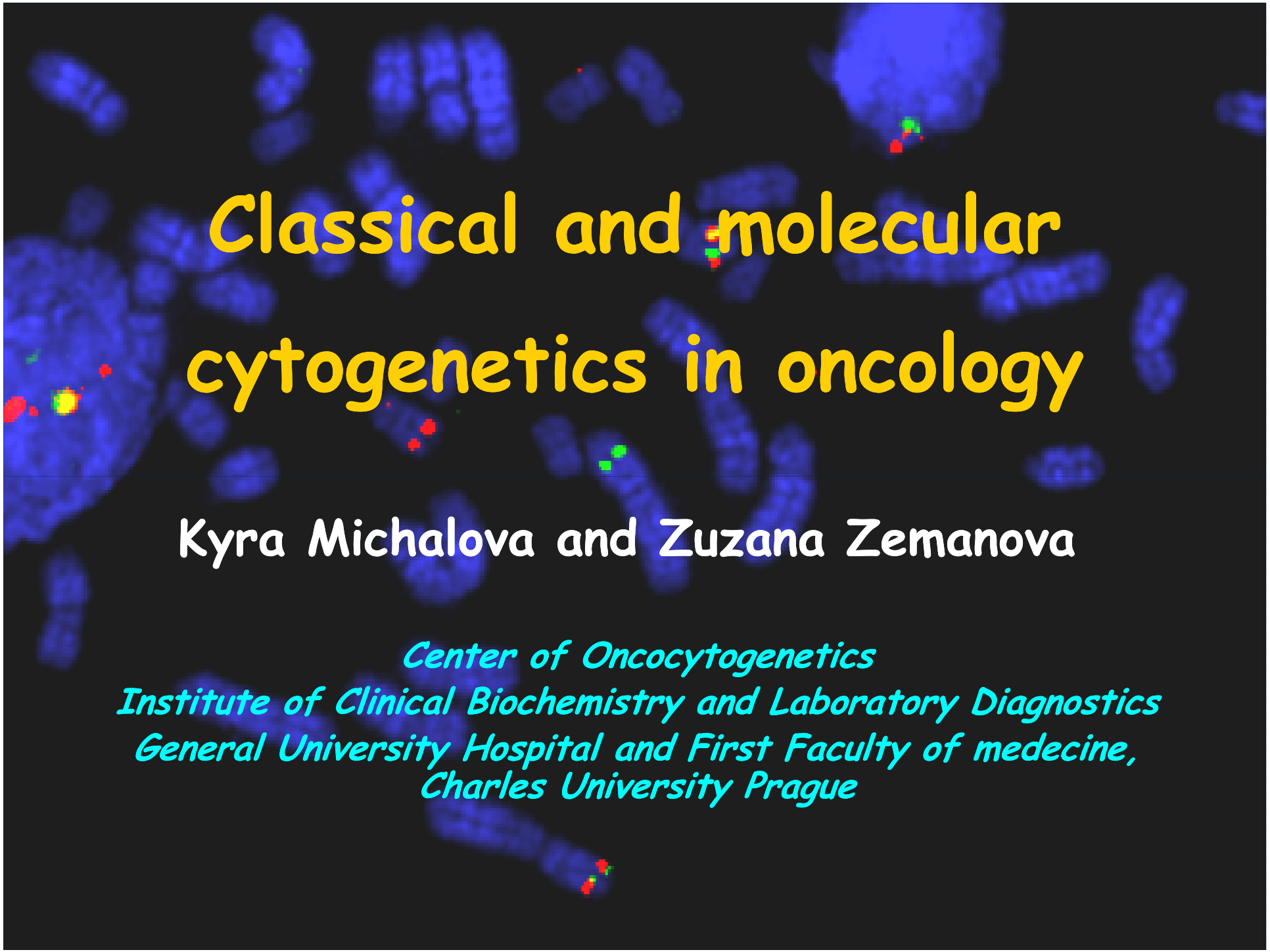

Classical and molecular cytogenetics in oncology

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Cytogenetic examination

Clinical cytogenetics → determination of karyotype of patients with inborn deffects

Prenatal diagnostics → examination of chromosomal complement of embryos, in vitro fertilization

Oncocytogenetics → specification of diagnosis and prognosis of malignant diseases

Services of hygiene laboratories - testing of mutagenicity of chemicals on human organism on chromosomal level, radiation cytogenetics etc.

Chromosomal aberrations

→ Changes of number or structure of chromosomes

I. Inborn (constitutional) aberrations are fundamental for origin of syndromes due to anomalies of chromosomes (for example Down´s sy); changes of chromosomes are present in all cells of the body.

II. Acquired changes of chromosomes in malignant cells; they have clonal character (only certain cellular clones are involved).

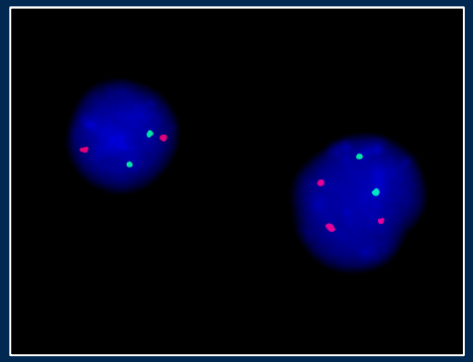
Cancer cells

- high genome instability - one of the most important events in the malignant process
- gene mutations and numerical and/or structural chromosomal aberrations

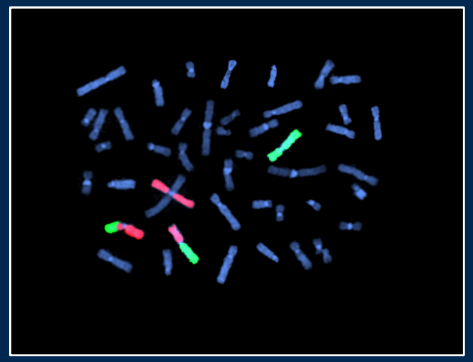
Nonrandom chromosomal aberrations are associated with specific disease subtypes and have a clear prognostic implications.

Methods

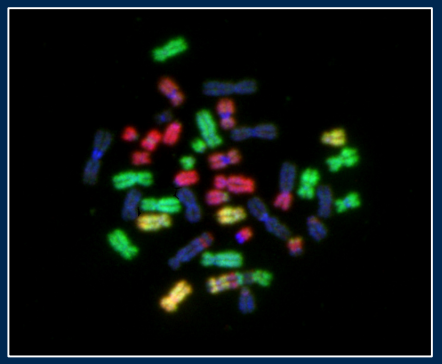
Conventional cytogenetic analysis



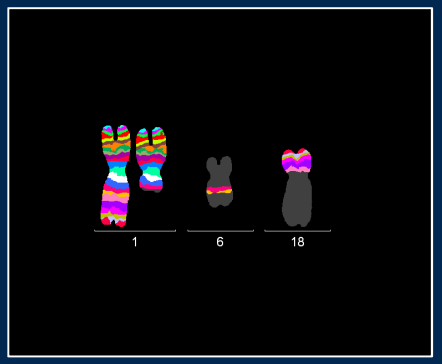
I-FISH



WCP-FISH



mFISH

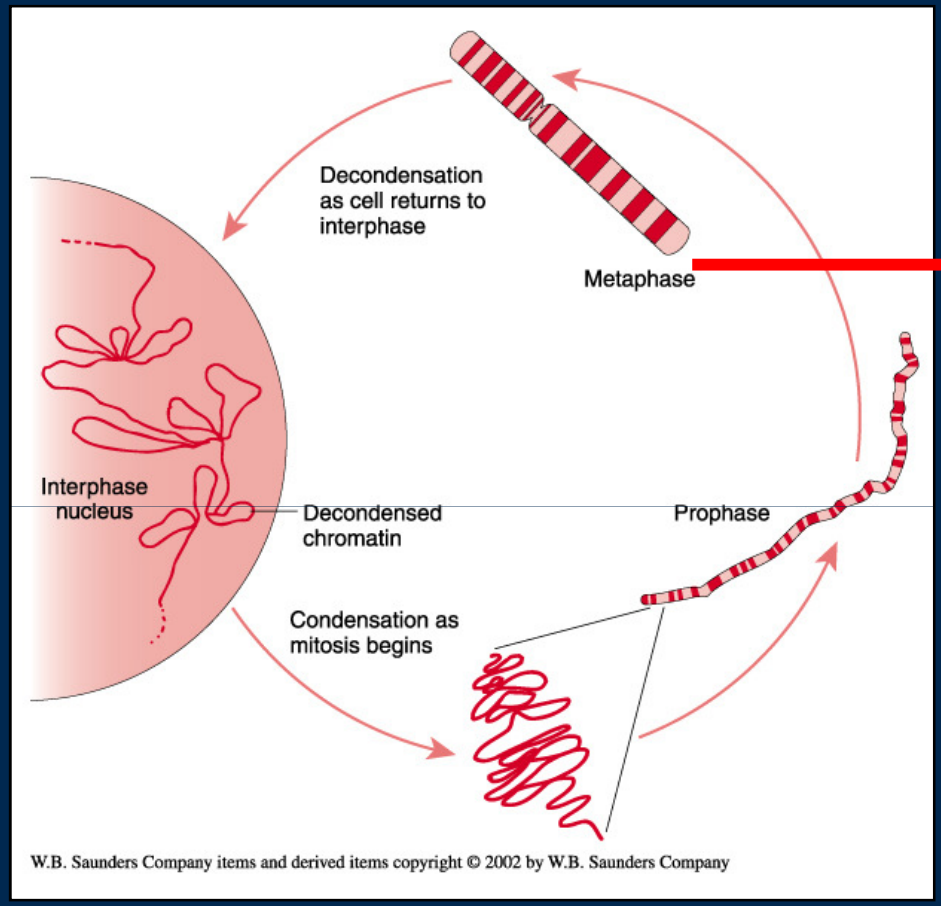


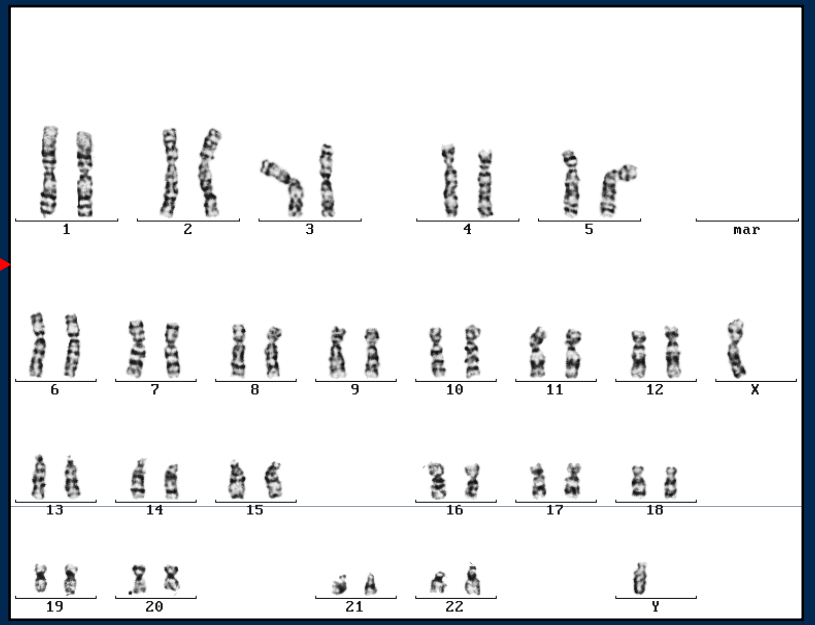
mBAND

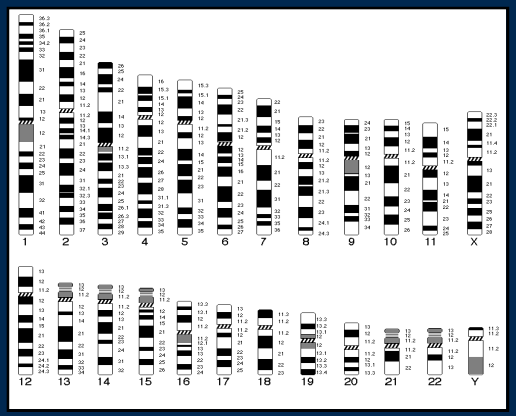
CGH

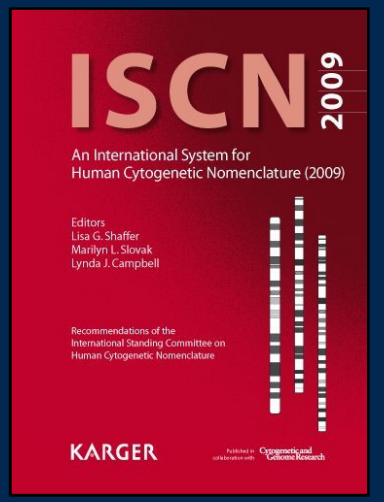
Array CGH

Conventional cytogenetic analysis

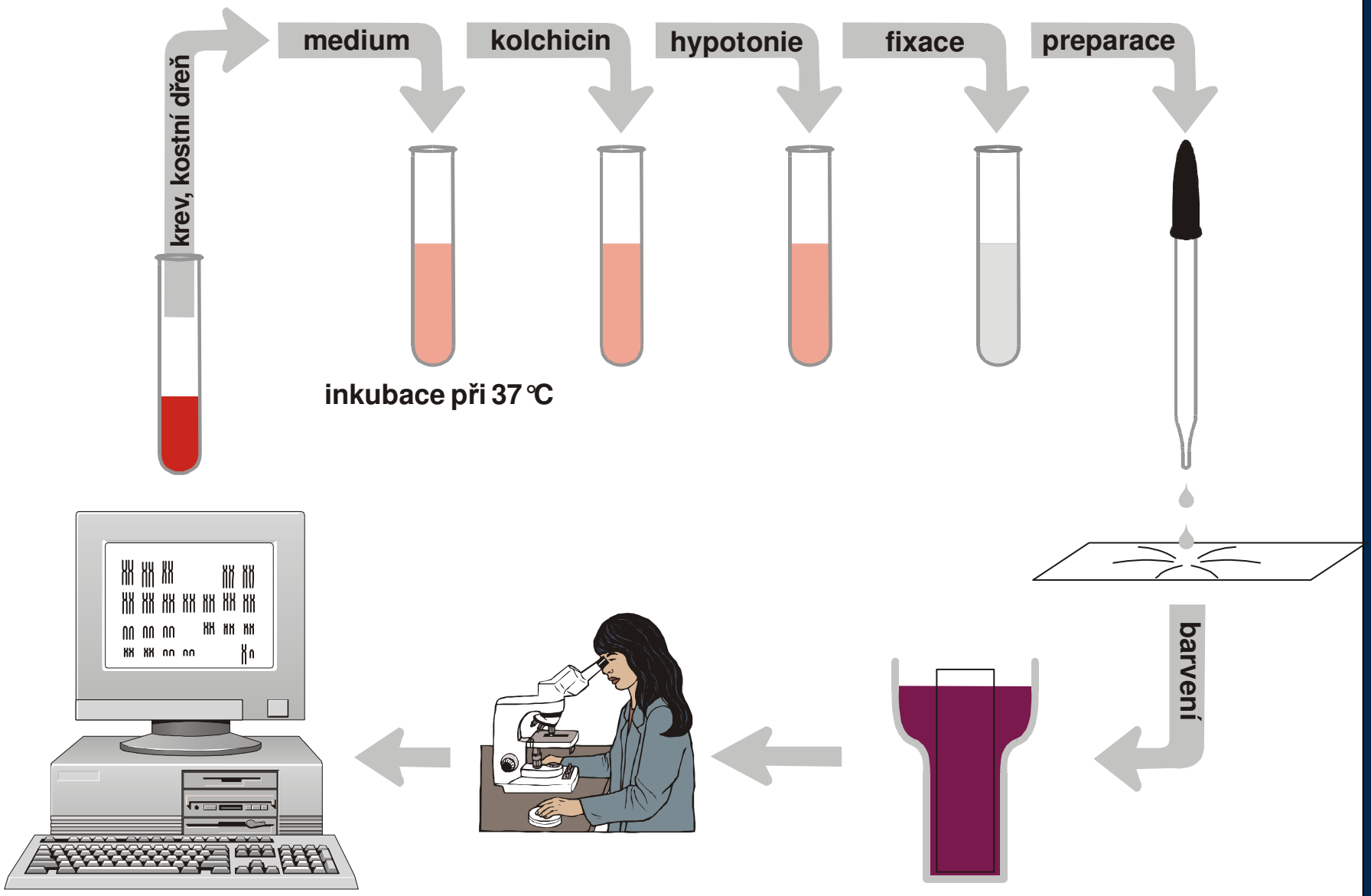






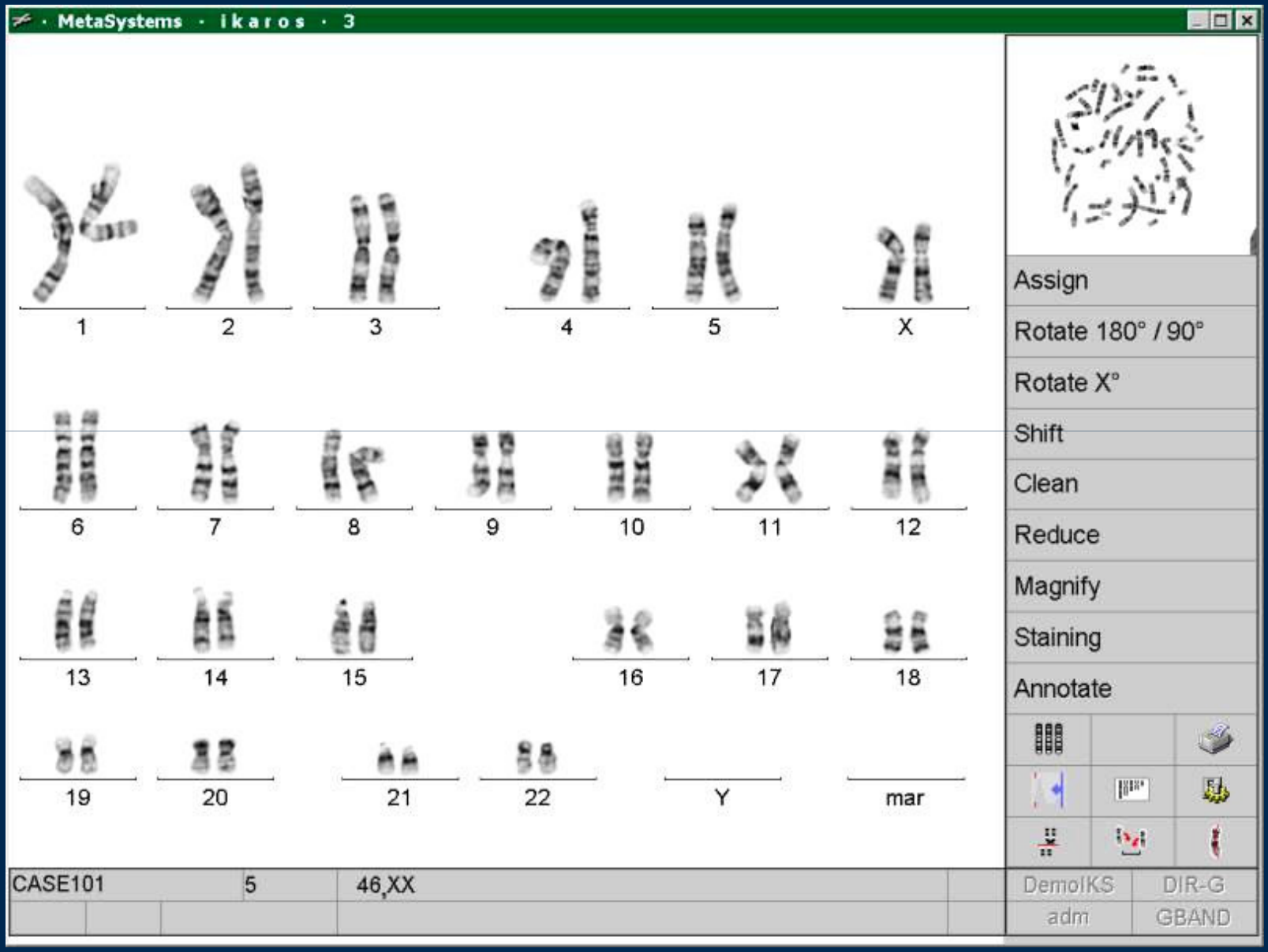


- Karyotype analysis
- Detection of chromosomal aberrations (numerical x structural)



Postup při kultivaci a přípravě preparátů k cytogenetickému vyšetření

Chromosomal banding:



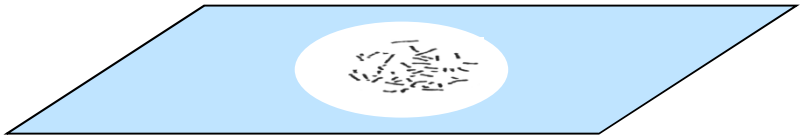
Limitations of classical cytogenetics

- sensitivity of chromosomal banding techniques is limited
- these techniques require a high rate of dividing cells with good chromosomal morphology (resolution limit of 10 Mb)
- in some leukemias malignant cells are not proliferating in the cell culture (only the normal cells are dividing) → the results of cytogenetic examination is not representative for malignant process
- chromosomal changes in leukemic cells are very often complex or could be cryptic under the limit of light microscopy

Fluorescence *in situ* hybridization (FISH)

- permits detection of selected acquired genetic changes in dividing (metaphase) and nondividing (interphase nuclei) cells
- is useful in establishing the percentage of neoplastic cells at the time of diagnosis and after therapy
- FISH studies are used to investigate the origin and progression of hematologic malignancies and to establish which hematopoietic compartments are involved in neoplastic processes

Fluorescence *in situ* hybridization (FISH)



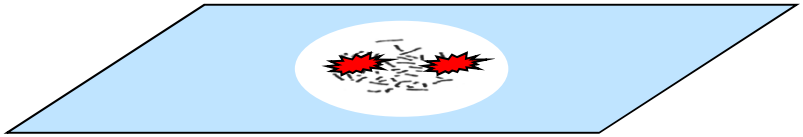
Cytogenetický preparát

Dvouřetězcová značená DNA

Denaturace buněčné DNA fixované na cytogenetickém preparátu

Denaturace DNA sondy

Hybridizace DNA sondy ke komplementárním úsekům cílové DNA fixované na cytogenetickém preparátu



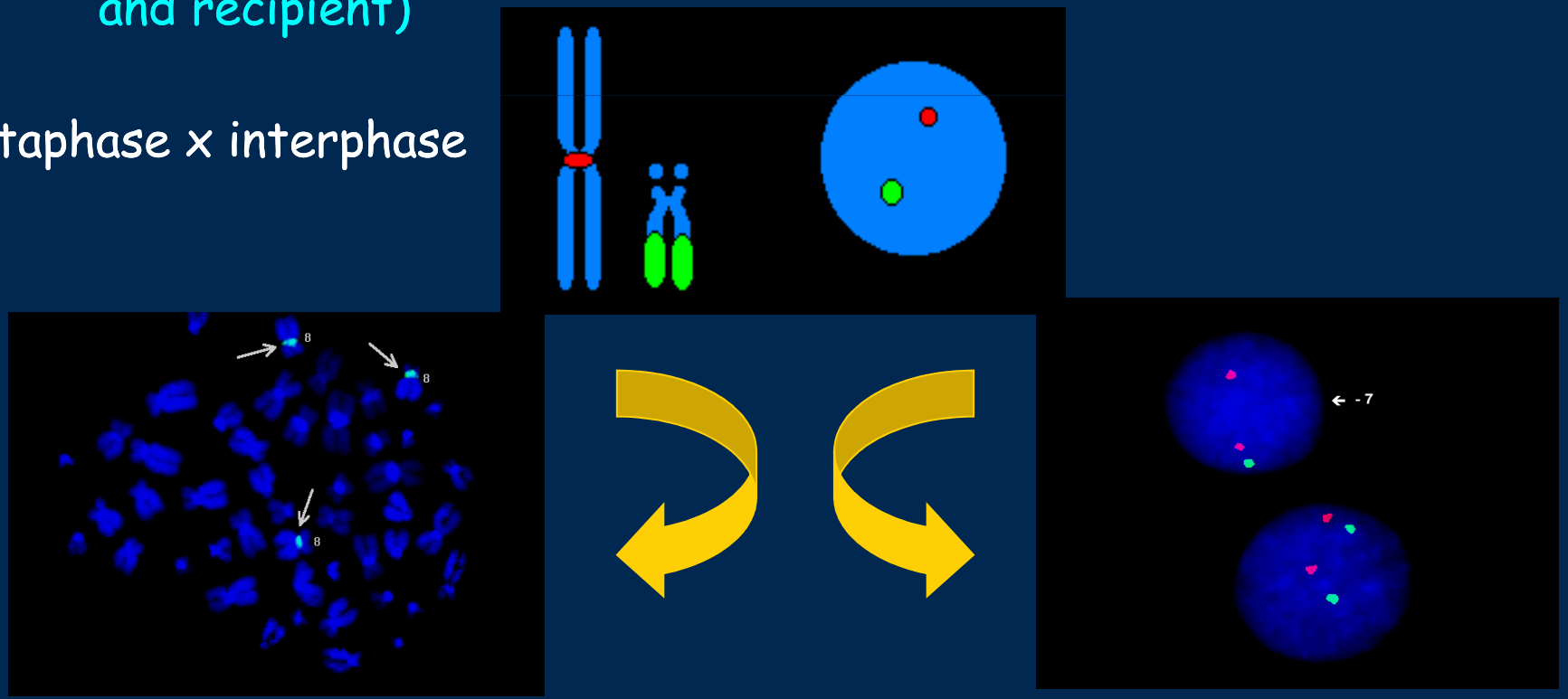
Analýza fluorescenčních signálů ve fluorescenčním mikroskopu

Probes for specific chromosomal structures:

α-satellite DNA - centromeres

- Determination of numerical aberrations, identification of the origin of cenromeres in marker-chromosomes, specification of cells after bone marrow transplantation (opposite sex of donor and recipient)

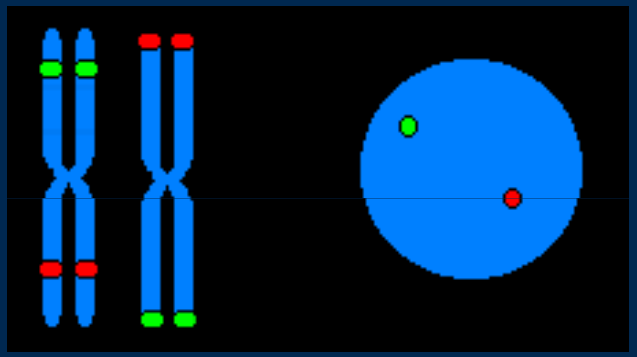
metaphase x interphase

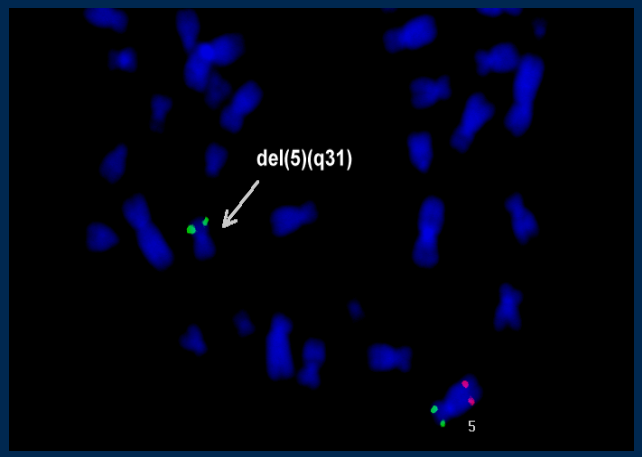


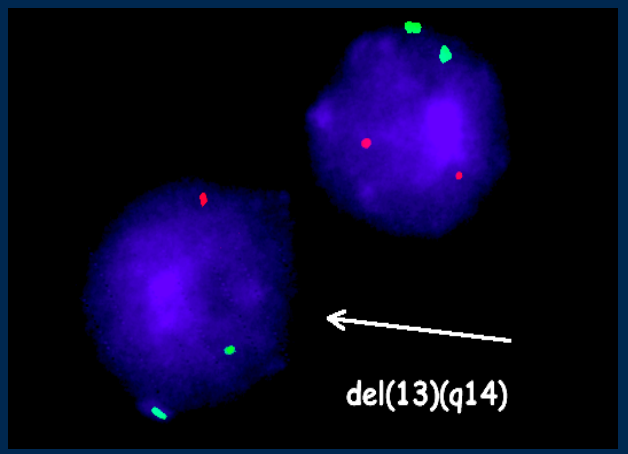
Locus specific DNA probes:

- Mapping of genes on chromosomes, detection of structural rearrangements (translocations, deletions)

metaphase x interphase





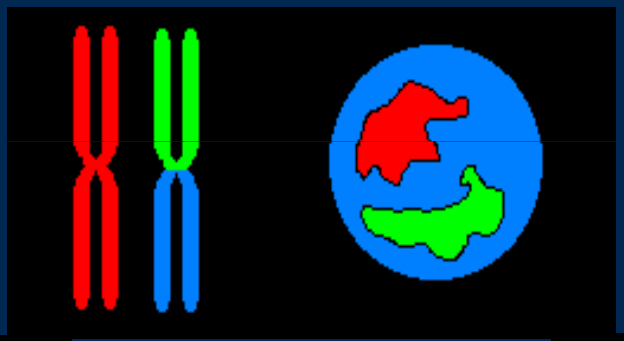


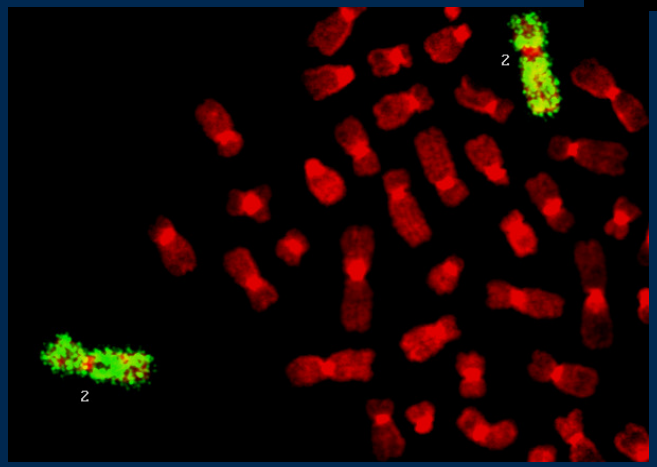
Chromosome painting probes:

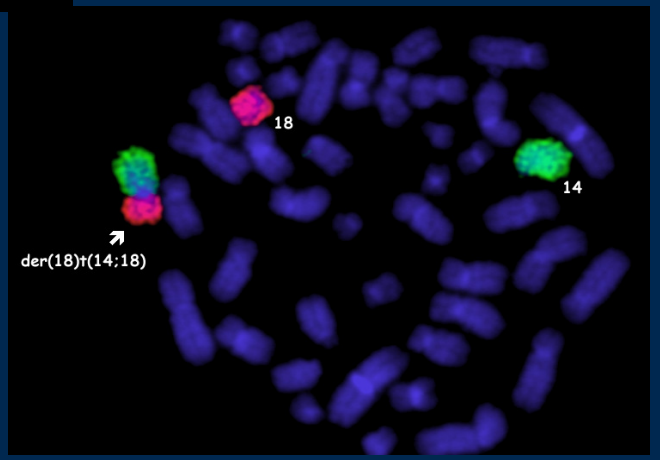
They contain sequences from whole chromosomes or chromosomal parts (partial probes)

➡ Determination of structural rearrangements (translocations and deletions of large extent), identification of origin of marker-chromosomes

Metaphase only

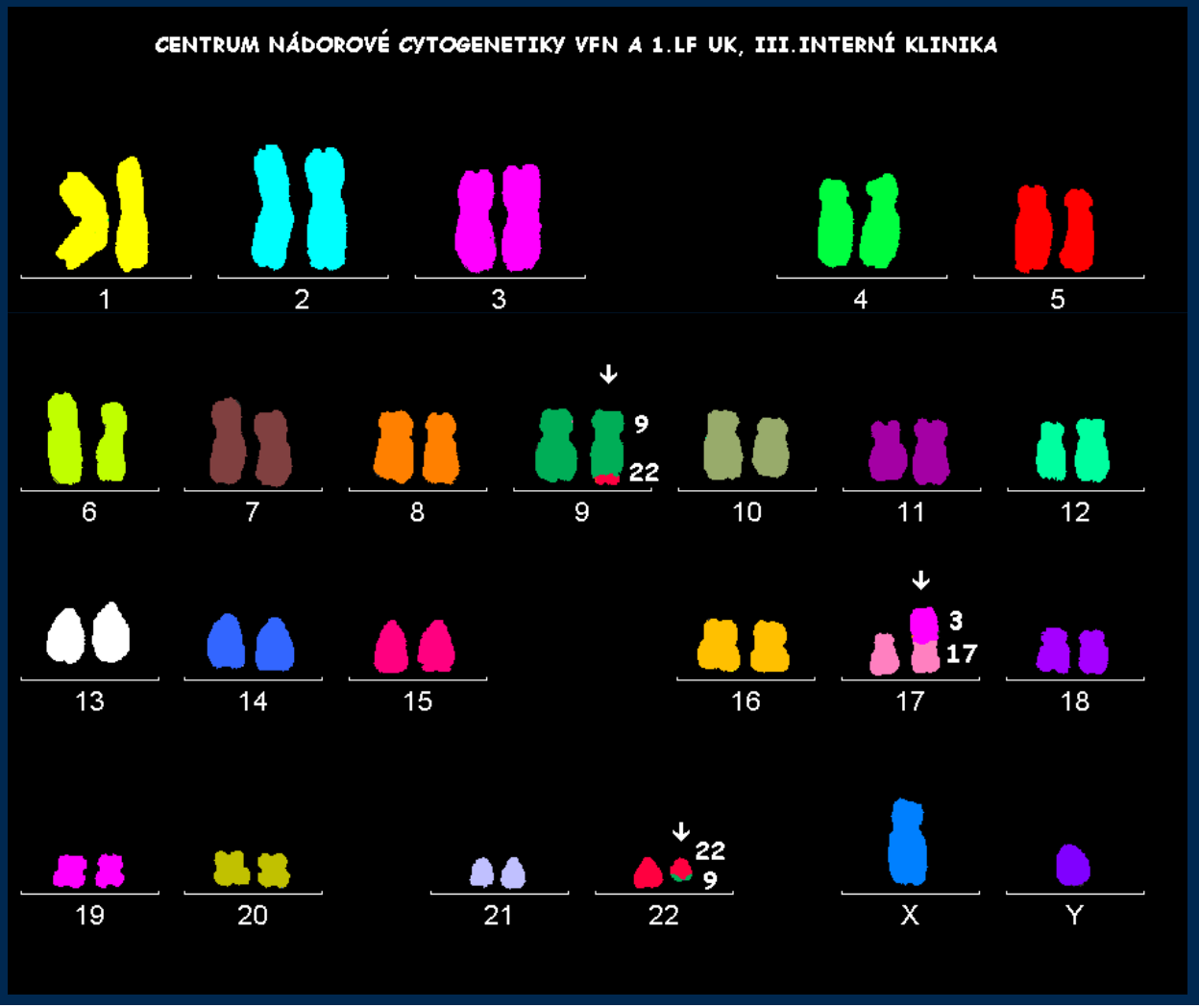






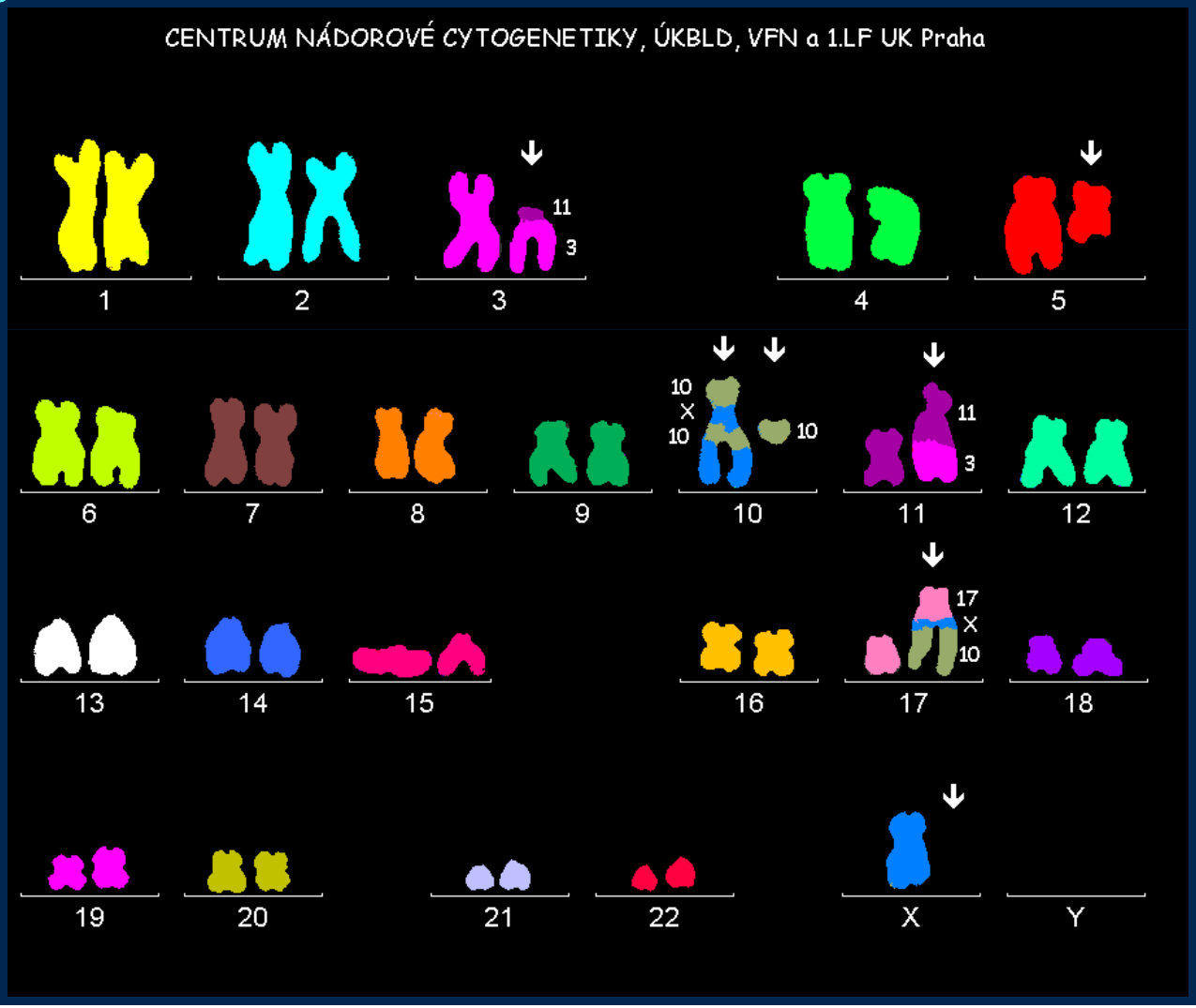
Multicolor FISH - mFISH

- allows in one hybridization experiment distinguish according to different color every pair of autosomes and sex chromosomes and then it is possible to make analyses of the whole genome and every structural and numerical rearrangement



Multicolor FISH - mFISH

- analyses of complex chromosomal rearrangements in bone marrow cells of patients with hematological malignancies will bring us detailed informations about involvement of specific chromosomes or their regions into rearrangements



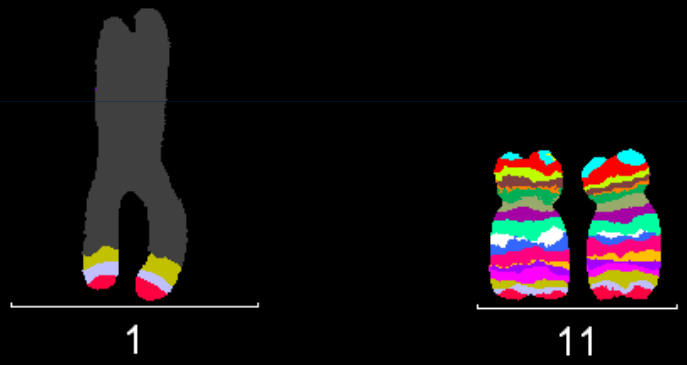
Multicolor banding with high resolution - mBAND

- enables determination of exact breakpoints of chromosomal aberrations with much higher resolution than classical banding

mBAND 1

der(1)(1;11)(pter->q31::q22.2q23.3)

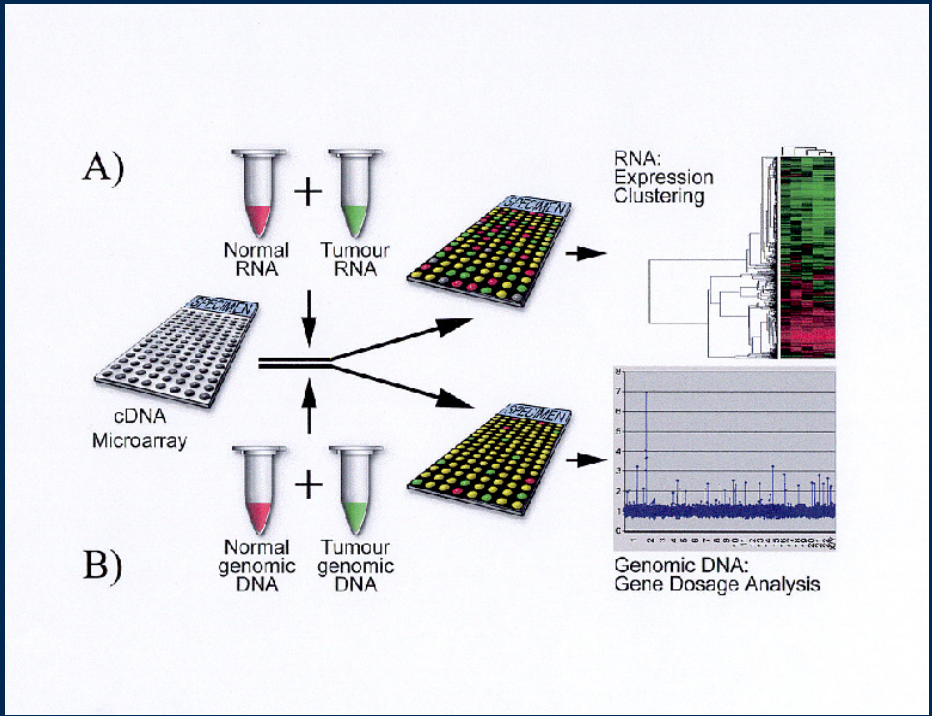
mBAND 11

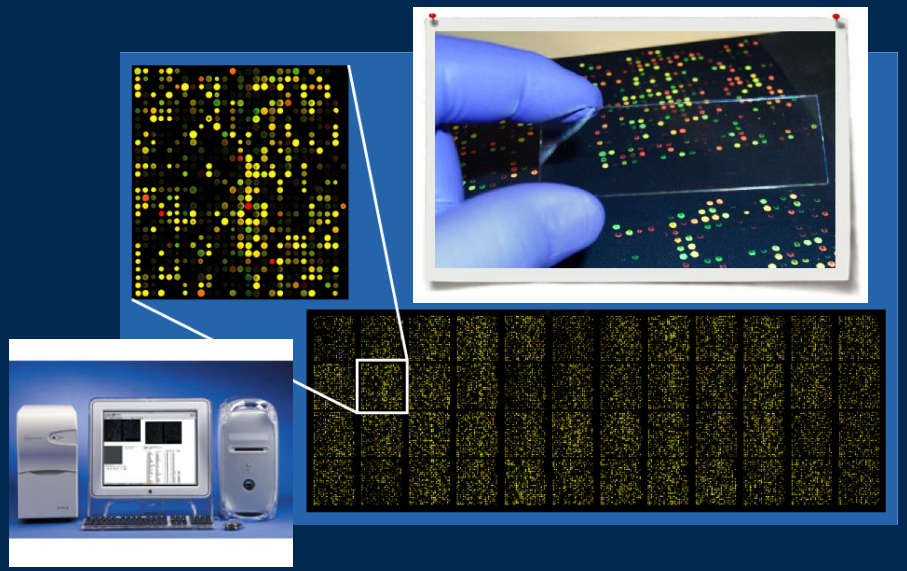


der(1)(1;11)(pter->q31::q22.2q23.3)

Array-based comparative genomic hybridization (aCGH)

- new tool to search for recurrent gains or loss of chromosomal regions throughout the genome according to detection with very high resolution of copy number changes at DNA level
- only recently is aCGH successfully utilised in diagnostics of leukemias and the results revealed a large spectrum of genomic imbalancies, including novel recurrent deletions and amplifications



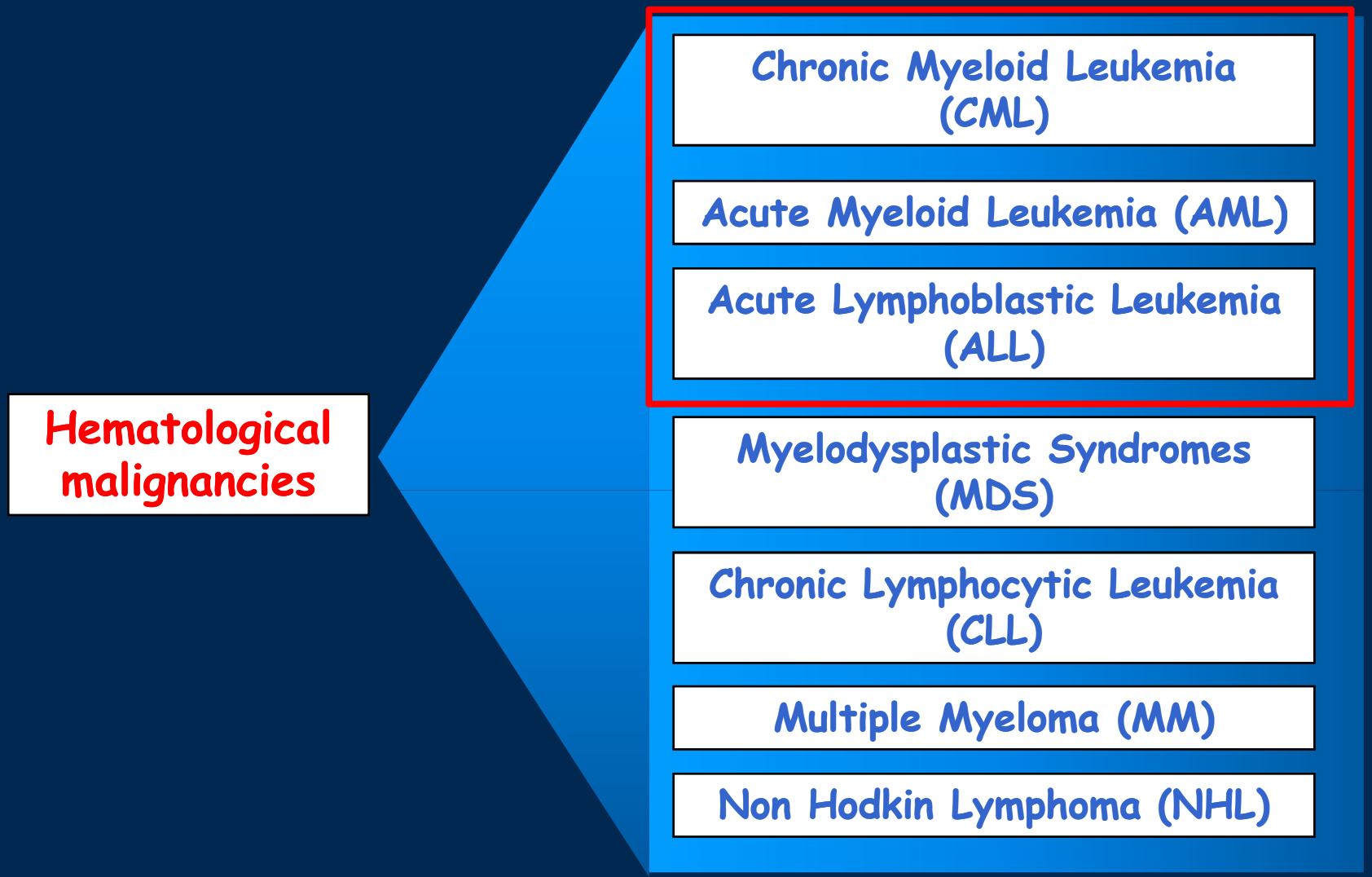


BAC arrays ~1MB
Oligo arrays ~100 kb (maximal resolution ~ 35 kb)

The impact of conventional and molecular cytogenetic analysis in oncohematology

- Is part of the work up at diagnosis
- Provides comprehensive information on the karyotype

  - ✓ help to specify diagnosis
  - ✓ help to determine the prognosis
  - ✓ help monitor effectiveness of treatment



Recommended examination procedures - depending on the type of disease and treatment protocol

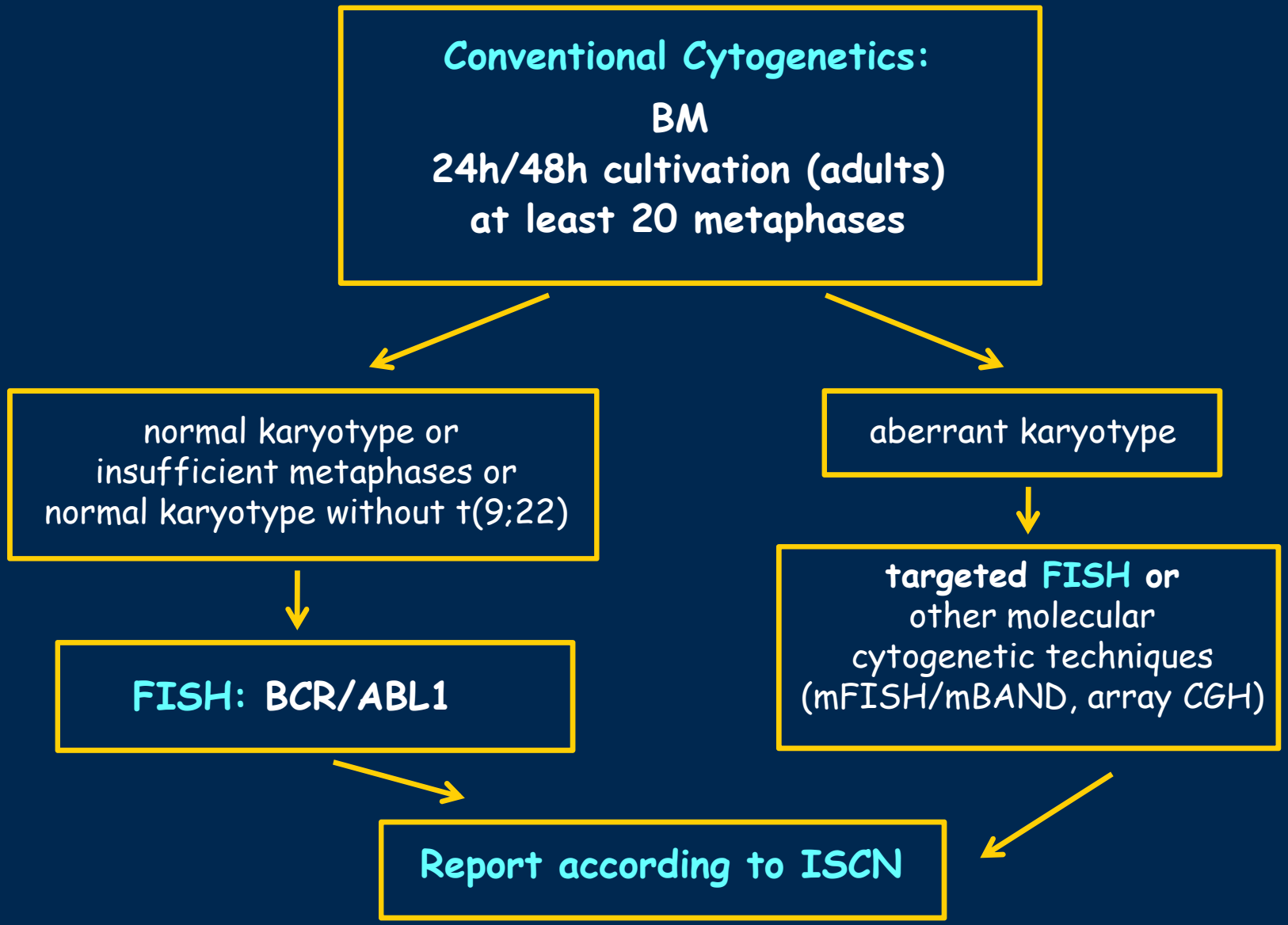
CML

Chronic Myeloid Leukemia (CML)

- ✓ 15-20% of all leukemia cases
- ✓ Mainly in adults (median age 65 years)
- ✓ Tri-phasic disease: CP - chronic phase (relatively benign, 3-9 years)
  AP - More malignant accelerated phase
  BC - Terminal blast crisis
- ✓ Blasts increase rapidly (crowd out healthy cells)
- ✓ One of the best-studied malignancies - has served as a paradigm for elucidation how genetic changes cause cells to become malignant: Ph chromosome t(9;22)(q34;q11) - BCR/ABL fusion
- ✓ One of the first malignancies in which a therapy targeting the underlying molecular defect has improved the clinical outcome of patients: Gleevec™ (Novartis) used for therapy
- ✓ t(9;22)(q34;q11) in 90-95% of patients (detectable by CC) - *BCR/ABL* fusion gene

CML

Chronic Myeloid Leukemia (CML)



AML

Acute Myeloid Leukemia (AML)

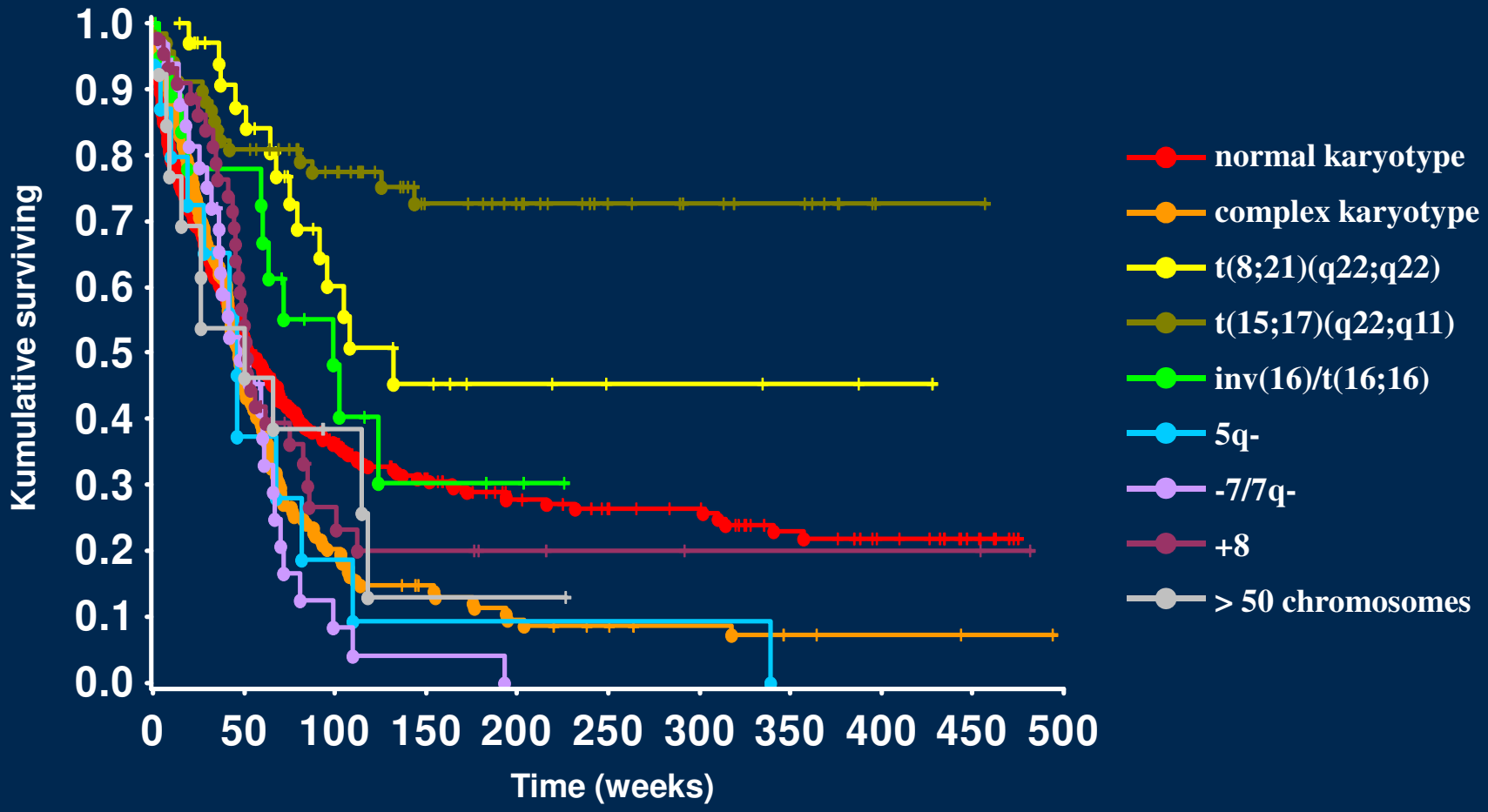
- ✓ Heterogenous group of malignant diseases of haemopoiesis
- ✓ Accumulation of immature myeloid cells (myeloblasts) in bone marrow
- ✓ Diagnosed in all age groups
- ✓ Most commonly affects people older than 60 years (median age 64-68 years)
- ✓ Secondary AML, therapy-related AML
- ✓ Aggressive disease - median OS 2-3 monts
- ✓ Specific chromosomal aberrations with clear prognostic significance → stratification of therapy according to cytogenetic findings

Cytogenetic findings in AML

Chromosomal aberration	Genes	Prognosis
t(8;21)(q22;q22)	*RUNX1-RUNX1T1*	good
inv(16)(p13.1q22) or t(16;16)(p13.1;q22)	*CBFB-MYH11*	good
t(15;17)(q22;q12)	*PML-RARA*	good
t(9;11)(p22;q23)	*MLLT3-MLL*	intermedial
t(6;9)(p23;q34)	*DEK-NUP214*	poor
inv(3)(q21q26.2) or t(3;3)(q21;q26.2)	*RPN1-EVI1*	worst
t(1;22)(p13;q13)	*RBM15-MKL1*	good
Rearrangements of *MLL* gene	*MLL*	poor
monosomy 7 or deletion of 7q31		poor
deletion of 5q31	*?*	poor
complex chromosomal aberrations	*?*	worst

AML

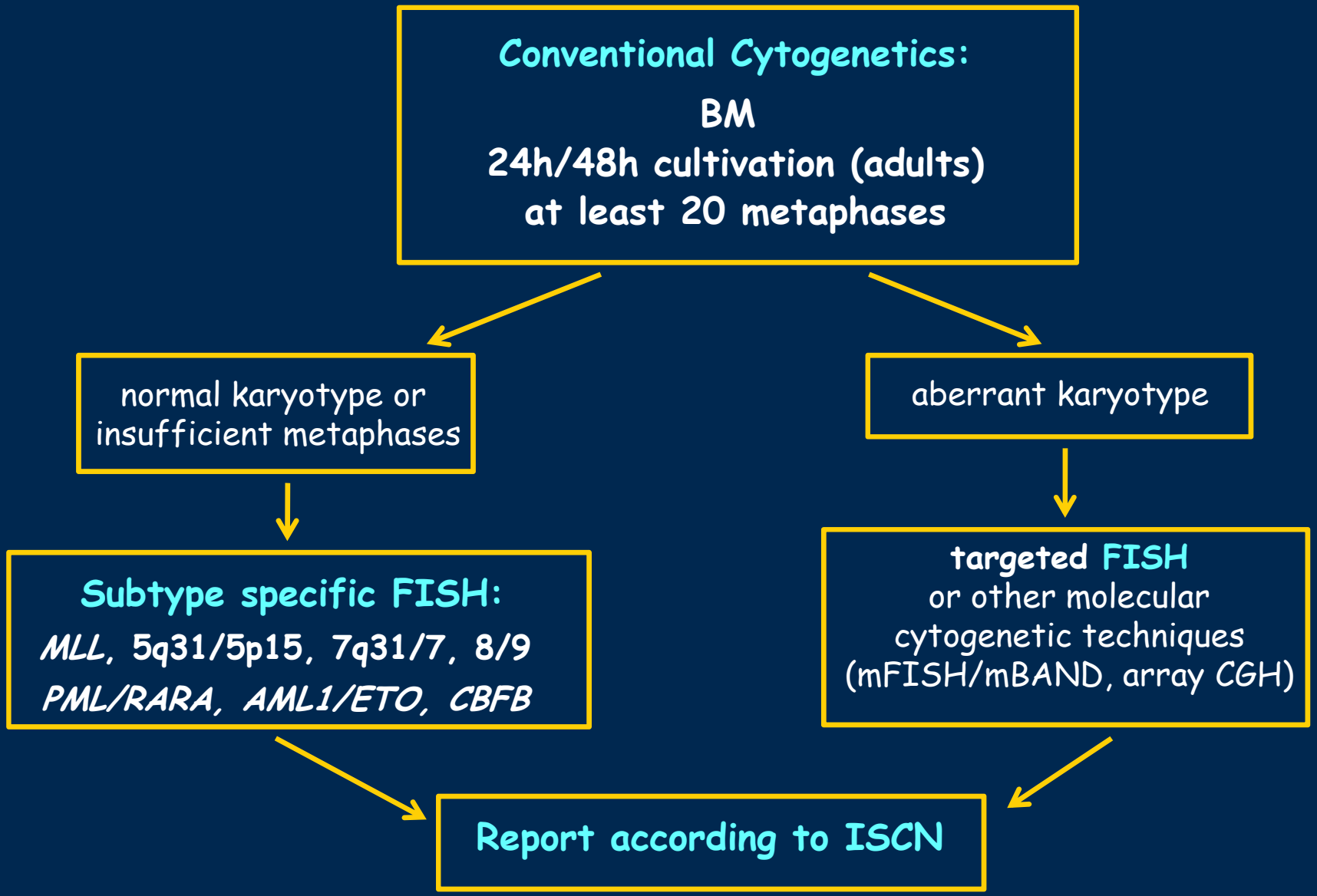
Prognostic impact of specific chromosomal aberrations in acute myeloid leukemia (AML)



Alert 2006

AML

Acute Myeloid Leukemia (AML)



Acute Lymphoblastic Leukemia (ALL) Overview

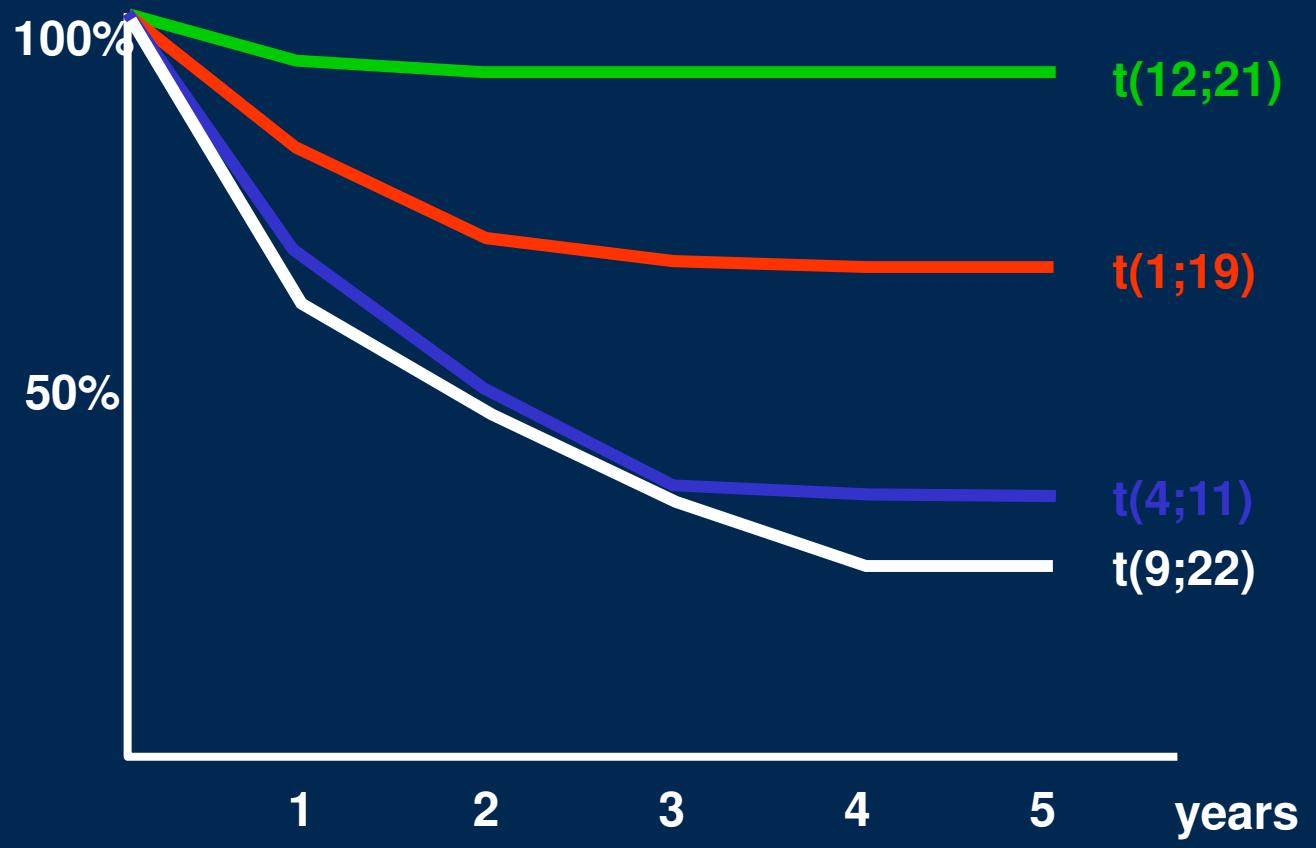
- ✓ Accumulation of malignant, immature lymphoid cells in the bone marrow and mostly also in peripheral blood
- ✓ Most common malignancy in children – representing 25% of pediatric cancer
- ✓ 80% of pediatric leukemias
- ✓ Heterogenous disease - distinct therapeutic and prognostic implications
- ✓ Chromosomal aberrations:
  - found up to 90% of cases
  - one of the most important prognostic factors!!
- ✓ Conventional chromosomal analysis are limited by the low mitotic activity of malignant B-lymphocytes → I-FISH for the most common recurrent chromosomal aberrations

Cytogenetic findings in ALL

Risk Group	Cytogenetic finding
Low risk	High hyperdiploidy (51-65 chromosomes)
	ETV6-RUNX1
Standard risk	t(1;19)(q23;p13)
	IGH-CEBP
	IGH-ID4
	del(6)(q)
	Aberration of 9p
	Aberration of 11q
	dup(1q)
	-7
	dic(9;20)(p13;q11)
	dic(9;12)(p11-21;p11-13)
	Any other aberration
	normal karyotype
High risk	t(9;22)(q34;q11)
	iAMP21
	MLL translocations
	„near" haploidy (<30 chromosomes)
	Low hypodiploidy (30-39 chromosomes)
	t(17;19)(q23;p13)
	Aberration of 17p
	Loss of 13q

Moorman et al., Lancet Oncol 2010

Prognostic impact of specific chromosomal aberrations in childhood acute lymphoblastic leukemia (ALL)



- Today, karyotype remains the gold standard for classification of patients with childhood ALL into risk group for treatment.

ALL

Acute Lymphoblastic Leukemia (ALL)

Conventional Cytogenetics:

BM
direct/24h cultivation (childhood)
24h/48h cultivation (adults)
at least 20 metaphases

↓

aberrant karyotype

↓

targeted FISH or
other molecular
cytogenetic techniques
(mFISH/mBAND, array CGH)

↓

Report according to ISCN

I-FISH:

B-ALL:

„triple test"

ETV6/RUNX1
MLL
Hyperdiploidy

or

T-ALL:

TCR genes

TCRαδ (14q11), TCRβ (7q34), TCRγ (7p14)

TP16 (9p21)

ABL1 (9q34)

CONCLUSIONS

- The impact of cytogenetic analyses on clinical diagnostics of hematological malignancies has increased dramatically during recent years → laboratory techniques have to be optimized to provide reliable results for optimal patient care.

- Quick and correct results save time and money by preventing unnecessary additional diagnostics and suboptimal treatment approaches.

- Standardization of cytogenetic diagnostic protocols may help to improve diagnosis, and hence treatment outcome of hematologic malignancies.