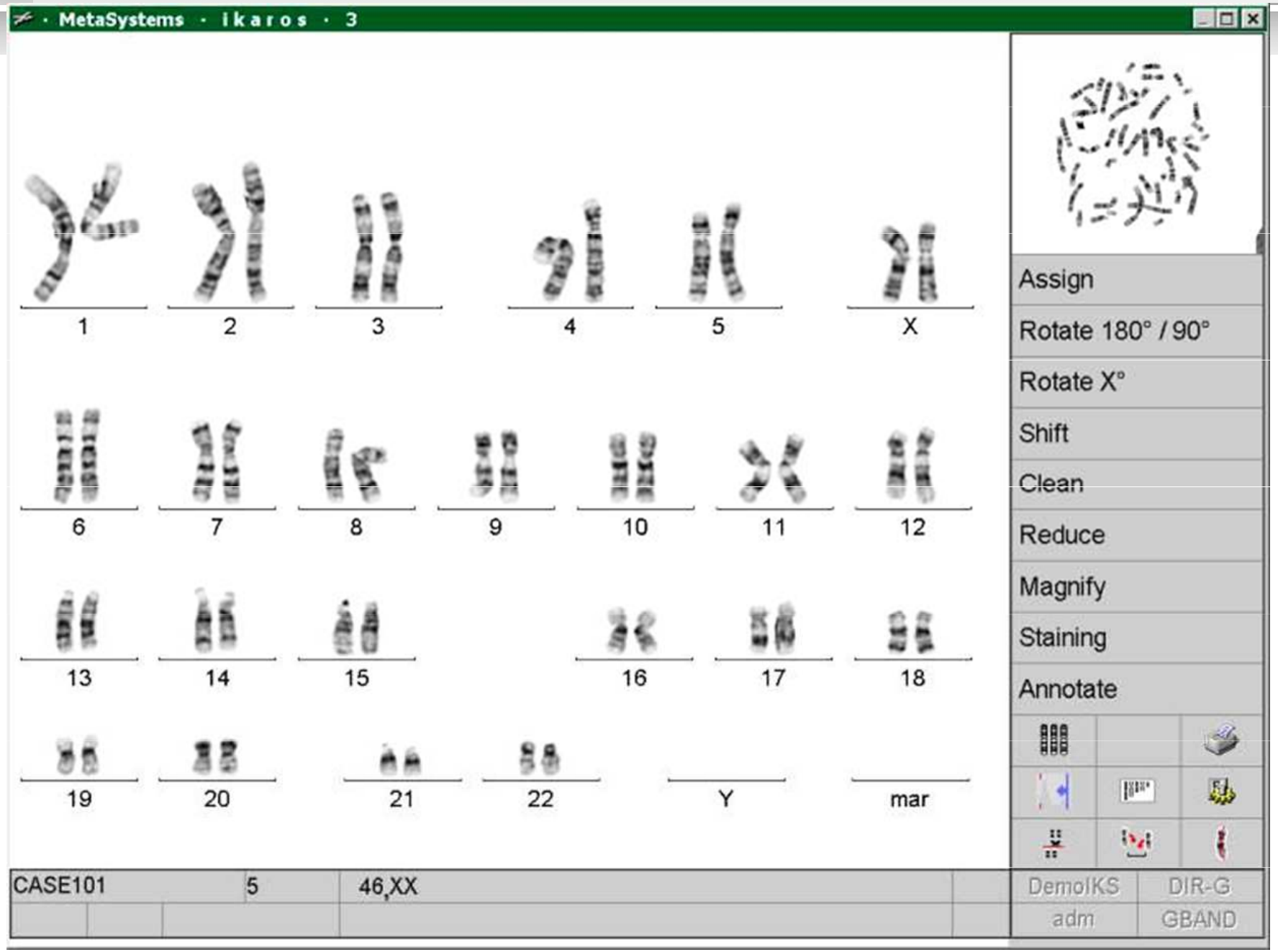
PŘÍPRAVA PREPARÁTŮ
KAPÁNÍM BB SUSPENZE
NA SKLO



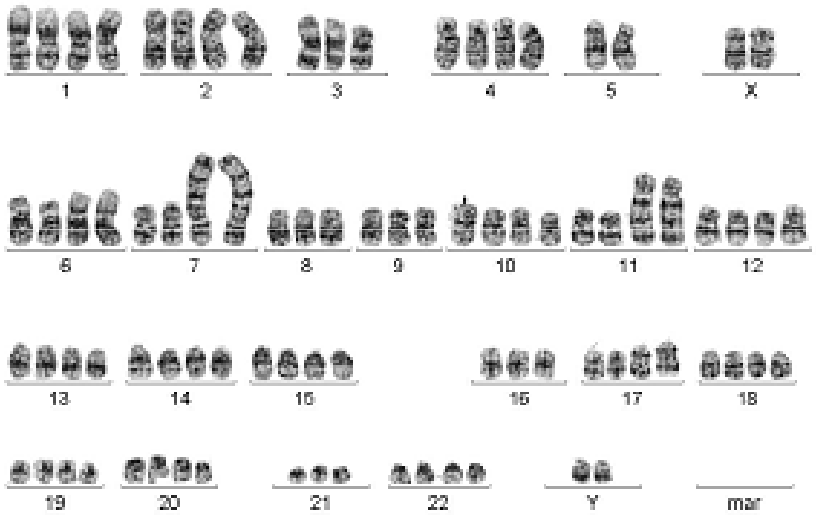
BARVENÍ A HODNOCENÍ
V MIKROSKOPU


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

Klasická cytogenetika - karyotyp



Cytogenetické vyšetření



G-banding; rozlišení 5-10Mb, např. chr 8 : 146Mb; ~500 genů,cMYC ~600kb,

E.C.A. - EUROPEAN CYTOGENETICISTS ASSOCIATION NEWSLETTER No. 31 January 2013

Guidelines and Quality Assurance
for Acquired Cytogenetics

A common European framework for quality assessment for banded chromosome studies and molecular cytogenetic investigations of acquired abnormalities.

E.C.A. Permanent Working Group for Cytogenetics and Society

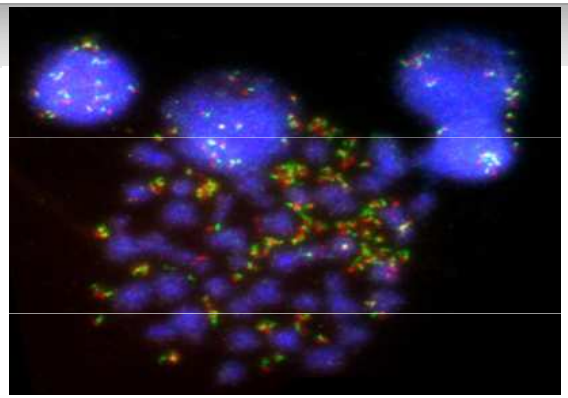
Authors:
Ros Hastings, Rod Howell, David Betts, Sarah Porter, Claudia Haferlach, Nicole Dastugue, Isabelle Radford-Weiss, H.Berna Beverloo, Annet Simons, Clemens Mellink, Simone Snijder, Eva van den Berg-de Ruiter, Jacqueline Schoumans, Blanca Espinet, Reiner Siebert, Jerome Couturier, Alain Bernheim, Francesc Solé, Isabelle Luquet, Sabine Stioui, Simona Cavani.

In the first instance, banding analysis must be undertaken and, if an abnormal karyotype is found, a minimum of five abnormal metaphases must be fully analysed with a further five clonal metaphases counted and scored for additional structural changes if available. In the event of anormal karyotype 20 metaphases must be examined with at least ten fully analysed and the remainder counted and scored for structural abnormalities before the issue of a normal report. If 20 metaphases cannot be examined the normal report must be qualified (see section 5 on reporting).

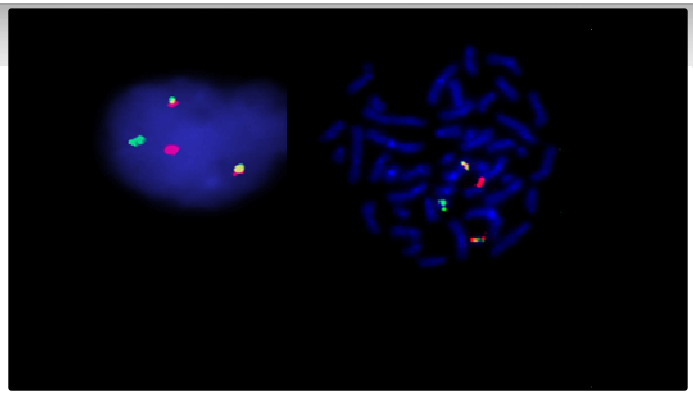
Cytogenetics and molecular genetics European recommendations and quality assurance for cytogenomic analysis of haematological neoplasms.

Rack et al. Leukemia (2019) 33:1851–1867

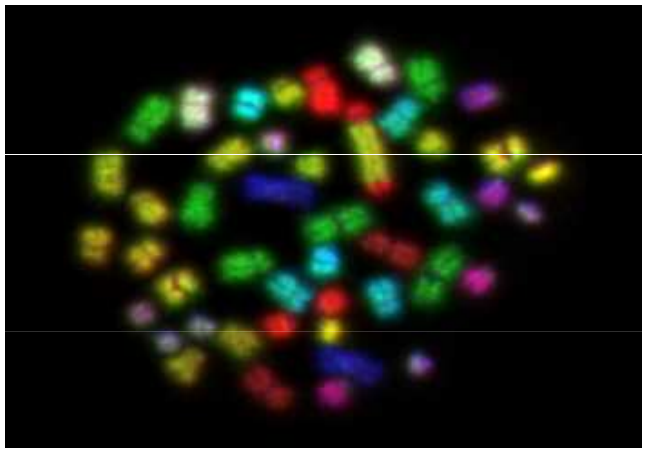
Molekulární cytogenetika



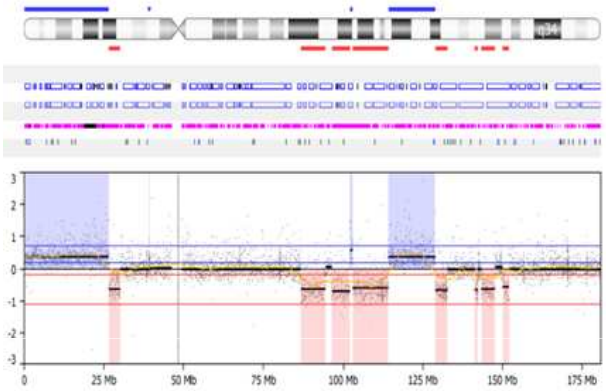
FISH



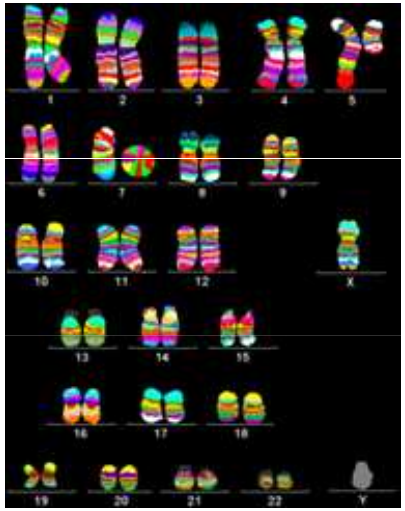
mFISH



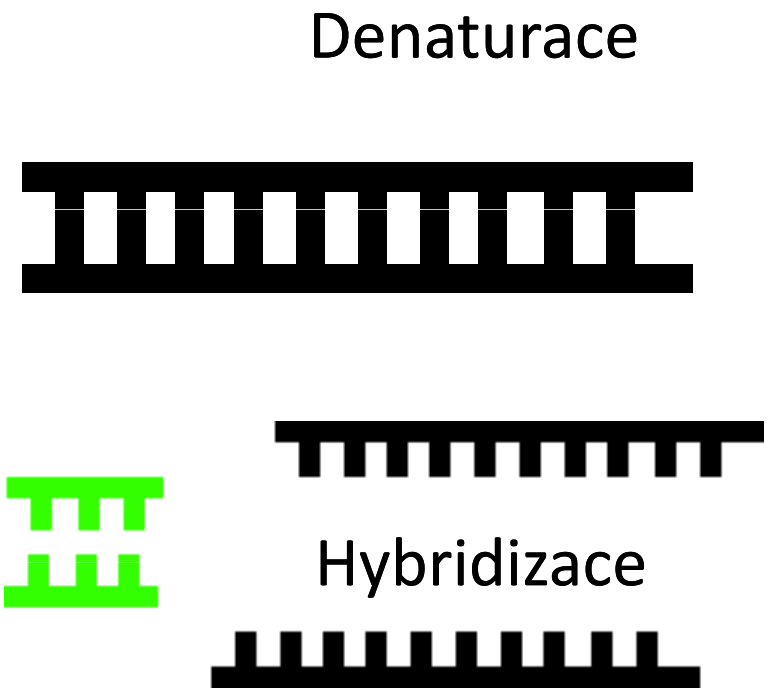
arrayCGH/SNP array



mBAND

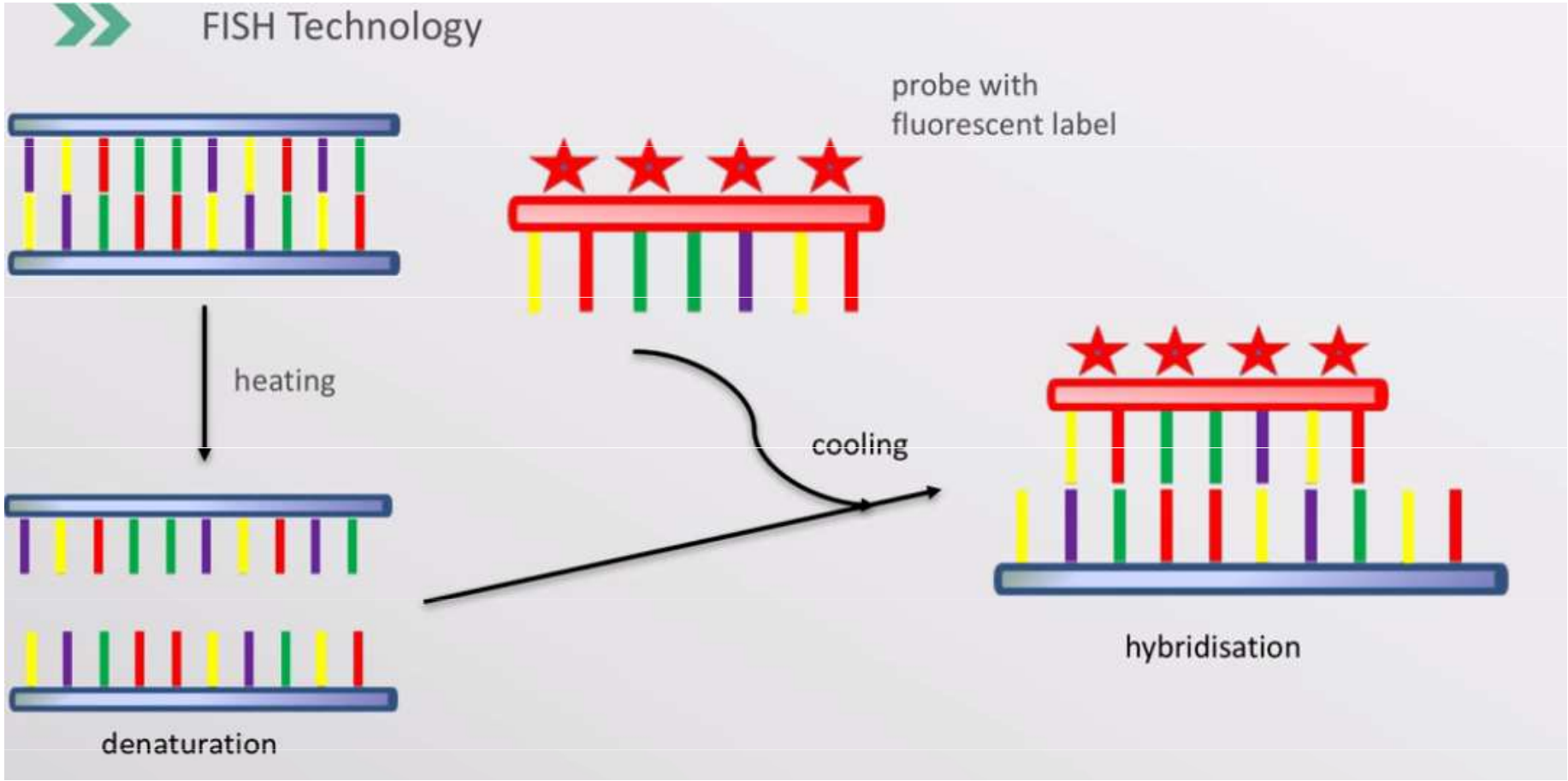


Molekulární cytogenetika

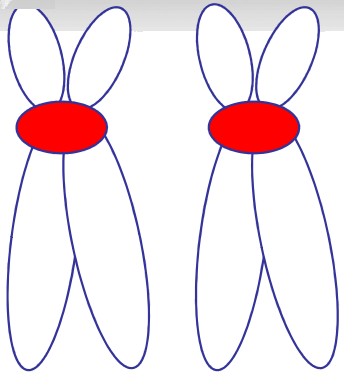


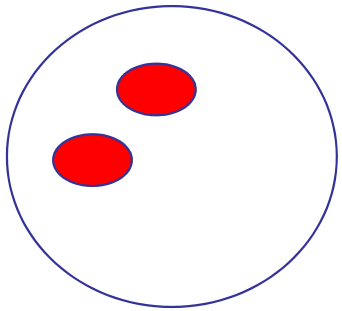
- Metody založené na fluorescenční in situ hybridizaci (FISH) vytváří spojení mezi metodami molekulární genetiky a klasické cytogenetiky

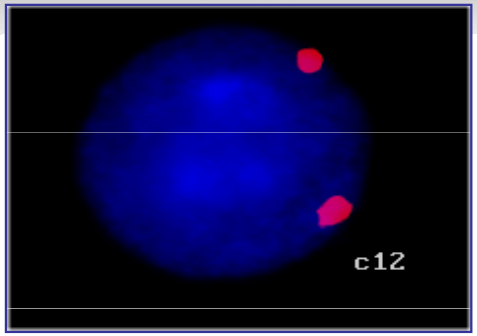
- Metody využívající základní vlastnosti jednořetězcové DNA vzájemně se vázat na základě komplementarity bazí


FISH Technology
probe with
fluorescent label
heating
cooling
hybridisation
denaturation

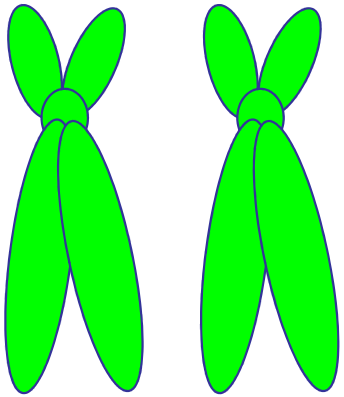
Typy sond

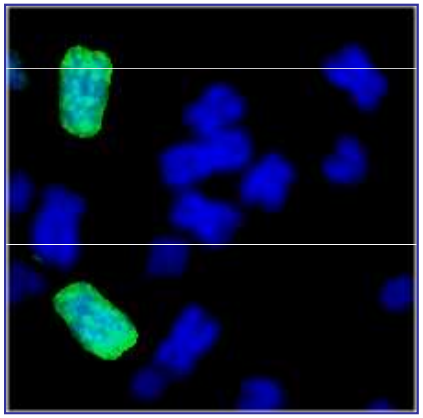




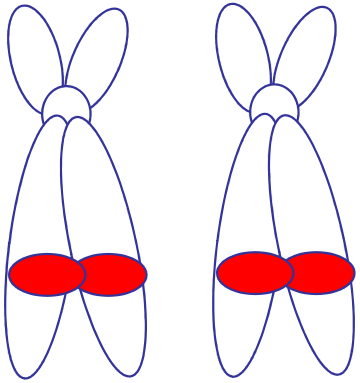


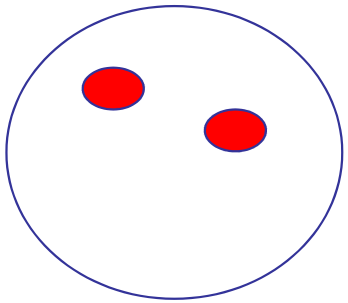
centromerické

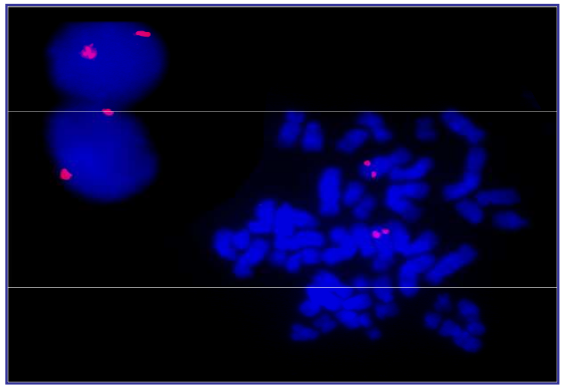




celochromosomové







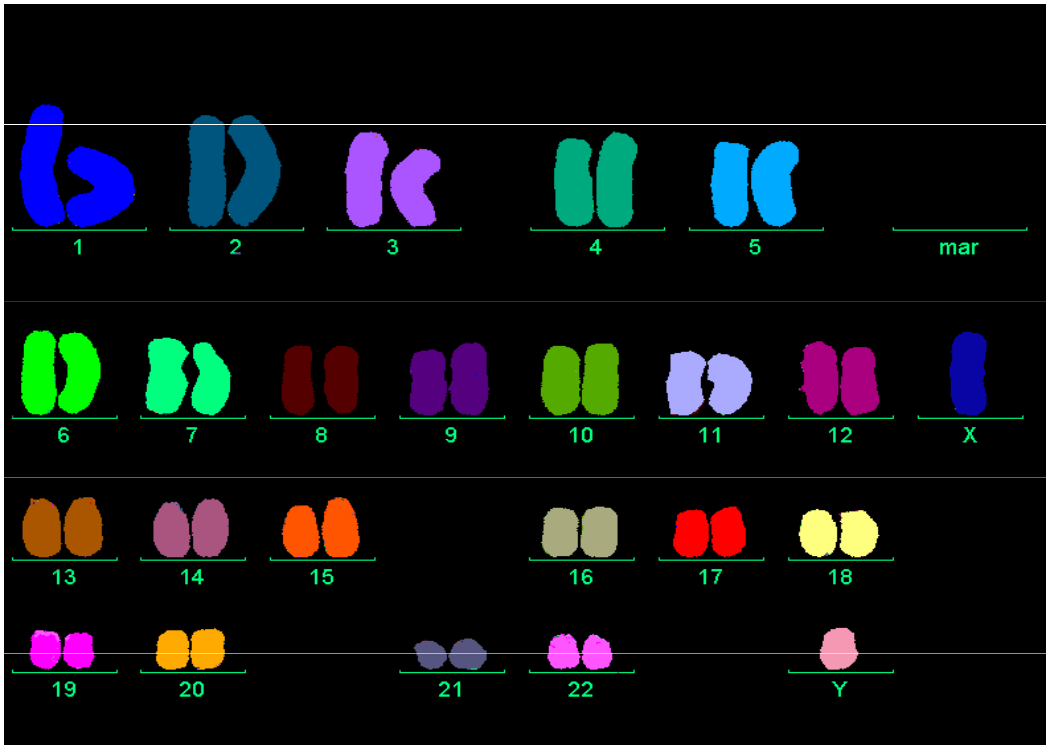
genové

Mnohobarevná fluorescenční in situ hybridizace (mFISH)

Mnohobarevná fluorescenční in situ hybridizace (M-FISH) je molekulárně cytogenetická metoda založená na hybridizaci 24 fluorescenčně značených celochromosomových sond, které dovolují současně obarvení všech chromosomových párů odlišnými barvami.

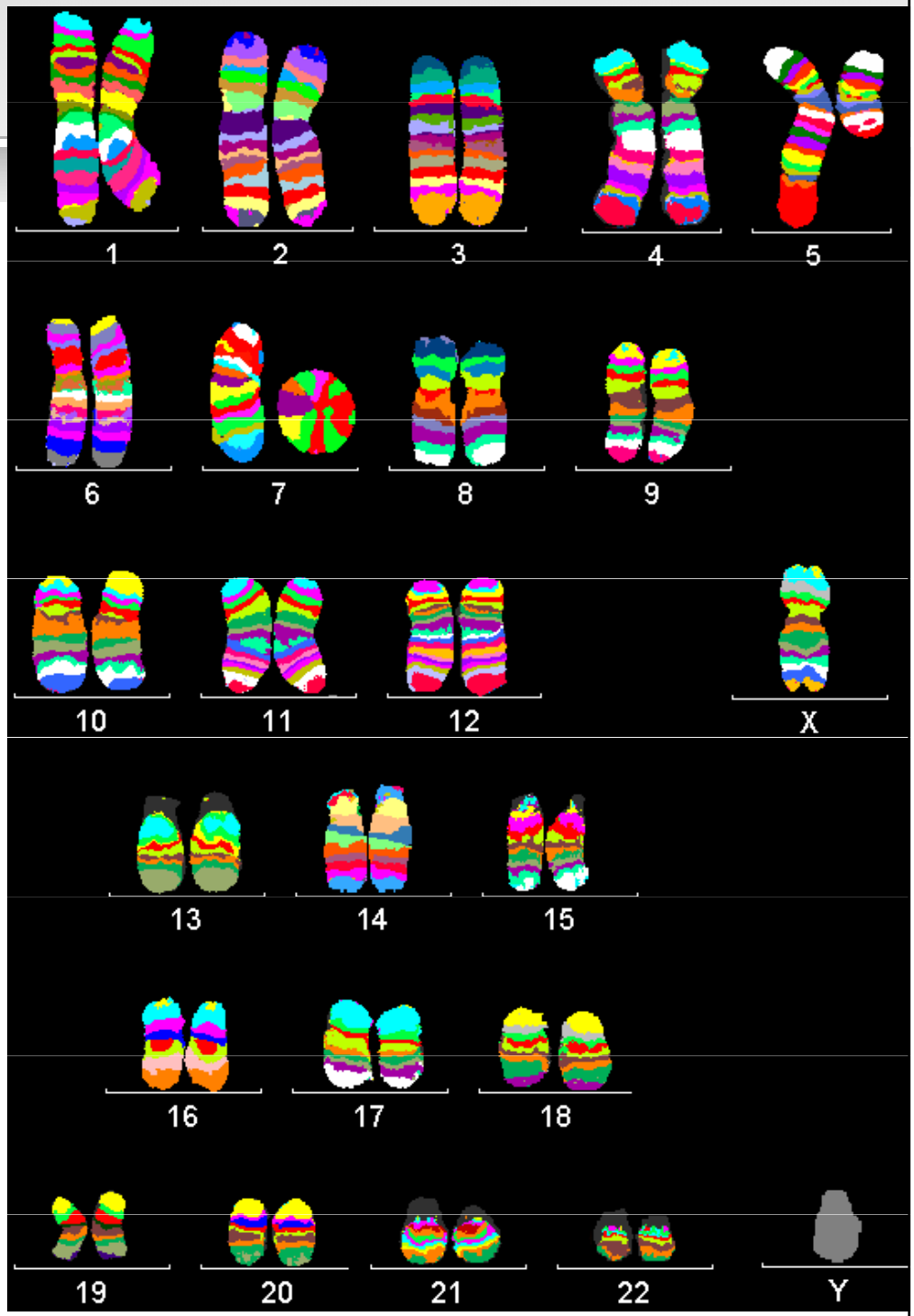
24 color karyotyping *hybridization and detection kit*

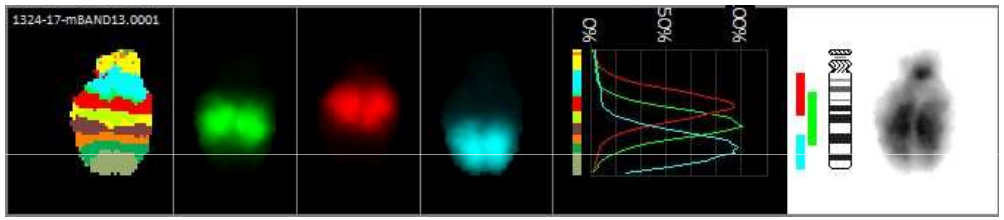
Chr.	FITC	Spectrum Orange	Texas Red	Cy5	DEAC
1					
2					
3					
4					
5					
6					
7					
8					
9					
10					
11					
12					
13					
14					
15					
16					
17					
18					
19					
20					
21					
22					
X					
Y					



Mband FISH

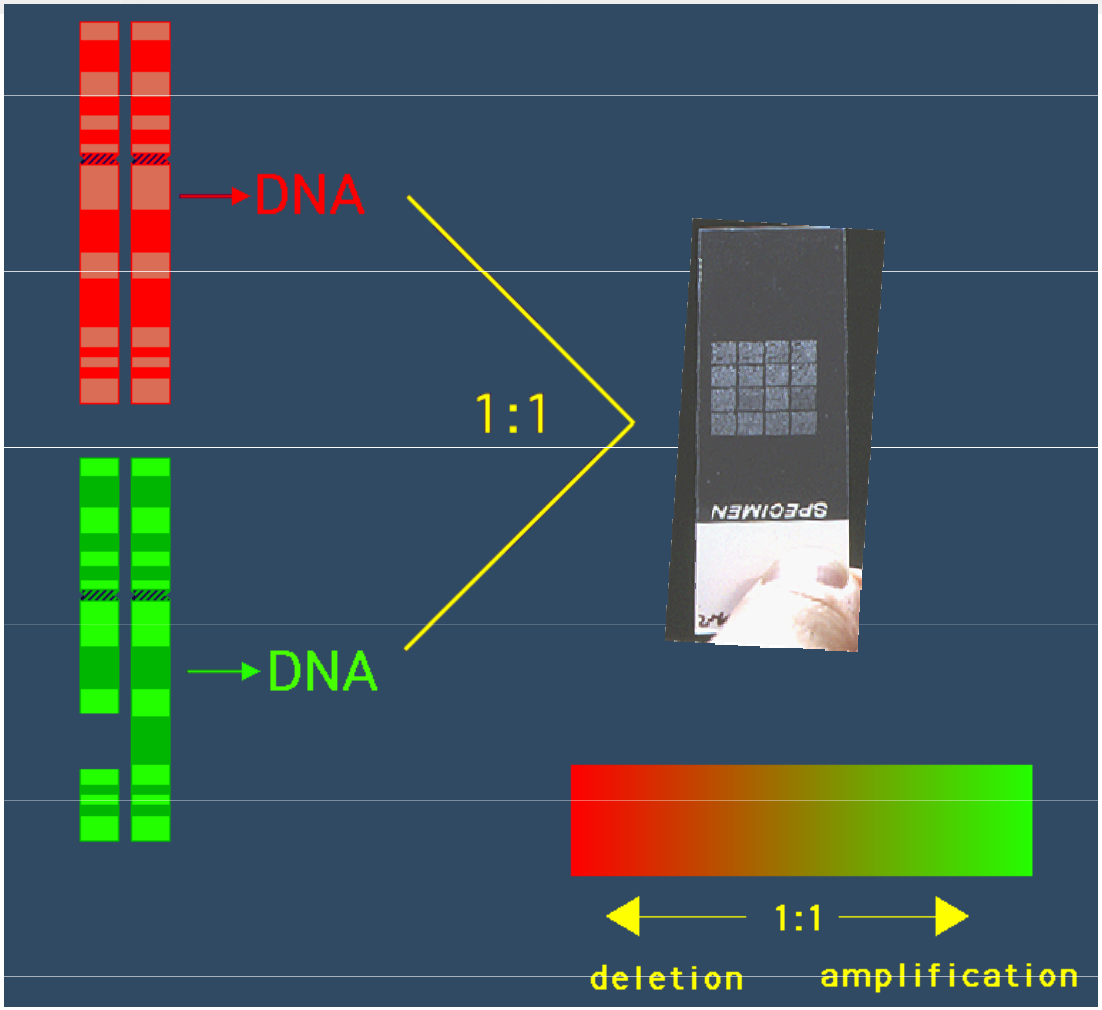
- Kombinuje paintingové proby specifické pro danou oblast chromosomu
- Sondy připravené mikrodisekcí chromosomových oblastí
- Pruhování pokrývá celý chromosom





Převzato I.Chudoba

ArrayCGH – komparativní genomová hybridizace



- Nádorová DNA je hybridizována společně s kontrolní DNA k hybridizačnímu sklu, na kterém jsou fragmenty genomické DNA/oligonukleotidy

arrayCGH/SNPs array

A

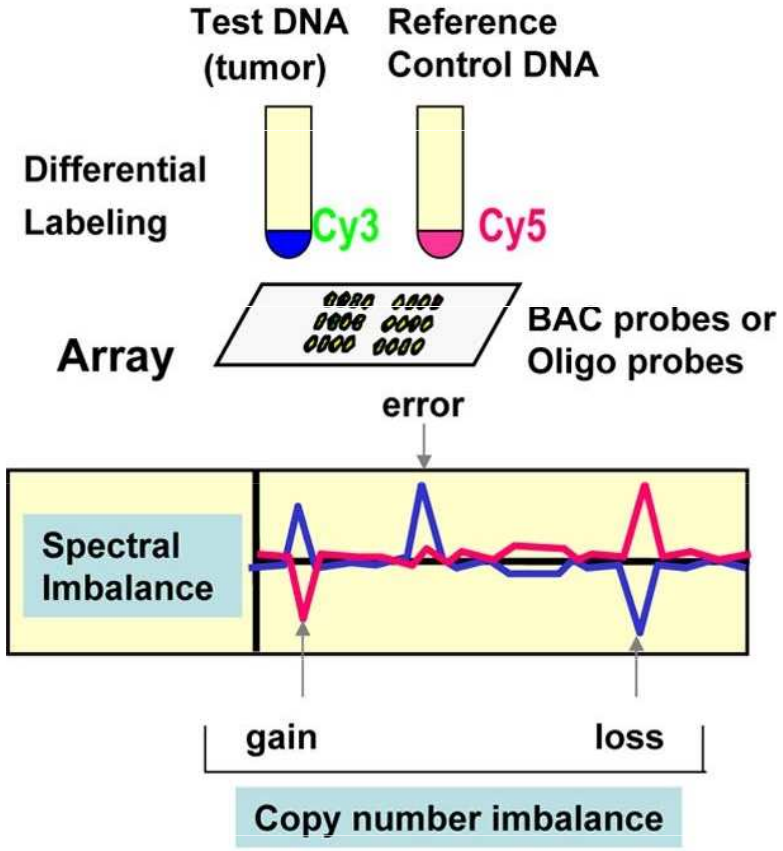
CGH-A

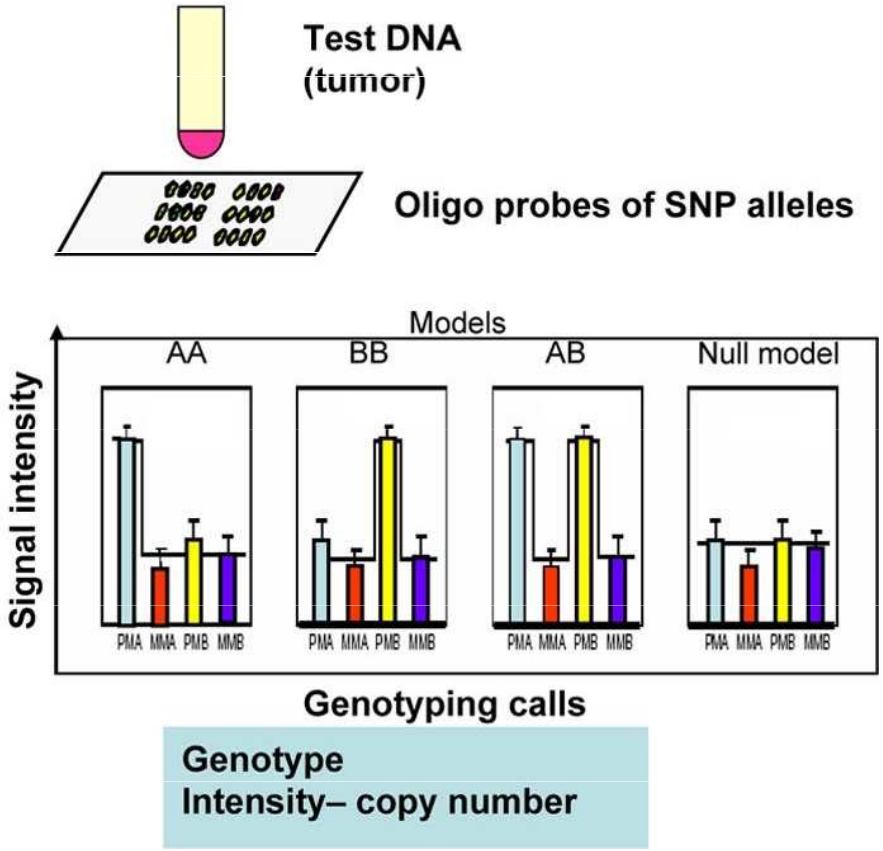
- BAC CGH-A
- Oligo CGH-A

B

SNP-A

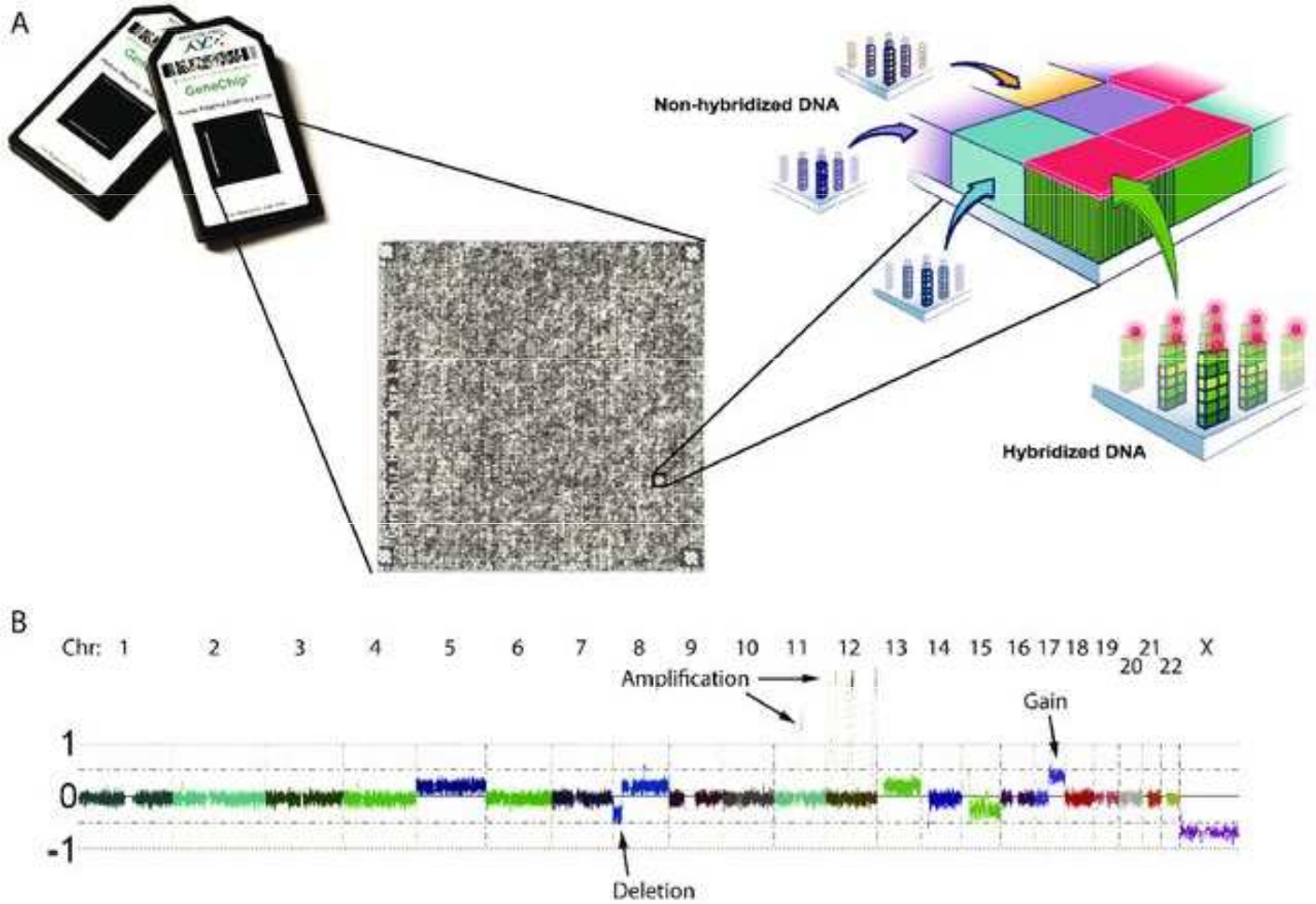
- Combined CN/SNP-A





Rozlišení aCGH/SNPs ~400kb (25–85-mer oligonucleotides)

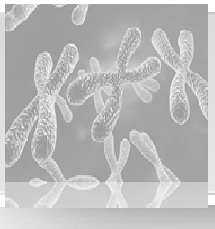
GeneChip 6.0 (906 600 SNP sequences a 900 000 nonpolymorphic oligonucleotides, an average spacing of 0.7 Kb, tj 700bp).



The microarray-principle, as exemplified by the Genechip® 250k NSP SNP-array (Affymetrix). The array contains assays against approximately 250.000 SNPs, distributed in small rectangular features on the array. Fluorescently labeled DNA is hybridized to the array, and the fluorescent intensity for each feature is then detected. The photograph of array cartridges and the schematic representation of the array features are reproduced with courtesy of Affymetrix

Genetické změny u hematologických malignit

- 90-95% nemocných s chronickou myeloidní leukémií (CML)
- 60-80% nemocných s akutní myeloidní leukémií (AML)
- 60% nemocných s myelodysplastickým syndromem (MDS)
- 50-80% nemocných s chronickou lymfocytární leukémií (CLL)
- 70-90% nemocných s akutní lymfoblastickou leukémií (ALL)
- 60-90% nemocných s nehodgkinským lymfomem (NHL)
- 90% nemocných s mnohočetným myelomem (MM)

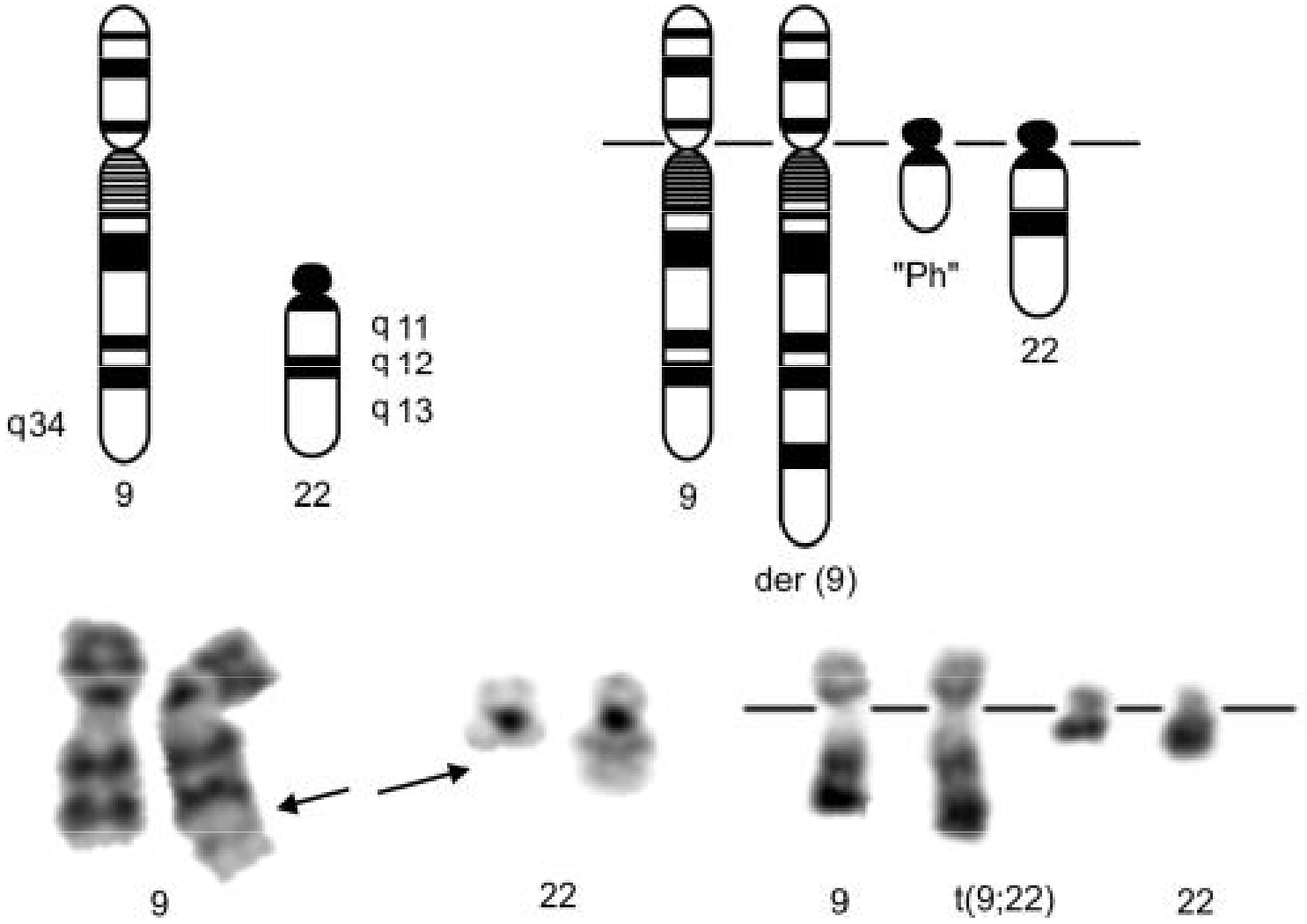


Cytogenetika v hematologii

1. Diagnosa
2. Prognosa
3. Léčebné rozhodování

Filadelfský chromosom (Ph)

První specifická chromosomová změna u nádoru člověka

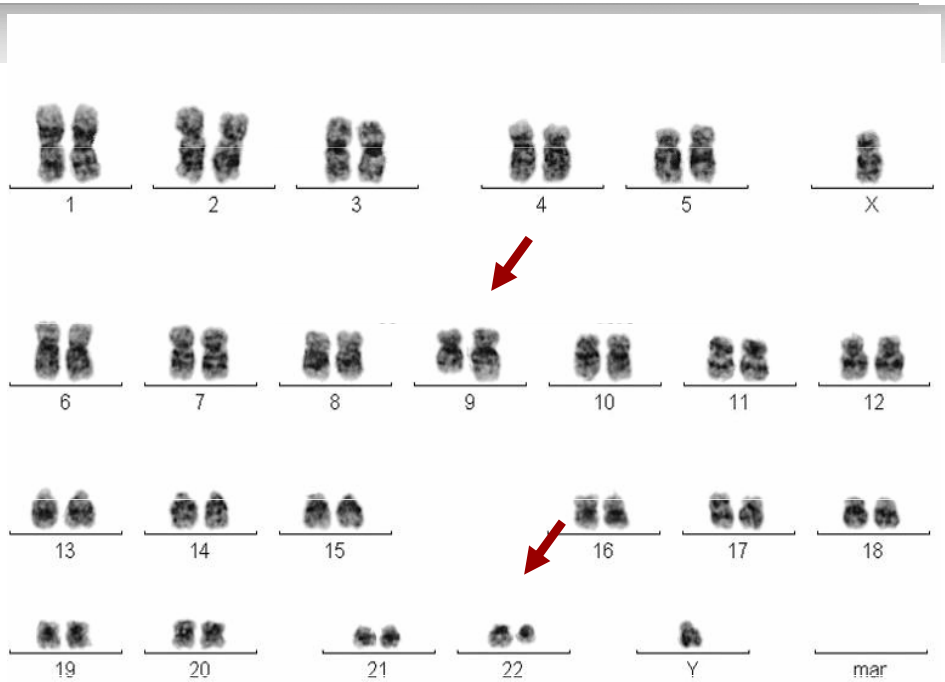


Převzato: www.jaypeedigital.com

Cytogenetika CML

Diagnóza

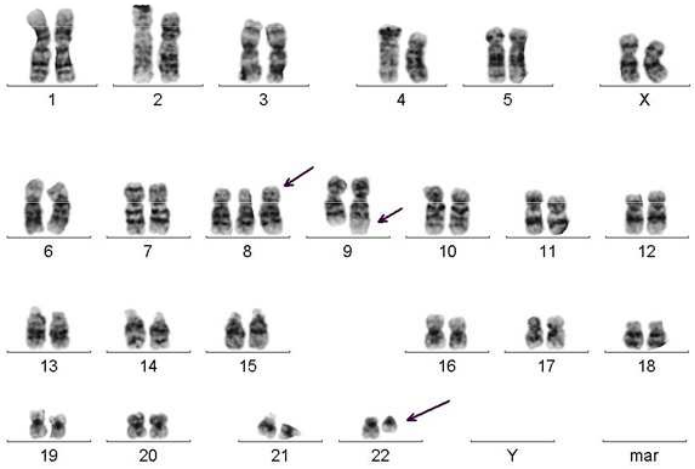
90-95% Ph chromosom výsledek translokace t(9;22)(q34;q21)

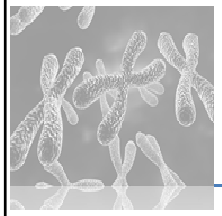


Prognóza

- Přídatné chromosomové změny

Diagnosa CHF: ~12%
Akcelerovaná fáze: ~30%
Blastická zvrat : ~70%





Přídatné chromosomové změny u CML

Aberace	Frekvence %
+8	38
+Ph	30
i(17q)	20
+19	13
-Y	8
+21	7
+17	5
-7	5
t(3;21)	2
Komplexní změny	1

- "major" route změny
  +8
  +der(22)t(9;22)
  +19
  i(17)(q10)

- "minor" route změny
  + 17, + 21
  - Y, -7, -17
  t(3;21)
  t(4;6), t(2;16), t(1;21)

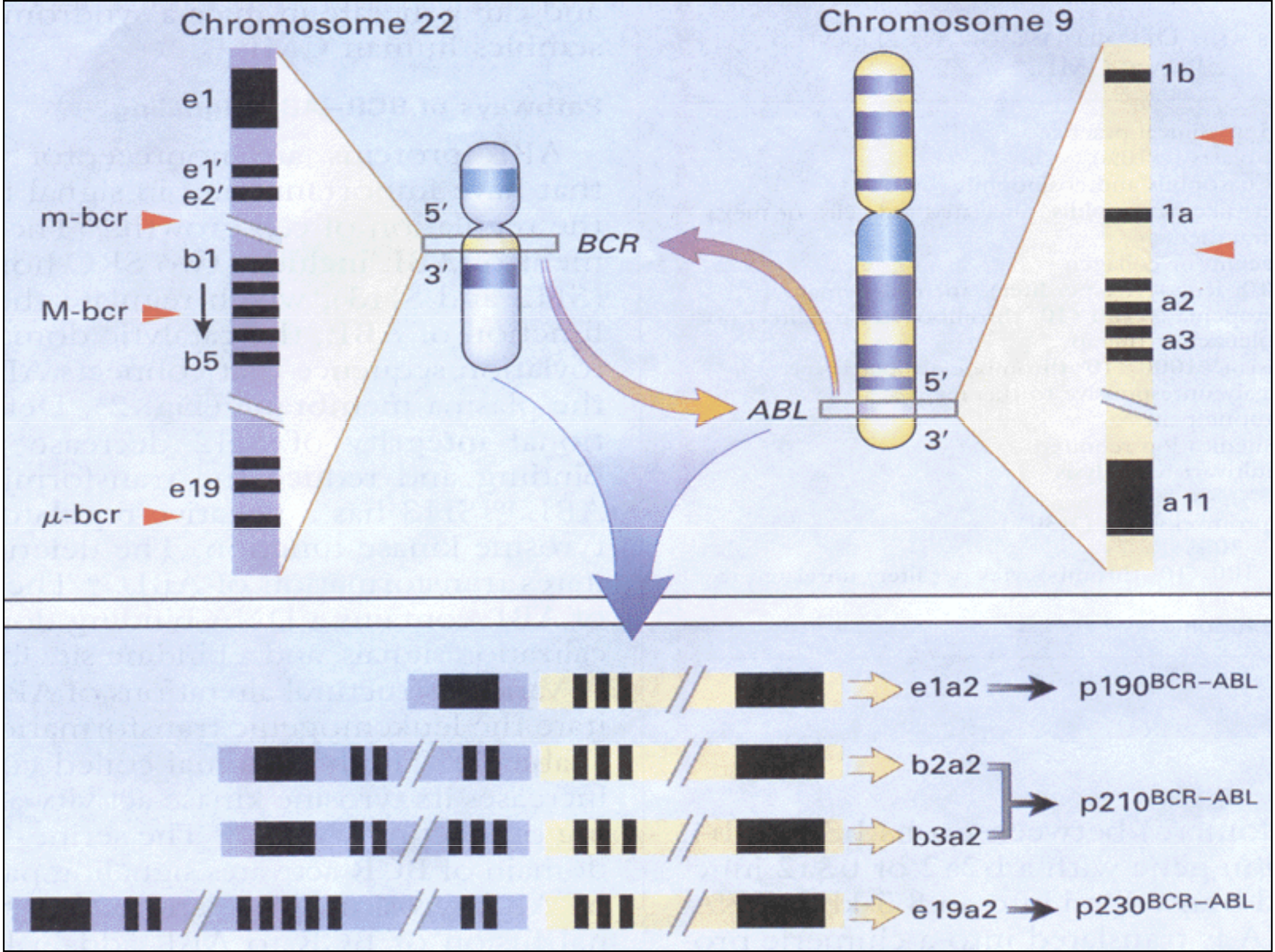
Mitelman ,Leuk Lymphoma 1993

Prognosa

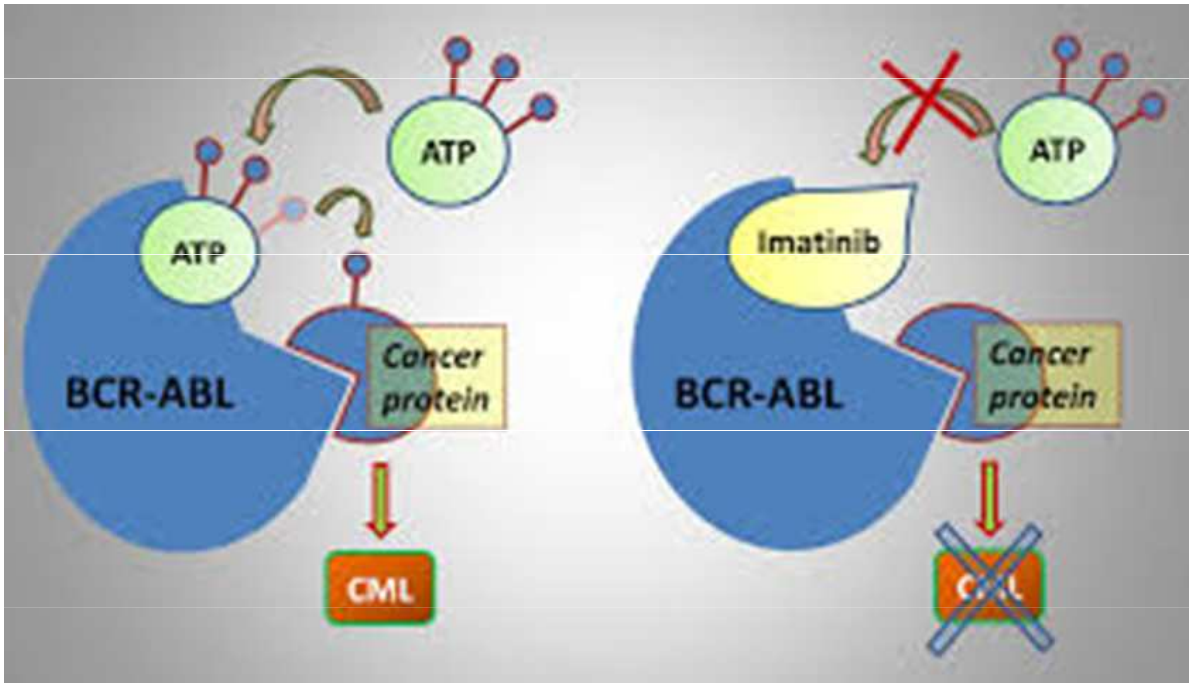
Relativně dobrá:
+8,+Ph,-Y

Relativně špatná:
i(17)
Aberace 3q26.3
-7/del7q

Wang et al, Blood 2016

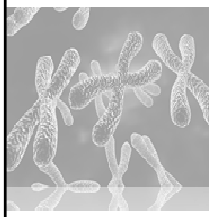

Chromosome 22
Chromosome 9
e1
e1′
e2′
m-bcr
b1
M-bcr
b5
e19
μ-bcr
5′
3′
BCR
ABL
5′
3′
1b
1a
a2
a3
a11
e1a2
p190BCR–ABL
b2a2
b3a2
p210BCR–ABL
e19a2
p230BCR–ABL

Glivec (Imatinib)



ELN sledování MRN CML cytogenetika

	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)



CML – v době léčby inhibitory

Generation	TKI	Approbation		
		1st line	2nd line	3rd line
1st	Imatinib	2003	2001	
2nd	Nilotinib	2011	2008	
	Dasatinib	2011	2007	
3rd	Bosutinib	Clinical trial	Clinical trial	2014
	Ponatinib	Clinical trial		

NIL and DAS have significantly increased apoptosis more than IM by involving both intracellular calcium signaling as well as oxidative stress.

WHO Classification

- Od roku 2008 je cytogenetika součástí diagnostiky a klasifikace řady hematologických malignit
  - Cytogenetika je součástí WHO klasifikace AML
  - Společně s cytomorfologií stratifikuje nemocné s MDS a MPN
  - Klasifikace lymfomů- histologie, cytogenetika a FISH potvrzují klasifikační zařazení
  - Je součástí prognostické stratifikace u MM

WHO Classification 2022

REVIEW ARTICLE OPEN

Check for updates

The 5th edition of the World Health Organization Classification of Haematolymphoid Tumours: Myeloid and Histiocytic/ Dendritic Neoplasms

Joseph D. Khoury[1✉], Eric Solary[2✉], Oussama Abla[3], Yassmine Akkari[4], Rita Alaggio[5], Jane F. Apperley[6], Rafael Bejar[7], Emilio Berti[8], Lambert Busque[9], John K. C. Chan[10], Weina Chen[11], Xueyan Chen[12], Wee-Joo Chng[13], John K. Choi[14], Isabel Colmenero[15], Sarah E. Coupland[16], Nicholas C. P. Cross[17], Daphne De Jong[18], M. Tarek Elghetany[19], Emiko Takahashi[20], Jean-Francois Emile[21], Judith Ferry[22], Linda Fogelstrand[23], Michaela Fontenay[24], Ulrich Germing[25], Sumeet Gujral[26], Torsten Haferlach[27], Claire Harrison[28], Jennelle C. Hodge[29], Shimin Hu[1], Joop H. Jansen[30], Rashmi Kanagal-Shamanna[1], Hagop M. Kantarjian[31], Christian P. Kratz[32], Xiao-Qiu Li[33], Megan S. Lim[34], Keith Loeb[35], Sanam Loghavi[1], Andrea Marcogliese[19], Soheil Meshinchi[36], Phillip Michaels[37], Kikkeri N. Naresh[38], Yasodha Natkunam[39], Reza Nejati[39], German Ott[40], Eric Padron[41], Keyur P. Patel[1], Nikhil Patkar[42], Jennifer Picarsic[43], Uwe Platzbecker[44], Irene Roberts[45], Anna Schuh[46], William Sewell[47], Reiner Siebert[48], Prashant Tembhare[42], Jeffrey Tyner[49], Srdan Verstovsek[31], Wei Wang[1], Brent Wood[50], Wenbin Xiao[51], Cecilia Yeung[35] and Andreas Hochhaus[52✉]

WHO klasifikace 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]

2016

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

2022

Table 7. Acute myeloid leukaemia

- **Acute myeloid leukaemia with defining genetic abnormalities**
  - Acute promyelocytic leukaemia with *PML::RARA* fusion
  - Acute myeloid leukaemia with *RUNX1::RUNX1T1* fusion
  - Acute myeloid leukaemia with *CBFB::MYH11* fusion
  - Acute myeloid leukaemia with *DEK::NUP214* fusion
  - Acute myeloid leukaemia with *RBM15::MRTFA* fusion
  - Acute myeloid leukaemia with *BCR::ABL1* fusion
  - Acute myeloid leukaemia with *KMT2A* rearrangement
  - Acute myeloid leukaemia with *MECOM* rearrangement
  - Acute myeloid leukaemia with *NUP98* rearrangement
  - Acute myeloid leukaemia with *NPM1* mutation
  - Acute myeloid leukaemia with *CEBPA* mutation
  - Acute myeloid leukaemia, myelodysplasia-related
  - Acute myeloid leukaemia with other defined genetic alterations
- **Acute myeloid leukaemia, defined by differentiation**
  - Acute myeloid leukaemia with minimal differentiation
  - Acute myeloid leukaemia without maturation
  - Acute myeloid leukaemia with maturation
  - Acute basophilic leukaemia
  - Acute myelomonocytic leukaemia
  - Acute monocytic leukaemia
  - Acute erythroid leukaemia
  - Acute megakaryoblastic leukaemia

Diagnosis and management of AML in adults: 2022 recommendations from an international expert panel on behalf of the ELN

Hartmut Döhner,[1] Andrew H. Wei,[2] Frederick R. Appelbaum,[3] Charles Craddock,[4] Courtney D. DiNardo,[5] Hervé Dombret,[6] Benjamin L. Ebert,[7] Pierre Fenaux,[8] Lucy A. Godley,[9] Robert P. Hasserjian,[10] Richard A. Larson,[11] Ross L. Levine,[12] Yasushi Miyazaki,[13] Dietger Niederwieser,[14] Gert Ossenkoppele,[15] Christoph Röllig,[16] Jorge Sierra,[17] Eytan M. Stein,[18] Martin S. Tallman,[18] Hwei-Fang Tien,[19] Jianxiang Wang,[20] Agnieszka Wierzbowska,[21] and Bob Löwenberg[22]

Table 1. AML and related neoplasms and acute leukemias of ambiguous lineage

AML and related neoplams	
AML with recurrent genetic abnormalities (requiring ≥10% blasts in BM or PB)* • APL with t(15;17)(q24.1;q21.2)/*PML*::*RARA*† • 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* • AML with t(9;11)(p21.3;q23.3)/*MLLT3*::*KMT2A*‡ • AML with t(6;9)(p22.3;q34.1)/*DEK*::*NUP214* • AML with inv(3)(q21.3q26.2) or t(3;3)(q21.3;q26.2)/*GATA2, MECOM(EVI1)*§ • AML with other rare recurring translocations‖ • AML with mutated *NPM1* • AML with in-frame bZIP mutated *CEBPA*¶ • AML with t(9;22)(q34.1;q11.2)/*BCR*::*ABL1**	**Myeloid sarcoma** **Acute leukemia of ambiguous lineage** • Acute undifferentiated leukemia • MPAL with t(9;22)(q34.1;q11.2)/*BCR*::*ABL1* • MPAL with t(v;11q23.3)/*KMT2A*-rearranged • MPAL, B/myeloid, not otherwise specified • MPAL, T/myeloid, not otherwise specified
Categories designated AML (if ≥20% blasts in BM or PB) or MDS/AML (if 10-19% blasts in BM or PB) • AML with mutated *TP53*# • AML with myelodysplasia-related gene mutations Defined by mutations in *ASXL1*, *BCOR*, *EZH2*, *RUNX1*, *SF3B1*, *SRSF2*, *STAG2*, *U2AF1*, and/or *ZRSR2* • AML with myelodysplasia-related cytogenetic abnormalities** • AML not otherwise specified	**Myeloid proliferations related to Down syndrome** • Transient abnormal myelopoiesis associated with Down syndrome • Myeloid leukemia associated with Down syndrome **Blastic plasmacytoid dendritic cell neoplasm**
Diagnostic qualifiers†† Therapy-related‡‡ • Prior chemotherapy, radiotherapy, immune interventions Progressed from MDS • MDS should be confirmed by standard diagnostics and >3 mo prior to AML diagnosis Progressed from MDS/MPN (specify type) • MDS/MPN should be confirmed by standard diagnostics and >3 mo prior to AML diagnosis Germline predisposition (specify type)	

WHO prognostická stratifikace AML

Table 5. 2017 European LeukemiaNet risk stratification by genetics[a]

Risk Category[b]	Genetic Abnormality
Favorable	t(8;21)(q22;q22.1); *RUNX1-RUNX1T1* inv(16)(p13.1q22) or t(16;16)(p13.1;q22); *CBFB-MYH11* Mutated *NPM1* without *FLT3*-ITD or with *FLT3*-ITD$^{low(c)}$ Biallelic mutated *CEBPA*
Intermediate	Mutated *NPM1* and *FLT3*-ITD$^{high(c)}$ Wild type *NPM1* without *FLT3*-ITD or with *FLT3*-ITD$^{low(c)}$ (w/o adverse-risk genetic lesions) ( )(p q) Cytogenetic abnormalities not classified as favorable or adverse
Adverse	t(6;9)(p23;q34.1); *DEK-NUP214* t(v;11q23.3); *KMT2A* rearranged t(9;22)(q34.1;q11.2); *BCR-ABL1* inv(3)(q21.3q26.2) or t(3;3)(q21.3;q26.2); *GATA2,MECOM(EVI1)* -5 or del(5q); -7; -17/abn(17p) Complex karyotype,[e] monosomal karyotype[f] Wild type *NPM1* and *FLT3*-ITD$^{high(c)}$ Mutated *RUNX1*[g] Mutated *ASXL1*[g] Mutated *TP53*[h]

[a] Frequencies, response rates and outcome measures should be reported by risk category, and, if sufficient numbers are available, by specific genetic lesions indicated.

[b] Prognostic impact of a marker is treatment-dependent and may change with new therapies.

[c] Low, low allelic ratio (<0.5); high, high allelic ratio (≥0.5); semi-quantitative assessment of *FLT3*-ITD allelic ratio (using DNA fragment analysis) is determined as ratio of the area under the curve (AUC) "*FLT3*-ITD" divided by AUC "*FLT3*-wild type"; recent studies indicate that acute myeloid leukemia with *NPM1* mutation and *FLT3*-ITD low allelic ratio may also have a more favorable prognosis and patients should not routinely be assigned to allogeneic hematopoietic-cell transplantation.[57-59,77]

[d] The presence of t(9;11)(p21.3;q23.3) takes precedence over rare, concurrent adverse-risk gene mutations.

[e] Three or more unrelated chromosome abnormalities in the absence of one of the World Health Organization-designated recurring translocations or inversions, i.e., t(8;21), inv(16) or t(16;16), t(9;11), t(v;11)(v;q23.3), t(6;9), inv(3) or t(3;3); AML with *BCR-ABL1*.

[f] Defined by the presence of one single monosomy (excluding loss of X or Y) in association with at least one additional monosomy or structural chromosome abnormality (excluding core-binding factor AML).[116]

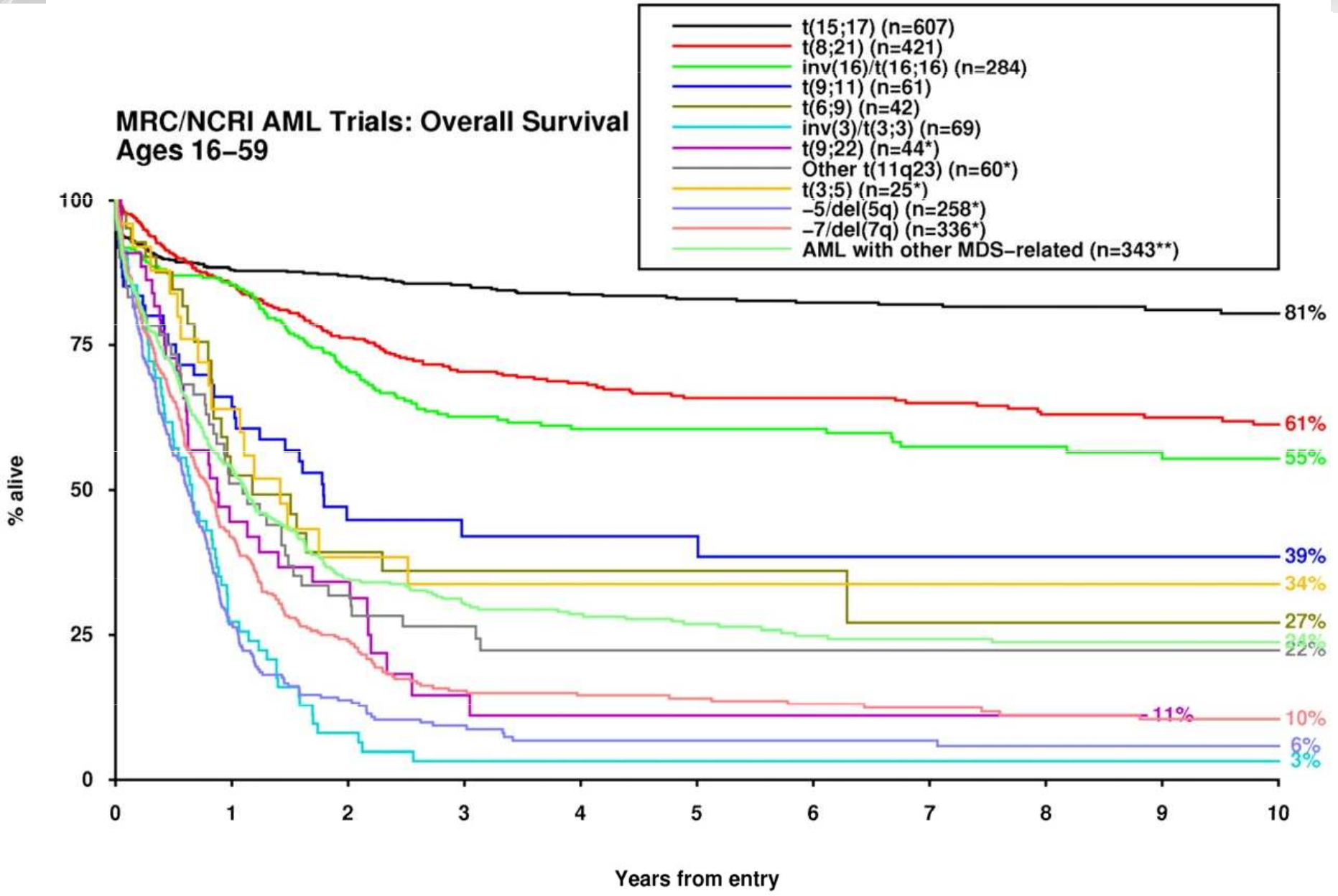
[g] These markers should not be used as an adverse prognostic marker if they co-occur with favorable-risk AML subtypes.

[h] *TP53* mutations are significantly associated with AML with complex and monosomal karyotype.[37,66-69]

Table 6. 2022 ELN risk classification by genetics at initial diagnosis*

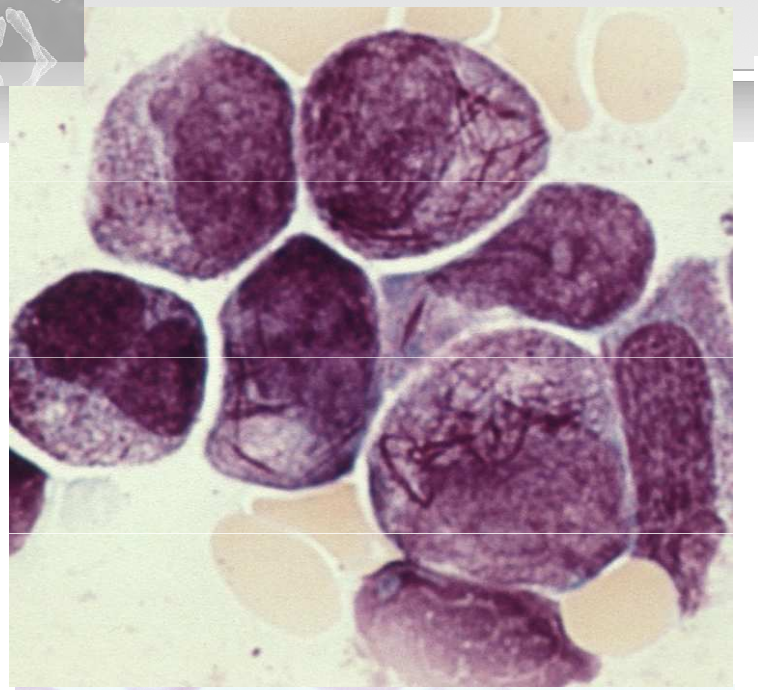
Risk category†	Genetic abnormality
Favorable	• t(8;21)(q22;q22.1)/*RUNX1::RUNX1T1*†,‡ • inv(16)(p13.1q22) or t(16;16)(p13.1;q22)/*CBFB::MYH11*†,‡ • Mutated *NPM1*†,§ without *FLT3*-ITD • bZIP in-frame mutated *CEBPA*‖
Intermediate	• Mutated *NPM1*†,§ with *FLT3*-ITD • Wild-type *NPM1* with *FLT3*-ITD (without adverse-risk genetic lesions) • t(9;11)(p21.3;q23.3)/*MLLT3::KMT2A*†,¶ • Cytogenetic and/or molecular abnormalities not classified as favorable or adverse
Adverse	• t(6;9)(p23.3;q34.1)/*DEK::NUP214* • t(v;11q23.3)/*KMT2A*-rearranged# • t(9;22)(q34.1;q11.2)/*BCR::ABL1* • t(8;16)(p11.2;p13.3)/*KAT6A::CREBBP* • inv(3)(q21.3q26.2) or t(3;3)(q21.3;q26.2)/*GATA2, MECOM(EVI1)* • t(3q26.2;v)/*MECOM(EVI1)*-rearranged • −5 or del(5q); −7; −17/abn(17p) • Complex karyotype,** monosomal karyotype†† • Mutated *ASXL1, BCOR, EZH2, RUNX1, SF3B1, SRSF2, STAG2, U2AF1, and/or ZRSR2*‡‡ • Mutated *TP53*[a]

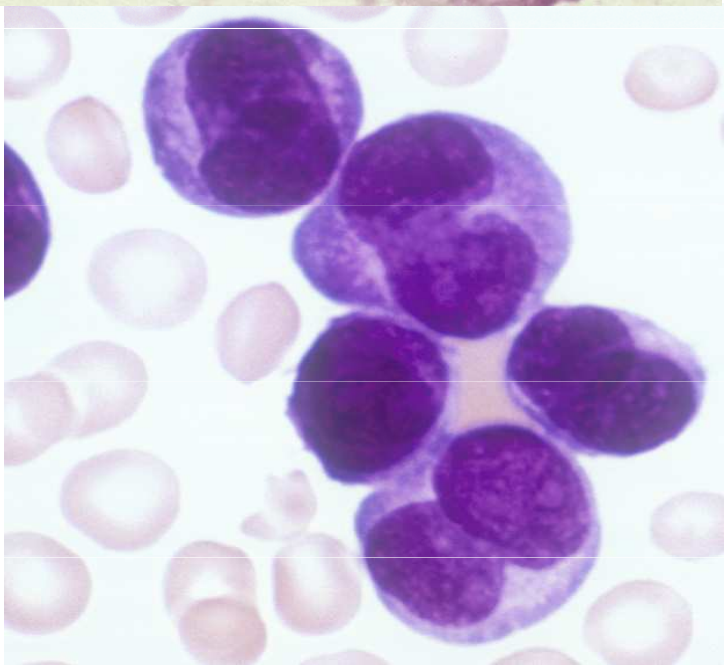
Stratifikace podle cytogenetických nálezů



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

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





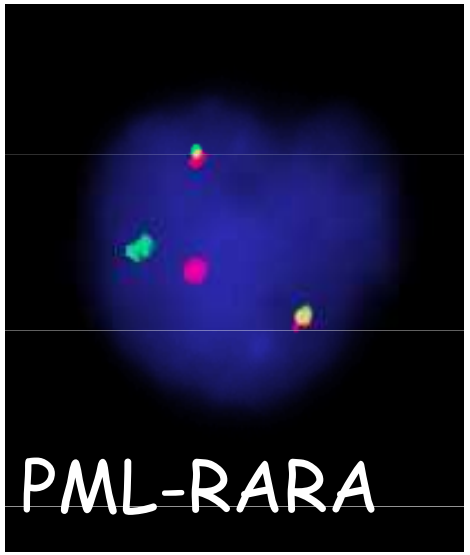
15/17 TRANSLOCATION, A CONSISTENT CHROMOSOMAL CHANGE IN ACUTE PROMYELOCYTIC LEUKAEMIA

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.

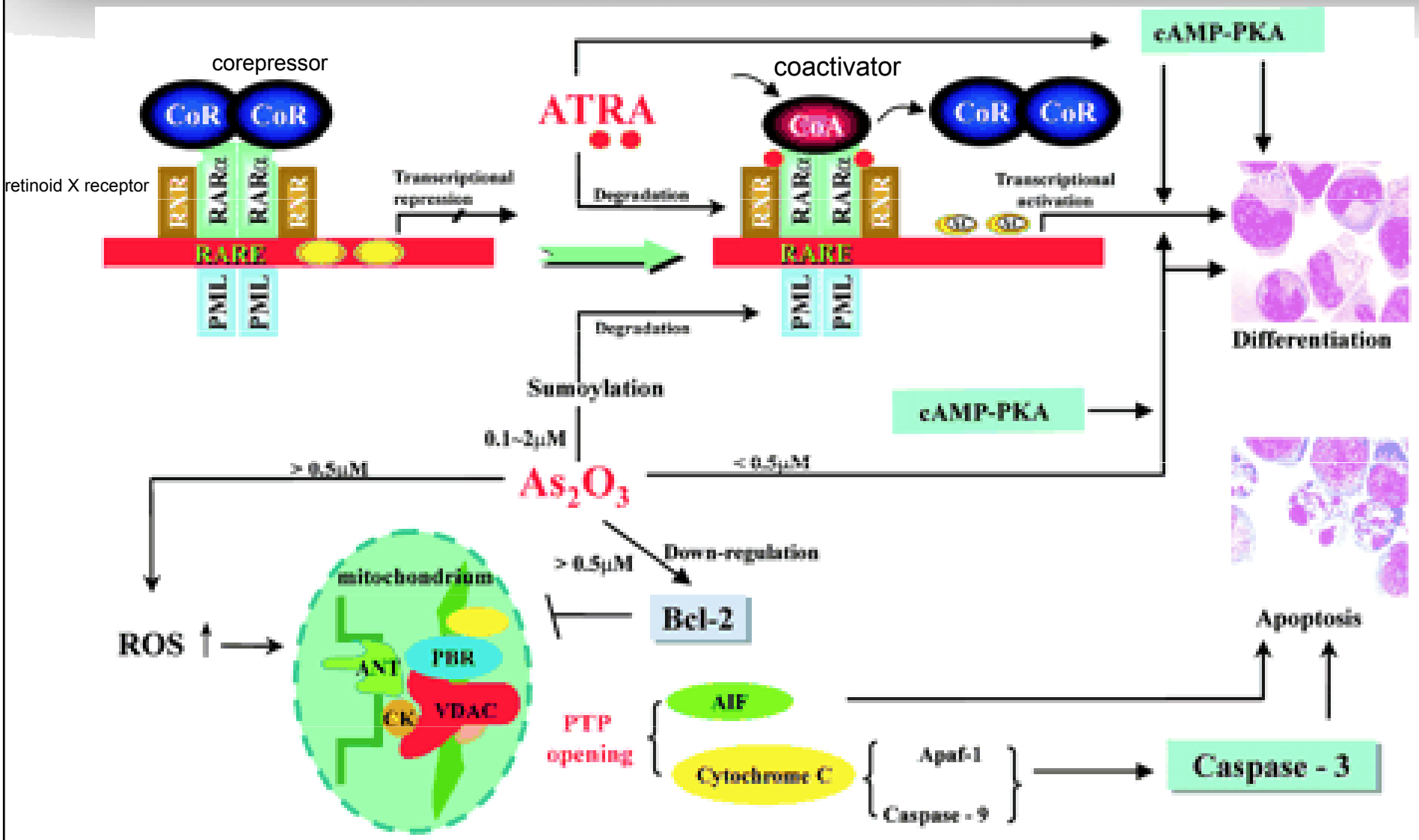
JANET D. ROWLEY
HARVEY M. GOLOMB
CHARLOTTE DOUGHERTY

t(15;17)(q22;q12)



Janet D Rowley et al. Lancet 1977

Cílená léčba nemocných s APL



AKUTNÍ LYMFOBLASTICKÁ LEUKEMIE (ALL)

ALL – heterogenní onemocnění s monoklonální proliferací a expanzí nezralých lymfoidních buněk v KD, PK a dalších orgánech

- Cytogenetika má prognostický význam
- Diagnostický význam - imunofenotyp

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

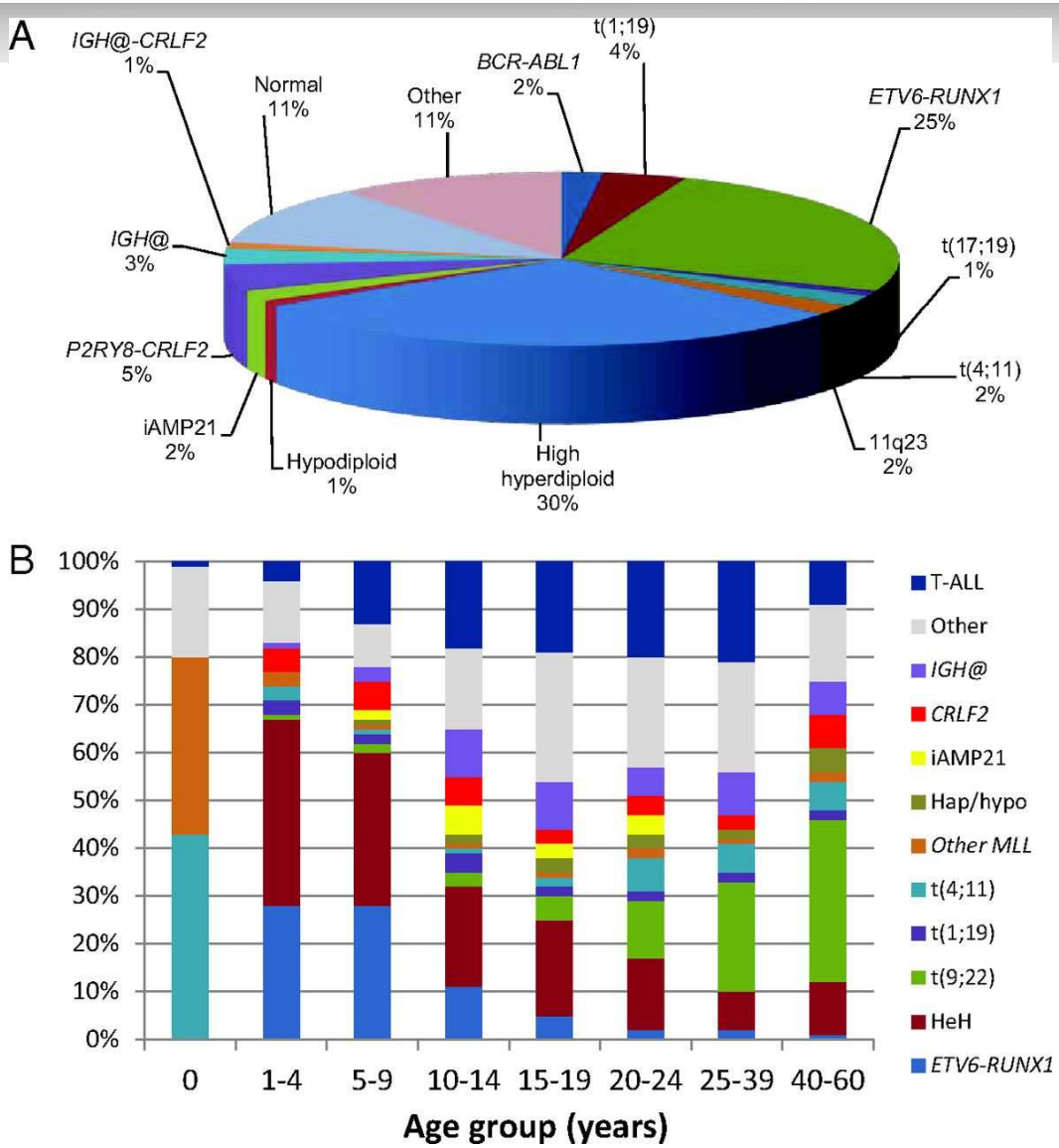
Precursor lymphoid neoplasms
B-cell lymphoblastic leukemia/lymphoma, not otherwise specified
B-cell lymphoblastic leukemia/lymphoma with recurrent genetic abnormalities
B-cell lymphoblastic leukemia/lymphoma with t(9;22)(q34;q11.2); *BCR-ABL1*
B-cell lymphoblastic leukemia/lymphoma with t(v;11q23); *MLL* 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.

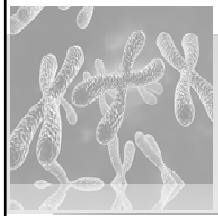
Dětské ALL

Tvoří 30% všech dětských nádorů



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

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



Myelodysplastický syndrom (MDS)

WHO klasifikace

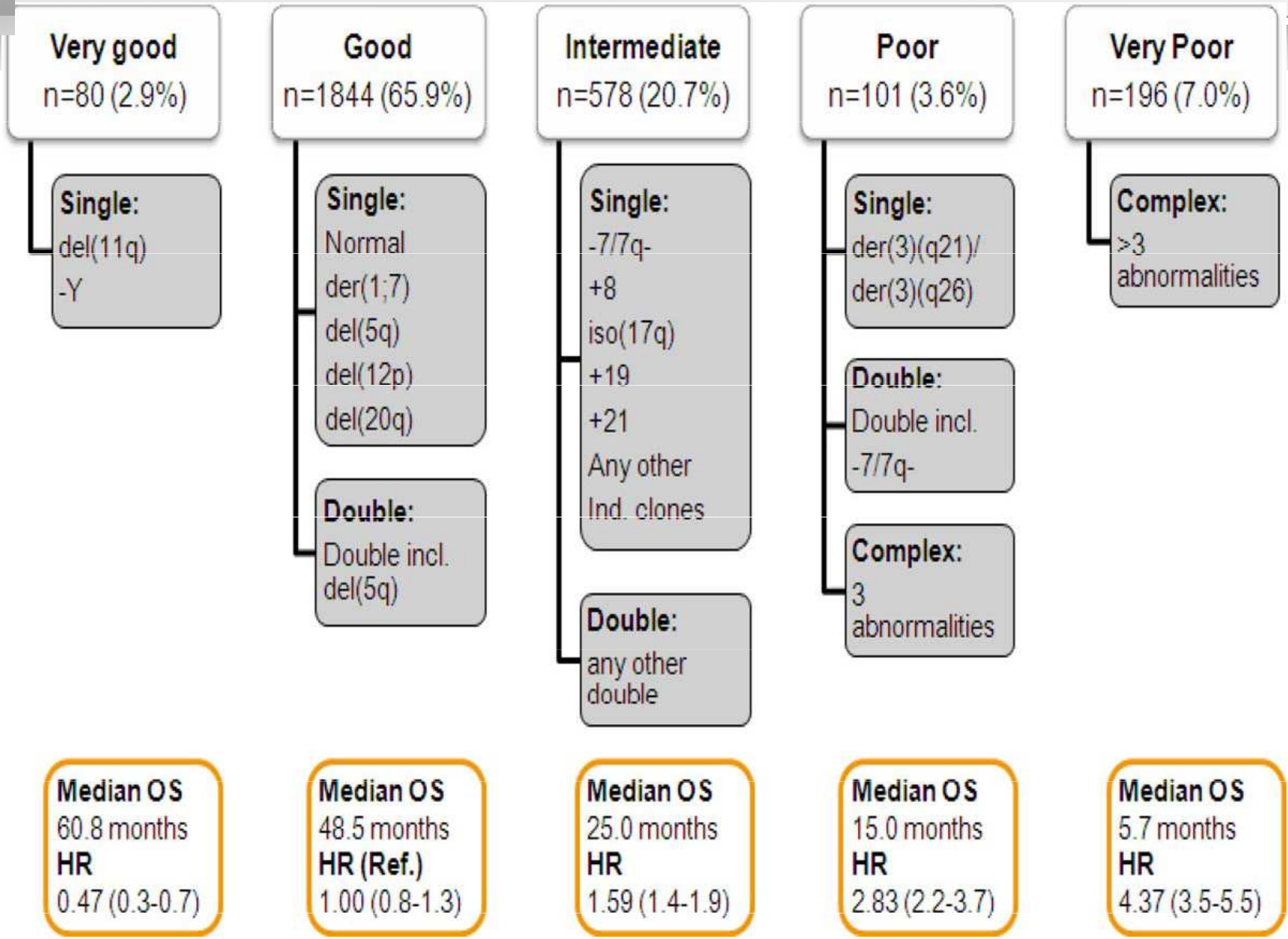
- Refractory cytopenia with unilineage dysplasia (RCUD)
- Refractory anemia with ringed sideroblasts (RARS)
- Refractory cytopenia with multilineage dysplasia (RCMD)
- Refractory anemia with excess blasts-1 (RAEB-1)
- Refractory anemia with excess blasts-2 (RAEB-2)
- Myelodysplastic syndrome, unclassified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

Klinická heterogenita MDS je odrazem heterogenity získaných somatických genetických změn

Chromosomové změny u MDS

- de novo MDS 40-60%
- t-MDS nebo sekundární MDS 90%
- SNPs+arrayCGH 70%

Prognostická stratifikace MDS



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LYMPHOMA

The 5th edition of the World Health Organization Classification of Haematolymphoid Tumours: Lymphoid Neoplasms

Table 1. WHO Classification of Haematolymphoid Tumours, 5th edition: B-cell lymphoid proliferations and lymphomas.

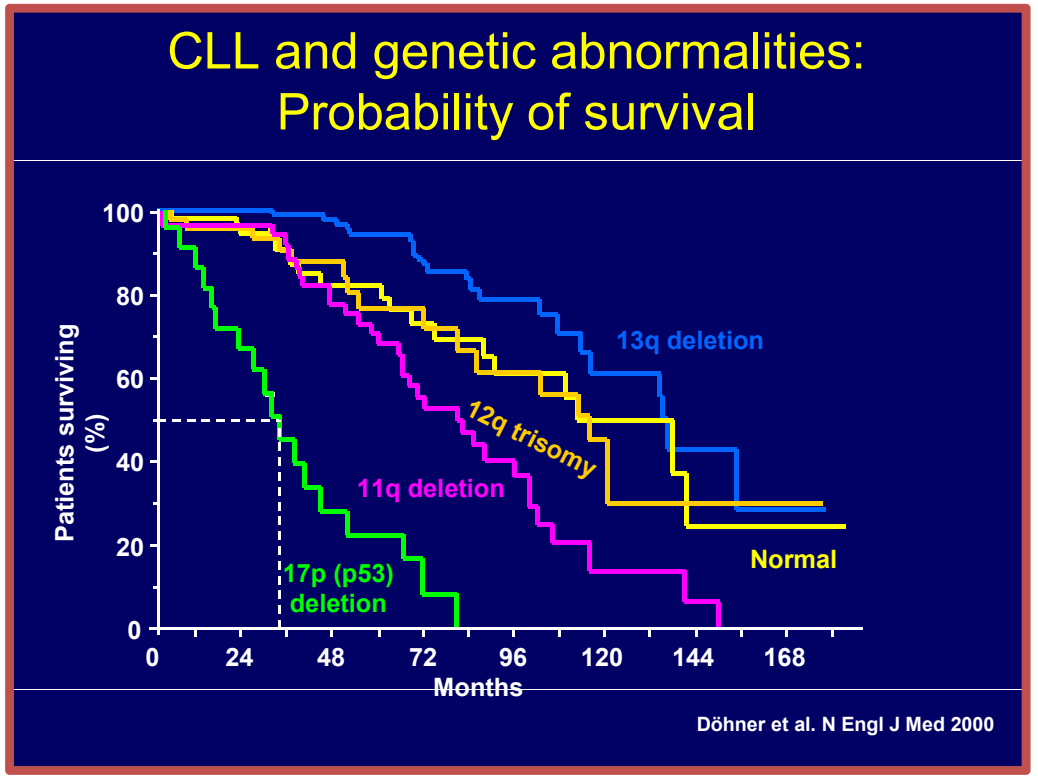
WHO Classification, 5th edition	WHO Classification, revised 4th edition
Tumour-like lesions with B-cell predominance	
Reactive B-cell-rich lymphoid proliferations that can mimic lymphoma	*Not previously included*
IgG4-related disease	*Not previously included*
Unicentric Castleman disease	*Not previously included*
Idiopathic multicentric Castleman disease	*Not previously included*
KSHV/HHV8-associated multicentric Castleman disease	Multicentric Castleman disease
Precursor B-cell neoplasms	
B-cell lymphoblastic leukaemias/lymphomas	
B-lymphoblastic leukaemia/lymphoma, NOS	(Same)
B-lymphoblastic leukaemia/lymphoma with high hyperdiploidy	B-lymphoblastic leukaemia/lymphoma with hyperdiploidy
B-lymphoblastic leukaemia/lymphoma with hypodiploidy	(Same)
B-lymphoblastic leukaemia/lymphoma with iAMP21	(Same)
B-lymphoblastic leukaemia/lymphoma with *BCR::ABL1* fusion	B-lymphoblastic leukaemia/lymphoma with t(9;22)(q34;q11.2); *BCR-ABL1*
B-lymphoblastic leukaemia/lymphoma with *BCR::ABL1*-like features	B-lymphoblastic leukaemia/lymphoma, *BCR-ABL1*-like
B-lymphoblastic leukaemia/lymphoma with *KMT2A* rearrangement	B-lymphoblastic leukaemia/lymphoma with t(v;11q23.3); *KMT2A*-rearranged
B-lymphoblastic leukaemia/lymphoma with *ETV6::RUNX1* fusion	B-lymphoblastic leukaemia/lymphoma with t(12;21)(p13.2;q22.1); *ETV6-RUNX1*
B-lymphoblastic leukaemia/lymphoma with *ETV6::RUNX1*-like features	*Not previously included*
B-lymphoblastic leukaemia/lymphoma with *TCF3::PBX1* fusion	B-lymphoblastic leukaemia/lymphoma with t(1;19)(q23;p13.3); *TCF3-PBX1*
B-lymphoblastic leukaemia/lymphoma with *IGH::IL3 fusion*	B-lymphoblastic leukaemia/lymphoma with t(5;14)(q31.1;q32.1); *IGH/IL3*
B-lymphoblastic leukaemia/lymphoma with *TCF3::HLF* fusion	*Not previously included*
B-lymphoblastic leukaemia/lymphoma with other defined genetic abnormalities	(Same)
Mature B-cell neoplasms	
Pre-neoplastic and neoplastic small lymphocytic proliferations	
Monoclonal B-cell lymphocytosis	(Same)
Chronic lymphocytic leukaemia/small lymphocytic lymphoma	(Same)
(Entity deleted)	B-cell prolymphocytic leukaemia
Splenic B-cell lymphomas and leukaemias	
Hairy cell leukaemia	(Same)
Splenic marginal zone lymphoma	(Same)
Splenic diffuse red pulp small B-cell lymphoma	(Same)
Splenic B-cell lymphoma/leukaemia with prominent nucleoli	*Not previously included* (encompassing hairy cell leukaemia variant and some cases of B-cell prolymphocytic leukaemia)

CYTOGENETIKA CLL

Prognostický význam chromosomových změn u 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.**

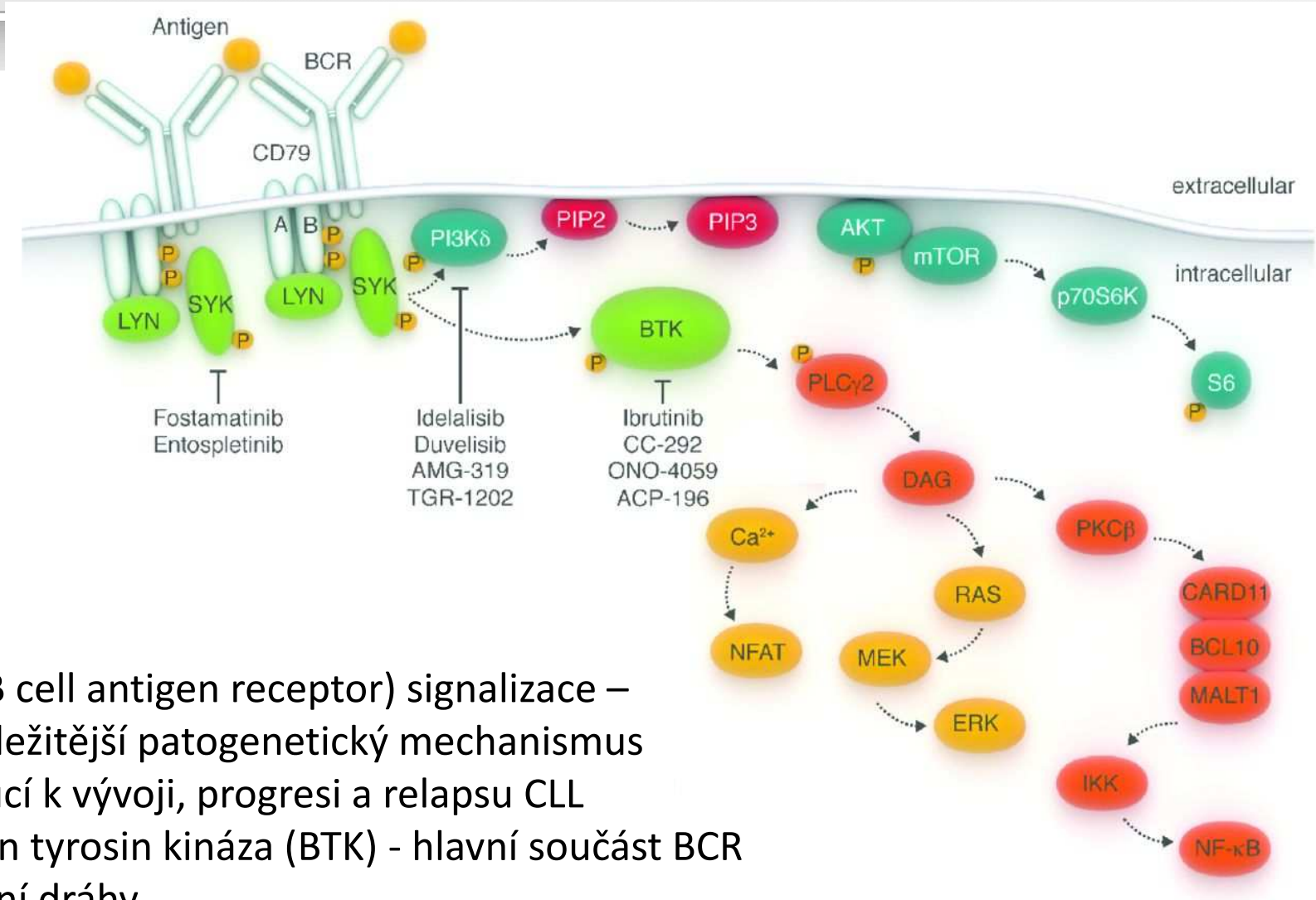


CLL – prognostická a léčebná stratifikace

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

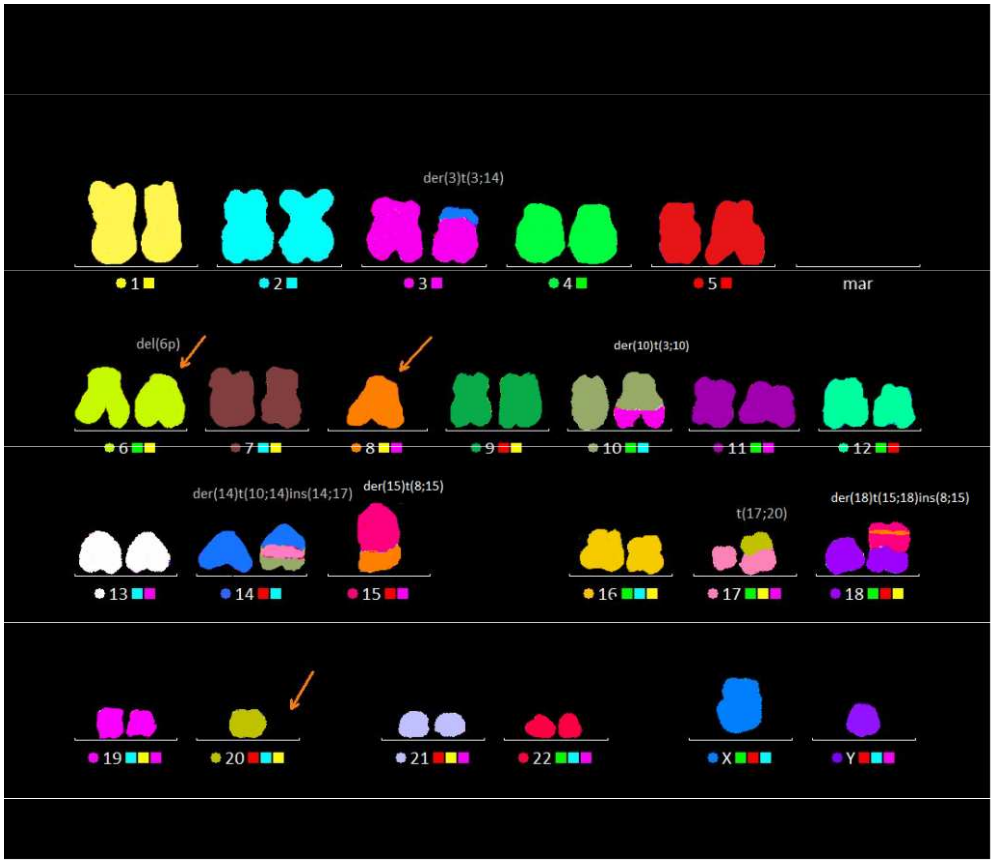
BCR signalizace u CLL

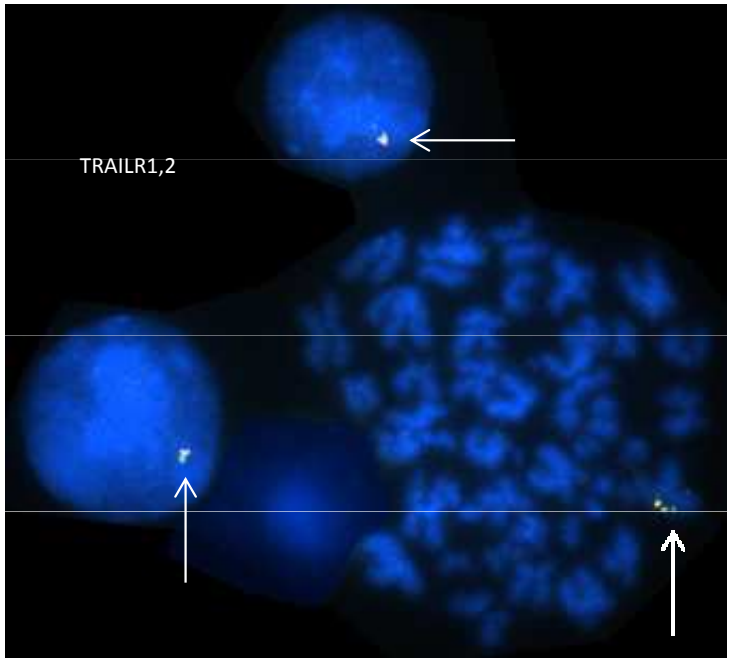


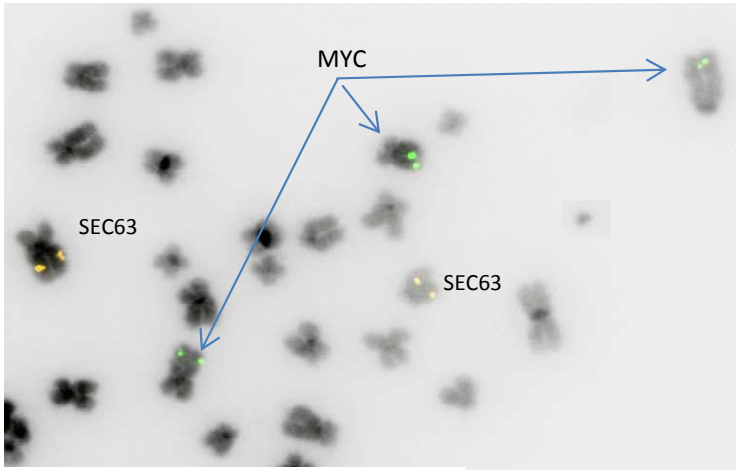
- BCR (B cell antigen receptor) signalizace – nejdůležitější patogenetický mechanismus vedoucí k vývoji, progresi a relapsu CLL
- Bruton tyrosin kináza (BTK) - hlavní součást BCR signální dráhy
- Konstitutivní aktivace BCR signalizace – buněčná proliferace, přežívání a migrace
- Inhibitor BTK blokuje BCR signální dráhu a indukuje apoptózu

Wiestner, Haematologica 2015

Pacient CLL05

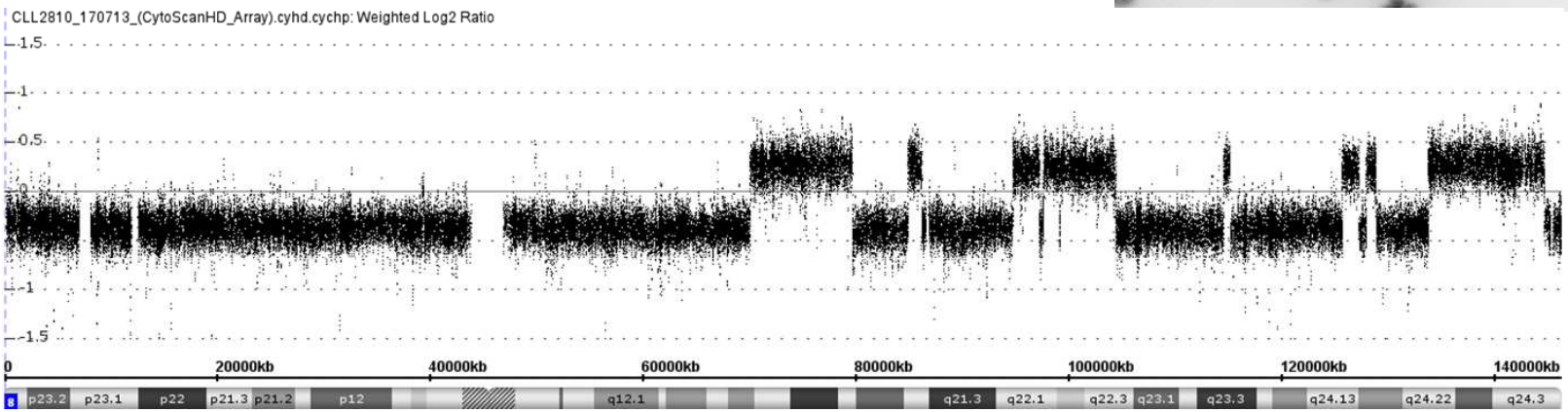






Mutace *TP53* a *BTK*

cth chr8 – CLL05



Nehodgkinské lymfomy - NHL

- Maligní lymfomy jsou heterogenní skupina nádorů lymfatické tkáně
- Vznikají na základě genetických změn v původně normálních buňkách
- Klasifikace lymfomů- histopatologie - WHO klasifikace lymfomů 2008
- Cytogenetika a FISH potvrzují klasifikační zařazení

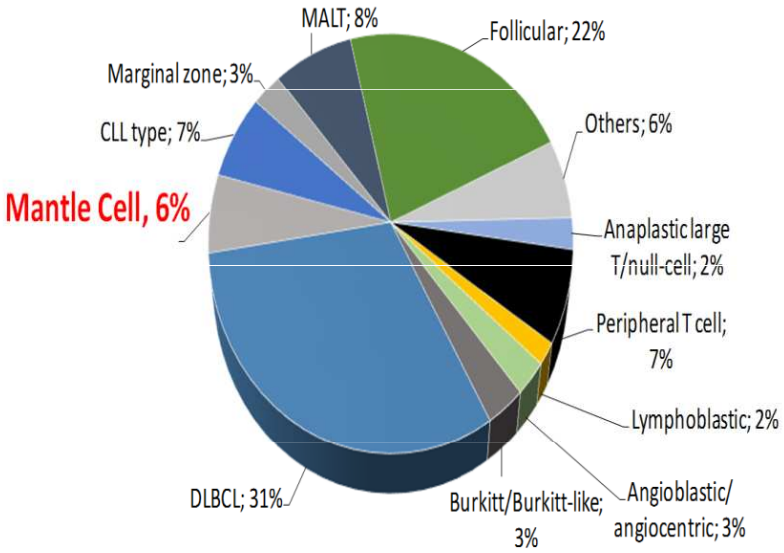
Folikulární lymfom (FL)

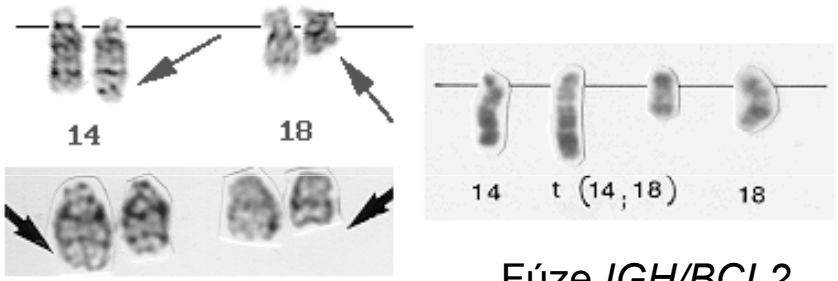
indolentní B buněčný lymfom

~20 % všech lymfomů

heterogenní klinický průběh , os několik roků až 20 l

90% nemocných má translokaci t(14;18)(q32;q21)

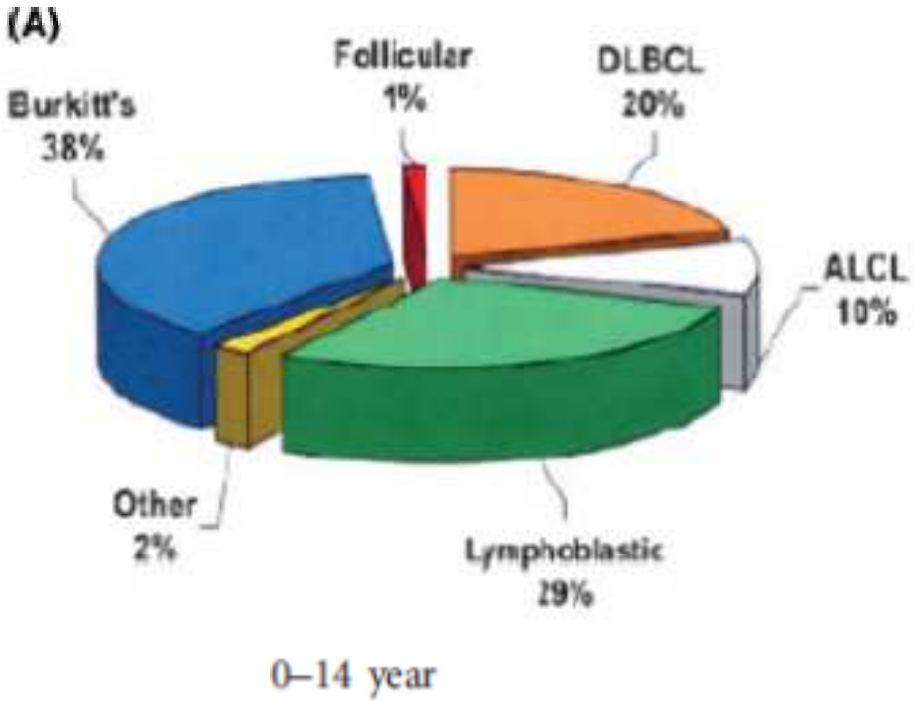




Fúze *IGH/BCL2*

Nehodgkinské lymfomy (NHL) u dětí

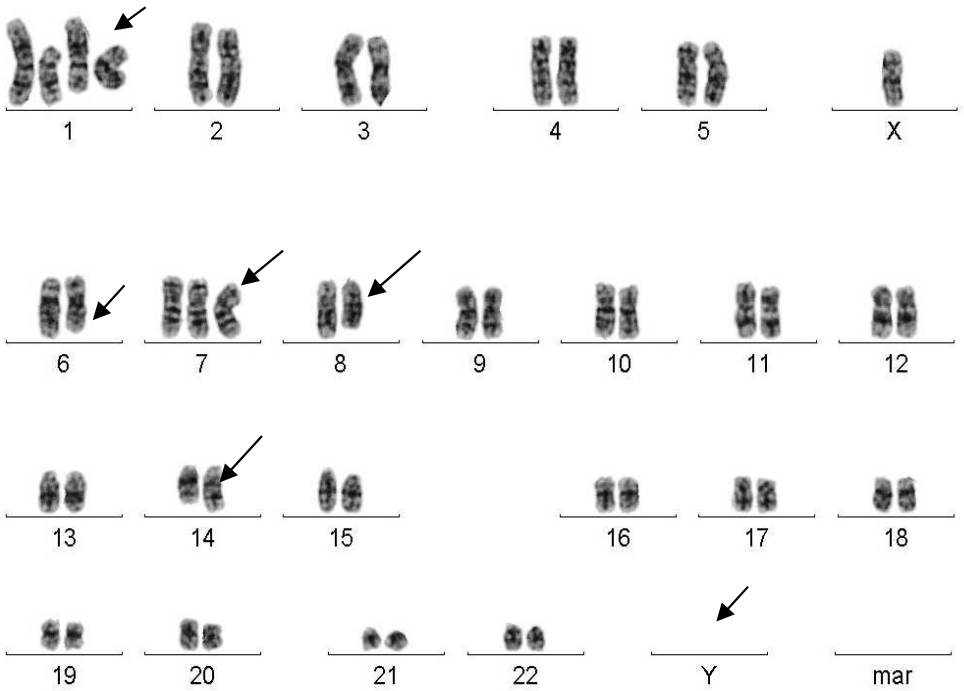
- 4-7% nádorů u dětí a mladistvých
- incidence vzrůstá s věkem
- zvýšené riziko děti s imunodeficitem (např. AT)
- WHO klasifikace 2008
- Frekvence histologických subtypů odlišná od dospělých

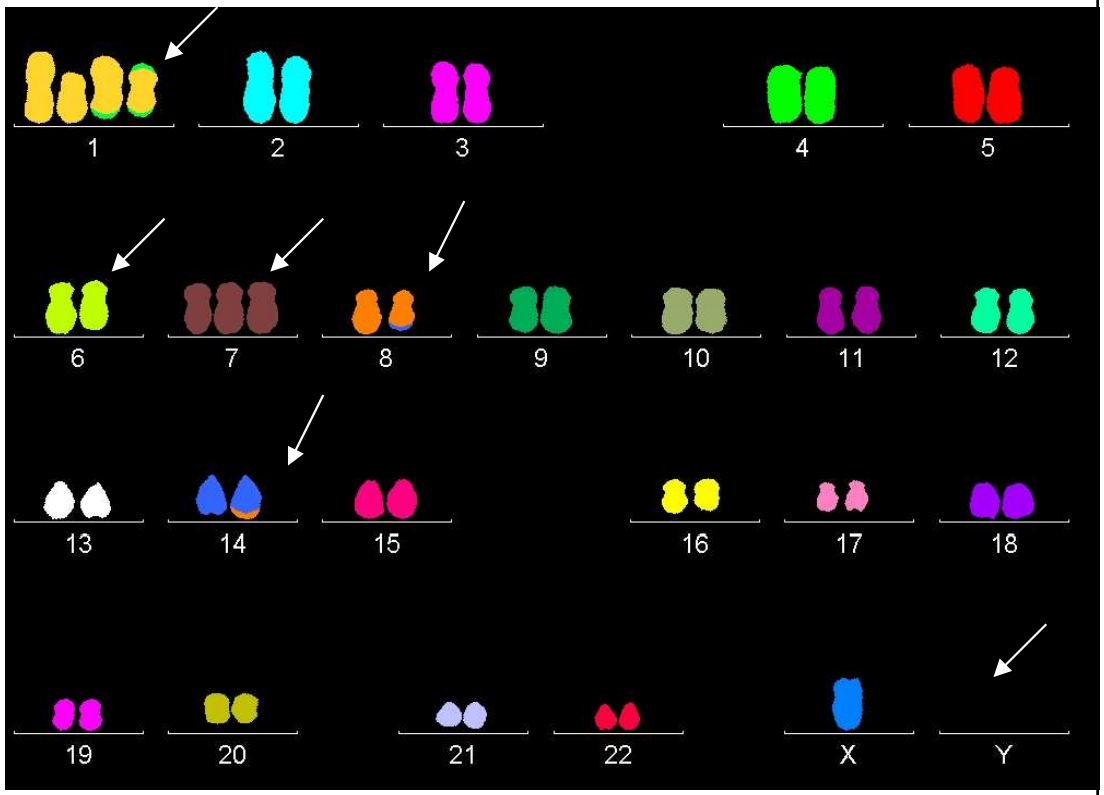


Převzato: Hochberg et al, BJH 2008

BURKITT LYMFOM (BL)

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%)

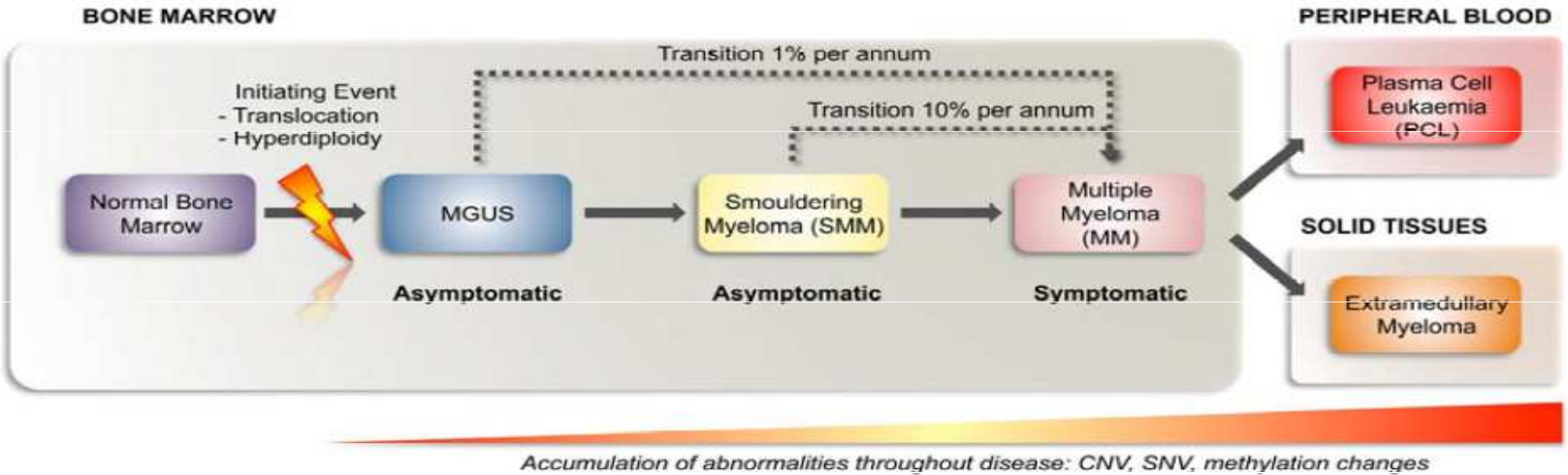




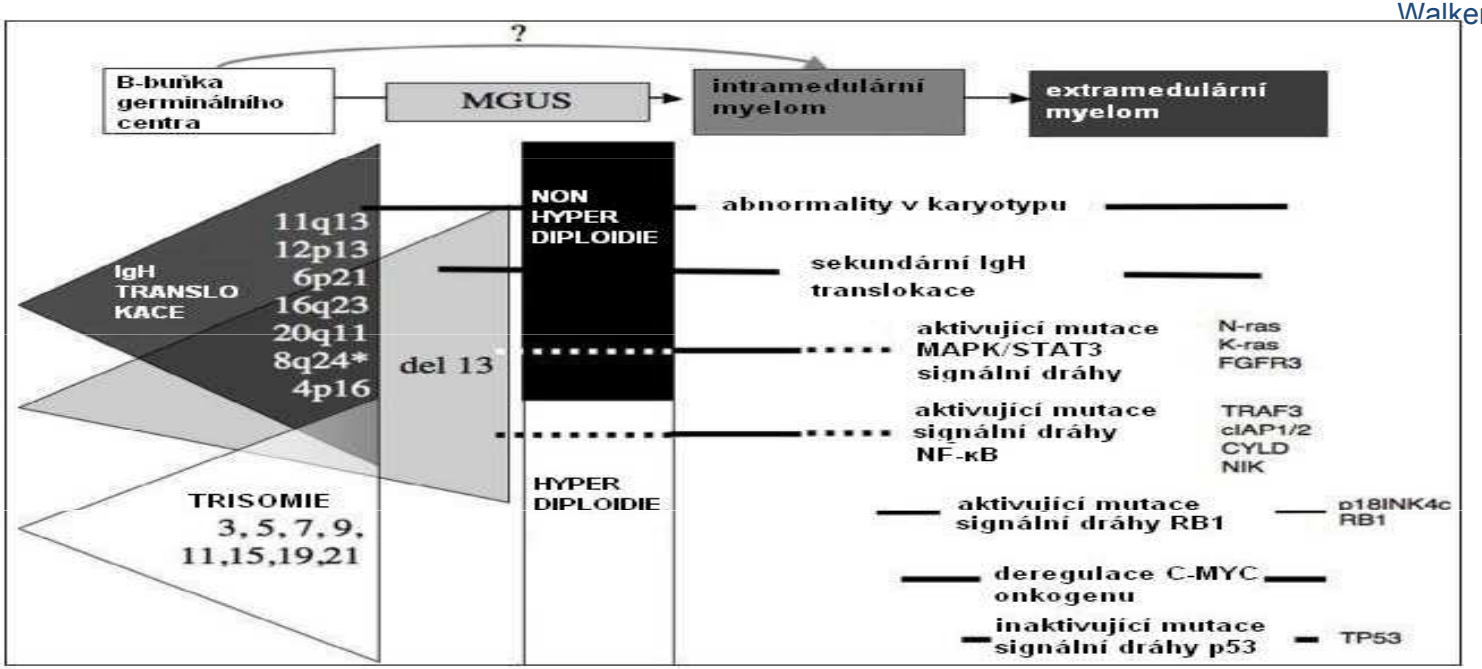
11/2011

MNOHOČETNÝ MYELOM

MM je B-buněčné nádorové onemocnění, charakterizované nekontrolovatelnou proliferací abnormálních plasmatických buněk v kostní dřeni.



Walker B,

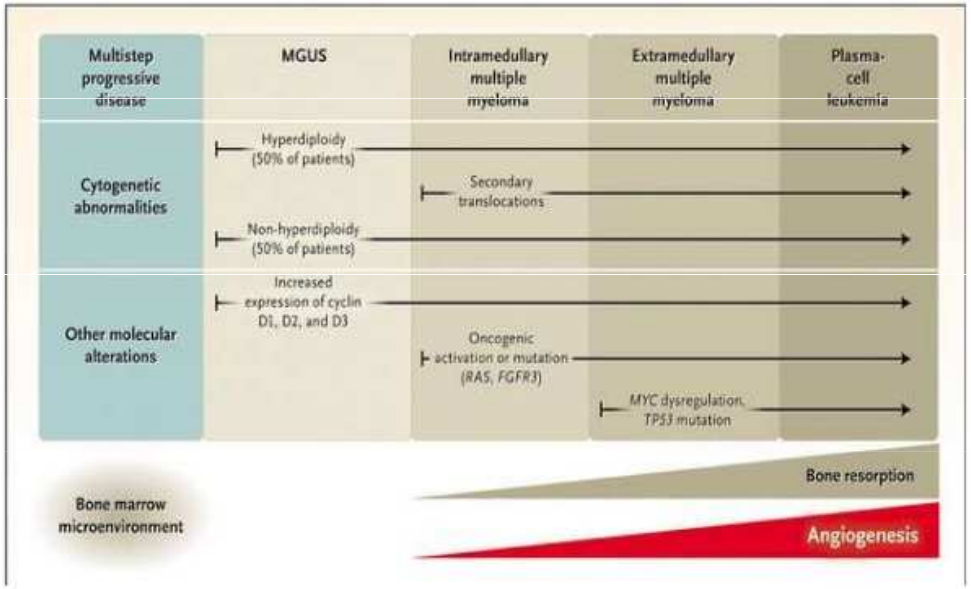


MNOHOČETNÝ MYELOM

2015

Revised International Staging System for Multiple Myeloma: A Report From International Myeloma Working Group

Multistep Pathogenesis of Multiple Myeloma

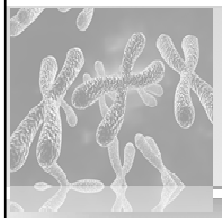


N Engl J Med 2011; 364:1046-1060 March 17,2011

Table 1. Standard Risk Factors for MM and the R-ISS

Prognostic Factor	Criteria
ISS stage	
I	Serum β_2-microglobulin < 3.5 mg/L, serum albumin ≥ 3.5 g/dL
II	Not ISS stage I or III
III	Serum β_2-microglobulin ≥ 5.5 mg/L
CA by iFISH	
High risk	Presence of del(17p) and/or translocation t(4;14) and/or translocation t(14;16)
Standard risk	No high-risk CA
LDH	
Normal	Serum LDH < the upper limit of normal
High	Serum LDH > the upper limit of normal
A new model for risk stratification for MM	
R-ISS stage	
I	ISS stage I and standard-risk CA by iFISH and normal LDH
II	Not R-ISS stage I or III
III	ISS stage III and either high-risk CA by iFISH or high LDH

Abbreviations: CA, chromosomal abnormalities; iFISH, interphase fluorescent in situ hybridization; ISS, International Staging System; LDH, lactate dehydrogenase; MM, multiple myeloma; R-ISS, revised International Staging System.



ZÁVĚR

- Cytogenetika je nedílnou součástí diagnostických a prognostických stratifikací hematologických malignit i dětských solidních nádorů
- V jednom vyšetření analyzuje celý genom
- Dovoluje potvrdit klinickou diagnosu nálezem specifických chromosomových změn
- Nenáhodné rekurentní změny určují prognosu onemocnění
- Určení změny dovoluje monitorovat účinnost léčby