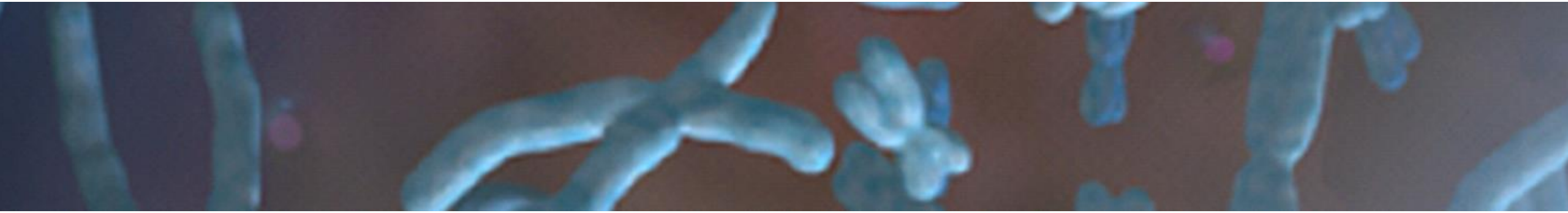
BHS training course

Laboratory Hematology
Cytogenetics



Jolien De Bie
Center for Human Genetics Leuven
14/10/2023

Organization of the Lecture

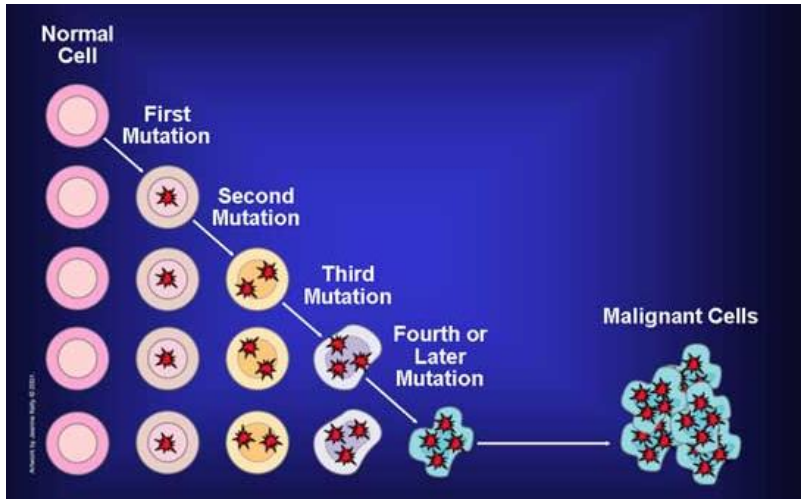
1. Definition and principles
2. Different techniques used
3. Applications of cytogenetic analyses
   - Diagnosis
   - Prognosis
   - Contribution to therapeutic developments

1. Cytogenetics: definition

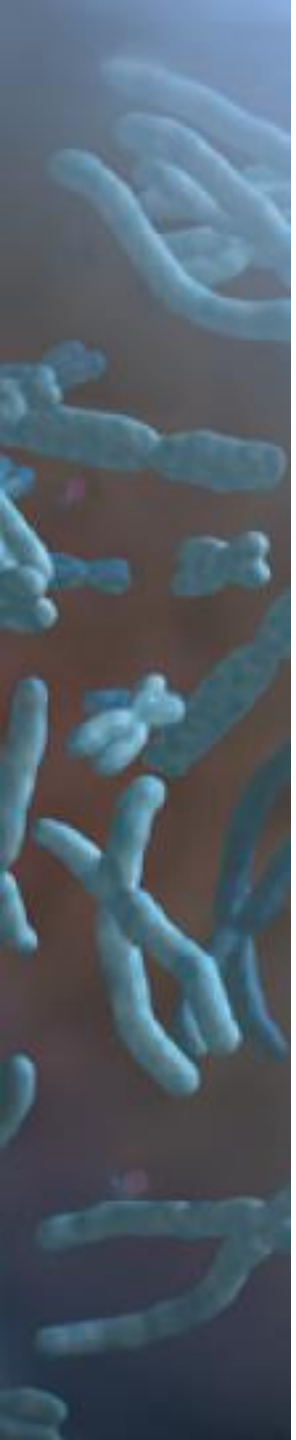
“Branch of genetics which correlates the structure and number of chromosomes present to the genotype and phenotype of individuals or neoplasia.”

Applicable to constitutional and acquired disorders

1. Acquired cytogenetics: principles



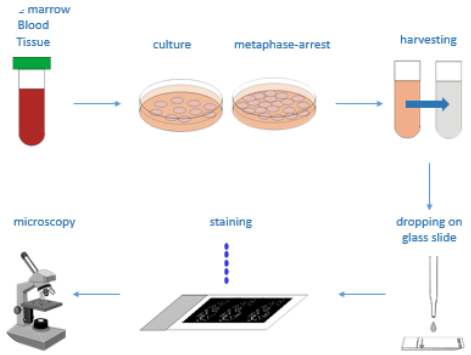
- Acquired malignant hemopathies are characterized by primary genetic aberrations which are present in all neoplastic cells demonstrating that these are clonal disorders
- Secondary abnormalities accumulate throughout the course of the disease (= clonal evolution)
- Aberrations can be chromosome abnormalities and/or gene mutations



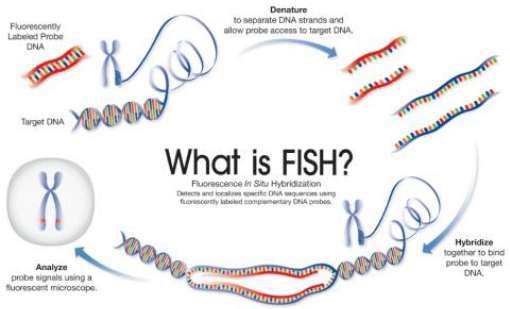
1. Acquired cytogenetics

- Identification of clonal abnormalities can
  - ❖ confirm or orientate a diagnosis
  - ❖ Provide prognostic information
  - ❖ Provide a marker for monitoring disease progression and efficacy of treatment
- Clonal abnormalities are recurrent within a disease entity
  - ❖ Some aberrations are disease-specific, e.g. t(15;17) in APL
  - ❖ Most are found in more than one entity, e.g. t(9;22) in CML and ALL
  - ❖ Each entity is defined by a characteristic profile of recurrent abnormalities
- Clonality ≠ always malignancy

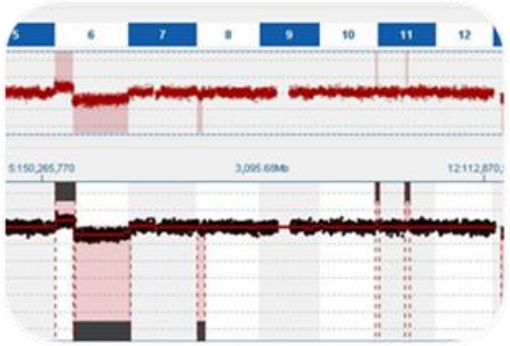
2. Cytogenetic methods to detect clonal abnormalities



Karyotyping

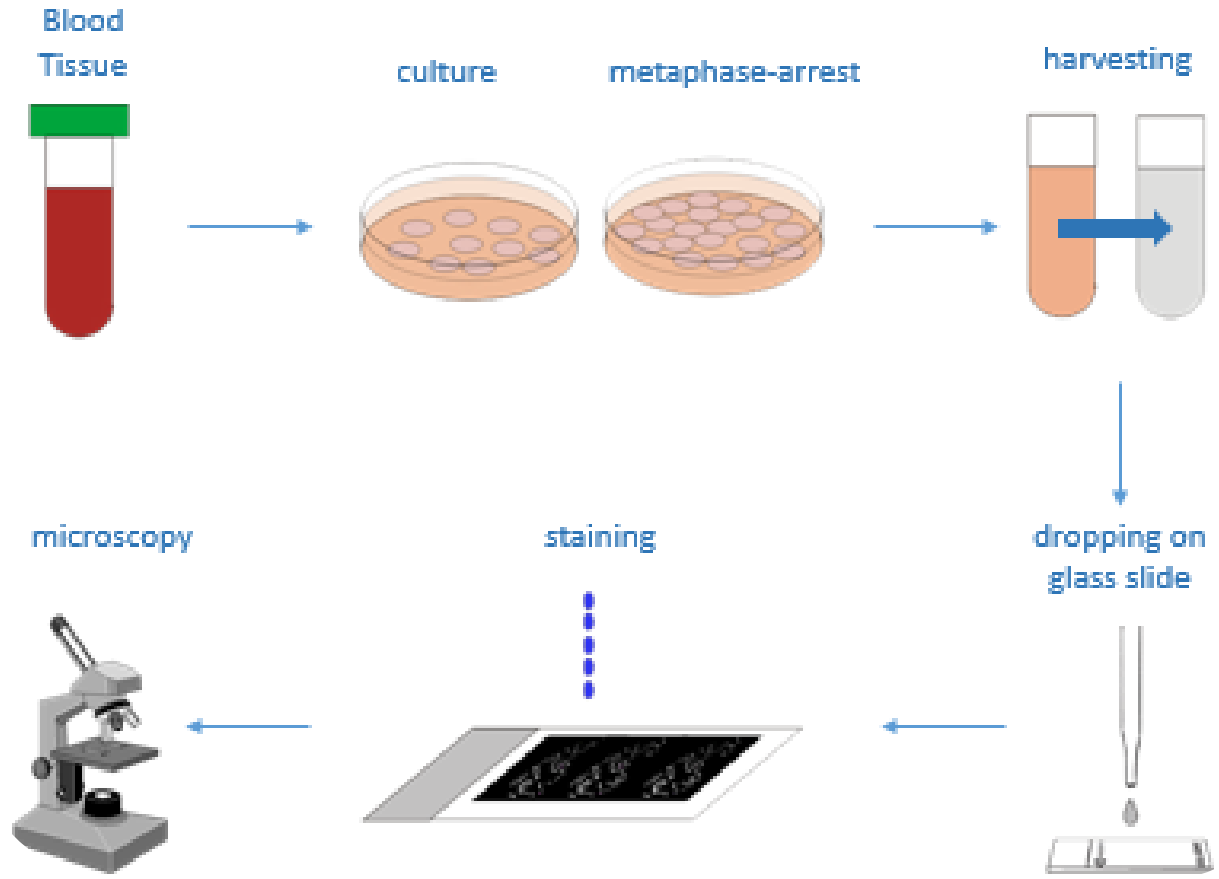


FISH



DNA based methods providing
a molecular karyotype

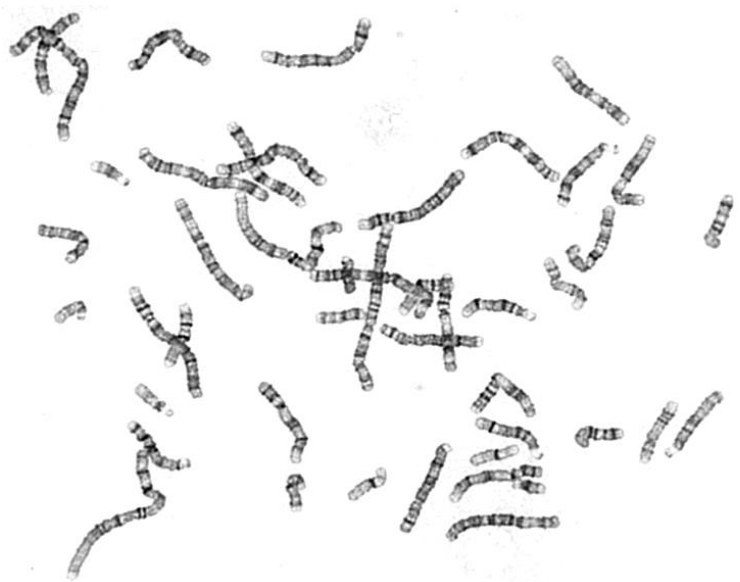
2.1. Karyotype



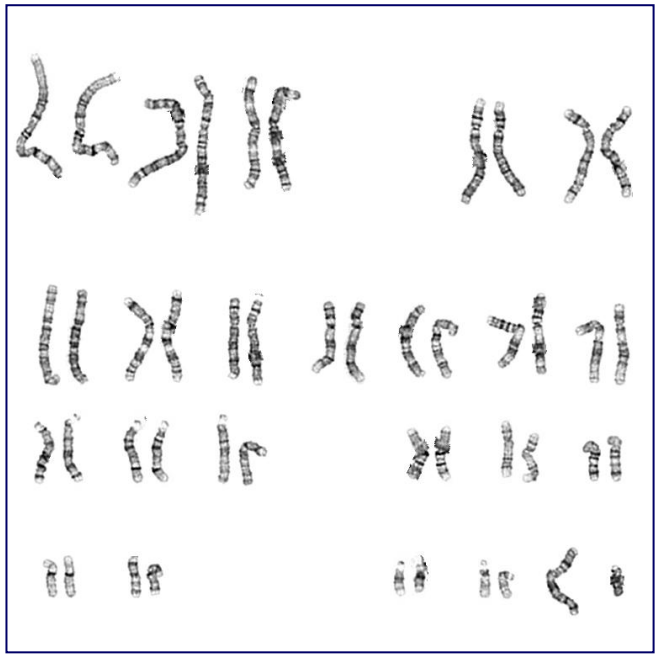
+ detection of structural and numerical variants genome wide

- failure of the karyotype, resolution is low

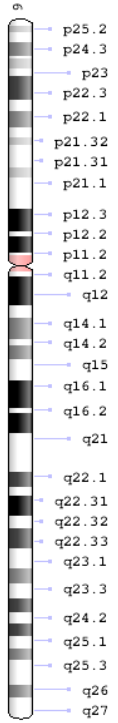
2.1 Establishment karyotype



Metaphase

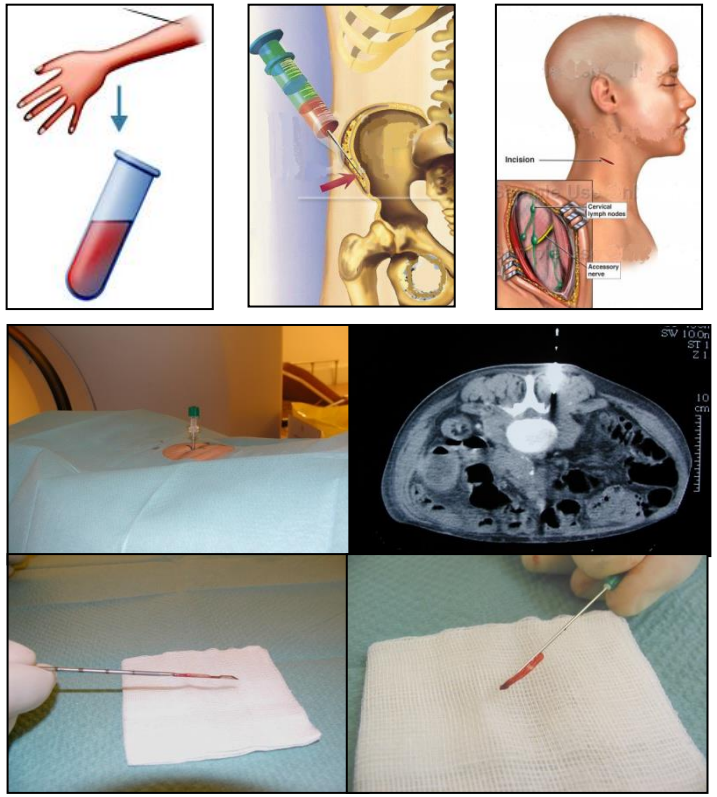


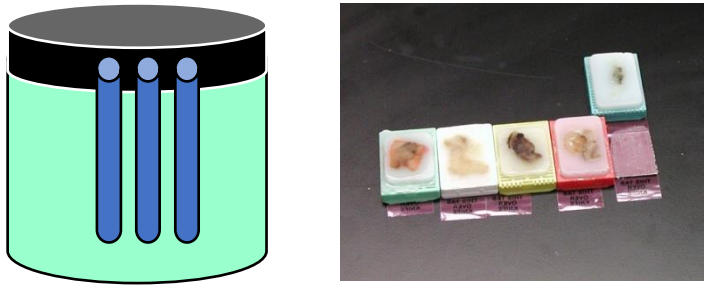
Karyogram



Idiogram

Sample types





All invaded tissues are suitable...*but* tissues must be viable, and the target cell capable of proliferation

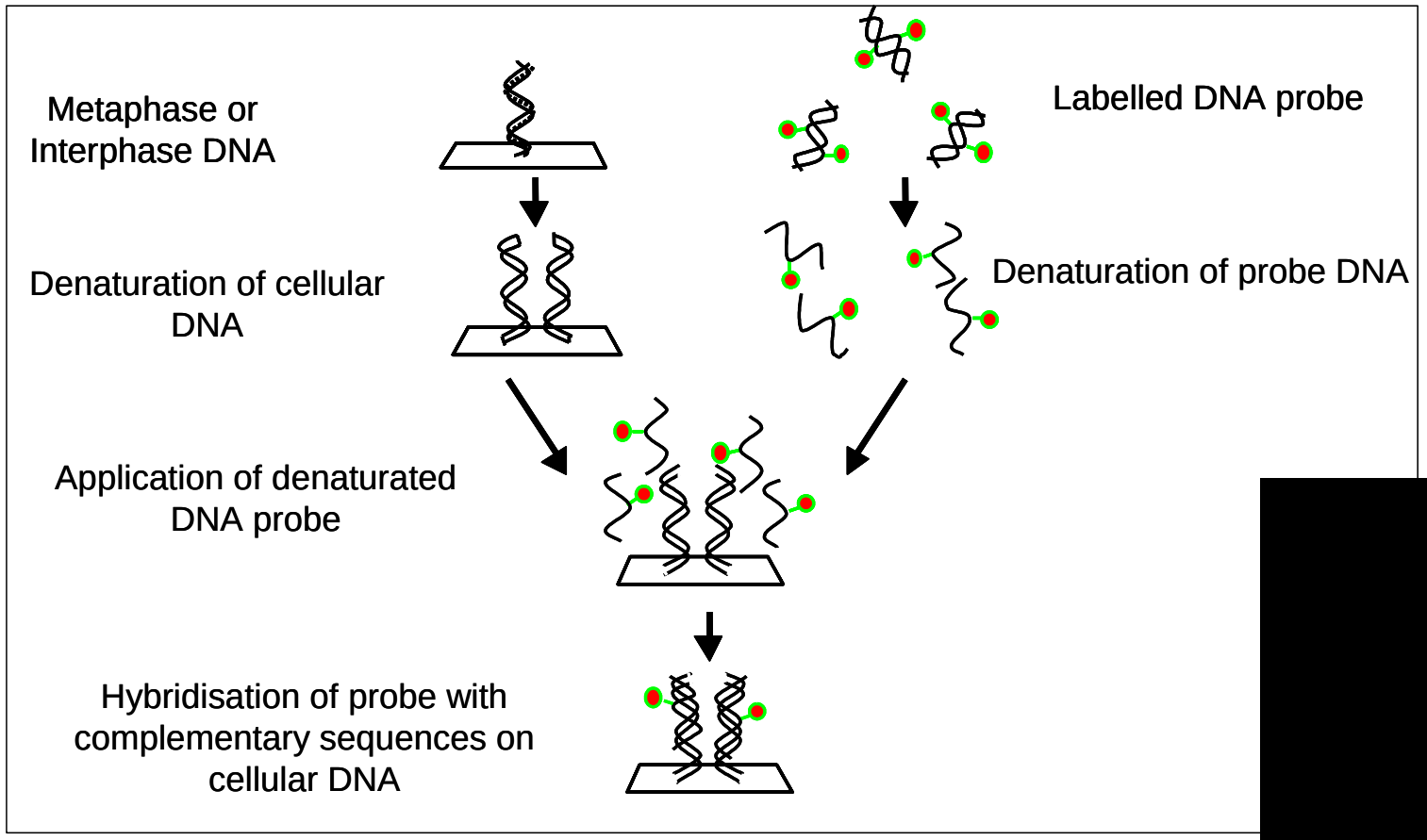
2.1 Karyotype result

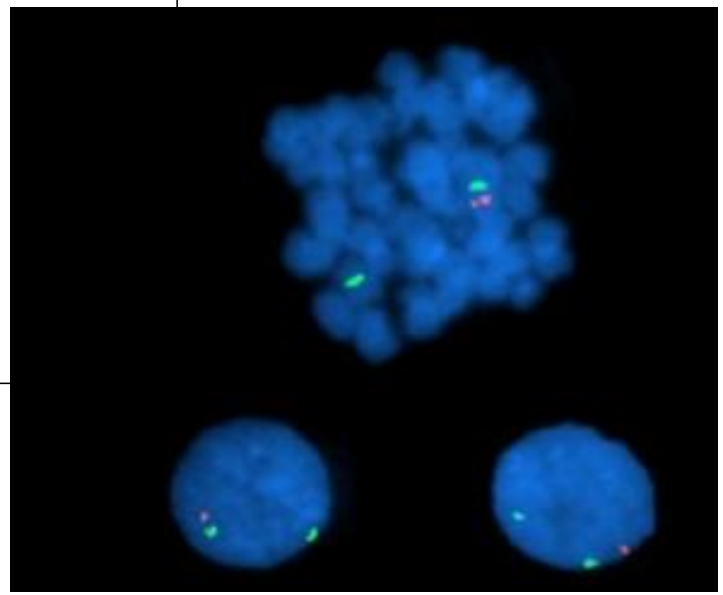
Result: karyotype = summary of several mitoses, expressed as a formula, according to rules and nomenclature (ISCN 2020)

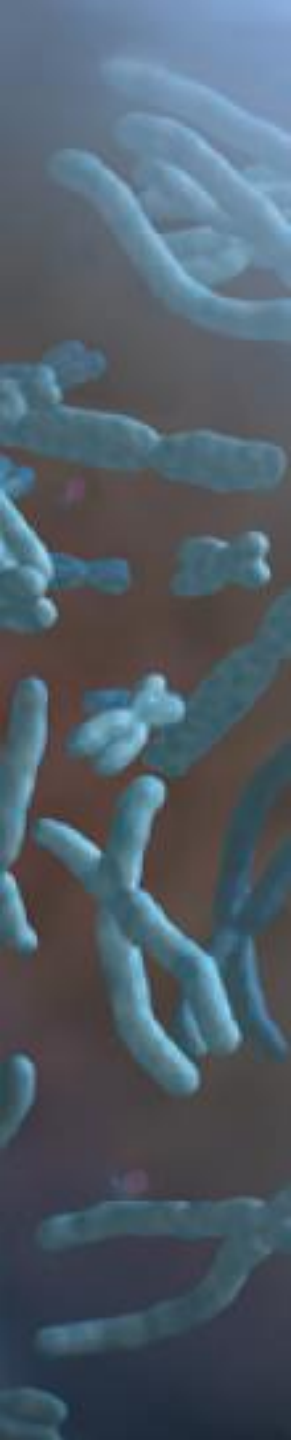
- Number of chromosomes («modal» number) of the clone
- Gonosomes (according to ploïdy) and abnormalities
- Autosomes (ascending order: 1→ 22) and abnormalities
- Abbreviation for each type of abnormality
- Number of cells in the clone : [ ]
- Each clone is described separately (« / » between clones)

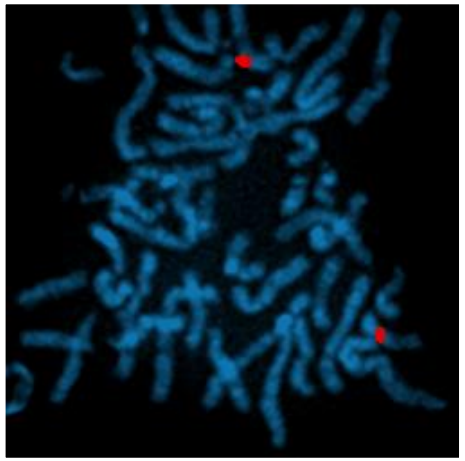
46,XY,t(9;22)(q34;q11)[4]/47,idem,+8[3]/46,XY[10]

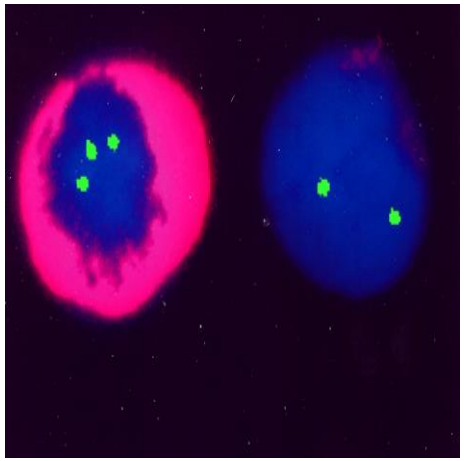
2.2 FISH (Fluorescence In Situ Hybridization)





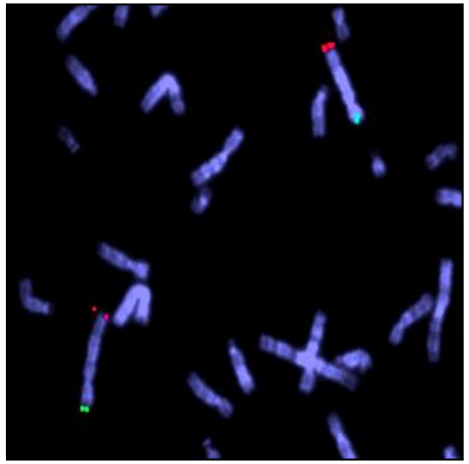


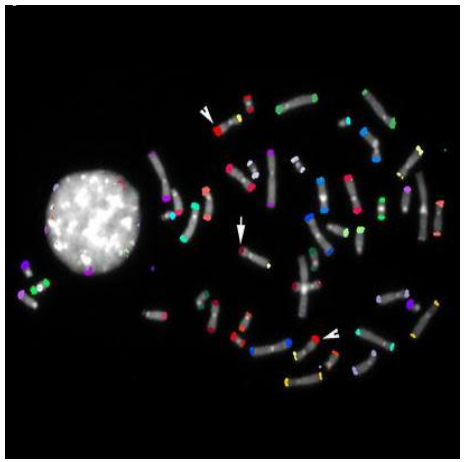




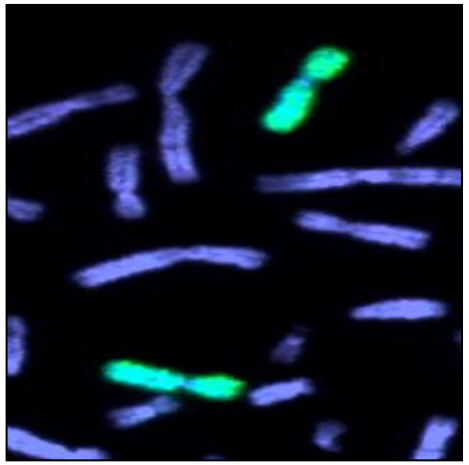
Different probes:

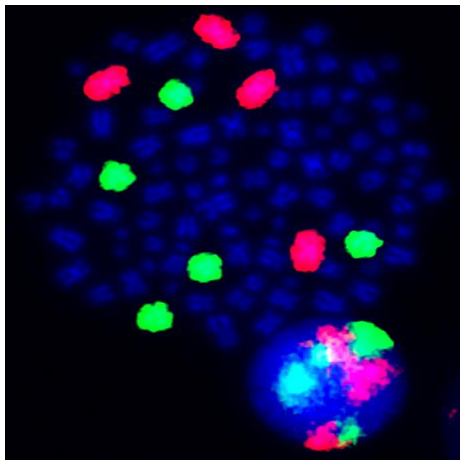
- centromeric





- telomeric



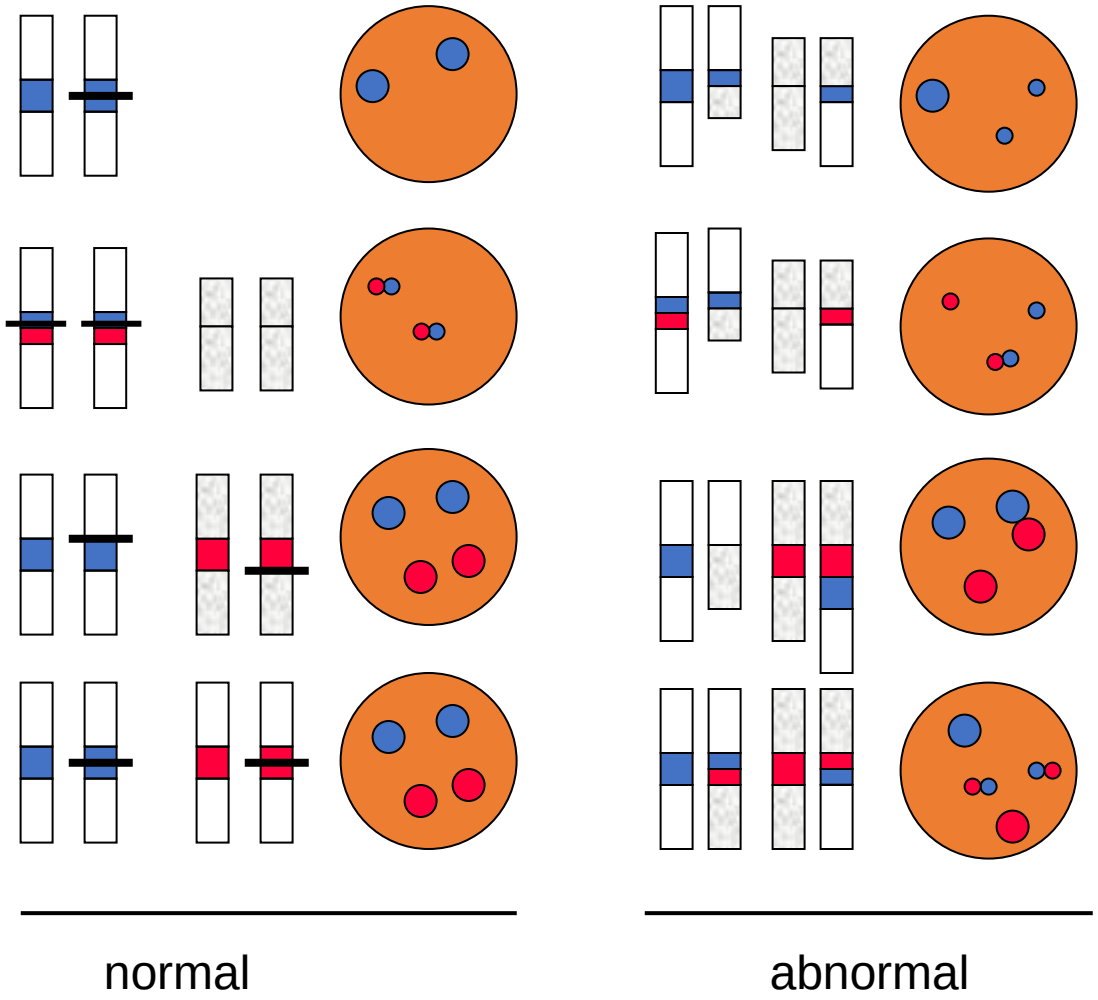


- painting (wcp)

  Metaphase only

Locus-specific probes:
translocation probes
microdeletions probes (*TP53*)

- Breakapart
- Single fusion
- Dual fusion



FISH allows the identification of cryptic abnormalities such as small deletions

–Conventional karyotype (smallest band) 5-10 Mb

–FISH on Interphase nuclei ± 50-100 Kb

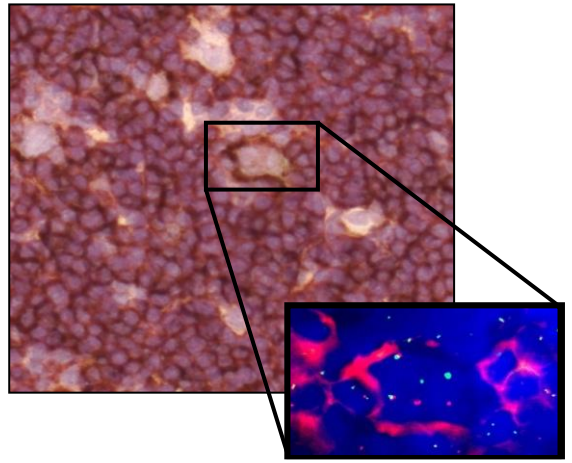
+ detection of specific structural or numerical variants at high resolution

FISH on interphase nuclei:

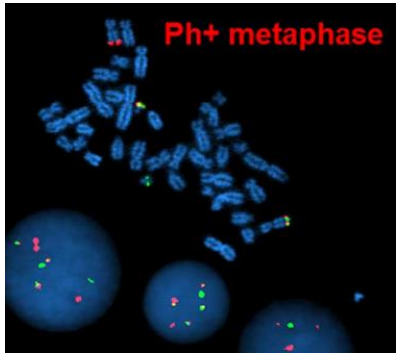
→ no cell culture required,

→ **more sensitive** than karyotype (more cells can be scored),

→ in combination with morphology & immunology to allow analysis of specific cell types



Metaphase FISH advantage: information on chromosome structure and probe location



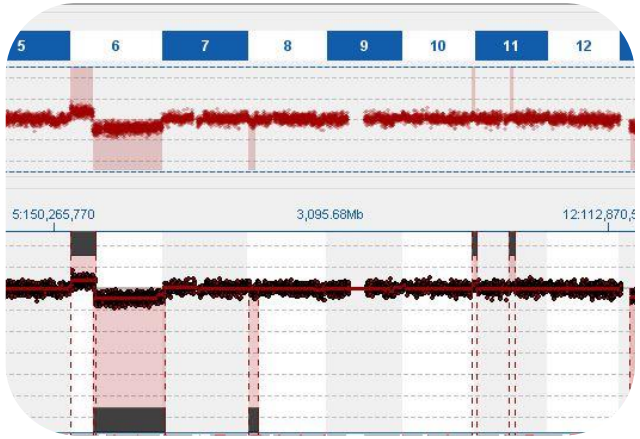
- targeted analysis

2.3 Molecular based virtual karyotypes

Several DNA based technologies are available - different technologies and commercial platforms

Based on either microarray or NGS technologies.

Compare the number of copies of genomic regions in the test sample compared to a normal genome to identify gains and losses



high number = Gain

low number = Loss

Regions of gains and loss mapped to reference genome sequence identifying precise genomic content (genes, genomic co-ordinates, exons..)

2.3 Advantages and limitations of molecular based karyotypes

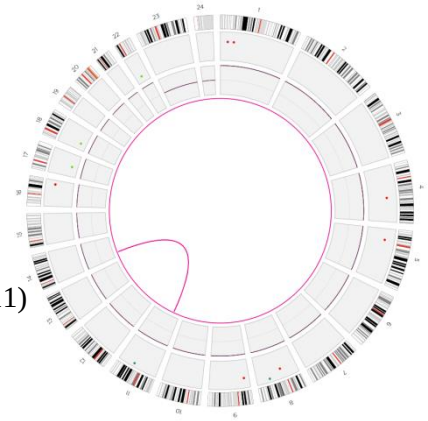
+	Overcomes limitation of karyotyping - No requirement for proliferating cells Overcomes limitation of FISH - Whole genome Higher resolution than karyotype and FISH – resolution defined by the number of probes and spacing
-	Does not detect balanced rearrangements Not widely implemented into routine acquired cytogenetics as need for complementary analyses

2.4 New emerging technologies for molecular based karyotypes, e.g. Optical Genome Mapping

+ Can detect copy number and structural abnormalities genome-wide

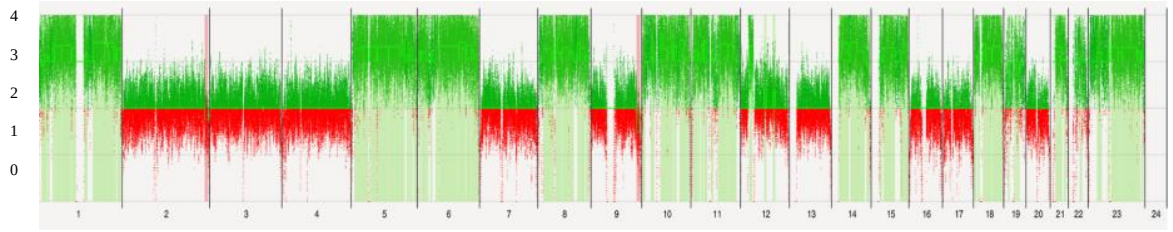
- Difficulties to detect hypo/hyperdiploidy as well as aberrations in certain genomic regions

Whole genome circos plot – Structural variants



t(11;14)(p13;q11)
LTRA::LMO2

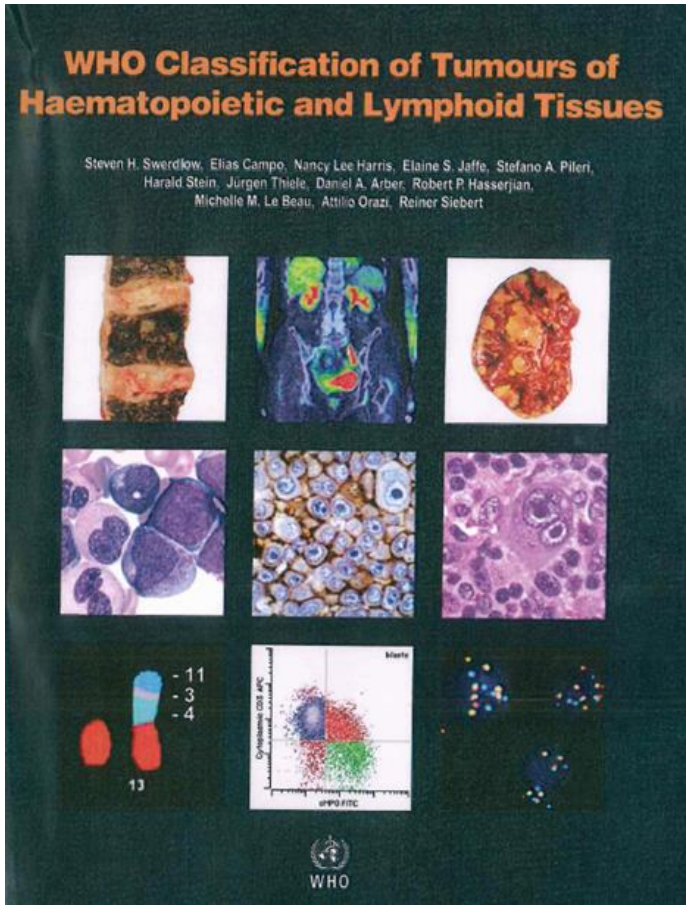
Whole genome CNV view



3.1 Cytogenetics: diagnostic value

The World Health Organization (WHO) classification of malignant hemopathies includes cytogenetics in the classification system for some entities

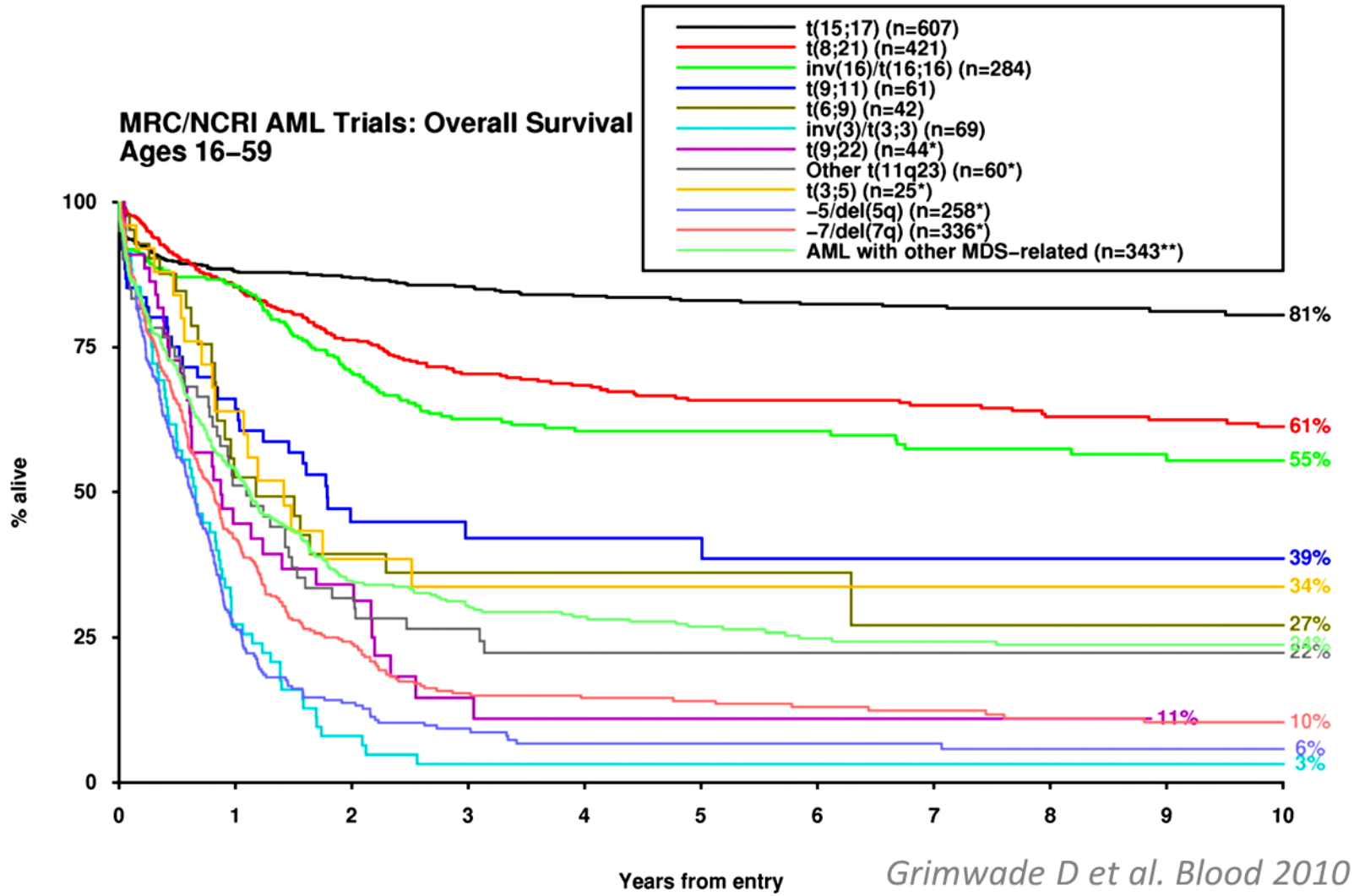
- Mandatory at diagnosis: acute leukemia, MPN (CML), MDS
- Mandatory in follow-up: CML, CLL before treatment
- Recommended at diagnosis: MM
- Useful at diagnosis: NHL



5th Edition,
Khoury et al, Leukemia 2022 (myeloid)
Alaggio et al, Leukemia 2022 (lymphoid)

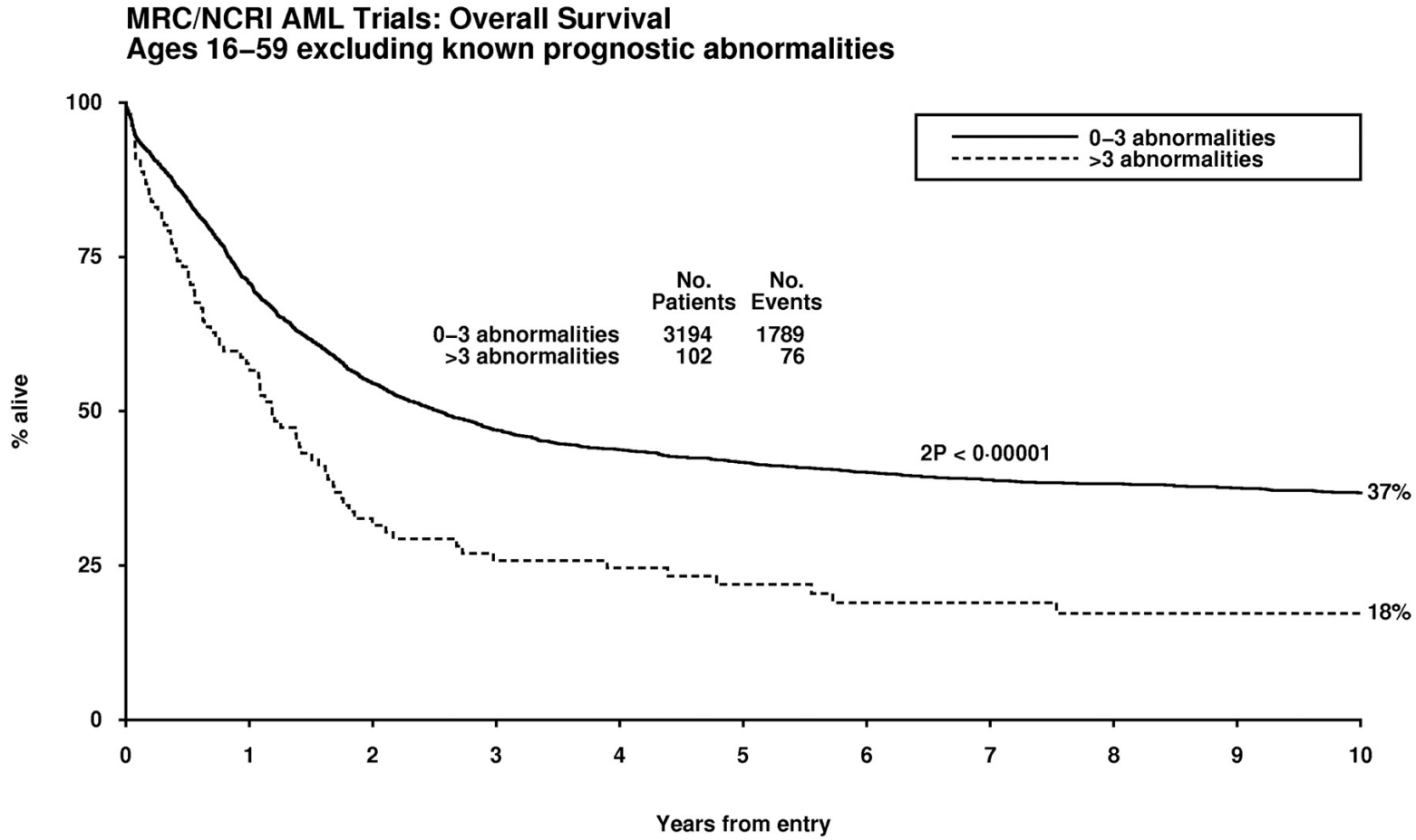
3.2 Cytogenetics: prognostic value

Example: prognostic value of the specific cytogenetic aberrations seen at diagnosis in AML. Used to stratify patients into cytogenetic risk groups



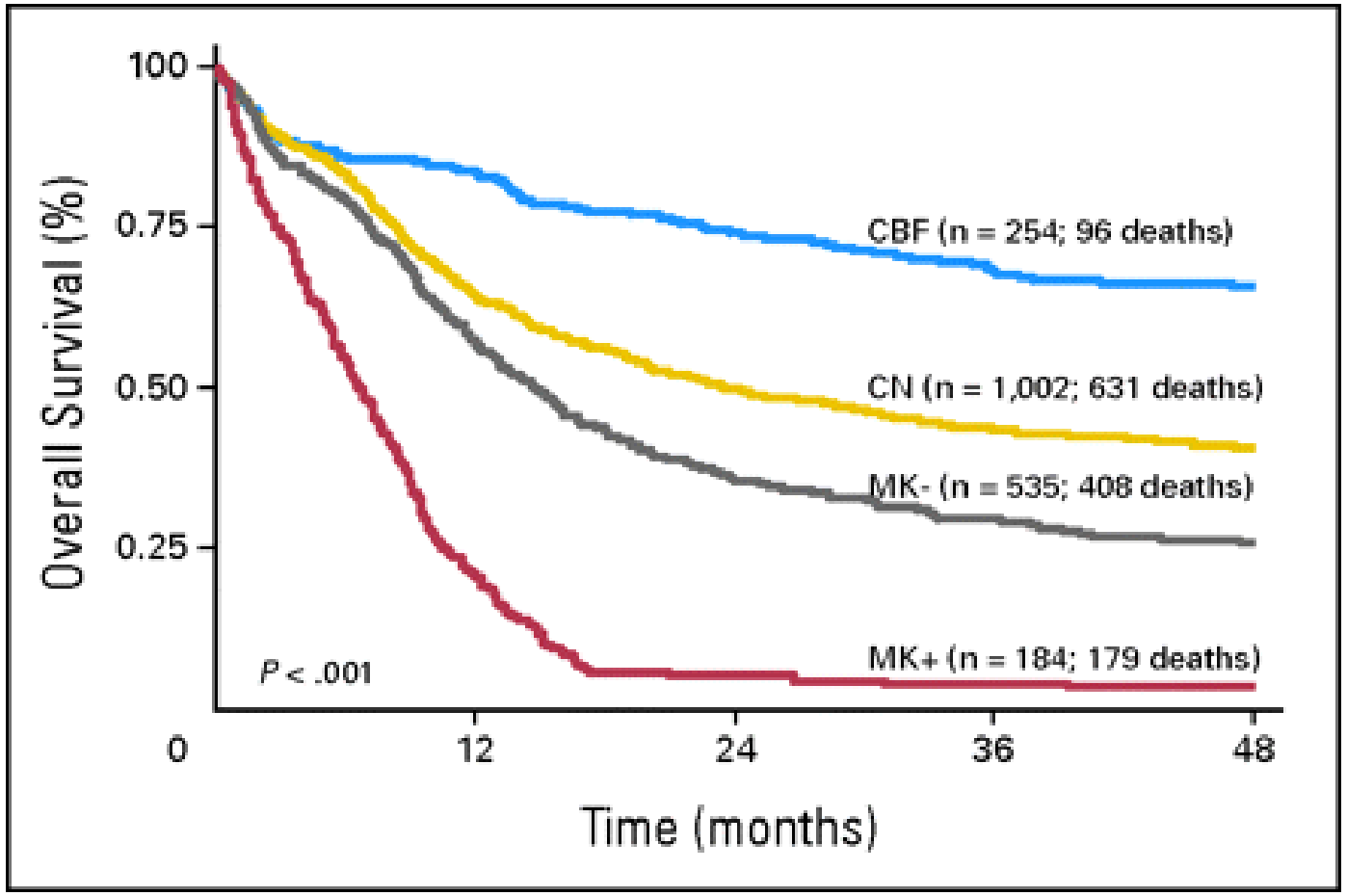
Grimwade D et al. Blood 2010

Impact of karyotype complexity on survival in AML
for patients not belonging to favorable/unfavorable subgroups
(multivariate analysis)

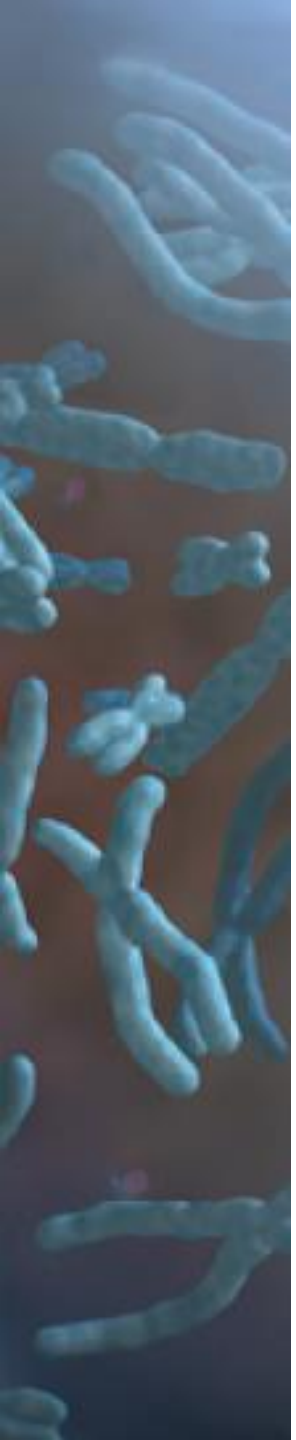


Grimwade D et al. Blood 2010

Impact of the monosomal karyotype in AML (presence of two or more distinct monosomies (excluding loss of X or Y), or one single autosomal monosomy in combination with at least one structural chromosome abnormality (excluding corebinding factor AML).



Breems, D. A. et al. J Clin Oncol 2008

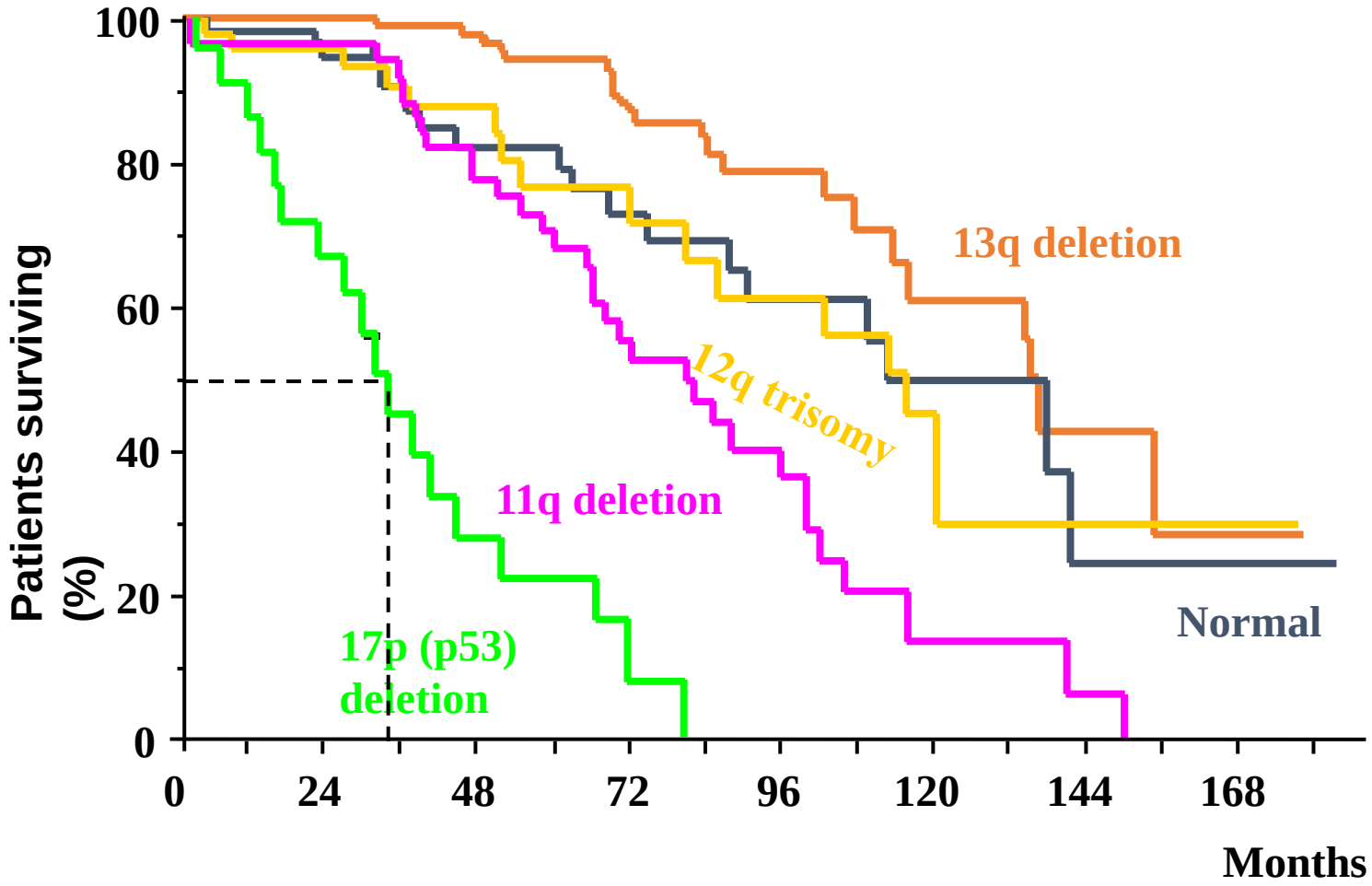


2022 ELN risk stratification by genetics at diagnosis

Risk Category[b]	Genetic Abnormality
Favorable	• t(8;21)(q22;q22.1)/*RUNX1*::*RUNX1T1*[b,c] • inv(16)(p13.1q22) or t(16;16)(p13.1;q22)/*CBFB*::*MYH11*[b,c] • Mutated *NPM1*[b,d] without *FLT3*-ITD • bZIP in-frame mutated *CEBPA*[e]
Intermediate	• Mutated *NPM1*[b,d] with *FLT3*-ITD • Wild-type *NPM1* with *FLT3*-ITD • t(9;11)(p21.3;q23.3)/*MLLT3*::*KMT2A*[b,f] • Cytogenetic and/or molecular abnormalities not classified as favorable or adverse
Adverse	• t(6;9)(p23;q34.1)/*DEK*::*NUP214* • t(v;11q23.3)/*KMT2A*-rearranged[g] • t(9;22)(q34.1;q11.2)/*BCR*::*ABL1* • t(8;16)(p11;p13)/*KAT6A*::*CREBBP* • inv(3)(q21.3q26.2) or t(3;3)(q21.3;q26.2)/*GATA2*, *MECOM(EVI1)* • t(3q26.2;v)/*MECOM*(*EVI1*)-rearranged • -5 or del(5q); -7; -17/abn(17p) • Complex karyotype,[h] monosomal karyotype[i] • Mutated *ASXL1*, *BCOR*, *EZH2*, *RUNX1*, *SF3B1*, *SRSF2*, *STAG2*, *U2AF1*, or *ZRSR2*[j] • Mutated *TP53*[k]

Döhner et al, Blood 2022.

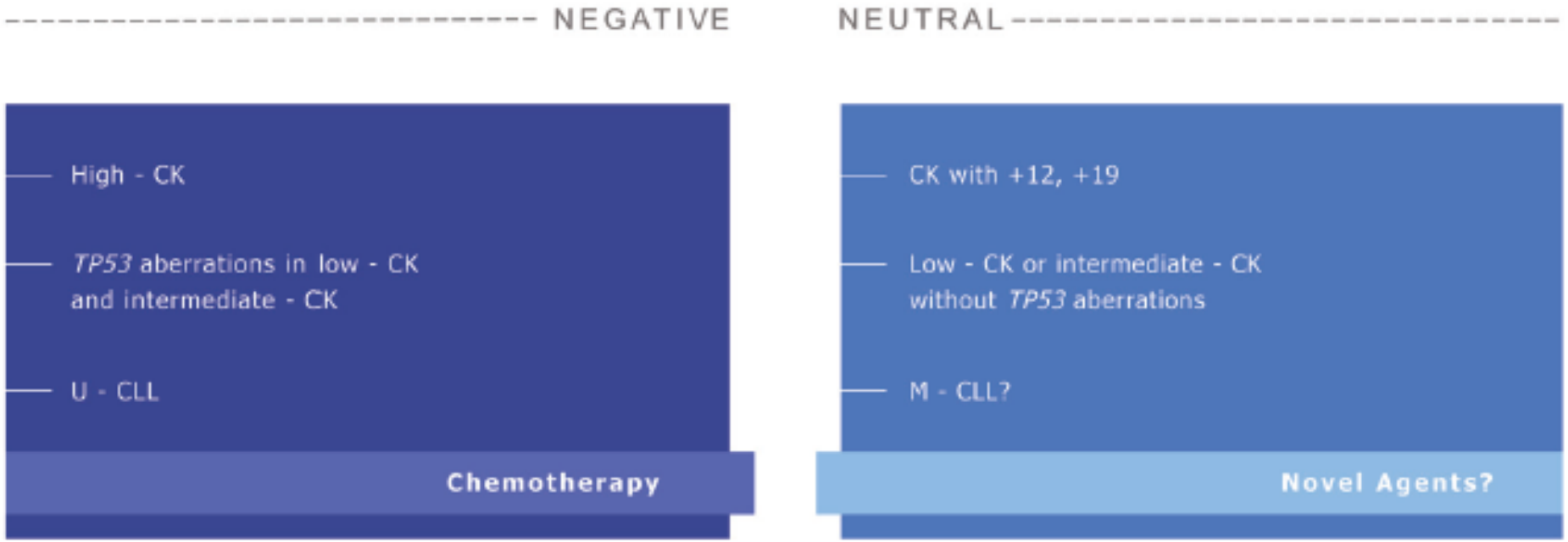
Example: type of aberrations in CLL (by FISH) prognostic impact



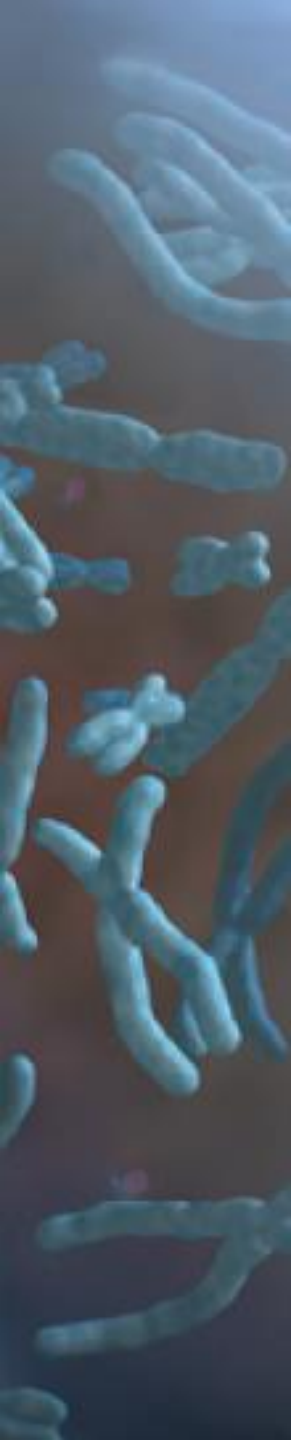
Döhner et al. N Engl J Med 2000

Landmark paper

Example: ERIC recommendations for cytogenetics in CLL



Baliakas et al. Hemasphere 2022



3.3 Cytogenetics: therapeutic value

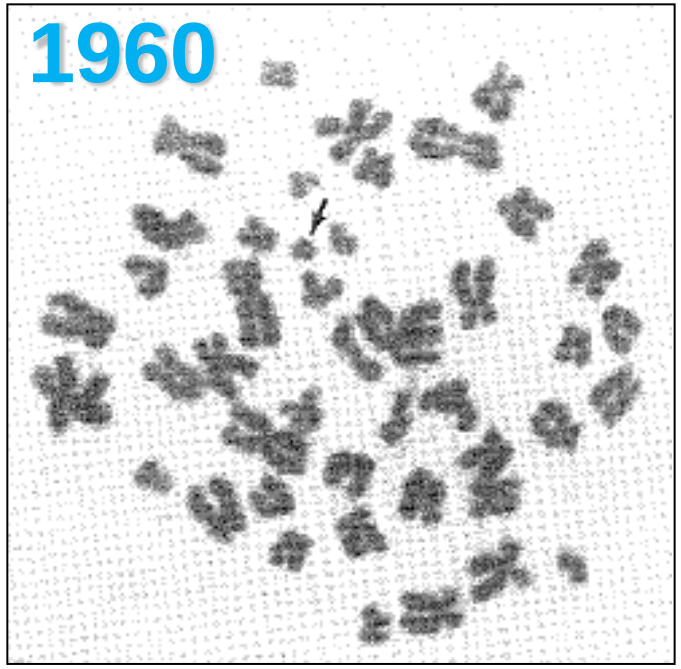
Chromosomal aberrations → potential therapeutic target / influence treatment choice

- ✓ example of CML (TKI)
- ✓ example of CLL (17p deletion)

Nowell and Hungerford, J Natl Canc Inst

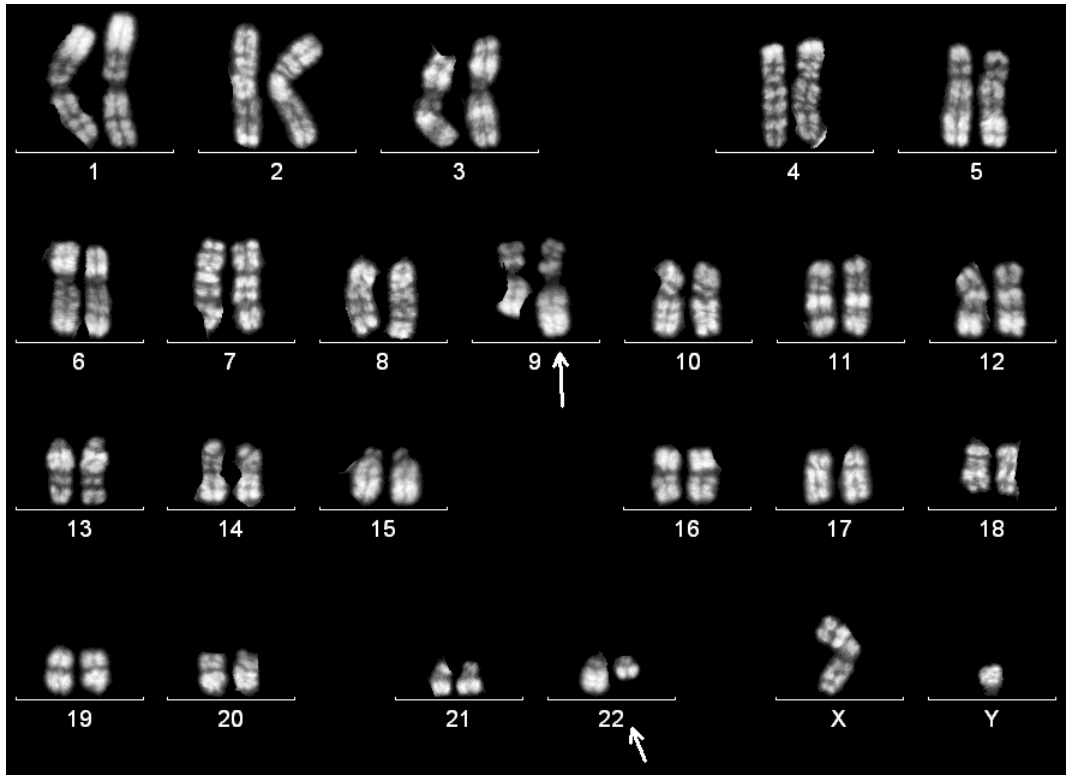
University of Pennsylvania in Philadelphia

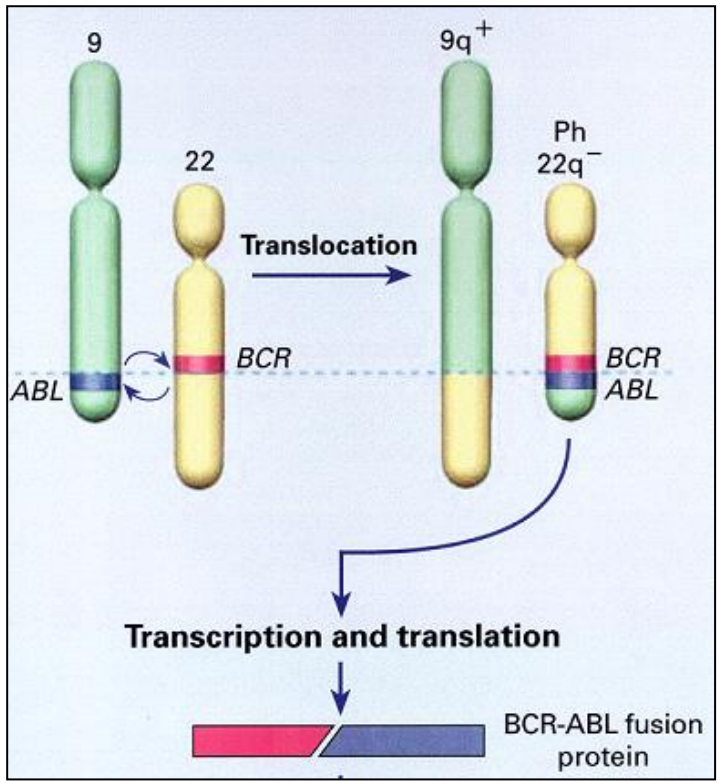
1960



A Minute Chromosome in Human Chronic Granulocytic Leukemia

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





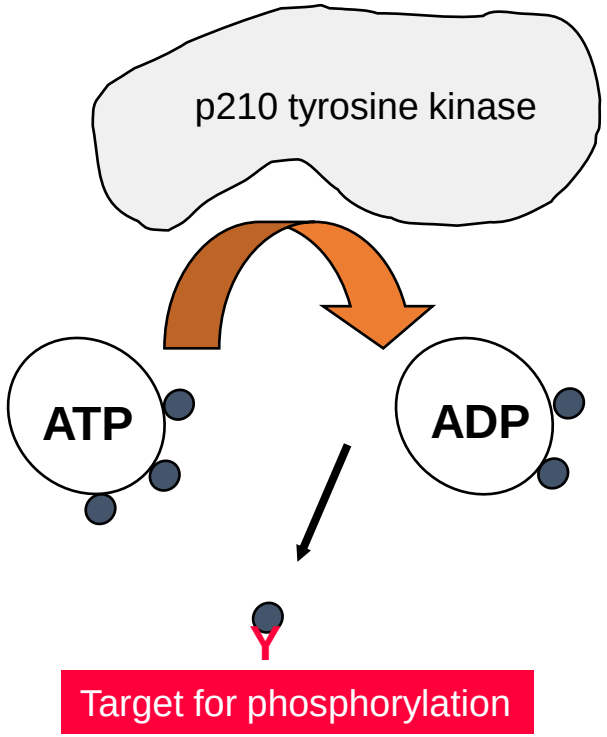
1985: *BCR::ABL1* fusion protein

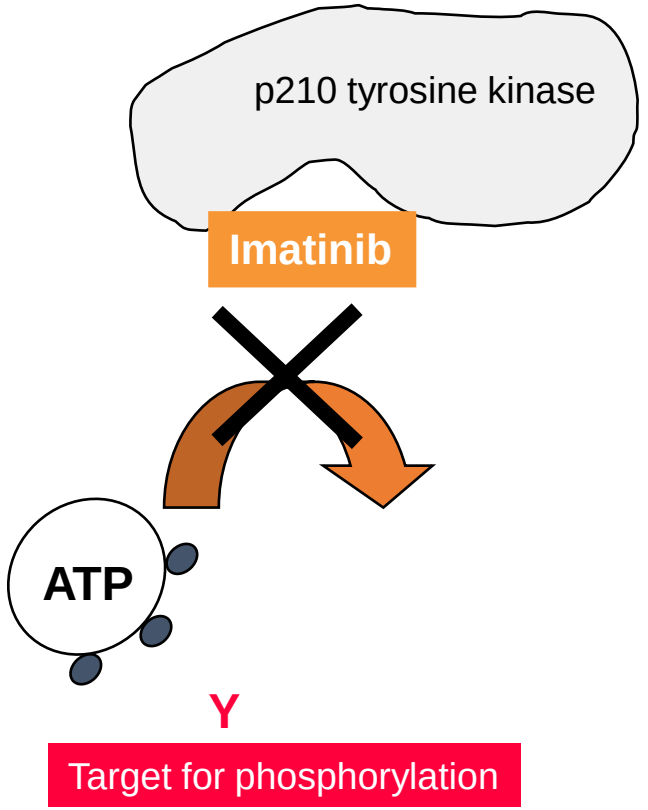
1990: Proof of the pathogenetic role of *BCR::ABL1*

Constitutive activation of ABL TK leading to malignant transformation

Development of TK inhibitor

- 1996: *In vitro* effect of Imatinib
- 1999: *In vivo* effect of Imatinib
- 1999: Clinical efficacy





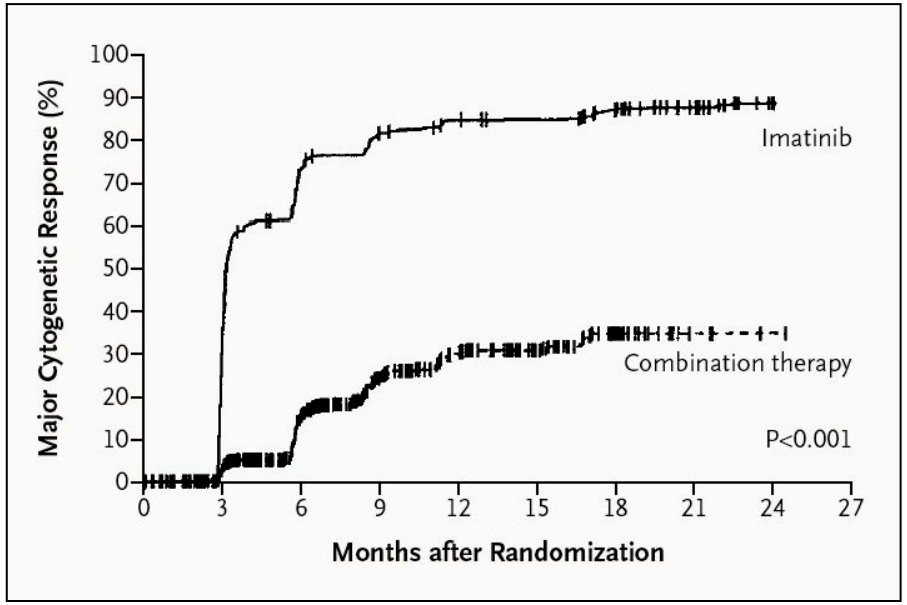
Imatinib inhibits the abnormal increased phosphorylation by blocking binding of ATP to ABL1 tyrosine kinase

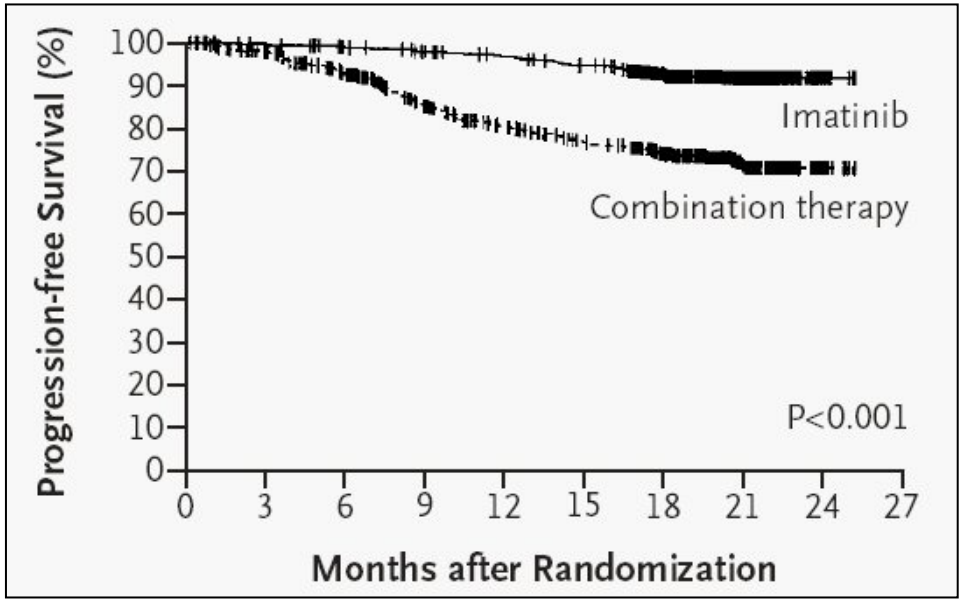
The New England Journal of Medicine

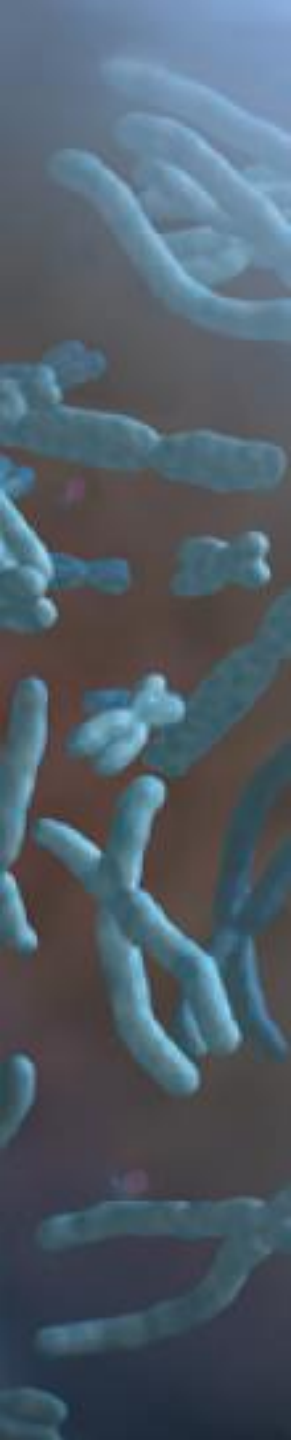
VOLUME 344 APRIL 5, 2001 NUMBER 14

EFFICACY AND SAFETY OF A SPECIFIC INHIBITOR OF THE BCR-ABL TYROSINE KINASE IN CHRONIC MYELOID LEUKEMIA

BRIAN J. DRUKER, M.D., MOSHE TALPAZ, M.D., DEBRA J. RESTA, R.N., BIN PENG, PH.D., ELISABETH BUCHDUNGER, PH.D., JOHN M. FORD, M.D., NICHOLAS B. LYDON, PH.D., HAGOP KANTARJIAN, M.D., RENAUD CAPDEVILLE, M.D., SAYURI OHNO-JONES, B.S., AND CHARLES L. SAWYERS, M.D.







Conclusion

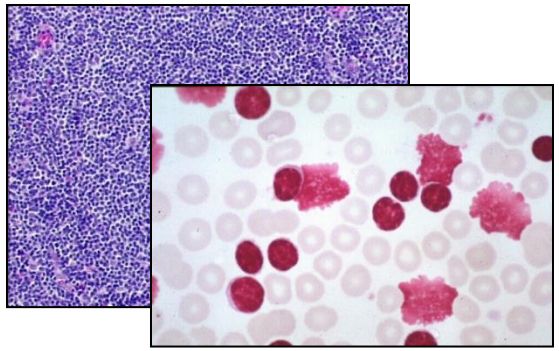
Cytogenetic analyses in hematological malignancies:

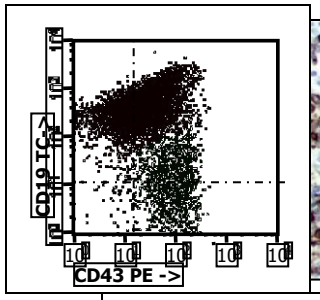
- Useful for diagnostic and prognostic purposes and mandatory in some disorders:
  - Mandatory at diagnosis: acute leukemia, MPN (CML), MDS
  - Recommended at diagnosis : MM
  - Useful at diagnosis: NHL
  - Mandatory in follow-up: CML, CLL before treatment

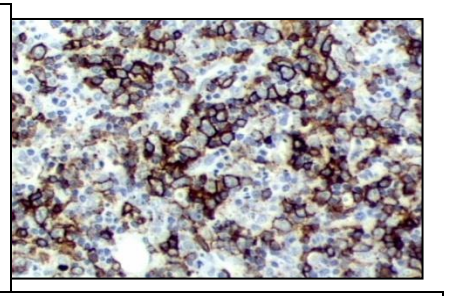
- Conventional cytogenetics historically very useful for research

- Molecular cytogenetics: expanding but expensive tools

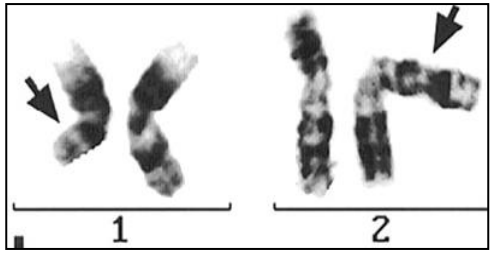
Cytogenetics
= part of **multidisciplinary** approach







Immunophenotype:
- **flow cytometry**
- **immunohistochemistry**

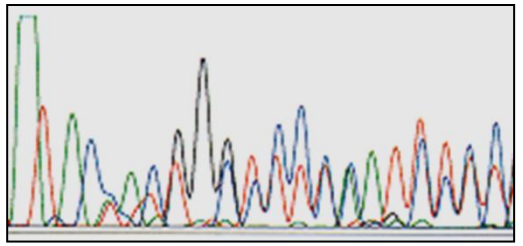


Cytogenetics

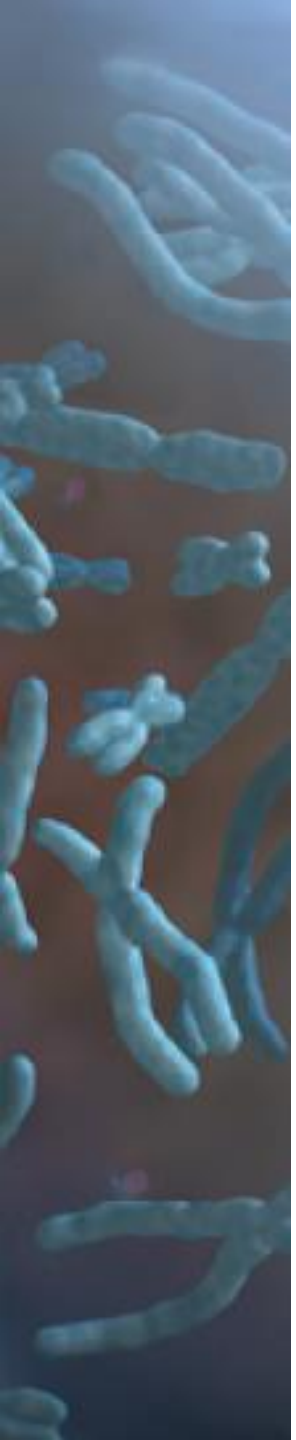
Clinics

Morphology
- **cytology**
- **histology**

Molecular biology



DIAGNOSIS
- **Entity**
- **Prognosis**
- **Therapy**



Suggested reading

- Atlas of cytogenetics: http://atlasgeneticsoncology.org (contains informations on clinico-biological entities and on specific chromosome aberrations)

- Catalog of genetic anomalies in cancer: http://cgap.nci.nih.gov/Chromosomes/Mitelman
  (useful in case of very rare aberrations)

- The 5th edition of the World Health Organization Classification of Haematolymphoid Tumours: Myeloid and Histiocytic/Dendritic Neoplasms. Khoury JD, et al. Leukemia, 2022.

- The 5th edition of the World Health Organization Classification of Haematolymphoid Tumours: Lymphoid Neoplasms. Alaggio R, et al. Leukemia, 2022.

- International Consensus Classification of Myeloid Neoplasms and Acute Leukemia: Integrating Morphological, Clinical, and Genomic Data. Arber D, et al. Blood, 2022.

- The International Consensus Classification of Mature Lymphoid Neoplasms: A Report from the Clinical Advisory Committee. Campo E, et al. Blood, 2022.

- Diagnosis and Management of AML in Adults: 2022 ELN Recommendations from an International Expert Panel. Döhner H, et al. Blood, 2022.

- European recommendations and quality assurance for cytogenomic analysis of haematological neoplasms. Rack KA, et al. Leukemia, 2019.