

Nádorová cytogenomika

(CYTOGENETIKA A MOLEKULÁRNÍ CYTOGENETIKA)

HEMATOLOGICKÝCH MALIGNIT

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Interní hematoonkologická klinika FN a LF MU Brno



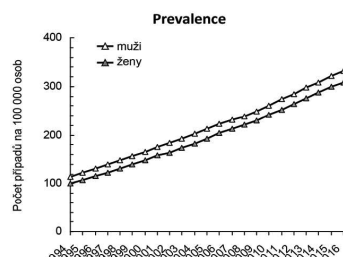
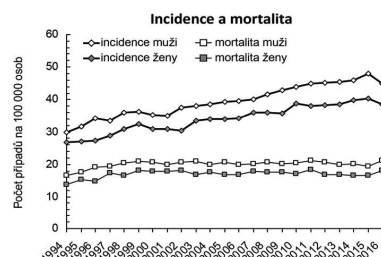
Genetika nádorů

- *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 krevetvorné 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 %



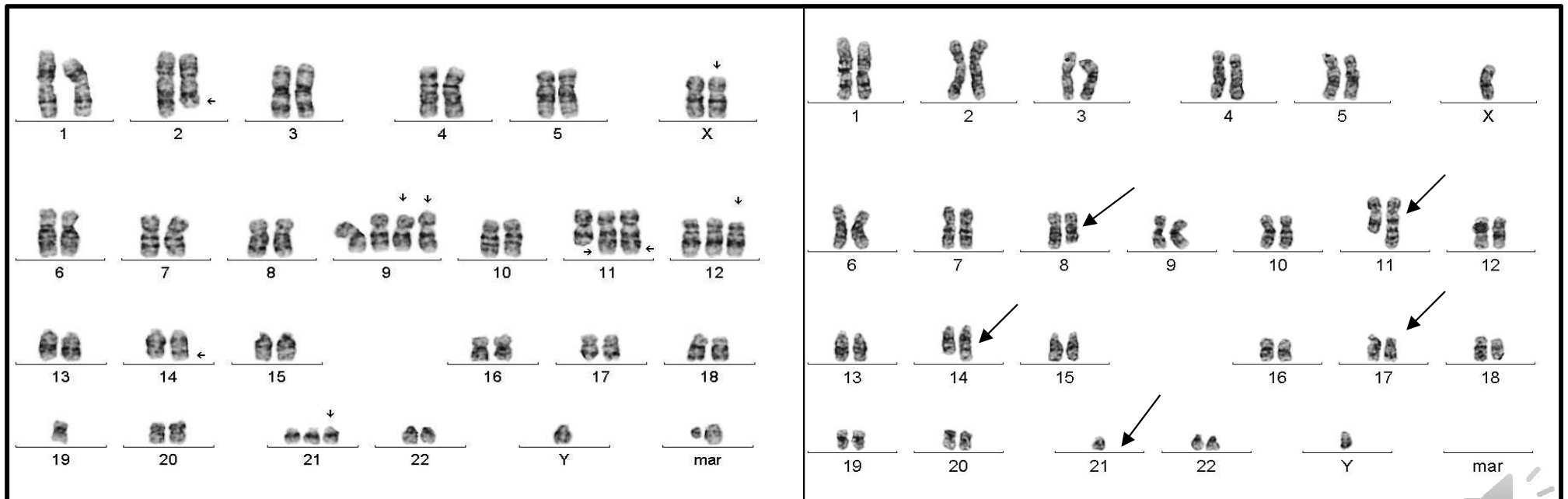
Zdroj: Národní onkologický registr, ÚZIS ČR

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 ročně průměrně roste o +4,1 %.



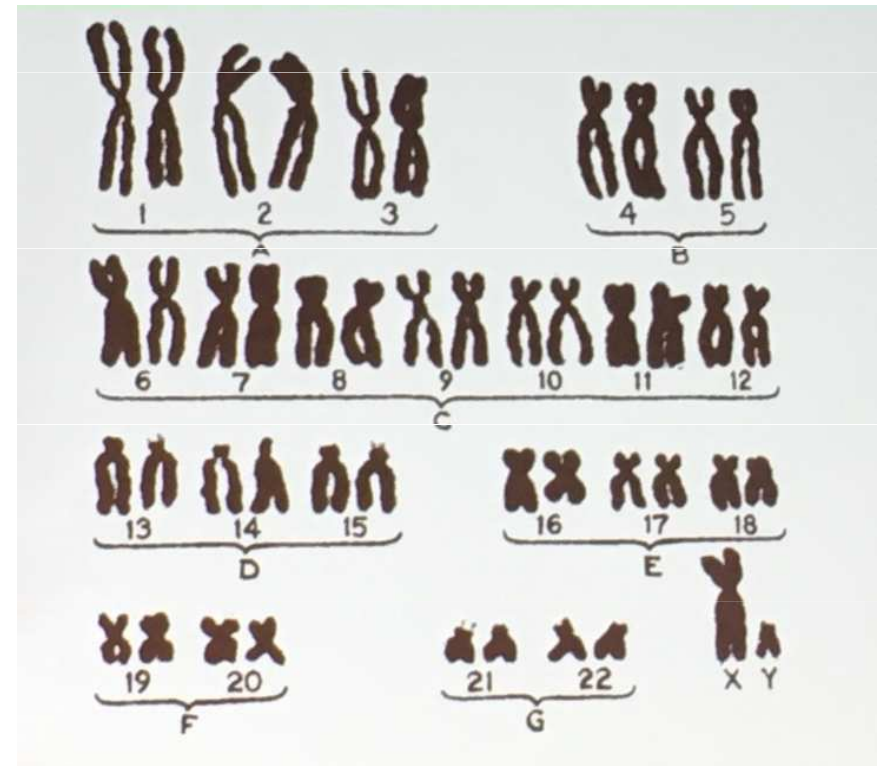
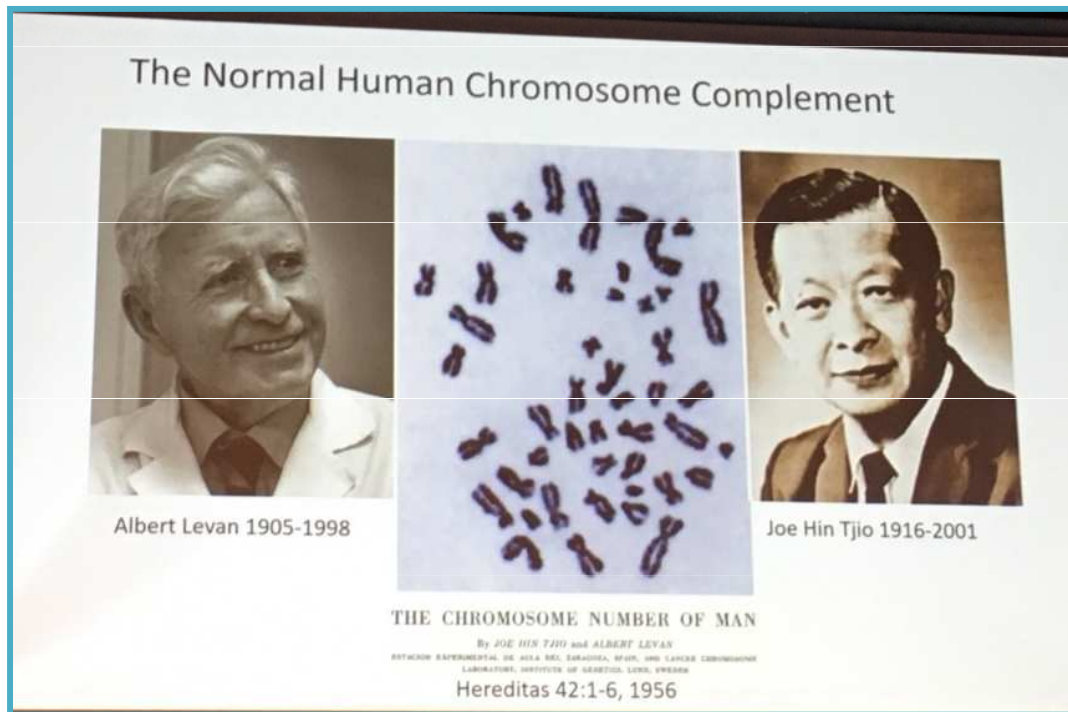
Nádorová cytogenomika

■ 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)



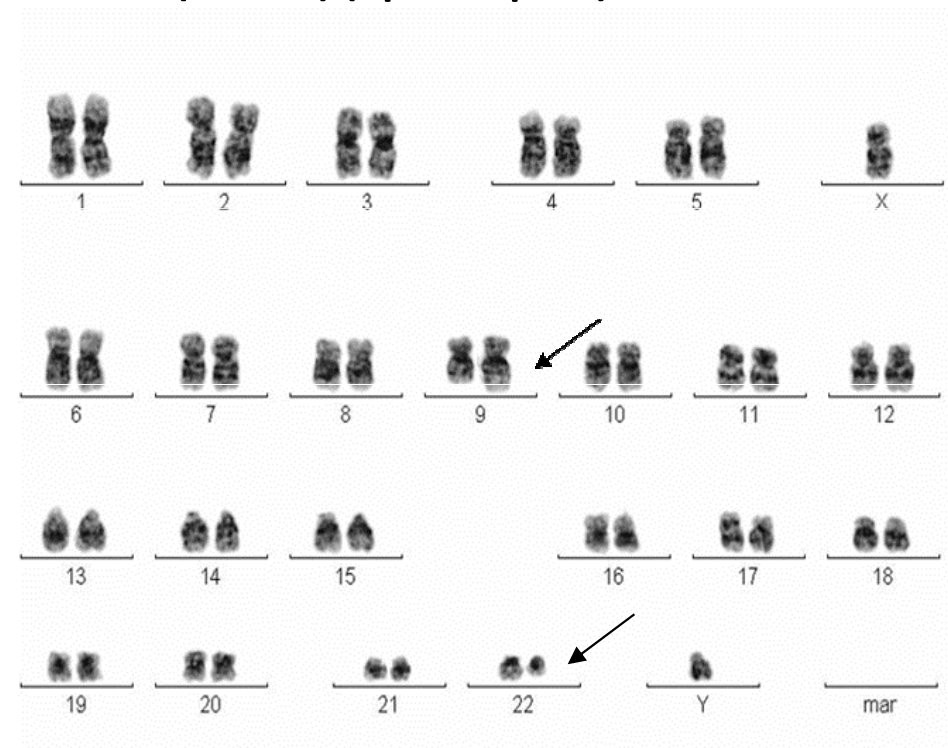
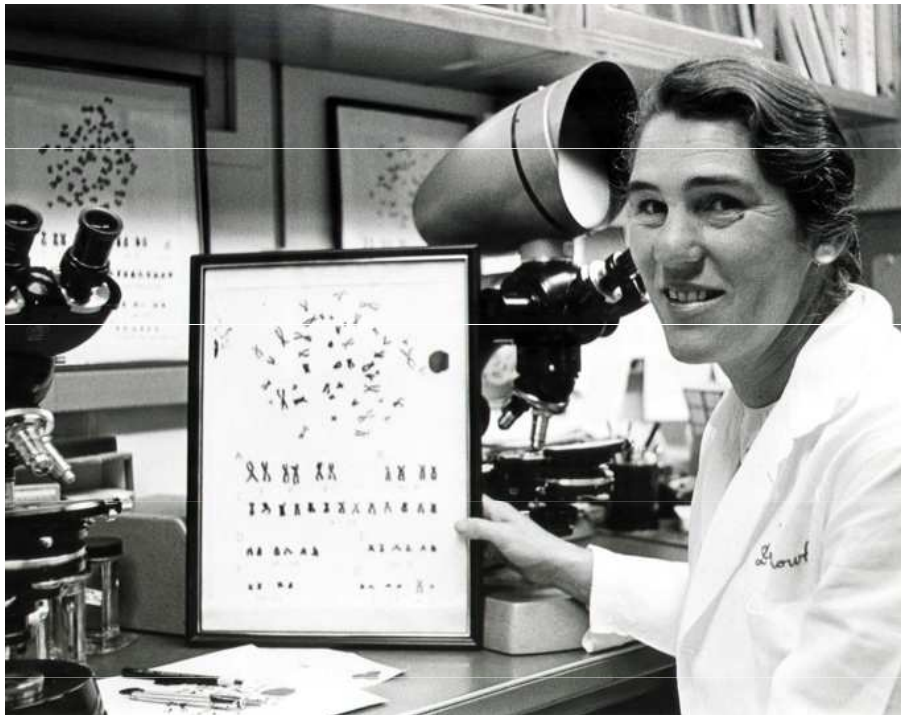
Peter Nowell & David Hungerford
Science 1960,132:1497



Historie nádorové cytogenetiky

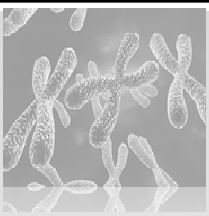
Philadelphia chromosome (Ph1)

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



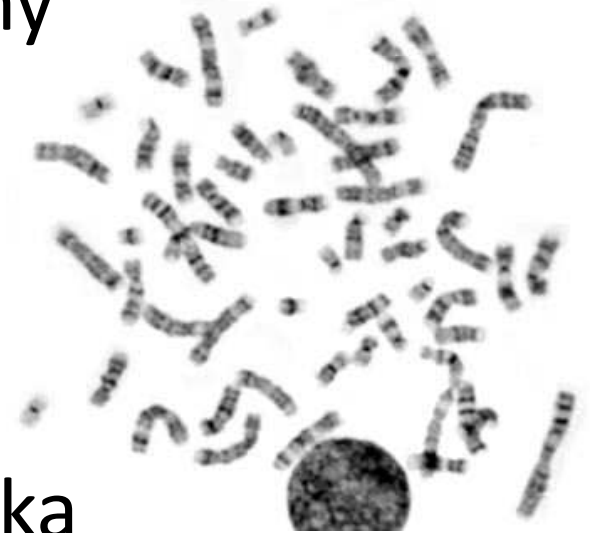
Dr. Rowley received the Lasker Award, given for distinguished contributions to medical science; the National Medal of Science from President Bill Clinton; and the Presidential Medal of Freedom from President Obama, among many other honors (1925-2013)





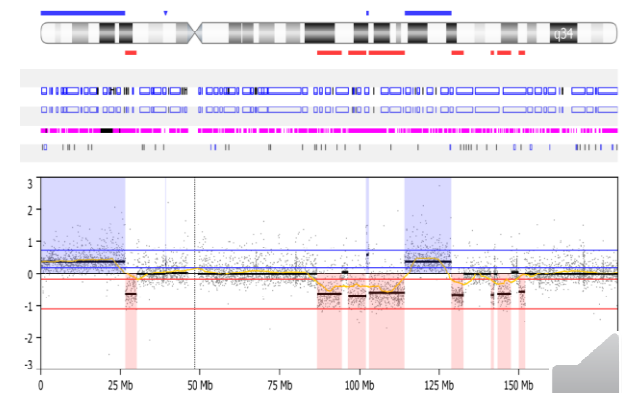
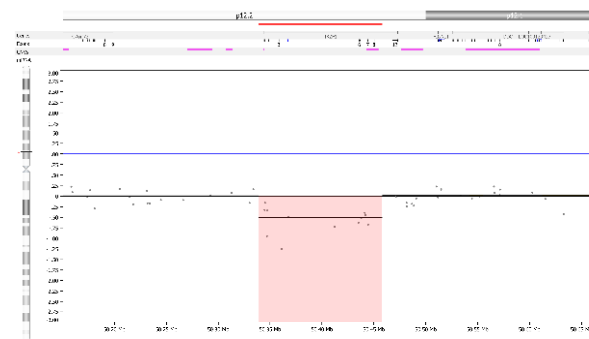
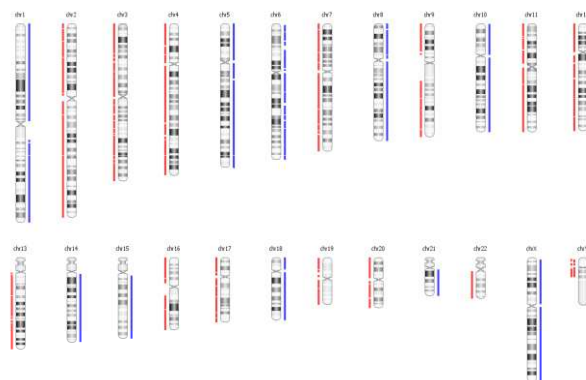
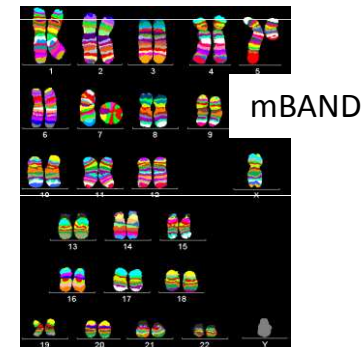
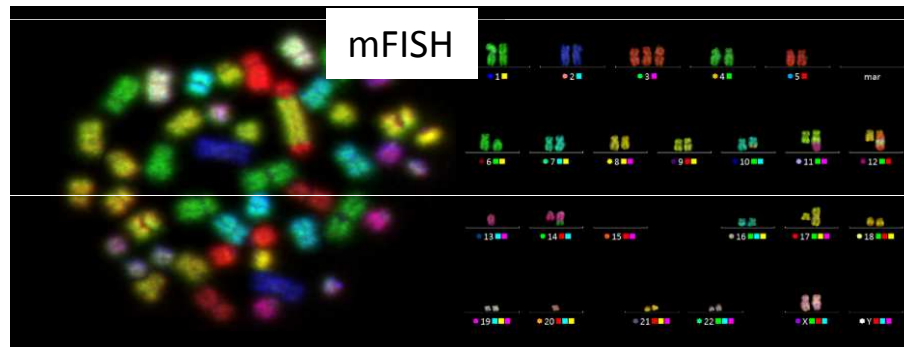
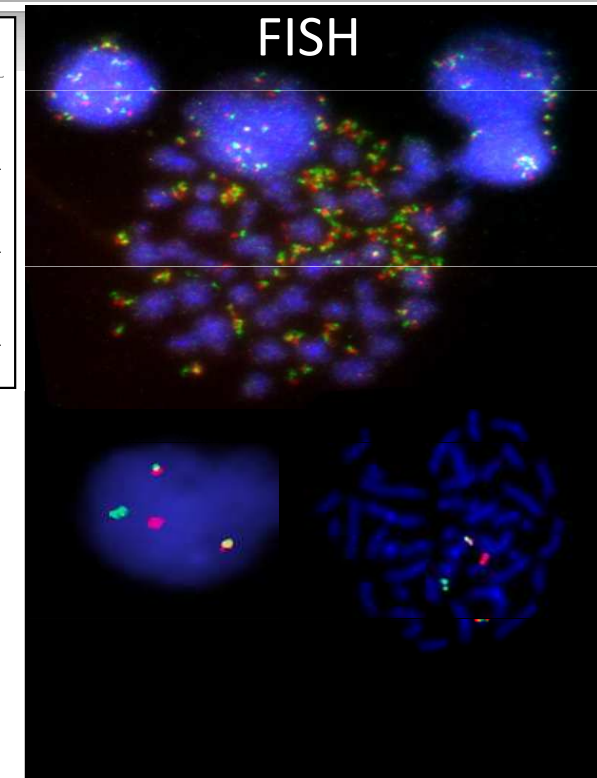
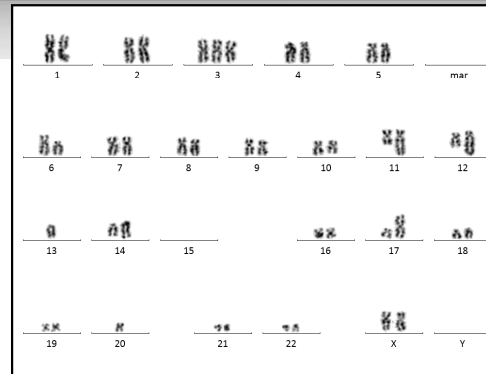
Nádorová cytogenetika

- 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

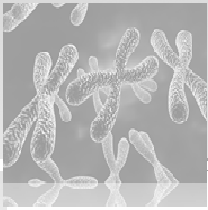


Nádorová cytogenomika - metody

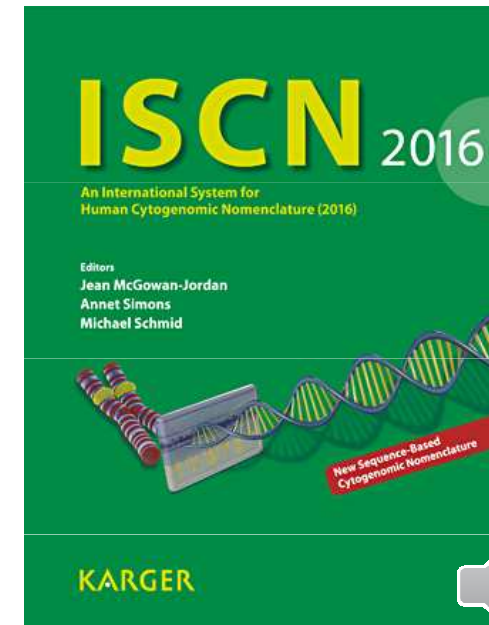
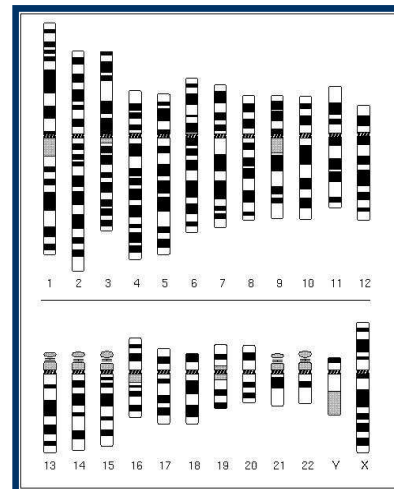
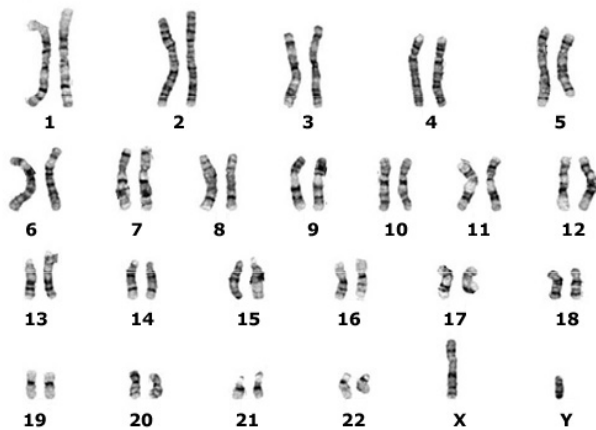
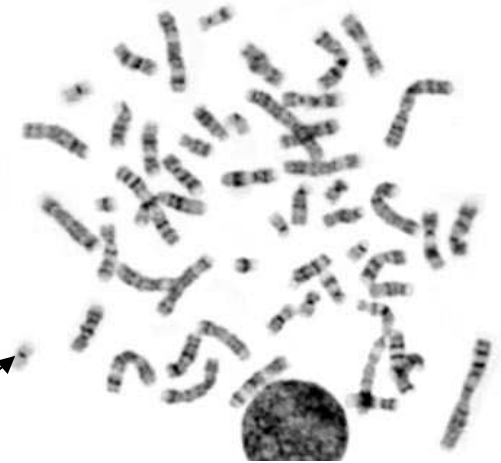
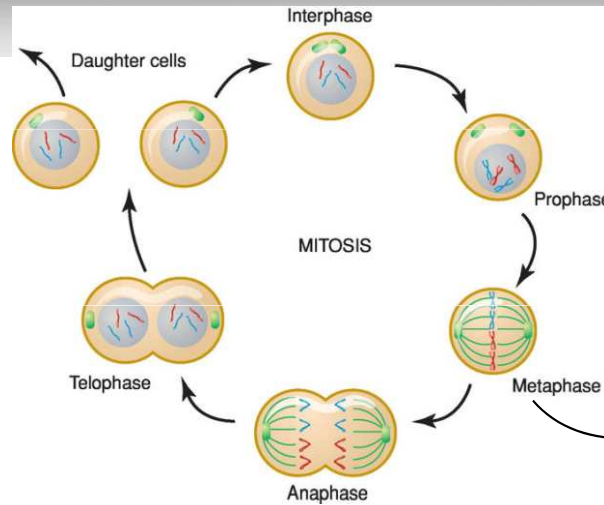
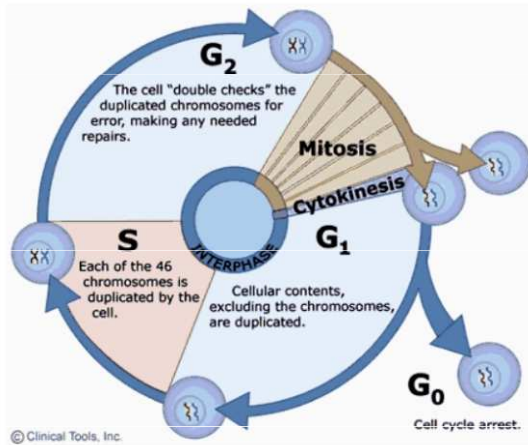
Konvenční
cytogenetická
analýza

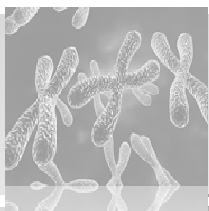


arrayCGH/SNP array

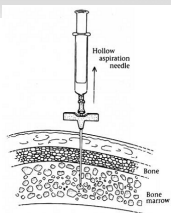


Konvenční cytogenetika

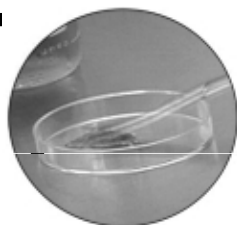
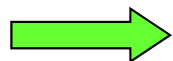




Postup kultivace buněk nádorů



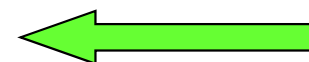
1-2ml



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

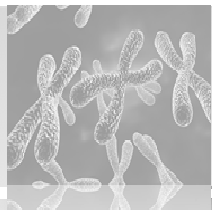


37°C/ 5%CO₂



Kultivace
2/24/72hod/týdny

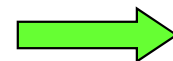




ZPRACOVÁNÍ BUNĚČNÉ KULTURY



www.shutterstock.com · 58307962

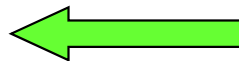


HYPOTONIZACE
0,075M KCl

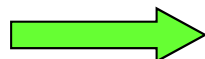
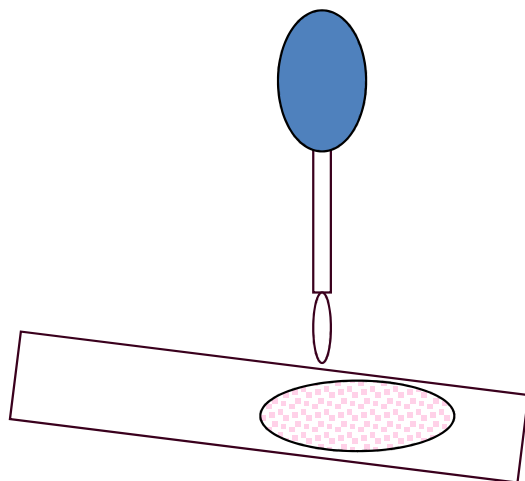


COLCEMIDE BLOKUJE MITÓZY V METAFÁZI

PŘÍPRAVA PREPARÁTŮ
KAPÁNÍM BB SUSPENZE
NA SKLO

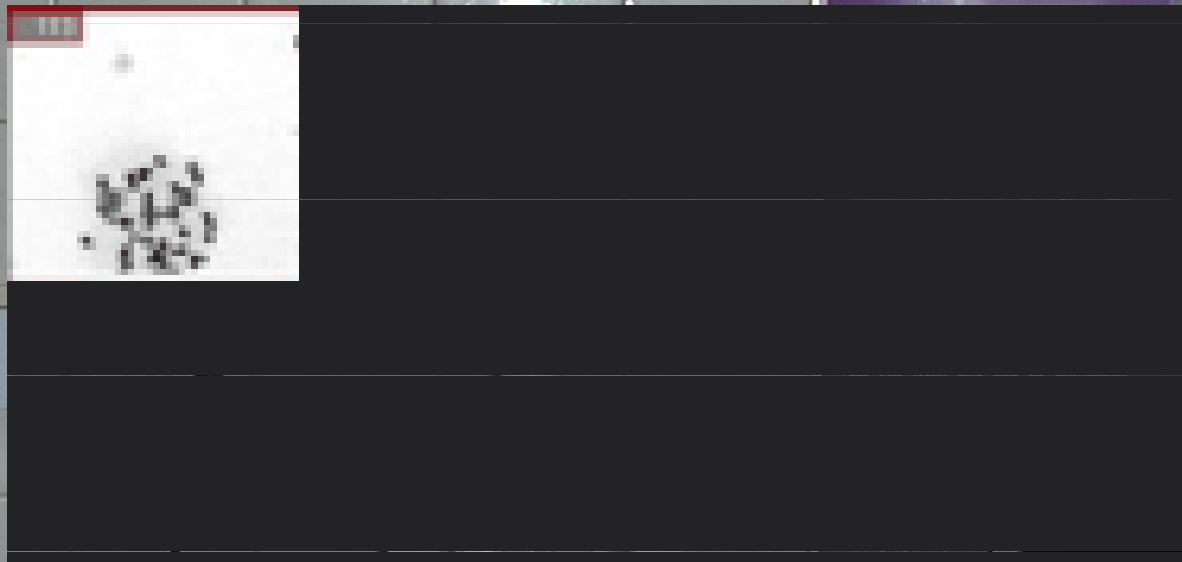
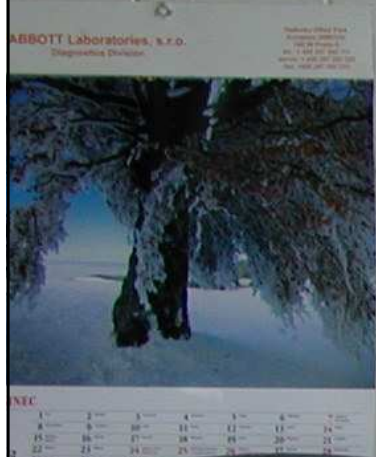


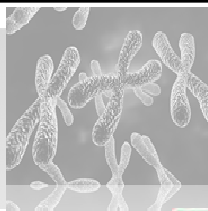
FIXACE ROZTOKEM
KYS.OCTOVÉ A METANOLU v poměru
1:3



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

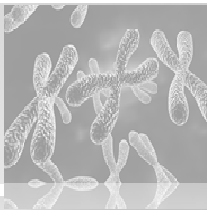
Magnify

Staining

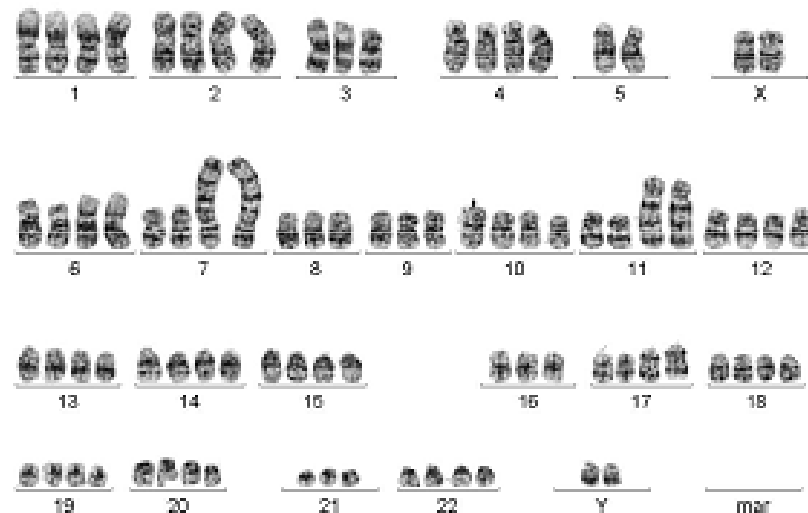
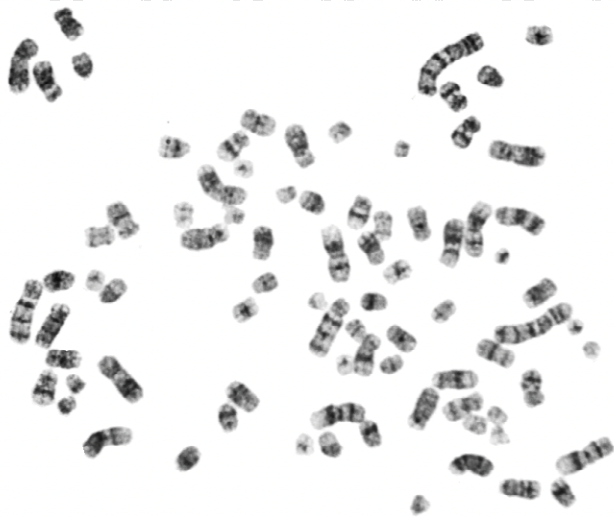
Annotate

DemolKS DIR-G
adm GBAND

CASE101 5 46,XX



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:

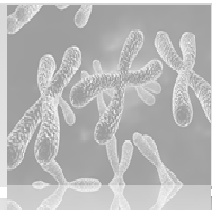
Ros Hastings, Rod Howell, David Betts, Sarah Porter, Claudia Haeflrich,
Nicole Dastugue, Isabelle Radford-Weiss, H. Berna Beverloo, Annet Simons,
Clemens Mellink, Simone Snijder, Eva van den Berg-de Ruyter, Jacqueline Schoumans,
Blanca Espinet, Reiner Siebert, Jerome Couturier, Alain Bernheim, Francesc 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 an abnormal 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



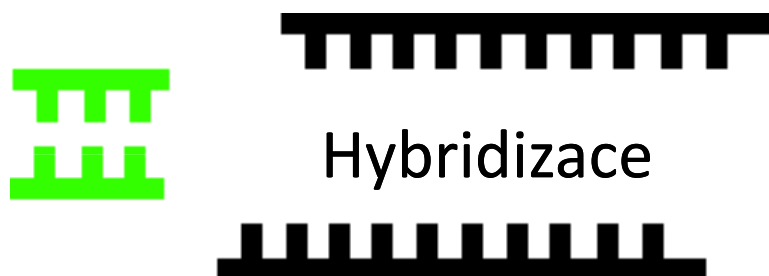


Molekulární cytogenetika

Denaturace



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

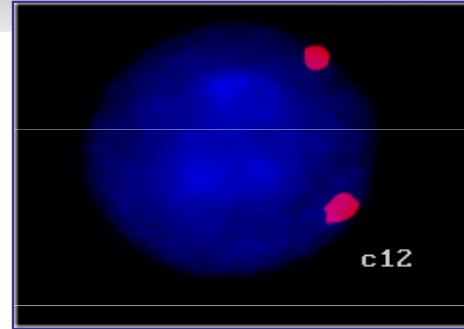
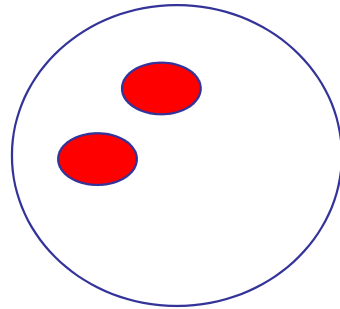
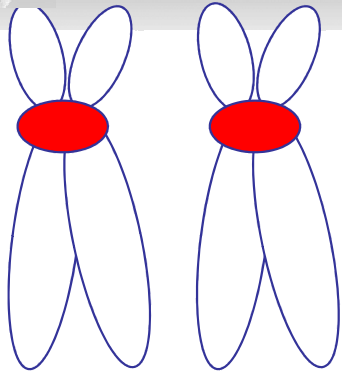
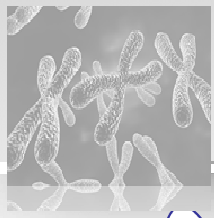


Hybridizace

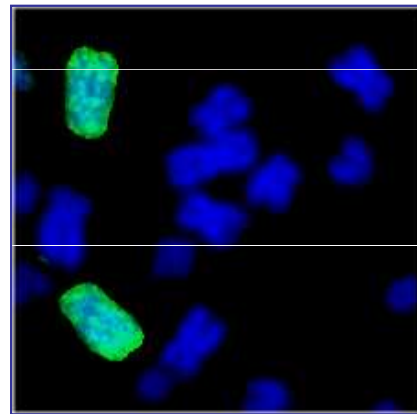
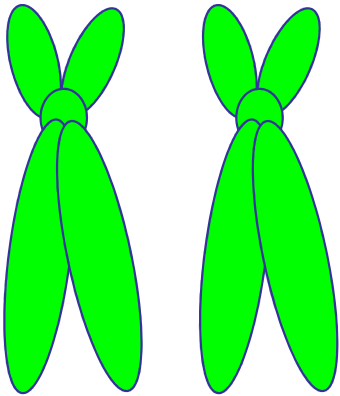
- Metody využívající základní vlastnosti jednořetězcové DNA vzájemně se vázat na základě komplementarity bazí



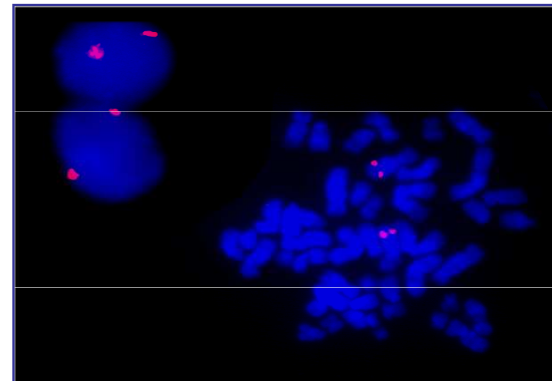
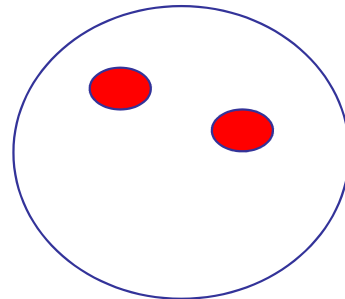
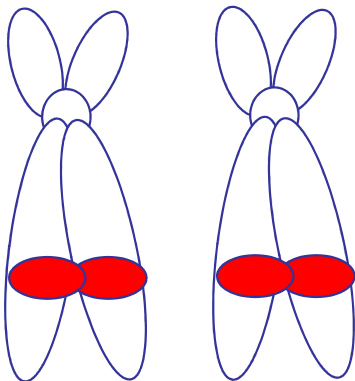
Typy sond



centromerické

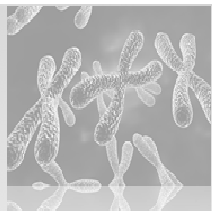


celochromosomové



genové



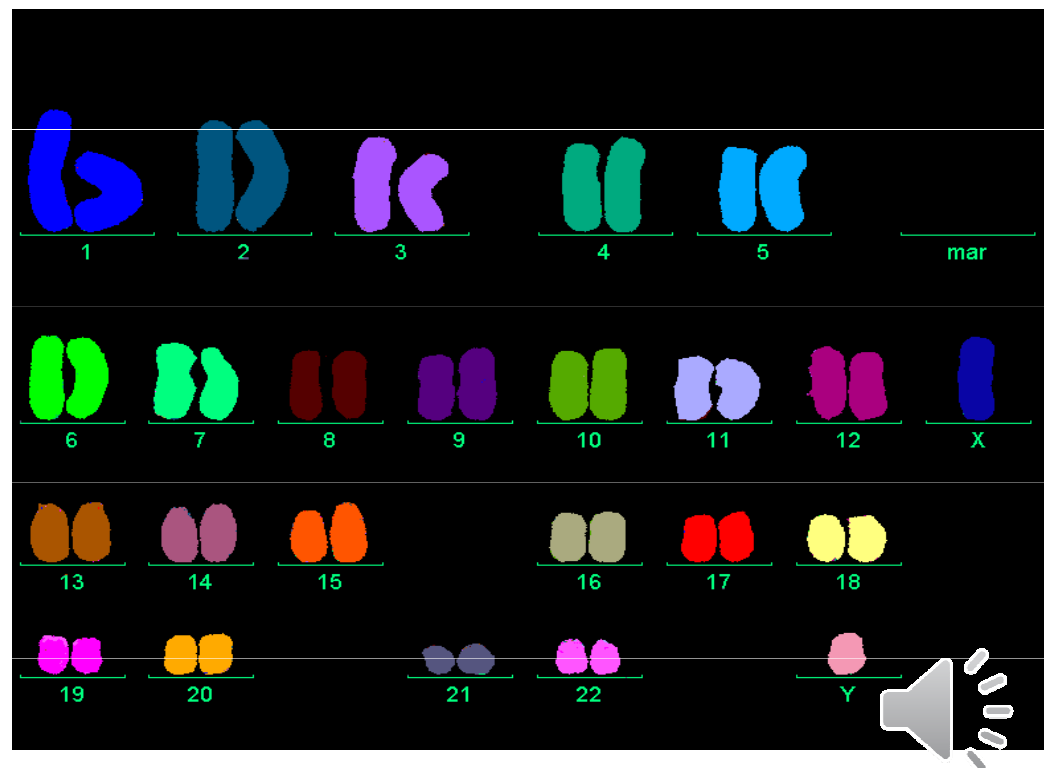


Mnohobarevná fluorescenční in situ hybridizace (mFISH)

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

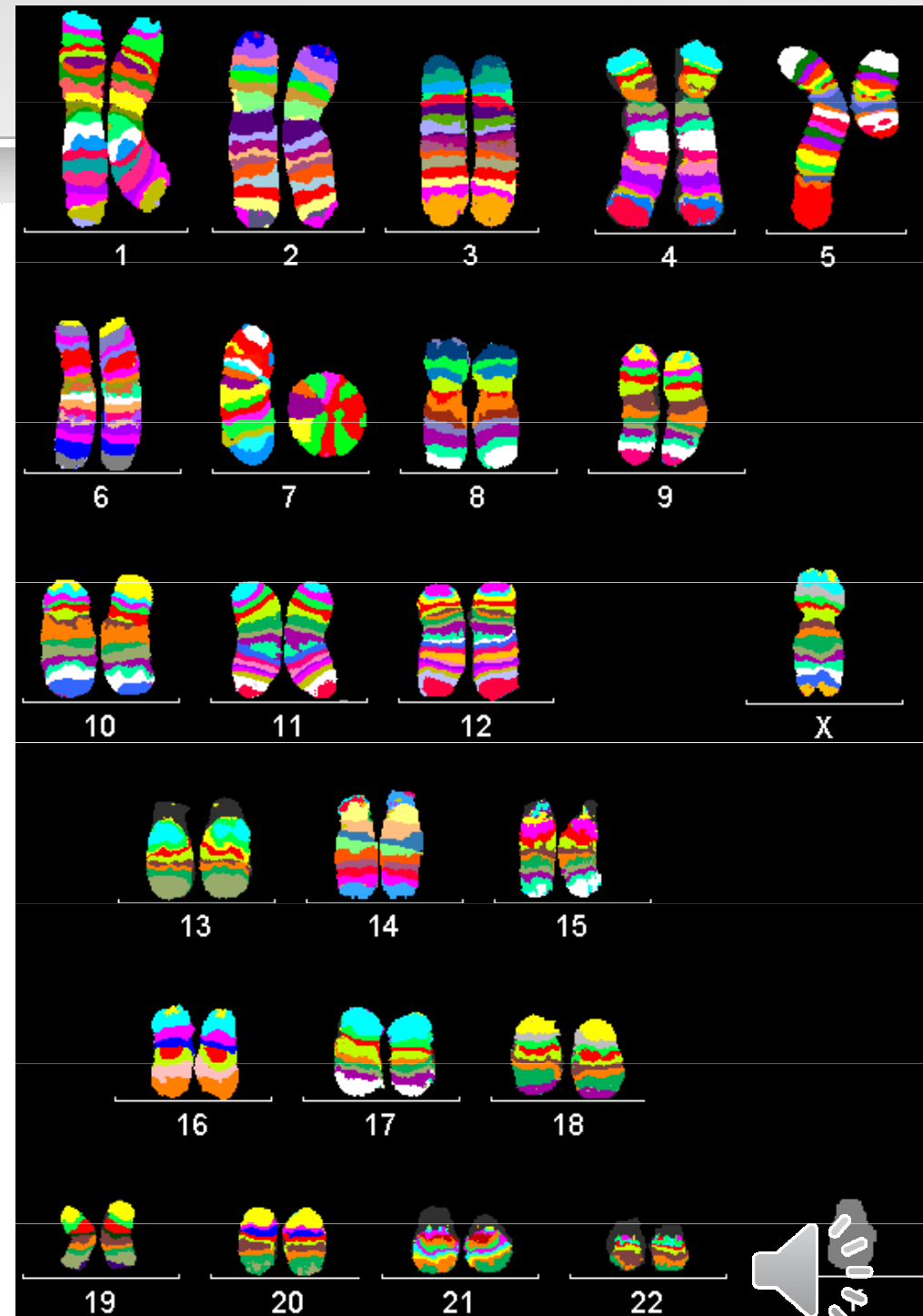
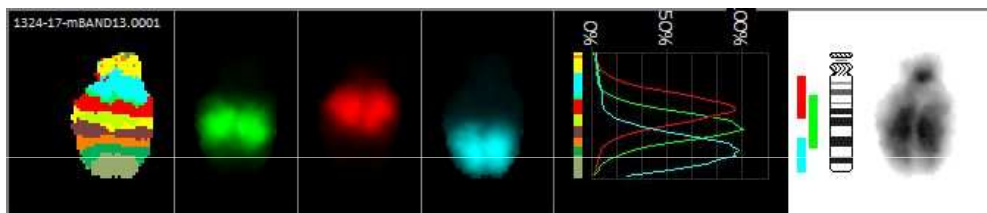
Chr.	FITC	Spectrum Orange	Texas Red	Cy5	DEAC
1					
2					
3					
4					
5					
6					
7					
8					
9					
10					
11					
12					
13					
14					
15					
16					
17					
18					
19					
20					
21					
22					
X					
Y					

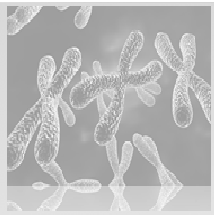




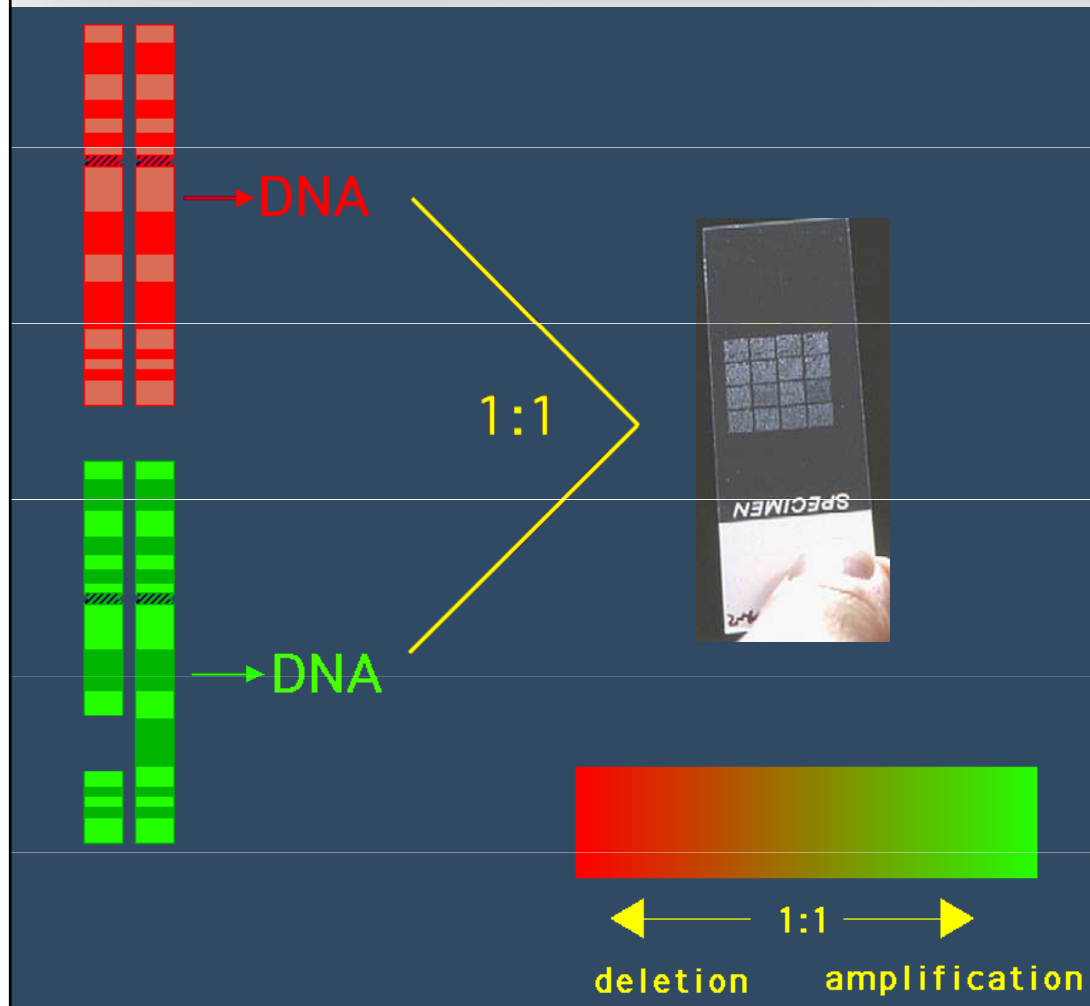
Mband FISH

- Kombinuje paintingové proby specifické pro danou oblast chromosomu
- Sondy připravené mikrodisekcí chromosomových oblastí
- 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

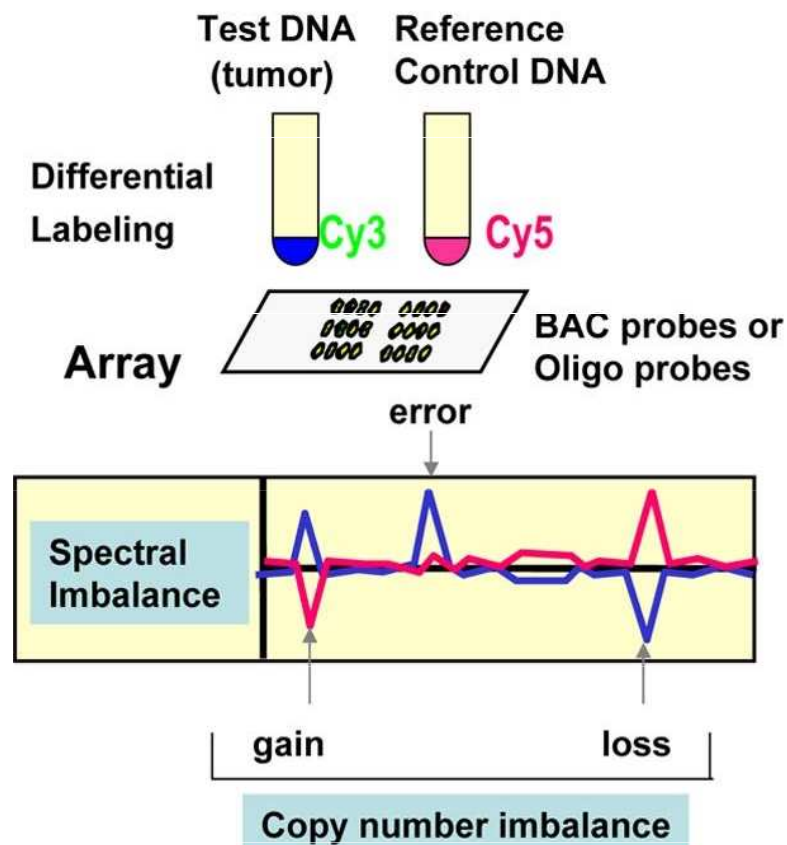


arrayCGH/SNPs array

A

CGH-A

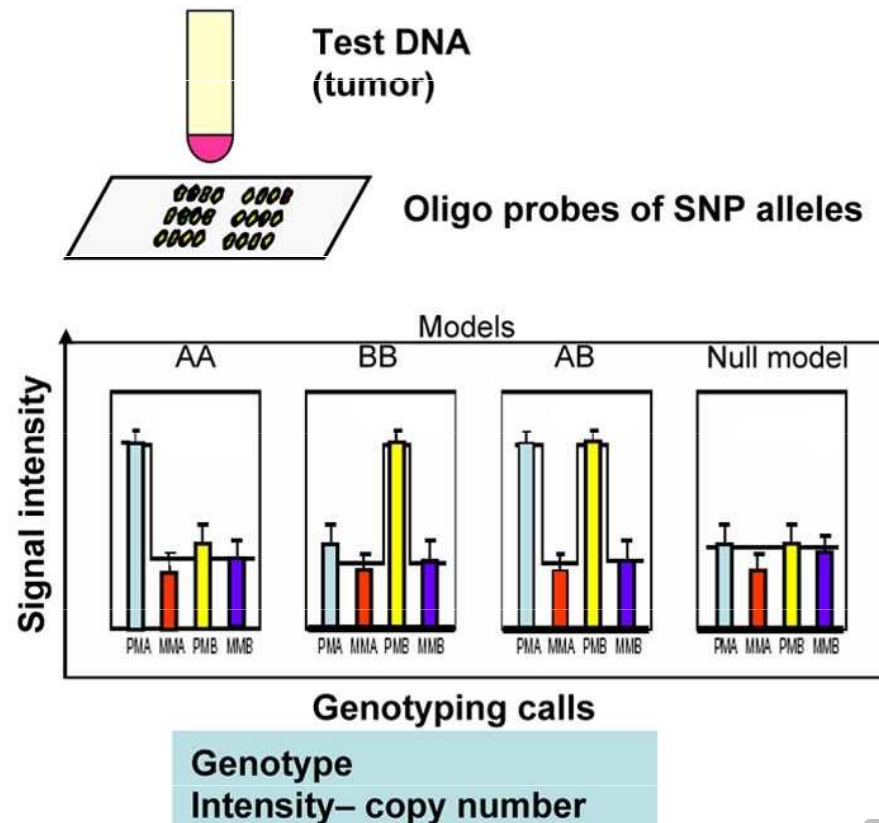
- BAC CGH-A
- Oligo CGH-A



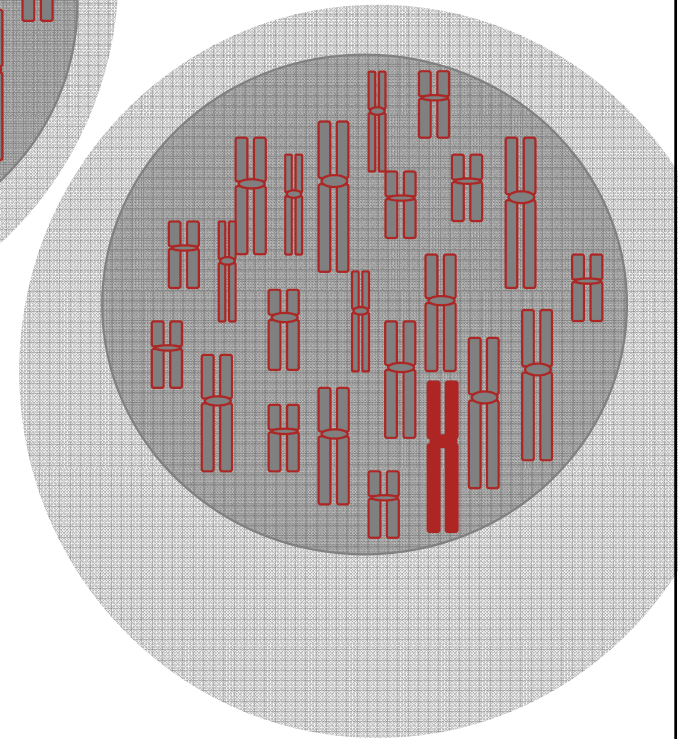
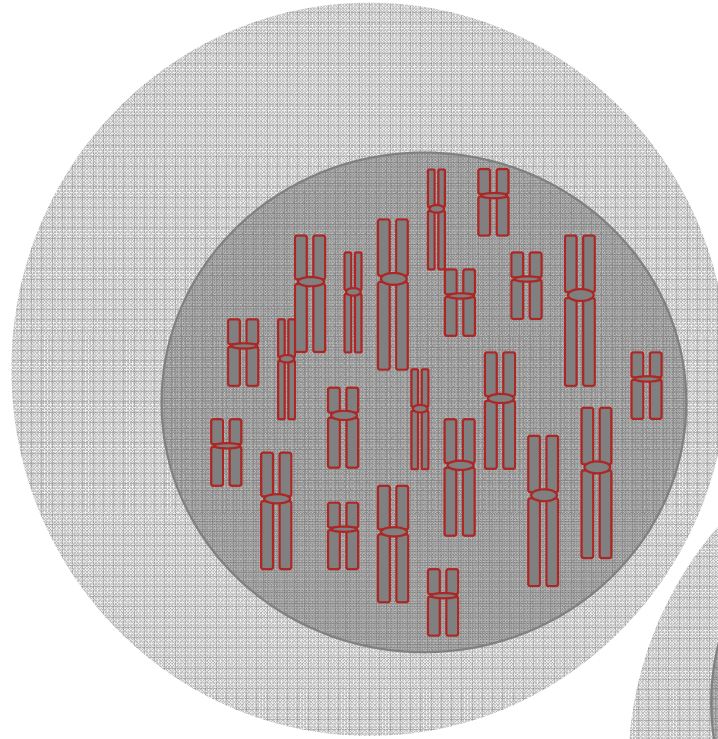
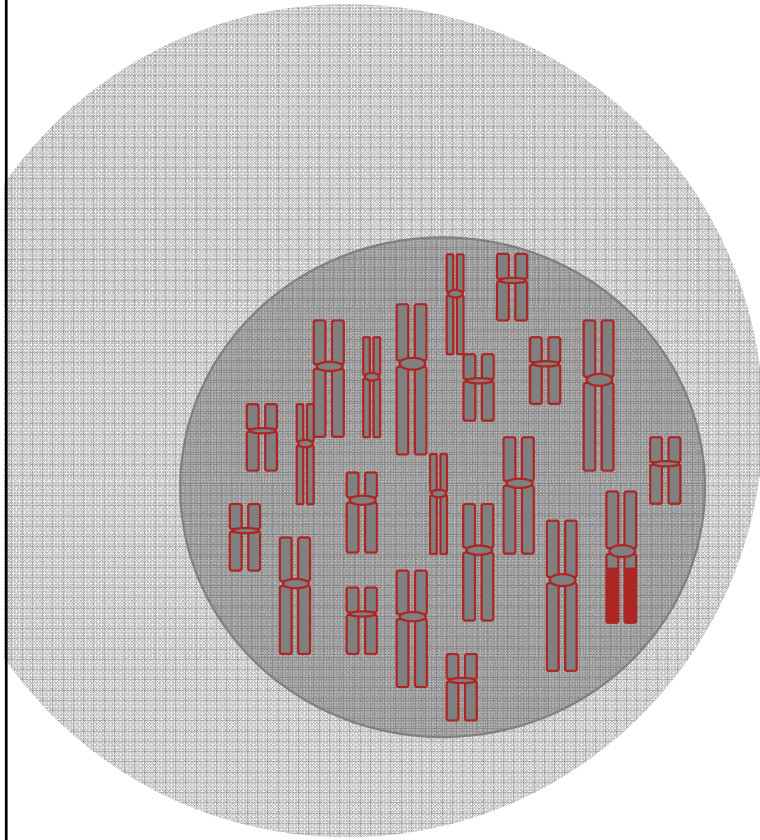
B

SNP-A

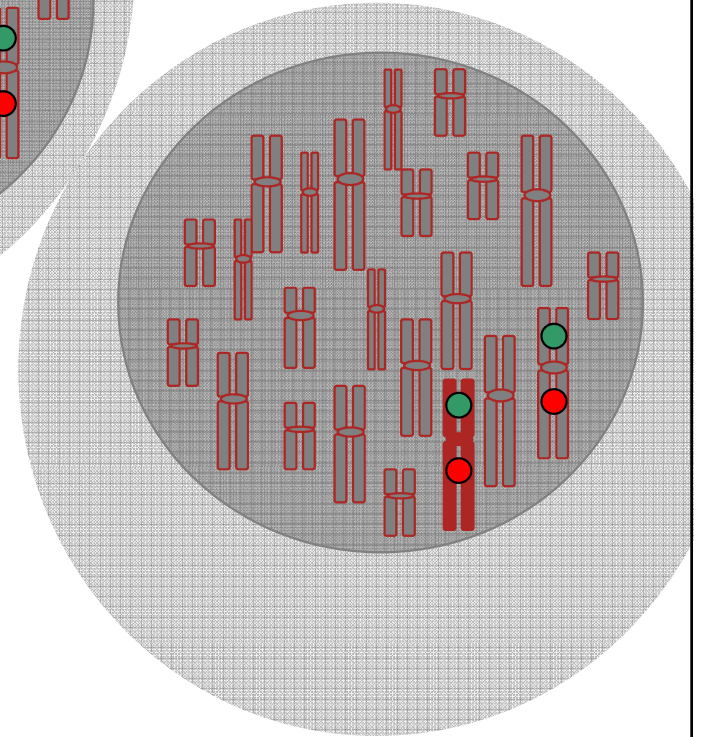
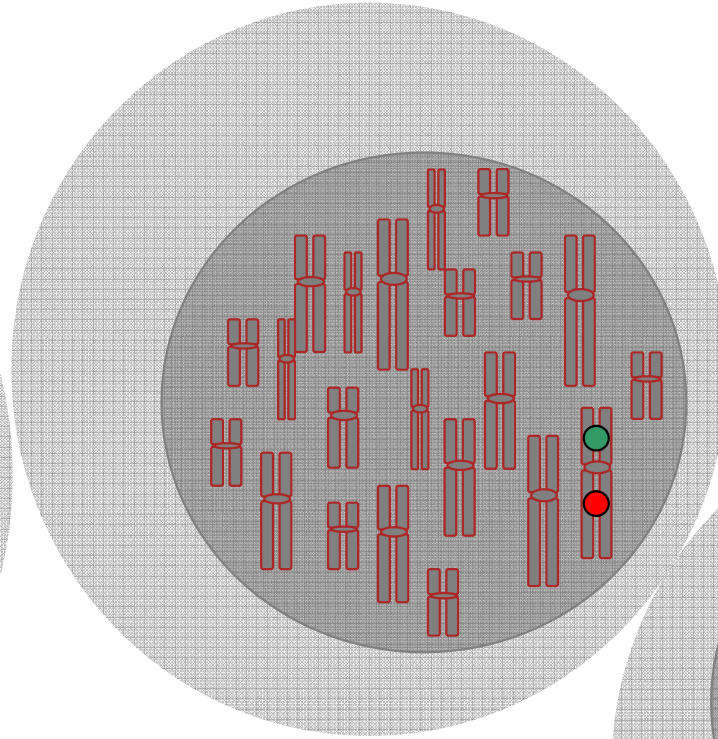
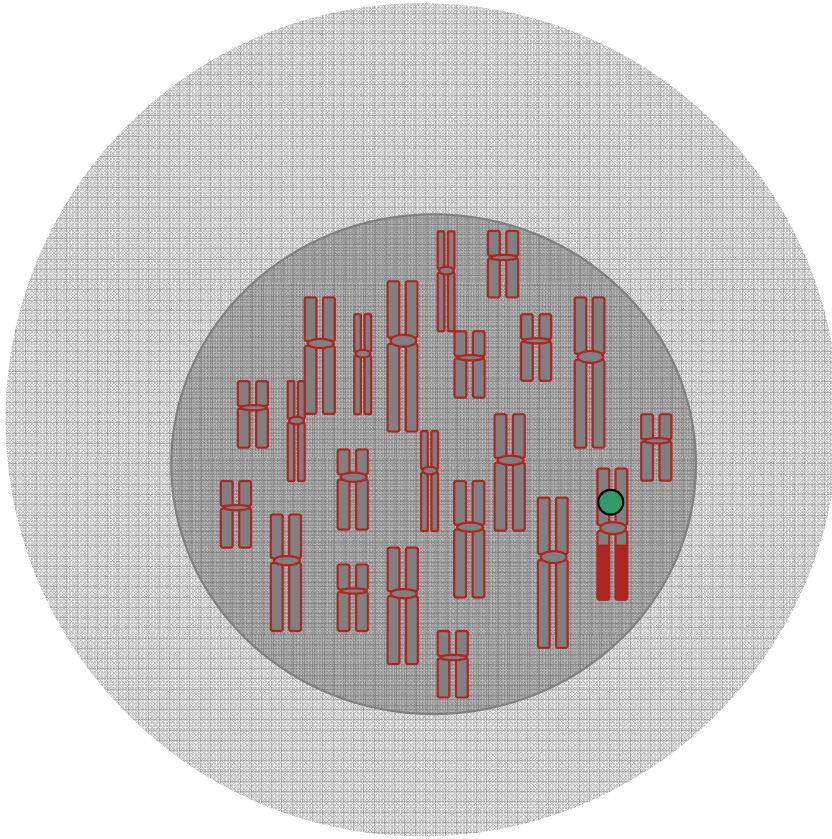
- Combined CN/SNP-A



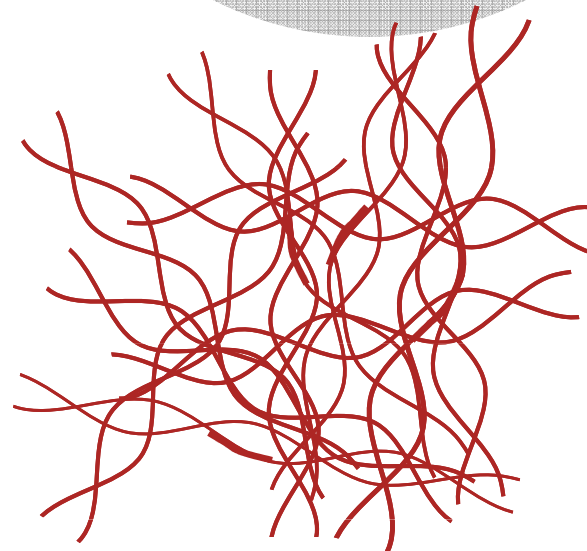
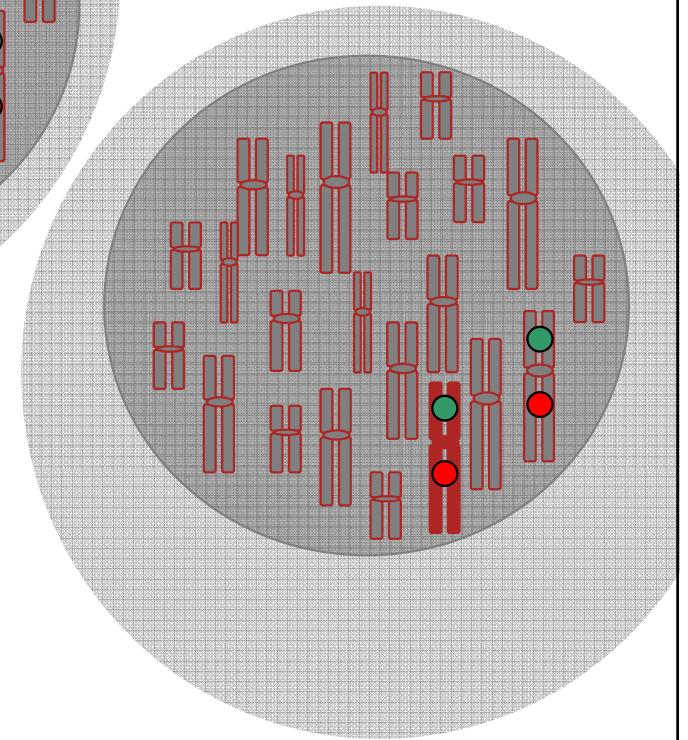
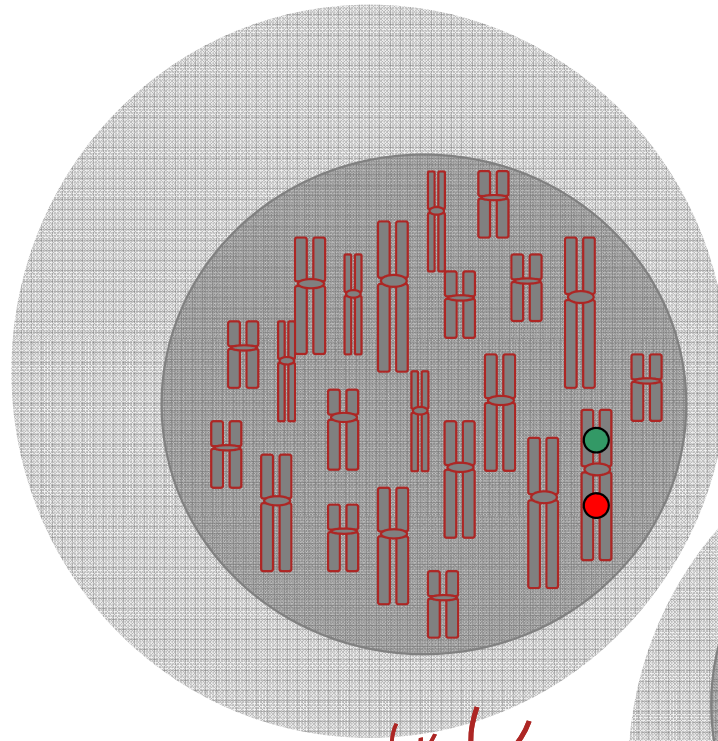
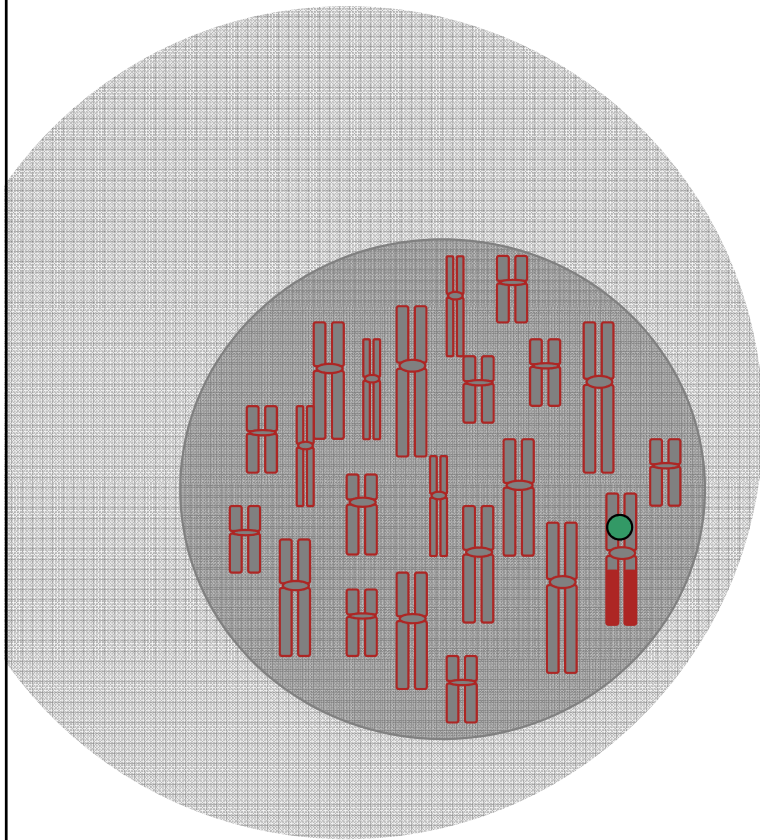
Cytogenetics

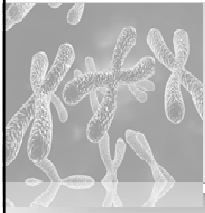


FISH



Molecular genetics

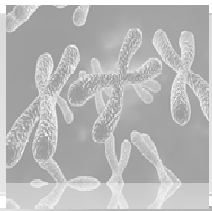




Genetické změny u hematologických malignit

- 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)





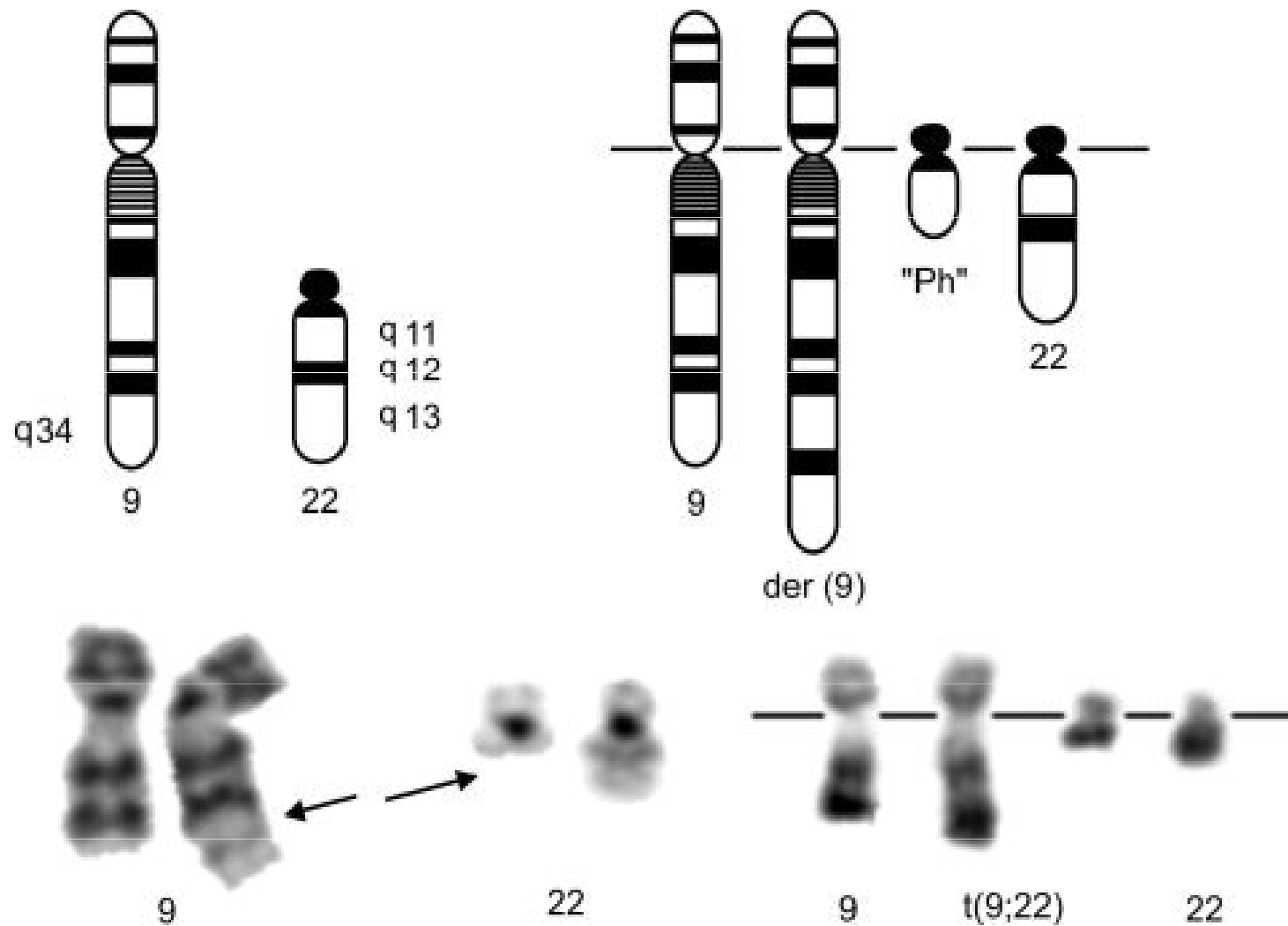
Cytogenetika v hematologii

- 1.Diagnosa
- 2.Prognosa
- 3.Léčebné rozhodování



Filadelfský chromosom (Ph)

První specifická chromosomová změna u nádoru člověka



Diagnóza

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

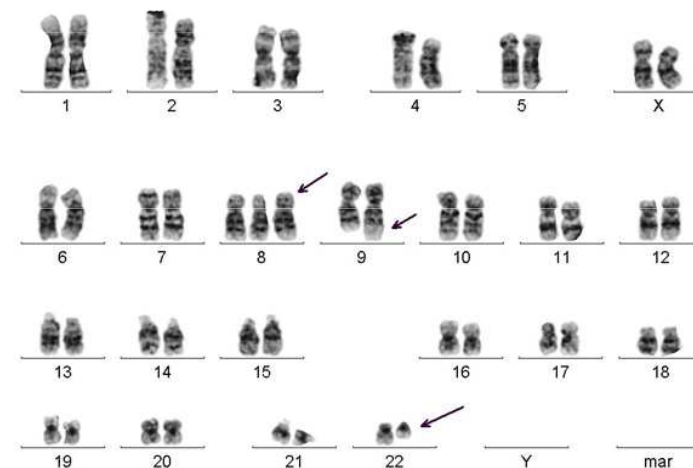
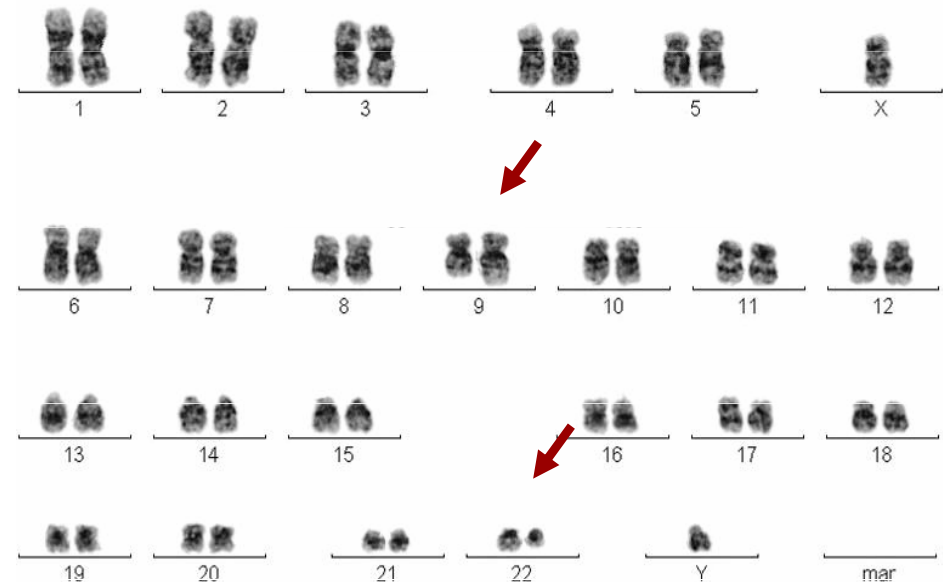
Prognóza

- Přídavné chromosomové změny

Diagnosa CHF: ~12%

Akcelerovaná fáze: ~30%

Blastická zvrát : ~70%





Přidatné 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
Komple xní 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)

Prognosa

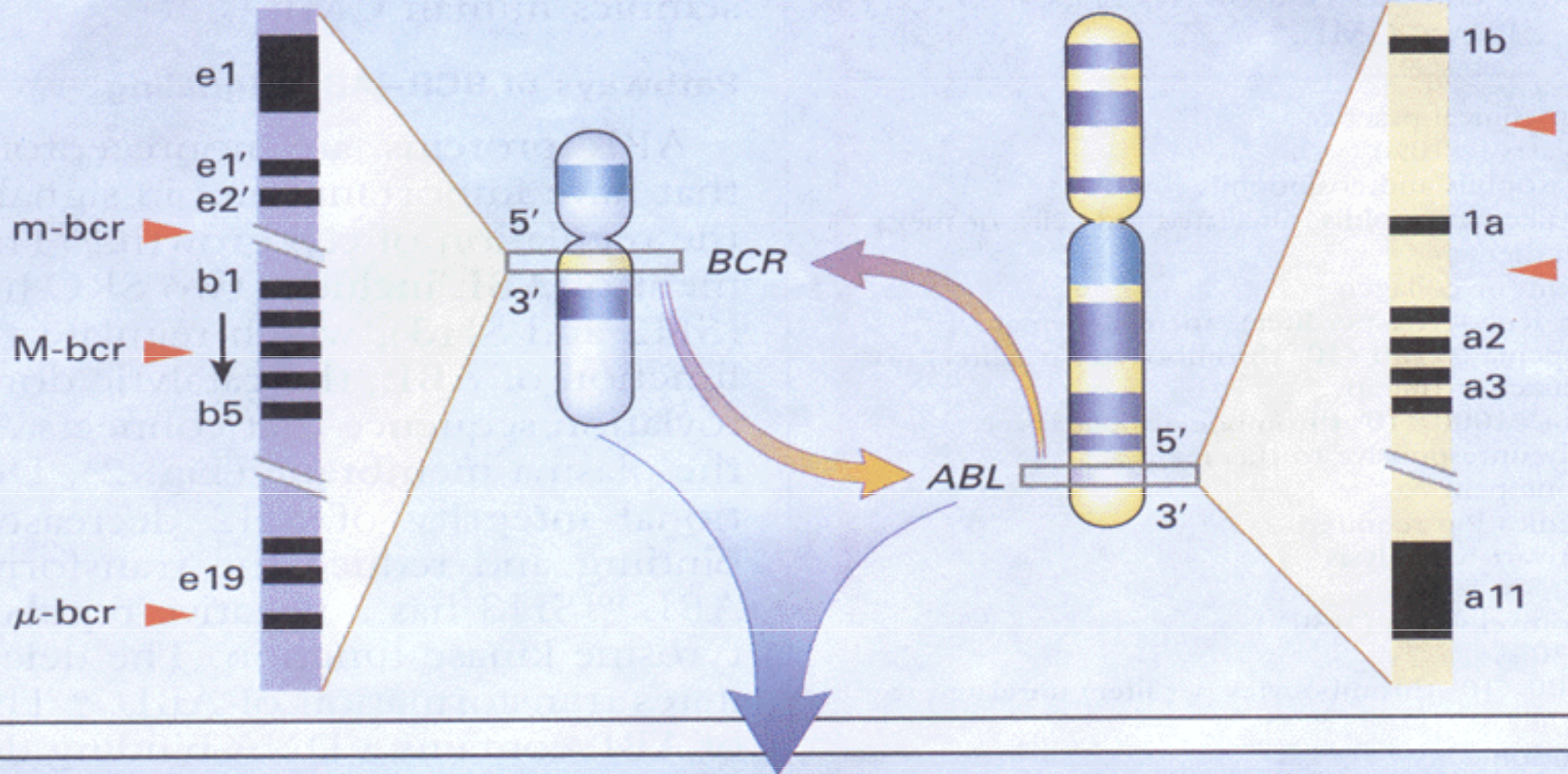
Relativně dobrá:
+8,+Ph,-Y

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



Chromosome 22

Chromosome 9



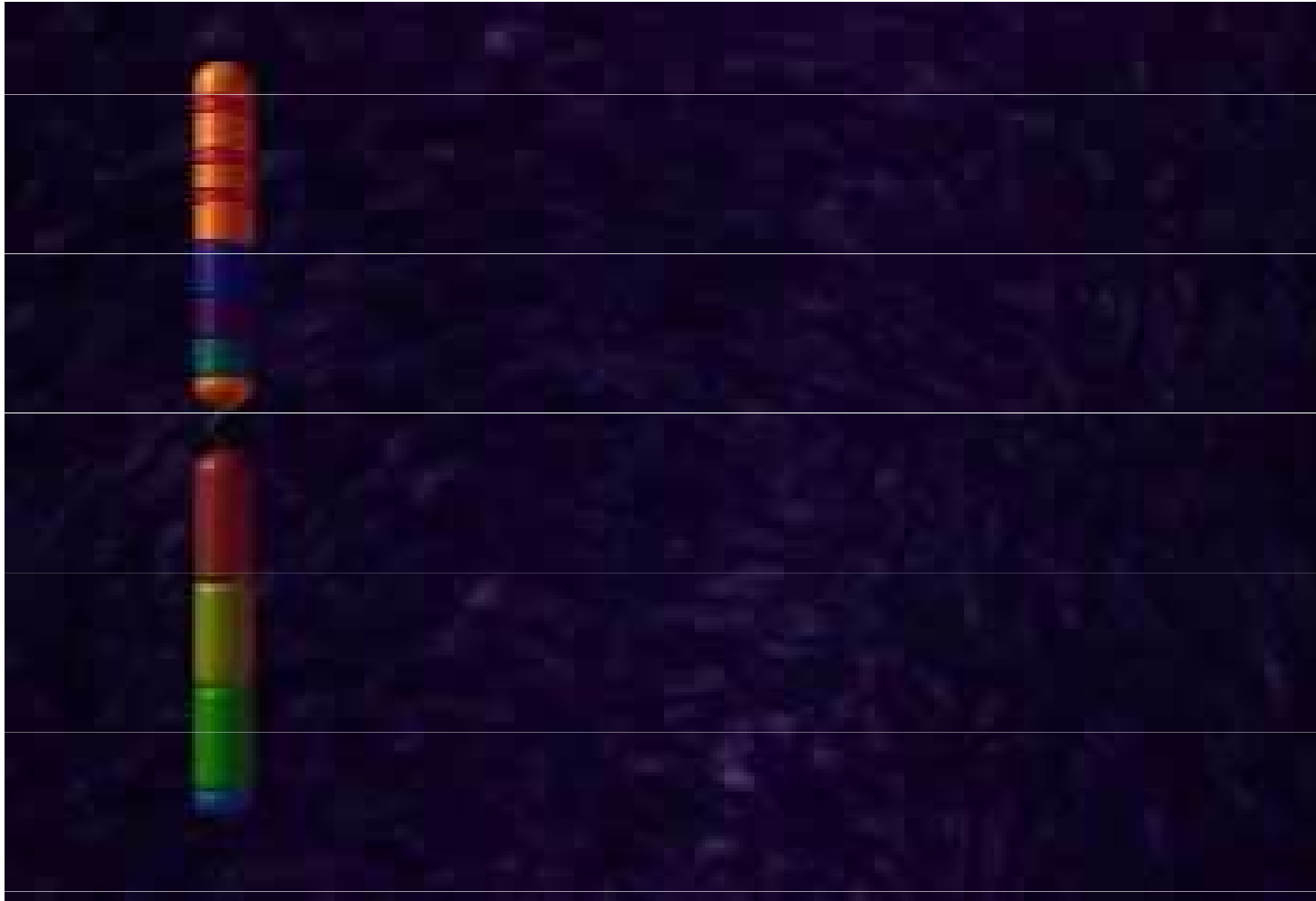
\Rightarrow e1a2 \Rightarrow p190^{BCR-ABL}

\Rightarrow b2a2 \Rightarrow p210^{BCR-ABL}

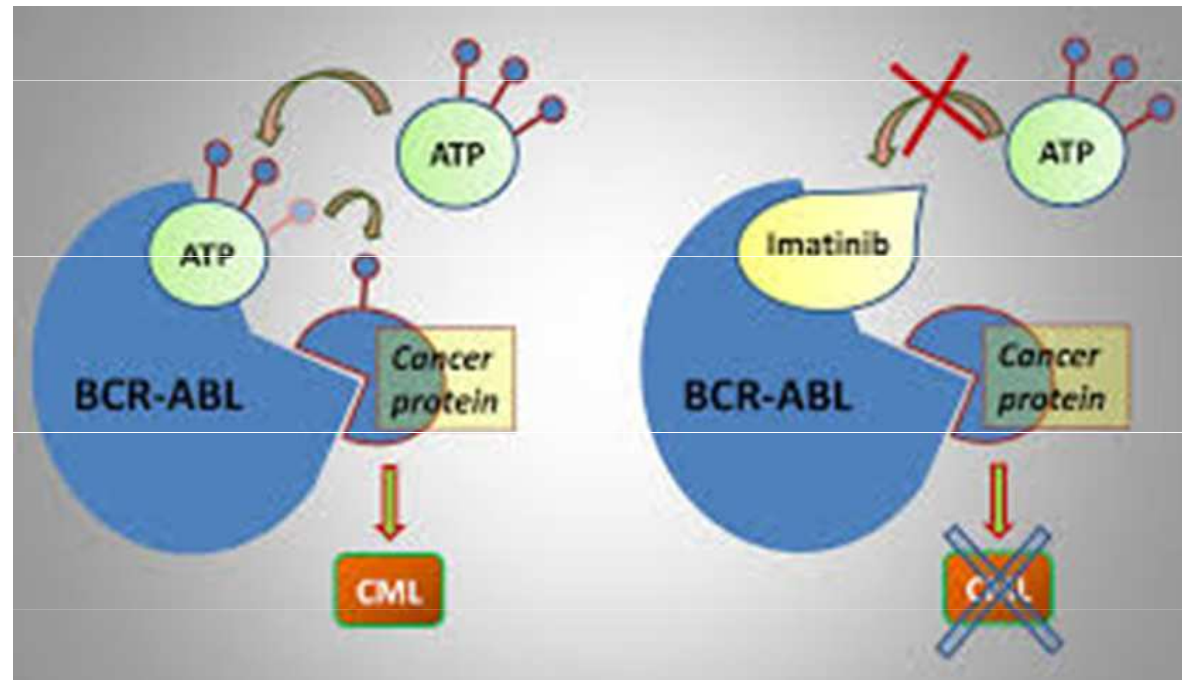
\Rightarrow b3a2 \Rightarrow p210^{BCR-ABL}

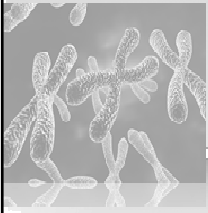
\Rightarrow e19a2 \Rightarrow p230^{BCR-ABL}

Glivec (Imatinib)



Glivec (Imatinib)

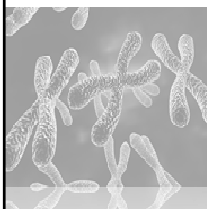




ELN sledování MRN CML cytogenetika

	Type of Response	Definition
CHR	Complete Hematologic Response	Normal differential, WBC & platelets \leq 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 \leq 0.1% (International Scale)
MR ^{4.0}		BCR-ABL/ABL \leq 0.001% (IS) “4-log reduction”
MR ^{4.5}		BCR-ABL/ABL \leq 0.003% (IS) “4.5-log reduction”
CMR	Complete Molecular Response	Undetectable BCR-ABL (test of sensitivity \geq 4.5 logs)





CML – v době léčby inhibitory

Generation	TKI	Approbation		
		1 st line	2 nd line	3 rd line
1 st	Imatinib	2003	2001	
2 nd	Nilotinib	2011	2008	
	Dasatinib	2011	2007	
3 rd	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.

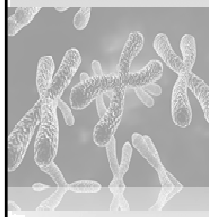




WHO Classification

- 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

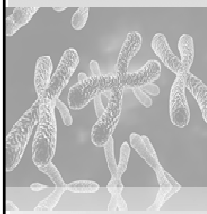
Provisional entity: B-lymphoblastic leukemia/lymphoma with iAMP21

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.

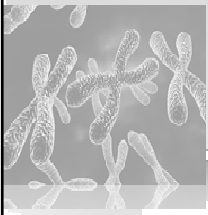
^e Three or more unrelated chromosome abnormalities in the absence of one of the World Health Organization-designated recurring translocations or inversions, i.e., t(8;21), inv(16) or t(16;16), t(9;11), t(v;11)(v;q23.3), t(6;9), inv(3) or t(3;3); AML with *BCR-ABL1*.

^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).¹¹⁶

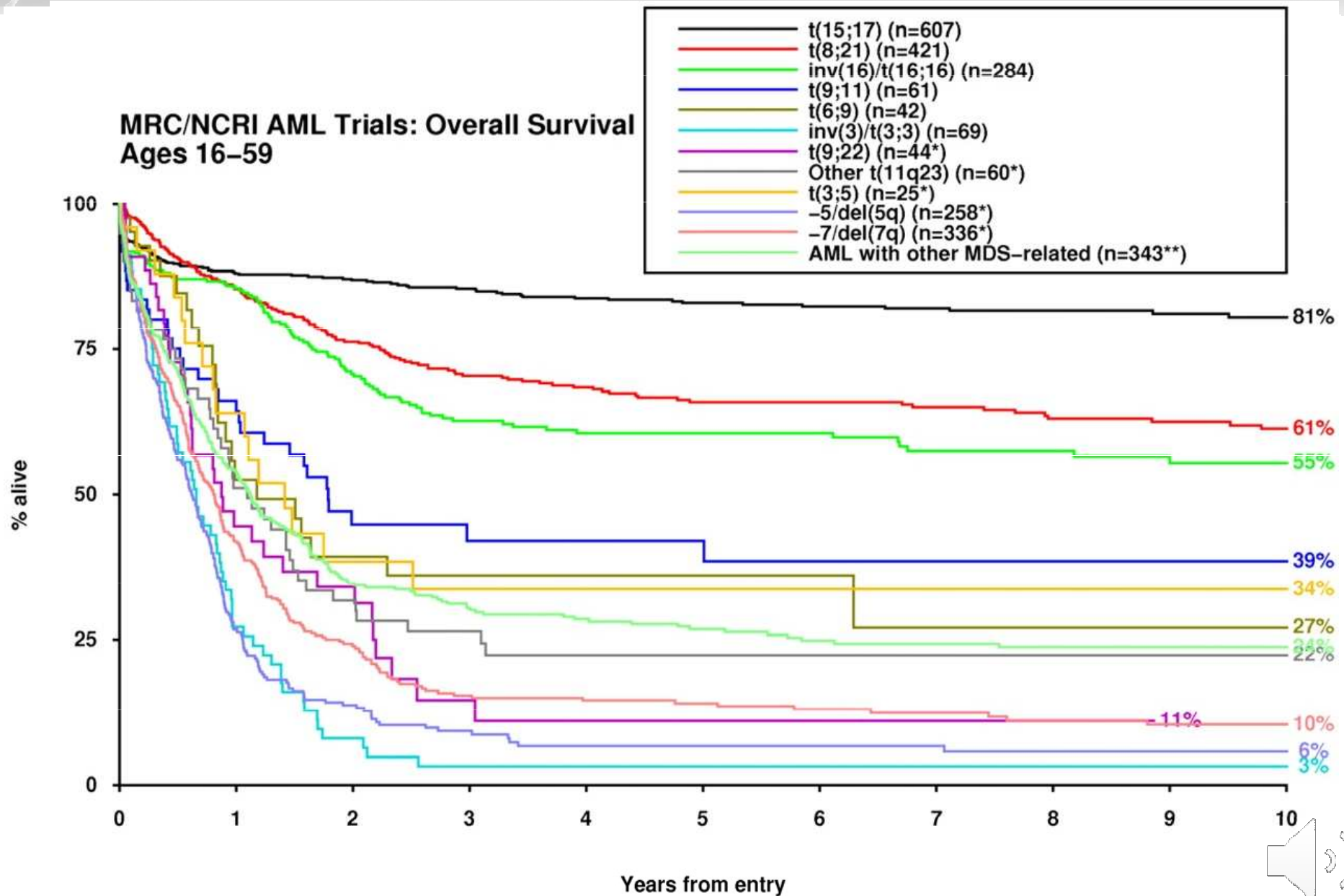
^g These markers should not be used as an adverse prognostic marker if they co-occur with favorable-risk AML subtypes.

^h *TP53* mutations are significantly associated with AML with complex and monosomal karyotype.^{37,66-69}

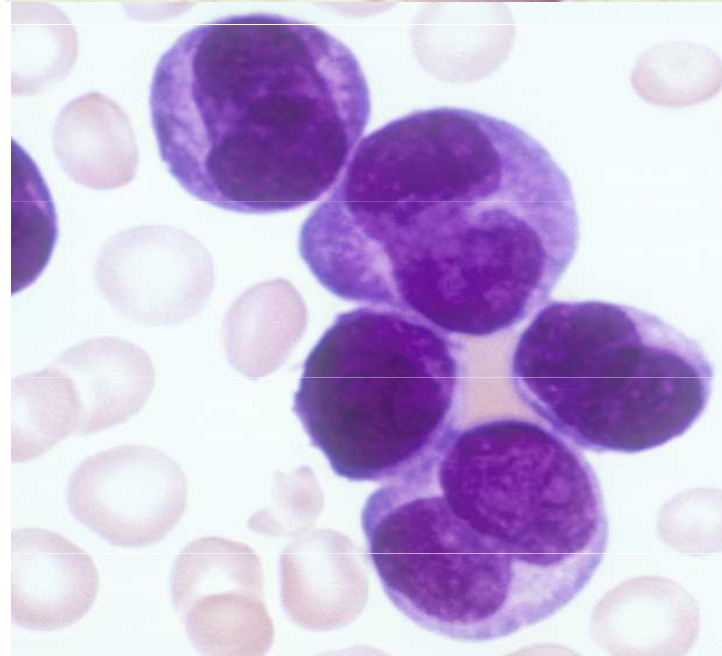
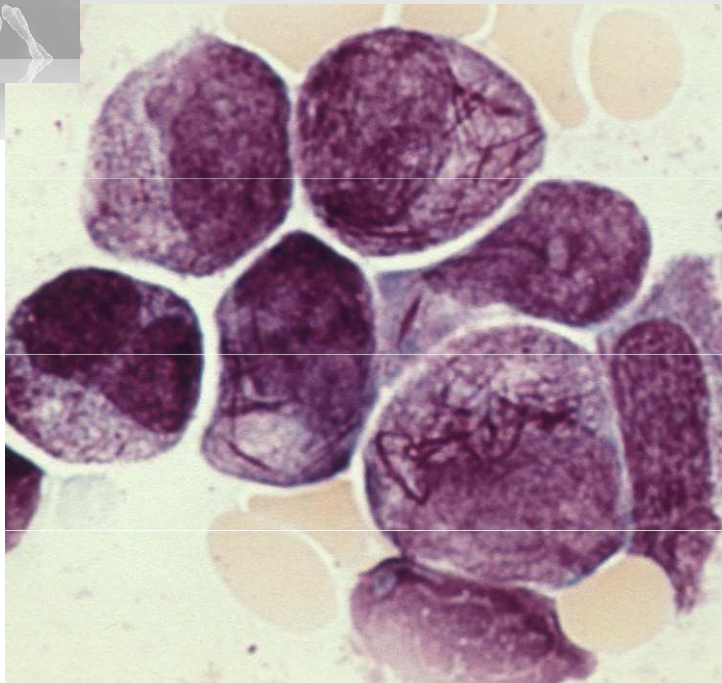
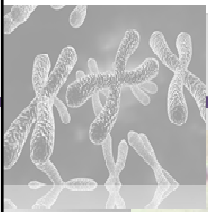




Stratifikace podle cytogenetických nálezů



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



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

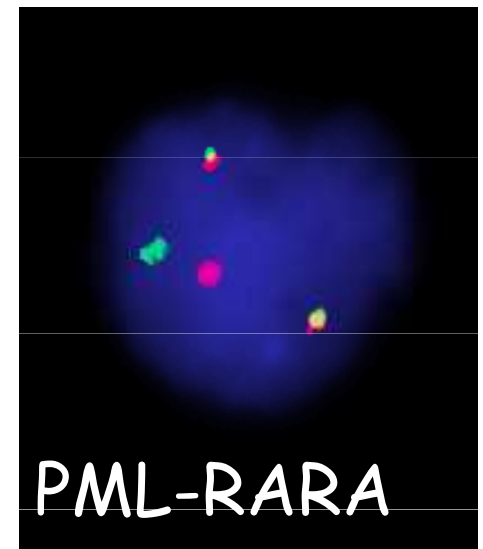
SIR,—We have described a similar chromosomal abnormality in two patients with acute promyelocytic leukaemia

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

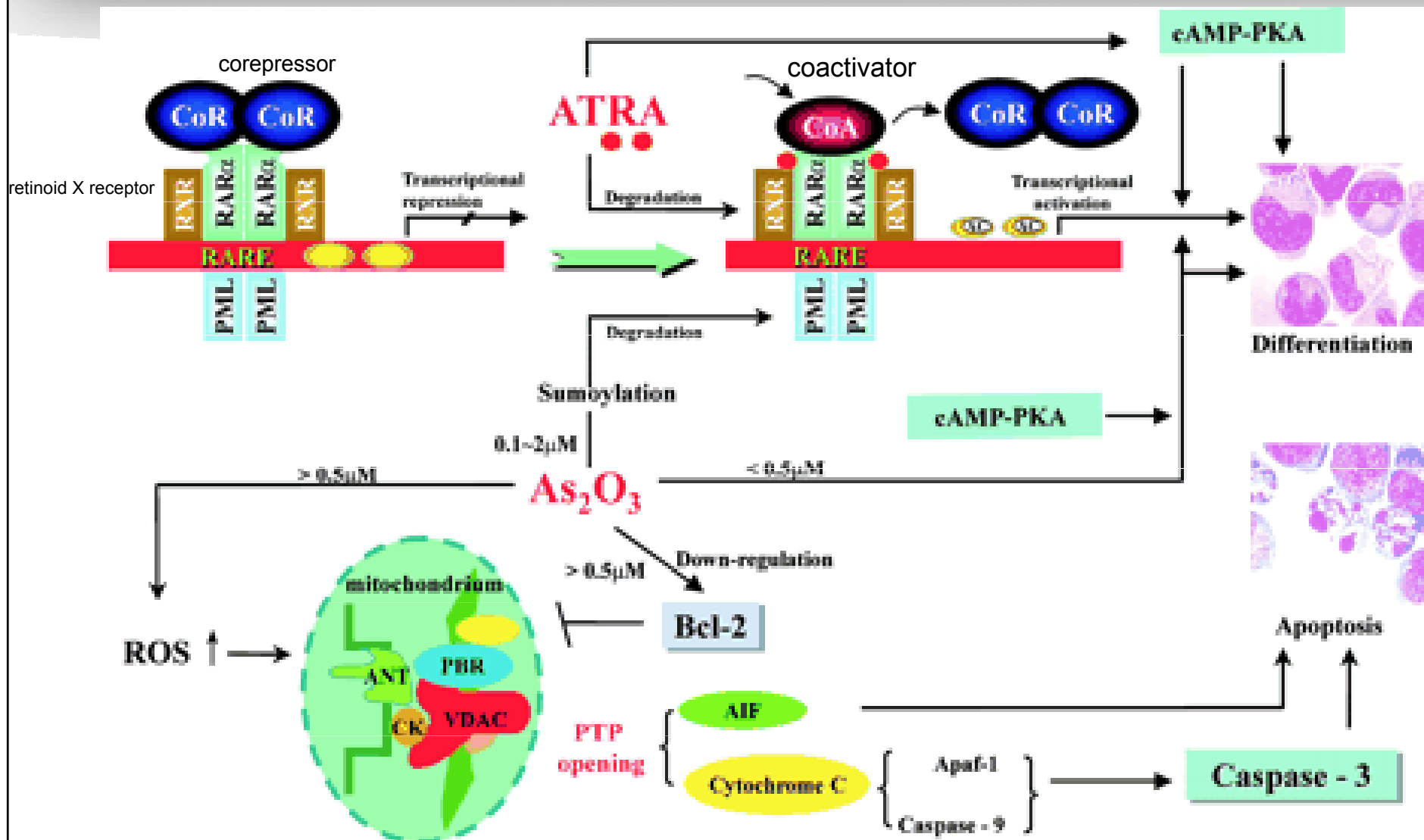


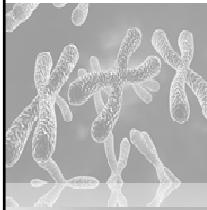
$t(15;17)(q22;q12)$



PML-RARA

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.

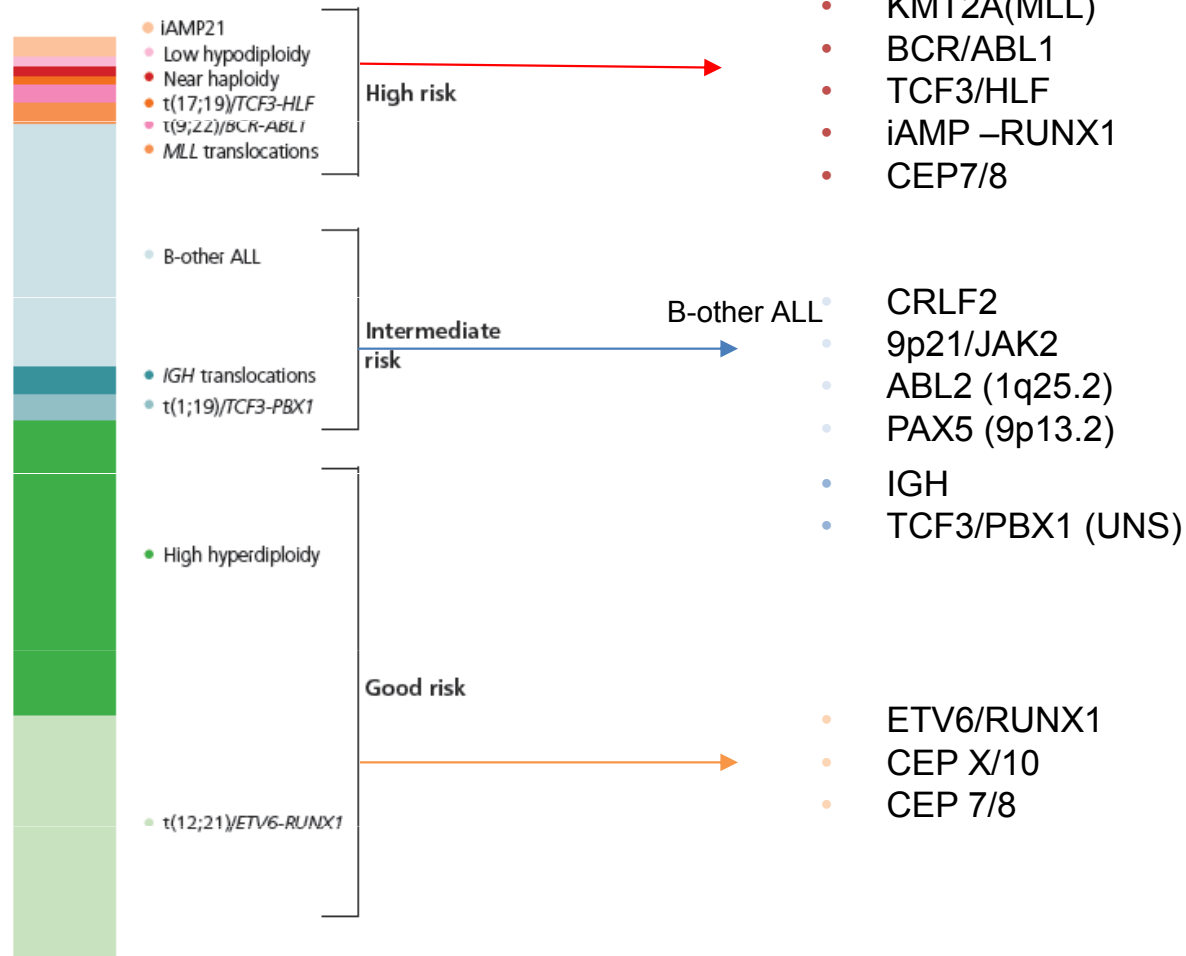


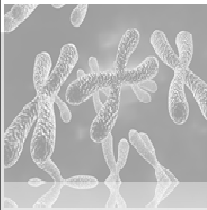
Doporučení pro vyšetřování dětských BCP ALL

Cytogenetika

FISH

CHILDREN & ADOLESCENTS





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)

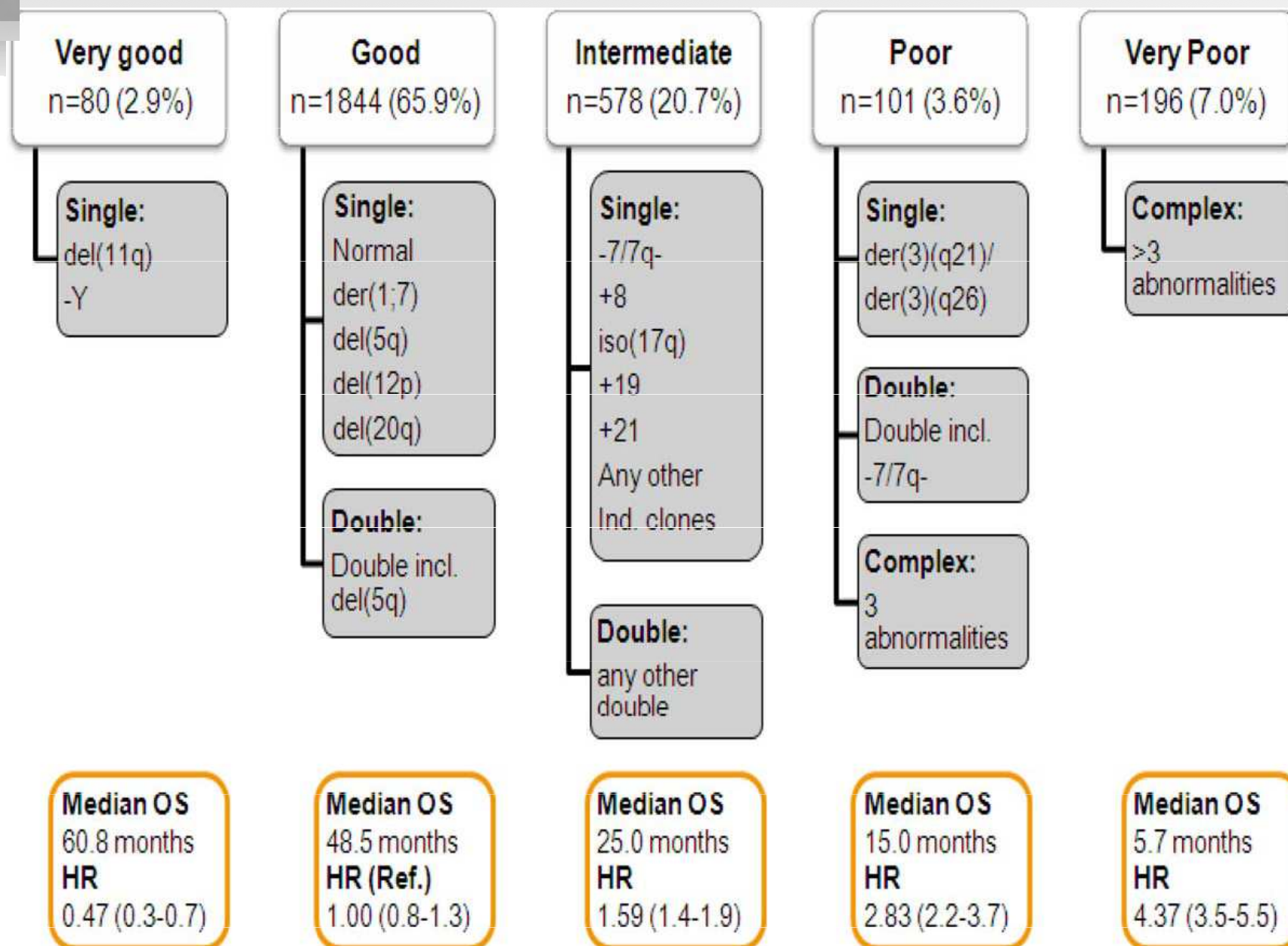
Klinická heterogenita MDS je odrazem heterogenity získaných somatických genetických změn

Chromosomové změny u MDS

- de novo MDS 40-60%
- t-MDS nebo sekundární MDS 90%

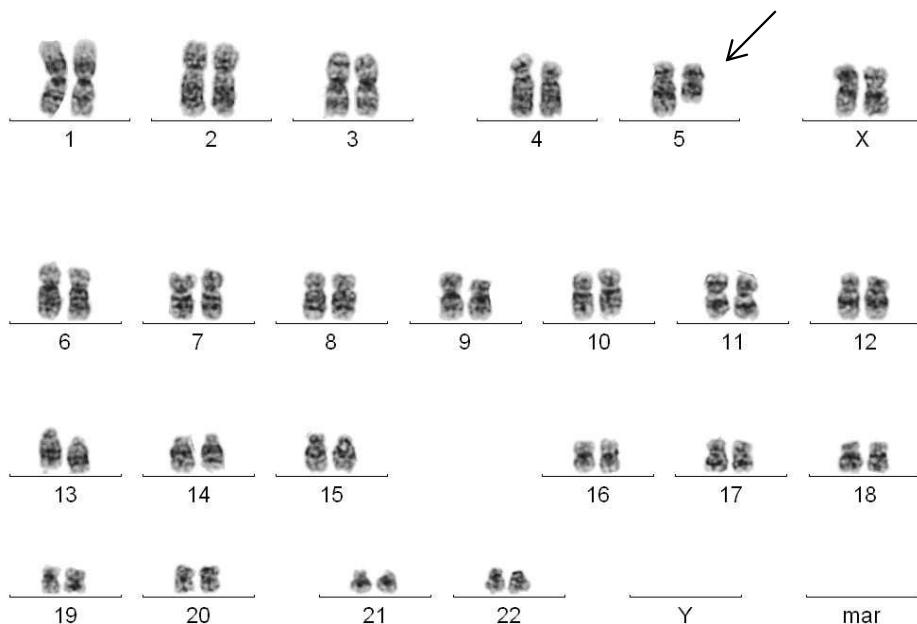


Prognostická stratifikace MDS



5q- SYNDROM

46,XX,del(5)(q31)



- 10 % nemocných
- dobrá prognóza
(5-16 % progrese do AML)
- intersticiální delece, 5q31,
5q32-5q33
- Cílená léčba: lenalidomid
azacytidin

Lenalidomid - imunimodulační
látka

Azacytidin (vidaza)- hypometylace
DNA

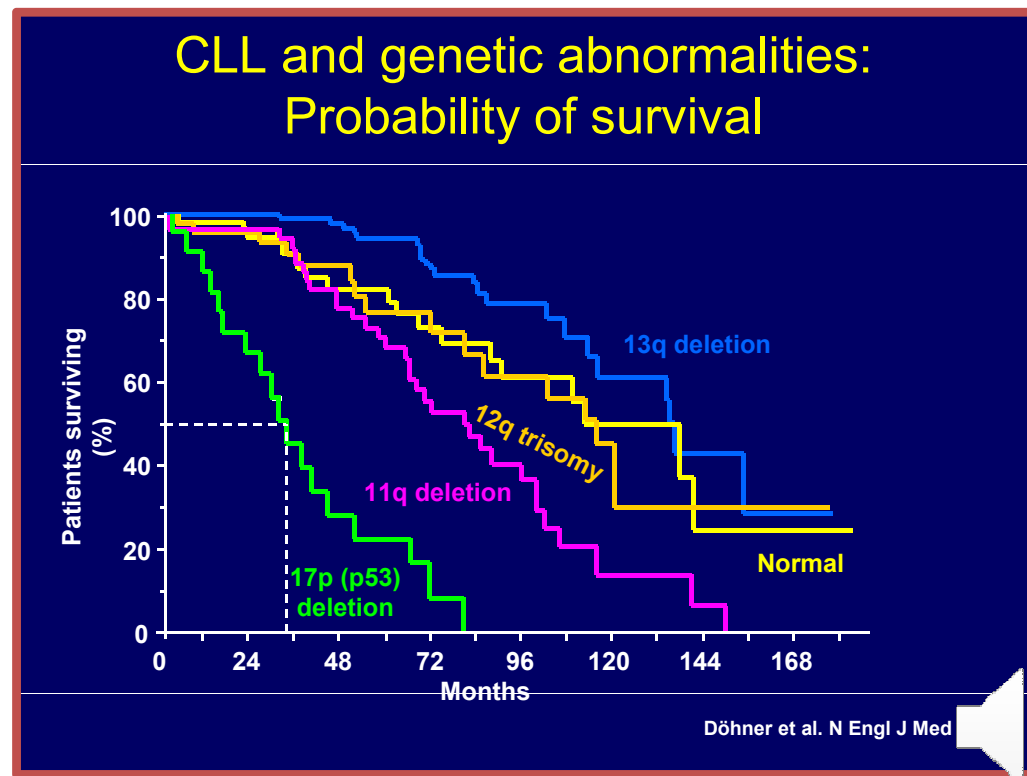


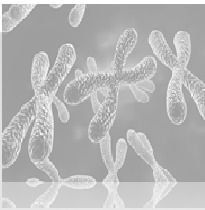
CYTOGENETIKA CLL

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.

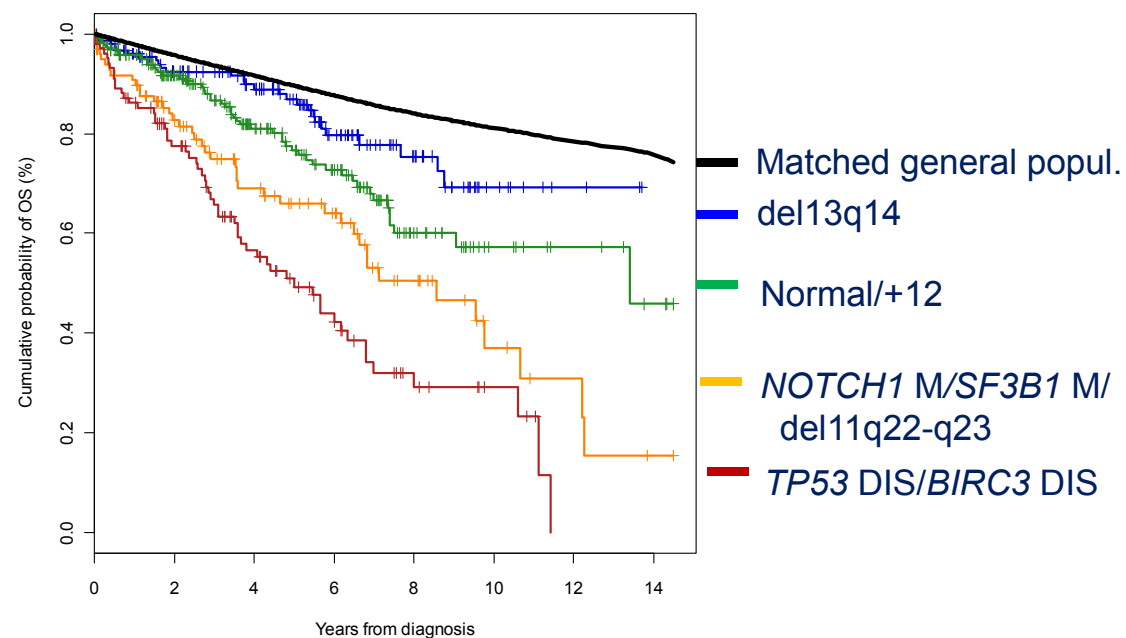
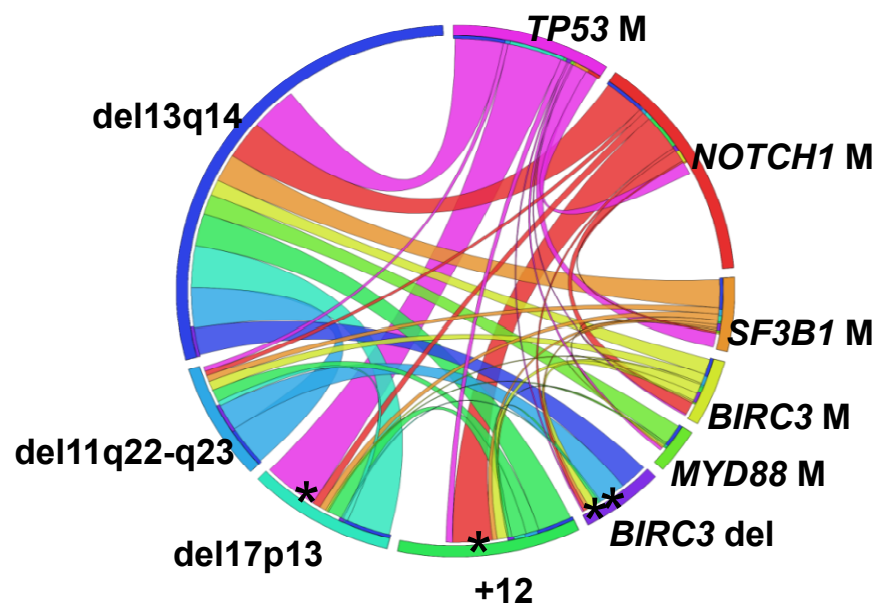
N Engl J Med 2000; 343:1910-1916.

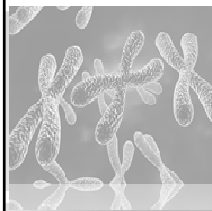




Chronická lymfocytární leukemie (CLL)

Mutační a cytogenetický model





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



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

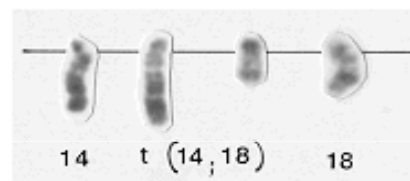
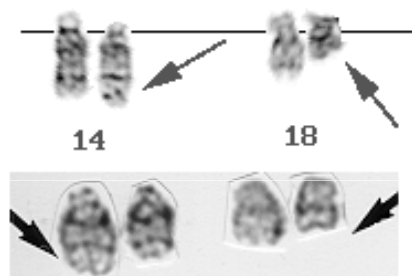
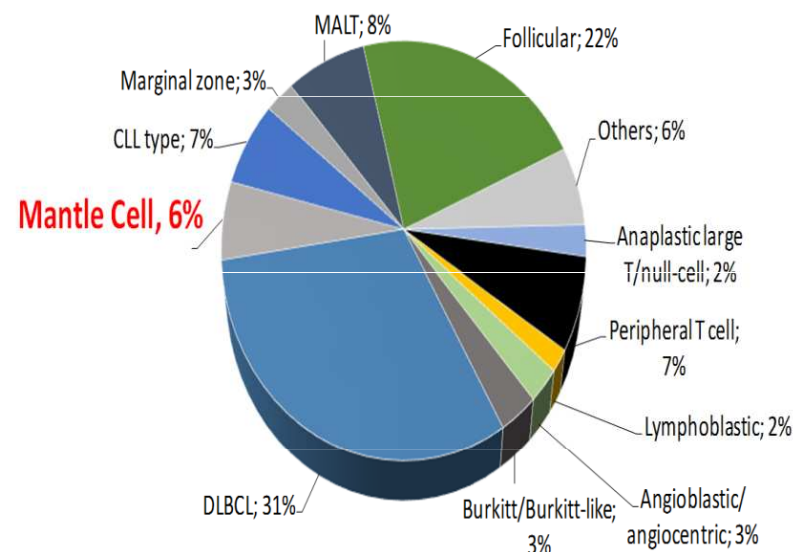
Folikulární lymfom (FL)

indolentní B buněčný lymfom

~20 % všech lymfomů

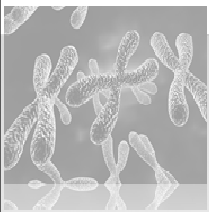
heterogenní klinický průběh , os několik roků až 20 let

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



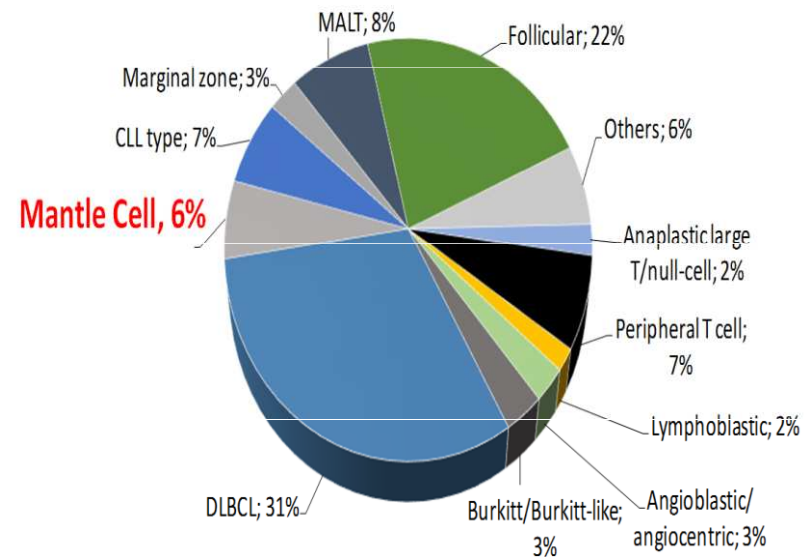
Fúze *IGH/BCL2*

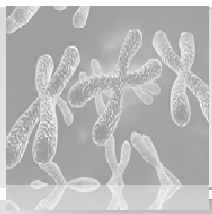




MCL (mantle cell lymphoma) lymfom plášťových buněk

- Agresivní onemocnění (OS 3-5 let)
- ~ 6 % všech NHL
- Diagnostika:
- Morfologie
- Imunohistochemie
- Imunofenotypizace
- Genetika:
 - cytogenetika
 - FISH
 - molekulární genetika - PCR

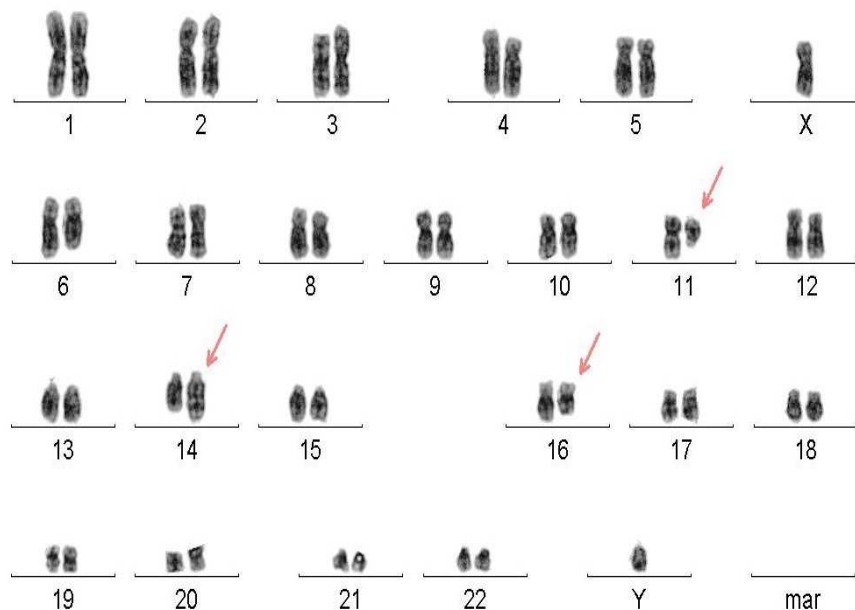




MCL (mantle cell lymphoma) lymfom plášťových buněk

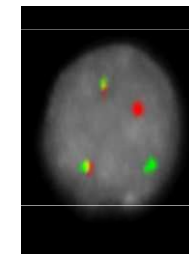
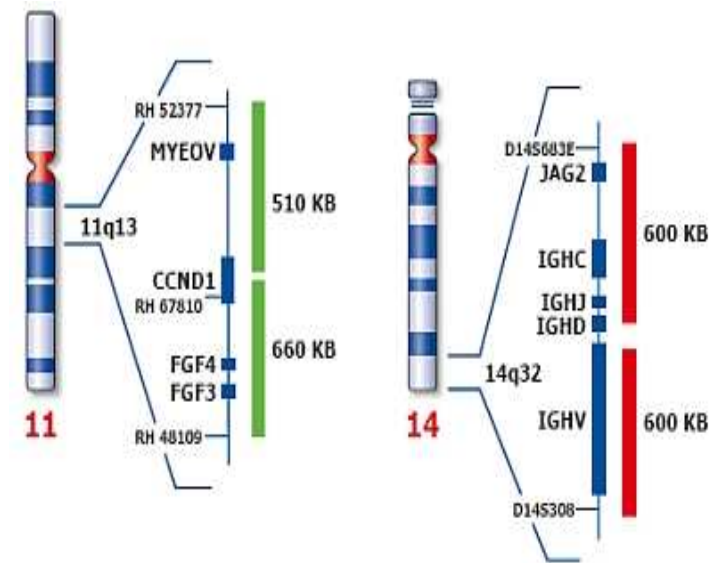
- Konvenční cytogenetika

t(11;14)

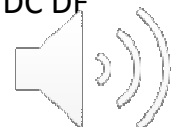


- FISH

dual color dual fusion probes

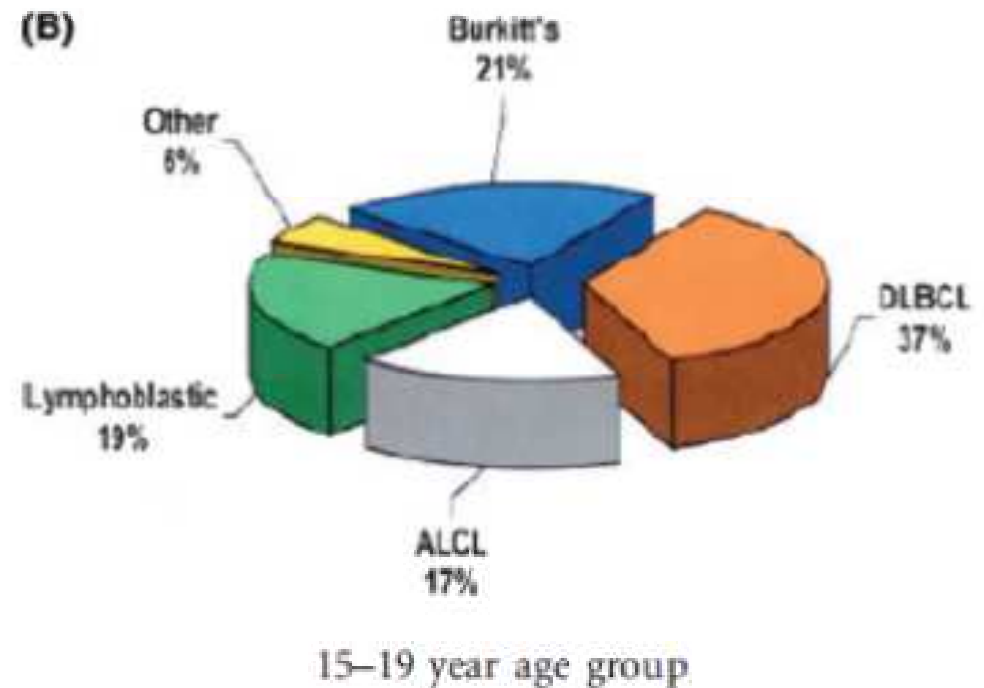
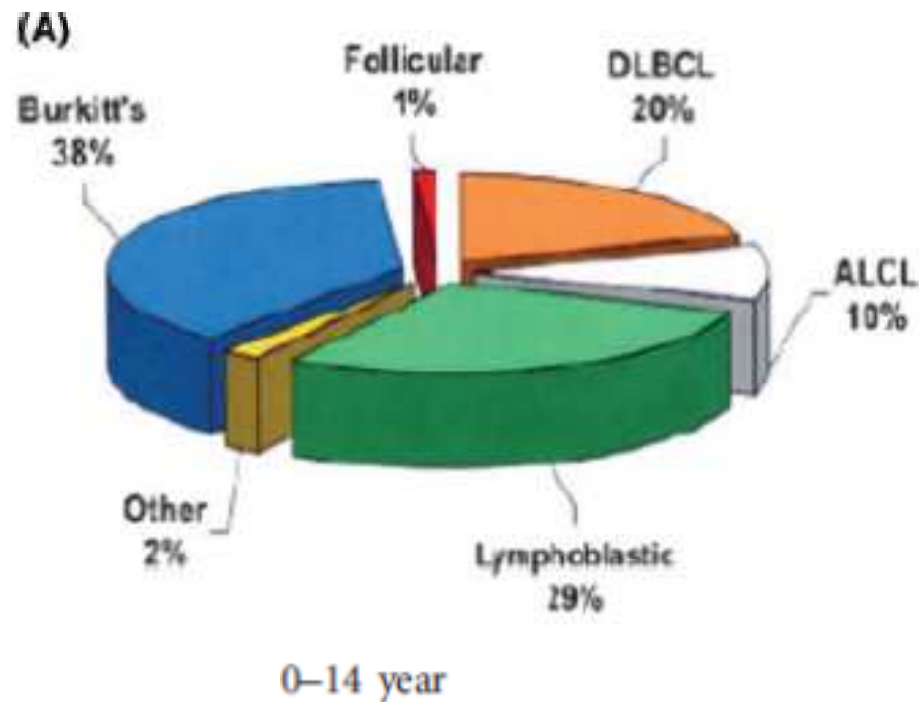


IgH/CCND1 DC DF
Kreatech



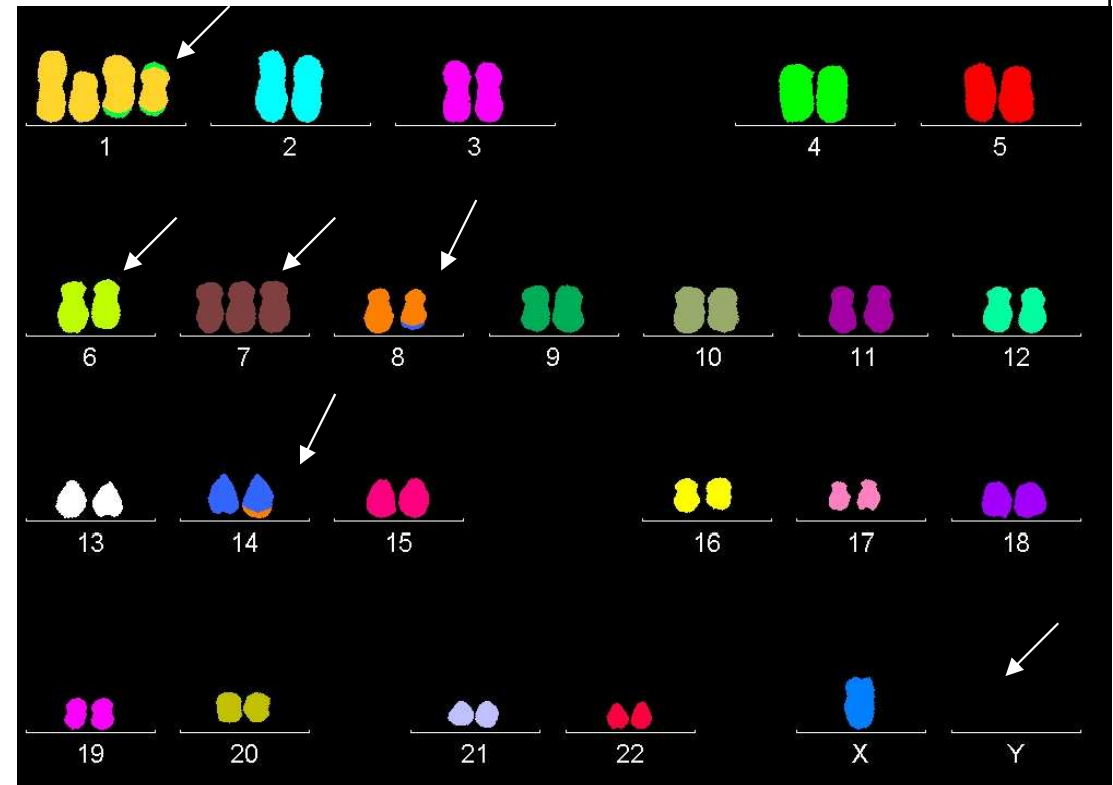
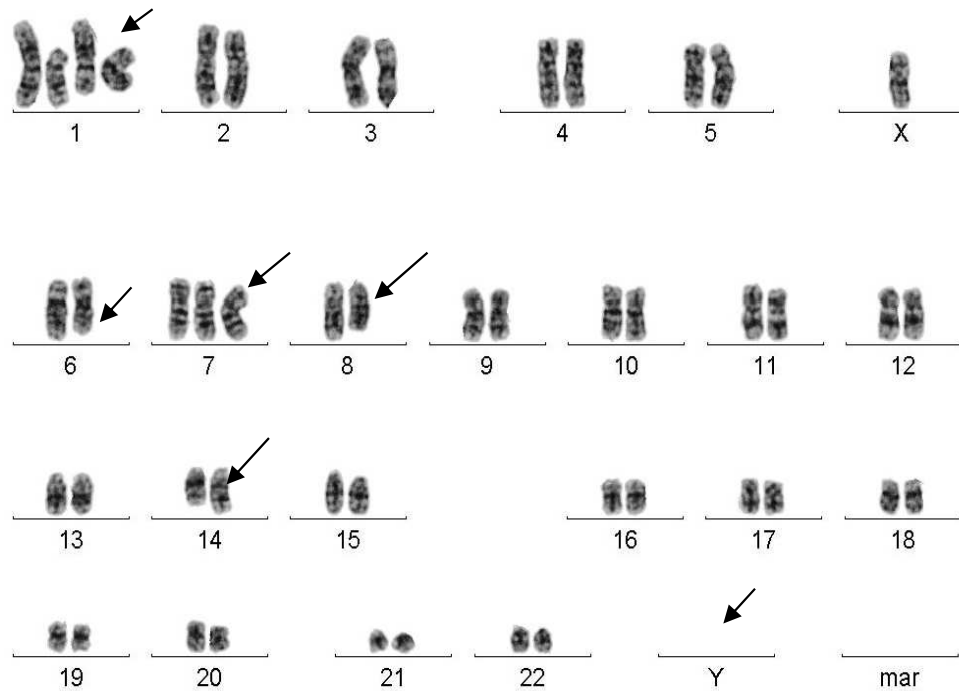
Nehodgkinské lymfomy (NHL) u dětí

- 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



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%)

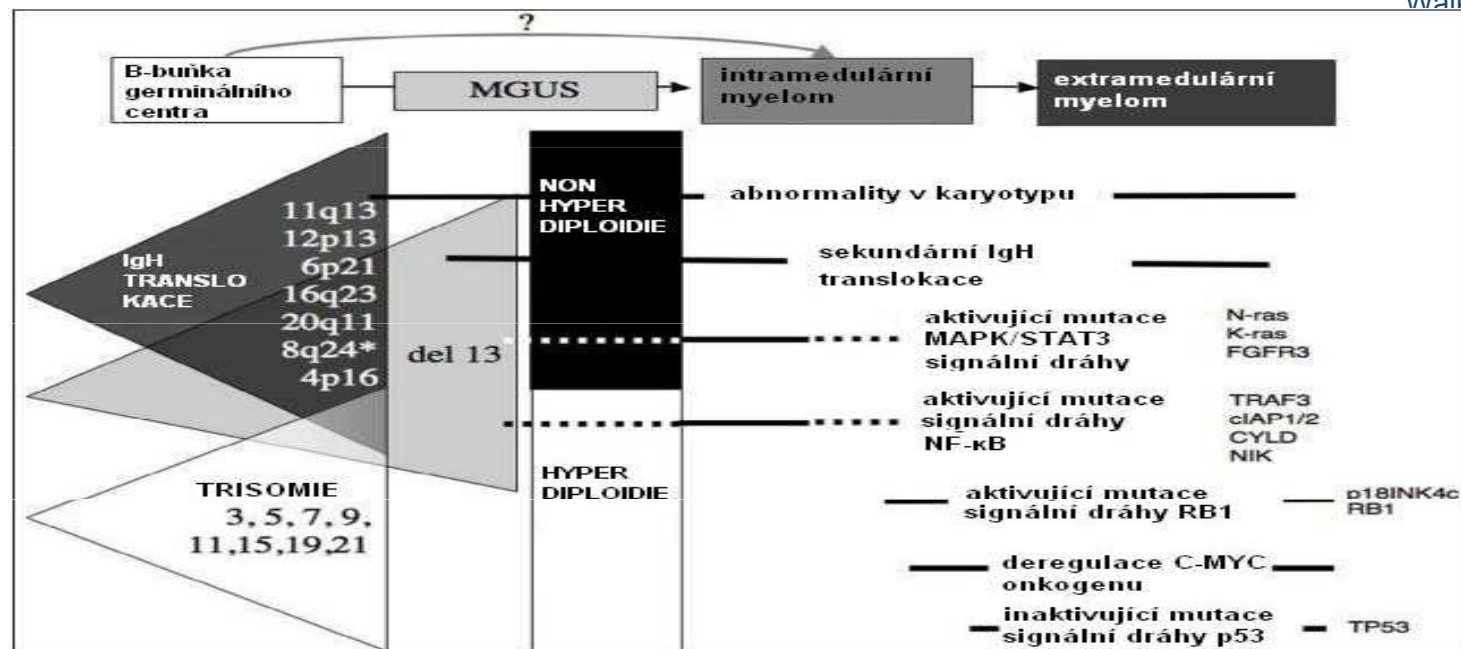
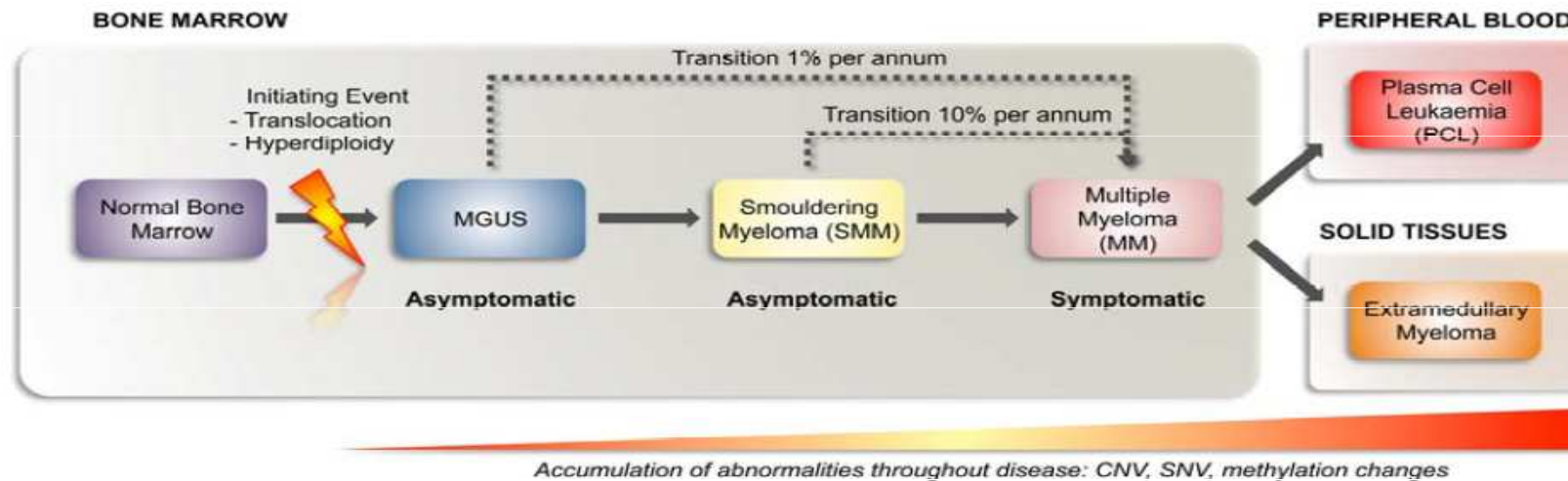


11/2011



MNOHOČETNÝ MYELOM

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



Walker B,

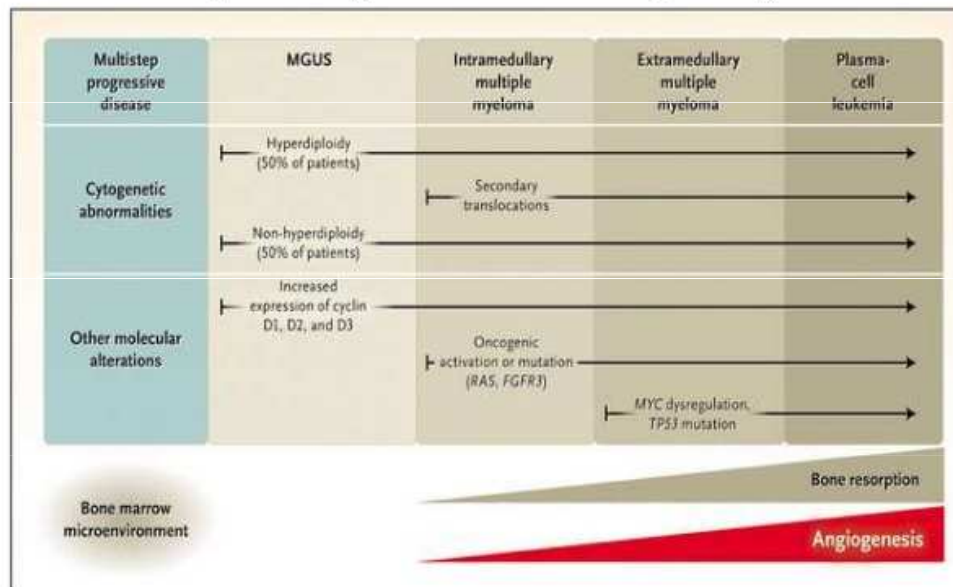


MNOHOČETNÝ MYELOM

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 \geq 3.5 g/dL
II	Not ISS stage I or III
III	Serum β_2 -microglobulin \geq 5.5 mg/L
CA by FISH	
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 FISH and normal LDH
II	Not R-ISS stage I or III
III	ISS stage III and either high-risk CA by FISH or high LDH

Abbreviations: CA, chromosomal abnormalities; FISH, 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
- 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

