Laboratory techniques: Cytogenetics

BHS training course

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Organization of the Lecture

- Definition and principles of cytogenetics
- Tools
- Applications of cytogenetic analyses
  - Diagnostic
  - Prognostic
  - Pathogenetic and Therapeutic

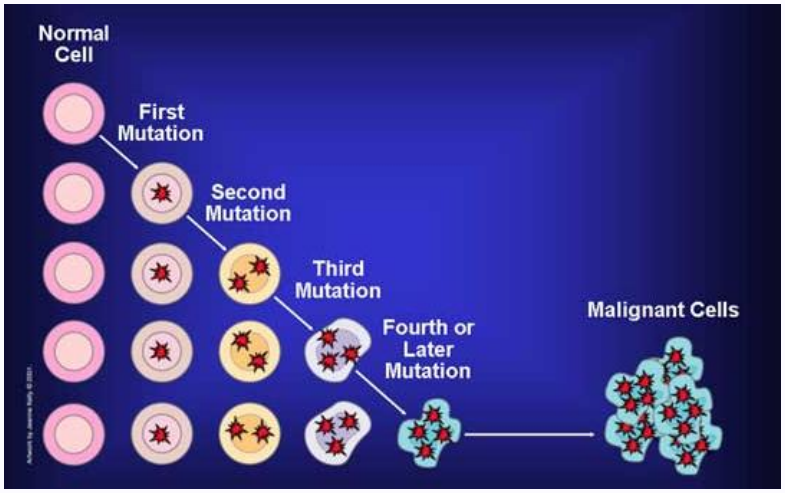
Cytogenetics: Definition

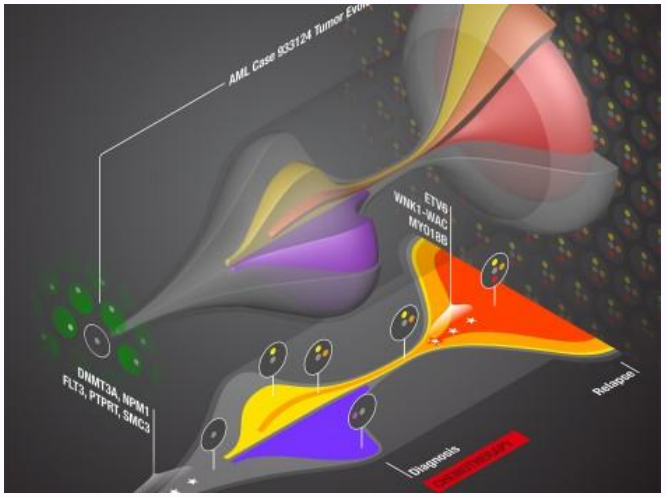
= Cellular Genetics

"Branch of genetics which correlates the structure and number of chromosomes as seen in isolated cells with variation in genotype and phenotype."

1. Conventional: karyotype (1950-…)
2. Molecular: isotopic → non isotopic techniques (1985-…):
   - immunoenzymatic,
   - immunofluorescence (FISH)

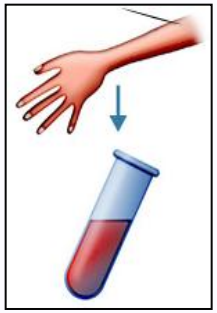
Cytogenetic analyses Principles

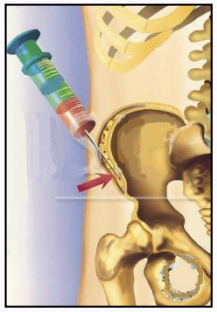


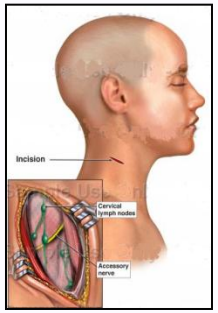


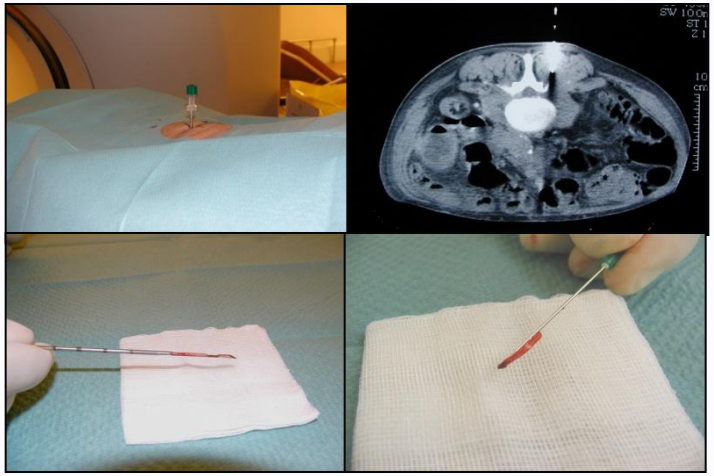
- Malignant hemopathies are acquired diseases characterized by genetic aberrations which persist (= clonality) and accumulate (= clonal evolution)
- Clonality detection is useful (☝: clonality ≠ always malignancy)
- Some aberrations are disease-specific

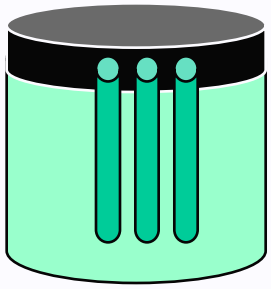
→ Clonality = diagnostic classifier & follow-up tool



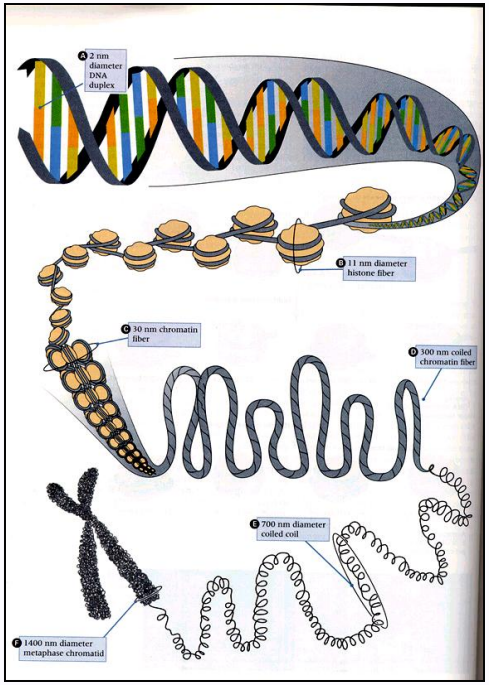








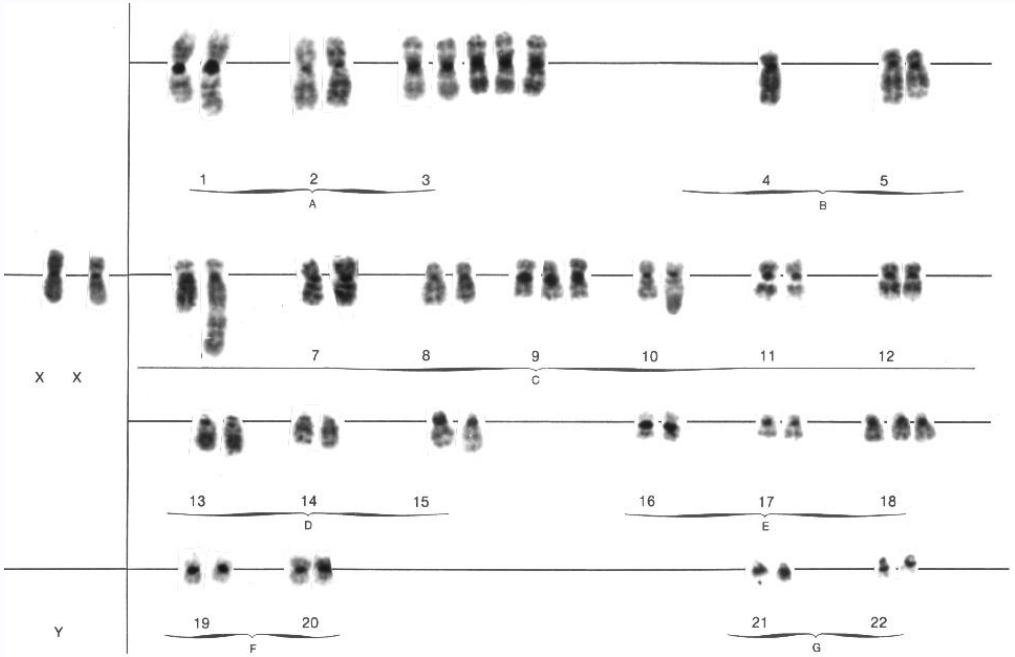
All invaded tissues are suitable...
but select the *appropriate tool*



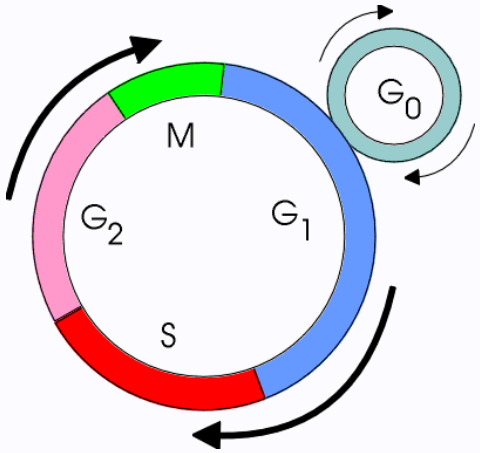
Tools

Karyotype

- Overview of genome
- Can miss subtle aberrations
- Requires “abnormal” cell division



M ≤ 1 hour

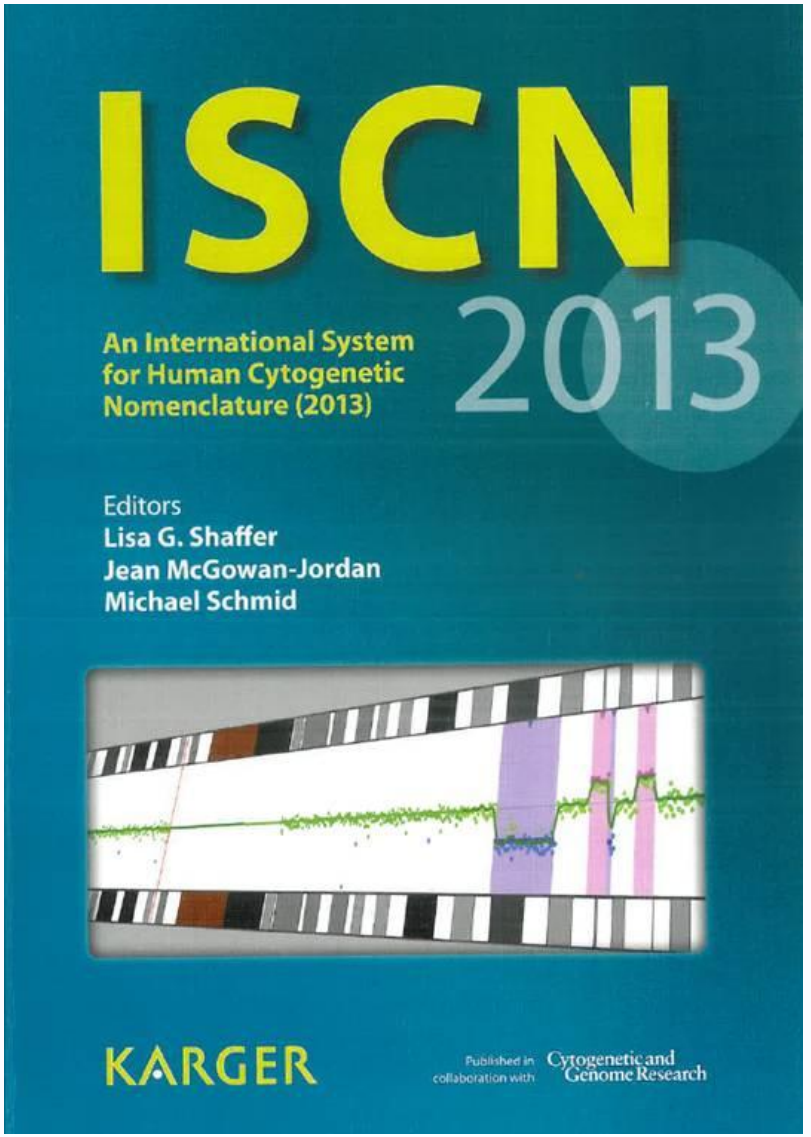


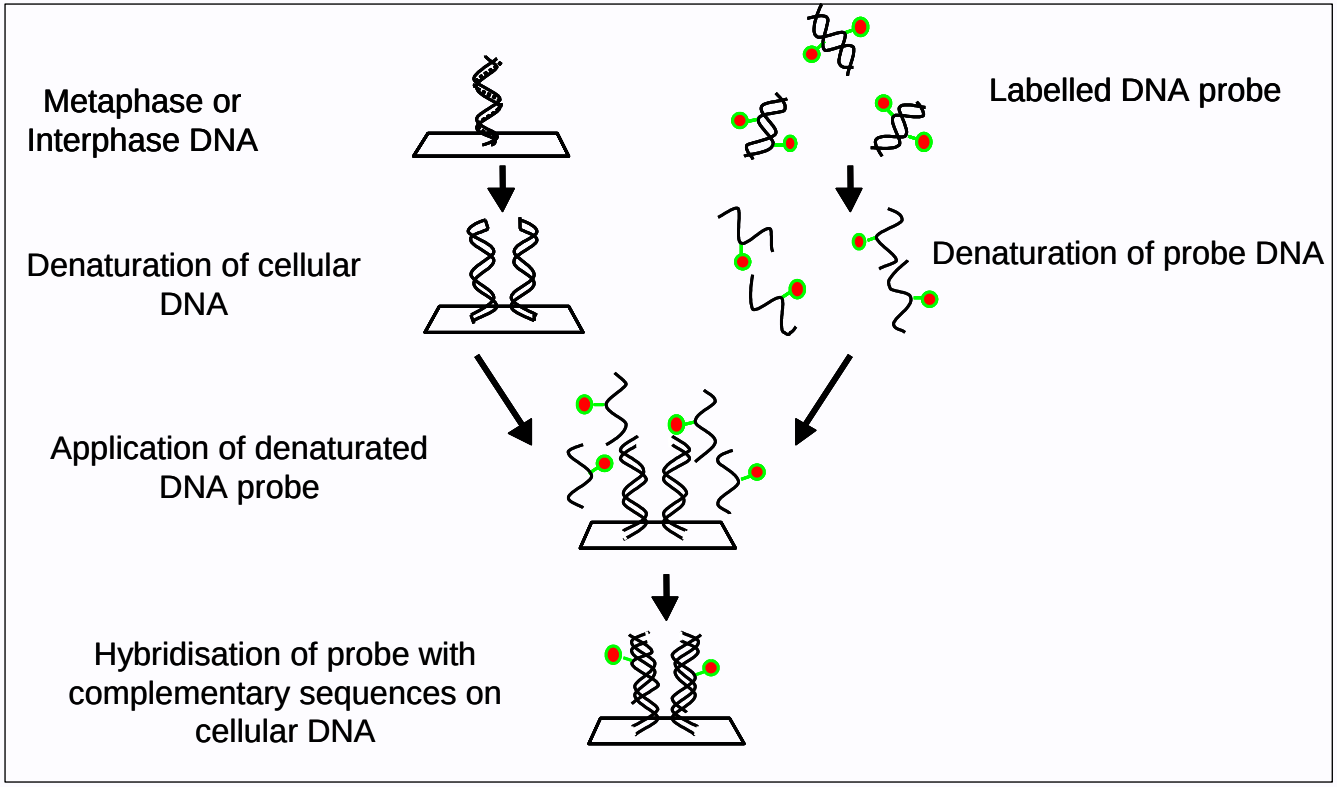
- Result op **karyotype = summary** of several analyzed mitoses
- Expressed as a **formula** (ISCN 2013)
  - Each clone is described (and different clones are separed by « / »)
  - Modal number of chromosomes
  - First: gonosomes (according to ploïdy) and their abnormalities
  - Second: autosomes, in increasing order (1→22) and their abnormalities
  - Number of cells in the clone: [ ]

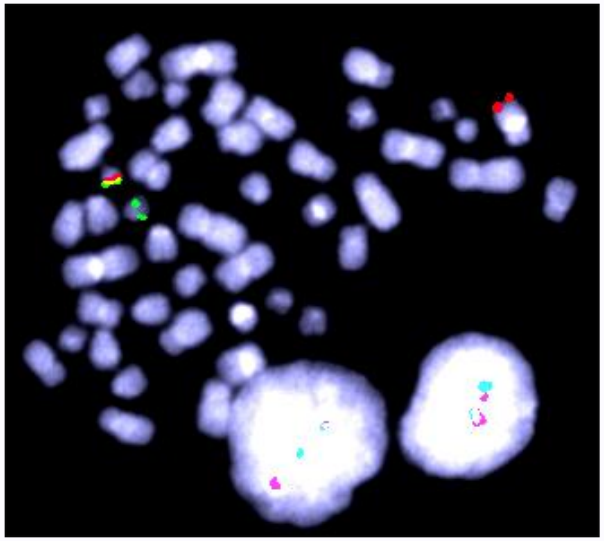
Example:

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

« **;** » and «, »





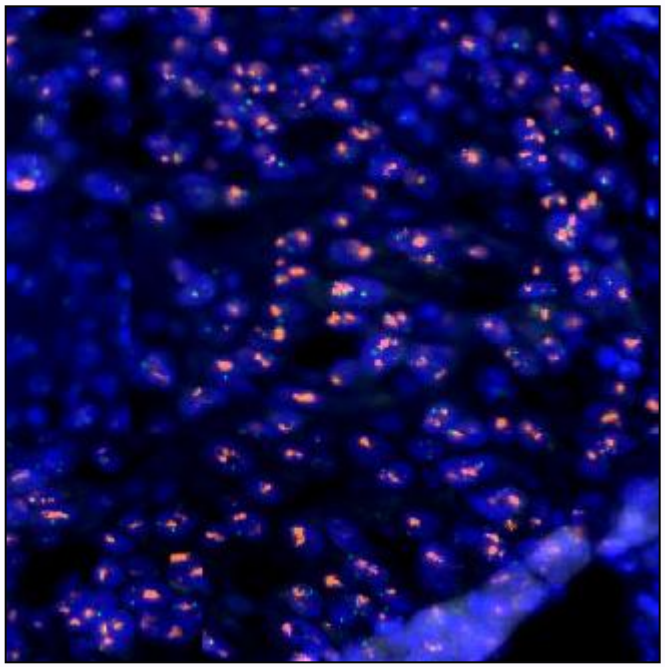


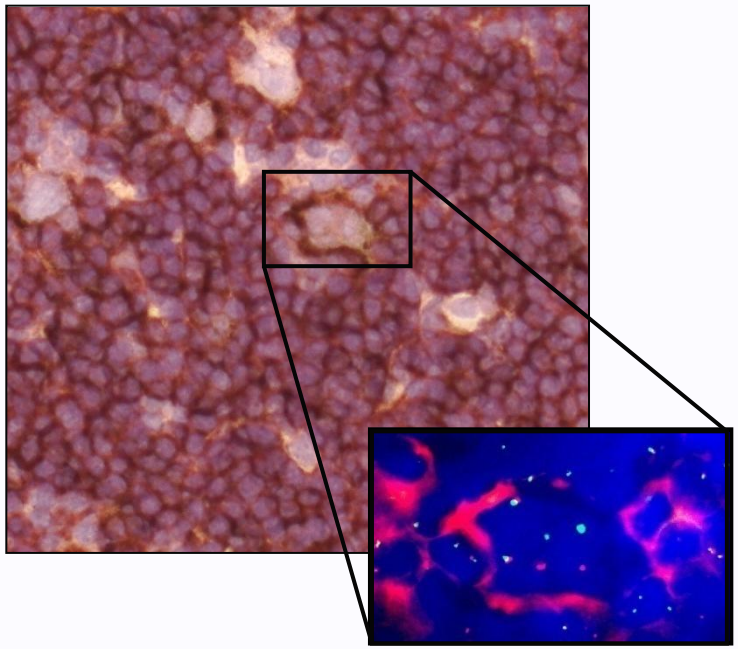
FISH

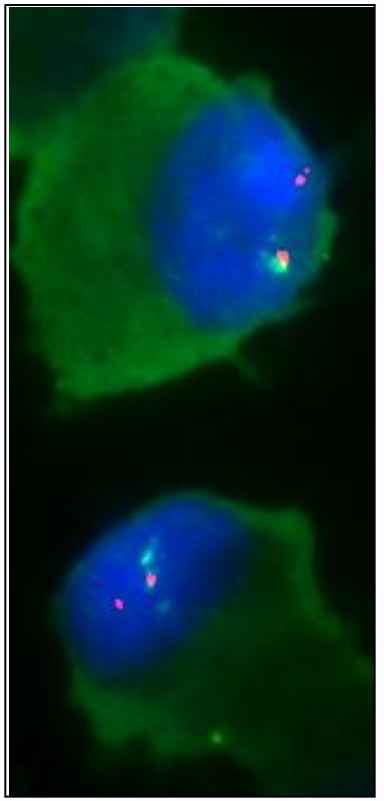
(Fluorescence In Situ Hybridization)

- Targeted analysis of region(s) of interest
- Does not necessarily require “abnormal” cell division

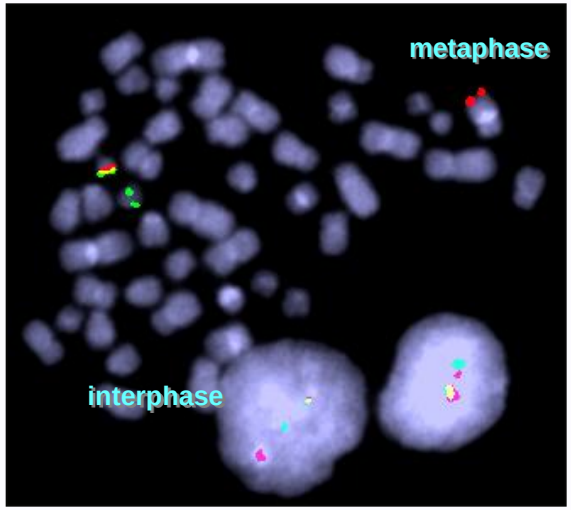
- FISH can be performed on interphase nuclei→ more sensitive than karyotype (more cells can be scored
- Interphase FISH is possible
  - on suspensions
  - on archival material
  - in combination with morphology & immunology (FICTion)

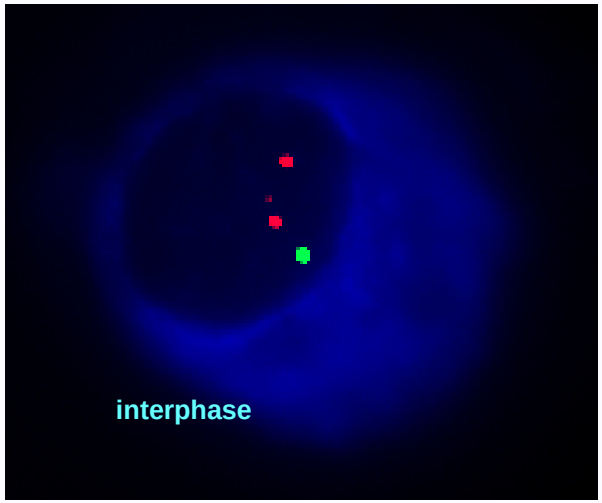


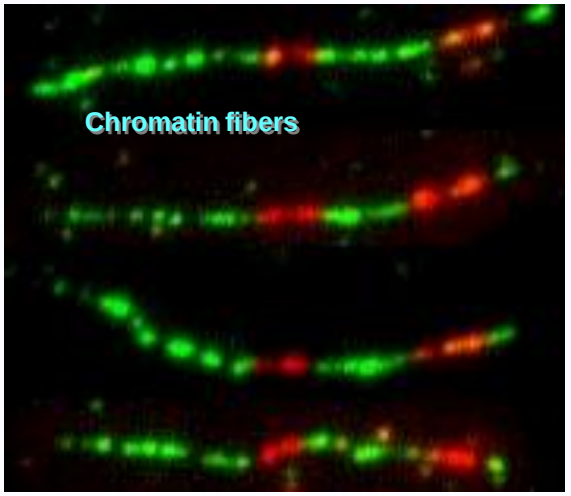


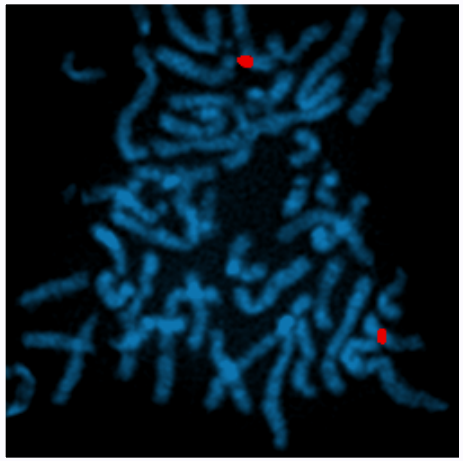


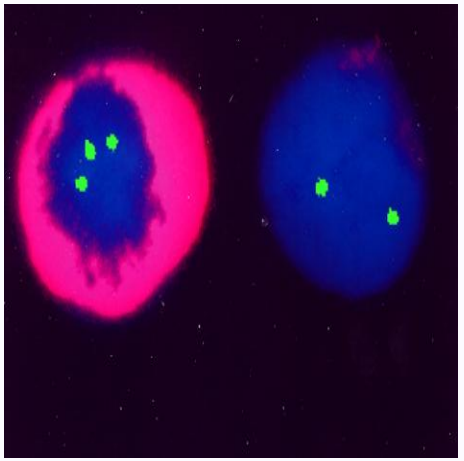
- FISH has a better resolution
  - Conventional karyotype (smallest band) 5-10 Mb
  - FISH on metaphase chromosomes ± 1 Mb
  - FISH on Interphase nuclei ± 100 Kb
  - FISH on chromatin fibers ("fiber FISH") ± 1 Kb

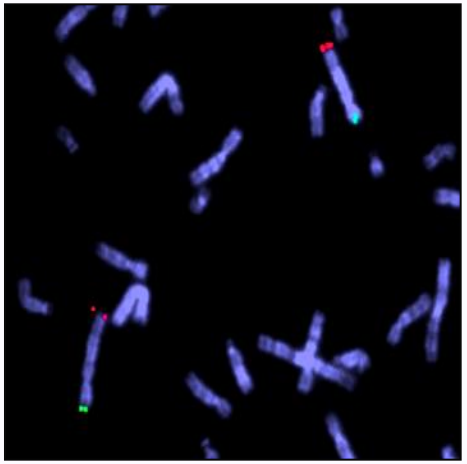


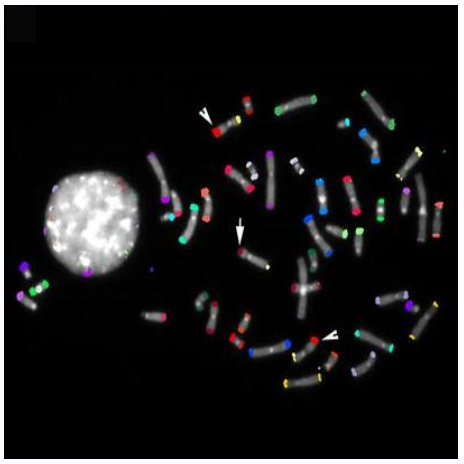


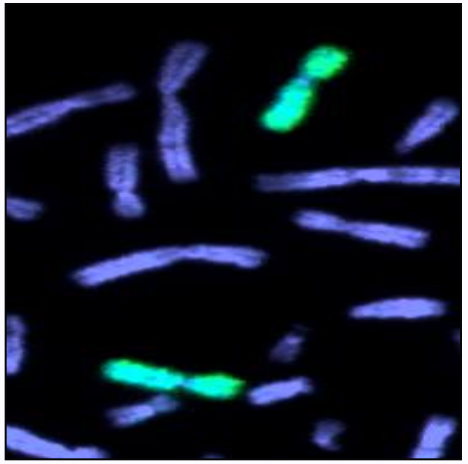


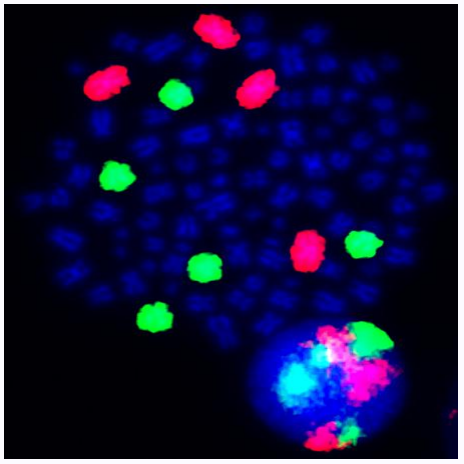










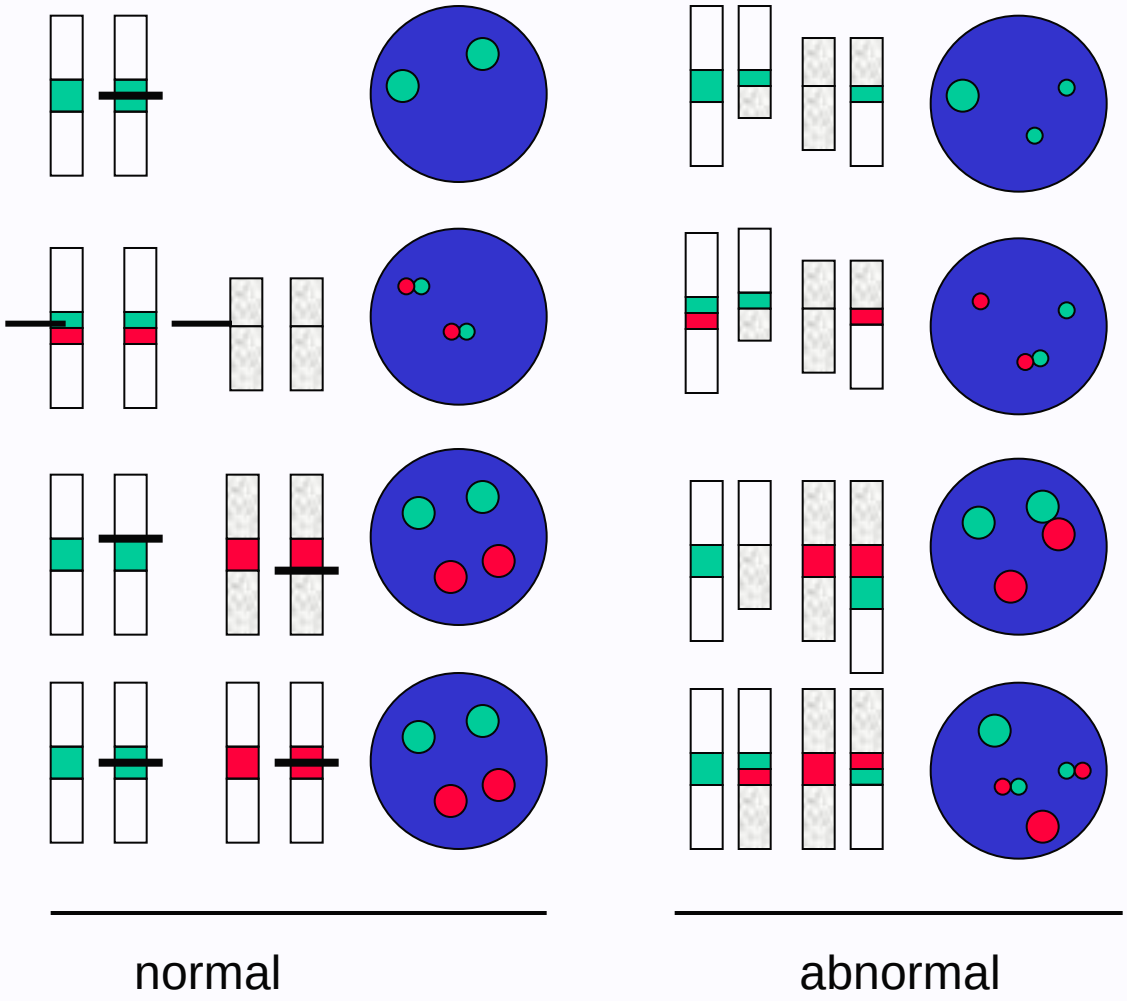


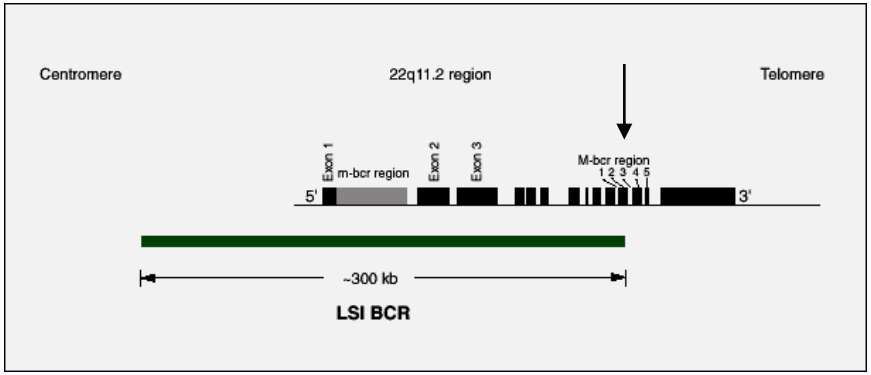
Different probes:

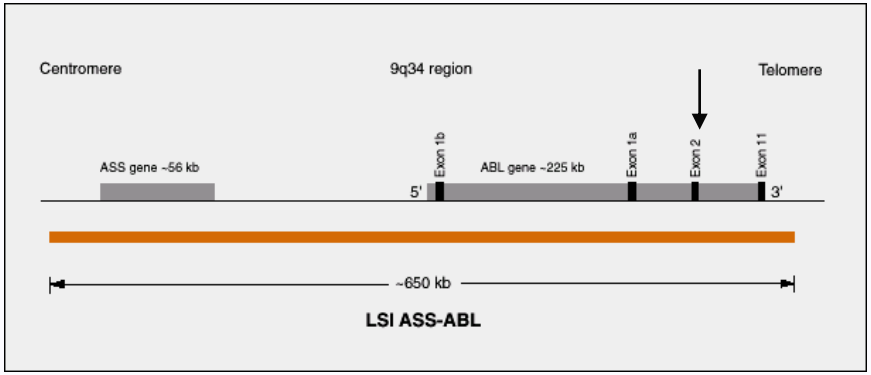
- centromeric
- telomeric
- painting (wcp)

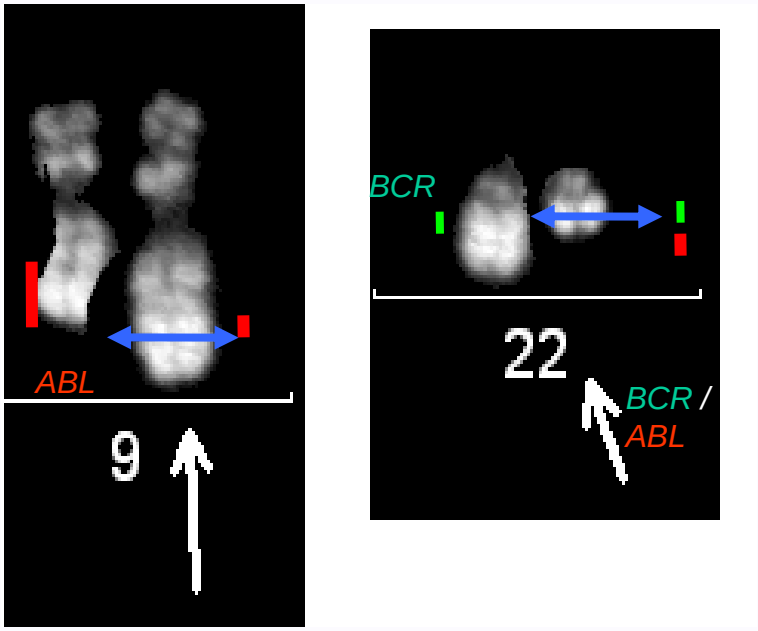
Locus-specific probes: strategies

- breakapart
- colocalization
- combination

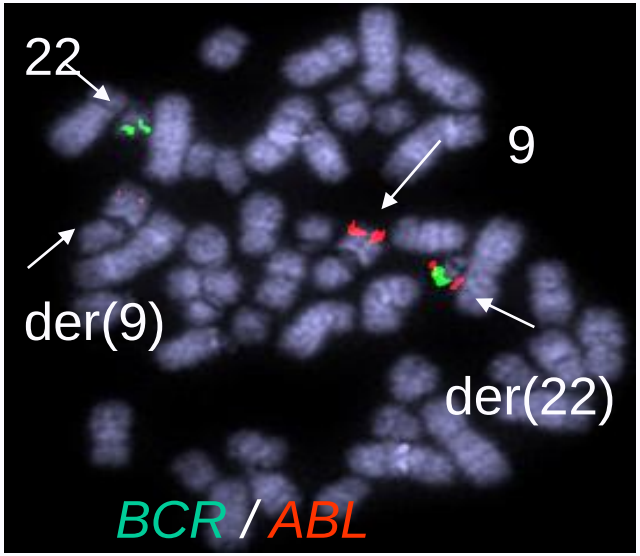


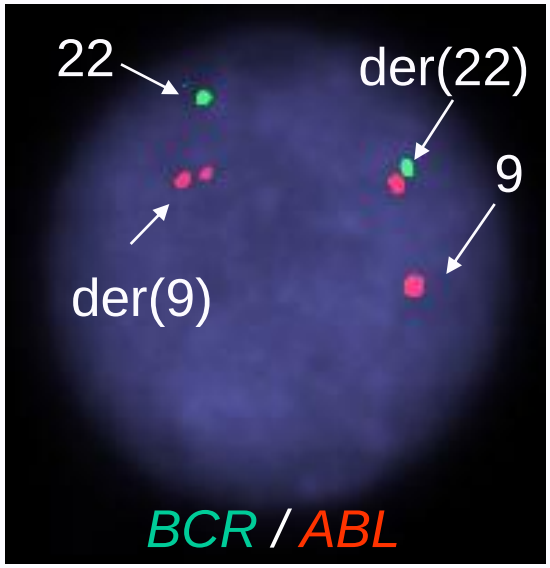


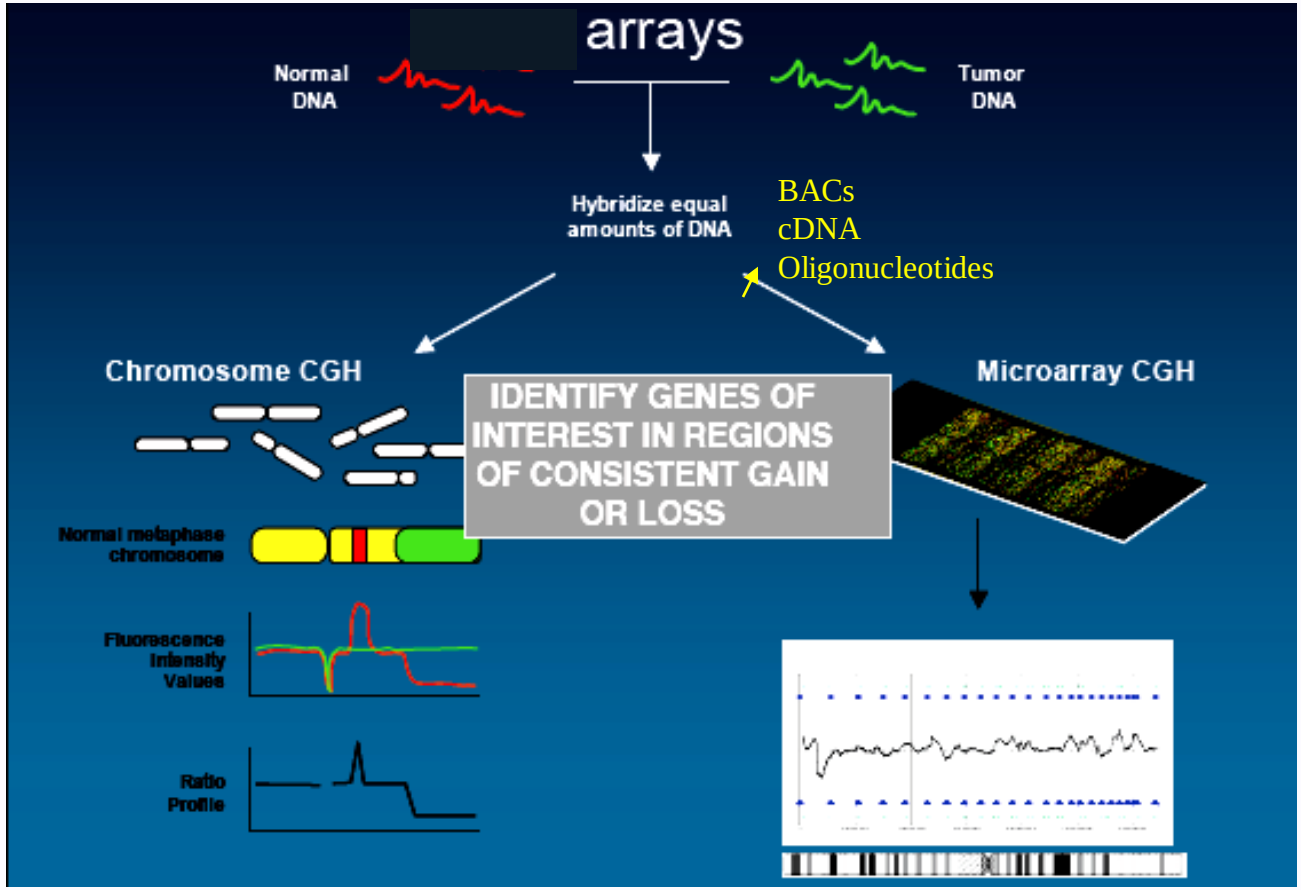


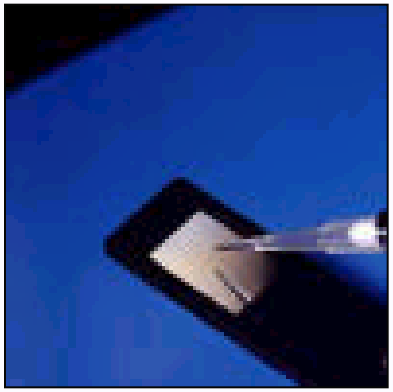


Example: Ph translocation in CML







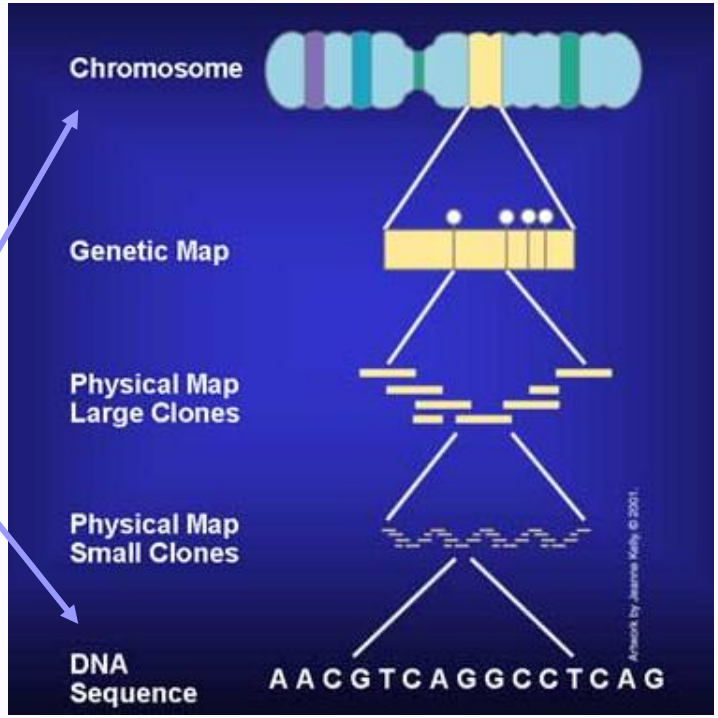


CGH: variant of FISH

- Screening of chromosomes or DNA for losses/gains
- Does not detect balanced aberrations

Cytogenetic analyses which tool?

- Selection based on
  - type of sample available (fresh/frozen or not, amount)
  - type of question (diagnostic set-up vs follow-up of MRD)
  - type of abnormality to screen for (point mutation / specific gene aberration vs genome wide screening)
  - Routine vs research



- Diagnosis → global technique on invaded tissue (+ targeted technique when indicated)
- Follow-up / staging → targeted search for anomalies identified in « index » sample (exception: CML→ global and targeted FU required)

Sometimes morphology+immunophenotyping are sufficient

- Fresh sample: everything is possible (!! transport delay, hierarchy of sample distribution, tissue conservation)
- Frozen sample: ~~karyotype~~
- EDTA: ~~karyotype~~
- Fixed tissue: ~~karyotype~~, molecular and FISH (!! duration of fixation)
- Small tissue: ~~karyotype~~
- Non/ minimally invaded sample: ~~karyotype~~

- Routine ≠ protocol / research!

Why to perform cytogenetic analyses?

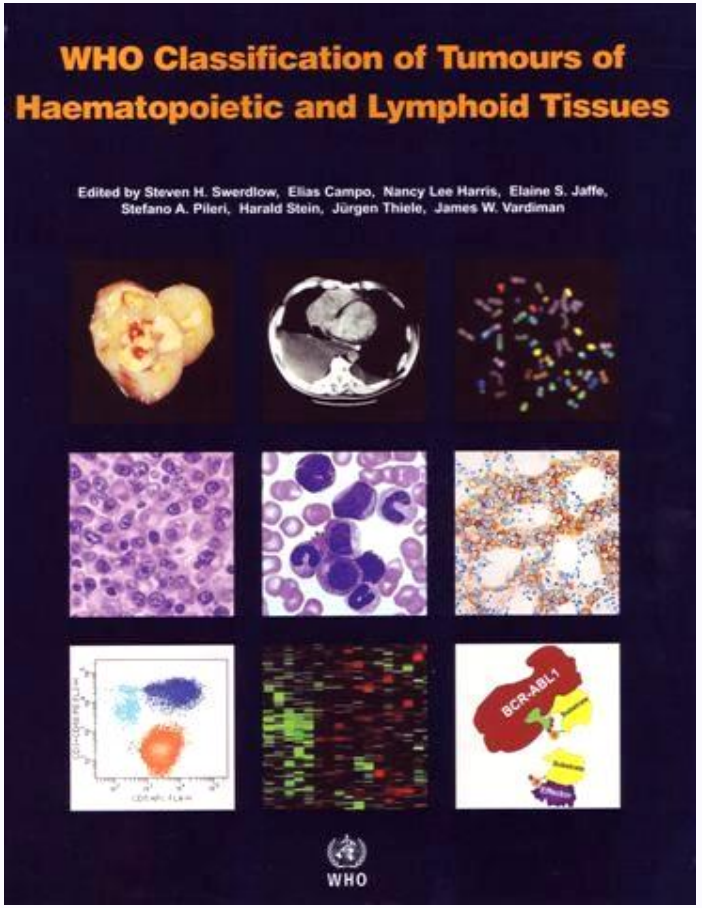
- ✓ Diagnostic accuracy: confirm & refine primary diagnosis
- ✓ Prediction of outcome
- ± Selection of “targeted” therapy
- ± Improvement of disease staging
- ± Monitoring of minimal residual disease
- ± Translation of new research insights into clinical tests
- ± Prediction of drug efficacy & toxicity
- • Definition of the patient’s disease profile
  - ✓ genomic
  - • proteomic
  - • pharmacogenomic

Why not to perform cytogenetic analyses?

- Time-consuming
  - especially karyotypes (culture time, microscopy, ….not fully automated)
- Expensive
  - Art 33
  - Art 33bis
- Not always informative
  - see morphology and immunology

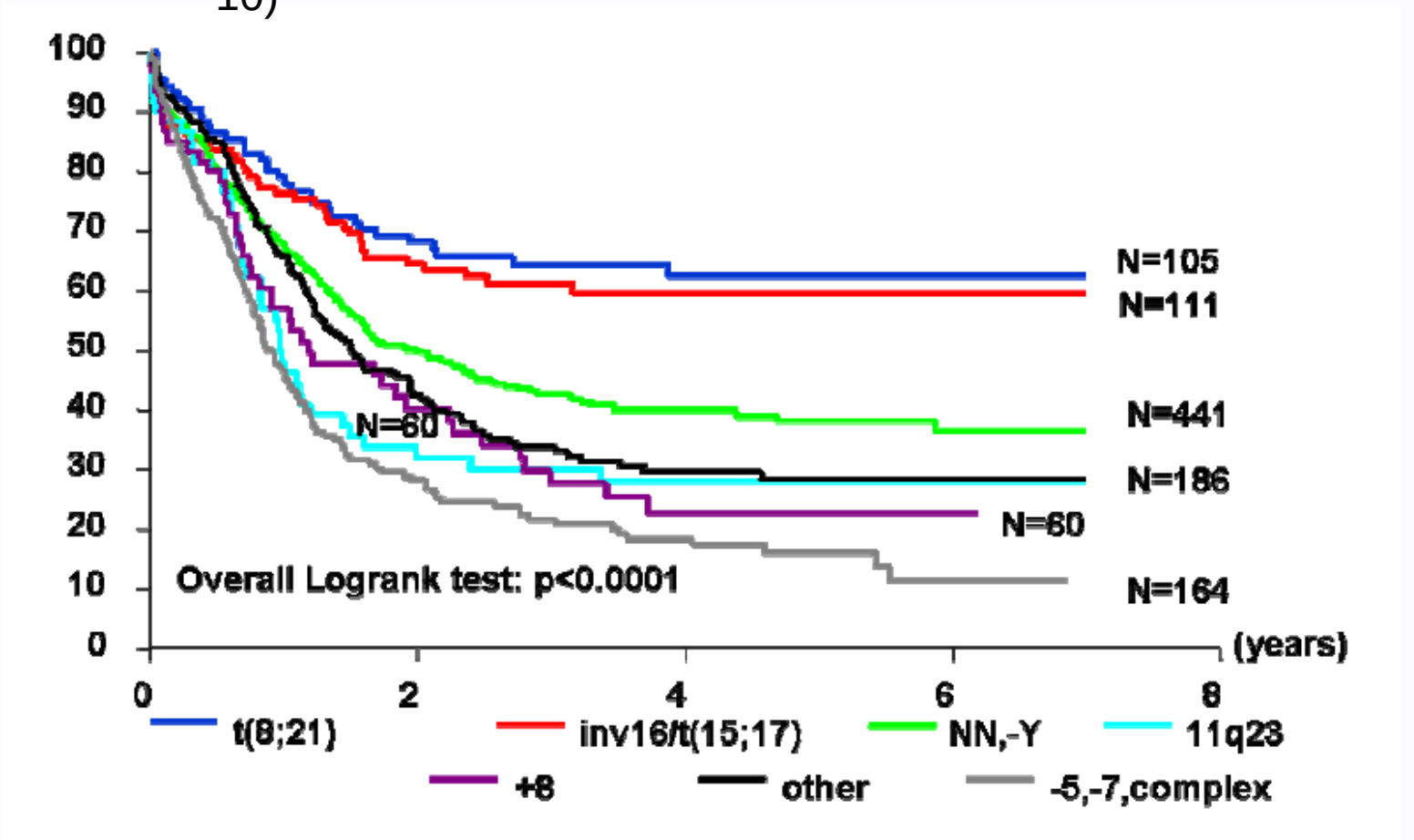
Diagnostic value of cytogenetic analyses

- The World Health Organization (WHO) classification of malignant hemopathies includes cytogenetics
  - Some aberrations are subtype specific
  - Some aberrations can indicate for the presence of a malignant disorder

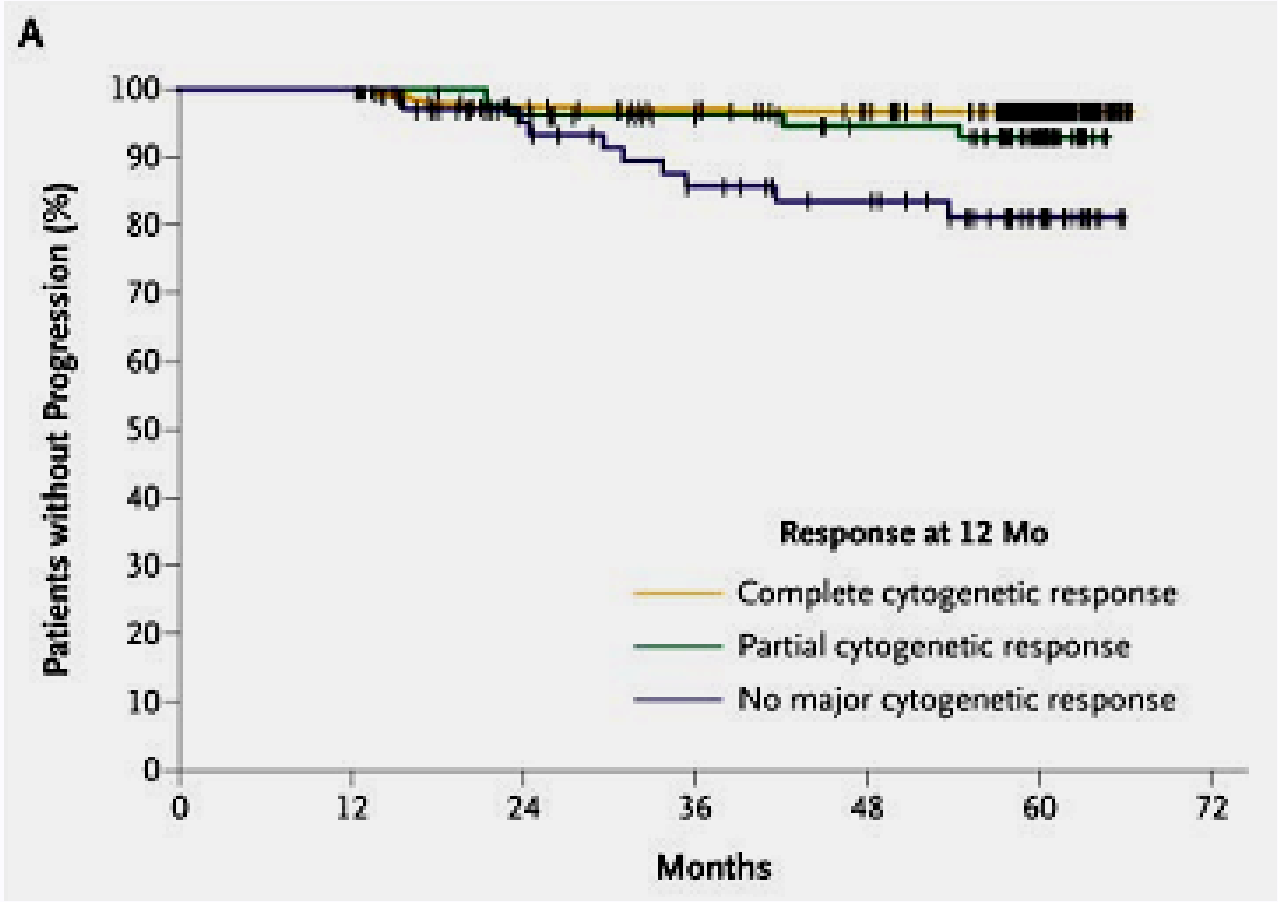


Prognostic value of cytogenetic analyses

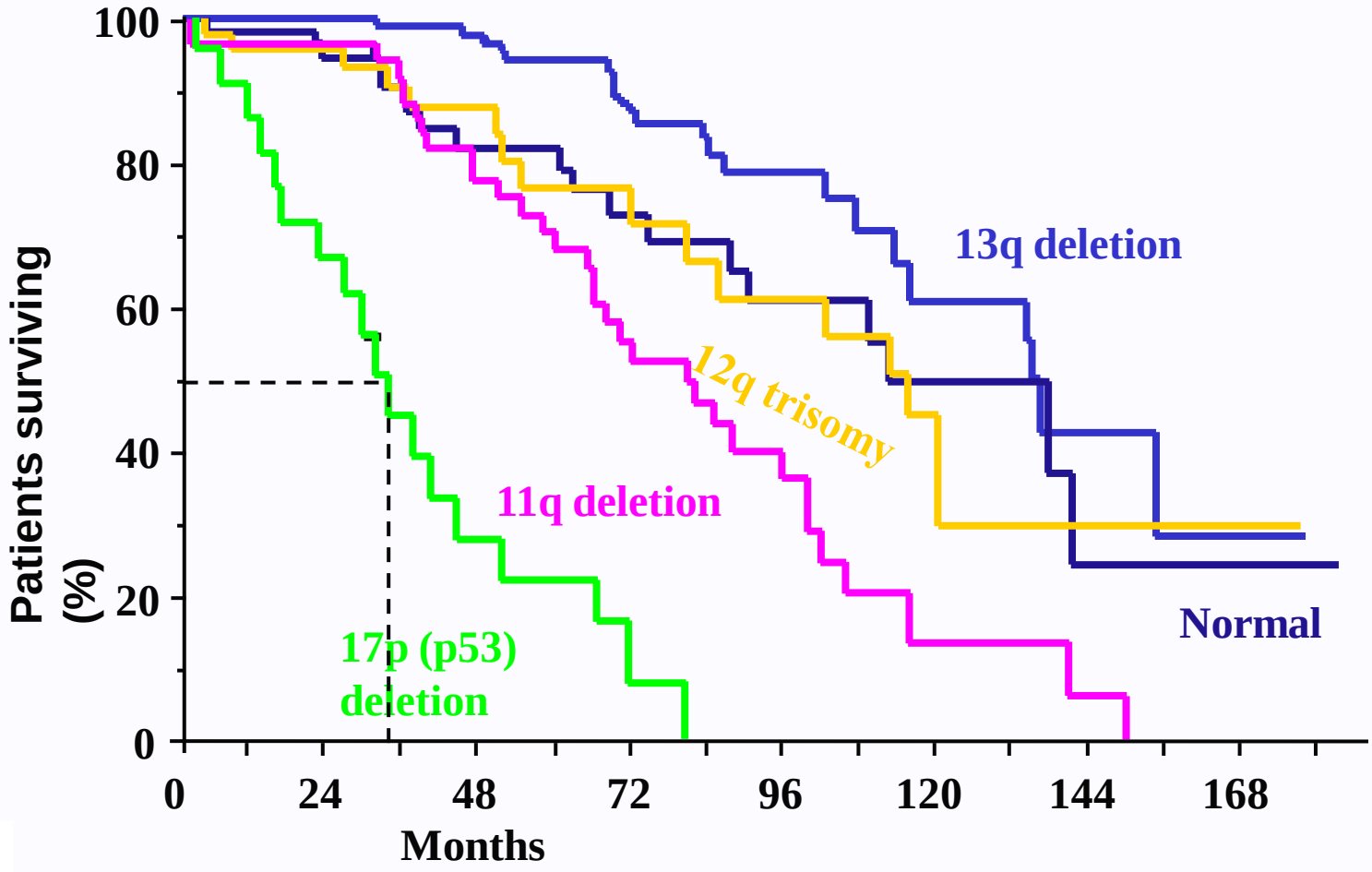
Example: prognostic value of the type of cytogenetic aberrations seen at diagnosis in AML (EORTC AML-10)



Example: prognostic value of cytogenetic response in CML (based on % of Ph positive metaphases in bone marrow during follow-up)



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



Döhner et al. N Engl J Med 2000

Pathogenetic value of cytogenetic analyses

Aberrations ⟶ genes located at breakpoint ⟶ function ⟶ aggressivity of disease

- « Specific » aberrations involved in disease onset, *helpful for classification*:

c-MYC	poliferation / apoptosis	Burkitt
BCL2	apoptosis	Follicular
BCL1	cell cycle	Mantle cell
BCL6	differenciation	Diffuse large B cell
REL	proliferation	Extra-nodal (GC)
AP1-MLT	apoptosis	MALT
PAX5/BSAP	differenciation	Lymphoplasmacytic
BCL10	apoptosis	MALT

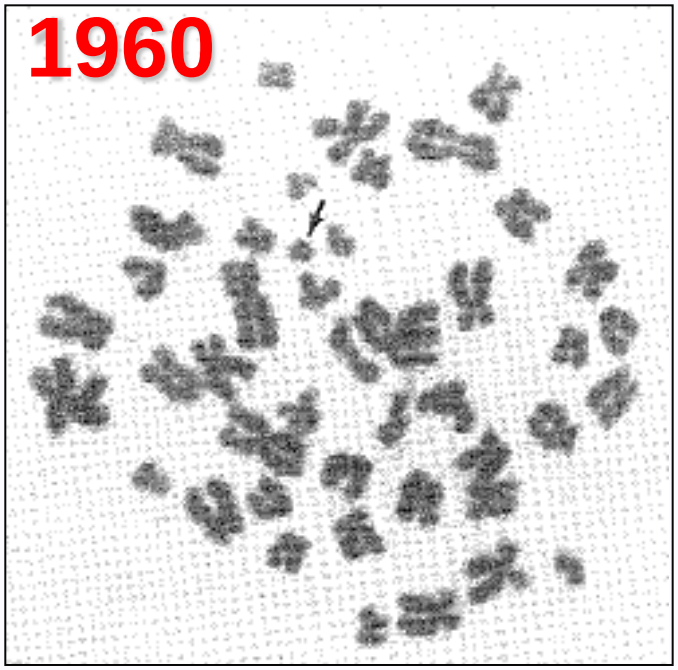
- « Additional » aberrations, may be associated with aggressive/progressive disease :

P53, P16	cell cycle	Low grade→ aggressive
FcγRIIB	immune receptor	Follicular : progression
dup(1q)		Burkitt, GC : aggressive?
enh(9p)		Mediastinal B

Nowell and Hungerford, J Natl Canc Inst

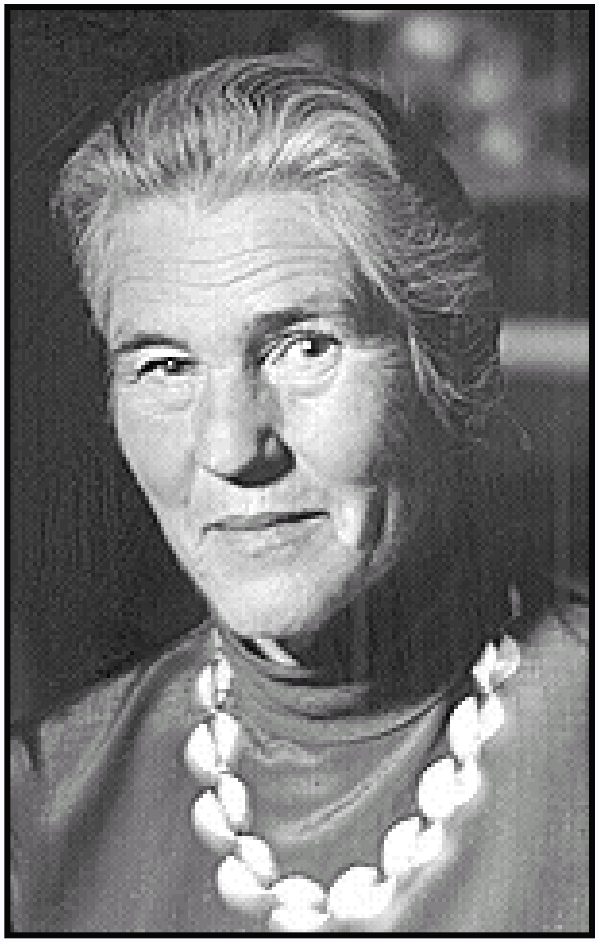
University of Pennsylvania in Philadelphia

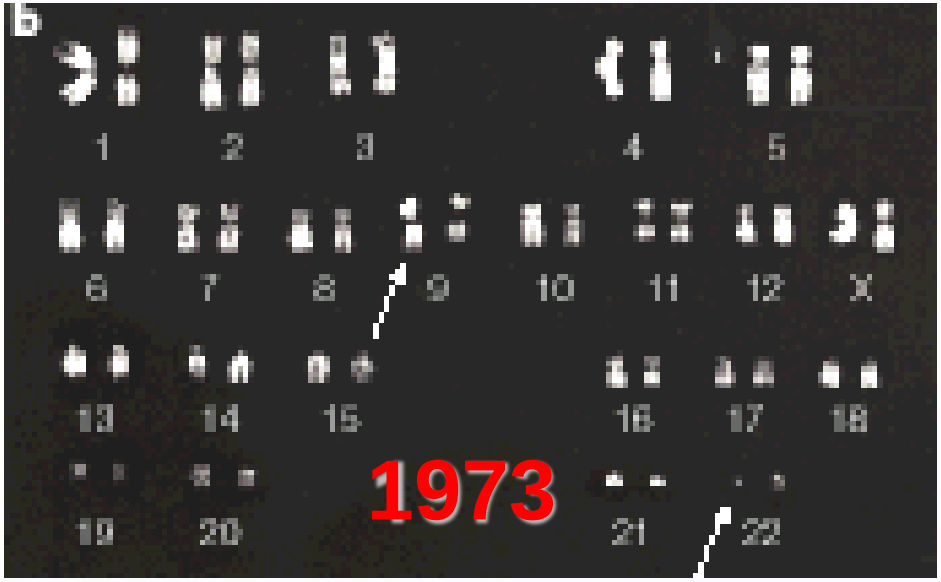
1960



A Minute Chromosome in Human Chronic Granulocytic Leukemia

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

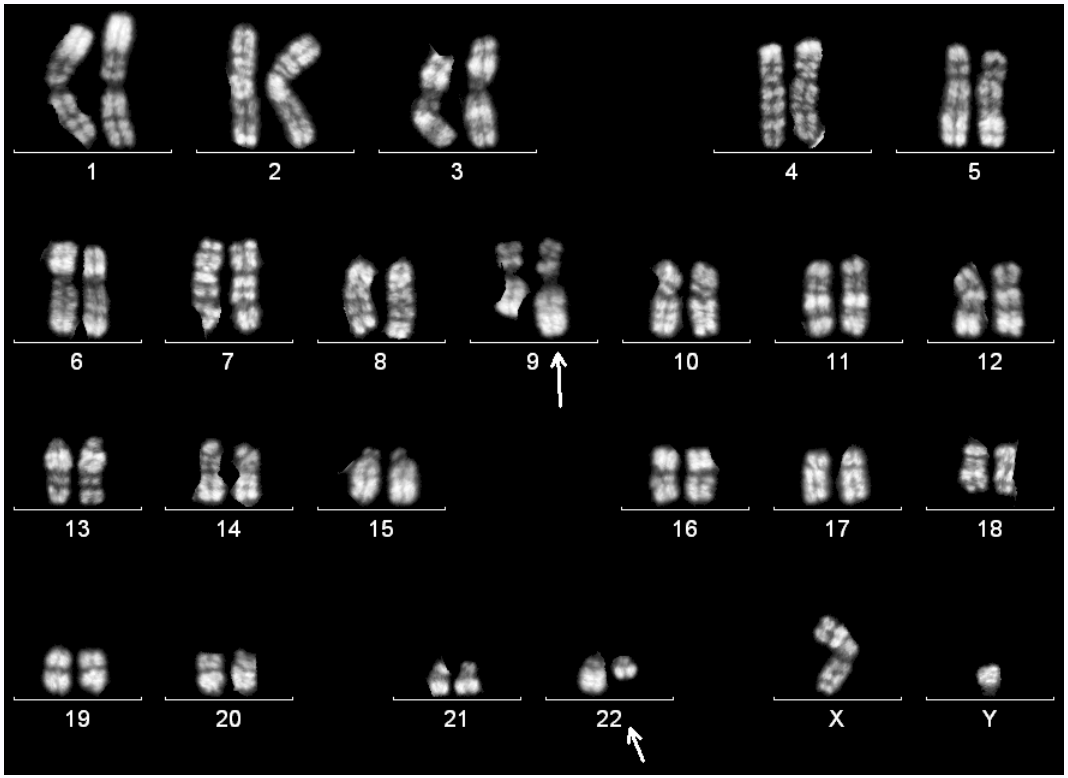


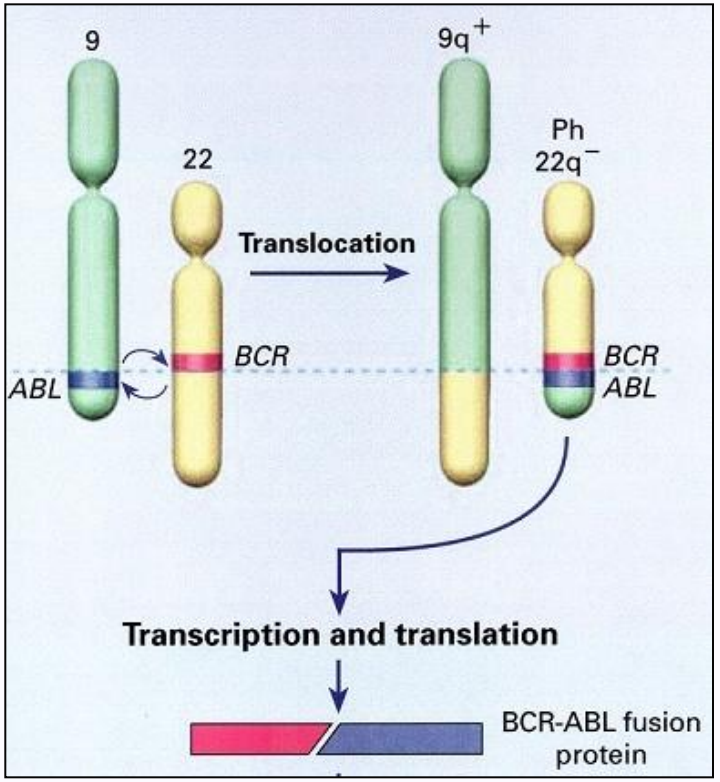


A new consistent chromosomal abnormality in chronic myelogenous leukemia identified by quinacrine fluorescence and Giemsa staining

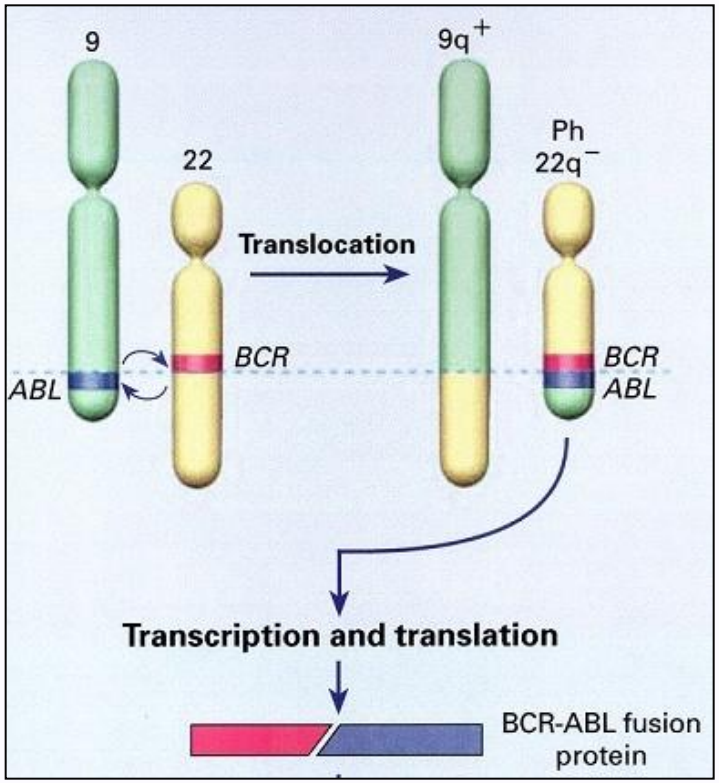
Rowley JD, Nature, 243, 290-293

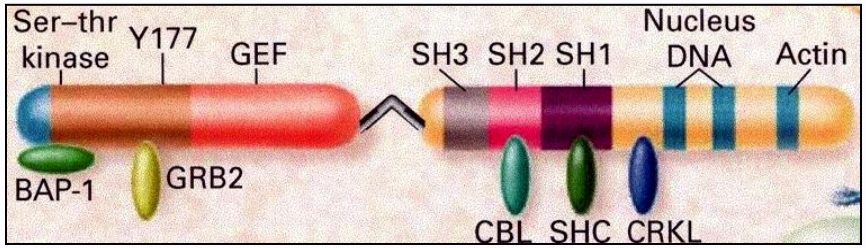
"…suggesting that there may be a hitherto undetected translocation between the long arm of 22 and the long arm of 9, producing the 9q+ chromosome…"

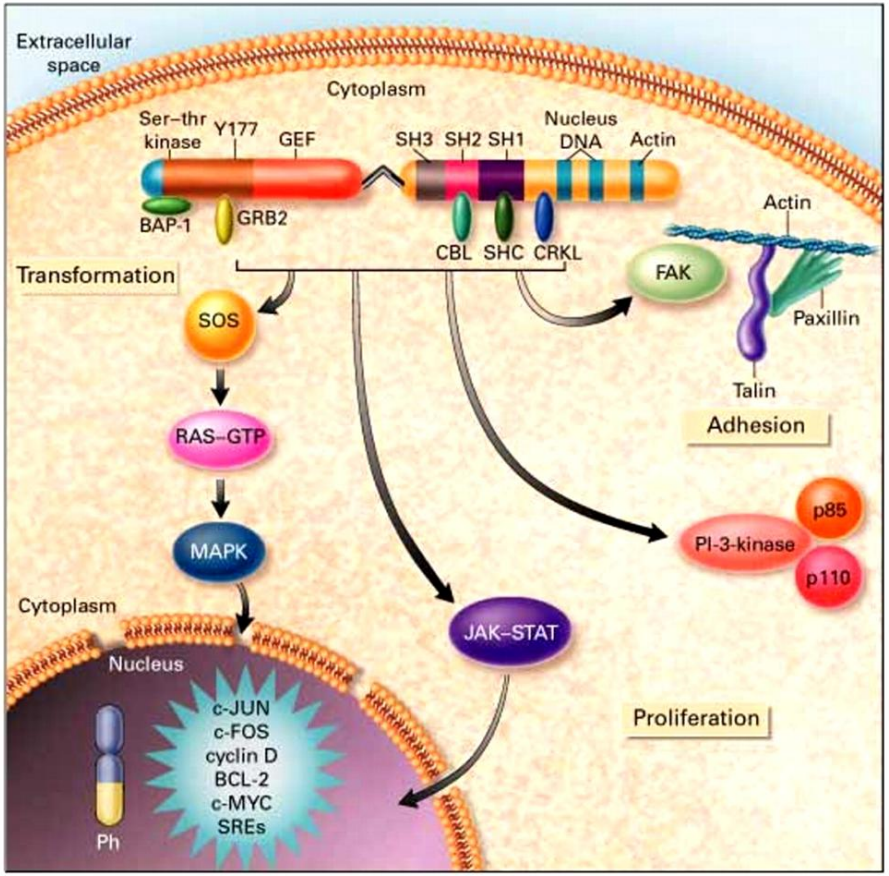




- 1982: ABL located on chromosome 9
- 1982: ABL involved in t(9;22)
- 1984: BCR located on chromosome 22







Faderl, S. et. al. N Engl J Med 1999;341:164-172

- 1984: ABL tyrosine kinase activity in cells with t(9;22)
- 1985: BCR/ABL fusion protein
- 1990: Proof of the pathogenetic role of BCR-ABL

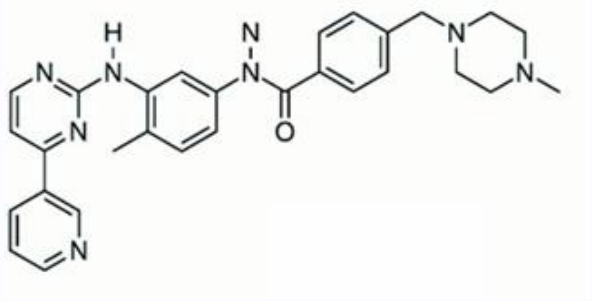
[CANCER RESEARCH 56, 100–104, January 1, 1996]

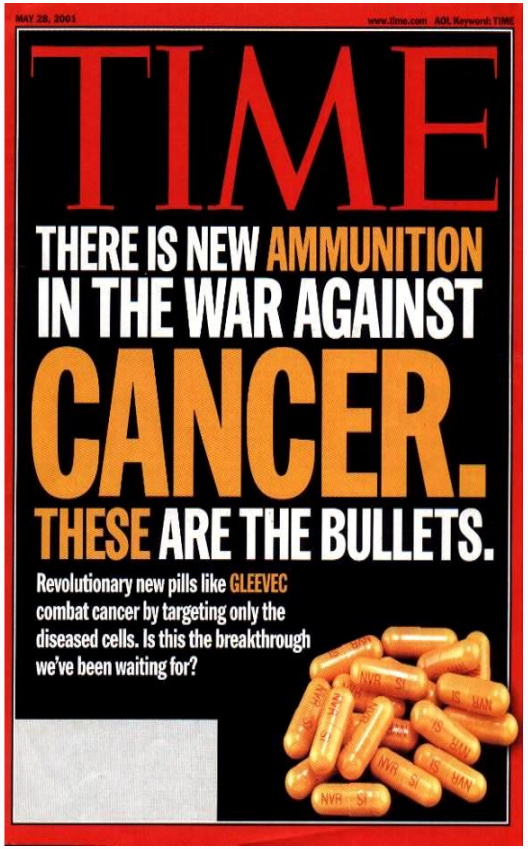
Inhibition of the Abl Protein-Tyrosine Kinase *in Vitro* and *in Vivo* by a 2-Phenylaminopyrimidine Derivative

Elisabeth Buchdunger,[1] Jürg Zimmermann, Helmut Mett, Thomas Meyer, Marcel Müller, Brian J. Druker, and Nicholas B. Lydon

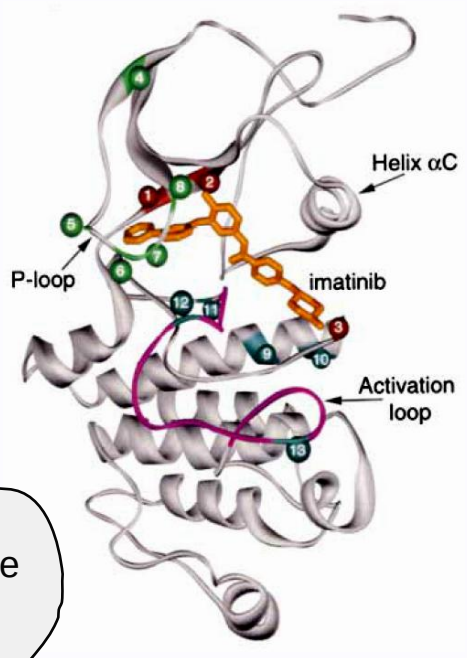
Ciba Pharmaceuticals Division, Oncology Research Department, Ciba-Geigy Limited, CH-4002 Basel, Switzerland [E. B., J. Z., H. M., T. M., M. M., N. B. L.], and Division of Hematology and Medical Oncology, Oregon Health Sciences University, Portland, Oregon 97201 [B. J. D.]

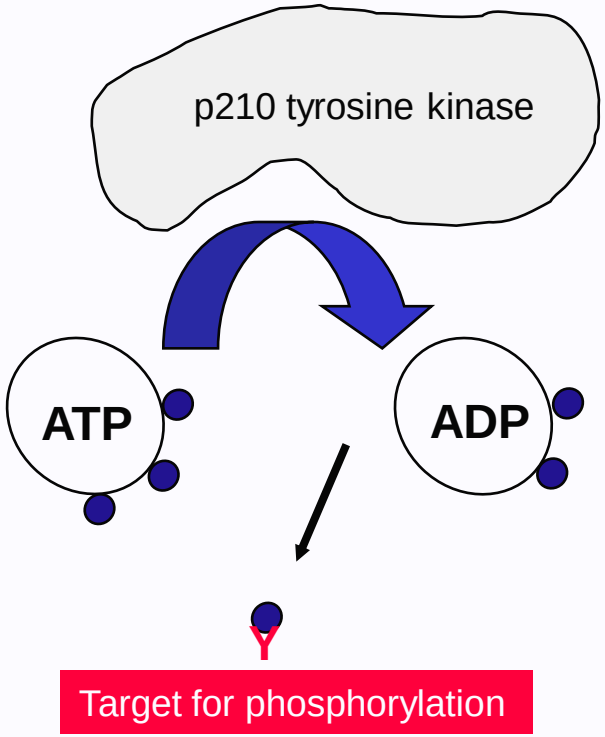
- 1996: *In vitro* effect of Imatinib
- 1999: *In vivo* effect of Imatinib
- 1999: Clinical efficacy

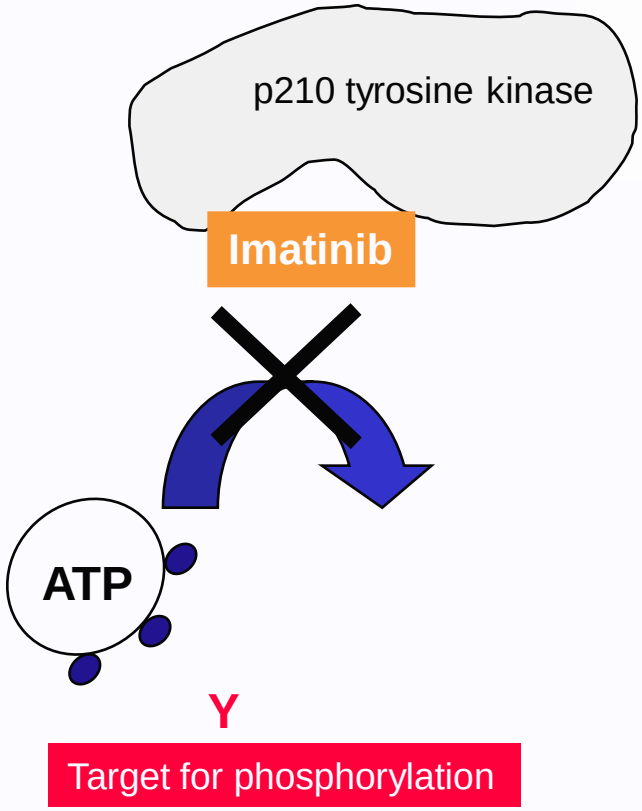




Imatinib inhibits the binding of ATP to abl tyrosine kinase







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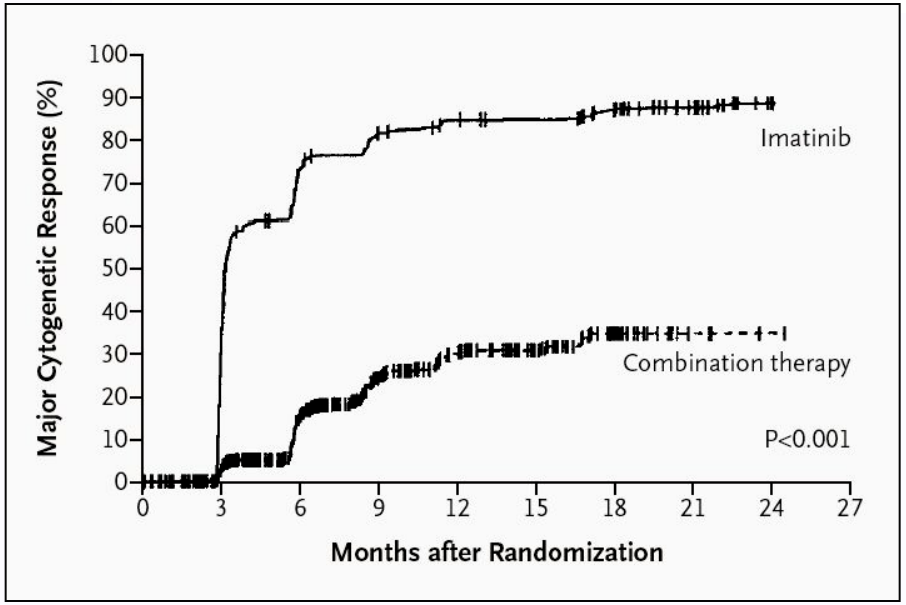
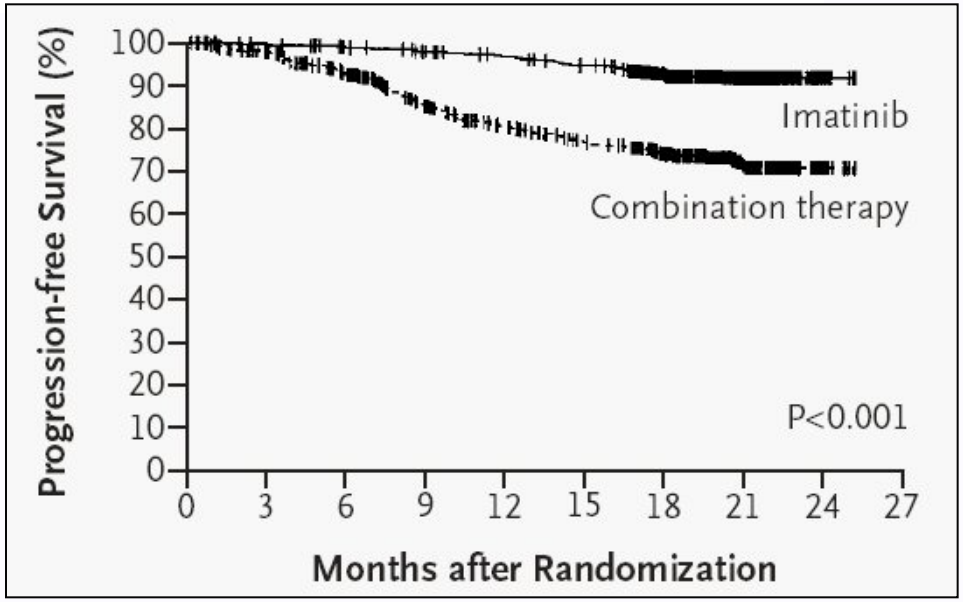
EFFICACY AND SAFETY OF A SPECIFIC INHIBITOR OF THE BCR-ABL TYROSINE KINASE IN CHRONIC MYELOID LEUKEMIA

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.



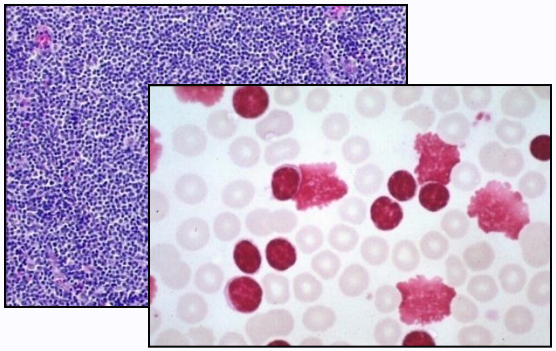


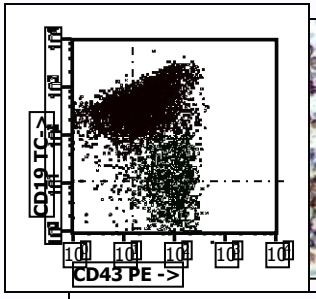
Conclusion

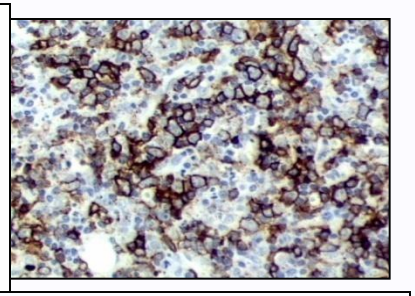
Cytogenetic analyses in malignant hemopathies

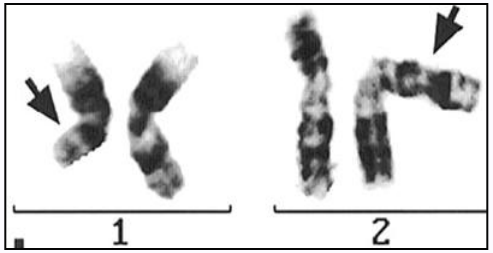
- are useful for diagnostic and prognostic purposes and mandatory in some disorders:
  - Mandatory at diagnosis: acute leukemias, MPD, MDS
  - Recommended at diagnosis : CLL
  - Useful at diagnosis: NHL, MM
  - Mandatory in follow-up: CML
- are very useful and promising for research purposes expanding but expensive tools)

Cytogenetics
= part of a **multidisciplinary** approach









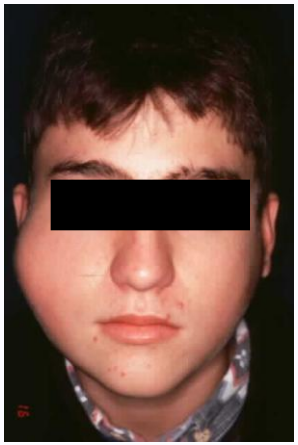
Immunophenotype:
- **flow cytometry**
- **immunohistochemistry**

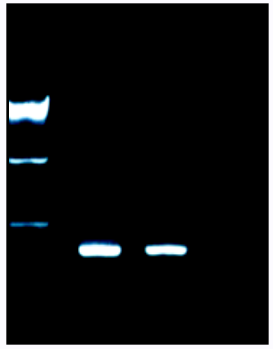
Morphology
- **cytology**
- **histology**

Clinics

Cytogenetics

Molecular biology





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

Suggested reading

- Atlas of cytogenetics: http://www.infobiogen.fr/services/chromcancer/ (contains informations on clinico-biological entities and on specific chromosome aberrations)
- WHO 2008
- Catalog of genetic anomalies in cancer: http://cgap.nci.nih.gov/Chromosomes/Mitelman (useful in case of very rare aberrations)