

# Nádorová cytogenomika (CYTOGENETIKA A MOLEKULÁRNÍ CYTOGENETIKA)

## HEMATOLOGICKÝCH MALIGNIT

*Marie Jarošová*

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



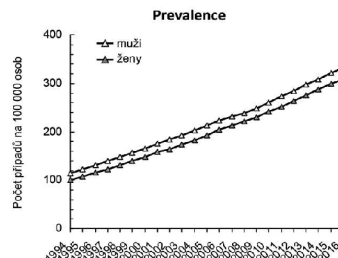
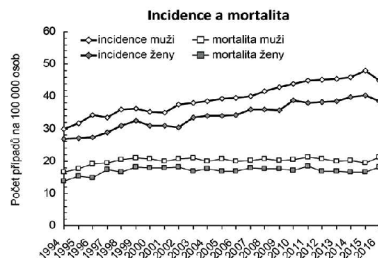
# Genetika nádorů

- *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 %                       |



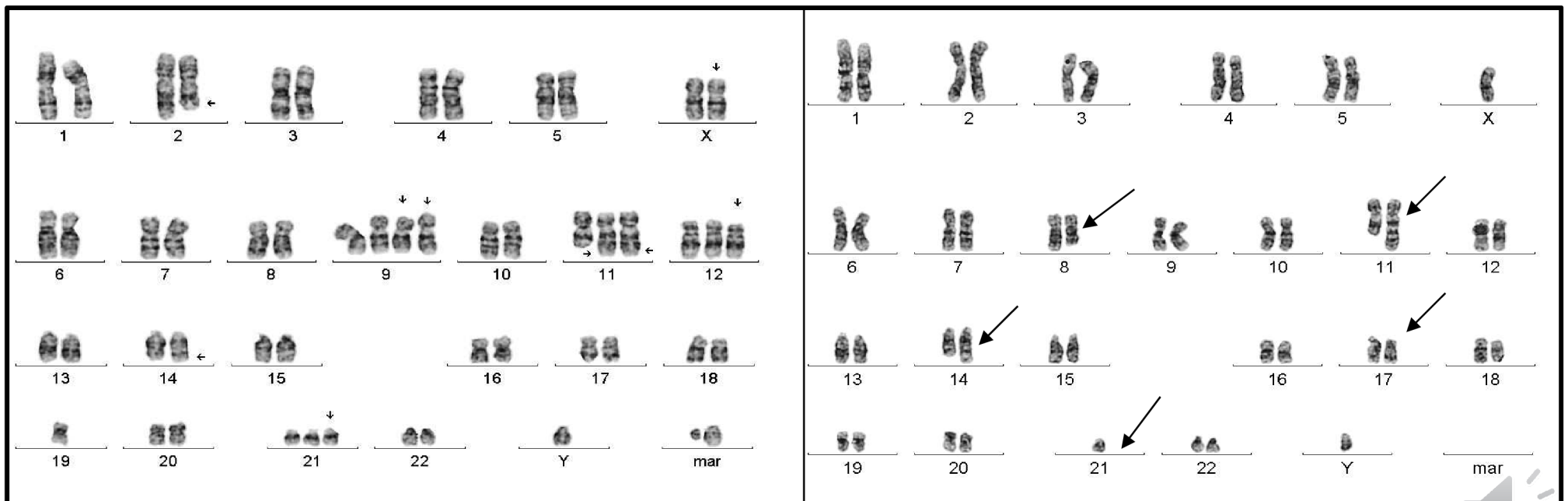
Zdroj: Národní onkologický registr, ÚZIS ČR

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 %.



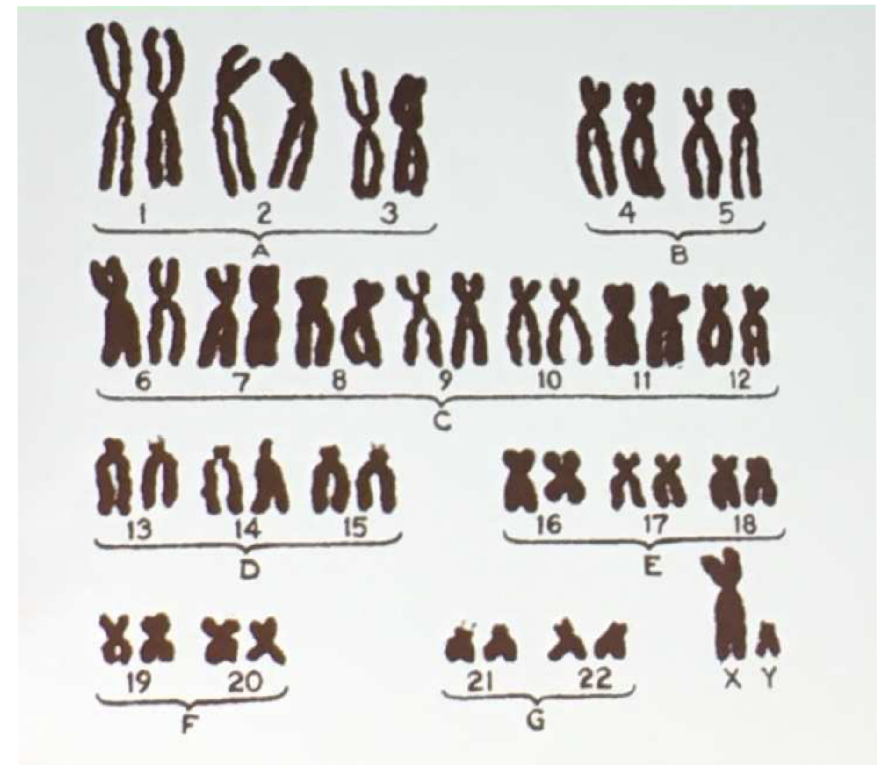
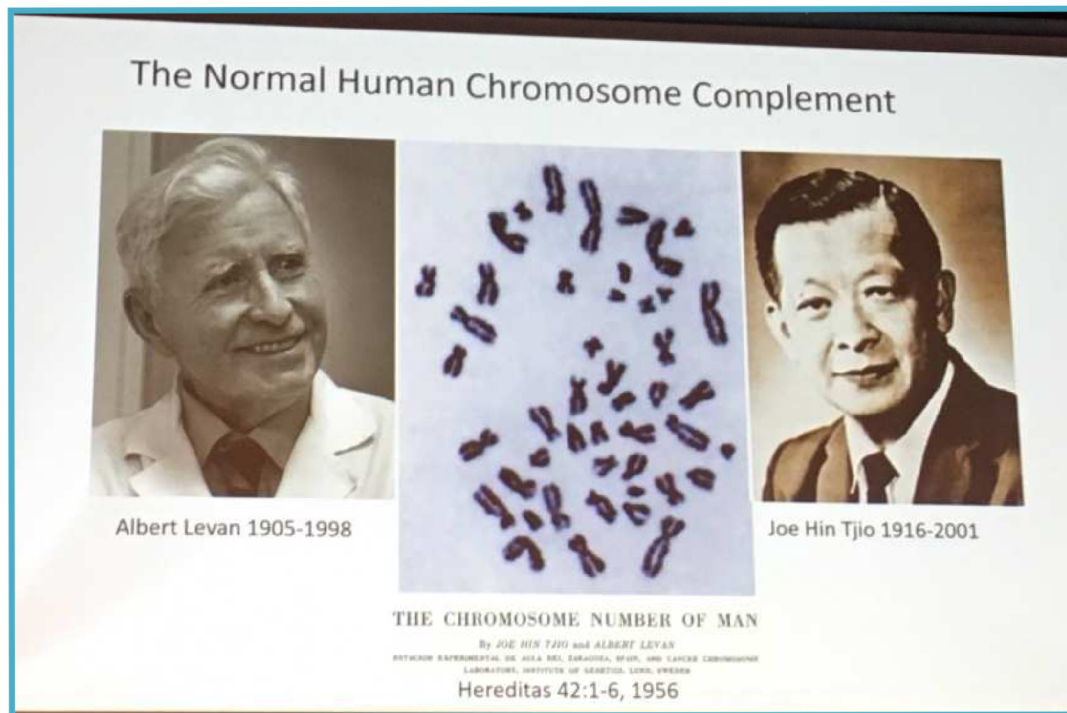
# Nádorová cytogenomika

- 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)



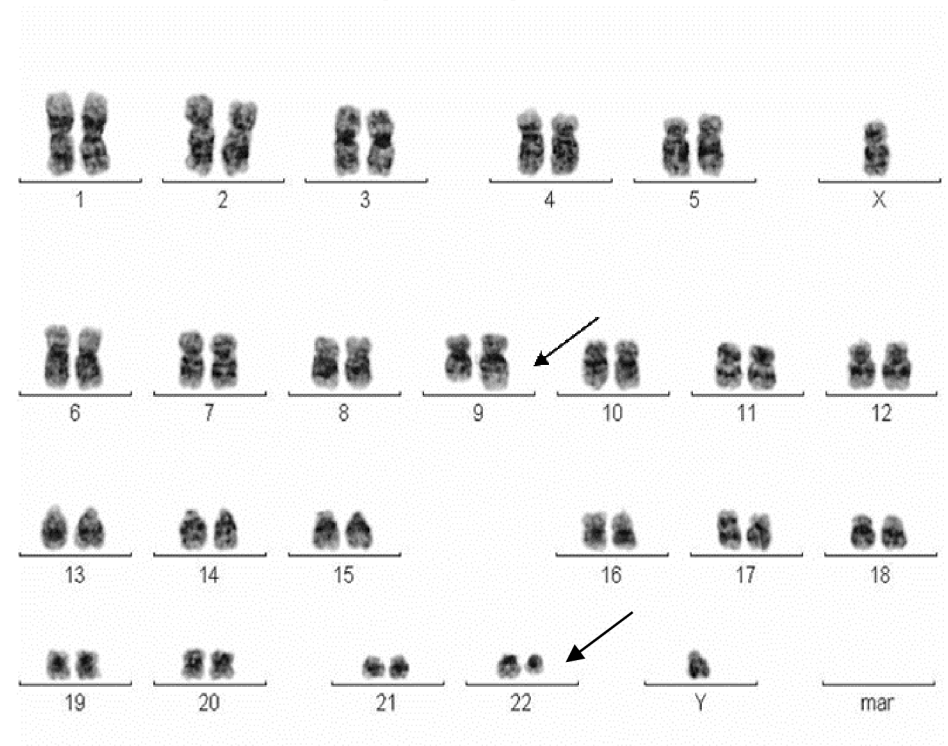
Peter Nowell & David Hungerford  
Science 1960,132:1497



# Historie nádorové cytogenetiky

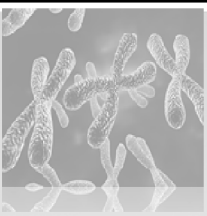
## Philadelphia chromosome (Ph1)

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



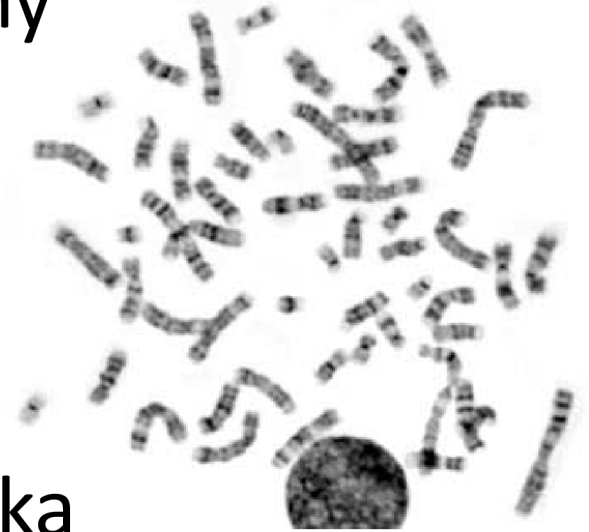
Dr. Rowley received the Lasker Award, given for distinguished contributions to medical science; the National Medal of Science from President Bill Clinton; and the Presidential Medal of Freedom from President Obama, among many other honors (1925-2013)



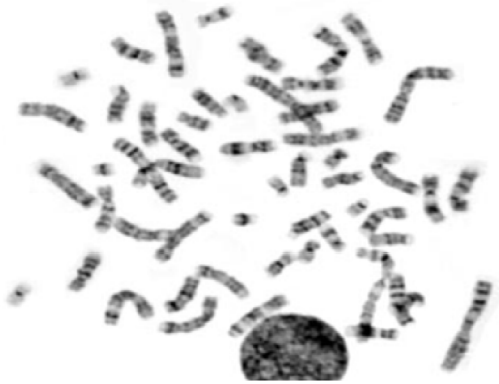


# Nádorová cytogenetika

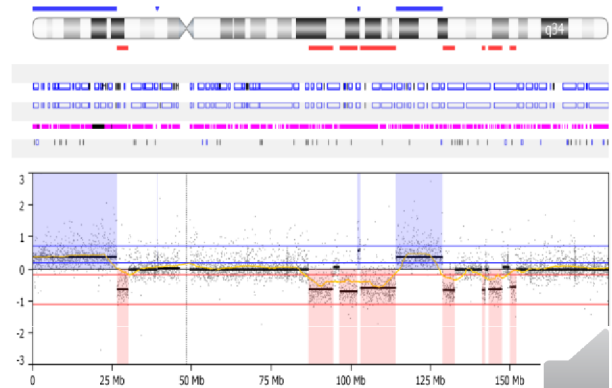
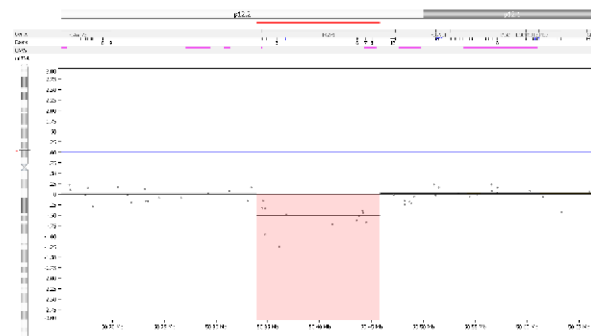
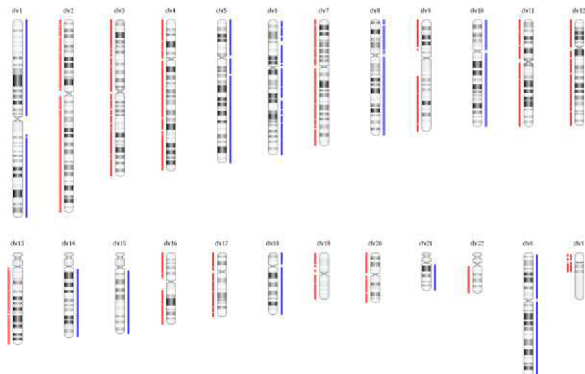
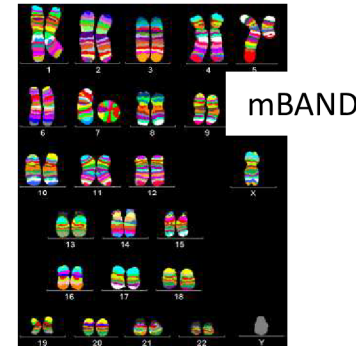
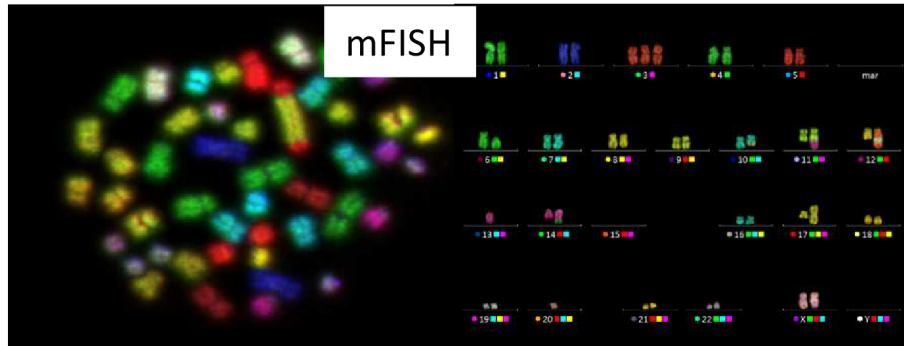
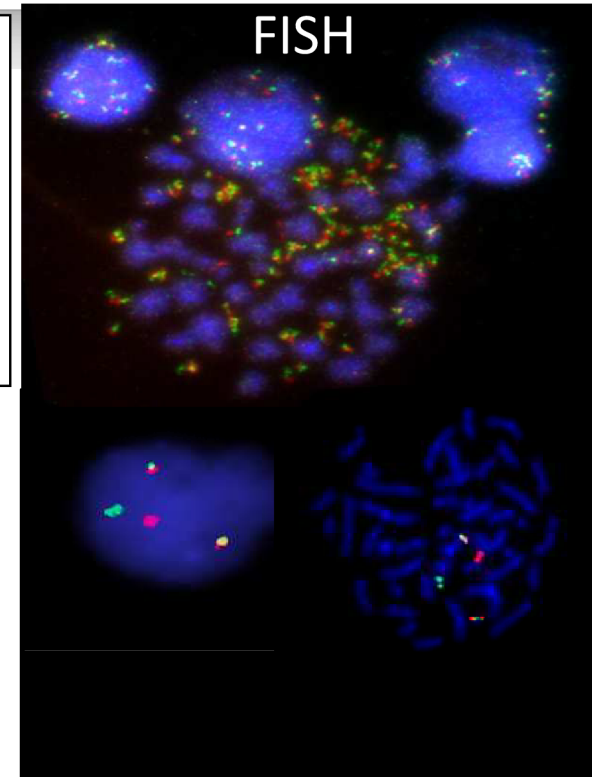
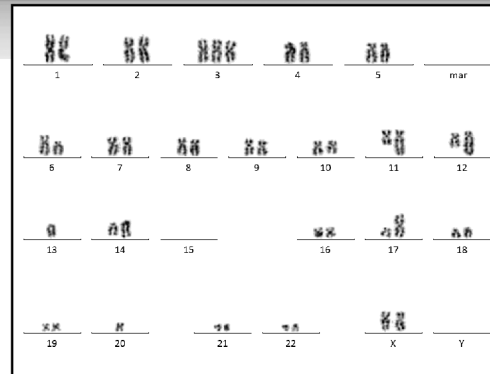
- Zkoumá získané chromosomové změny nádorových buněk
- Hodnotí početní a strukturní změny chromosomů
- Základní metoda – G-pruhovací technika (rozlišení kolem 3-5Mb)
- V jednom vyšetření analyzuje celý genom



# Nádorová cytogenomika - metody



Konvenční  
cytogenetická  
analýza

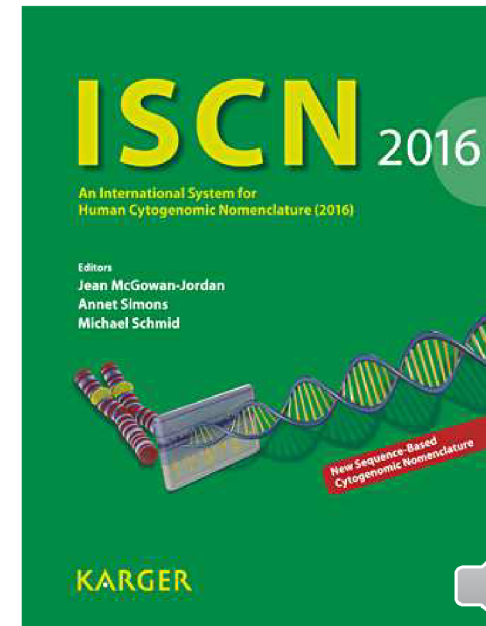
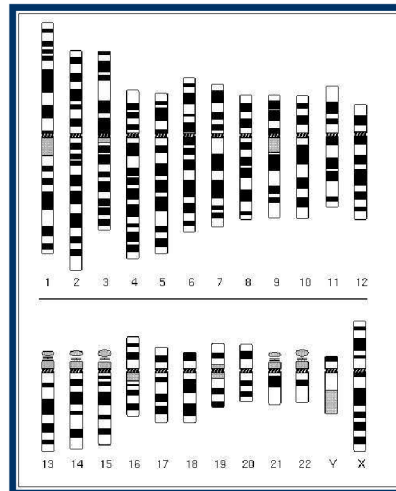
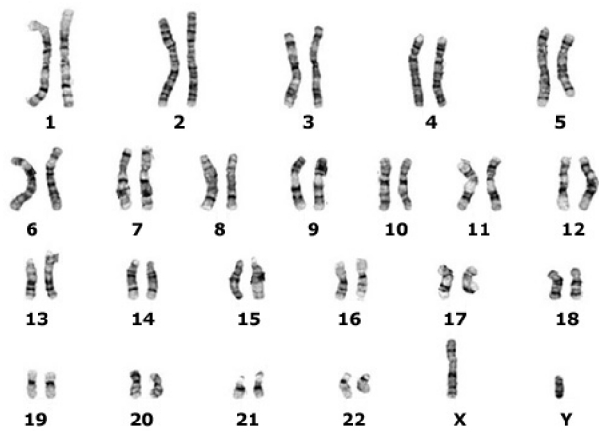
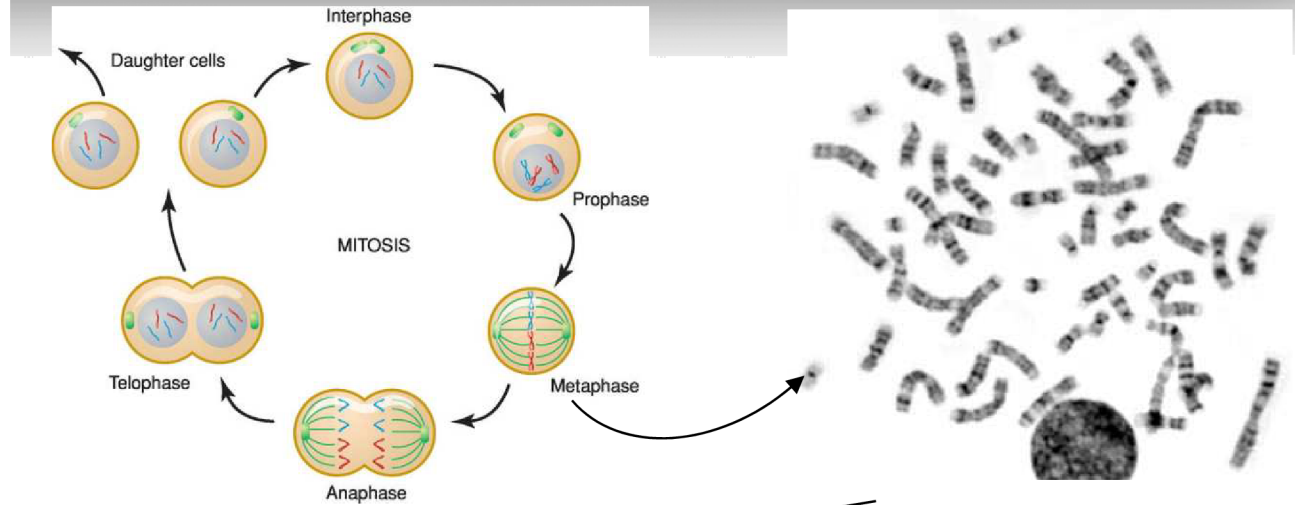
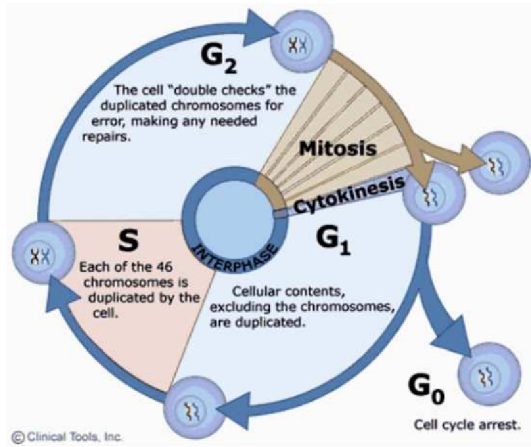
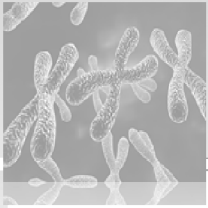


arrayCGH/SNP array

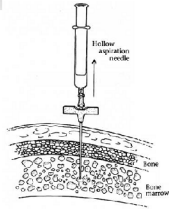




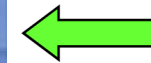
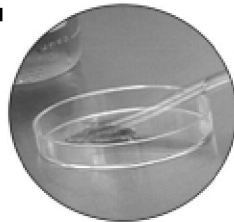
# Konvenční cytogenetika



# Postup kultivace buněk nádorů



1-2ml



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

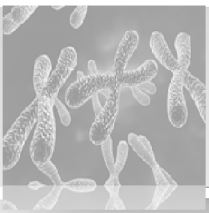


37°C/ 5%CO<sub>2</sub>



**Kultivace**  
**2/24/72hod/týdny**





# ZPRACOVÁNÍ BUNĚČNÉ KULTURY



www.shutterstock.com · 58307962



**HYPOTONIZACE  
0,075M KCl**

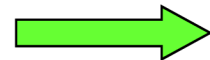
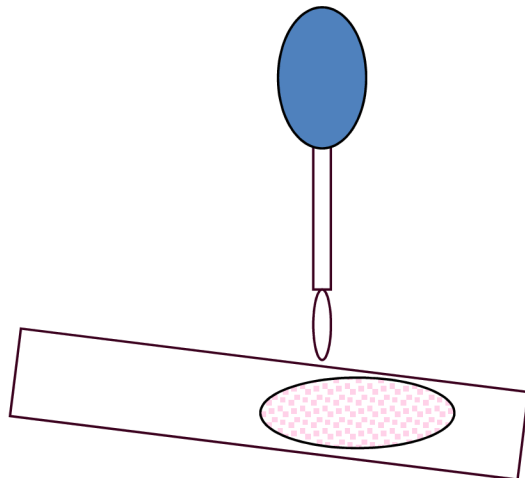


**COLCEMIDE BLOKUJE MITÓZY V METAFÁZI**

**PŘÍPRAVA PREPARÁTŮ  
KAPÁNÍM BB SUSPENZE  
NA SKLO**



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



**BARVENÍ A HODNOCENÍ  
V MIKROSKOPU**

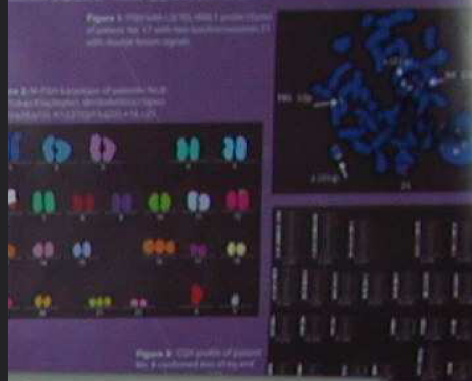
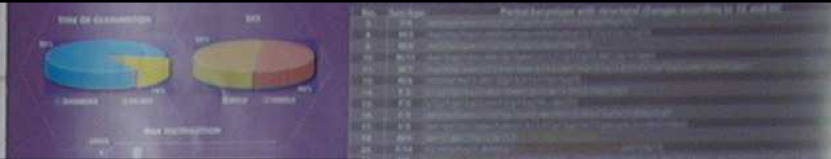
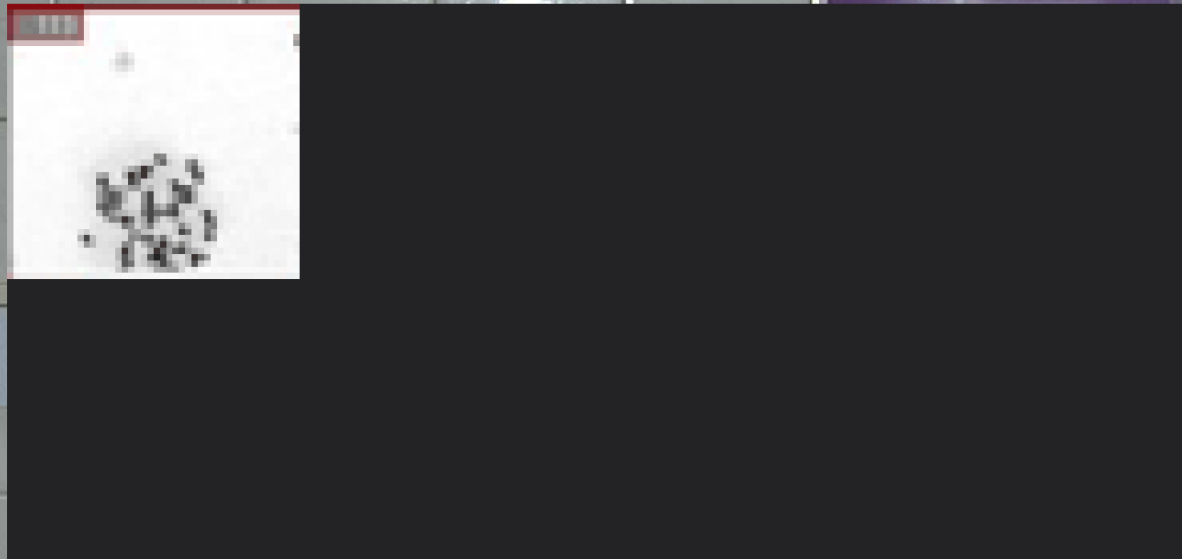


ABBOTT Laboratories, s.r.o.  
Diagnostic Division



NEC

|    |    |    |    |    |    |    |    |    |    |
|----|----|----|----|----|----|----|----|----|----|
| 1  | 2  | 3  | 4  | 5  | 6  | 7  | 8  | 9  | 10 |
| 11 | 12 | 13 | 14 | 15 | 16 | 17 | 18 | 19 | 20 |
| 21 | 22 | 23 | 24 | 25 | 26 | 27 | 28 | 29 | 30 |
| 31 | 32 | 33 | 34 | 35 | 36 | 37 | 38 | 39 | 40 |



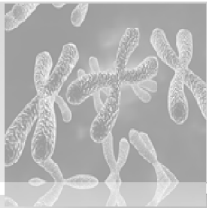
# Klasická cytogenetika - karyotyp

MetaSystems · Ikaros · 3

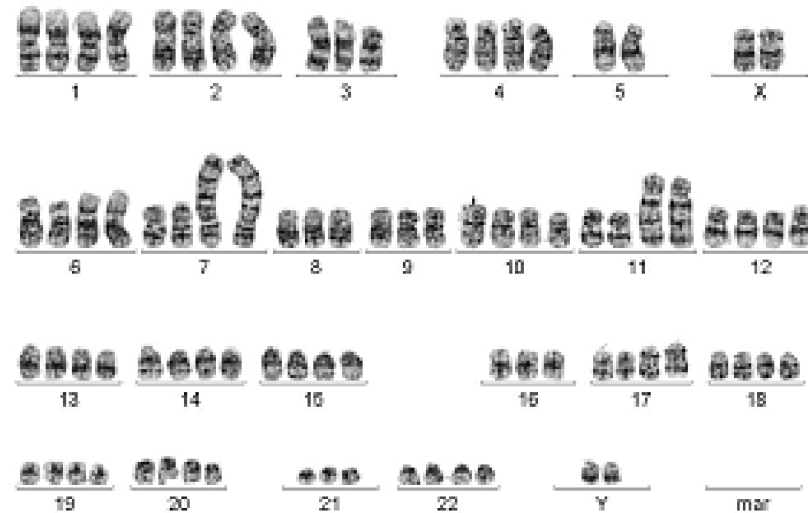
|         |   |       |
|---------|---|-------|
| CASE101 | 5 | 46,XX |
|         |   |       |
|         |   |       |

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

DemolKS DIR-G  
adm GBAND



# 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:

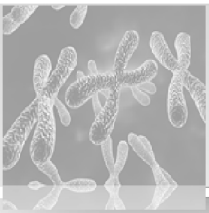
Ros Hastings, Rod Howell, David Betts, Sarah Porter, Claudia Haferlach,  
Nicole Dastugue, Isabelle Radford-Weiss, H. Berna Beverloo, Annet Simons,  
Clemens Mellink, Simone Snijder, Eva van den Berg-de Ruyter, Jacqueline Schoumans,  
Blanca Espinet, Reiner Siebert, Jerome Couturier, Alain Bernheim,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

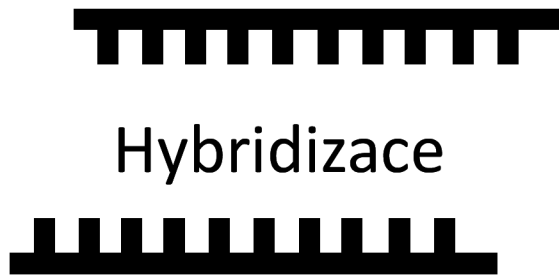
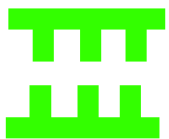
Rack et al. *Leukemia* (2019) 33:1851–1867





# Molekulární cytogenetika

Denaturace

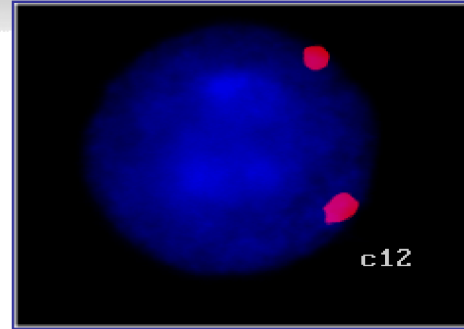
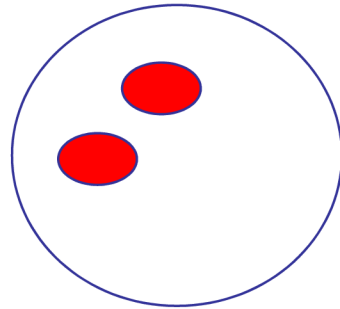
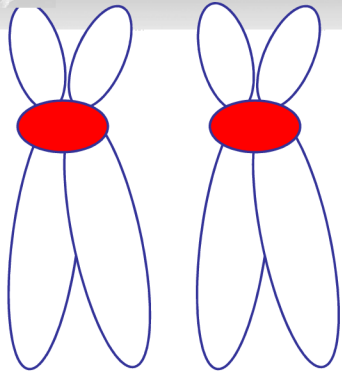
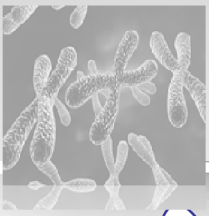


Hybridizace

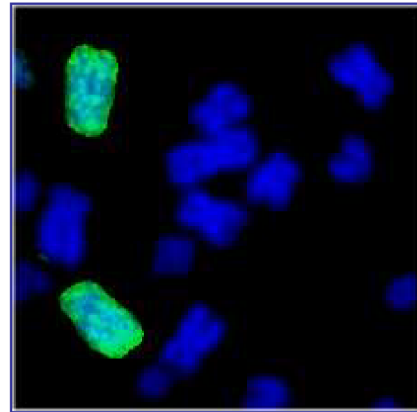
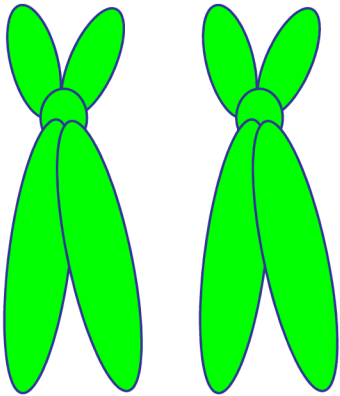
- Metody založené na fluorescenční in situ hybridizaci (FISH) vytváří spojení mezi metodami molekulární genetiky a klasické cytogenetiky
- Metody využívající základní vlastnosti jednořetězcové DNA vzájemně se vázat na základě komplementarity bazí



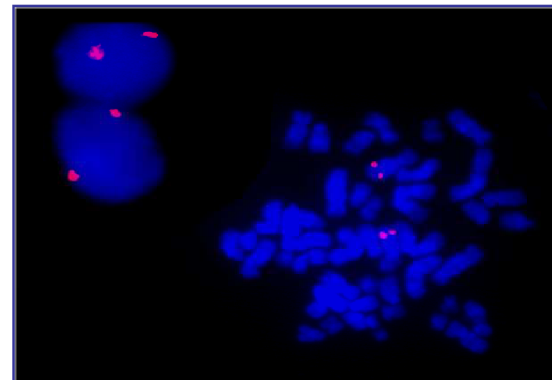
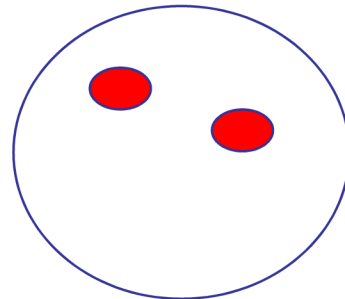
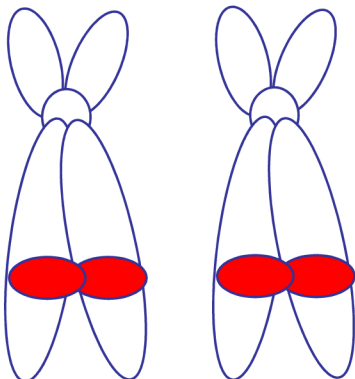
# Typy sond



centromerické



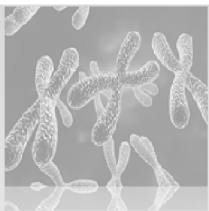
celochromosomové



genové





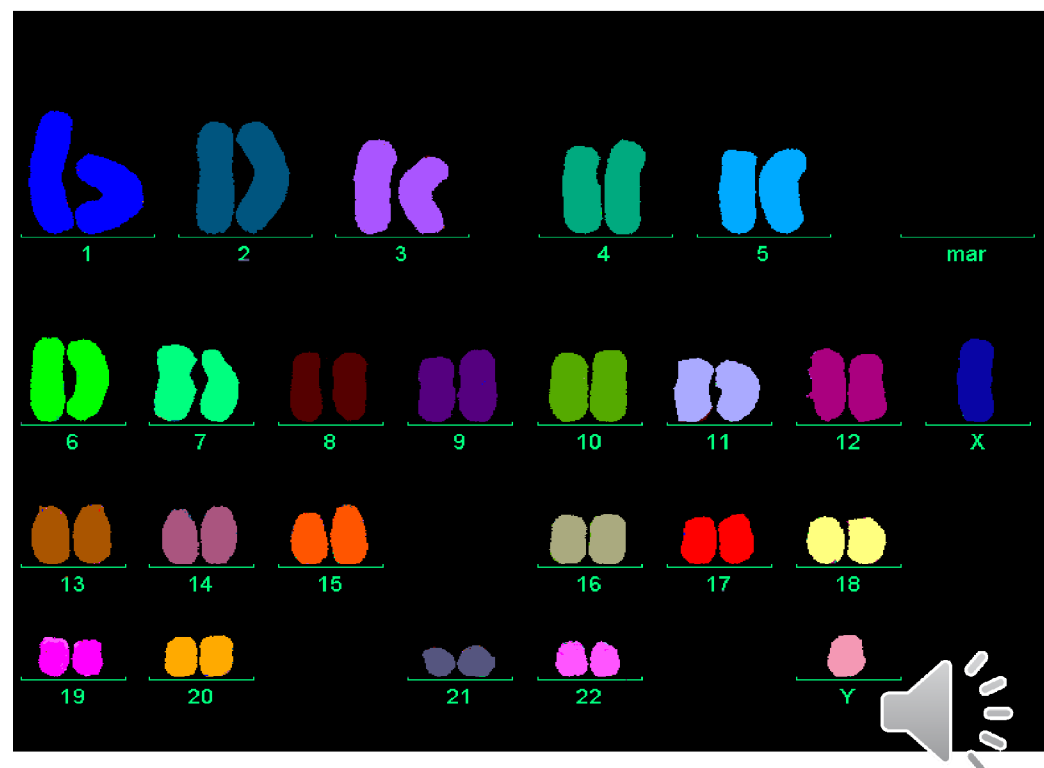


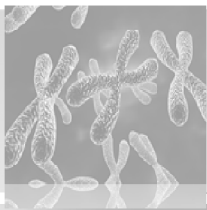
# Mnohobarevná fluorescenční in situ hybridizace (mFISH)

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

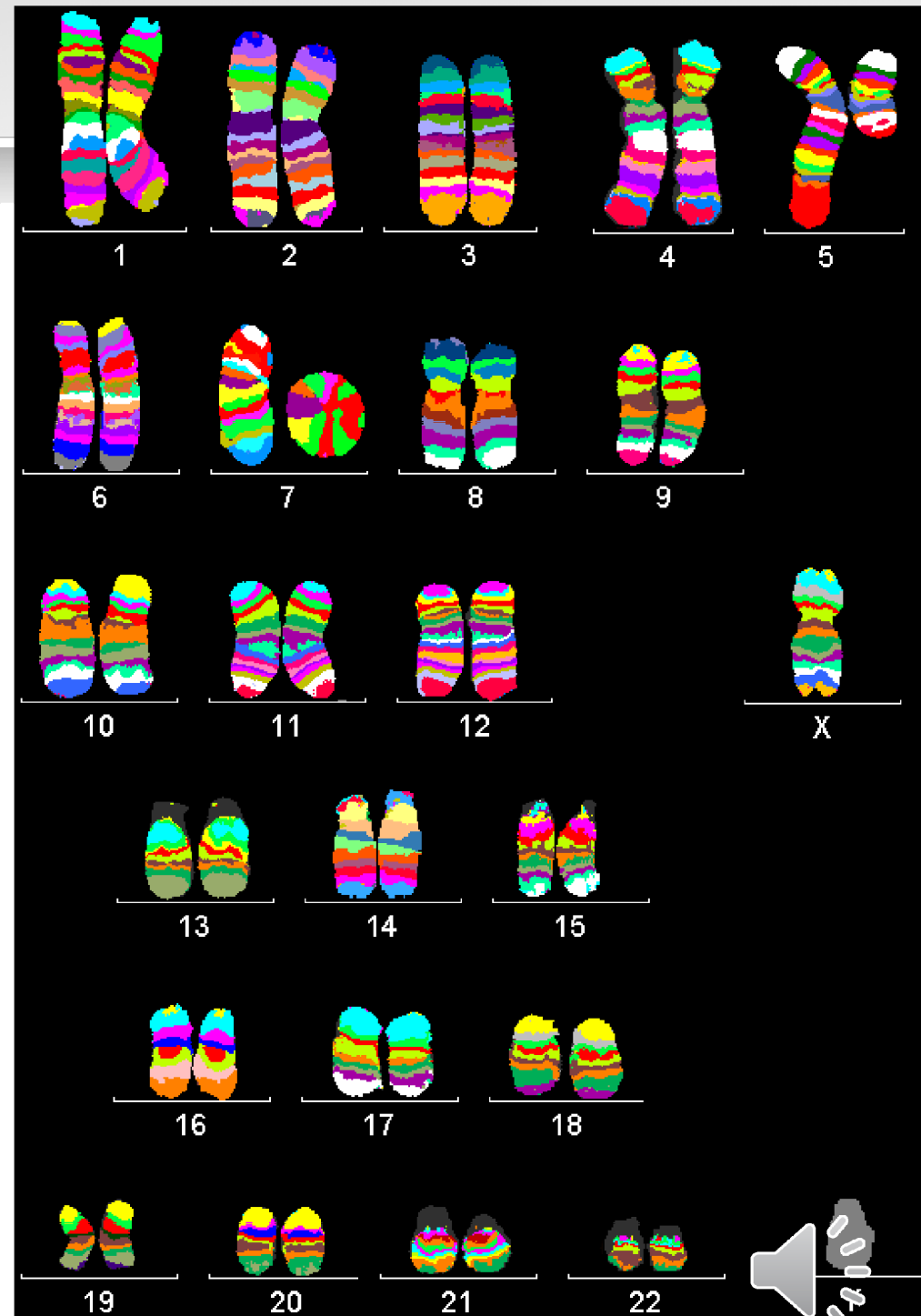
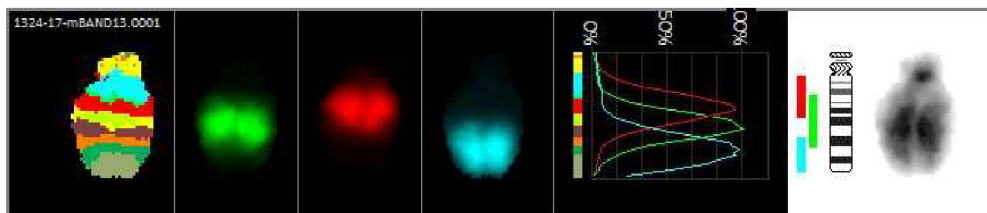
| Chr. | FITC  | Spectrum Orange | Texas Red | Cy5 | DEAC |
|------|-------|-----------------|-----------|-----|------|
| 1    |       |                 |           | Red | Blue |
| 2    |       |                 |           |     | Blue |
| 3    |       |                 | Red       |     |      |
| 4    | Green |                 |           |     |      |
| 5    |       | Yellow          |           |     |      |
| 6    | Green |                 |           | Red |      |
| 7    |       |                 |           | Red | Blue |
| 8    |       |                 | Red       | 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       | 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 |

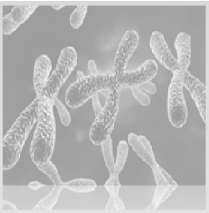




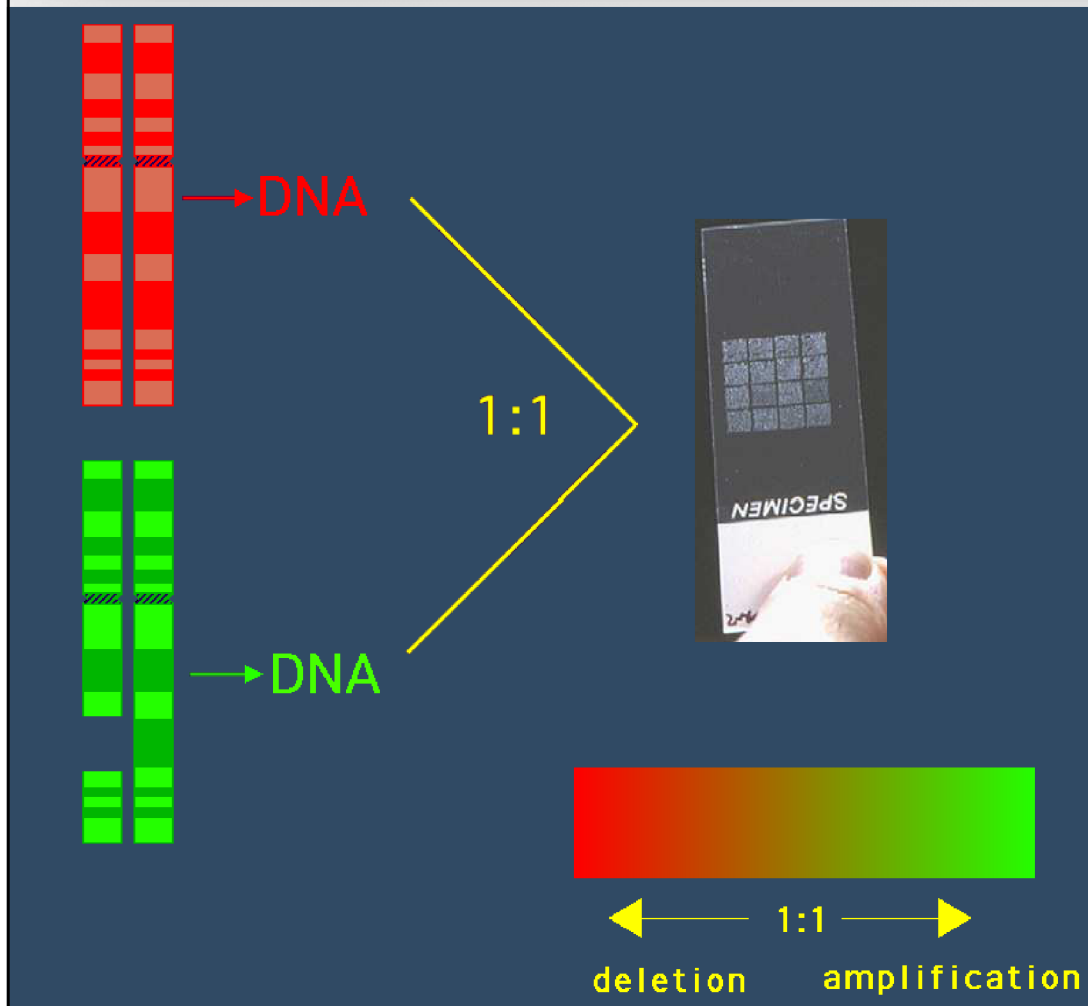
# Mband FISH

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

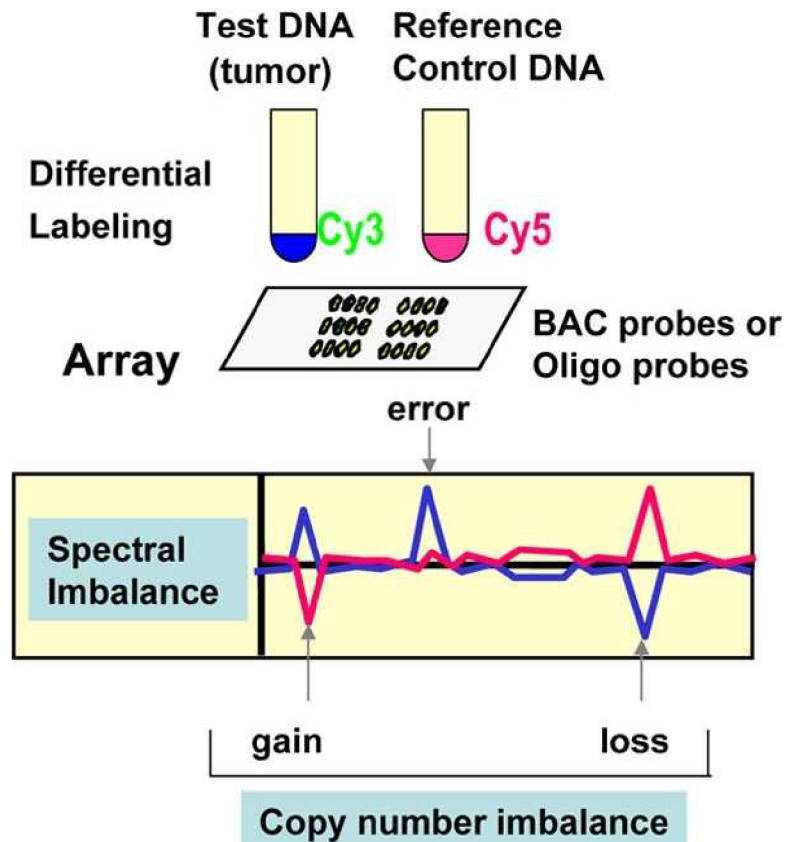


# arrayCGH/SNPs array

A

## CGH-A

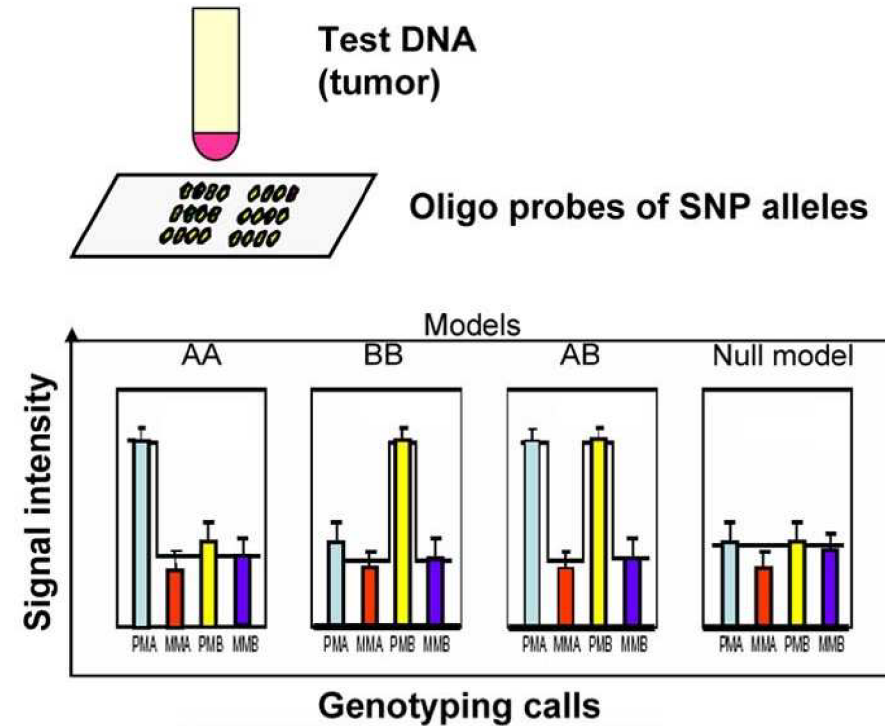
- BAC CGH-A
- Oligo CGH-A



B

## SNP-A

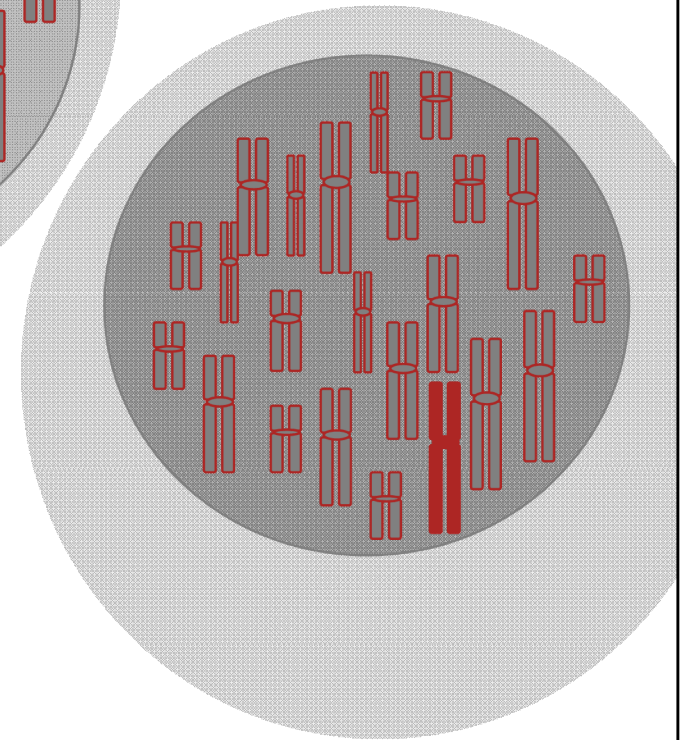
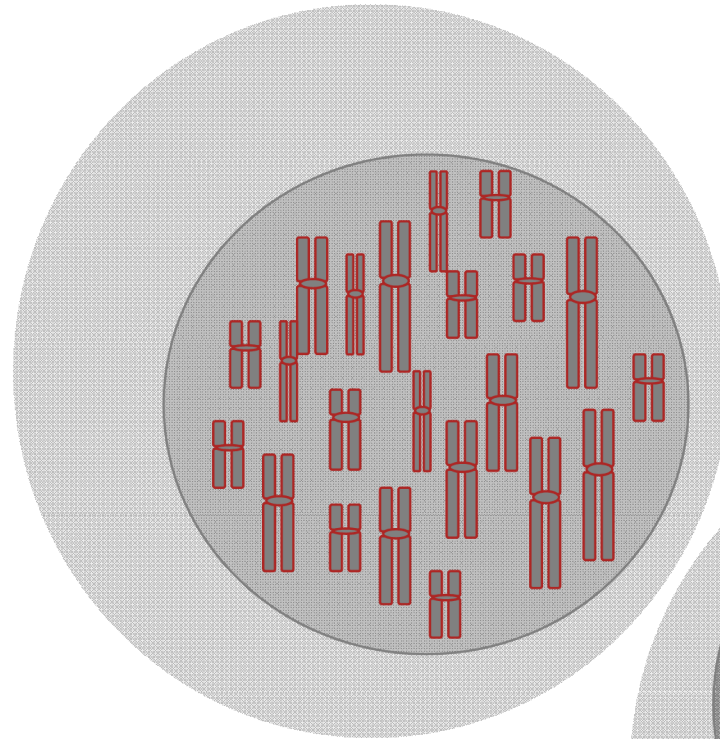
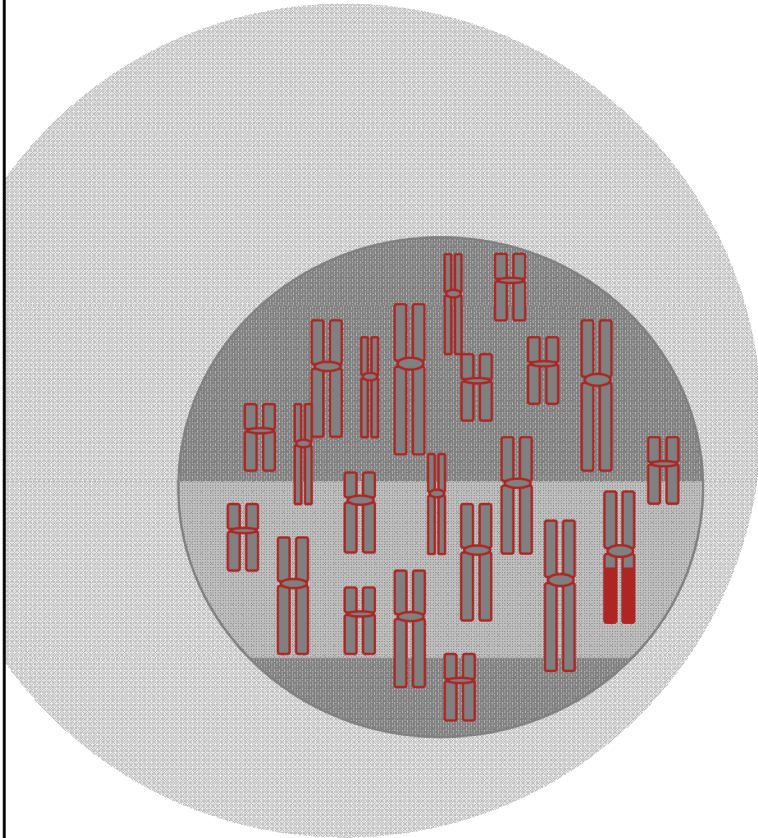
- Combined CN/SNP-A



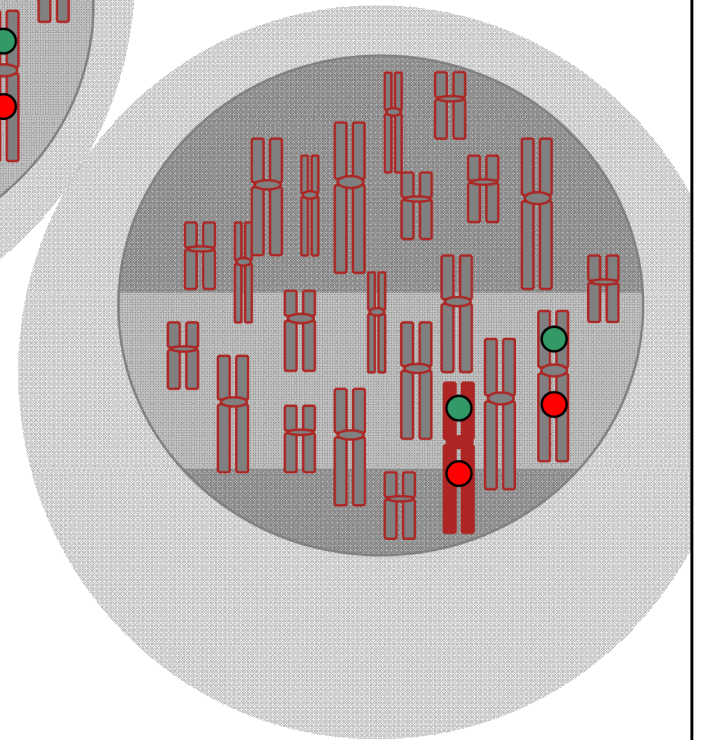
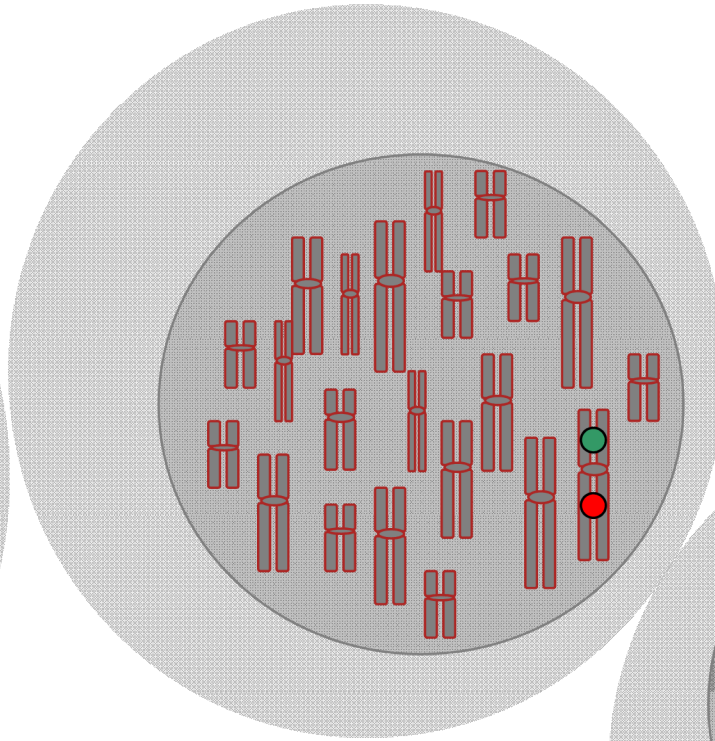
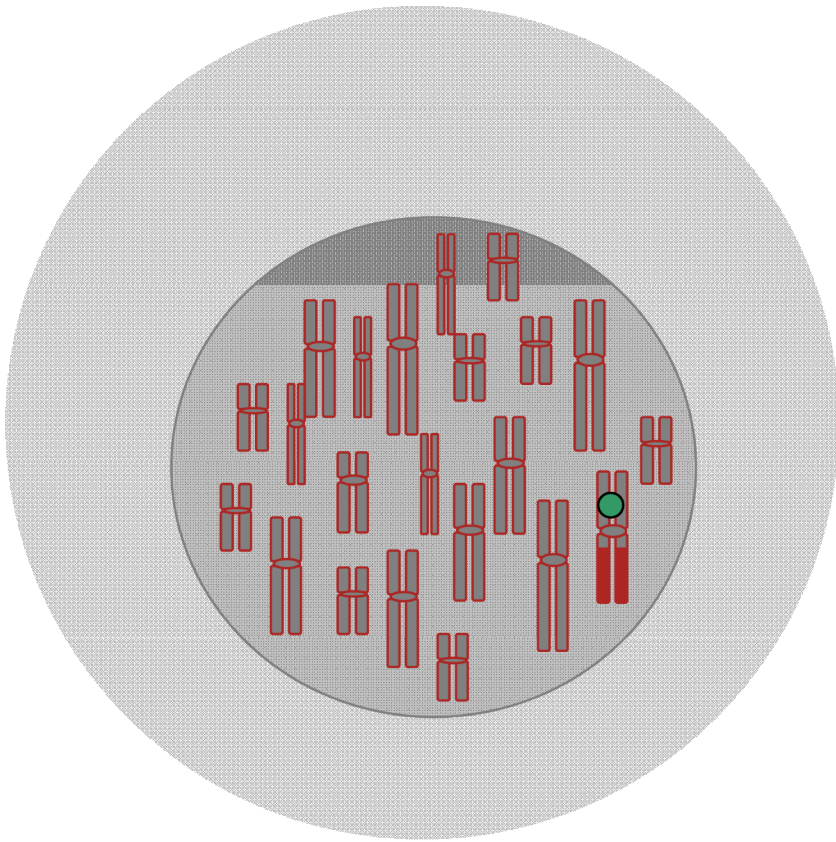
Genotype Intensity– copy number

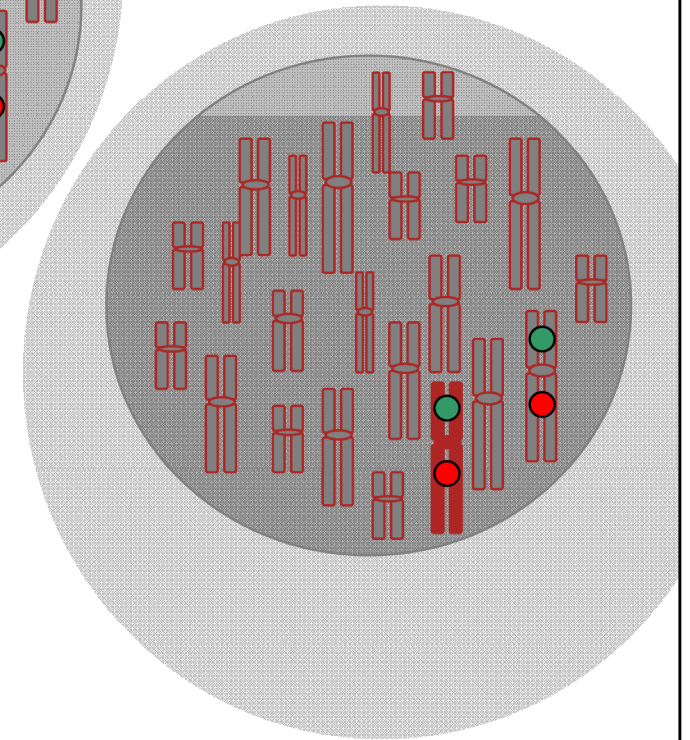
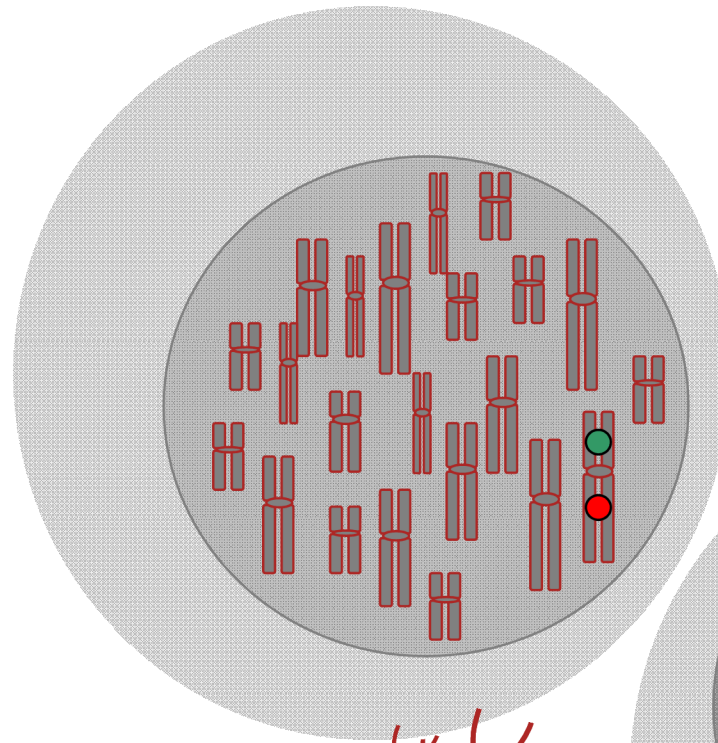
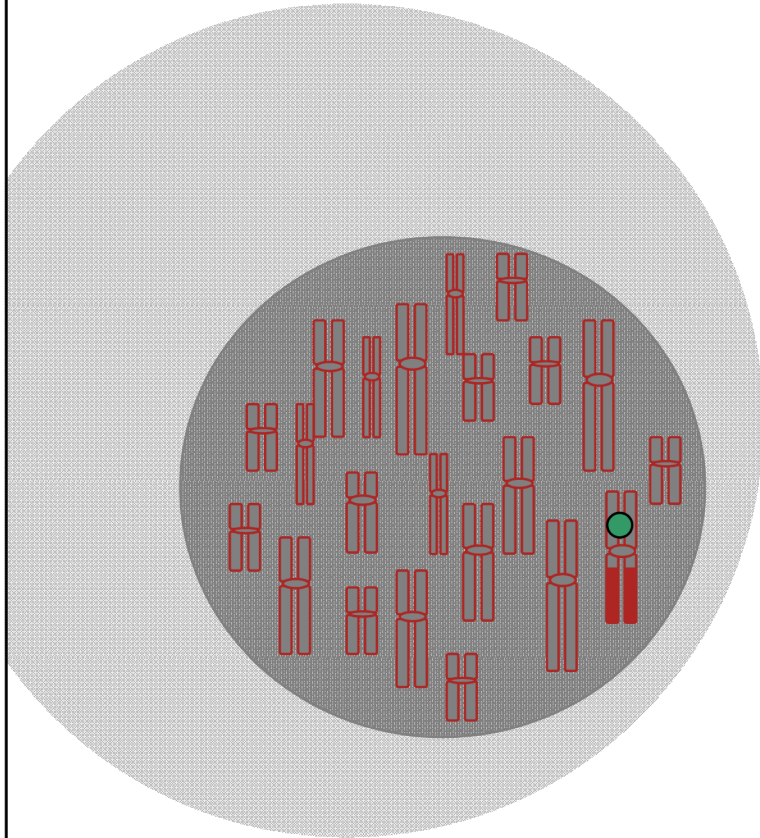


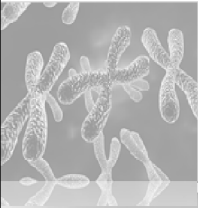
# Cytogenetics



# FISH







# 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

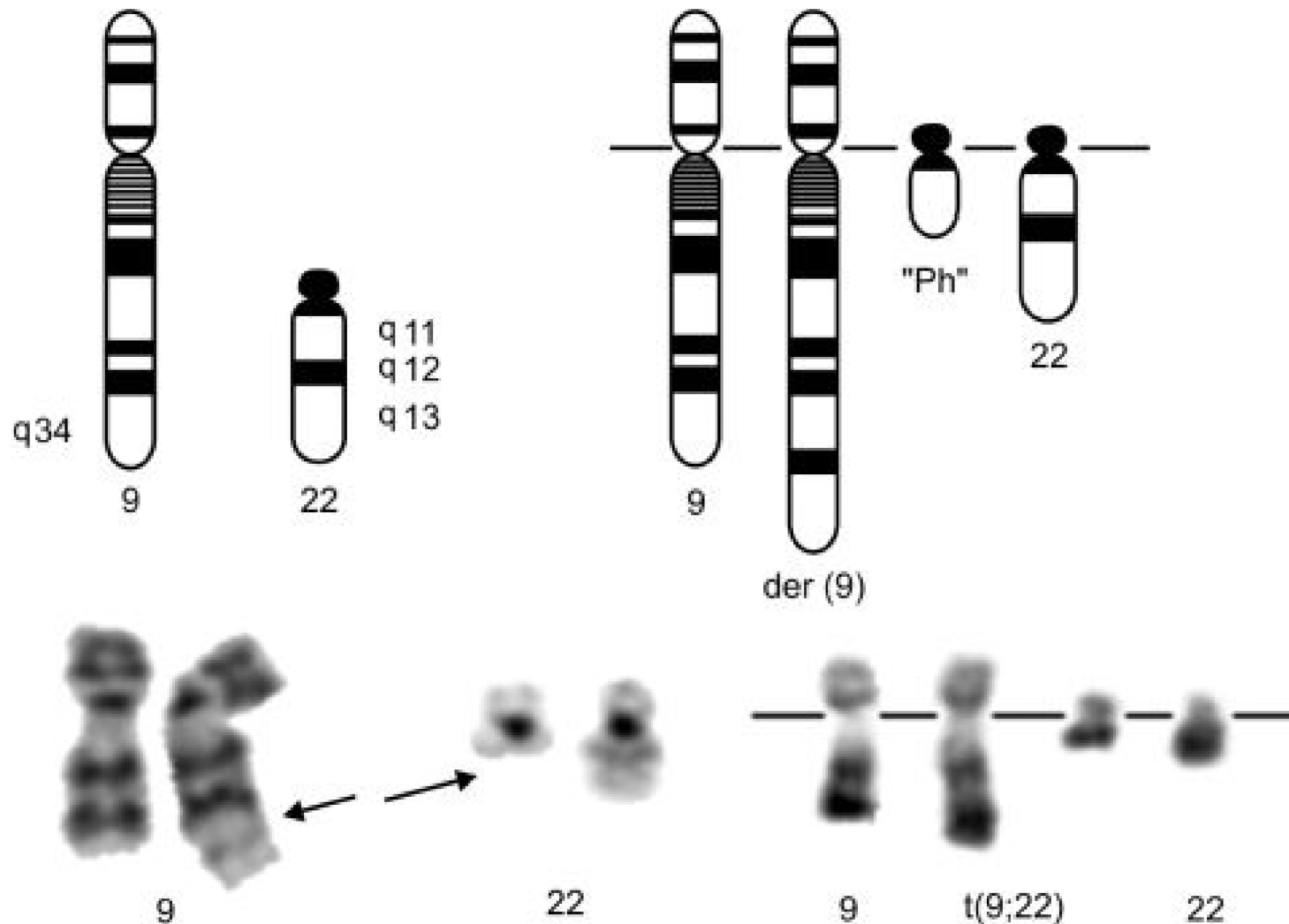
2.Prognosa

3.Léčebné rozhodování



# Filadelfský chromosom (Ph)

První specifická chromosomová změna u nádoru člověka



# Cytogenetika CML

## Diagnóza

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

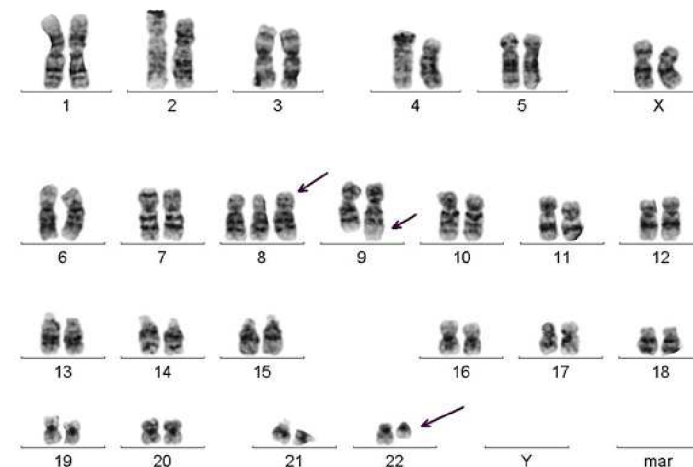
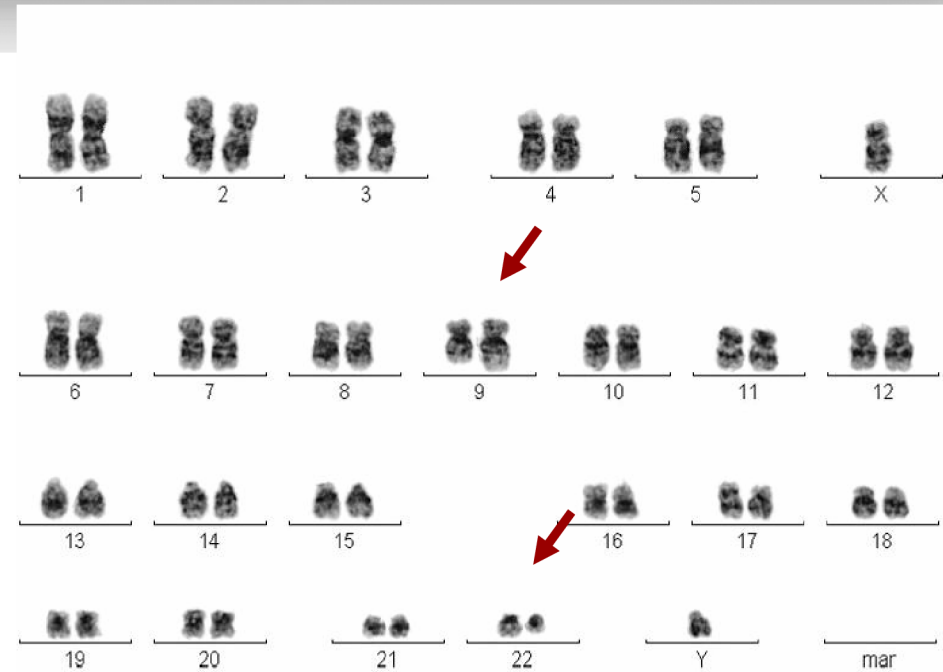
## Prognóza

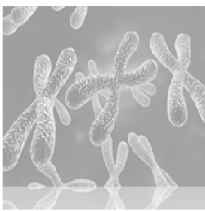
- Přidatné chromosomové změny

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<br>xní<br>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

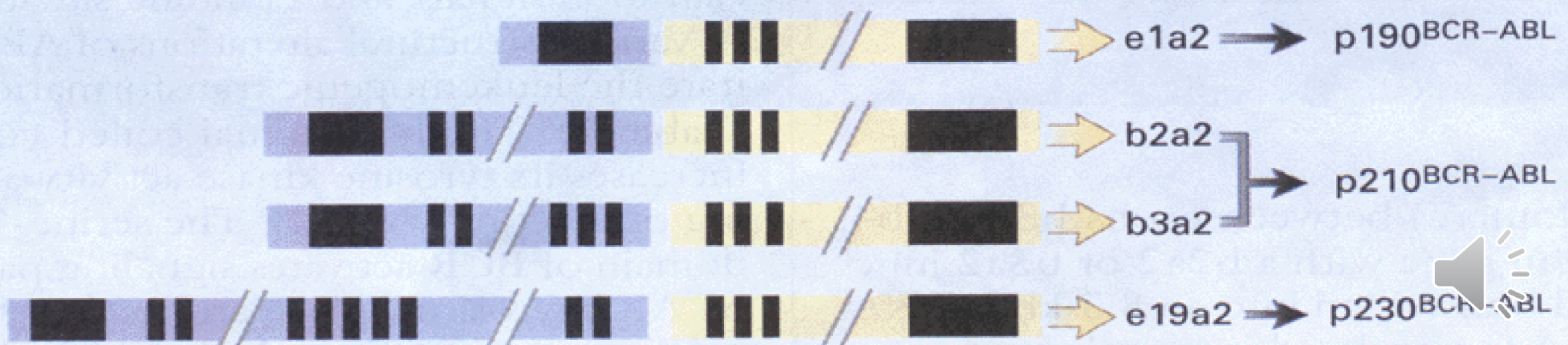
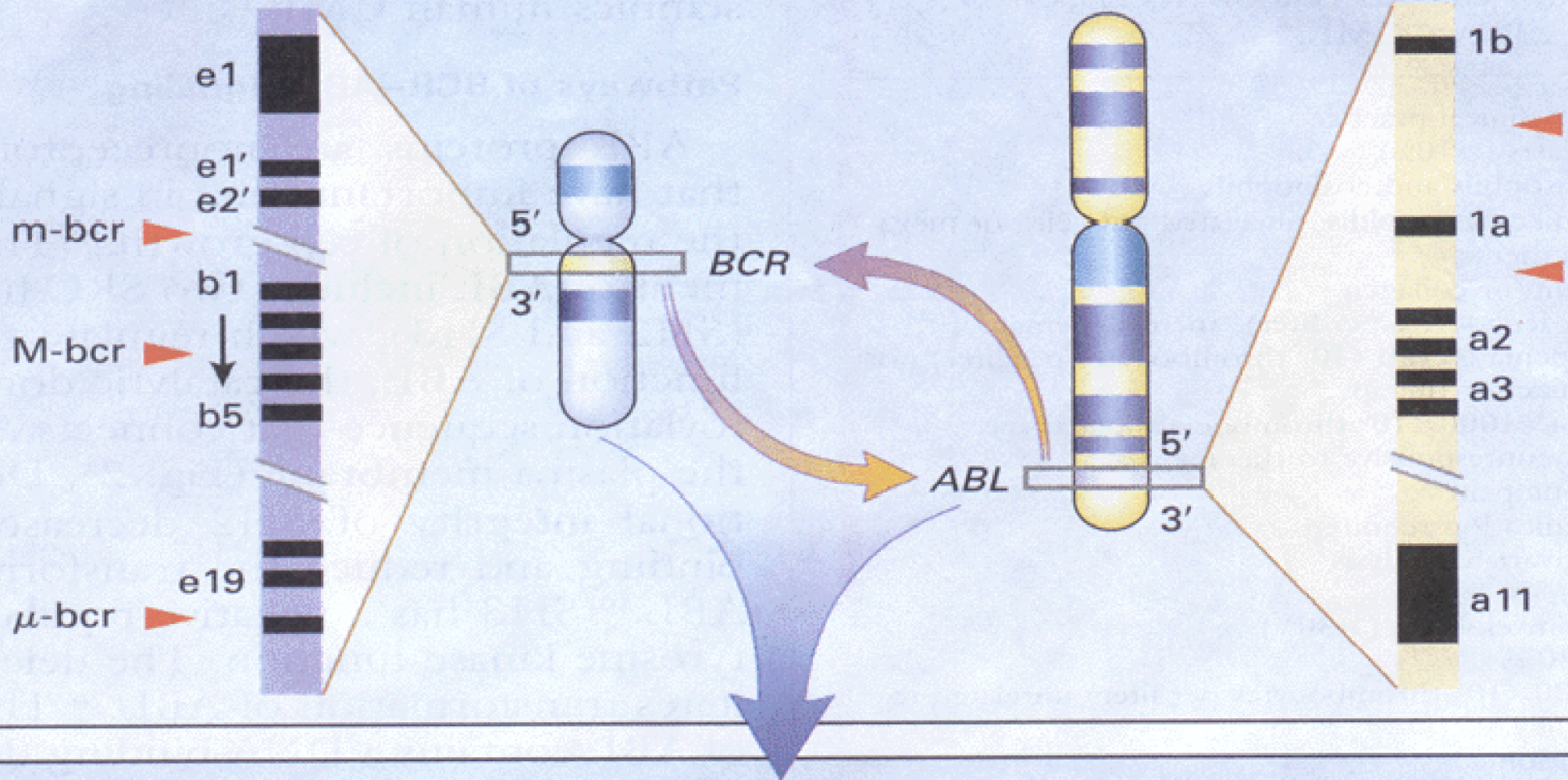
Relativně dobrá:  
+8, +Ph, -Y

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



Chromosome 22

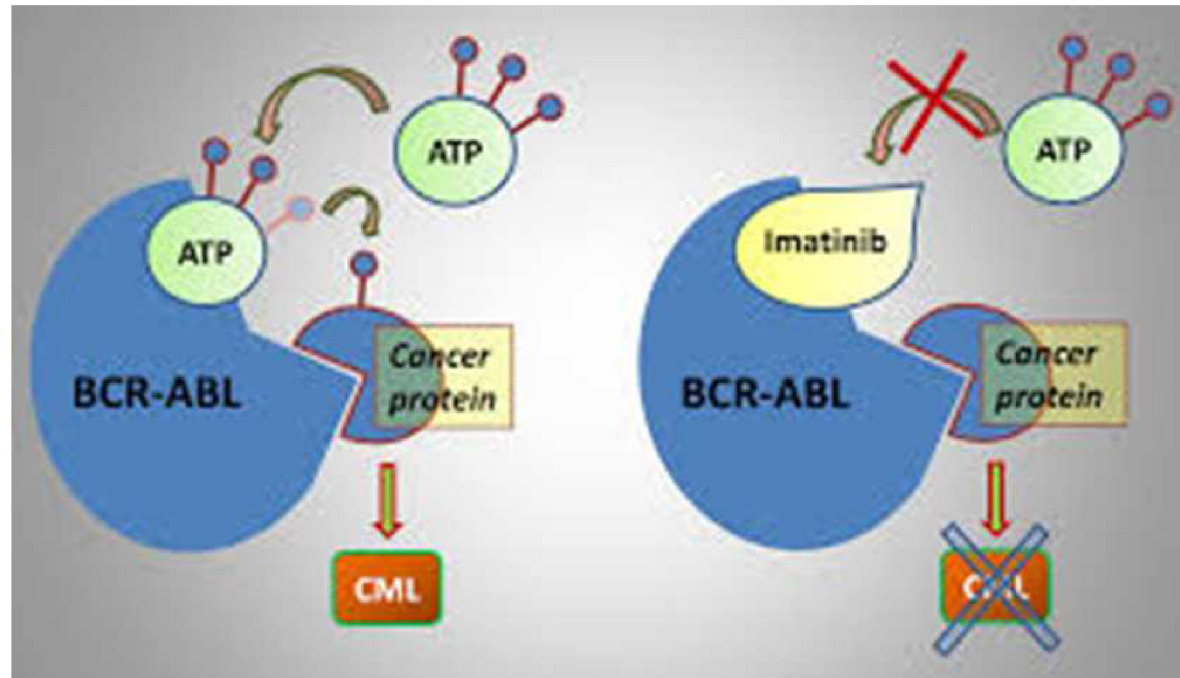
Chromosome 9

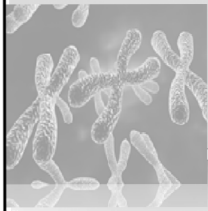


# Glivec (Imatinib)



# 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 <sup>4.0</sup> |                               | BCR-ABL/ABL $\leq$ 0.001% (IS) “4-log reduction”           |
| MR <sup>4.5</sup> |                               | 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 <sup>st</sup> line | 2 <sup>nd</sup> line | 3 <sup>rd</sup> line |
| 1 <sup>st</sup> | Imatinib  | 2003                 | 2001                 |                      |
| 2 <sup>nd</sup> | Nilotinib | 2011                 | 2008                 |                      |
|                 | Dasatinib | 2011                 | 2007                 |                      |
| 3 <sup>rd</sup> | 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,<sup>1</sup> Attilio Orazi,<sup>2</sup> Robert Hasserjian,<sup>3</sup> Jürgen Thiele,<sup>4</sup> Michael J. Borowitz,<sup>5</sup> Michelle M. Le Beau,<sup>6</sup> Clara D. Bloomfield,<sup>7</sup> Mario Cazzola,<sup>8</sup> and James W. Vardiman<sup>9</sup>

### 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<sup>a</sup>**

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

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

<sup>b</sup> Prognostic impact of a marker is treatment-dependent and may change with new therapies.

<sup>c</sup> 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.<sup>57-59,77</sup>

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

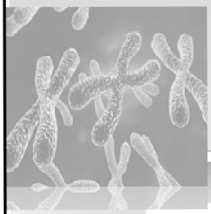
<sup>e</sup> Three or more unrelated chromosome abnormalities in the absence of one of the World Health Organization-designated recurring translocations or inversions, i.e., t(8;21), inv(16) or t(16;16), t(9;11), t(v;11)(v;q23.3), t(6;9), inv(3) or t(3;3); AML with *BCR-ABL1*.

<sup>f</sup> 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).<sup>16</sup>

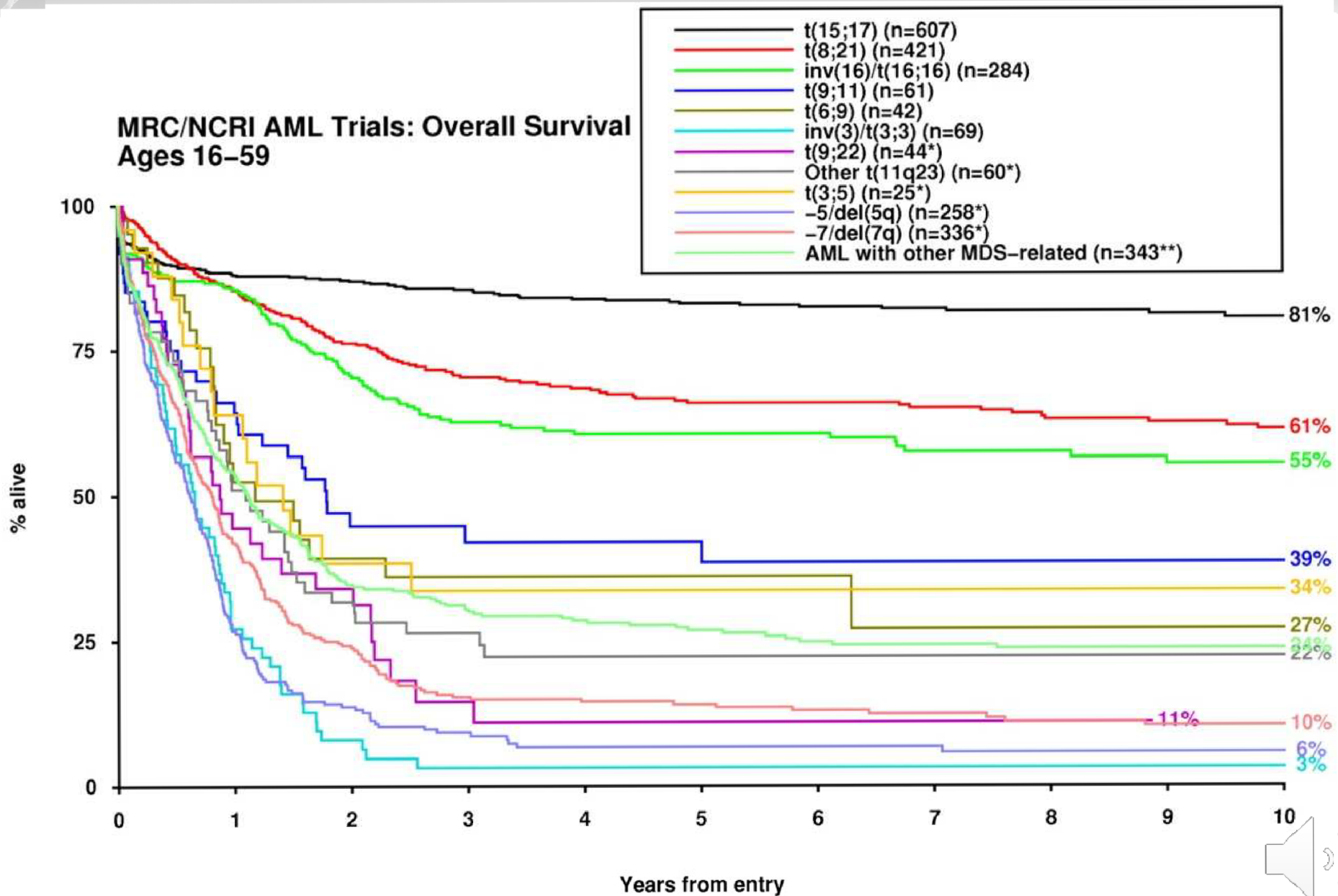
<sup>g</sup> These markers should not be used as an adverse prognostic marker if they co-occur with favorable-risk AML subtypes.

<sup>h</sup> *TP53* mutations are significantly associated with AML with complex and monosomal karyotype.<sup>37,86-89</sup>

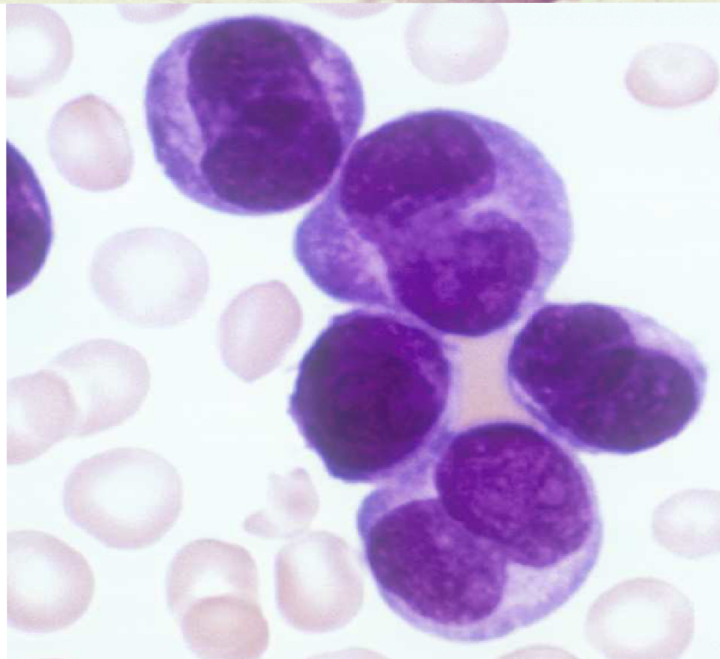
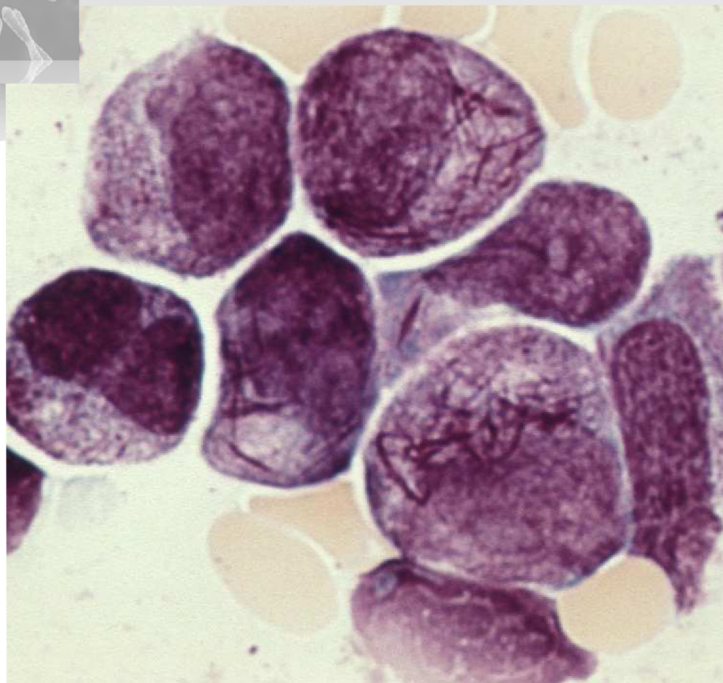




# Stratifikace podle cytogenetických nálezů



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



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

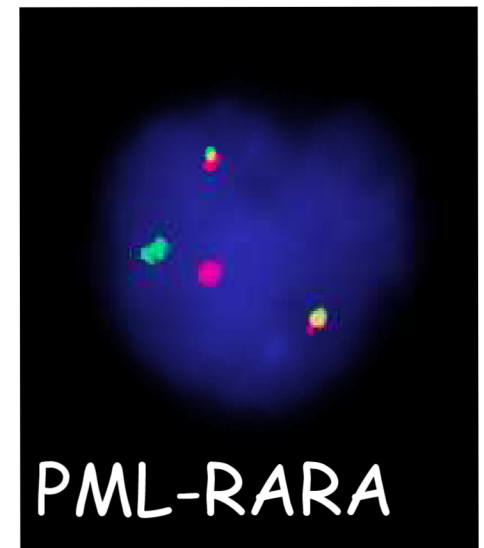
SIR,—We have described a similar chromosomal abnormality in two patients with acute promyelocytic leukaemia

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



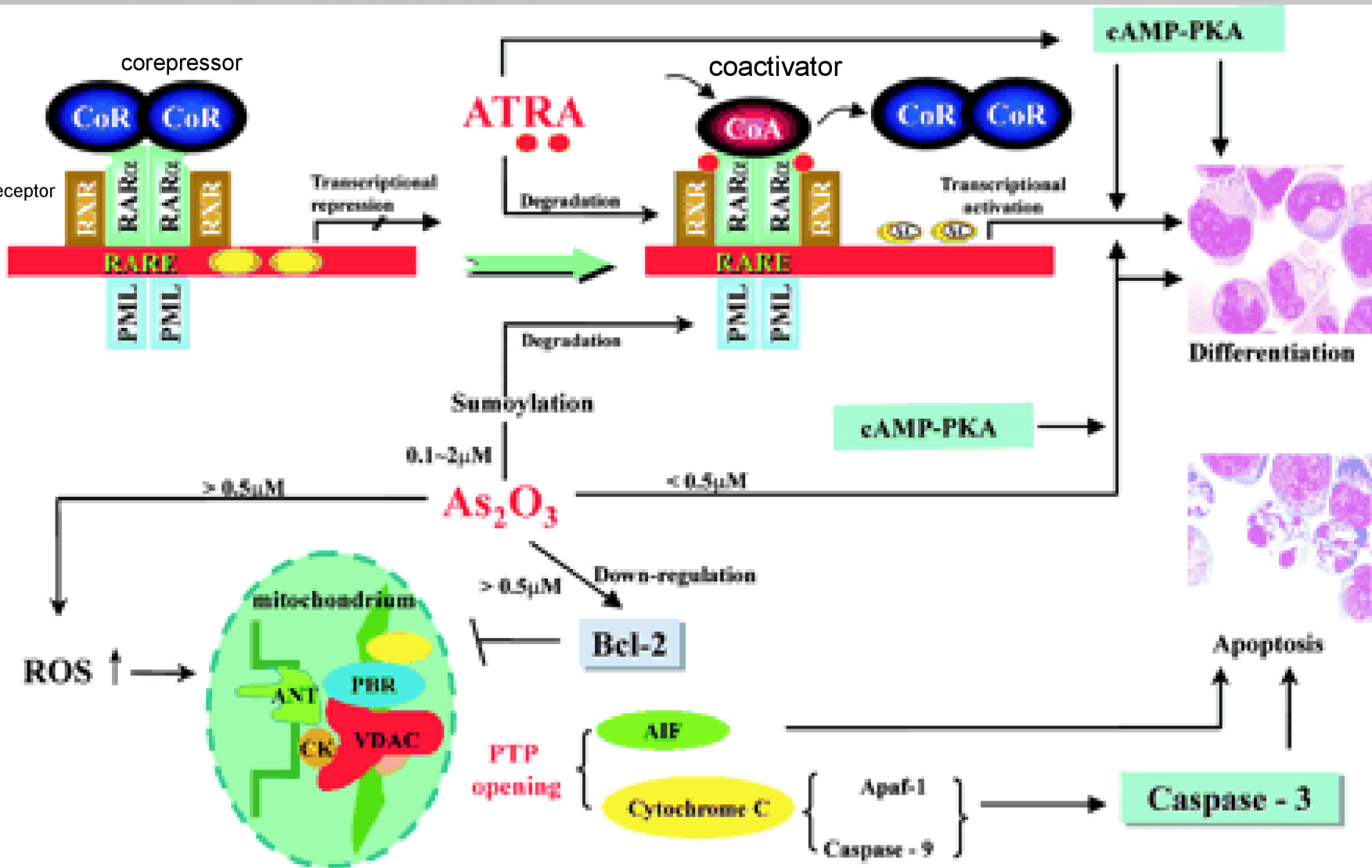
$t(15;17)(q22;q12)$



**PML-RARA**

# Cílená léčba nemocných s APL

retinoid X receptor



# 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.



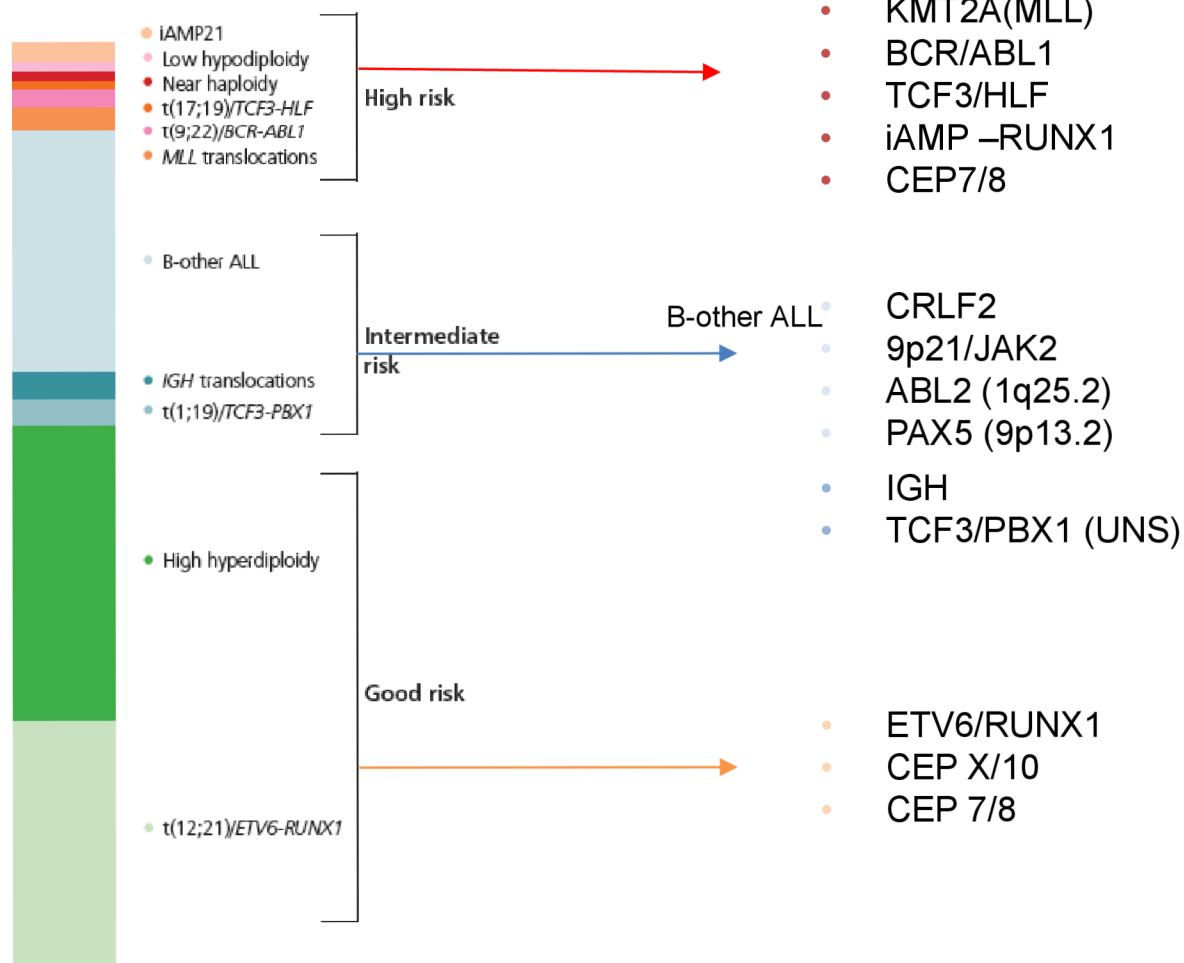


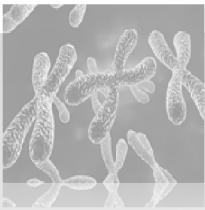
# Doporučení pro vyšetřování dětských BCP ALL

## 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)

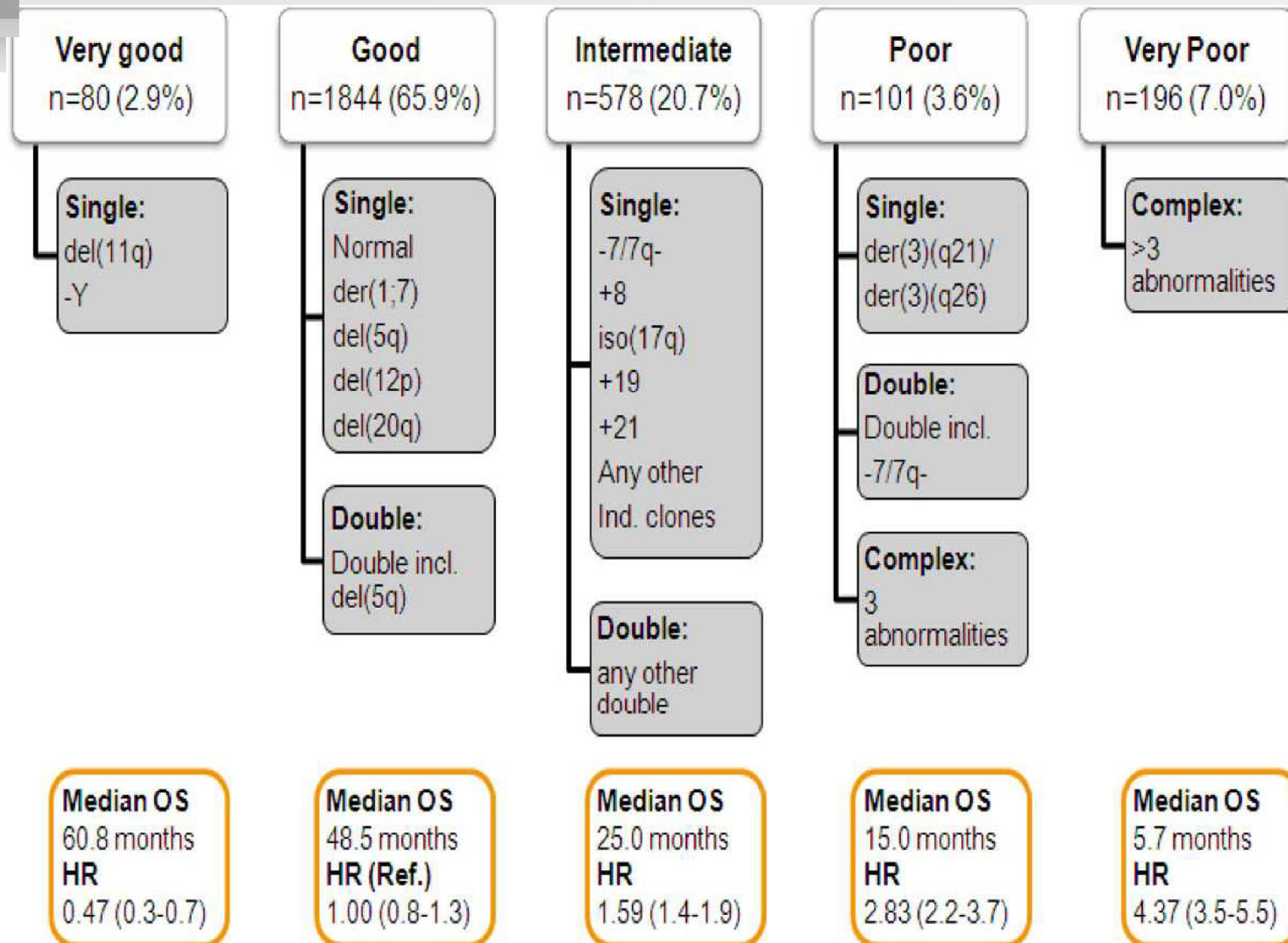
**Klinická heterogenita MDS je odrazem heterogenity získaných somatických genetických změn**

## Chromosomové změny u MDS

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

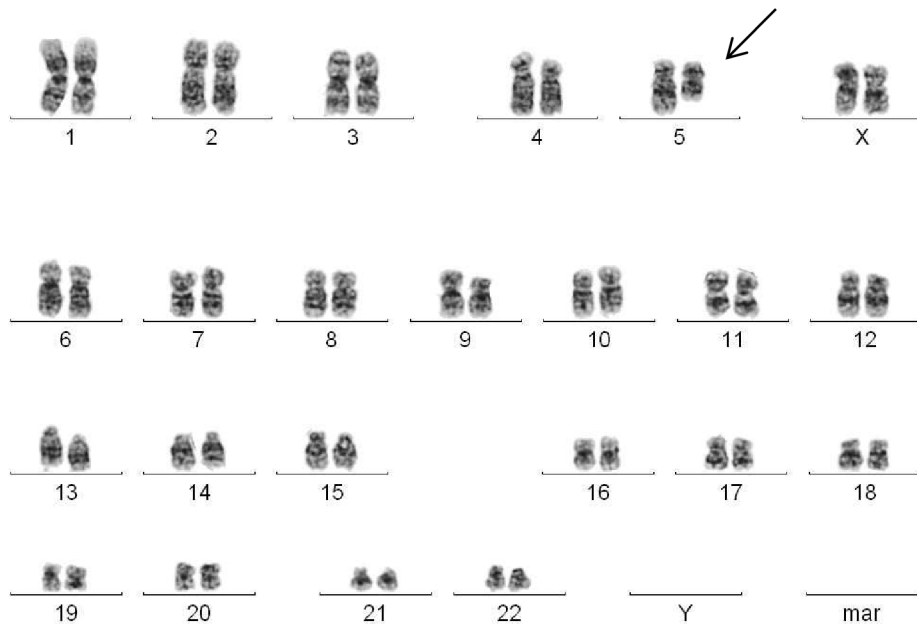


# Prognostická stratifikace MDS



# 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

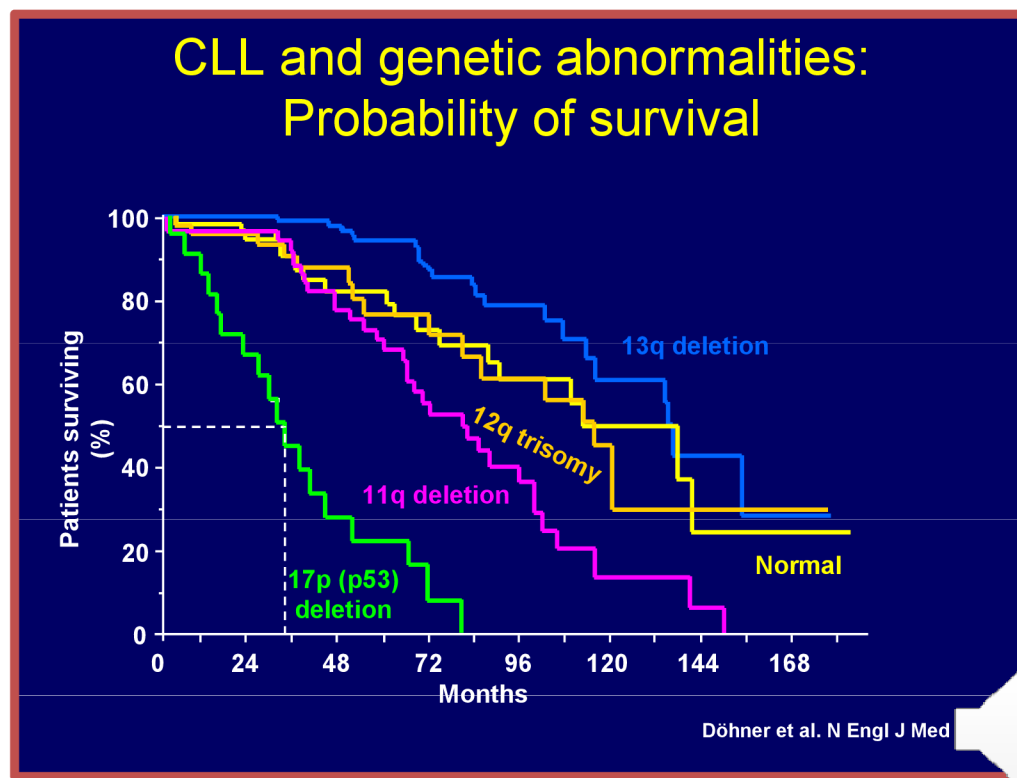


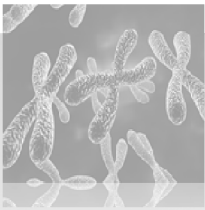
# CYTOGENETIKA CLL

## 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.

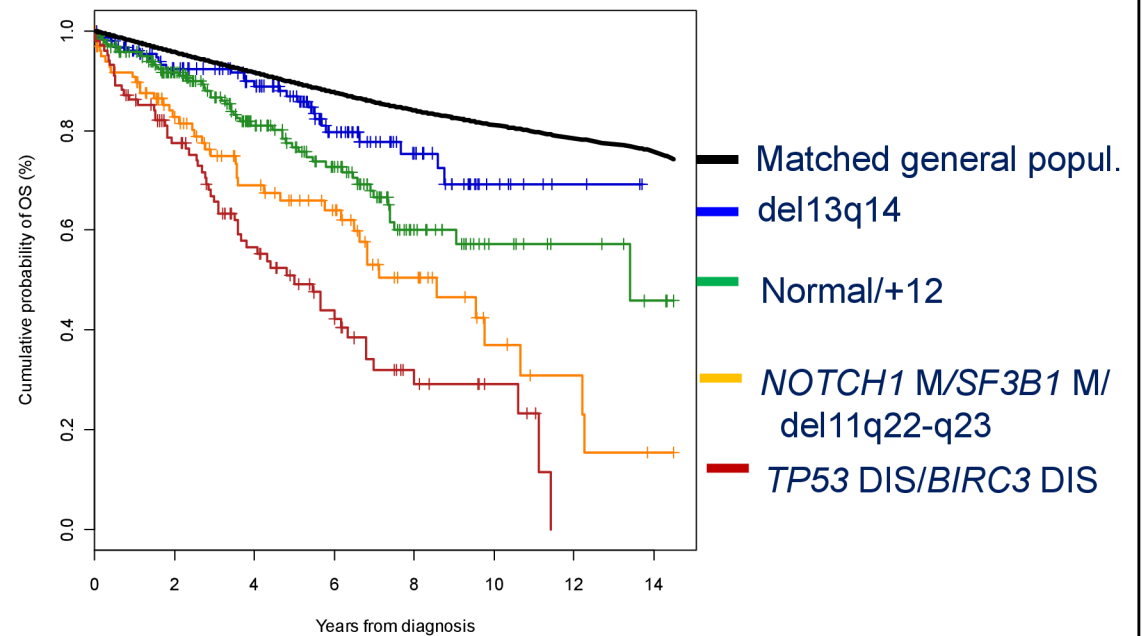
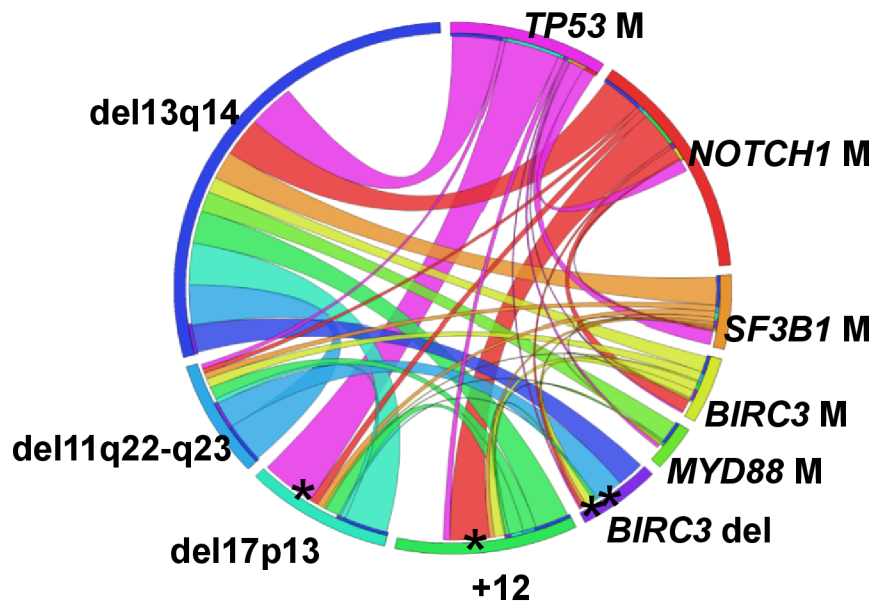
*N Engl J Med* 2000; 343:1910-1916.

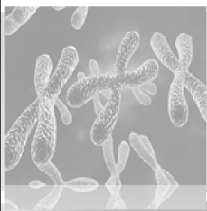




# Chronická lymfocytární leukemie (CLL)

## 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<br>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

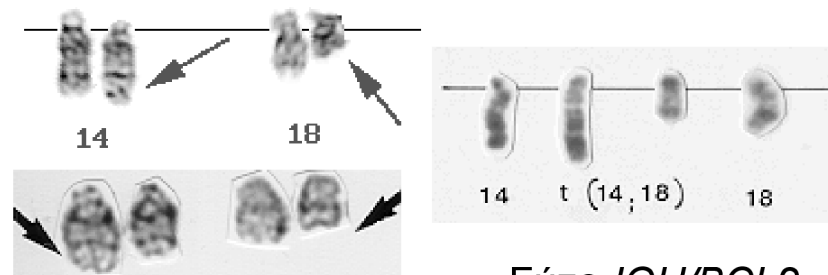
## Folikulární lymfom (FL)

indolentní B buněčný lymfom

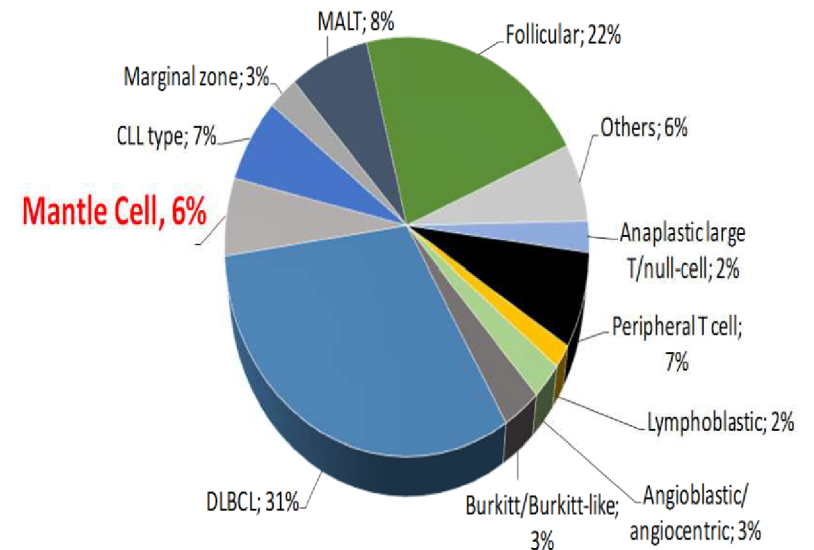
~20 % všech lymfomů

heterogenní klinický průběh , os několik roků až 20 let

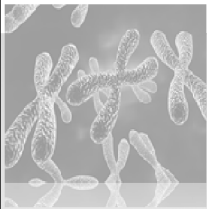
90% nemocných má translokaci t(14;18)(q32;q21)



Fúze *IGH/BCL2*

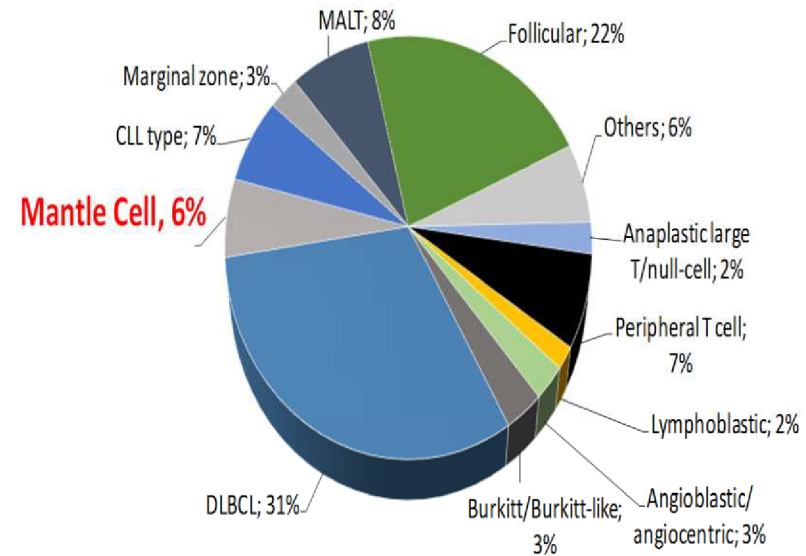






# MCL (mantle cell lymphoma) lymfom plášťových buněk

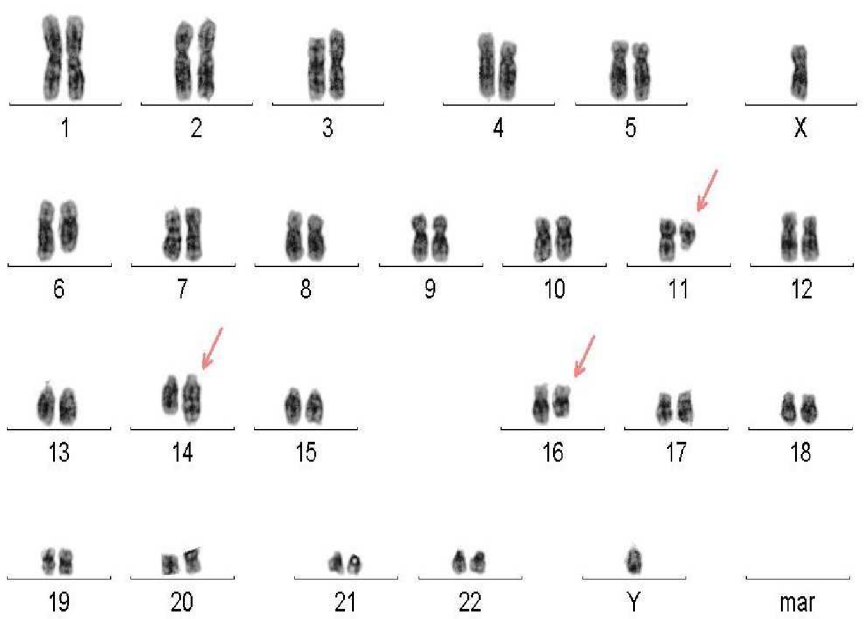
- Agresivní onemocnění (OS 3-5 let)
- ~ 6 % všech NHL
- Diagnostika:
- Morfologie
- Imunohistochemie
- Imunofenotypizace
- Genetika:
  - cytogenetika
  - FISH
  - molekulární genetika - PCR



# MCL (mantle cell lymphoma) lymfom plášťových buněk

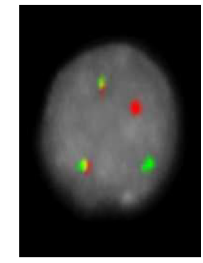
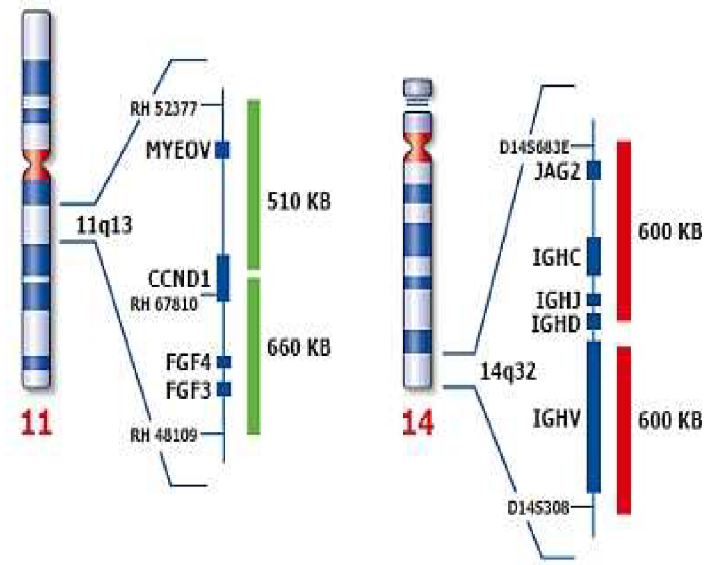
- Konvenční cytogenetika

t(11;14)

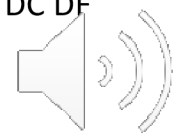


- FISH

dual color dual fusion probes

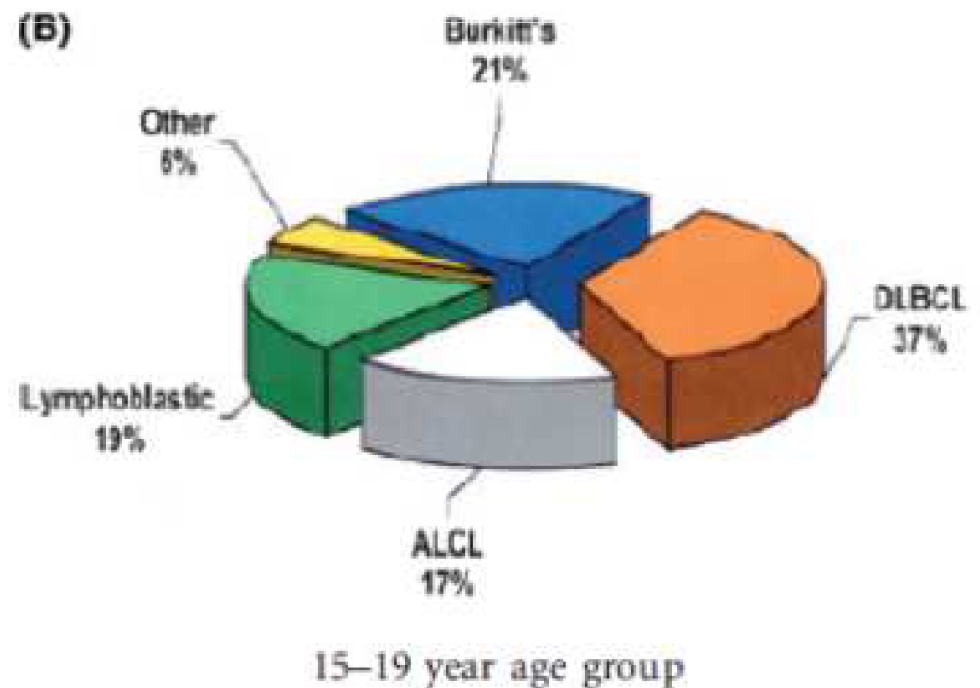
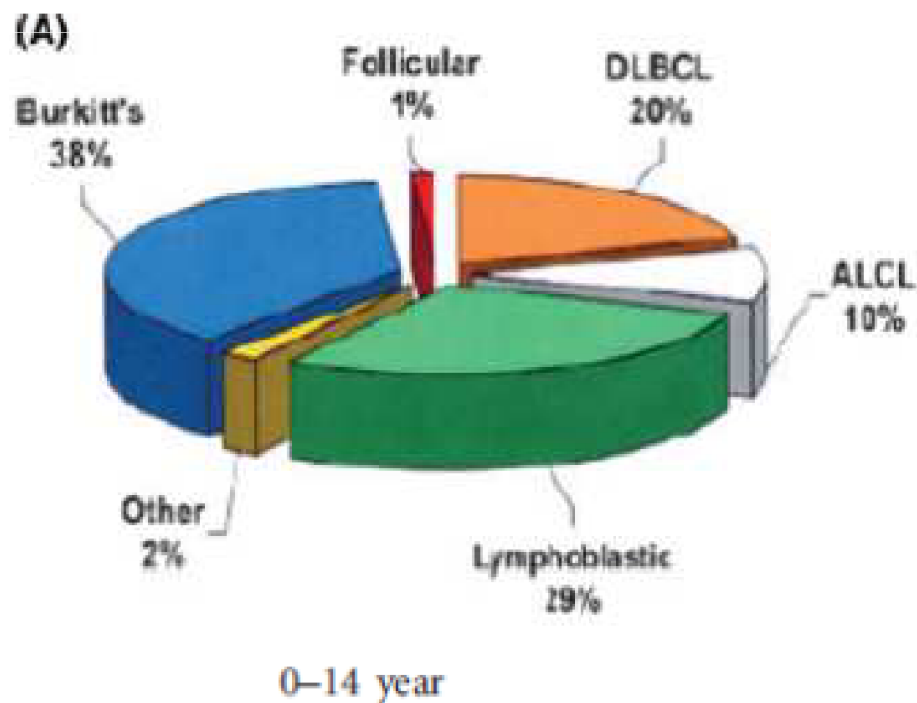


IgH/CCND1 DC DF  
Kreatech



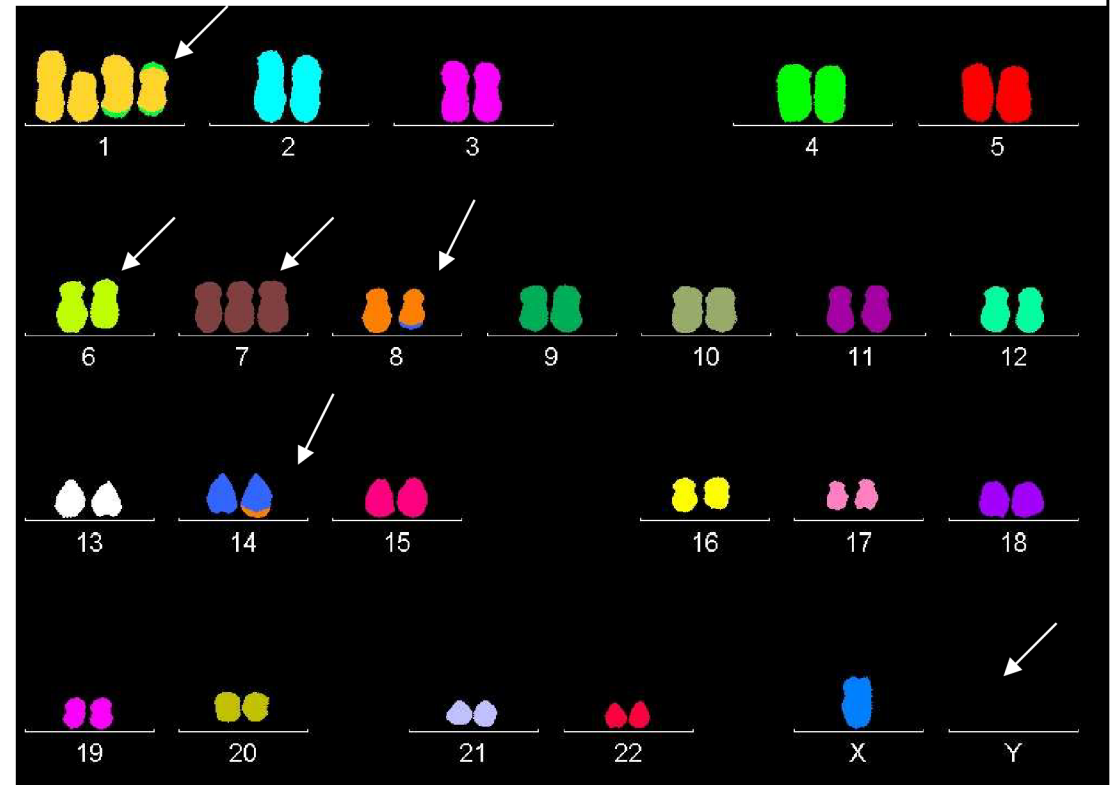
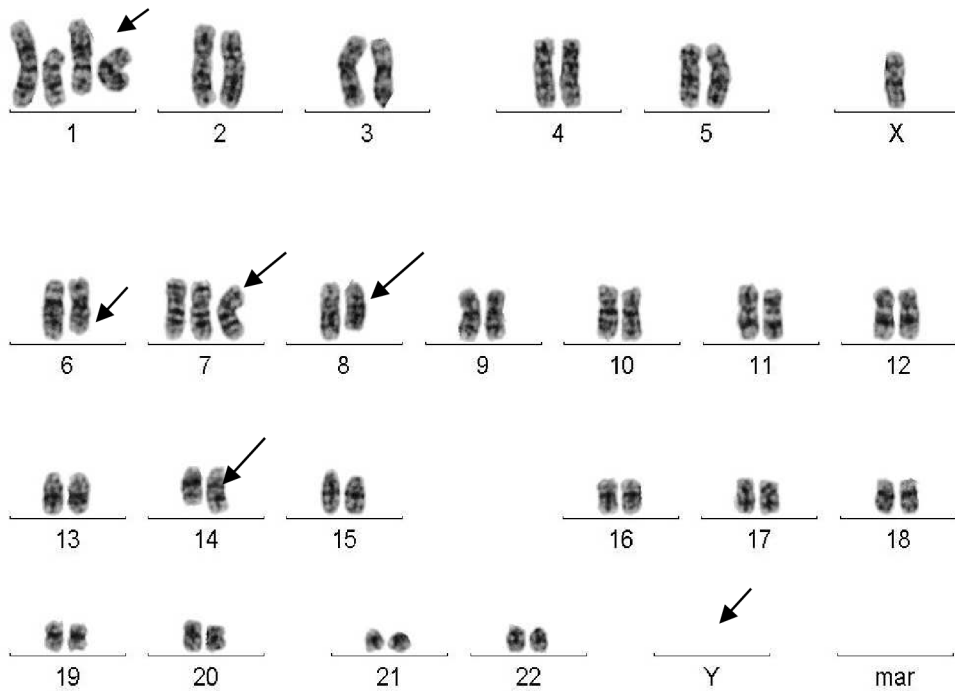
# Nehodgkinské lymfomy (NHL) u dětí

- 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%)

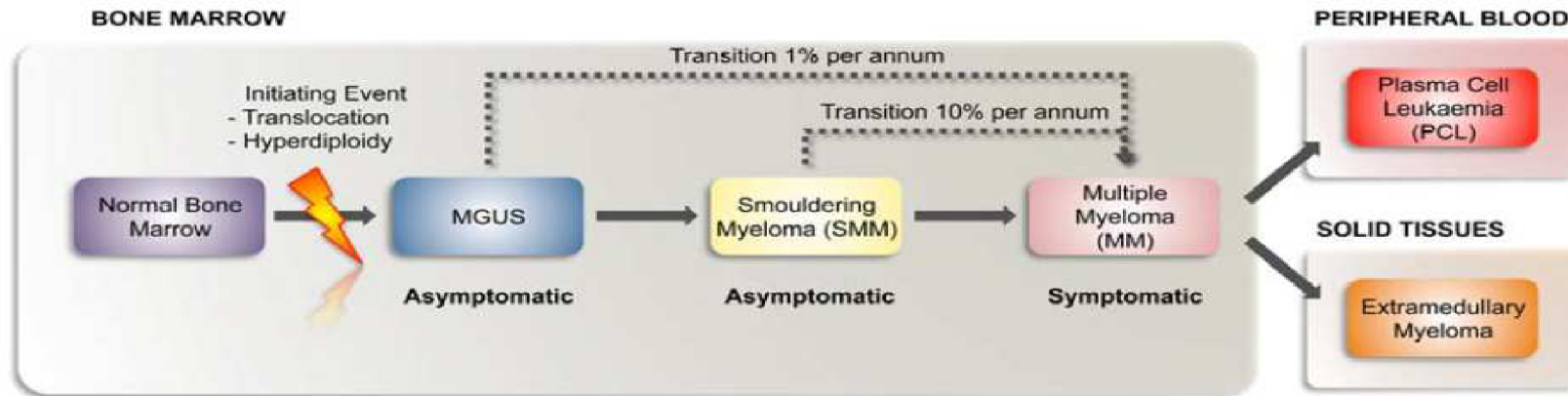


11/2011



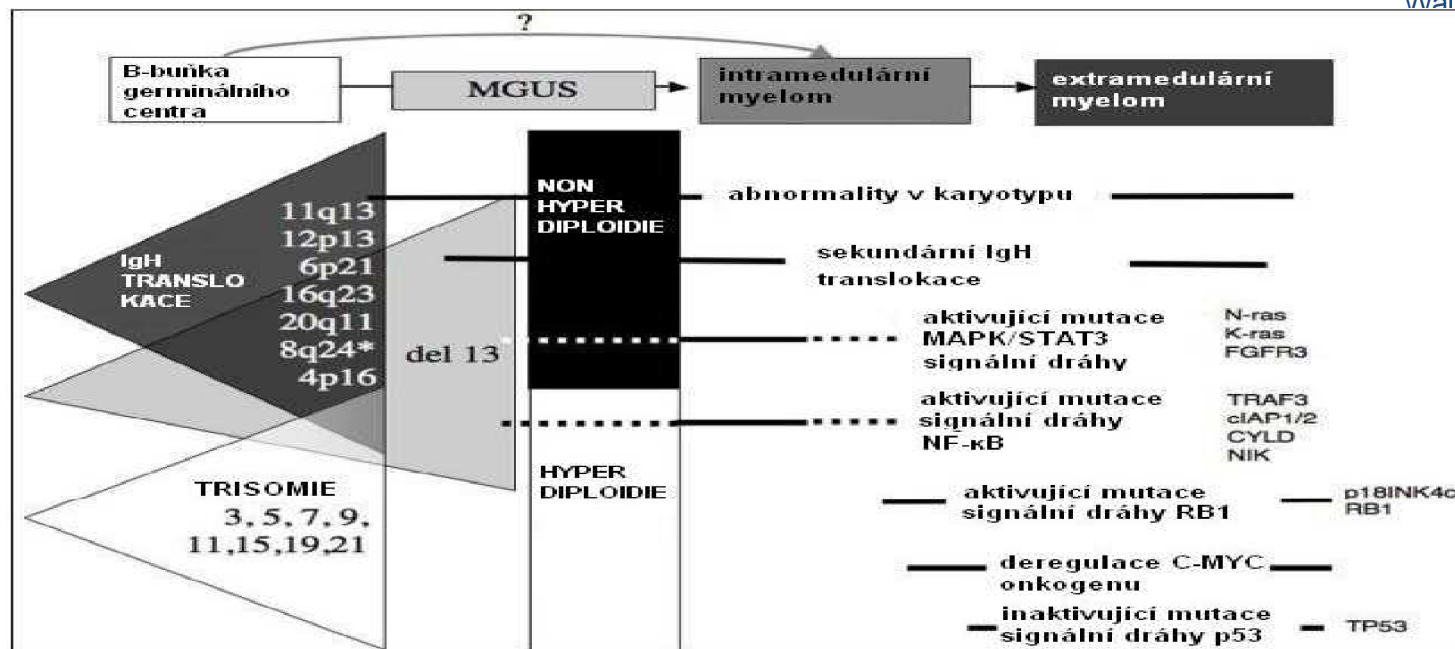
# MNOHOČETNÝ MYELOM

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



Accumulation of abnormalities throughout disease: CNV, SNV, methylation changes

Walker B,

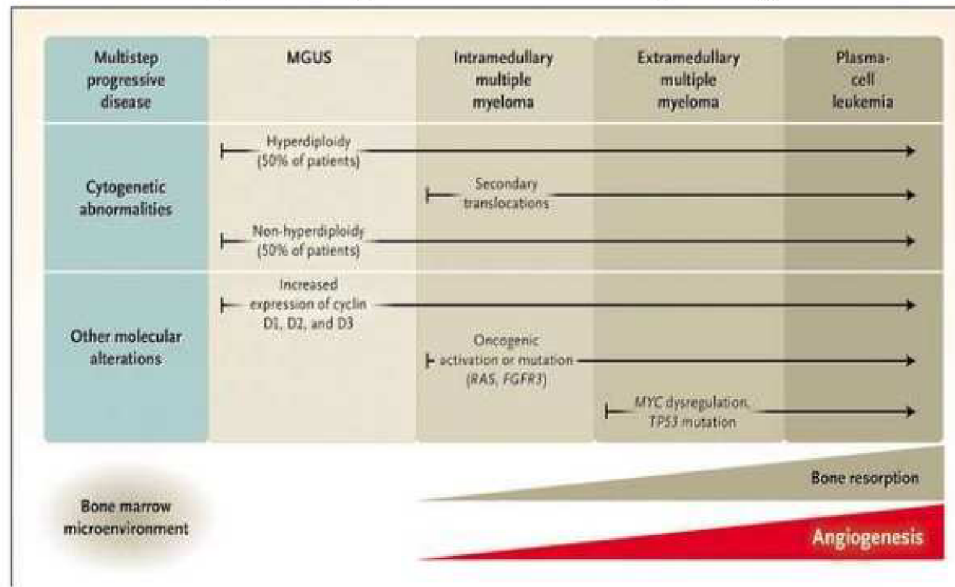


# MNOHOČETNÝ MYELOM

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 $\beta_2$ -microglobulin < 3.5 mg/L, serum albumin $\geq$ 3.5 g/dL        |
| II   | Not ISS stage I or III  |
| III  | Serum $\beta_2$ -microglobulin $\geq$ 5.5 mg/L                                  |
| CA by <i>t</i> 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 <i>t</i> FISH and normal LDH                |
| II   | Not R-ISS stage I or III  |
| III  | ISS stage III and either high-risk CA by <i>t</i> FISH or high LDH              |

Abbreviations: CA, chromosomal abnormalities; *t*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

