Genetic analyses in tumor diseases

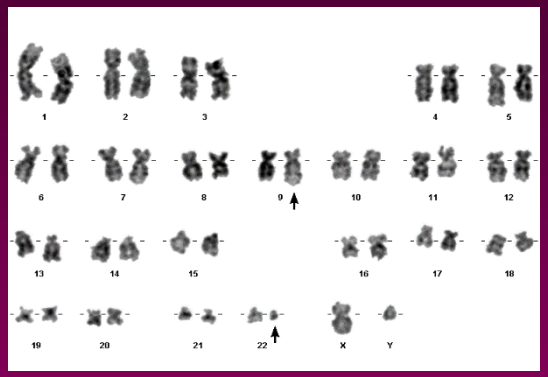
Developopmnet of tumor genetics
(cytogenetics)

David Hanseman (1890)
Theodor Boveri (1914)

The first theories on the relationship between mitotic disorders and tumorigenesis

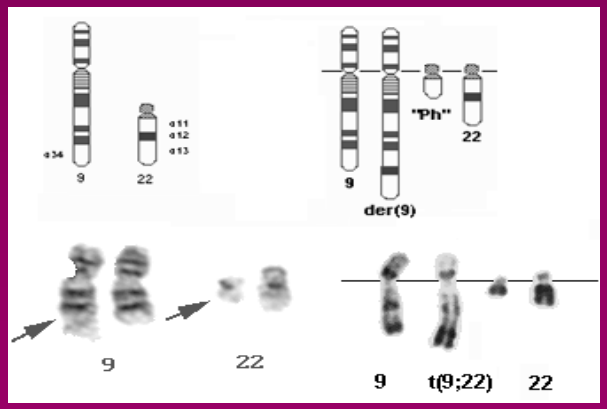
Nowell a Hungerford (1960)

Discovery of Ph chromozomu



Tjio and Levan (1956)

Rowley (1973)



A Minute Chromosome in Human Chronic Granulocytic Leukemia

In seven cases thus far investigated (five males, two females), a minute chromosome has been observed replacing one of the four smallest autosomes in the chromosome complement of cells of chronic granulocytic leukemia cultured from peripheral blood. No abnormality was observed in the cells of four cases of *acute* granulocytic leukemia in adults or of six cases of acute leukemia in children. There have been several recent reports of chromosome abnormalities in a number of cases of human leukemia [including two of the seven cases reported here: Nowell and Hungerford, *J. Natl. Cancer Inst.* 25, 85 (1960)], but no series has appeared in which there was a consistent change typical of a particular type of leukemia.

Cells of the five new cases were obtained from peripheral blood (and bone marrow in one instance), grown in culture for 24–72 hours, and processed for cytological examination by a recently developed air-drying technique (Moorhead, *et al.*, *Exptl. Cell Research*, in press). The patients varied from asymptomatic untreated cases to extensively treated cases of several years duration in terminal myeloblastic crisis. All seven individuals showed a similar minute chromosome, and none showed any other frequent or regular chromosome change. In most of the cases, cells with normal chromosomes were also observed. Thus, the minute is not a part of the normal chromosome constitution of such individuals.

The findings suggest a causal relationship between the chromosome abnormality observed and chronic granulocytic leukemia.

PETER C. NOWELL
School of Medicine, University of Pennsylvania
DAVID A. HUNGERFORD
Institute for Cancer Research

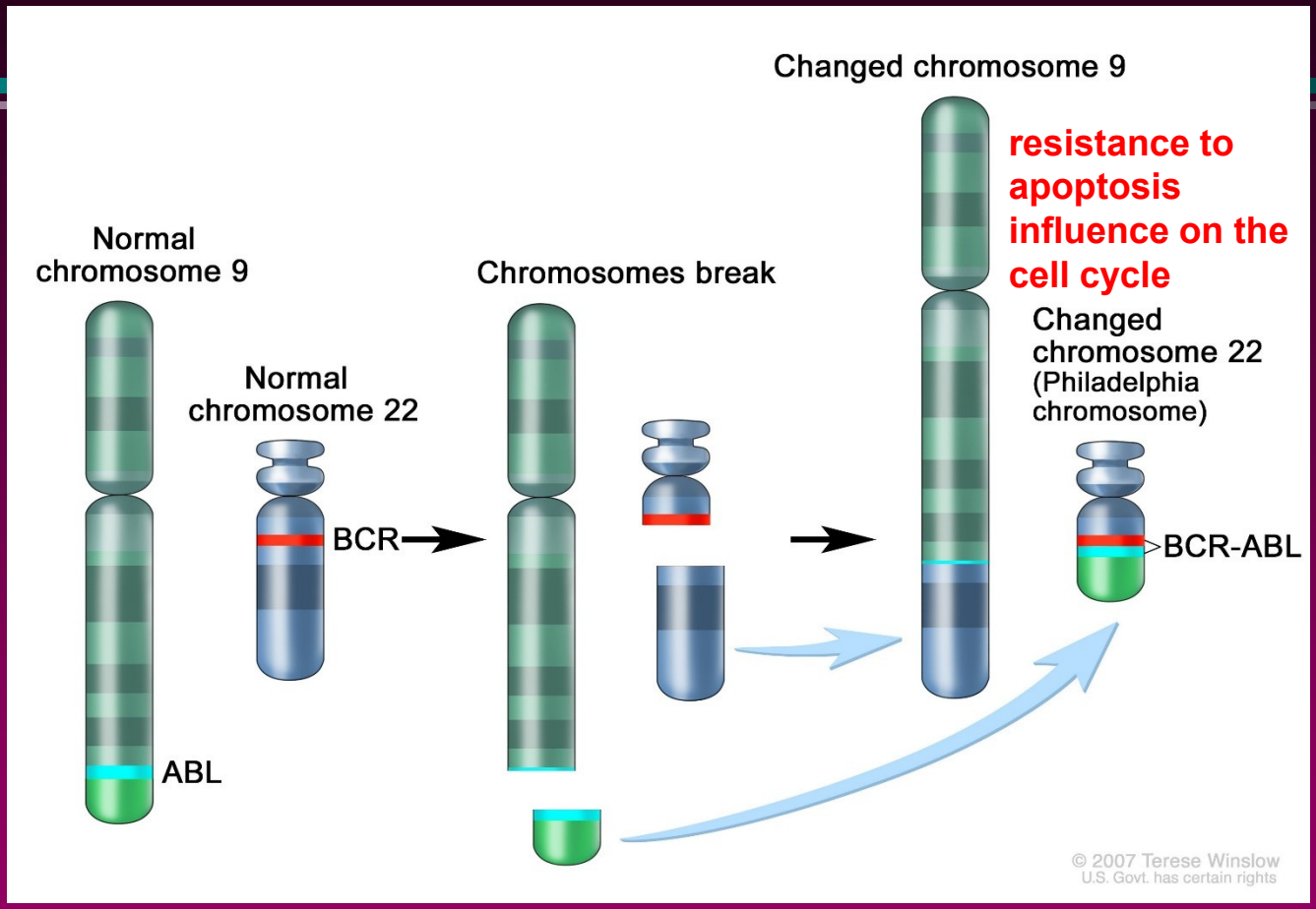
1960

A minute chromosome in human granulocytic leukemia. Science 132, 1960, 1497.

PC Nowell, DA Hungerford, University of Pennsylvania in Philadelphia

…the findings suggest a causal relationship between the chromosome abnormality observed and chronic granulocytic leukemia…

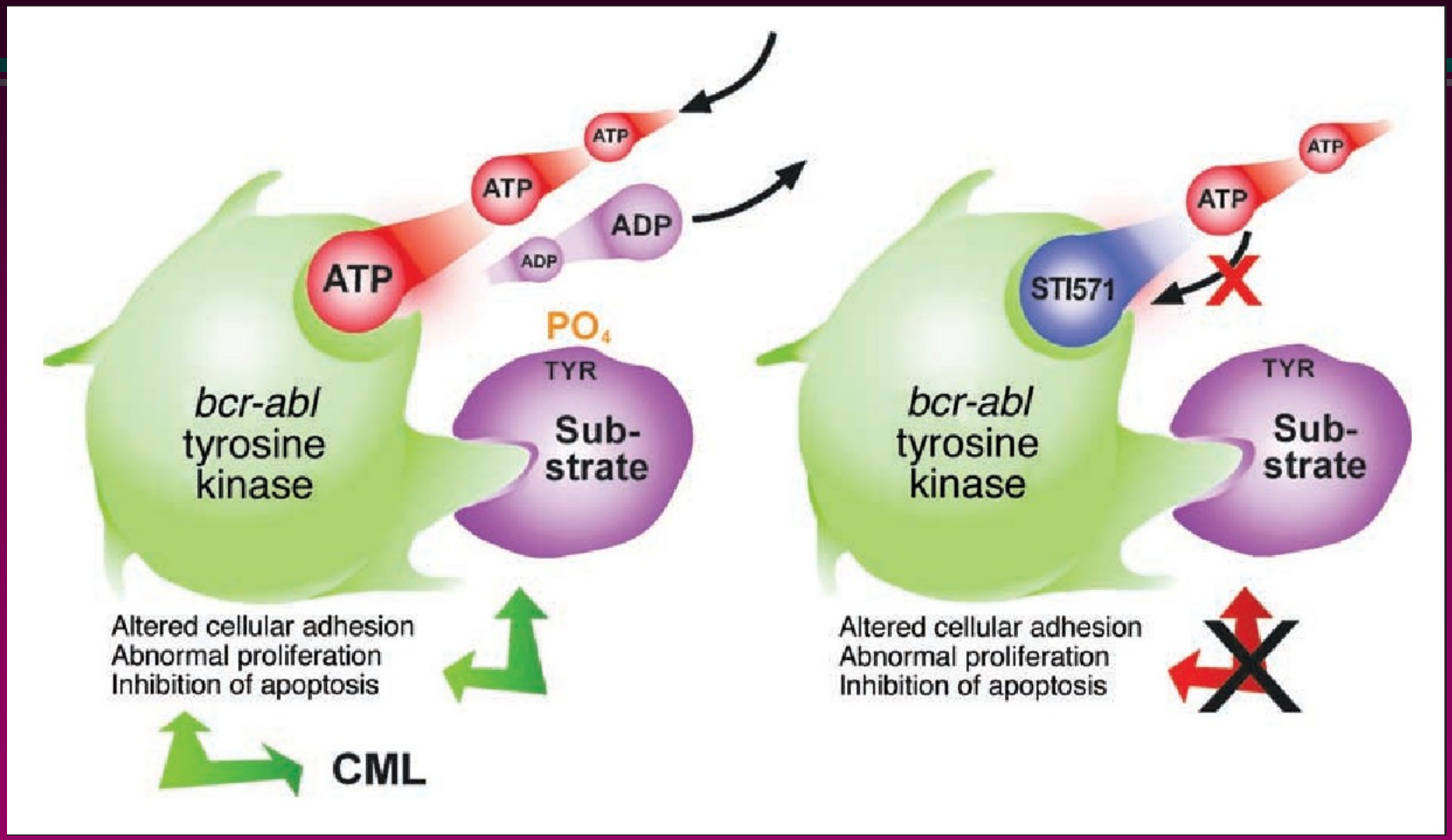
The origin of the Philadelphia chromosome (Ph) and the BCR - ABL fusion gene after translocation



Chronic myeloid leukaemia (CML) - 25% of all adult leukaemias with an incidence of 1-2 cases per 100,000 population

***Ph* chromozome - 95 % patients with CML**

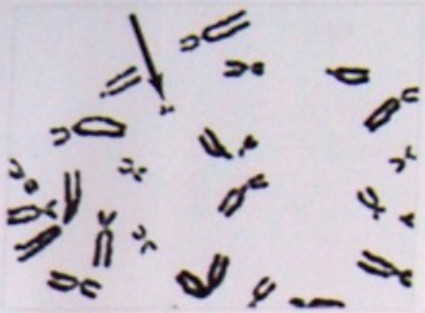
t(9;22) negative effects can be trated! 1998 - Clinical trilas of STI 571 agens(Glivec – Novartis) - inhibitor of TKs



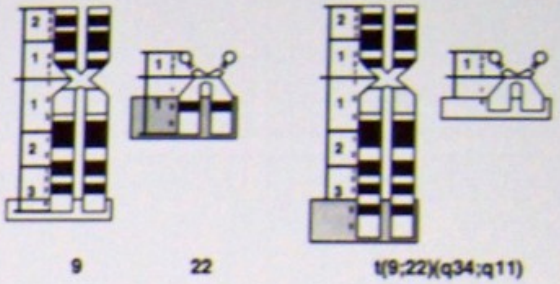
Tyrosine kinase inhibitors - imatinib, dasatinib and nilotinib - a model for modern non-cytostatic treatment of malignant diseases

!!Personalized medicine !!

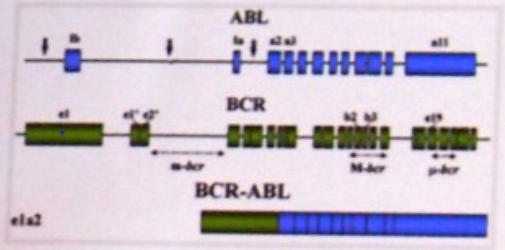
From Discovery to Targeted Treatment



1960: Discovery of the Philadelphia chromosome; the first recurrent cytogenetic change in neoplasia



1973: The Philadelphia chromosome results from a balanced translocation between chromosomes 9 and 22

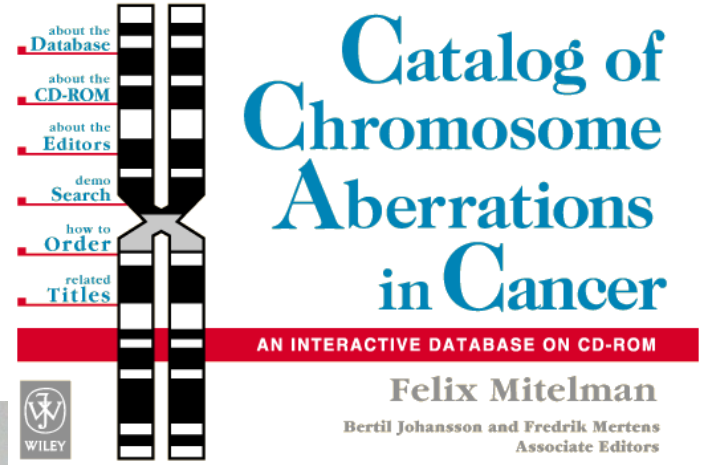


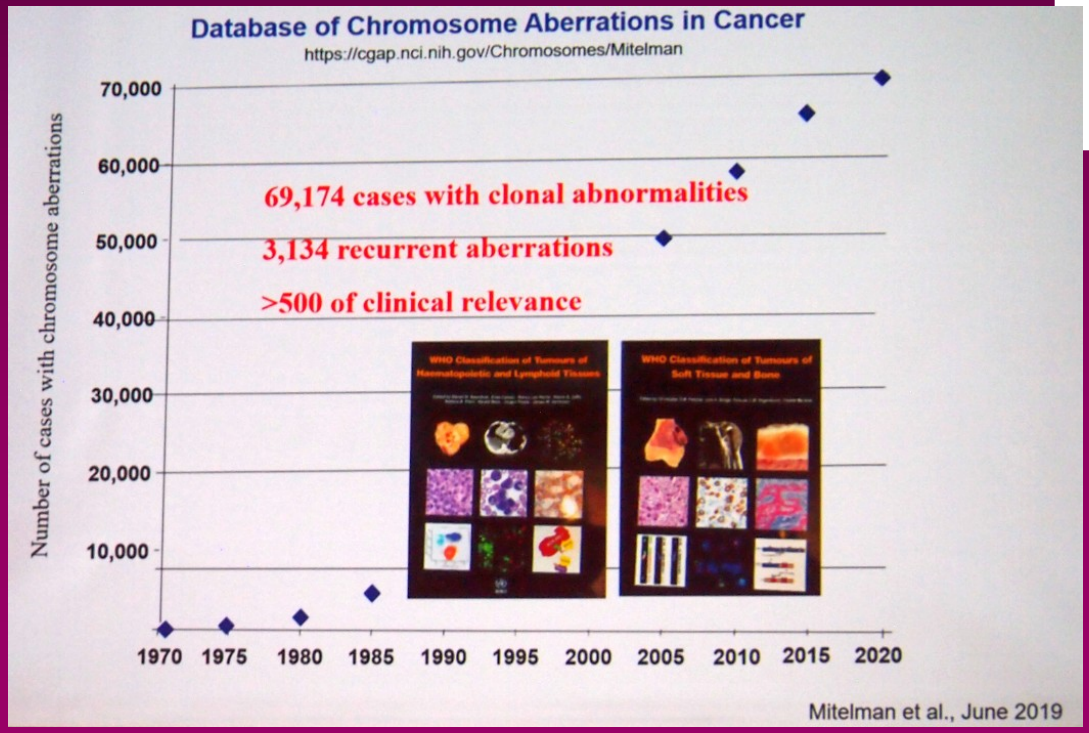
1985: The Ph chromosome results in the fusion of the BCR and ABL1 genes with increased tyrosine kinase activity

2001: Imatinib that specifically targets the BCR/ABL1 fusion gene is approved by the FDA

Database of chromosomal aberrations in cancer

Prof. Felix Mitelman





The role of cytogenetic testing in oncology

- an **integral part of oncohaematological** examinations (initial examinations) as well as examinations of **some solid tumours**
- cytogenetic and molecular genetic **methods** used in oncology are **part of the diagnosis** and treatment of many malignancies in the sense of:
  - **clarifying the diagnosis, determining the prognosis**
  - **stratification of patients, determination of treatment strategy**
  - **differential diagnosis**
  - **treatment monitoring (prediction of sensitivity to treatment)**
  - **monitoring residual disease after transplantation**
  - **prediction of the likely course of the disease**
  - **localization of proto-oncogenes and tumor suppressor genes**

- the **importance** of cytogenetic and molecular **genetic testing** in hematological malignancies is also **emphasized** in the classification of **myeloid certain cancers**, where separate entities with specific genetic changes **are distinguished**

Techniques used for detection of CHAs in tumor diseases

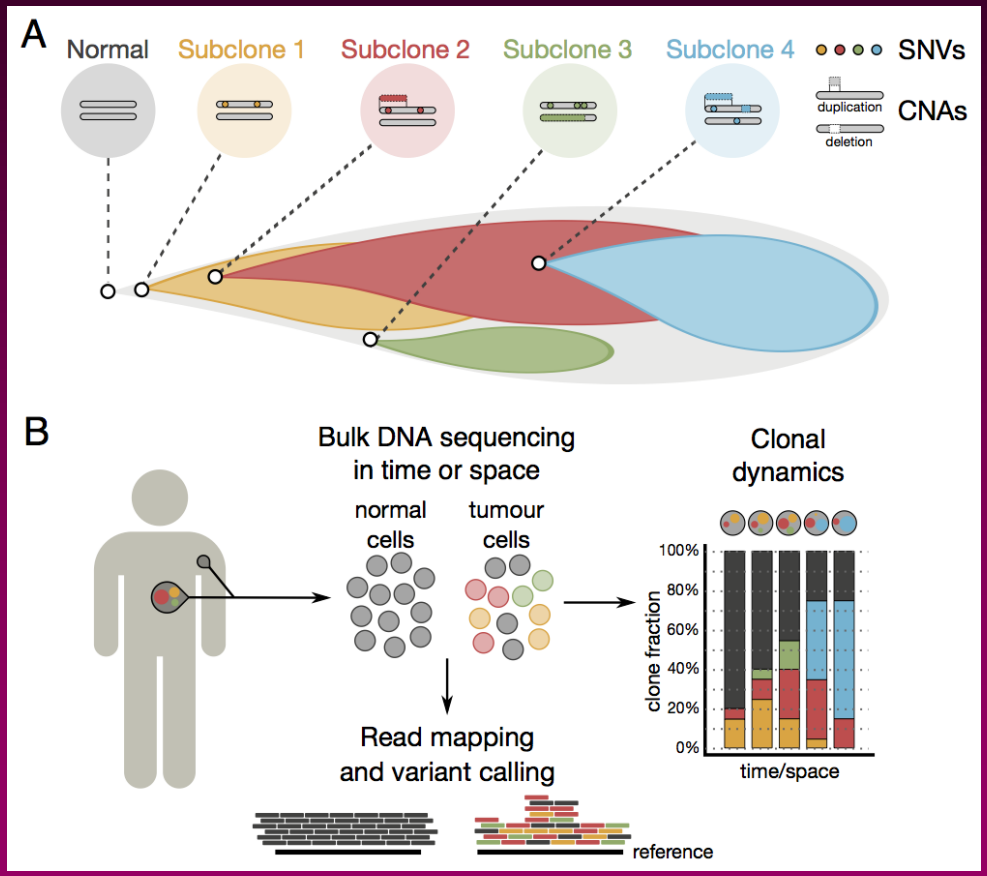
- Standard G- banding
- **Fluorescence in situ hybridization (FISH) - especially I-FISH - specific probes for tumor investigation - deletion, translocation, inversion....!!!**
- **-** always set a cut off for a given probe !
- **Additional methods**
- Multicolor FISH (M-FISH, SKY)
- Multicolor banding (M-BAND - complex rearrangements
- Microchip technology (array-CGH)

G-banding in tumor diseases

- basic examination method - tumor karyotype
- we use cells **cultured in vitro**, mainly from **bone marrow**, less from peripheral blood or other types of tissues
- cultured in culture medium at 37°C **without stimulation**
- success rate of cytogenetic examination of BM around 80%
- 150 - 200 bands (G-banding)
- **but...worse chromosome morphology, few mitoses**

- **Examination of karyotype in tumors is still of great importance- we evaluate individual mitoses, we can also detect small clones....**

Clonal CHAs in tumors



https://www.sanger.ac.uk/wp-content/uploads/140529-clonal_0.png

- **Tumor cell populations are composed of heterogeneous cells....important !!!!**
- **Most changes are clonal,** but we often find cells with normal karyotypes
- **The karyotype of a tumor evolves gradually ....we find several clones - selection advantage !!!**

- **<u>CLONE</u>**

= the same chromosome is missing in 3 mitoses

= 2 mitoses have the same supernumerary chromosome or the same structural aberration

Genome destabilization and the multistep process of tumor formation

Tumor formation is the result of mutations affecting genes:

1) cell aging (telomerase)

2) cellular proto-oncogenes –

e.g. *MYC, ERBB, MYB, INT1, RAS, NEU, ABL* (activation by insertional mutagenesis, point mutation, translocation, gene amplification) behave in a dominant manner

3) tumour suppressor genes *TP53, Rb1*

mutation, loss of gene or whole chromosome, loss of heterozygosty (LOH), recessive character

1% of tumours - gametic mutations

99% of tumours - somatic mutations

Chromosomal aberrations in tumours - basic classification

Primary	**basis for tumorigenesis, a single change, triggers a multistep process of carcinogenesis - e.g. t(9;22)**
Secondary	**secondary occur during the course of the disease, image of the stage of the disease, expression of tumour progression, sometimes a prognostic factor for disease progression**
Specific	**regularly in a certain type of tumour, the same chromosomes represent a specific tumour marker, genes involved in the tumour process have been identified at the breakpoints - influence on prognosis !!!**
Random	**they occur randomly, affecting different chromosomes**

Chromosomal changes in tumours - classification according to mechanism

loss of genetic material
(deletion, monosomy)

multiplication of genetic material
duplication, amplification, trisomy, polyploidy)

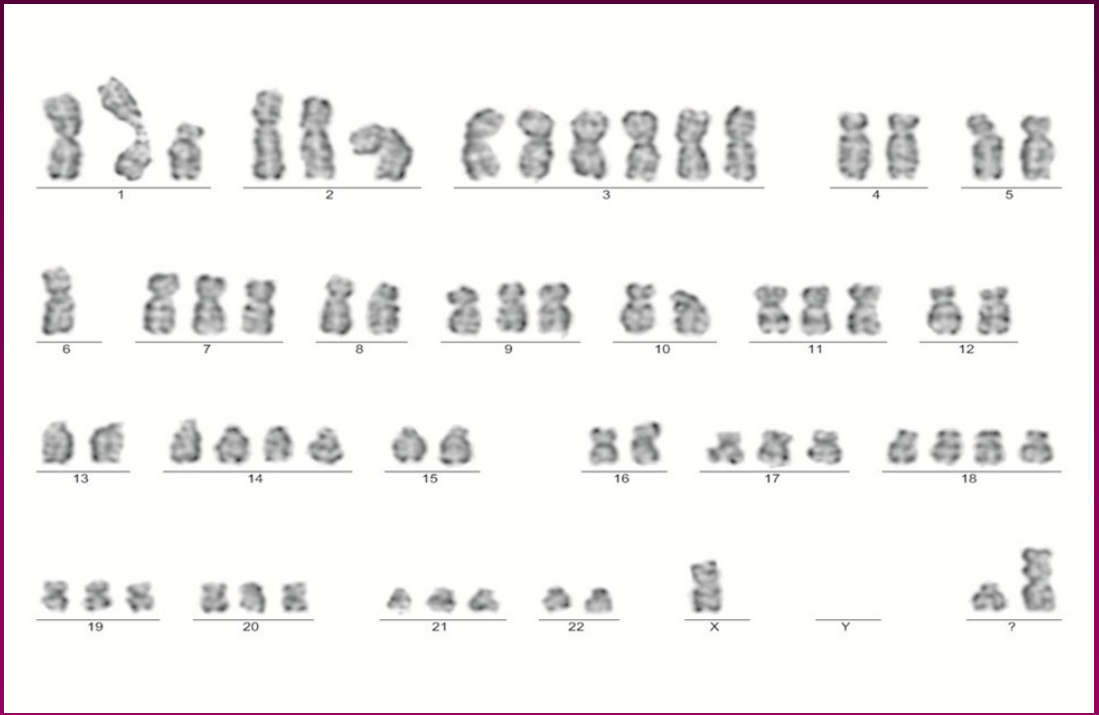
translocation without loss of material
(translocation, inversion, insertion)

Numerical CHAs in tumor diseases

- aneuploidy x polyploidy - **common in tumours !**
- **hyperdiploidy (more than 46 chromosomes**) - often better prognosis (ALL, multiple myeloma) ...more active tumor suppressor genes?
- **hypodiploidy - less than 46 chromosomes** - worse prognosis
- Methods of investigation - classical cytogenetics, I-FISH, flow cytometry, array-CGH (not ploidy)

Demonstration of hyperdiploid karyotype in a patient with myeloma - 63 chromosomes

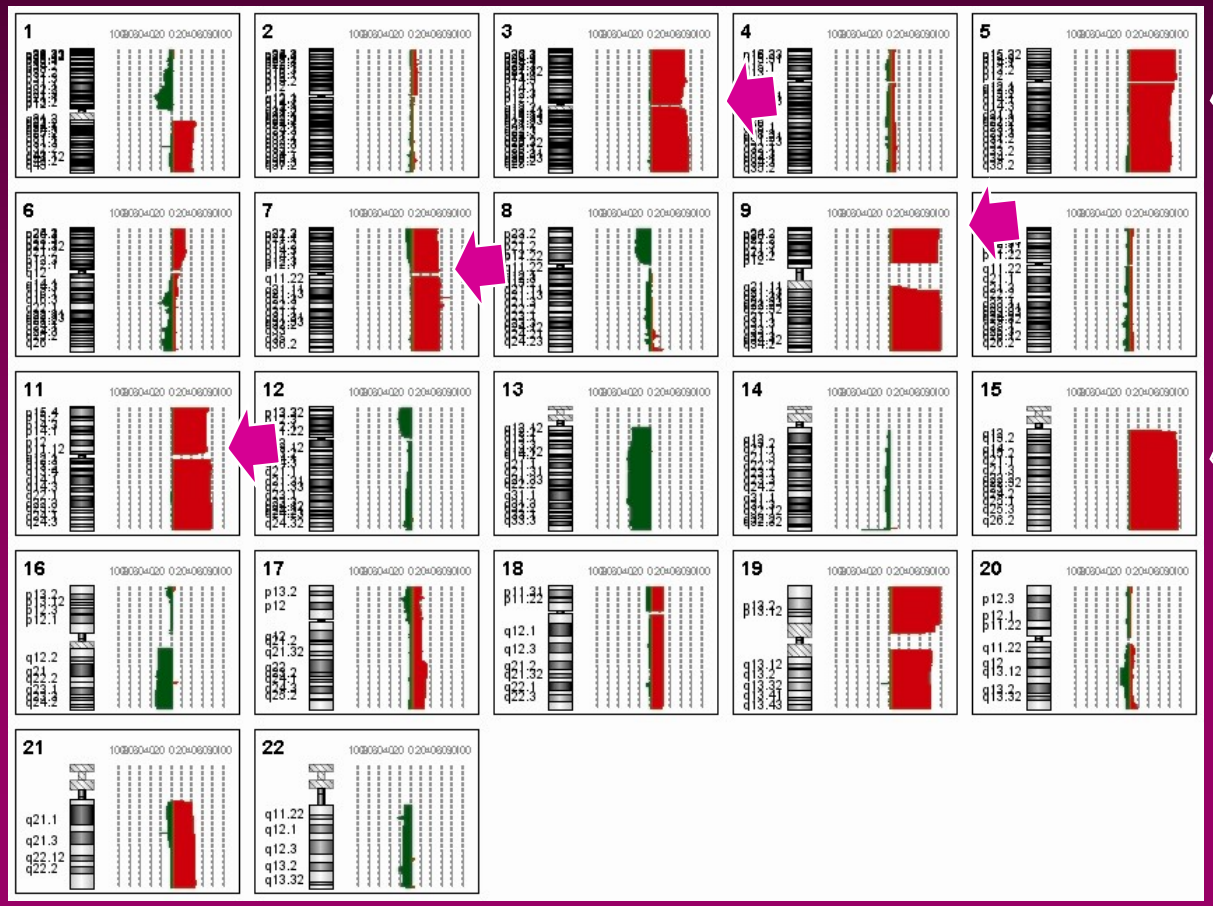
Multiple myeloma: hyperdiploid subgroup
non hyperdiploid subgroup



Hypotriploid karyotype from patient with extramedular myeloma relapse at the time of diagnosis detected by G-banding. ISCN: 63,X,-X,-X,der(1),+3,+3,+3,-4,-5,-6,-6,-8,-10,-12,-13,+14,-15,-16,+18,-22,+mar,+mar

Summary of whole genome profiles in 51 patients with multiple myeloma - hyperdiploid subtypes - "odd chromosome" trisomy array-CGH

Trisomy of chromosomes 3, 5, 7, 9, 11, 15, 17, 19 often occurs in MM - better prognosis !



Consequences of individual chromosomal aberrations in the process of carcinogenesis

Translocations

- **genes directly involved in the tumour process identified at the breakpoints**

There are two principal consequences of translocations and inversions:

- a break site within genes on each chromosome, rearranging them to create a fusion gene encoding a chimeric protein involved in the **malignant process**
- **deregulation of gene expression** by translocation to a strong promoter region (gene for T-cell receptor or immunoglobulin protein near proto-oncogene) ...often transcription factors

Translocations and tumors

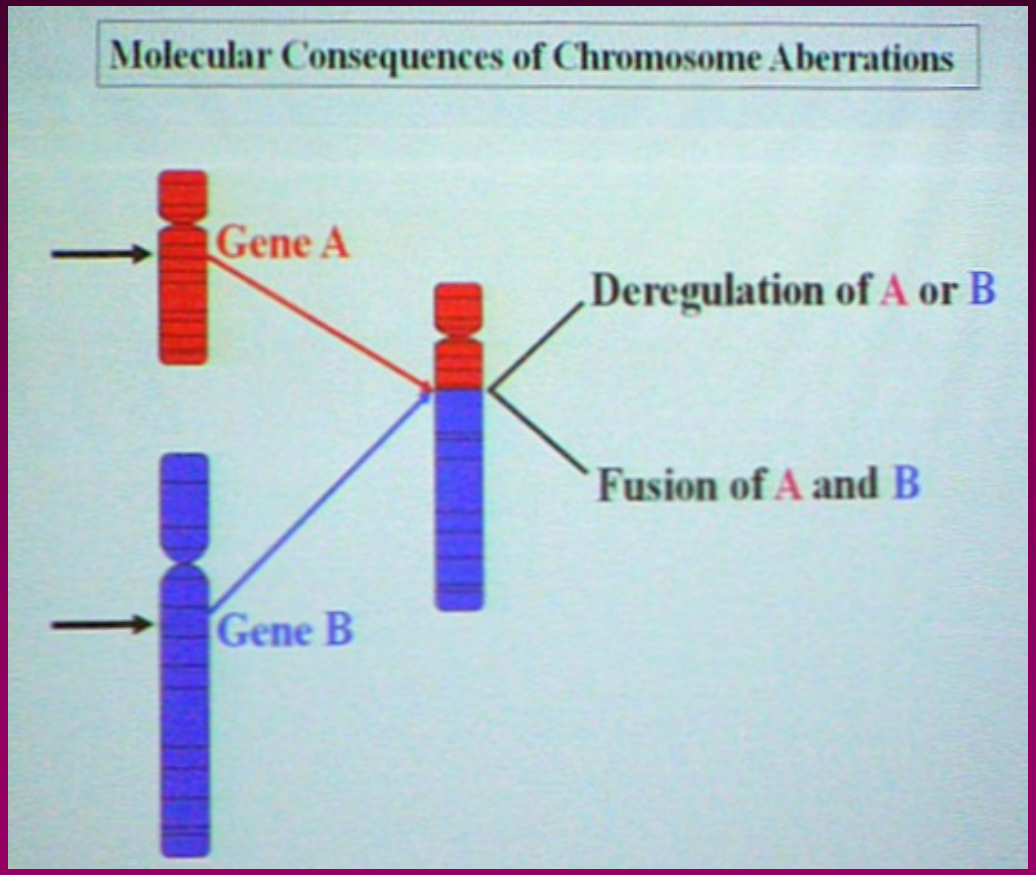


Table 1. Gene fusions in neoplasia reported 1980-2017, based on the Database of Chromosome Aberrations and Gene Fusions in Cancer (Mitelman et al., 2018).

Year	No. of reported gene fusions
1980-1984	5
1985-1989	18
1990-1994	69
1995-1999	140
2000-2004	216
2005-2009	442
2010-2014	2,778
2015-2017	8,914

Discovered fusion genes - counts
Hemat. l malignancies - 1231
Solid tumours - 9641

Consequences of individual chromosomal aberrations in the process of carcinogenesis

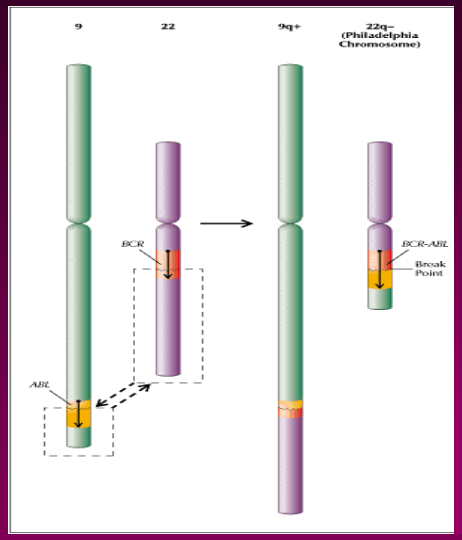
Translations associated with creation of chimeric proteins

t(9;22) – chronic myeloid leukemia (CML)

„PH“ chromosome

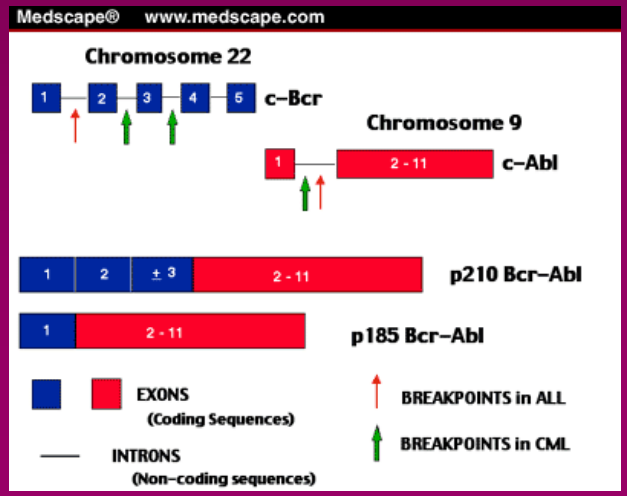
the cellular **proto-oncogene *ABL*** is localized on chromosome **9q34**, the ***BCR*** gene on chromosome **22q11**

- during translocation, mutual exchange of parts of both chromosomes cration of a **BCR/ABL** fusion gene is created which encodes the hybrid **protein p210**, which initiates the malignant process...several breakpoints



t(15;17) – acute myeloid leukemia (AML)

- **PML/RARA fusion protein** is likely the target protein in trans retinoic acid therapy
- direct relationship between treatment of genetic defect and malignancy



Consequences of individual chromosomal aberrations in the process of carcinogenesis

Translocations associted with protooncogen activation

t(8;14) - Acute Lymphoblastic Leukemia

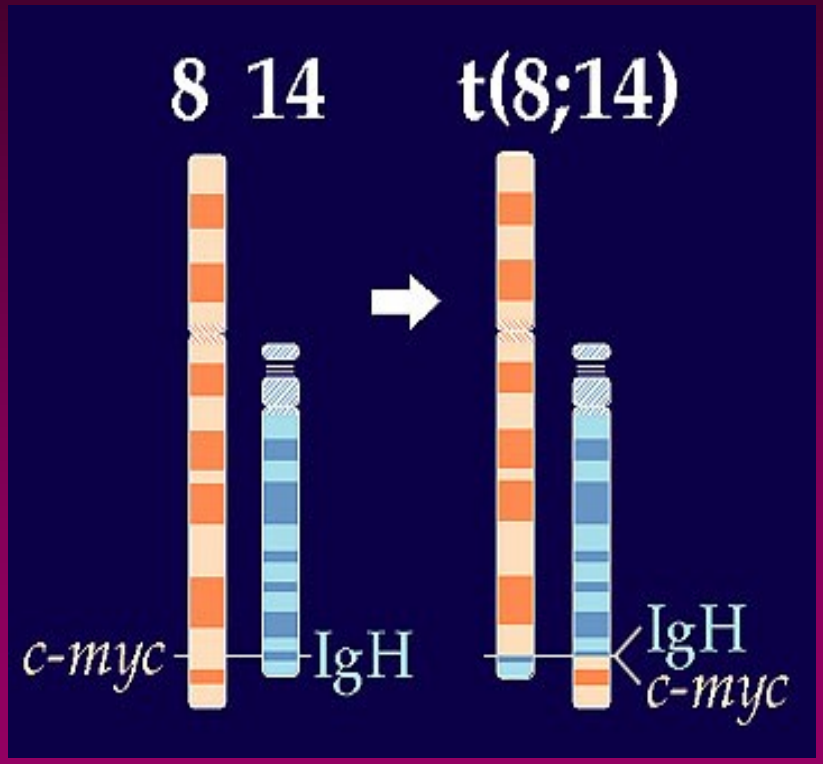
- c-MYC oncogene from 8q24 moved to the immunoglobulin heavy chain gene region on 14q32
- the relocation results in deregulation of c-MYC proto-oncogene expression

  deregulation triggers the process of carcinogenesis

t(2;8), t(8;22)

- variant translocation with a breakpoint in the c-MYC gene

  ...regions of the kappa (2p11) and lambda (22q11) light chain genes



Examples of translocations in B cell lymphomas – involvement of IgH locus 14q32!

MALT lymphomas (mucosa-associated lymphoid tissue)

- recurrent translocations associated with include **t(11;18)(q21;q21); t(1;14)(p22;q23); t(14;18)(q32;q21) and t(3;14)(p14.1;q32)**

Follicular lymphoma (FL)

- the recurrent translocation associated with FL is characterised by **t(14;18)(q32;q21)**

Mantle cell lymphoma (MCL)

- the recurrent translocation associated with is **t(11;14)(q13;q32)**

Burkitt lymphoma (BL)

- characterised typically by t(8;14)(q24;q32)

Consequences of individual chromosomal aberrations in the process of carcinogenesis

Deletions - examples

- loss of part of a chromosome, often affecting **tumour-suppressor genes** (***RB1, p53***) or genes for stimulatory and growth factors
- **LOH- loss of heterozygosity due to gene deletion**
- **specific changes in haematological malignancies**

del 5q - acute myeloid lekuemia, myelodysplatic syndrome

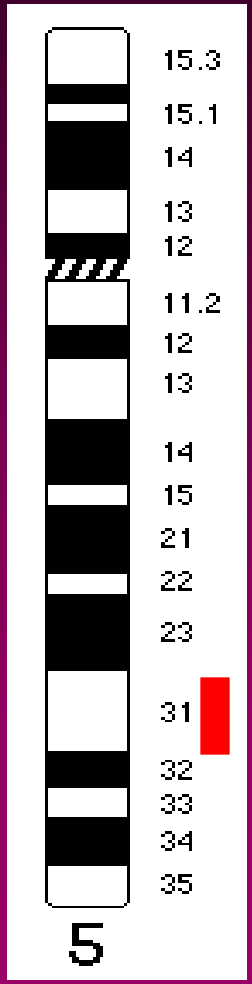
- in AML and MDS, the deletion is interstitial, the **extent is highly variable**,
- **5q31-critical region is always affected**, multiple genes controlling normal hematopoiesis are mapped in the region

del 11q23

- in AML and ALL, ***MLL*** gene is mapped in the 11q23 region, moreover *MLL* involved in numerous translocations (6q27, 9p21, 10p15, 17q11, 19p13) besides deletions

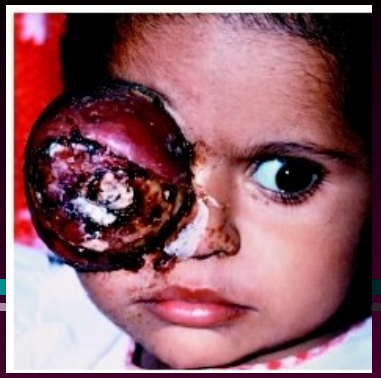
Monosomy

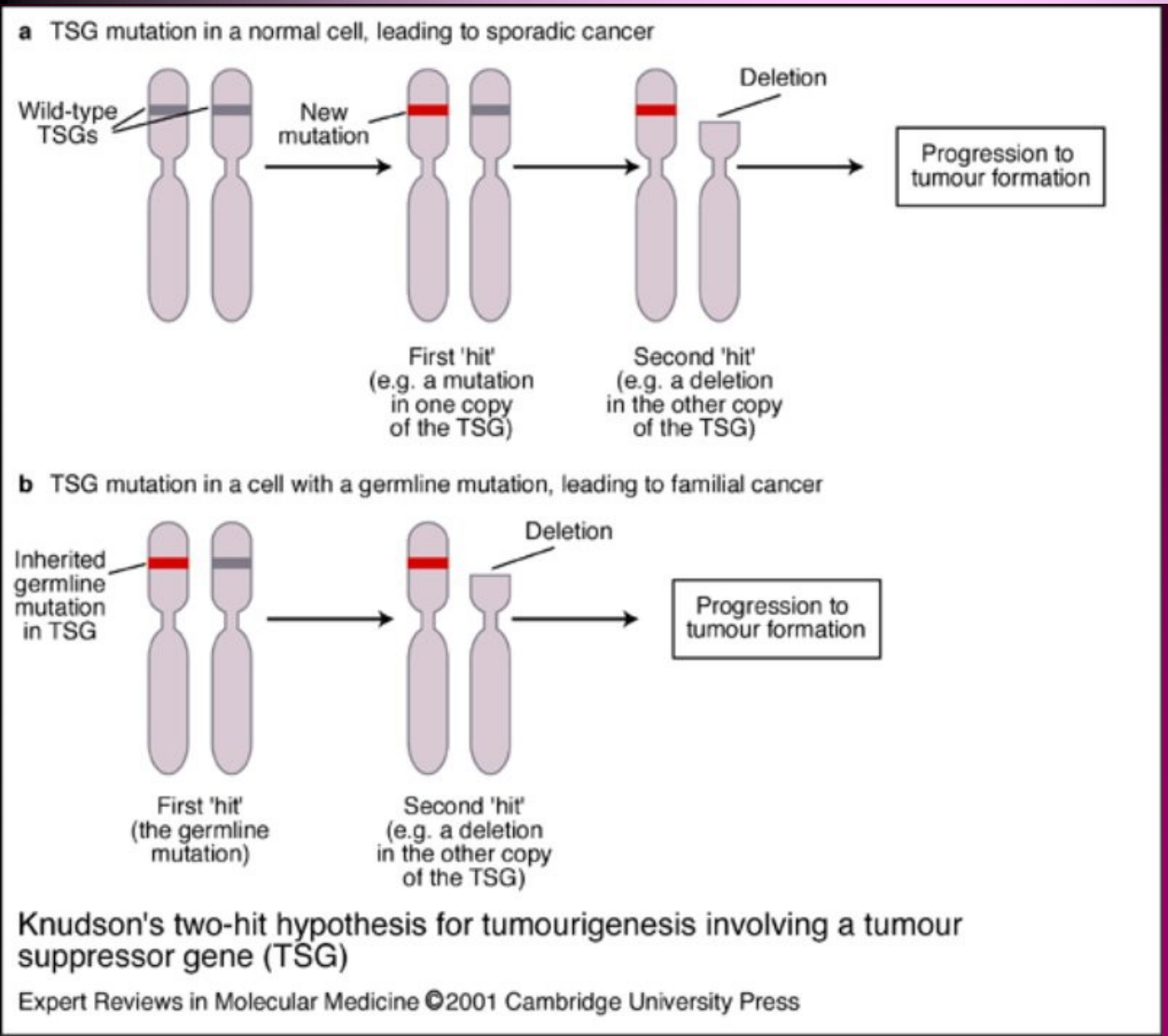
- **loss of whole chromosomes**, changes are rather secondary, frequent monosomy 5, 7 in MDS



RB1 gene deletion - loss of constitutive heterozygosity in retinoblastoma - LOH

Knuston hypothesis – (the double hit theory)



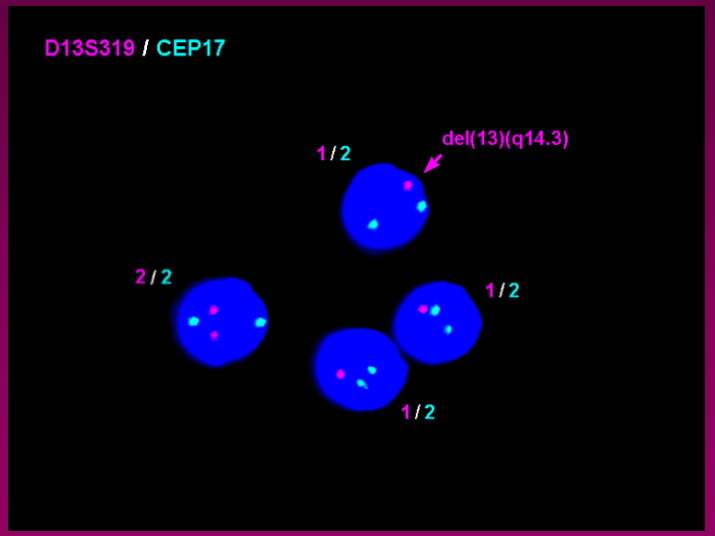


Knudson's two-hit hypothesis for tumourigenesis involving a tumour suppressor gene (TSG)

„The most tumor suppressor genes require both alleles to be inactivated, either through mutations or through epigenetic silencing, to cause a phenotypic change“
Alfred G. Knudson, 1971

Examples of significant deletions in tumor diseases

- **Retinoblastoma -** malignant eye tumor of children **del(13)(q14) - *RB1 gene*** - blocks progression to S phase



- **Wilms tumour - kidney tumour**
- del(11)(p) 11p13 - 11p15
- del(16)(q) WT1 gene
-
- **Neuroblastoma - del(1)(p36)**

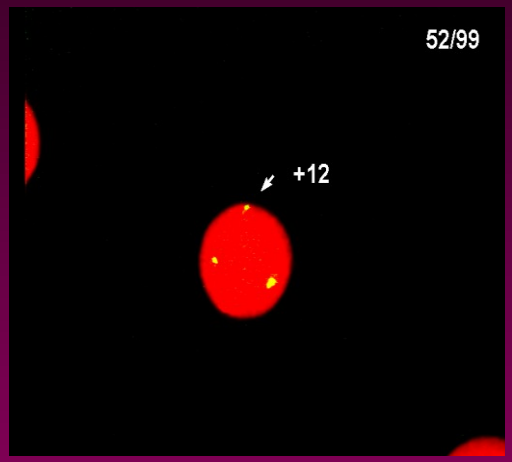
- ***TP53* gene deletion (17p13) - many tumors !!!**

Consequences of individual chromosomal aberrations in the process of carcinogenesis

Aberrations with gain of genetic material

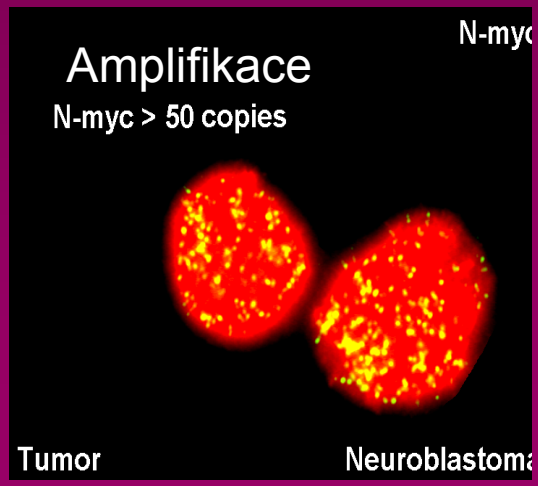
Duplication, trisomy

- multiplication of **whole or parts** of **chromosomes**
- frequent **secondary changes** in tumor cells
- multiplication of **gene dosage**
- cancer progression
- **in leukemias common +8, in CLL specific +12, other Ph copies**



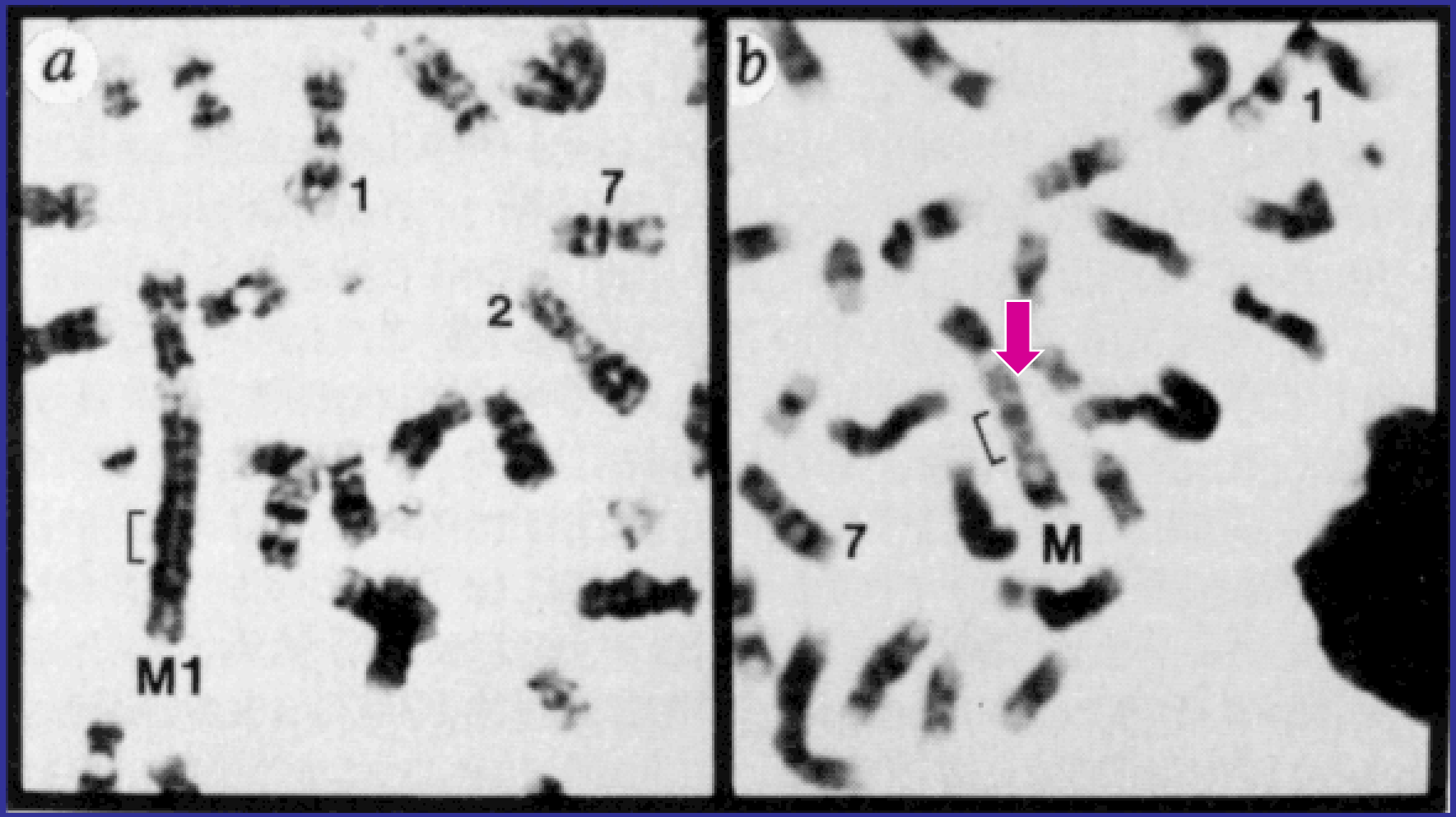
Amplification

- **frequent in solid tumors but also in leukemias**
- mostly **amplification of proto-oncogenes (*N-myc, c-myc, Her 2*)**
- **double minutes, homogeneously staining areas** (DMs, HSRs) **are seen in mitosis !!!**
- Detection: **I-FISH, array-CGH**
- **examination for the presence of amplification is of diagnostic and prognostic importance !!!**

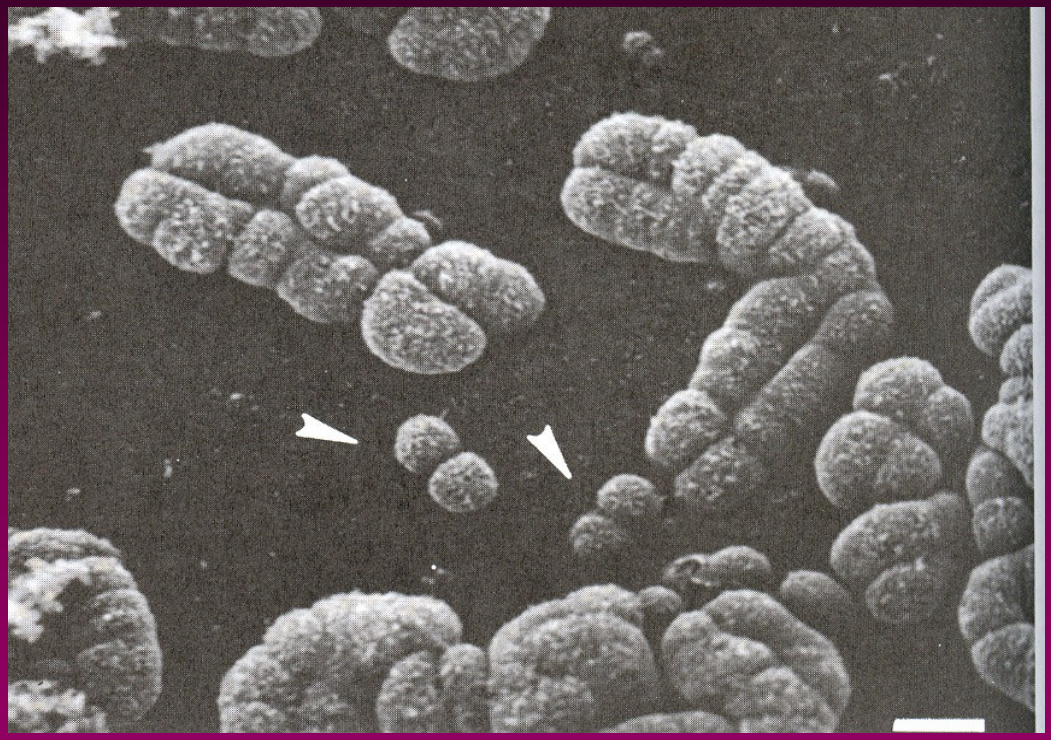


Homogeneously staining region (HSR)

regions of amplified genes in the karyotype (absence of banding)



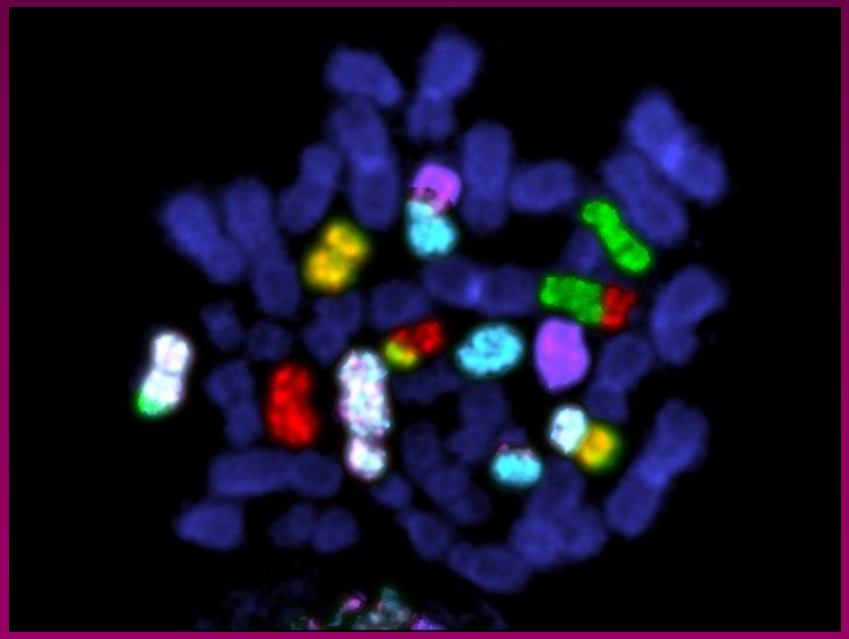
Double minutes DMs during mitosis



DMs are formed by HSR decay - they contain **several thousand copies** of a certain gene !!!

Complex karyotypes in tumor diseases

Complex karyotype - we detect numerical or numerical changes involving three or more chromosomes or structural changes involving three or more breaks



Poor prognosis!!!

Chromothripsis -

new view of tumour formation (new mechanism of complex changes - discovered in 2011)

Cell. 2011 Jan 7;144(1):27-40. doi: 10.1016/j.cell.2010.11.055.

Massive genomic rearrangement acquired in a single catastrophic event during cancer development.

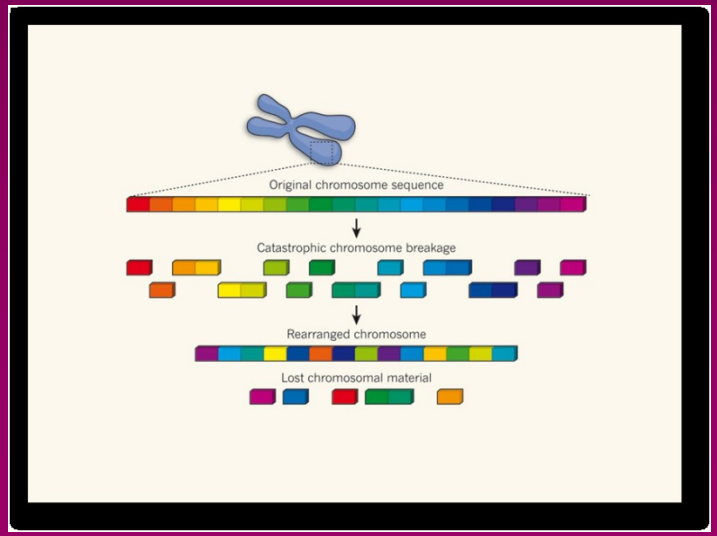
Stephens PJ[1], Greenman CD, Fu B, Yang F, Bignell GR, Mudie LJ, Pleasance ED, Lau KW, Beare D, Stebbings LA, McLaren S, Lin ML, McBride DJ, Varela I, Nik-Zainal S, Leroy C, Jia M, Menzies A, Butler AP, Teague JW, Quail MA, Burton J, Swerdlow H, Carter NP, Morsberger LA, Iacobuzio-Donahue C, Follows GA, Green AR, Flanagan AM, Stratton MR, Futreal PA, Campbell PJ.

Author information

Abstract

Cancer is driven by somatically acquired point mutations and chromosomal rearrangements, conventionally thought to accumulate gradually over time. Using next-generation sequencing, we characterize a phenomenon, which we term chromothripsis, whereby tens to hundreds of genomic rearrangements occur in a one-off cellular crisis. Rearrangements involving one or a few chromosomes crisscross back and forth across involved regions, generating frequent oscillations between two copy number states. These genomic hallmarks are highly improbable if rearrangements accumulate over time and instead imply that nearly all occur during a single cellular catastrophe. The stamp of chromothripsis can be seen in at least 2%-3% of all cancers, across many subtypes, and is present in ~25% of bone cancers. We find that one, or indeed more than one, cancer-causing lesion can emerge out of the genomic crisis. This phenomenon has important implications for the origins of genomic remodeling and temporal emergence of cancer.

- **Pacient WITH CLL**
- **NGS - 42 genomových přestaveb !**



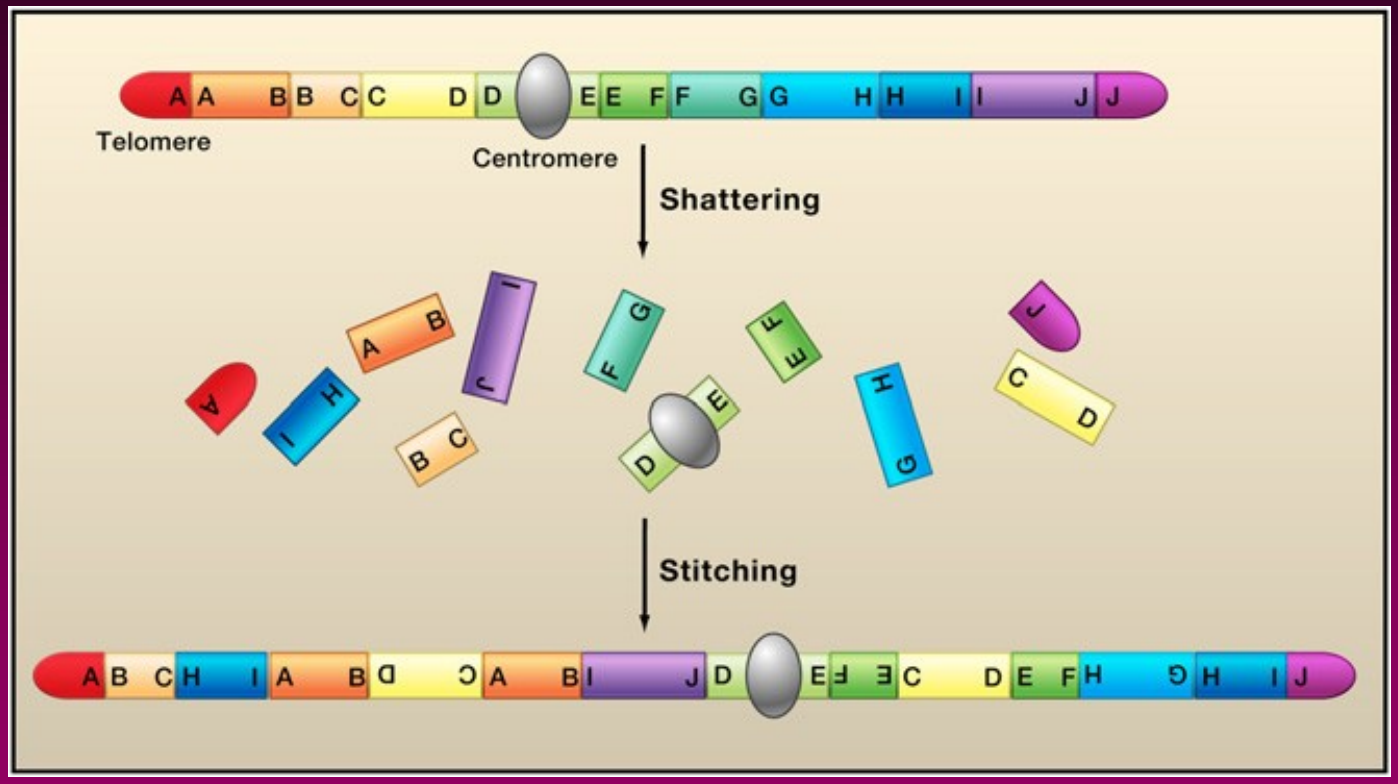
Chromothripsis

- chromothripsis (from Greek chromos - chromosome and thripsis - division into pieces)
- these are **tens or hundreds of rearrangements** on one or more **chromosomes**, occurring **suddenly** during a **single** catastrophic **event**
- a **chromosome** or a part of it is **broken** into small pieces (multiple double-strand breaks....) and these are **then randomly** put back **together** by reparative mechanisms
- however, this **assembly** is **not completely accurate**, some parts may be assembled in a **different order**, **missing or duplicated**

- **chromothripsis** has been demonstrated in **various types of tumours** (2-3%), but also in **congenital** genetic **diseases**

Chromothripsis

Cytogenetic consequences - complex rearrangements, deletions, duplications, insertions, inversions...



Chromothripsis is proposed to involve the shattering of a single chromosome, a small group of chromosomes, or a single chromosome arm. The fragments, or a subset of the fragments, are then stitched together by nonhomologous end-joining.

Meyerson and Pellman 2011

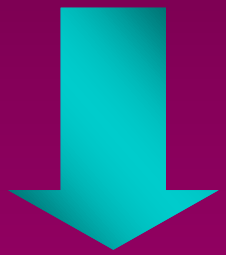
Chromothripsis and tumor diseases

- incidence of 2 - 3% of all cancers
- chromothripsis has been demonstrated in leukemia, multiple myeloma, colon cancer, medulloblastoma, neuroblastoma, etc.
- **frequent occurrence** in bone tumors (up to 25%) - osteosarcoma, Ewing's sarcoma, chondrosarcoma
- chromothripsis - worse prognosis !!! - **extensive rearrangements** - deletions, duplications, inversions, insertions - deregulation of a large number of genes....

Alghortim of cytogenetic/genomic analyses in cancer diseases:

G-banding, I-FISH, M_FISH, M-BAND array-CGH

Comprehensive analysis of cytogenetic changes in tumors



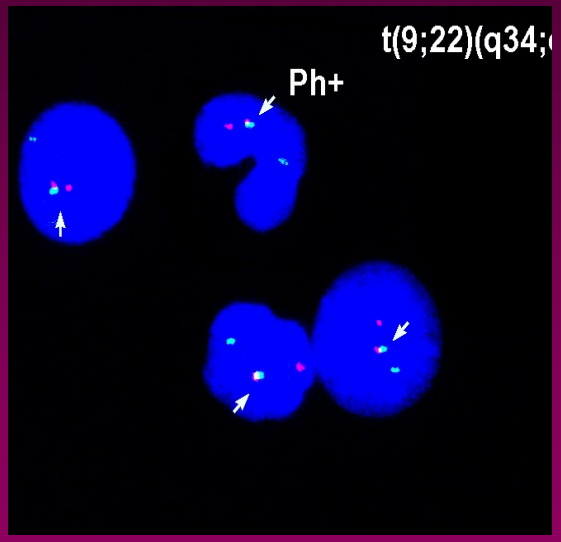
individualization of medical therapy, biological therapy"

Prognostic changes of chromosomal aberrations associated with negative prognosis in hematoncologic and solid cancer diseases

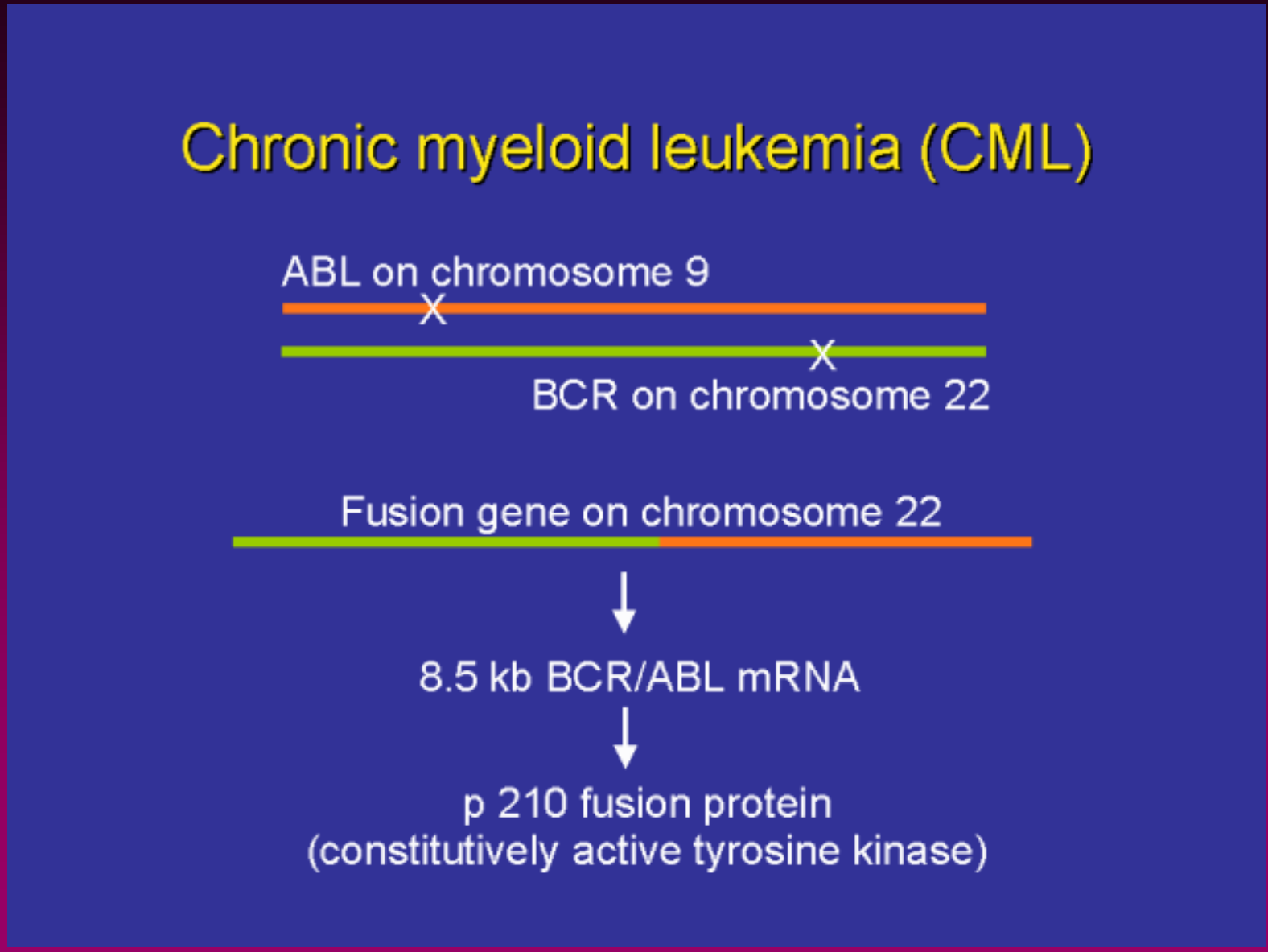
Chronic myeloid leukemia (CML) 1:100 000

- Ph is usually present in 95% of patients at the time of diagnosis, (where it is not, we usually find a masked Ph chromosome as insertion)
- there are **3 breakpoint regions** in the ***BCR*** gene

  the best prognosis is for patients who have a Ph chromosome as the only change at the time of diagnosis !!!
- at the time of diagnosis, presence of other chromosomal changes besides Ph, is an unfavourable prognostic feature
- at the time of blastic reversal, up to 70% of patients have additional chromosomal changes, most often +8, Ph duplication, +19, i(17q)
- **FISH detects BCR/ABL rearrangement, specific probe allows to investigate also interphase nuclei**



I – FISH translocation DNA probes



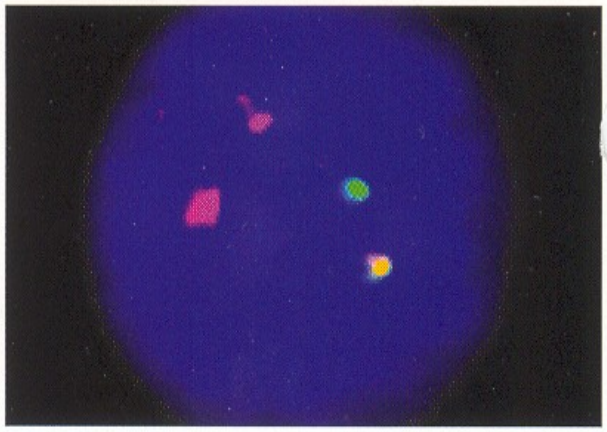
Translocation t(9;22) - bcr/abl – interphase

Translocation positive cells can be

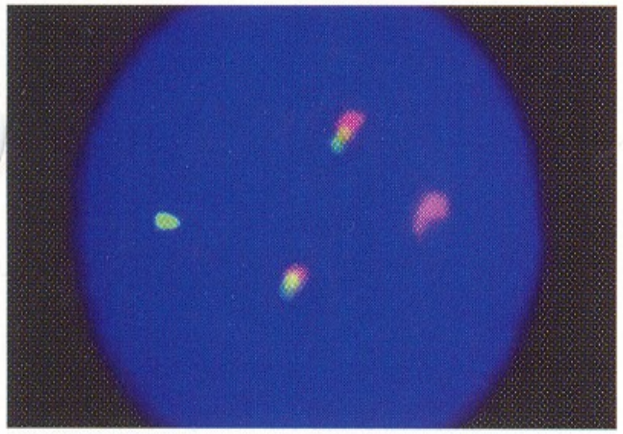
With additional signal

or

DUAL fusion s – double fusion signal



LSI BCR/ABL ES Dual Color Translocation Probe hybridized to a nucleus containing the t(9;22) showing one green (native BCR), one large orange (native ABL), one smaller orange (ES), and one fused orange/green (20IGIF) signal pattern.

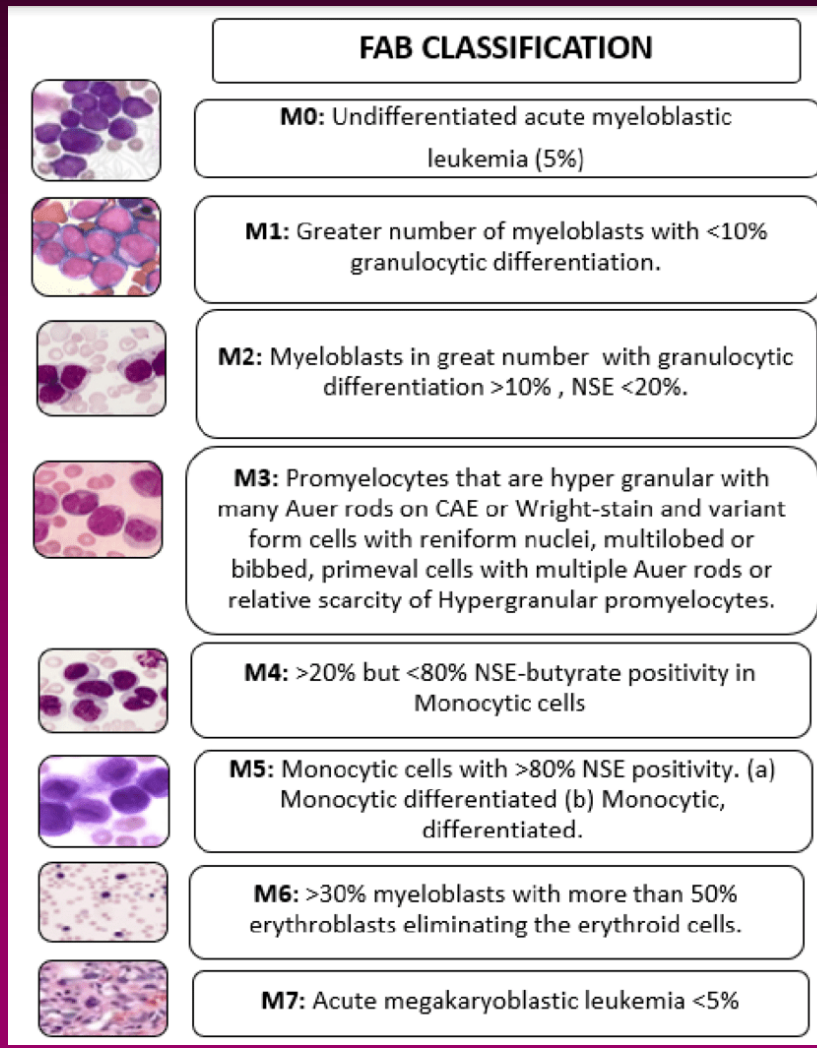


LSI BCR/ABL Dual Color, Dual Fusion Translocation Probe hybridized to a nucleus containing a simple balanced t(9;22). One orange, one green, and two orange/green fusion signals are observed (1O1G2F).

Acute myeloid leukemia (AML) 3/100 000

- **60-70%** of patients have a clonal chromosomal aberration
- the aberrant clone is often accompanied by a normal clone
- Chromosomal changes **present** at the time of **diagnosis disappear** after **achieving remission**, reappear in **relapse**, often with **other secondary** changes
- some changes specific to FAB subtypes of AML, diagnostic and prognostic significance, disease monitoring !
- The French-American-British (FAB) classification of AML:

M1 - M7, M0 according to aberrations



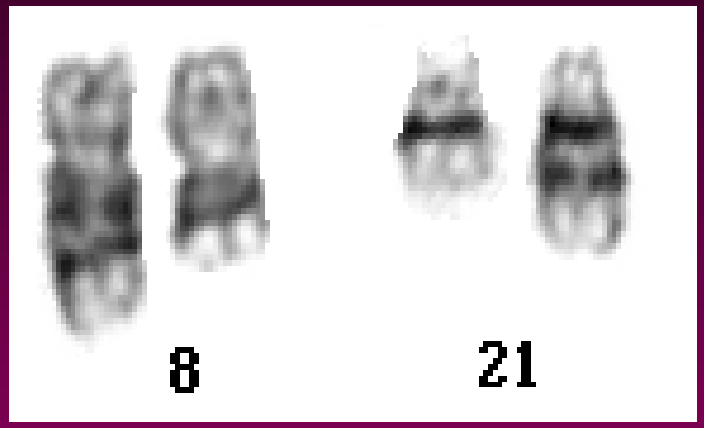
AML subgroups

Onemocnění	Chromozómová změna	Místa zlomu nebo delece
LEUKÉMIE		
Chronická myeloidní leukemie	t(9;22)	9q34;22q11
Akutní myeloidní leukemie M1	t(9;22)	9q34;22q11
M2	t(8;21)	8q22;21q22
M3	t(15;17)	15q22;17q11
M4	inv(16)	16p13q22
M4, M5	t(9;11)	9p22;11q23
M1, M2, M4, M5, M6	del(5q)	del(5)(q13q33)
	+8	
Chronická lymfocytární leukemie	+12, 13q-, 17p-	
	t(11;14)	11q13;14q32
Akutní lymfocytární leukemie		
L1 - L2	t(9;22)	9q34;22q11
L2	t(4;11)	4q21;11q23
L3	t(8;14)	8q24;14q32
	t(8;22)	8q24;22q11
LYMFOMY		
Burkittův lymfom	t(8;14)	8q24;14q32
SOLIDNÍ NÁDORY		
Diseminovaný neuroblastom	del(1p)	1p31p36
Malobuněčný karcinom plic	del(3p)	3p14p23
Retinoblastom, familiární, sporadický	del(13q)	13q14
Aniridie-Wilmsův tumor	del(11p)	11p13

AML aberrations

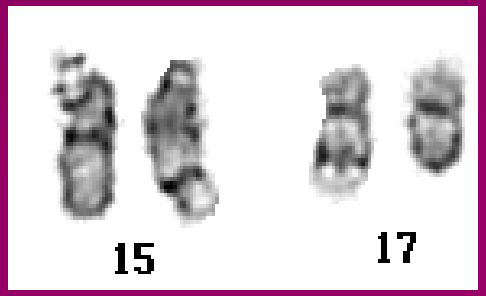
AML-M2 - t(8;21)

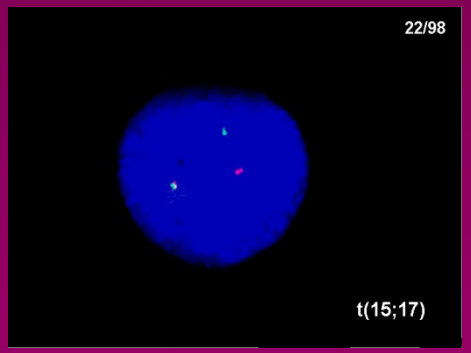
- mapped genes ***ETO, AML1***
- mainly in younger patients, often in children
- secondary changes -Y, -X, 9q-, 7q-, +8
- good prognosis



AML-M3: t(15;17)

- ***PML/RARA*** genes mapped
- Less frequently variant translocations
- most common **additive change +8**
- good prognosis

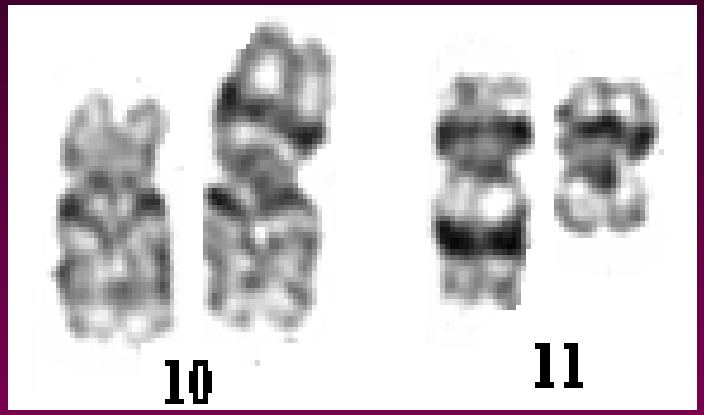




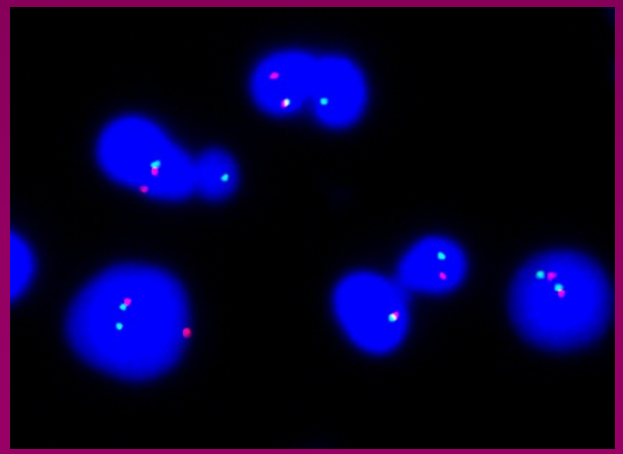
AML aberrations

AML-M5 disruption 11q23 DNA probe

- **11q23** aberrations involving deletions or translocation in **the break region** of the ***MLL*** gene
- up to 50% of patients have these changes
- most common t(1;11), t(6;11), t(9;11), t(10;11), t(11;19) and others (40 translocations)
- often also in paediatric AML
- **generally unfavourable prognosis**



Disruption in *MLL* genu



Myelodysplastic syndrome (MDS) 15/100 000

rare disease, occurs in the elderly, pancytonemia in the blood, often transitioning to acute leukemia

- rare disease, occurs in the elderly, pancytonemia in the blood, often transitioning to acute leukemia
- chromosomal changes in about **60% of patients**

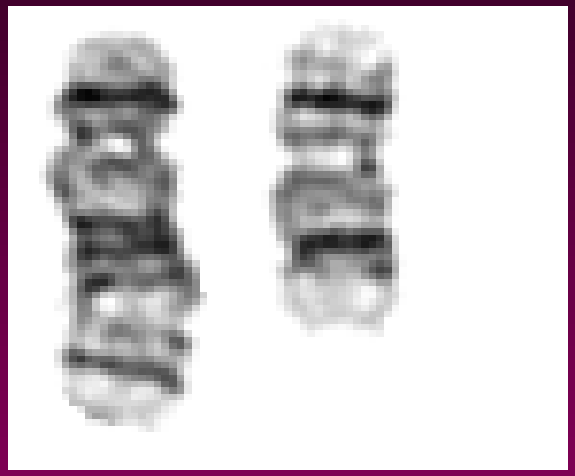
<u>del(5q) - 5q-syndrome</u>

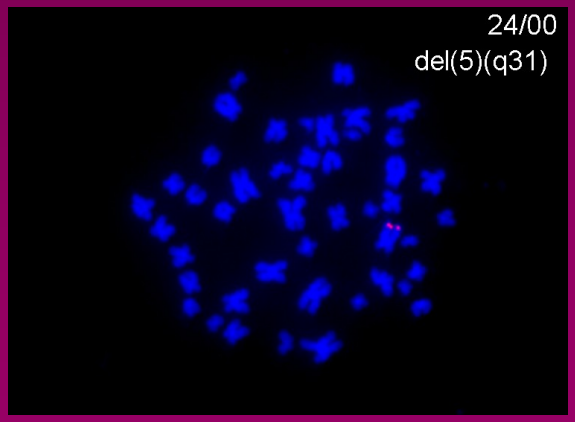
- interstitial deletion, extent of deletion varies, missing 5q31 band
- other changes: **-5, del(7q), -7, +8, del(11q), 12p-,**
- frequent **complex** changes

best prognosis in patients **with normal karyotype**, single 5q- favourable prognosis,

combination with other changes unfavourable,

-7 very unfavourable, **<u>patients with complex changes have the shortest survival !!!</u>**





Acute lymphocytic leukemia (ALL)

- ALL is the most common childhood malignancy; **3 : 100 000**
- **70-90% of patients** have an acquired chromosomal aberration

t(12;21) - *TEL/AML1* - better prognosis !

Numerical chromosome changes

a) **hyperdiploidy**

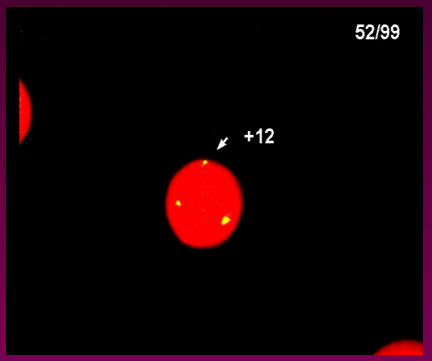
- most common, **more than 46 chromosomes**, **moderate** prognosis
- more than 50 chromosomes, good prognosis
- supernumerary chromosomes 4, 6, 10, 14, 17, 18, 20 and 21

b) hypodiploidy

- number less than 46, unfavourable prognosis
- the importance of I-FISH in detecting hyperdiploid and hypodiploid clones, the algorithm uses a panel of **the 10 most frequently included chromosomes**

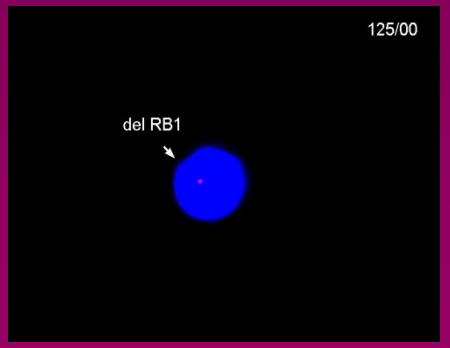
Chronic Lymphocytic Leukemia

- little cell proliferation activity *in vitro*, often normal karyotype found
- **CHAs** found in more than **40% of CLL** patients **with FISH**



Trisomy 12

- most common, in B-CLL, more than **20% of patients**
- often combined with **del(13q)**, del(6q), **del(17p),** or complex rearrangements
- median survival **3-4 years, better response to chemotherapy**



del(13q)

- ***RB1 gene*****,** the most common alteration after trisomy 12

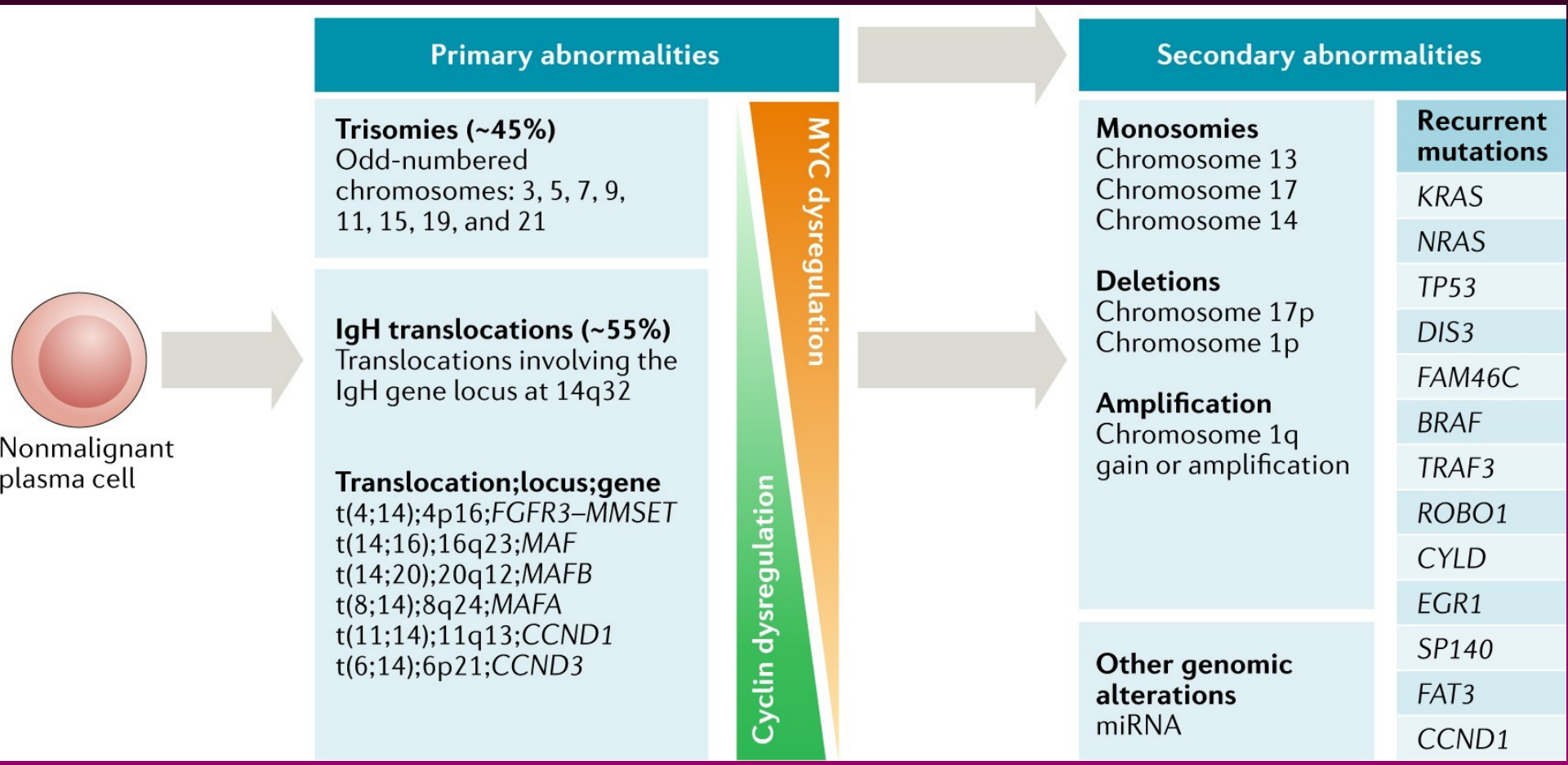
del(17p)

- ***TP53*** **gene,** prognostic significance, **survival 1.5-2 years**

Multiple myeloma

- A **clonal disease** that results from a **malignant mutation** in the development of the **B-lymphocytes**
- It accounts for about **10%** of all **oncohematological diseases**
- Affects mainly **the elderly**, rare before the age of 40
- Currently incurable, with variable survival and patient response to treatment
- Wide spectrum of clinical manifestations: bone destruction, pancytopenia, anaemia, impaired antibody immunity, myeloma nephropathy ...
- Optimization of treatment requires determination of prognostic factors

Mnohočetný myelom a cytogenetika



https://www.nature.com/articles/s41571-018-0018-y

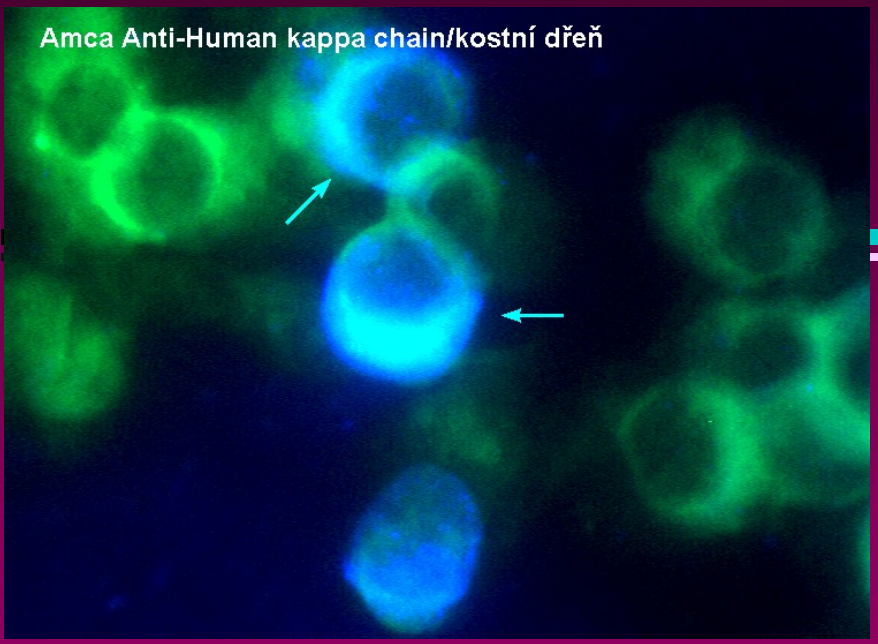
Multiple myeloma and CHAs

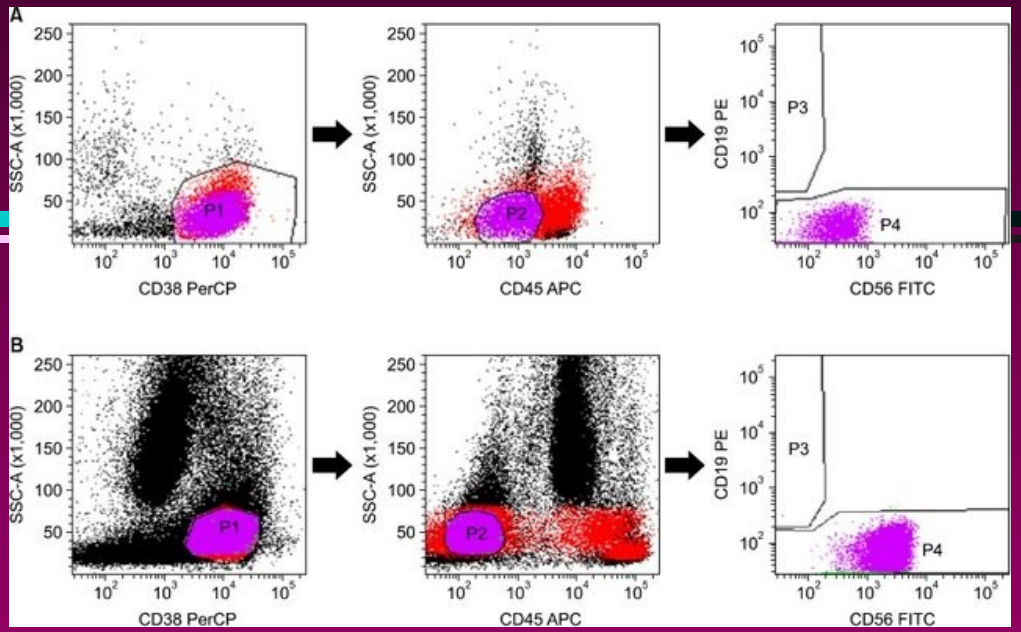
Cytogenetic changes - an important prognostic factor in MM !!!

- **Hypodiploidy: most often monosomy of chromosome 8, 13, 14, X** unfavourable prognosis
- **Hyperdiploidy: most often trisomy of chromosome 3, 5, 7, 9, 11, 15,19, 21** favorable prognosis
- **Translocation involving the IgH gene (14q32):**
  - **t(11;14) (q13;q32) favourable prognosis**
  - **t(4;14)(p16;q32), t(14;16) (q32;q23) unfavourable prognosis**
- **RB1 gene deletion (13q14) - intermediate prognosis**
- **Deletion of p53 gene (17p) poor prognosis**
- **gain 1q21 - poor prognosis**

Cell sorting in MM pacients

fluorescence immunophenotyping and interphase in situ hybridization





FICTION technique in MM

fluorescence immunophenotyping and interphase in situ hybridization

Simplified flow cytometric

immunophenotyping panel for multiple myeloma, CD56/CD19/CD138(CD38)/CD45

Prognosis of disease in MM patients

Effect of presence of aneuploidies

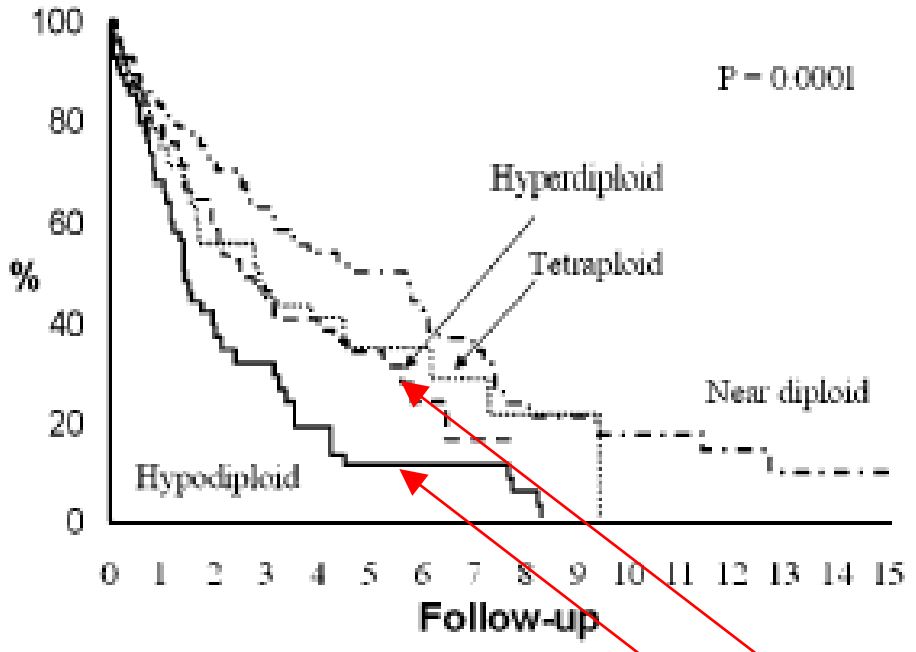
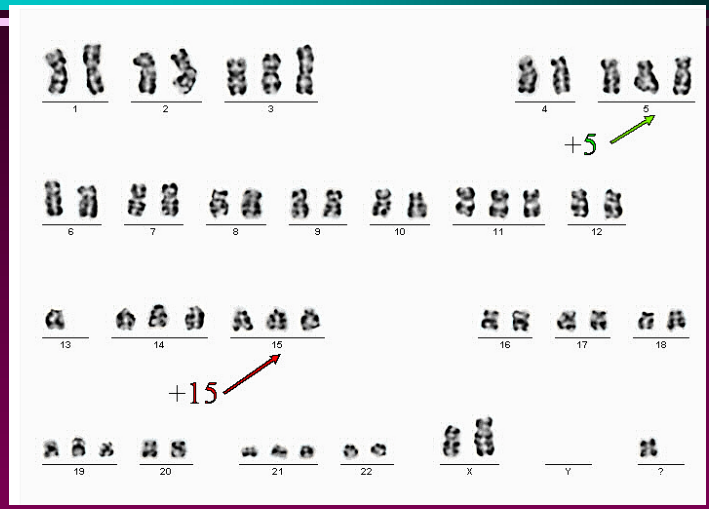


Figure 6 Overall survival of patients according to ploidy status. Kaplan–Meier survival analysis of patients according to ploidy category. The survival since diagnosis time is presented in the x-axis in years and the *P* value is the univariate log-rank probability. The chromosomal abnormality in question is always represented by the dotted line.



Status	5 Year Survival (%)
Pseudodiploid	49.9
Near-tetraploid	34.6
Hyperdiploid	**33.5**
Hypodiploid	**10**

Převzato z Debes –Marun *et al.*, 2003, Blood

Solid tumors

Type of tissue	Benign	Malignant
Epithelial tissue		
lining epithelium	papilloma	carcinoma
glands & lobules	adenoma	adenocarcinoma
Connective tissue & muscle tissue		
dense connective tissue	fibroma	fibrosarcoma
cartilage	chondroma	chondrosarcoma
bone	osteoma	osteosarcoma
smooth muscle	leiomyoma	leiomyosarcoma
skeletal muscle	rhabdomyoma	rhabdomyosarcoma
Neuronal tissue		
glial tissue	glioma	glioblastoma
meninges	meningioma	meningial sarcoma

https://www.researchgate.net/profile/Annette-Gylling-lindroos/publication/47931966/figure/tbl1/AS:669431457918989@1536616305664/Classification-of-solid-tumors-according-to-tissue-of-origin-modified-from.png

Cytogenetics of solid tumors

Biological material:

- effusion (ascitic tumors) - epithelial and tumor cells
- culture of tumor cells
- tumor imprints (FISH) ....confirmation by pathologist !
- paraffin sections (FISH)
- cytospins

Cytogenetics of solid tumors

Problems:

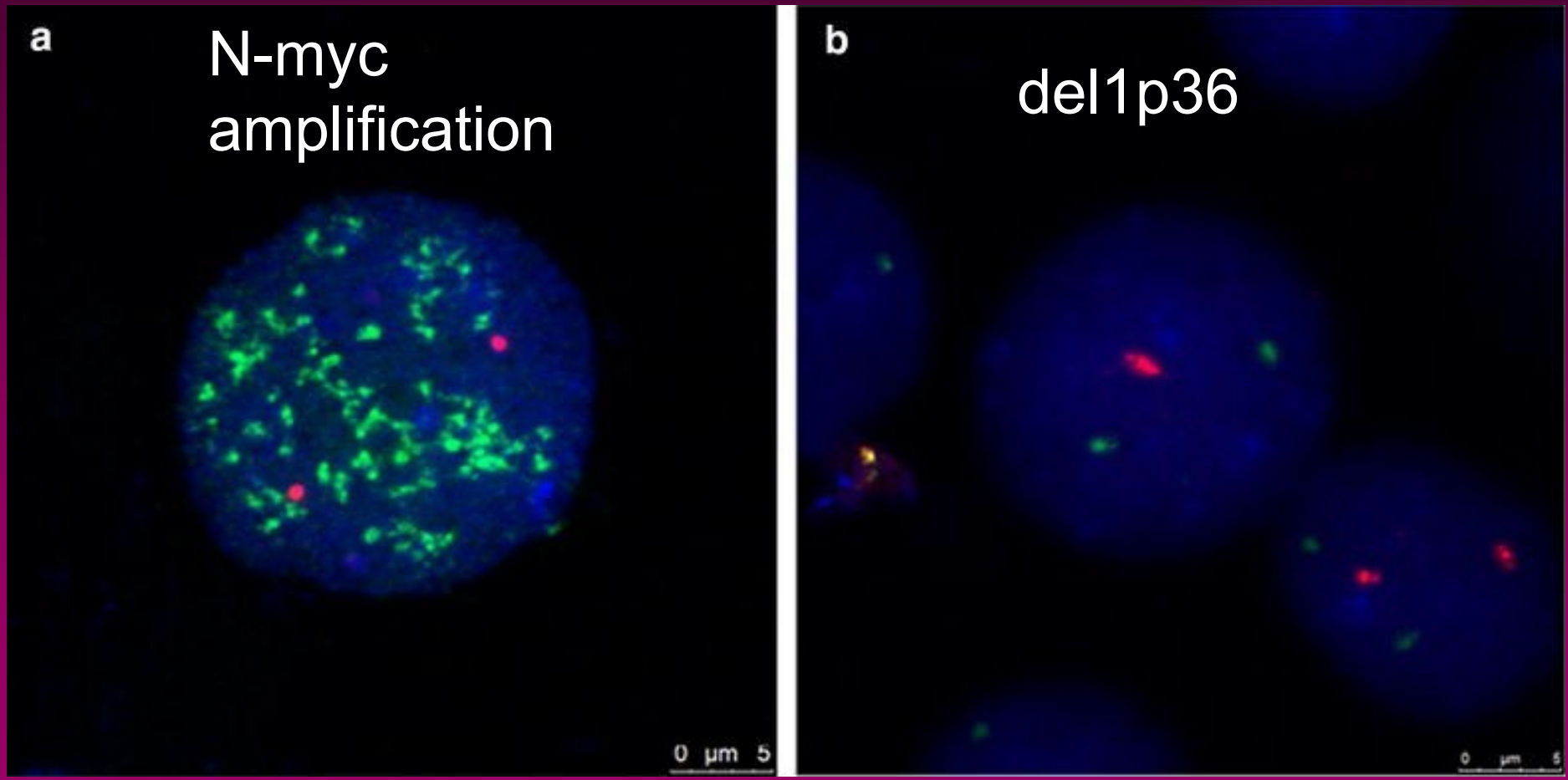
- Difficulty of long-term cultivation (will it **outgrow non-tumor** material ?)
- **lack of mitoses, quality of slides**
- **complexity of karyotype (unbalanced changes)**
- **heterogeneity of tumors**

individualization of patient treatment - cytogenetic and genetic testing is essential !

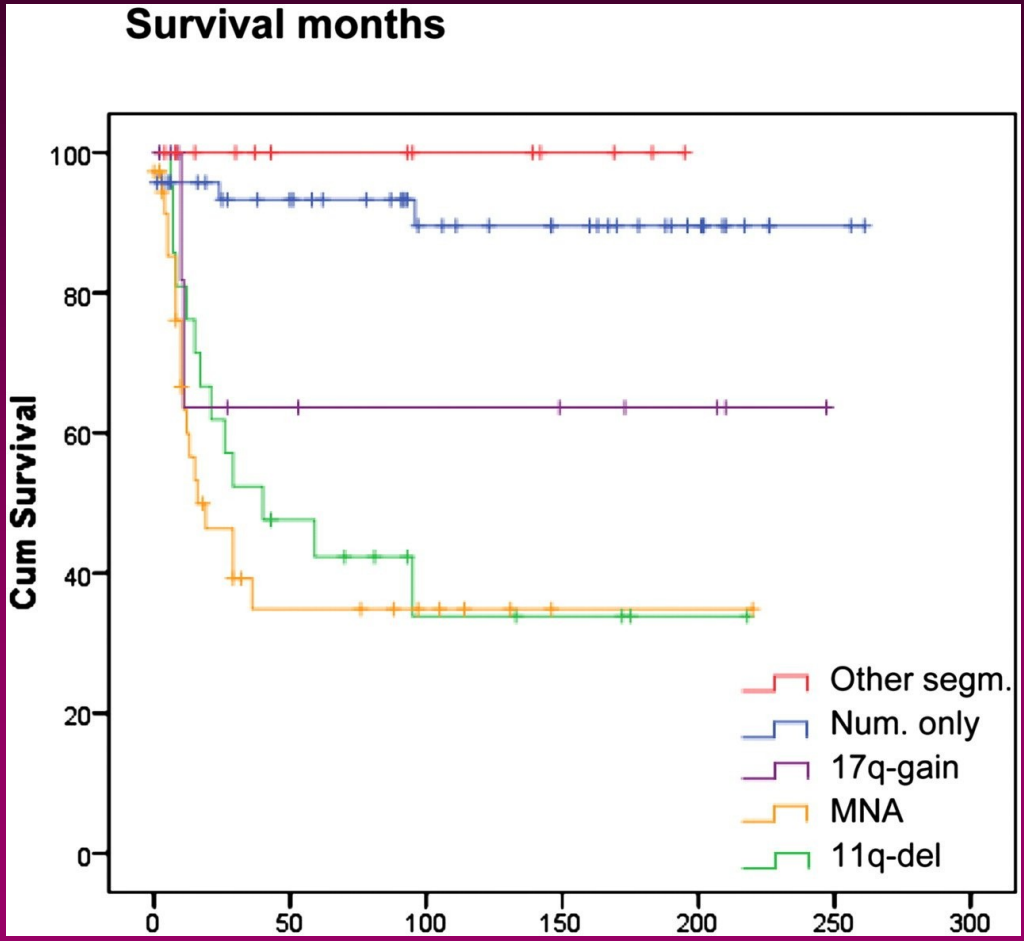
Cytogenetické změny u některých solidních nádorů dětí

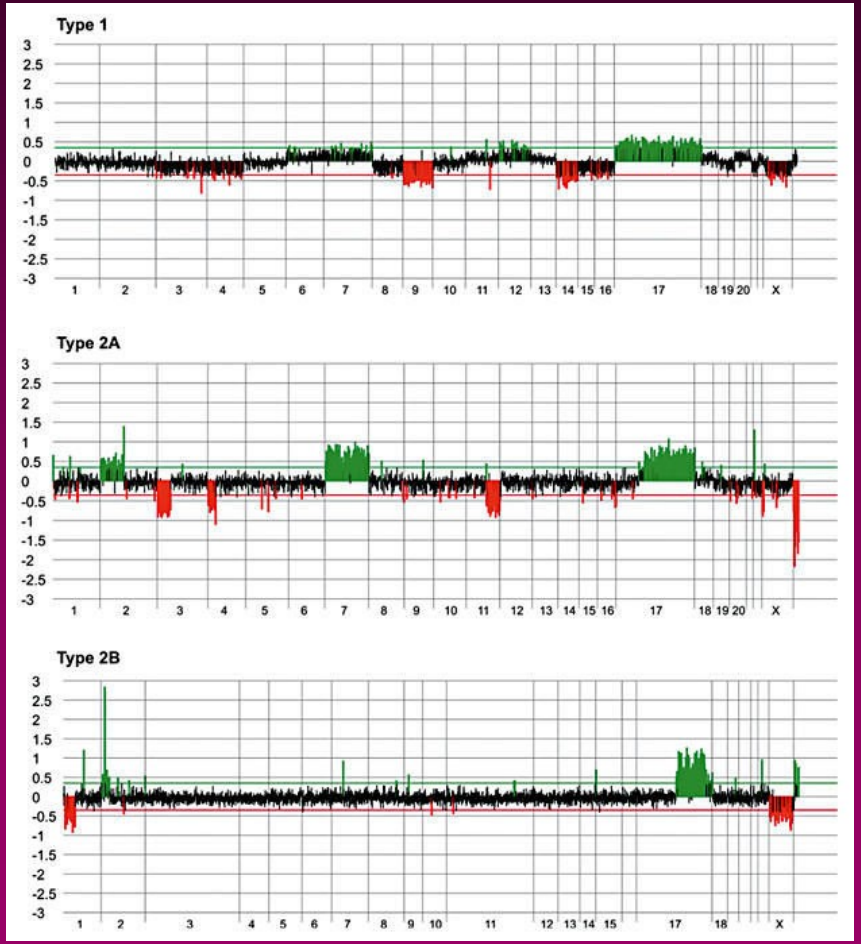
- **Retinoblastoma** - malignant eye tumour in children
  del(13)(q14) - *RB1 gene*
- **Wilms tumor** - kidney tumor
  del(11)(p) 11p13 - 11p15 - *WT1* gene
  del(16q)
- **Ewing's sarcoma** - t(11;22)(q24;q12)
  EWS-FLI1 fusion gene
- **Neuroblastoma** - amplification of *N-myc* gene,
  del(1)(p36), del 11q, gain(17)(q)
- **Medulloblastoma** - *c-myc* gene amplification

Neurobalstoma – FISH probes



Prognosis and therapy of neuroblastoma based on genomic profiles of tumor cells!





https://www.researchgate.net/profile/Bjorn-Menten/publication/6674618/figure/fig2/AS:267916767658004@1440887743724/ArrayCGH-visualisation-of-representative-neuroblastoma-tumors-each-belonging-to-a_W640.jpg

Examples of FISH analyses usedin solid tumors

E.C.A. - EUROPEAN CYTOGENETICISTS ASSOCIATION NEWSLETTER No. 34 July 2014

Disease	Chromosomal abnormality	Commercially available FISH probes
Medulloblastoma	i(17)(q10)	17p13.3 and RARA
Mucoepidermoid carcinoma and Hidradenoma	t(11;17)(q21;p13)	MAML2, BA
Myxoid liposarcoma	t(12;16)(q13;p11)	DDIT3, BA; FUS, BA
Myxoid liposarcoma	t(12;22)(q13;q12)	DDIT3, BA; EWSR1, BA
Neuroblastoma	*MYCN* amplification / del(1p) / del(11q)	Various, combinations available to determine *MYCN* copy number, 1p and 11q status
Non-small cell lung carcinoma	inv(2)(p21p23)	ALK, BA
Non-small cell lung carcinoma	t(6q22)	ROS1, BA
Oligodendroglioma	del(1p) / del(19q)	1p36/1q25, 19q13/19p13
Other Carcinomas		EGFR, MET, ALK, ROS1, RET
Papillary Renal Cell Carcinoma	Trisomy 7 and 17, disomy 1	Chromosome enumerator probes for chr. 1, 7 and 17
Pilocytic astrocytoma	putative inv(7)(q34)	BRAF BA*
Renal cell carcinoma with Xp11 translocation	t(Xp11.2), usually t(X;1)(p11.2;q21)	TFE3, BA
Schwannoma	22q deletion	22q11
Secretory carcinoma (breast, salivary gland)	t(12;15)(p13;q26)	ETV6, BA
Synovial sarcoma	t(X;18)(p11;q11)	SS18, BA

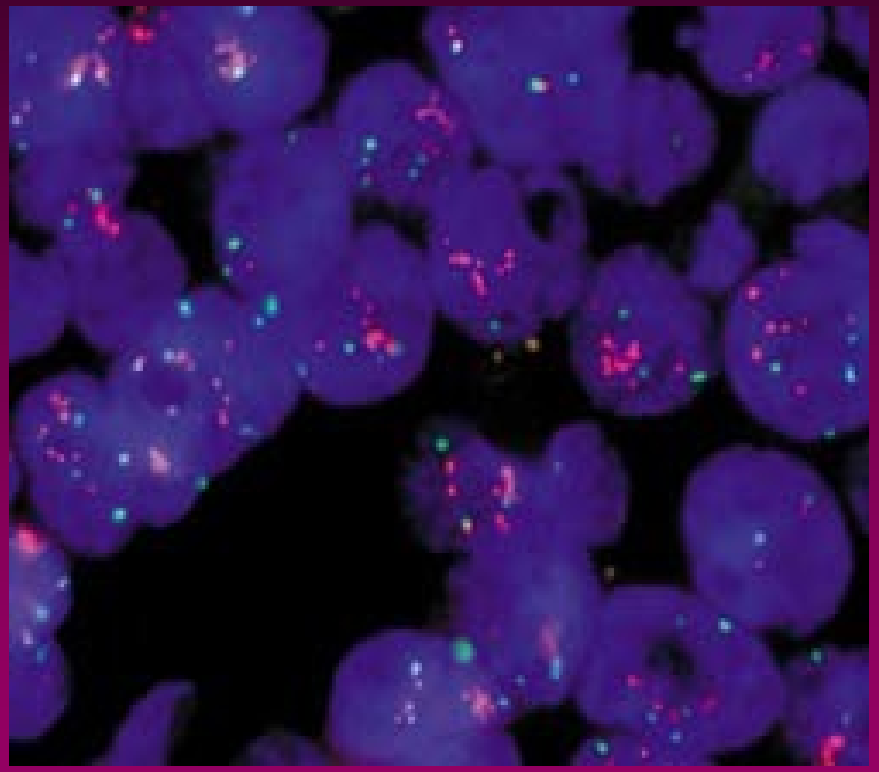
**BRAF* activation through the *KIAA1549-BRAF* fusion has also been described in other paediatric low-grade gliomas (e.g. pilomyxoid astrocytoma). *BRAF* point mutations (V600E) are observed in non-pilocytic paediatric low-grade gliomas as well, including approximately two-thirds of pleomorphic xanthoastrocytoma cases and in ganglioglioma and desmoplastic infantile ganglioglioma.

Abbreviations:
BA: break-apart; DF: dual fusion

Disease	Chromosomal abnormality	Commercially available FISH probes
Ewing tumour	t(2;22)(q33;q12)	EWSR1, BA
Ewing tumour	t(7;22)(p22;q12)	EWSR1, BA.
Ewing tumour	t(11;22)(q24;q12)	EWSR1, BA; EWSR1/FLI1, DF
Ewing tumour	t(17;22)(q21;q12)	EWSR1, BA
Ewing tumour	t(21;22)(q22;q12)	EWSR1, BA; EWSR1/ERG, DF
Ewing tumour	inv(22q)	EWSR1, BA
Ewing tumour	t(16;21)(p11;q22)	FUS, BA
Endometrial stromal sarcoma	t(7;17)(p15;q21)	JAZF1, BA
Extraskeletal myoepithelial tumours	t(22q12)	EWSR1, BA
Gastric carcinoma	*ERRB2* (*HER2*) amplification	ERBB2 (HER2) and D17Z1
Hyalinizing clear cell carcinoma (salivary gland)	t(12;22)(q13;q12)	EWSR1, BA
Infantile fibrosarcoma, congenital mesoblastic nephroma	t(12;15)(p13;q26)	ETV6, BA
Inflammatory myofibroblastic tumour	t(1;2)(q25;p23)	ALK, BA
Inflammatory myofibroblastic tumour	t(2;17)(p23;q23)	ALK, BA
Inflammatory myofibroblastic tumour	t(2;19)(p23;p13)	ALK, BA
Inflammatory myofibroblastic tumour	t(2;11)(p23;p15)	ALK, BA; CARS, BA
Liposarcoma	*MDM2* amplification	MDM2, D12Z1
Low grade myxoid fibrosarcoma	t(7;16)(q34;p11)	FUS, BA
Lung adenocarcinoma	inv(2)(p23p21) or other 2p23 rearrangements	ALK
Lung adenocarcinoma	6q22.1	ROS1
Lung adenocarcinoma	10q11	RET
Lung adenocarcinoma		EGFR, MET, ERBB2

Personalized medicine and HER -2 gene amplification in breast cancer

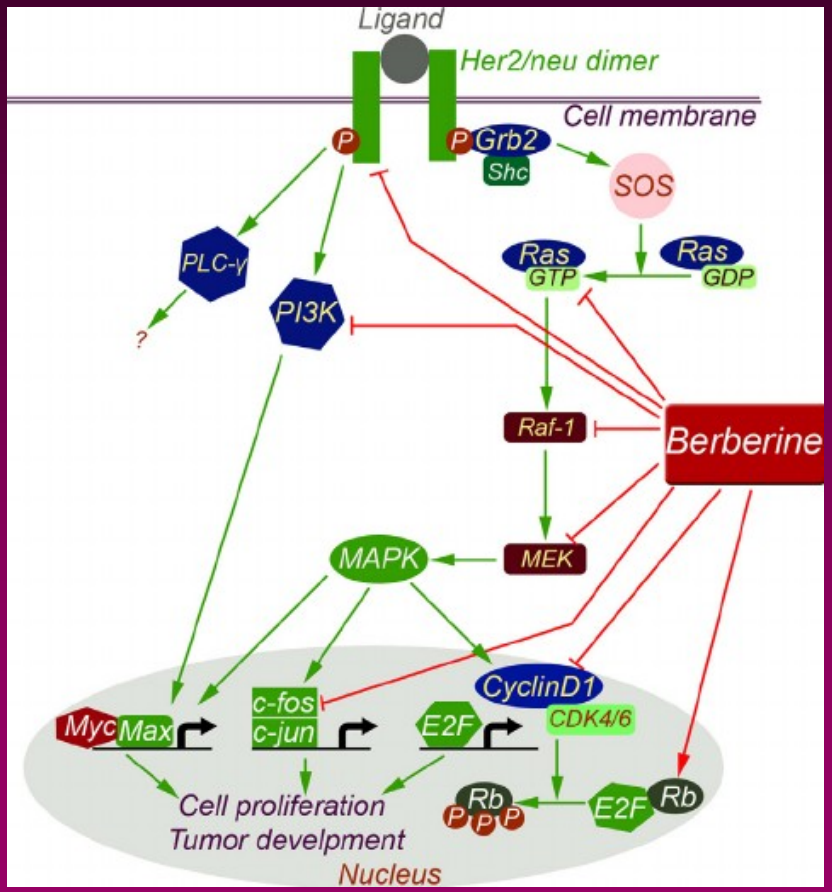
- **Breast CA...6000 women per year in the Czech Republic**
- ***Her 2* - an important prognostic and therapeutic marker**
- **Occurs in 25-30% of breast cancers**
- **Gene amplification is associated with an unfavorable prognosis (rapid proliferation, shortened survival time)**
- **treatment with Herceptin ...transtuzumab - blocking Her 2 receptor !**



Prediction of the course of the disease

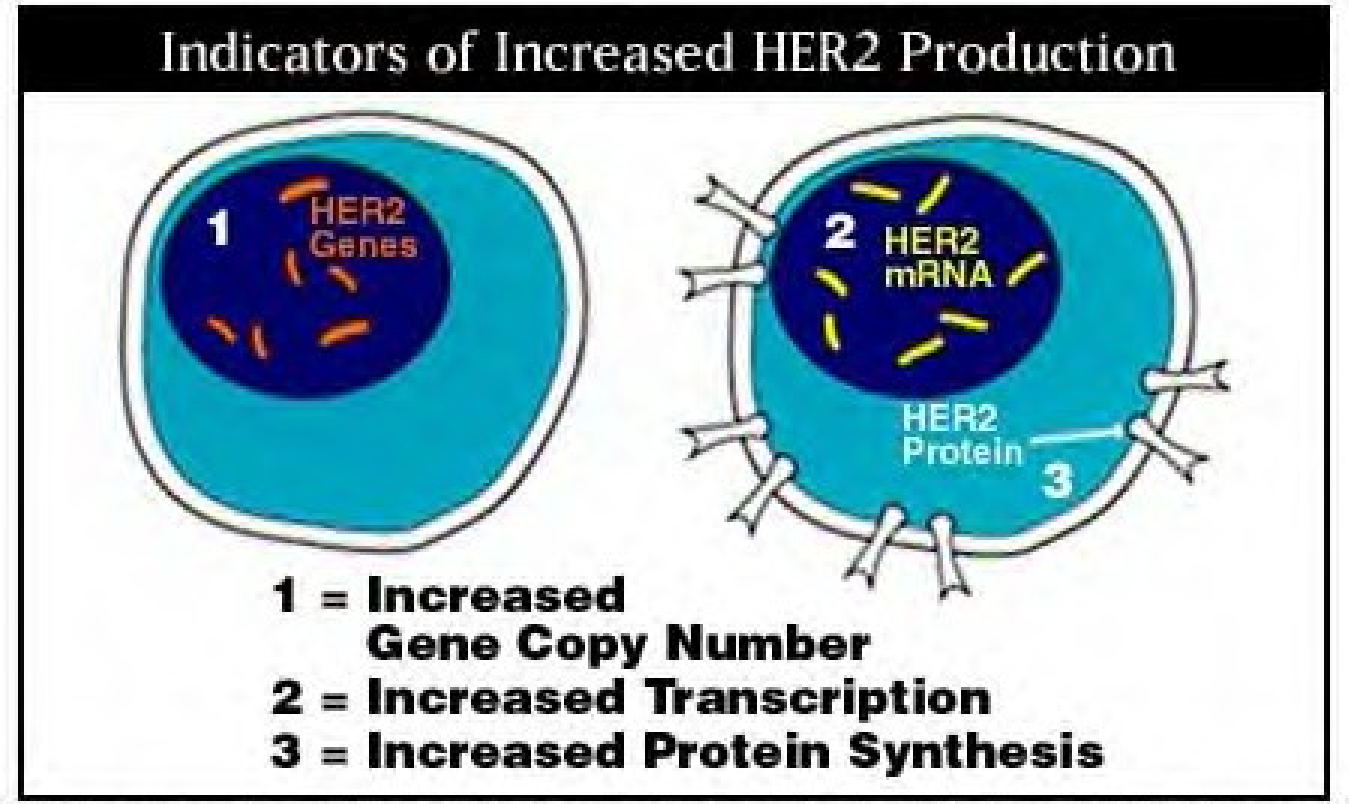
HER-2/*neu* Overview

- Human Epidermal Growth Factor Receptor-2
- Also known as:
  - c-*erb*B2
  - *neu* (rat homolog)
- Codes for a 185 Kd transmembrane cell surface receptor
- Member of the tyrosine kinase family



https://www.researchgate.net/profile/Parham-Jabbarzadeh-Kaboli-2/publication/264976148/figure/fig2/AS:295978197372932@1447578109798/Her2-neu-signaling-pathway-The-her2-neu-heterodimer-is-a-growth-factor-receptor-that.png

Indicators of Increased HER-2 production

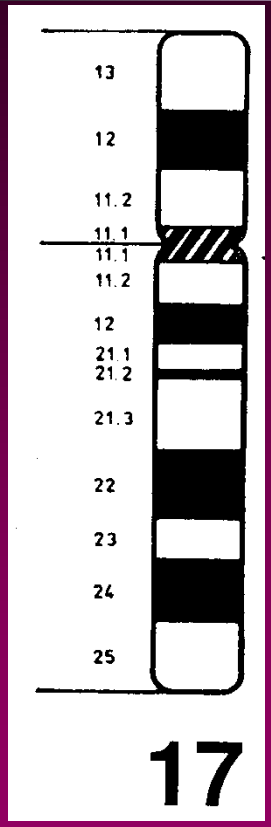


HER2 overexpression can be achieved by 1) gene alteration resulting in additional copies of the HER2 gene; 2) increased transcription of the HER2 gene, producing increased levels of HER2 mRNA; and/or 3) increased translation of HER2 mRNA. All three alterations can result in greater HER2 synthesis and expression on the cell surface as well as increased cell proliferation.

Image from Genetech

HER-2/*neu* DNA Probes

HER-2/neu & *chr. 17*



17p11.1-q11.1 17 alpha

17q11.2-q12 HER-2/neu

Centromere

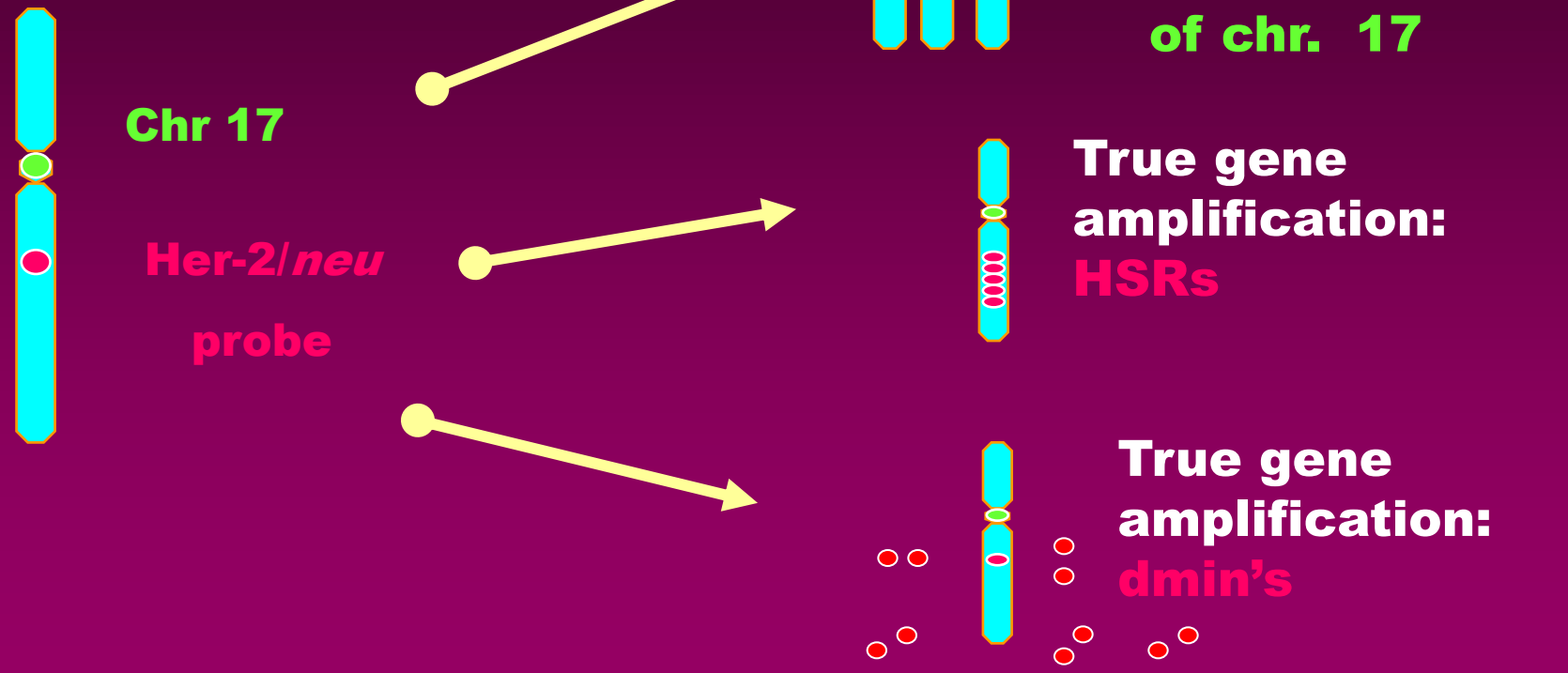
17q11.2 -q12

HER-2/*neu* gene

Telomere

~ 400 kb

HER-2/*neu* DNA Probes and Gene Amplification



ALK gene (2p23) disruption in Non-small cell lung pcancer (NSCLC)

- Lung cancer diagnosed annually **in 1.6 million** people worldwide ...
- **NSCLC - 80%** of cases; **5-year survival - about 15%**
- ***KRAS, EGFR, ALK* mutations**
- **Patients with *ALK gene* and disruption and *EML/ALK* fusion gene (5-7% of cases) benefit from targeted treatment with anaplastic lymphoma kinase inhibitor - crizotinib (Xalkori)**
- **for EGFR gene mutations - gefitinib and erlotinib...**

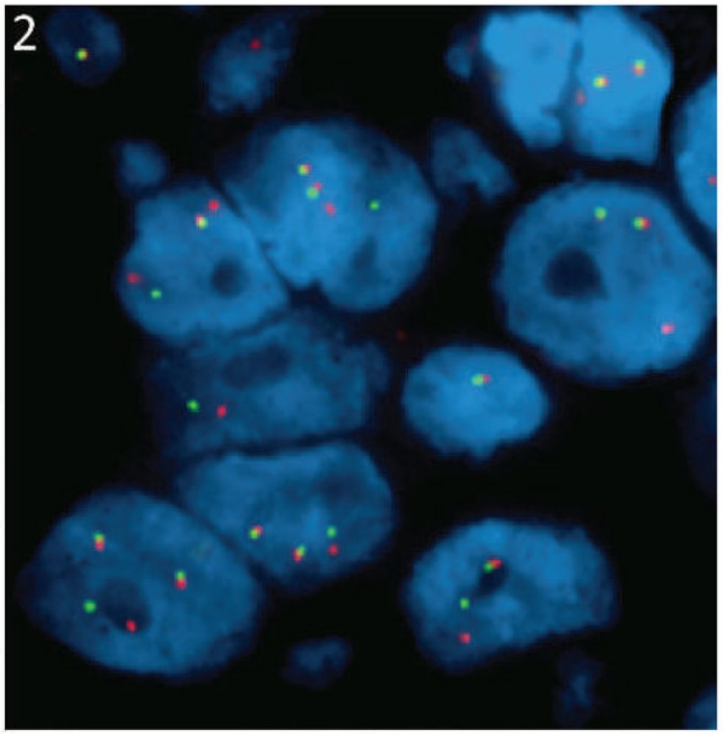


Figure 2.
Positive for *ALK* rearrangement (split 3′ *ALK*-5′ ALK) (original magnification × 1000).

Comparison of Probe Designs for Inversions