

Nádorová cytogenomika (CYTOGENETIKA A MOLEKULÁRNÍ CYTOGENETIKA)

HEMATOLOGICKÝCH MALIGNIT

Marie Jarošová

Centrum molekulární biologie a genové terapie
Interní hematoonkologická klinika FN a LF MU Brno



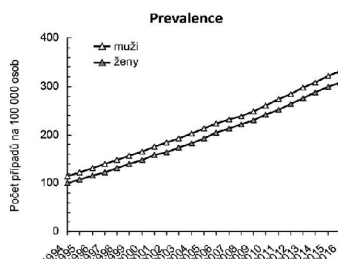
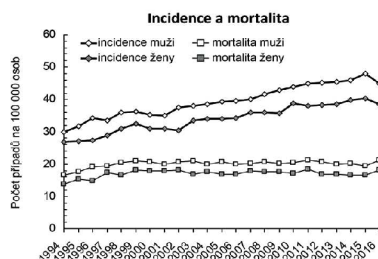
Genetika nádorů

- *Nádor je genetické onemocnění, které vzniká jako důsledek kumulace řady genetických změn*
- *V ČR je diagnostikováno ročně více jak 90 tis. nových nádorů*

(ÚZIS: V roce 2015 bylo do Národního onkologického registru ČR (NOR) nově nahlášeno celkem 94 462 případů zhoubných novotvarů (ZN) a novotvarů in situ (dg. C00–C97 a D00–D09 dle MKN-10), z toho 48 666 případů u mužů a 45 796 případů u žen.)

Novotvary mízní a krvetvorné tkáně v České republice

	2010	2011	2012	2013	2014	2015	2016	průměrná roční změna (trend)
Incidence	4 341	4 345	4 374	4 410	4 513	4 661	4 410	+0,8 %
Mortalita	1 965	2 082	1 972	1 931	1 934	1 900	2 068	-0,2 %
Prevalence	26 405	27 597	28 847	30 055	31 354	32 746	33 793	+4,1 %



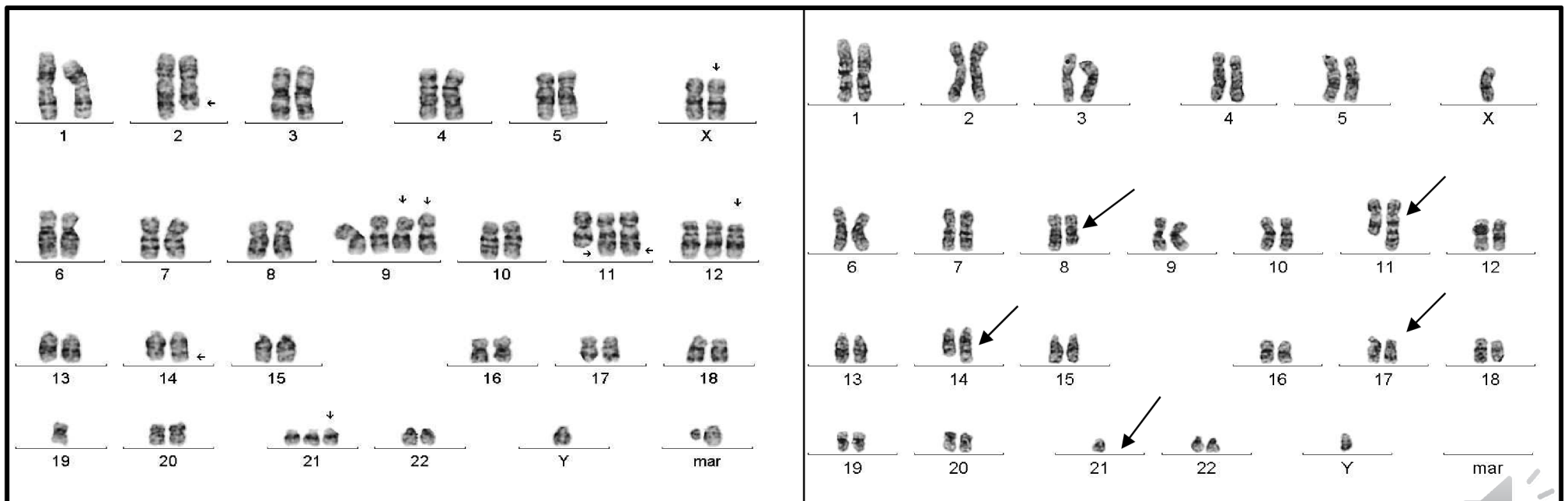
Zdroj: Národní onkologický registr, ÚZIS ČR

Celková incidence hematologických malignit přerušila v letech 2015–2016 hodnotu 4400 případů ročně, při dlouhodobě stabilním průměrném ročním růstu +0,8 %. Mortalita recentně mírně klesá (ročně -0,2 %) a dosahuje hodnoty přibližně 2000 úmrtí ročně. Důsledkem odlišného trendu ve vývoji incidence a mortality je prudce rostoucí prevalence těchto onemocnění, která v letech 2015–2016 dosáhla hodnoty téměř 34 000 osob a průměrně roste o +4,1 %.



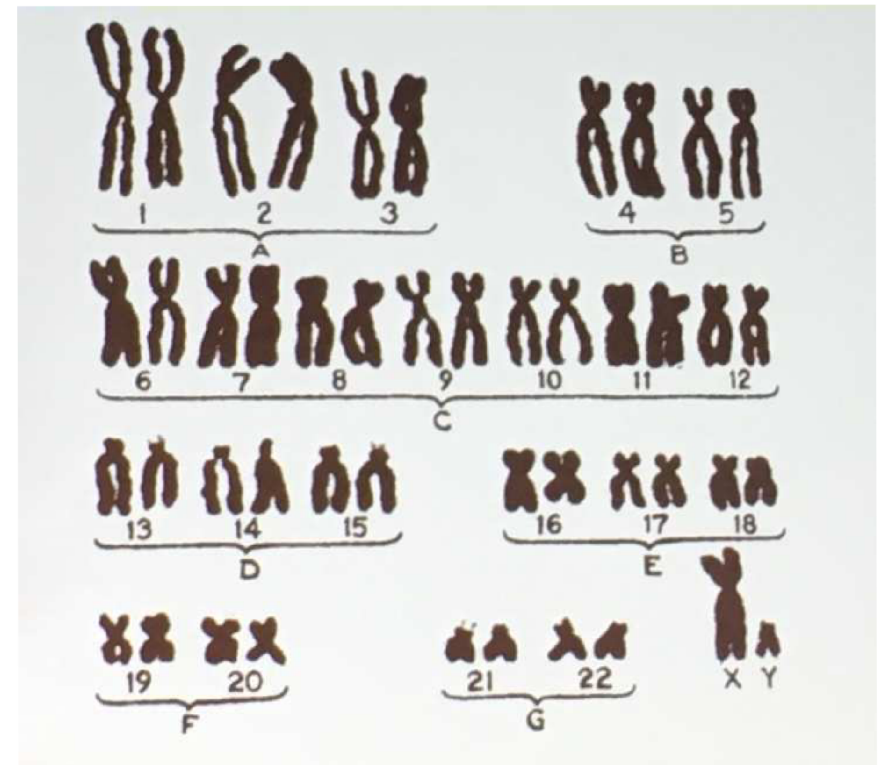
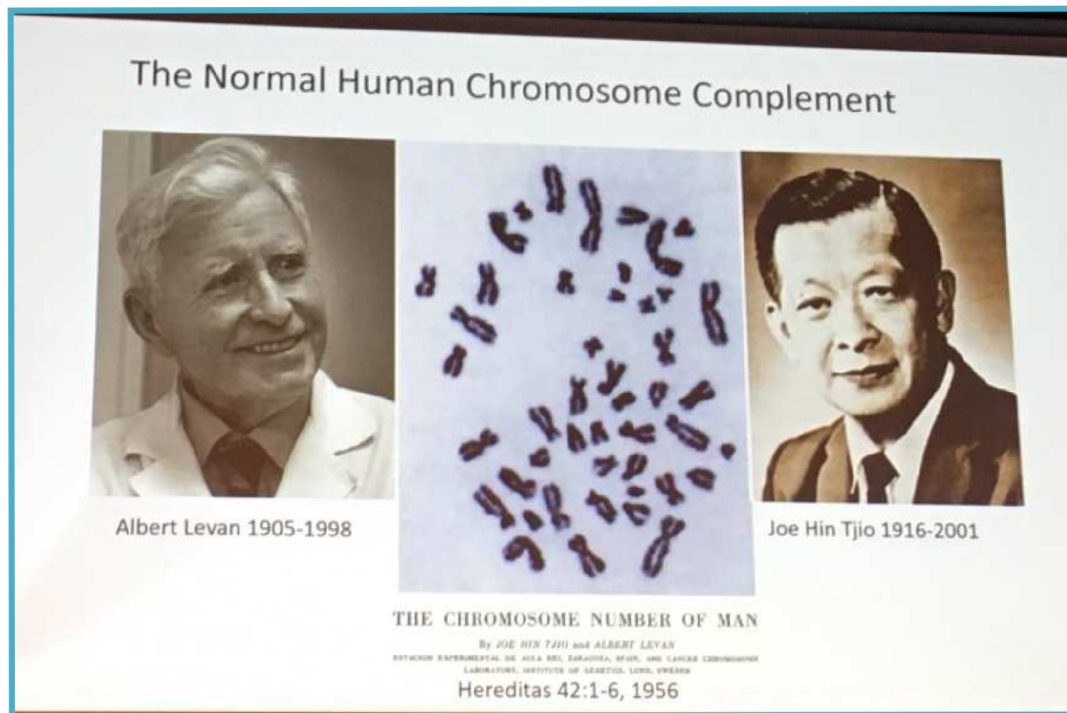
Nádorová cytogenomika

- 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 & Singh 1979



1956 - určen přesný počet 46 lidských chromosomů



Historie nádorové cytogenetiky

Philadelphia chromosome (Ph1)



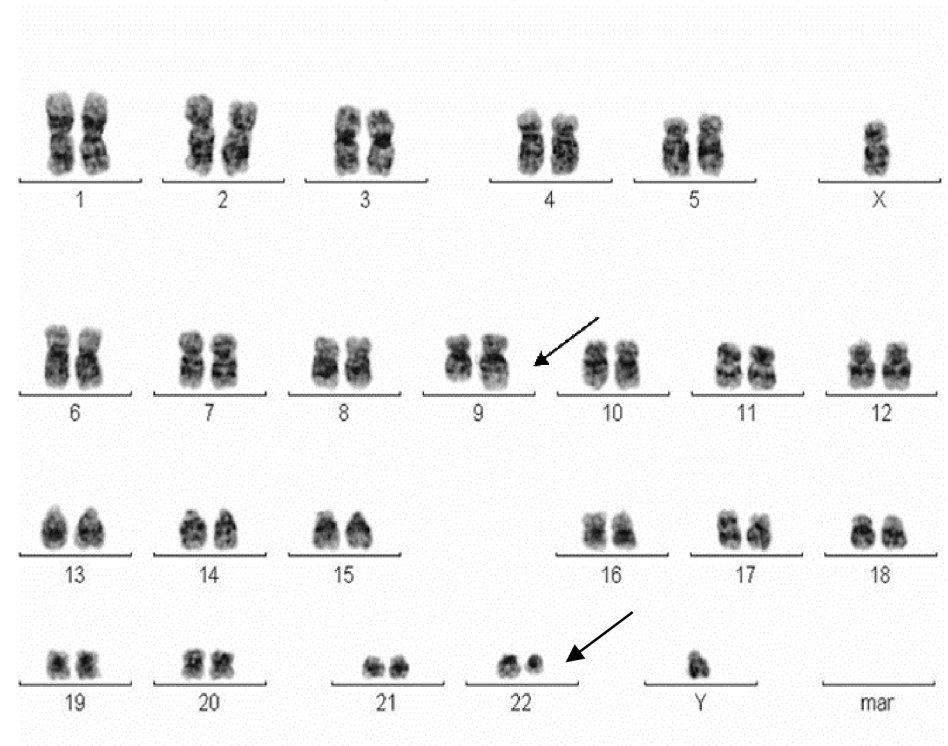
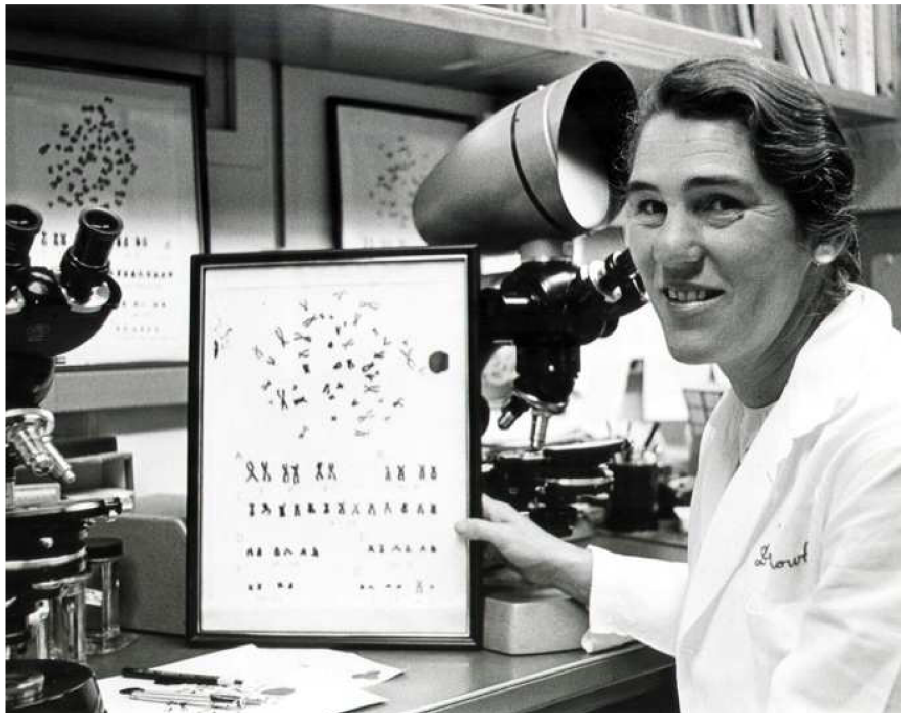
Peter Nowell & David Hungerford
Science 1960,132:1497



Historie nádorové cytogenetiky

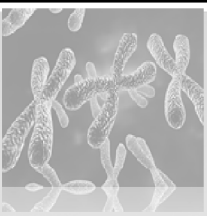
Philadelphia chromosome (Ph1)

$t(9;22)(q34;q11)$



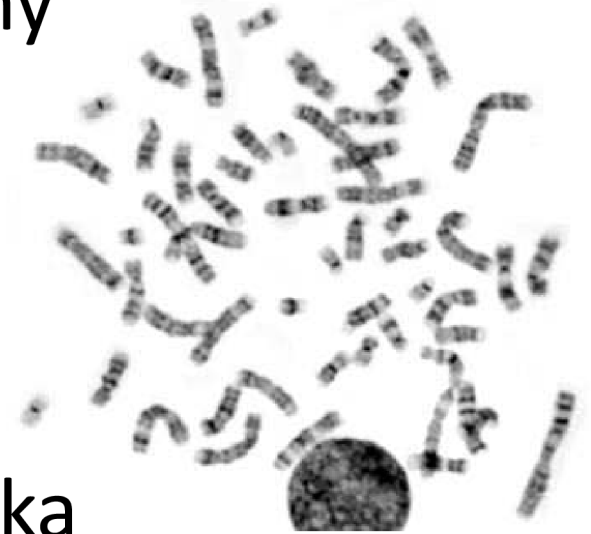
Dr. Rowley received the Lasker Award, given for distinguished contributions to medical science; the National Medal of Science from President Bill Clinton; and the Presidential Medal of Freedom from President Obama, among many other honors (1925-2013)



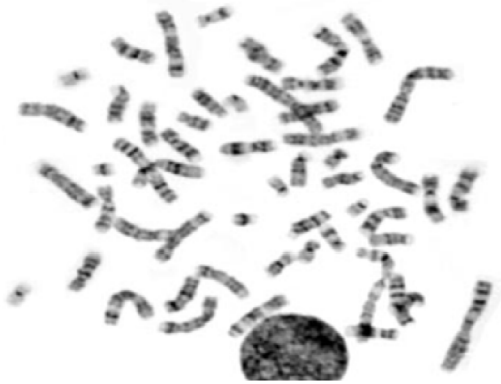


Nádorová cytogenetika

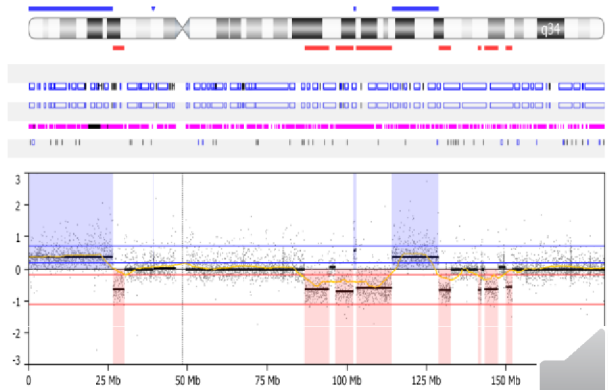
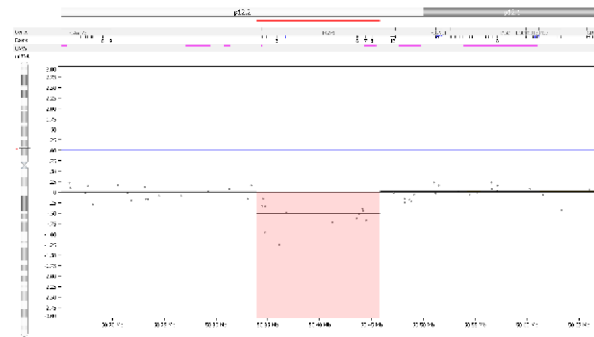
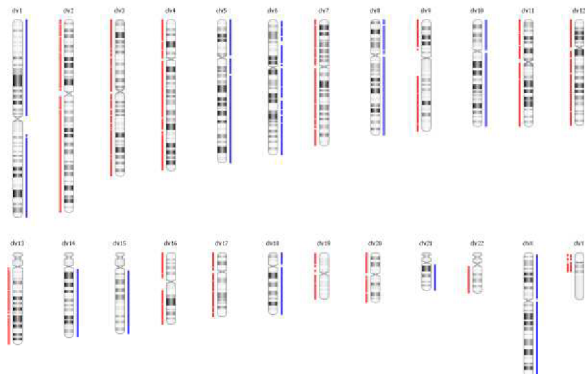
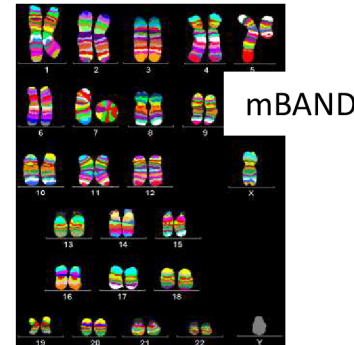
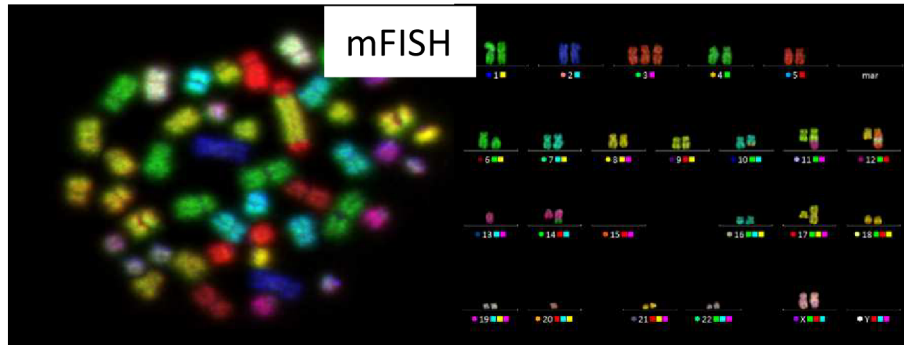
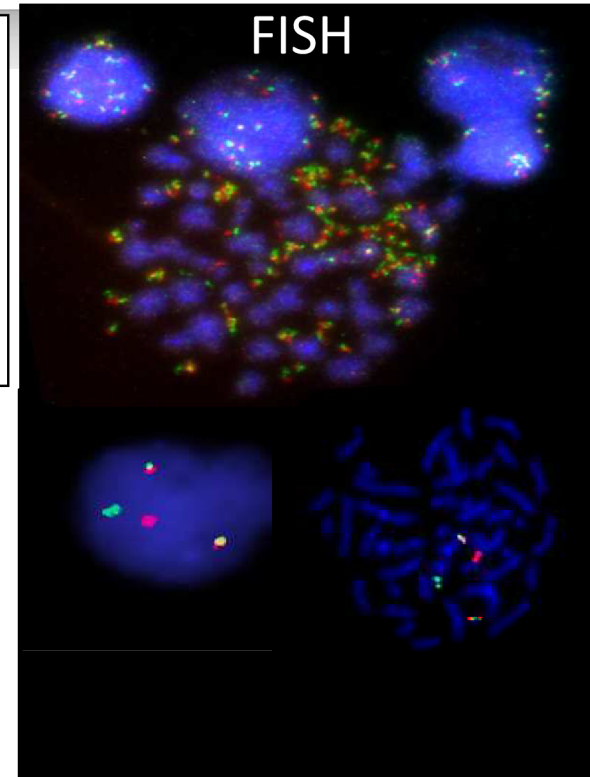
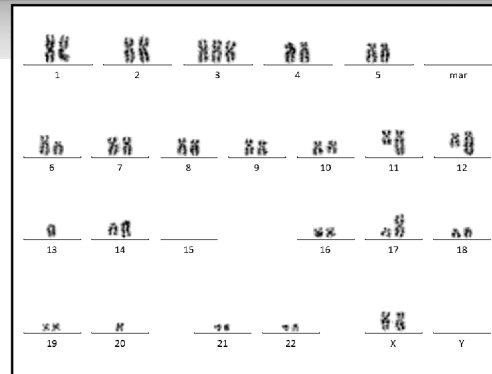
- Zkoumá získané chromosomové změny nádorových buněk
- Hodnotí početní a strukturní změny chromosomů
- Základní metoda – G-pruhovací technika (rozlišení kolem 3-5Mb)
- V jednom vyšetření analyzuje celý genom



Nádorová cytogenomika - metody



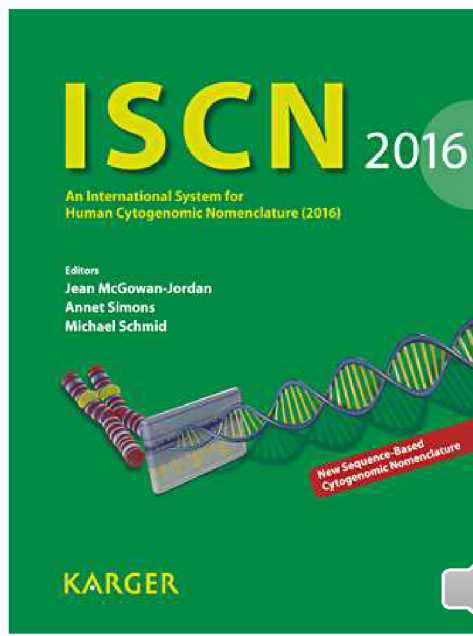
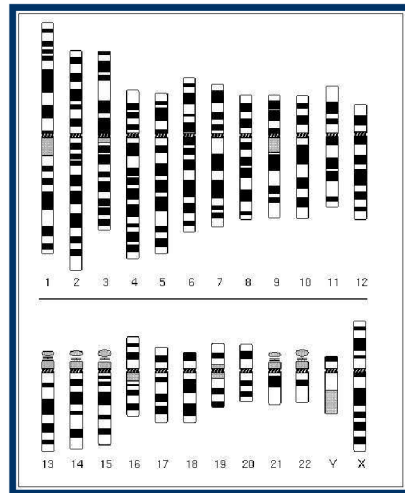
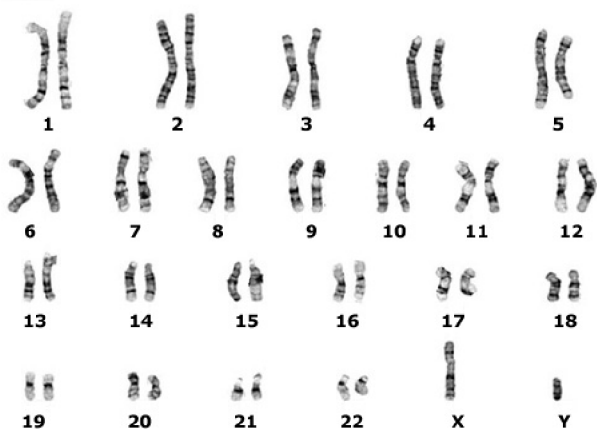
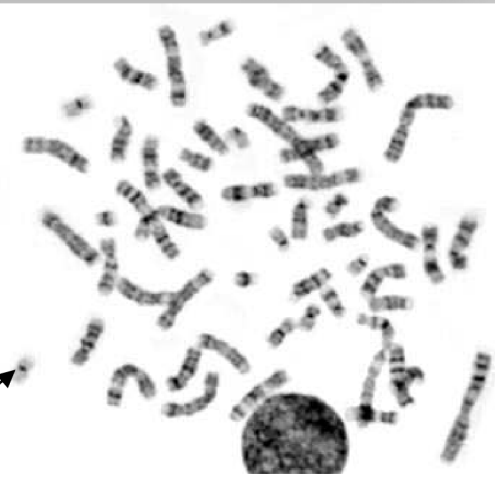
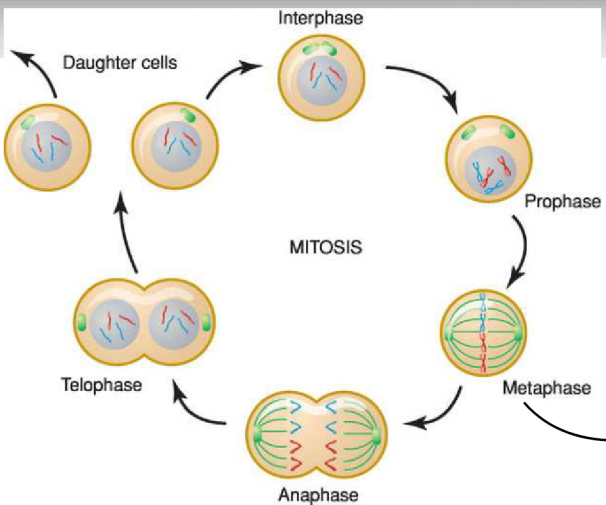
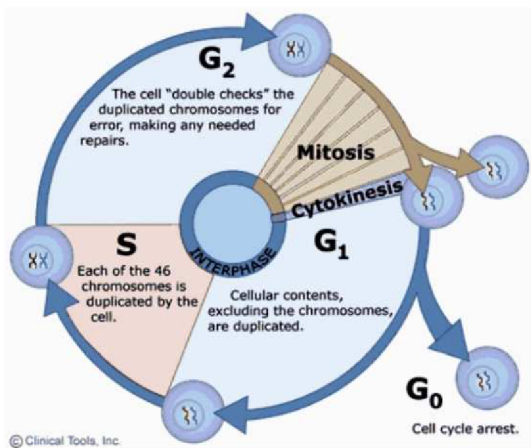
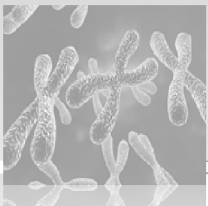
Konvenční
cytogenetická
analýza



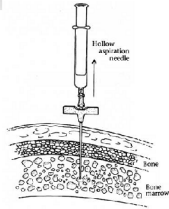
arrayCGH/SNP array



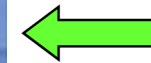
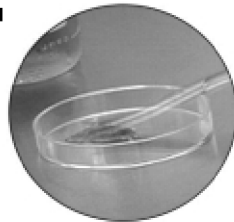
Konvenční cytogenetika



Postup kultivace buněk nádorů



1-2ml



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

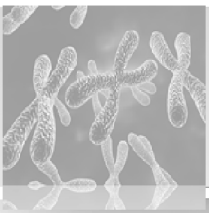


37°C/ 5%CO2



Kultivace
2/24/72hod/týdny





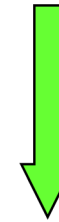
ZPRACOVÁNÍ BUNĚČNÉ KULTURY



www.shutterstock.com · 58307962



**HYPOTONIZACE
0,075M KCl**

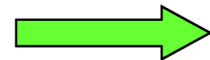
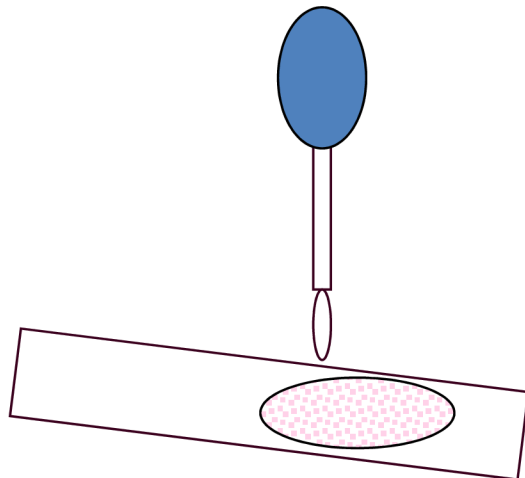


COLCEMIDE BLOKUJE MITÓZY V METAFÁZI

**PŘÍPRAVA PREPARÁTŮ
KAPÁNÍM BB SUSPENZE
NA SKLO**



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



**BARVENÍ A HODNOCENÍ
V MIKROSKOPU**



Klasická cytogenetika - karyotyp

MetaSystems · Ikaros · 3

1 2 3 4 5 X

6 7 8 9 10 11 12

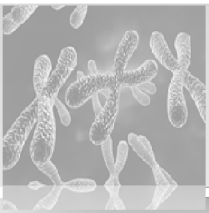
13 14 15 16 17 18

19 20 21 22 Y mar

Assign
Rotate 180° / 90°
Rotate X°
Shift
Clean
Reduce
Magnify
Staining
Annotate

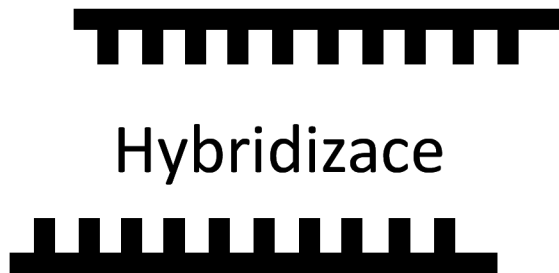
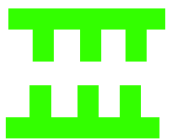
CASE101	5	46,XX
---------	---	-------

DemoIKS DIR-G
adm GBAND



Molekulární cytogenetika

Denaturace

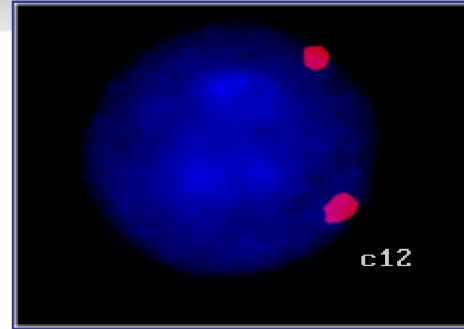
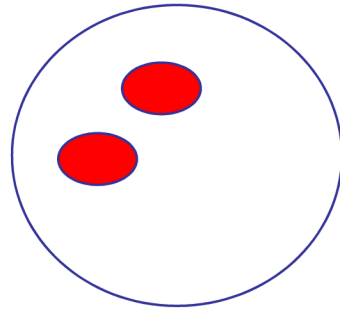
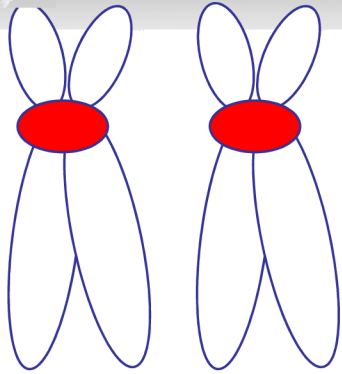
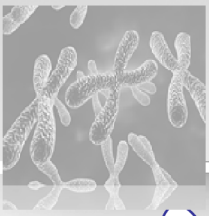


Hybridizace

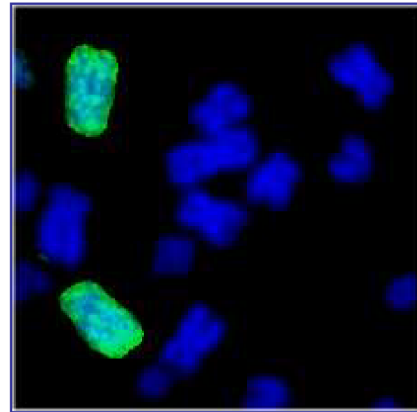
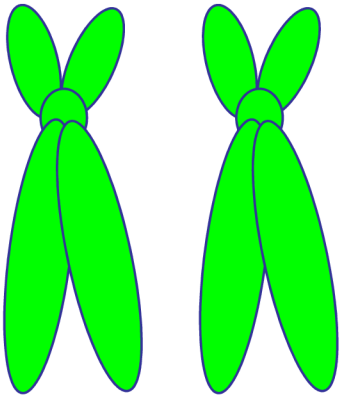
- Metody založené na fluorescenční in situ hybridizaci (FISH) vytváří spojení mezi metodami molekulární genetiky a klasické cytogenetiky
- Metody využívající základní vlastnosti jednořetězcové DNA vzájemně se vázat na základě komplementarity bazí



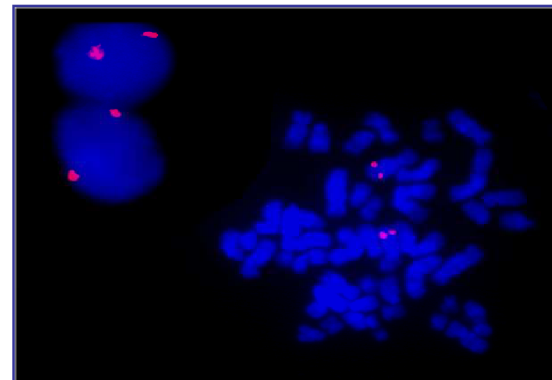
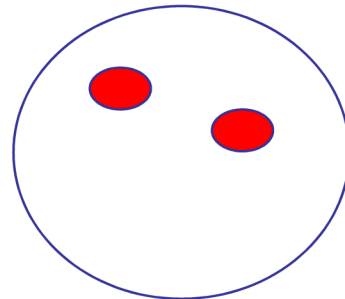
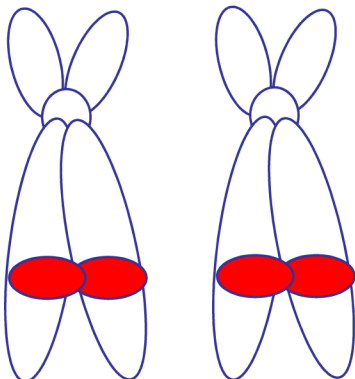
Typy sond



centromerické

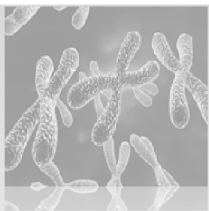


celochromosomové



genové



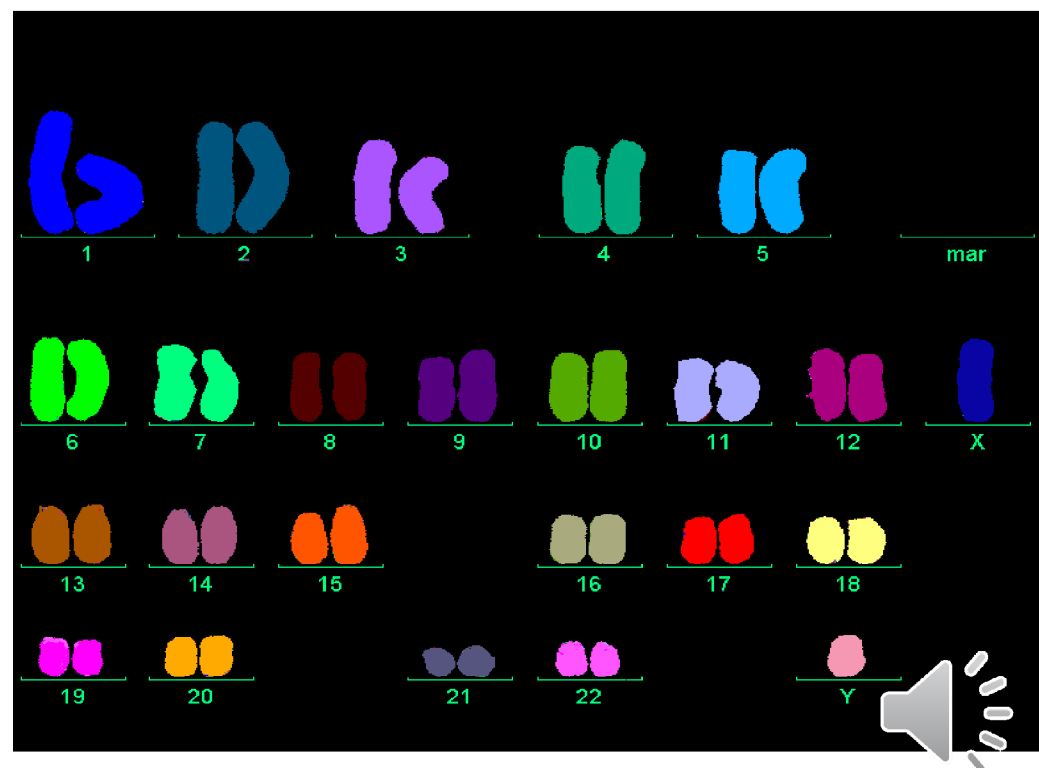


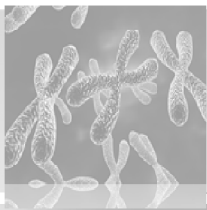
Mnohobarevná fluorescenční in situ hybridizace (mFISH)

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

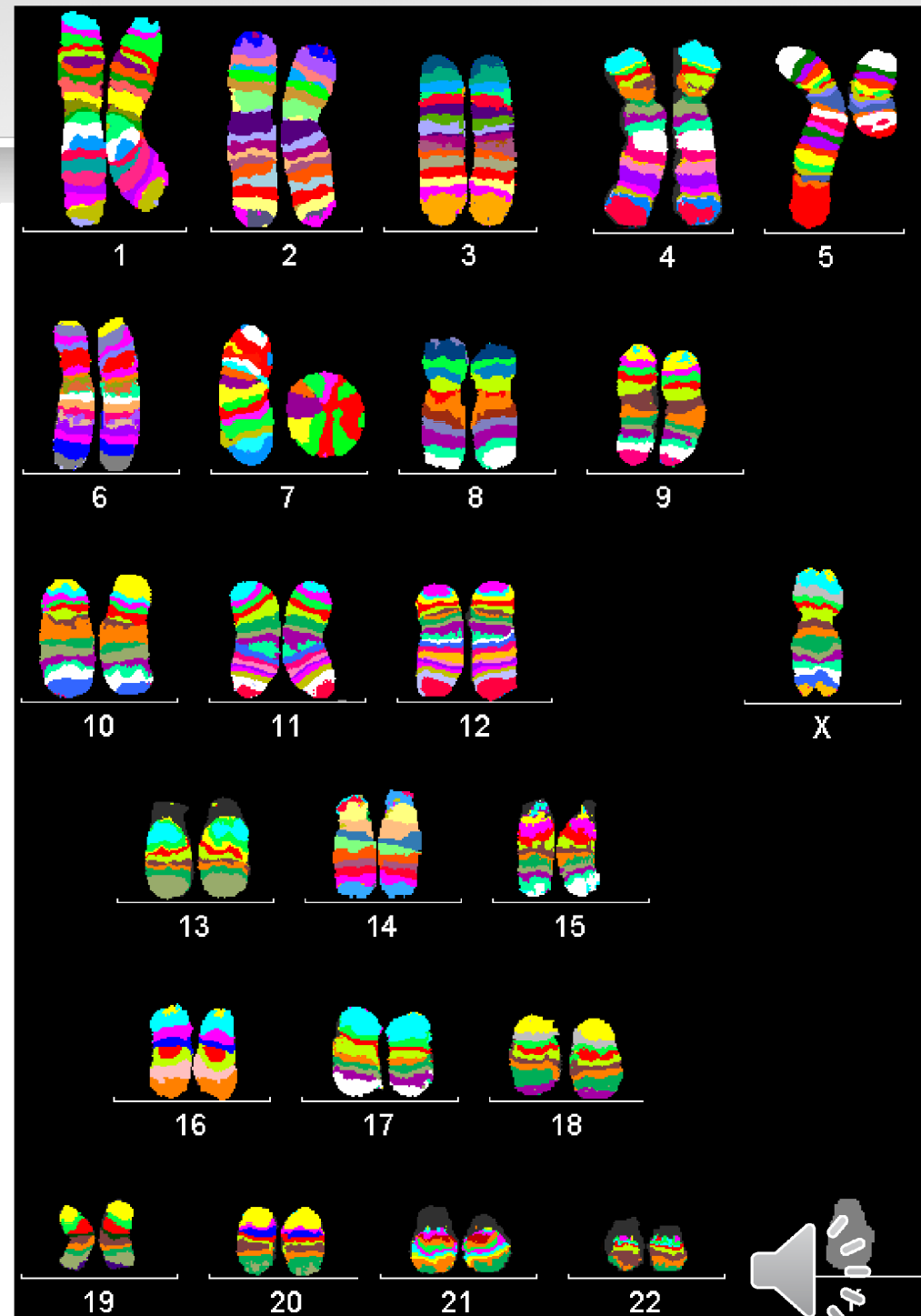
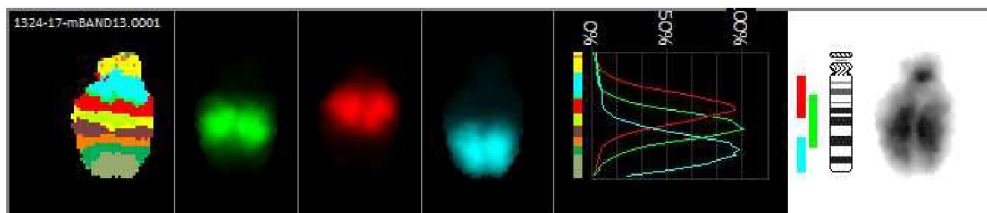
Chr.	FITC	Spectrum Orange	Texas Red	Cy5	DEAC
1				Red	Blue
2					Blue
3			Red		
4	Green				
5		Yellow			
6	Green			Red	
7				Red	Blue
8			Red		
9		Yellow	Red	Red	
10	Green				Blue
11	Green		Red		
12	Green	Yellow			
13			Red		Blue
14		Yellow			Blue
15		Yellow	Red		
16	Green			Red	Blue
17	Green		Red	Red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

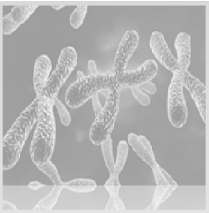




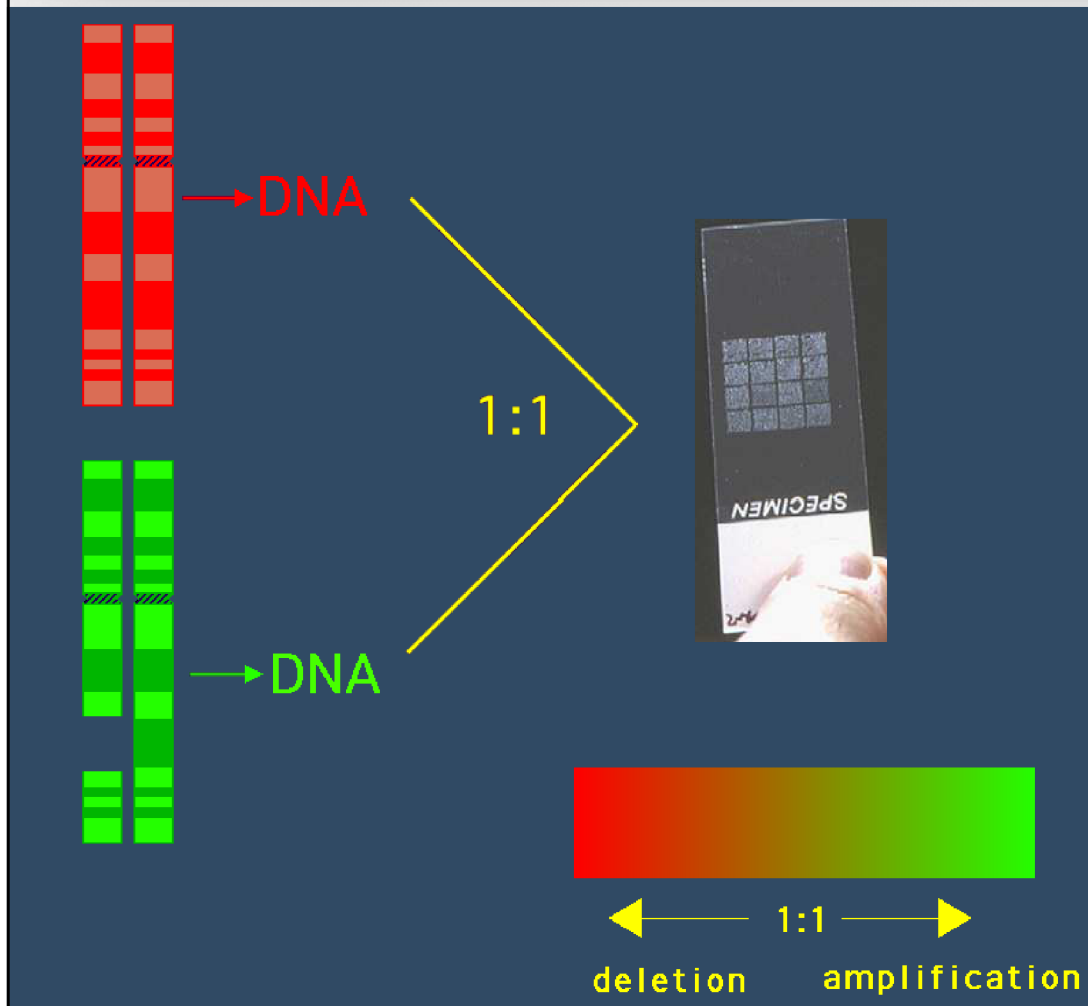
Mband FISH

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





Array CGH – komparativní genomová hybridizace



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

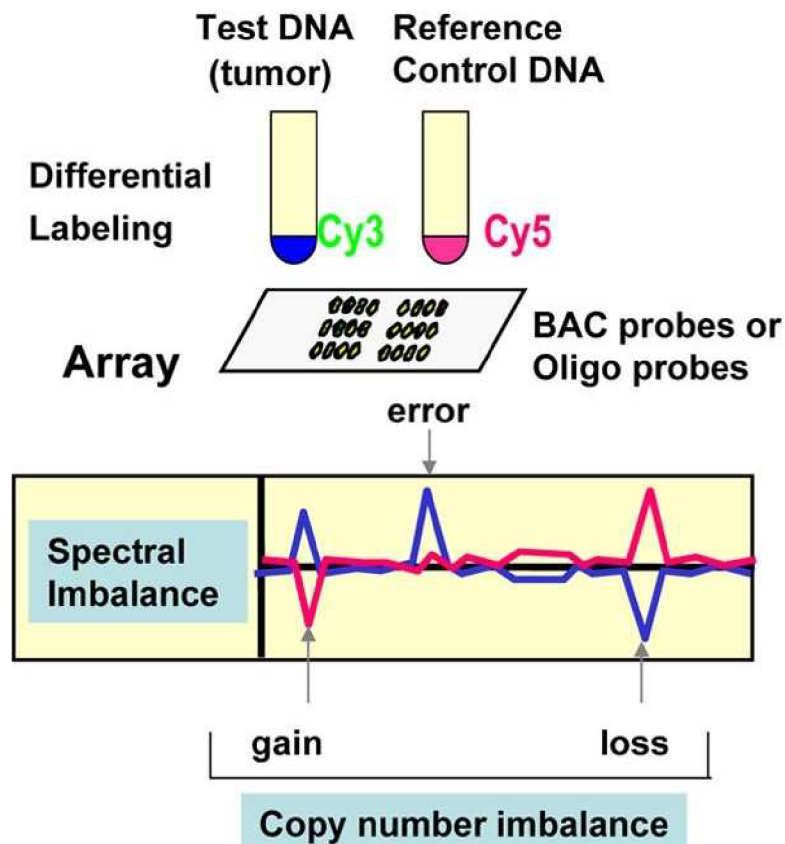


arrayCGH/SNPs array

A

CGH-A

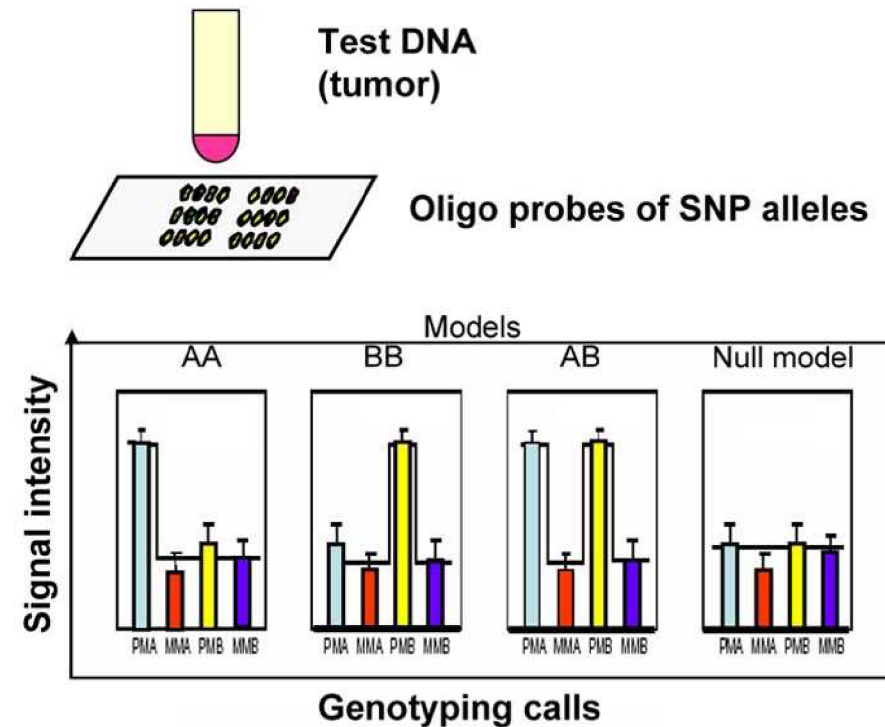
- BAC CGH-A
- Oligo CGH-A



B

SNP-A

- Combined CN/SNP-A



Genotype Intensity– copy number





Cytogenetika v hematologii

1.Diagnosa

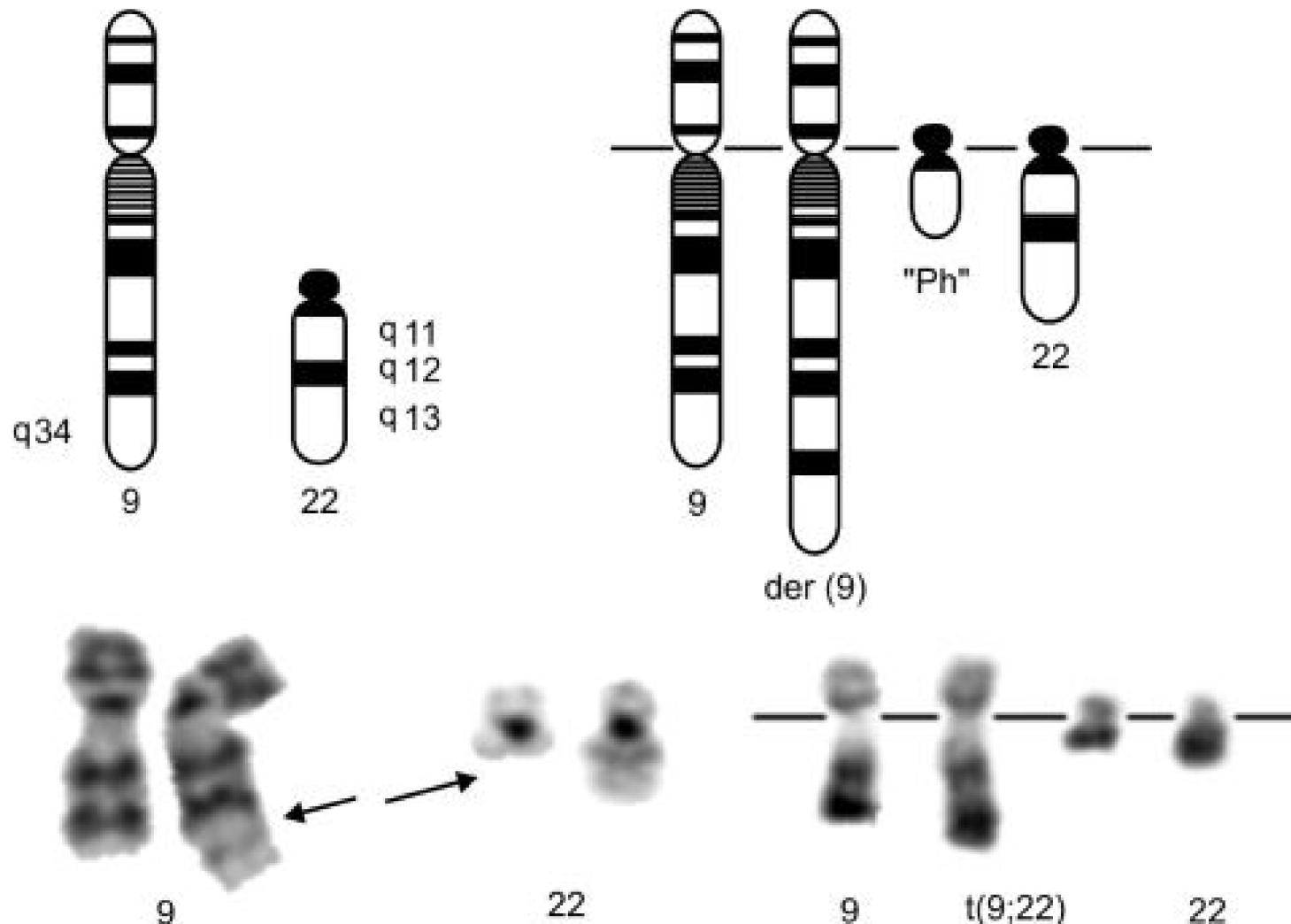
2.Prognosa

3.Léčebné rozhodování



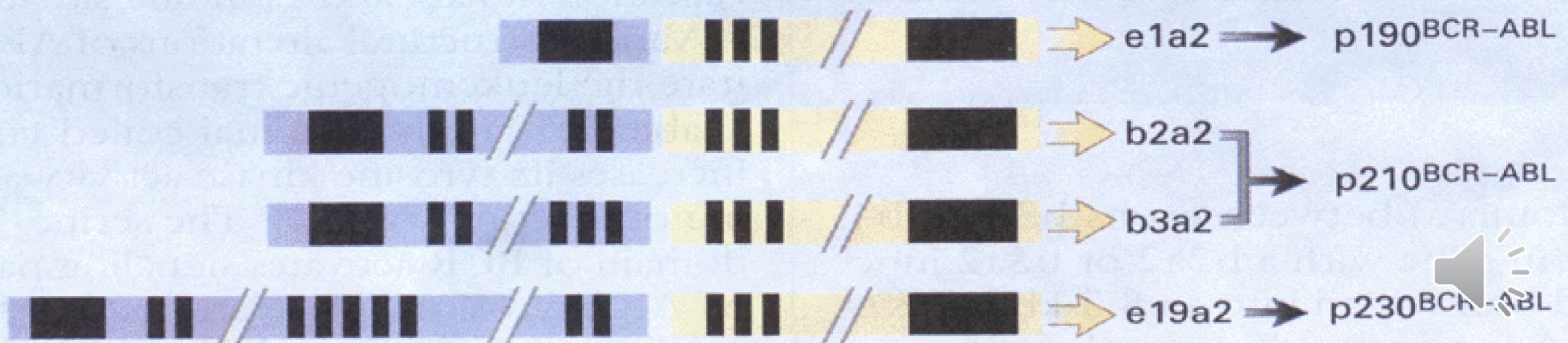
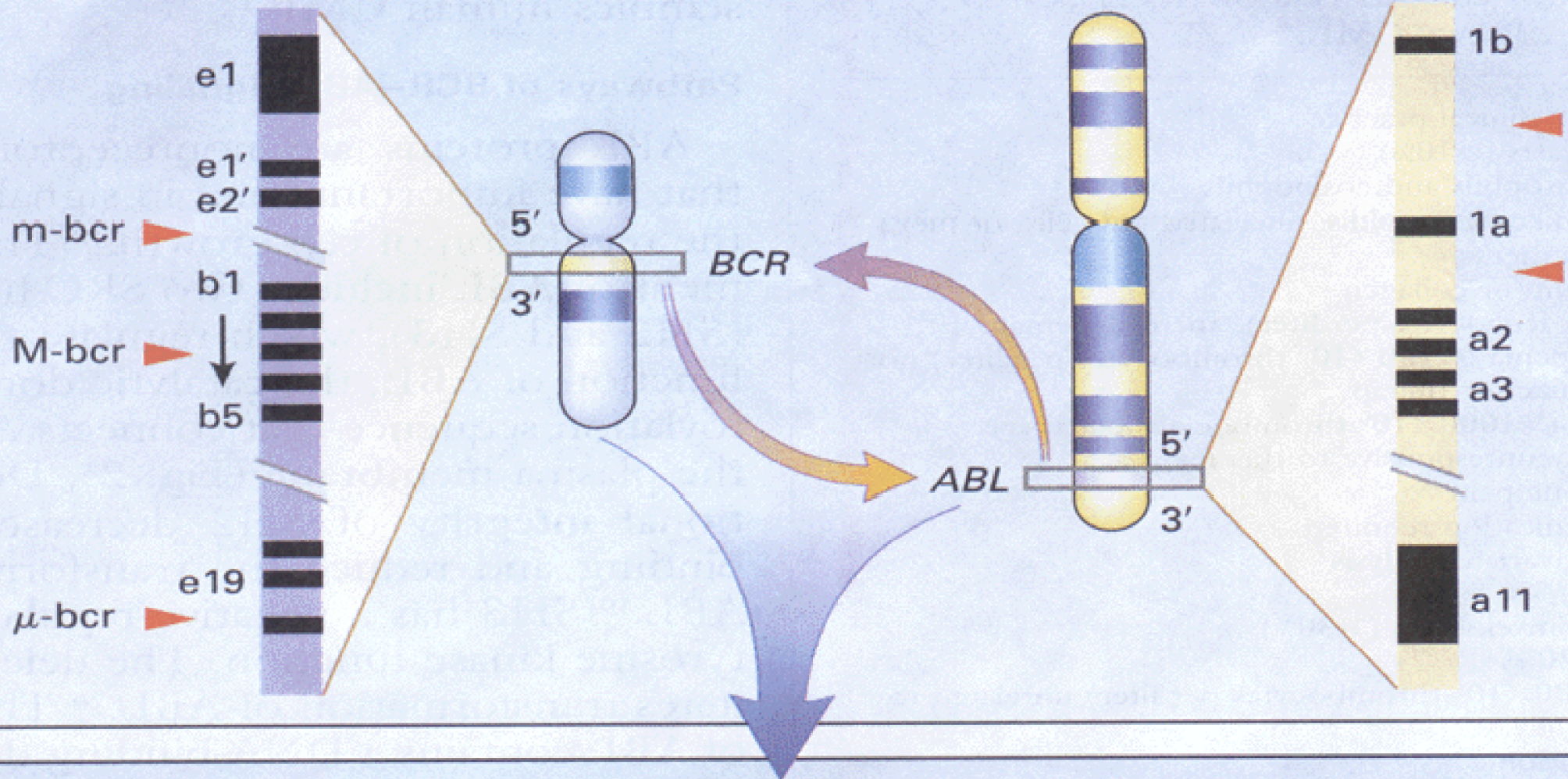
Filadelfský chromosom (Ph)

První specifická chromosomová změna u nádoru člověka



Chromosome 22

Chromosome 9





WHO Classification

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



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. Vardiman⁹

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

^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.^{37,86-89}



ZÁVĚR

- Cytogenetika je nedílnou součástí diagnostických a prognostických stratifikací hematologických malignit
- 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

