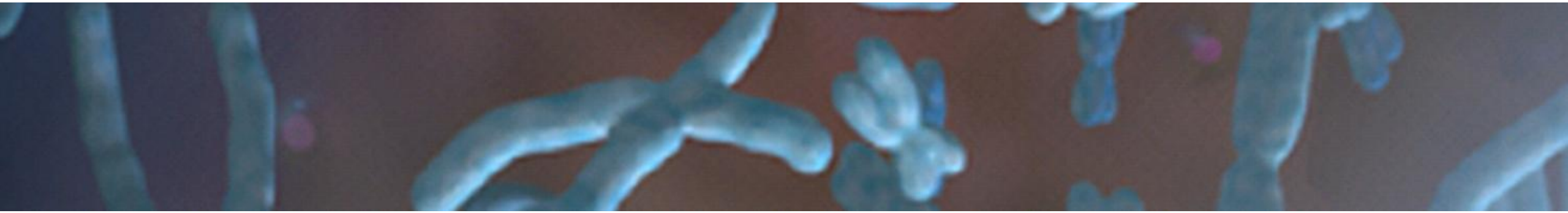
BHS training course

Laboratory Hematology
Cytogenetics



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Center for Human Genetics Leuven
10/11/2025

Organization of the Lecture

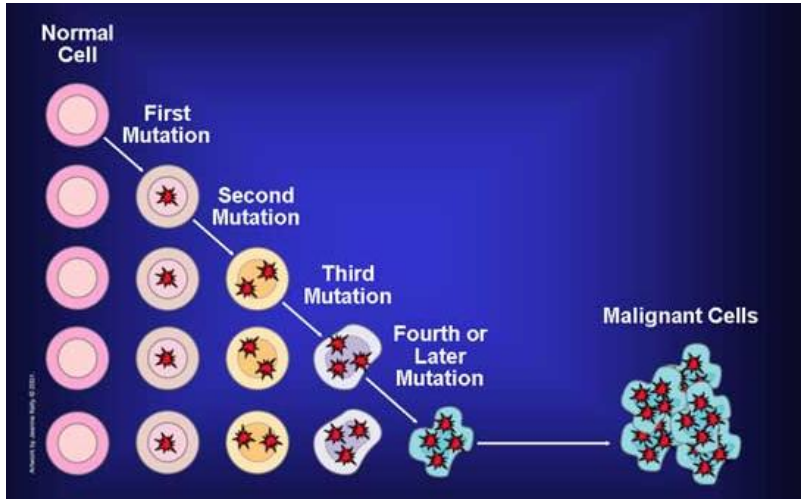
1. Definition and principles
2. Different techniques used
3. Applications of cytogenetic analyses
   - Diagnosis
   - Prognosis
   - Contribution to therapeutic developments

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

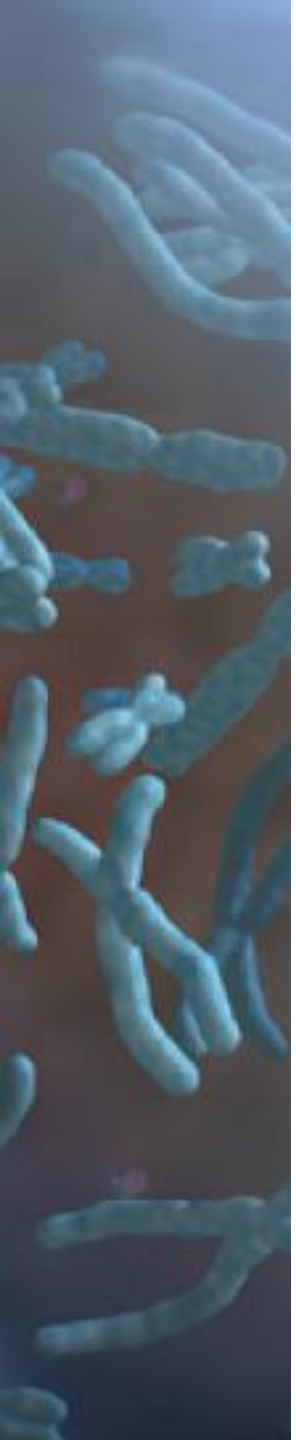


- Acquired malignant hemopathies are characterized by primary genetic aberrations which are present in all neoplastic cells demonstrating that these are clonal disorders
- Secondary abnormalities accumulate throughout the course of the disease (= clonal evolution)
- Aberrations can be chromosome abnormalities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

POLL - 1

Acquired cytogenetics are used in the clinic to...

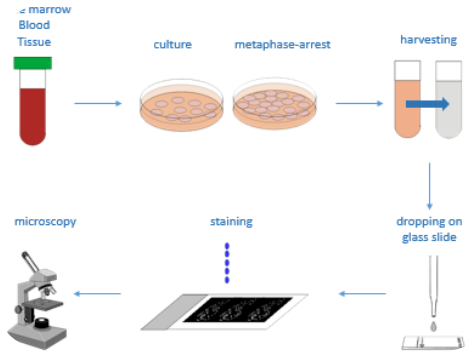
- establish a diagnosis of a hematopoietic malignancy
- determine the appropriate treatment strategy
- estimate prognosis
- provide a marker for follow-up or disease progression
- all four options are true



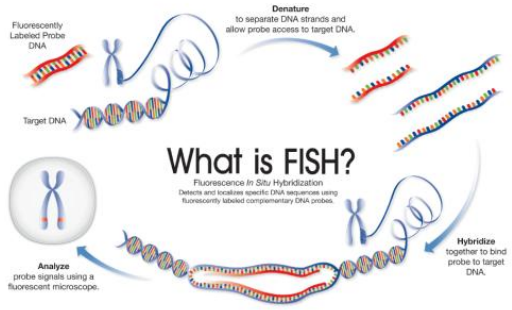
1. Acquired cytogenetics

- 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)/*PML::RARA* in APL
  - Most are found in more than one entity, e.g. t(9;22)/*BCR::ABL1* in CML and ALL
  - Each entity is defined by a characteristic profile of recurrent abnormalities
- Clonality ≠ always malignancy

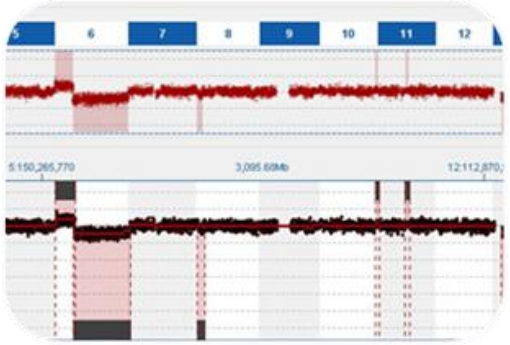
2. Cytogenetic methods to detect clonal abnormalities



Chromosome banding analysis

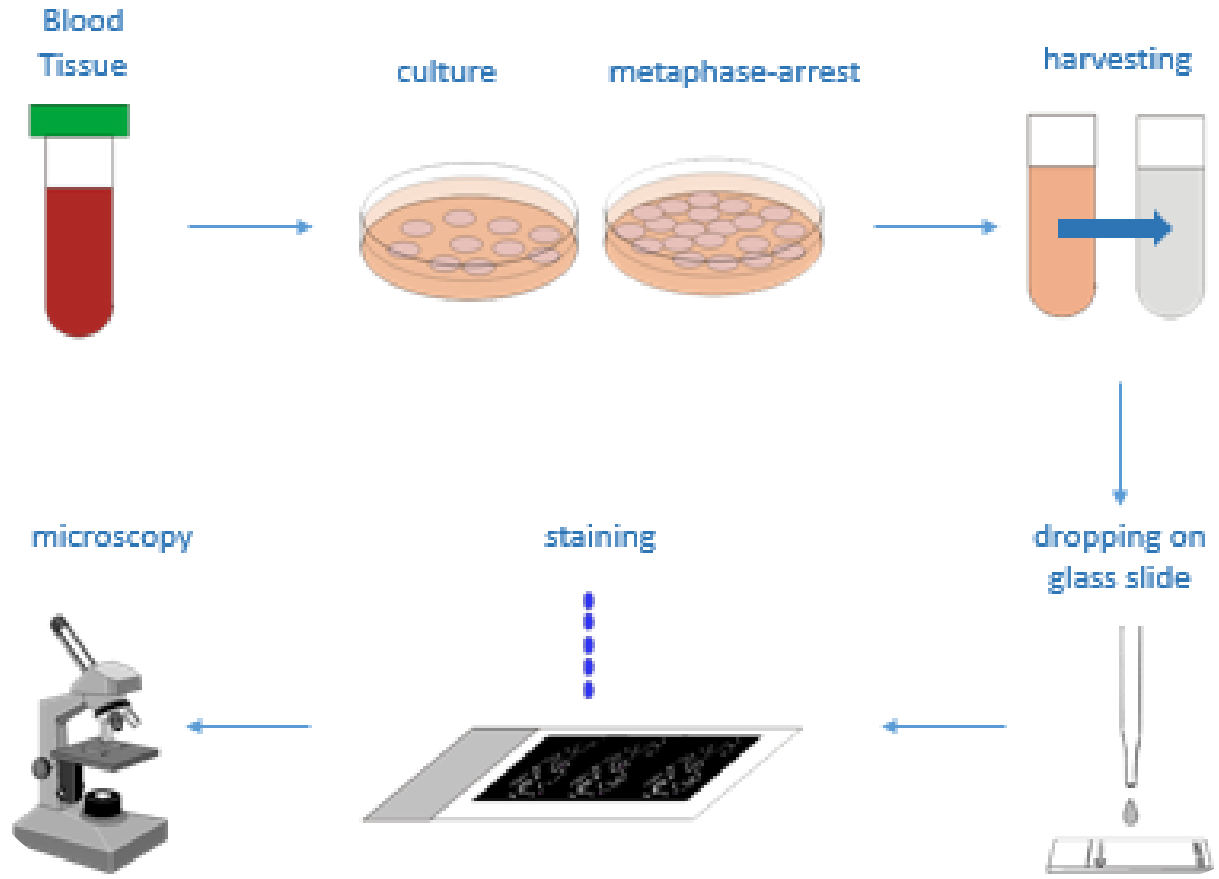


FISH



DNA based methods providing a molecular karyotype

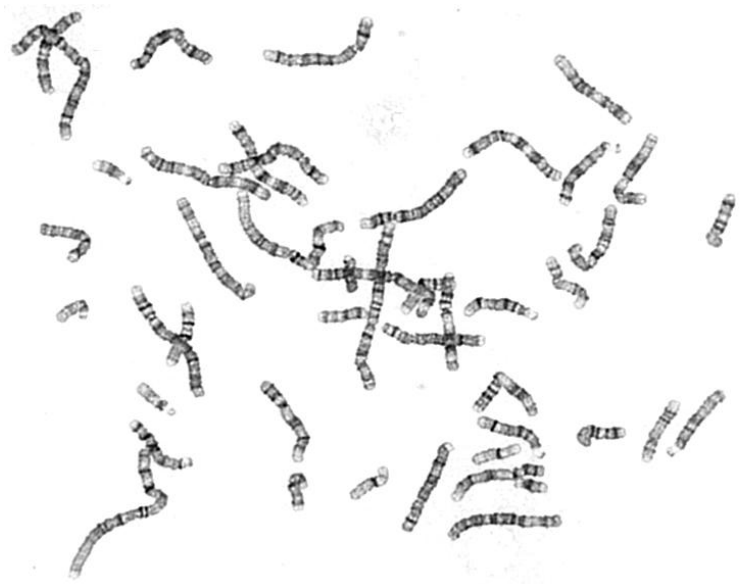
2.1. Karyotype



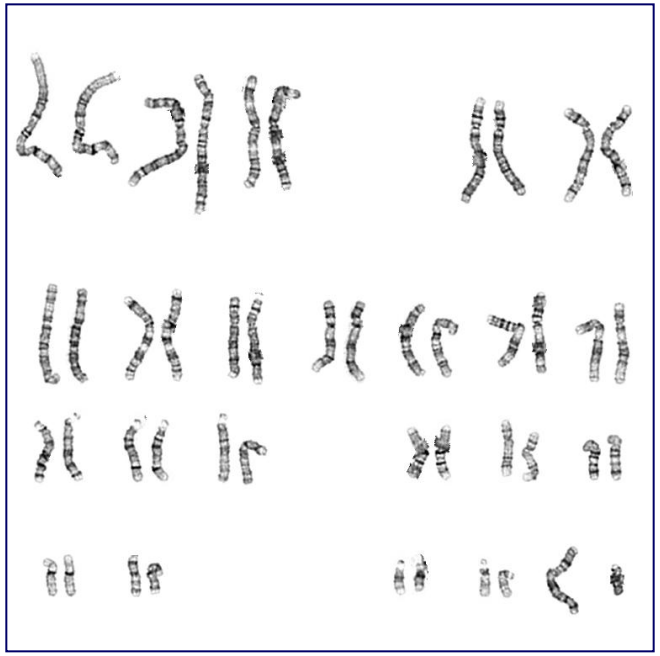
+ detection of structural and numerical variants genome wide

- failure of the karyotype, resolution is low

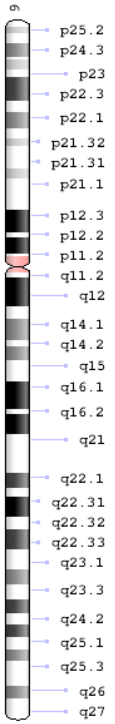
2.1.1 Establishment karyotype



Metaphase

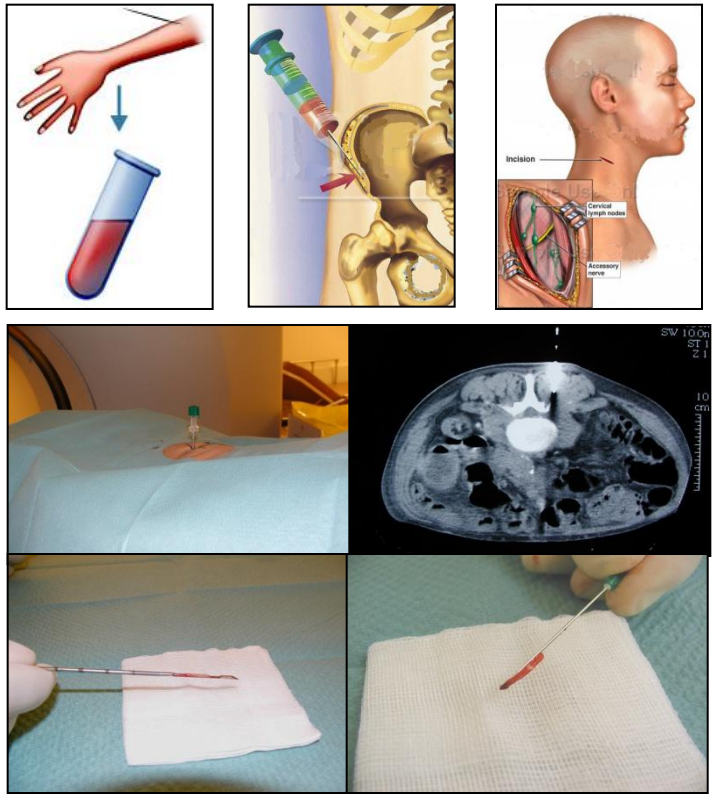


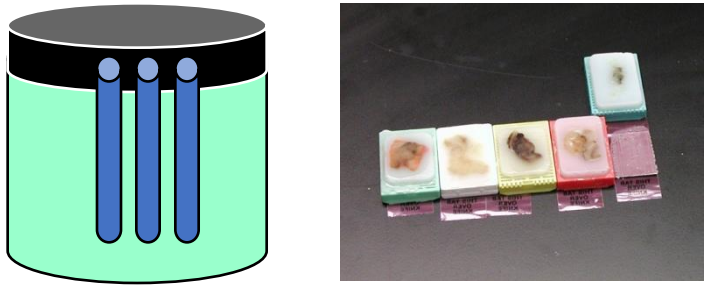
Karyogram



Idiogram

Sample types

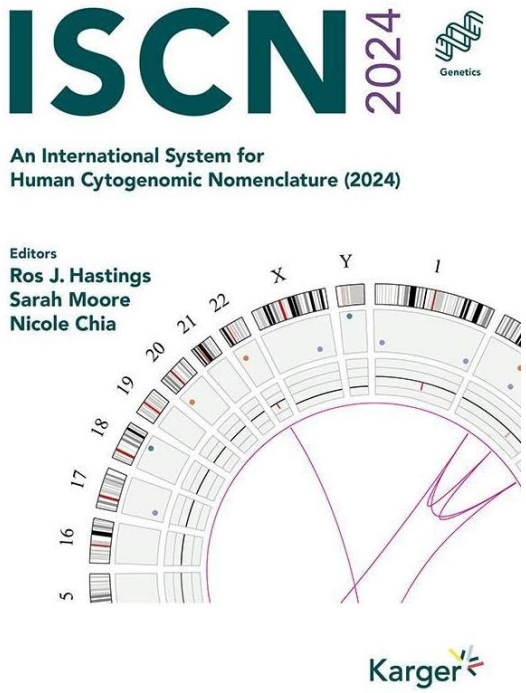




All invaded tissues are suitable...*but* tissues must be viable, and the target cell capable of proliferation

2.1.2 Karyotype result

Result: karyotype = summary of several mitoses, expressed as a formula, according to rules and nomenclature (ISCN 2024)



- Number of chromosomes («modal» number) of the clone
- Gonosomes (according to ploidy) 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)

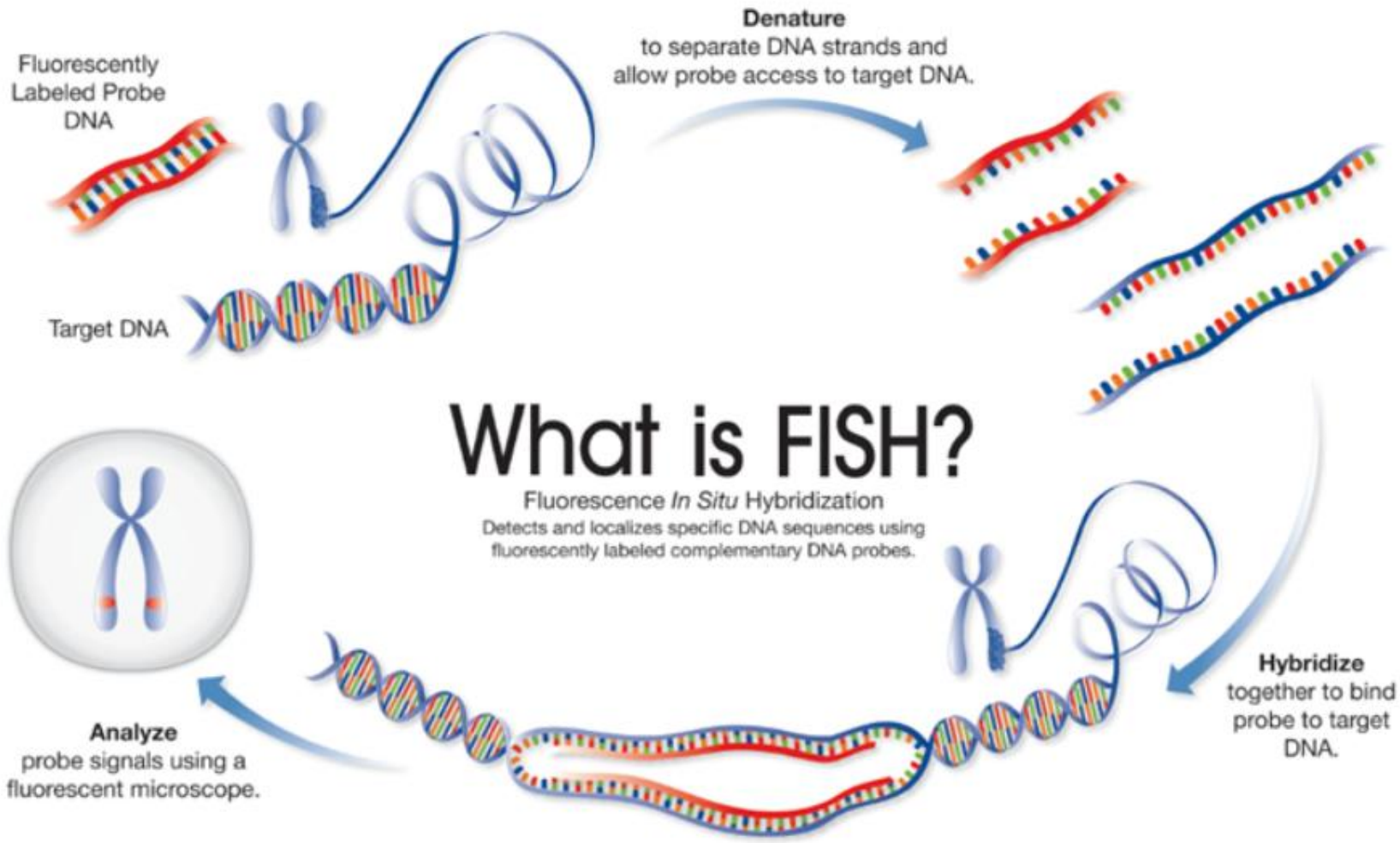
46,XY,t(9;22)(q34;q11)[7]/47,idem,+8[3]/46,XY[10]

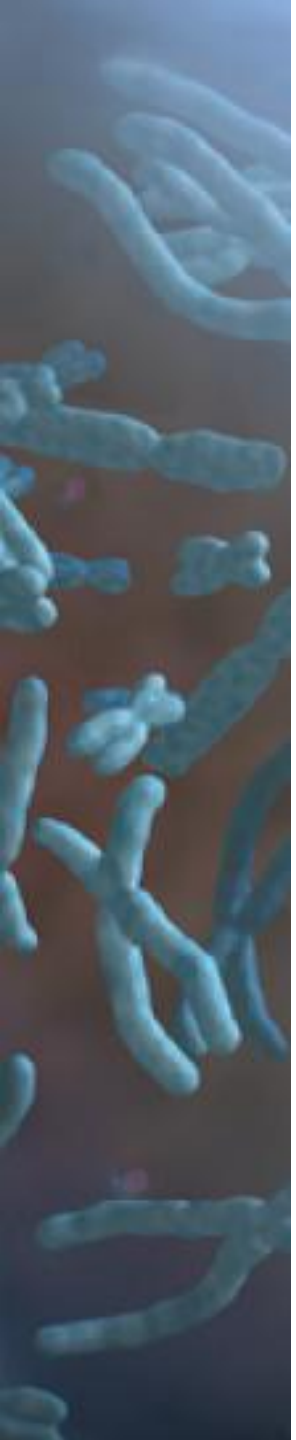
POLL - 2

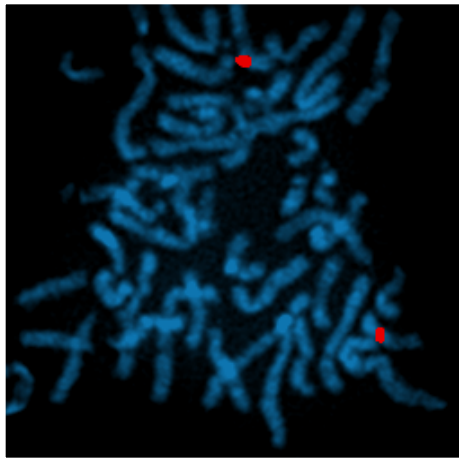
Patient X is referred to the hospital for unexplained cytopenia. Bone marrow investigation reveals dysplasia in the myeloid lineage and megakaryocytes. Chromosome banding analysis reveals a deletion on the long arm of chromosome 5 in 6 mitoses and a monosomy 7 in another 2 mitoses. What is the correct karyotype annotation?

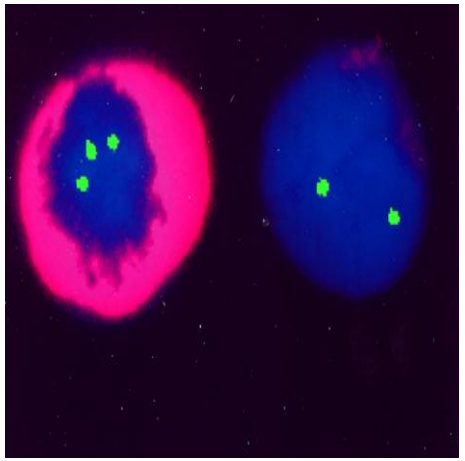
- 46,XY[12]/46,XY,del(5)(q13q31)[6]/45,XY,-7[2]
- 46,del(5)(q13q31),XY[6]/45,-7,XY[2]/46,XY[12]
- 46,XY,del(5)(q13q31)[6]/45,XY,-7[2]/46,XY[12]
- 45,XY,-7[2]/46,XY,del(5)(q13q31)[6]/46,XY[12]
- 46,XY,del(5)(q13q31)[6]/46,XY,-7[2]/46,XY[12]

2.2 FISH (Fluorescence In Situ Hybridization)

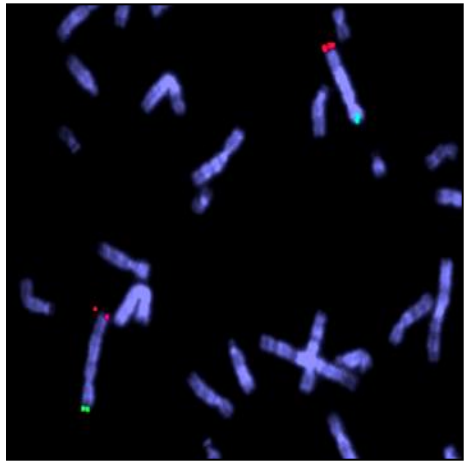


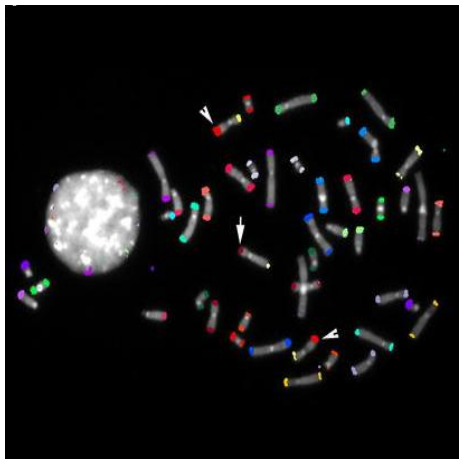




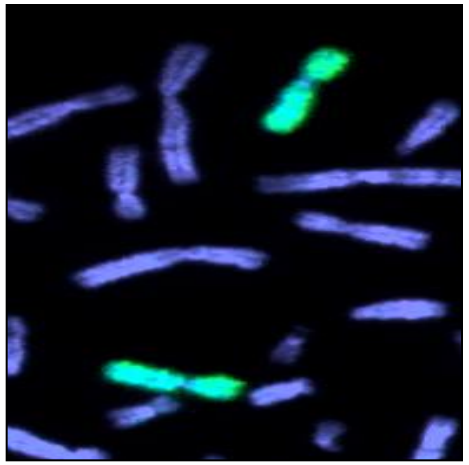


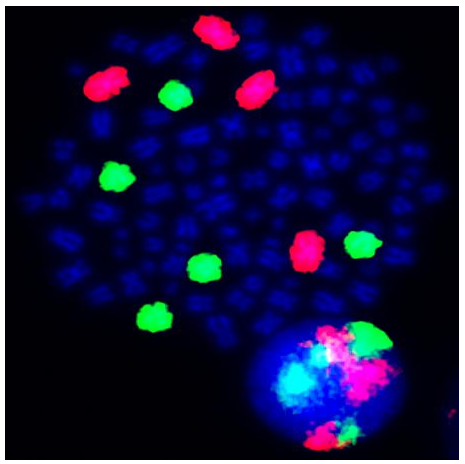
Different probes:





- centromeric

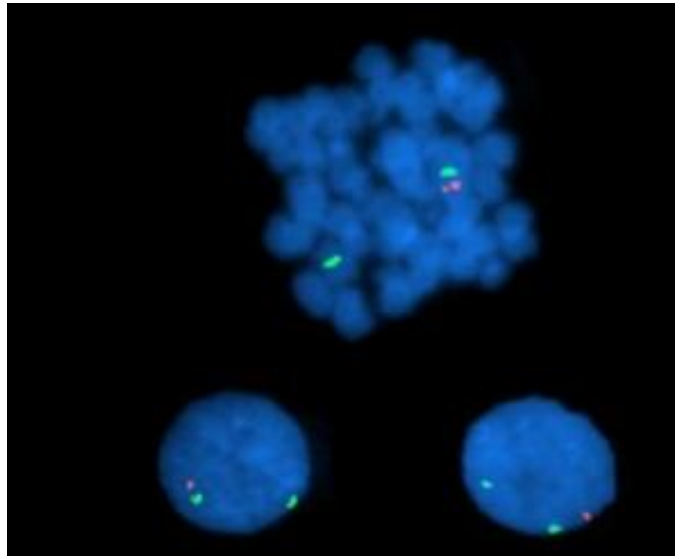


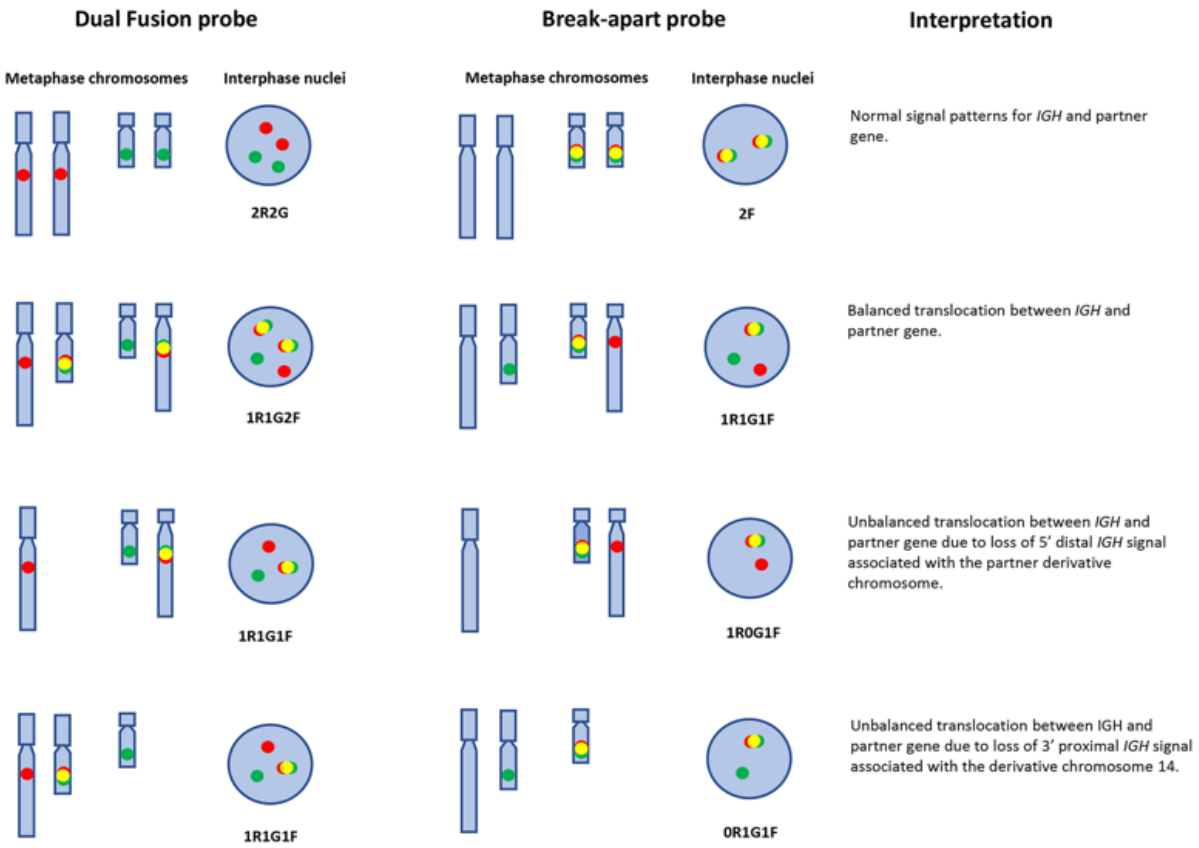


- telomeric
- painting (wcp)

  Metaphase only

Locus-specific probes:
translocation probes
microdeletions probes (e.g.,*TP53*)



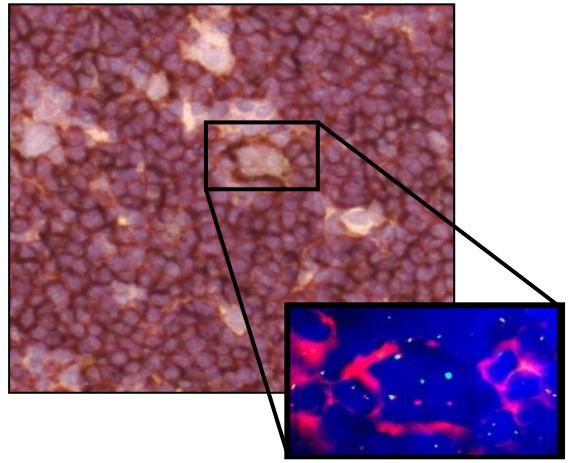


FISH allows the identification of cryptic abnormalities such as small deletions

–Conventional karyotype (smallest band) 5-10Mb

–FISH on Interphase nuclei ± 50-100kb

+ detection of specific structural or numerical variants at high resolution

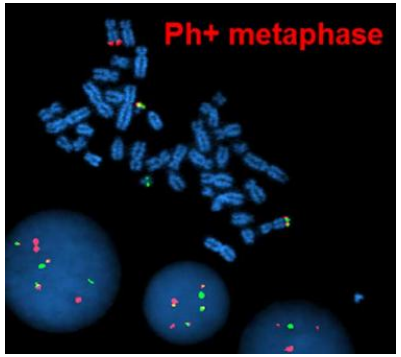


FISH on interphase nuclei:

→ 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



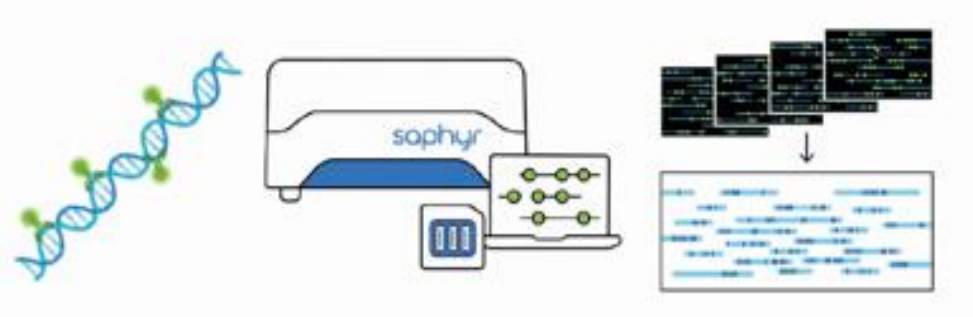
Metaphase FISH advantage: information on chromosome structure and probe location

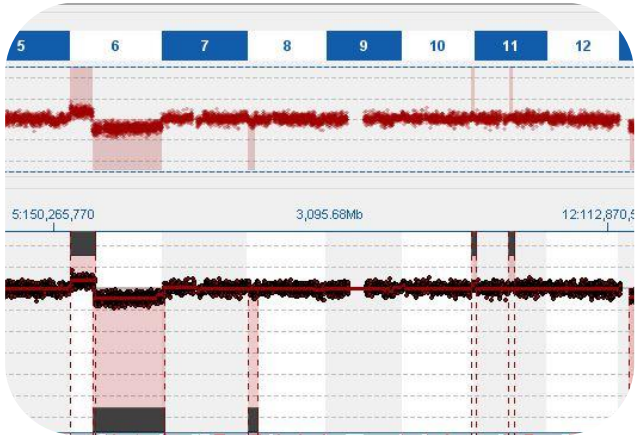
- targeted analysis

2.3 Molecular based virtual karyotypes

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

Based on either microarray or NGS or optical mapping technologies.



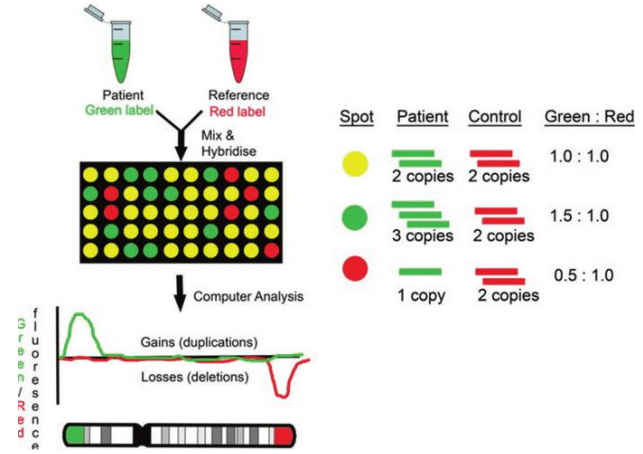


2.3.1 Arrays and low pass/shallow sequencing

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

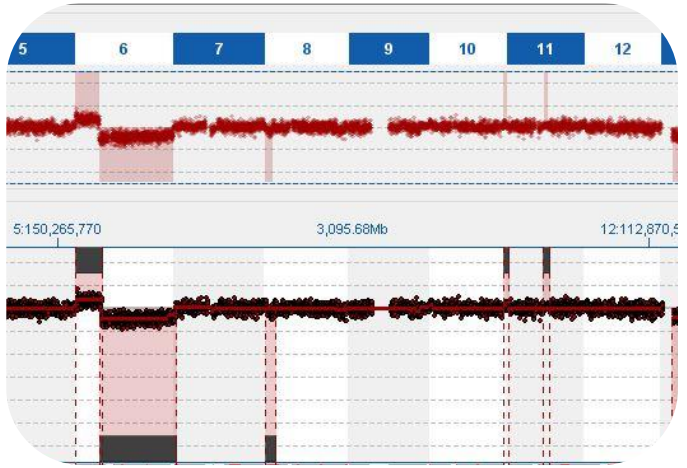
high number = gain

low number = loss



Array CGH (Comparative Genomic Hybridization)

+ sequencing identifies the precise genomic content (genes, genomic co-ordinates, exons..)



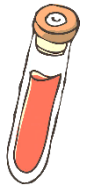
LPS (Low Pass/shallow Sequencing)

Advantages and limitations of arrays and low pass/shallow sequencing

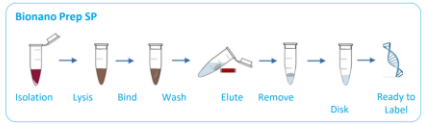
+	Overcomes limitation of karyotyping - No requirement for proliferating cells Overcomes limitation of FISH - Whole genome Higher resolution than karyotype and FISH – resolution defined by the number of probes and spacing
-	Does not detect balanced rearrangements Not widely implemented into routine acquired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

2.3.2 Optical Genome Mapping

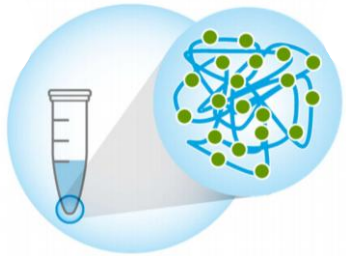
Fresh (frozen) material

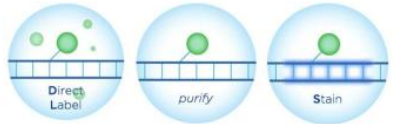


Extraction of UHMW DNA

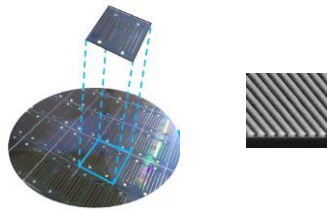


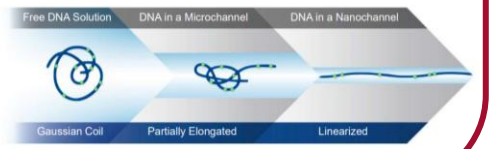
Label DNA at specific motifs





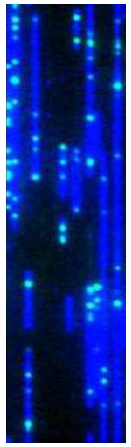
Transfer labeled DNA into Chip



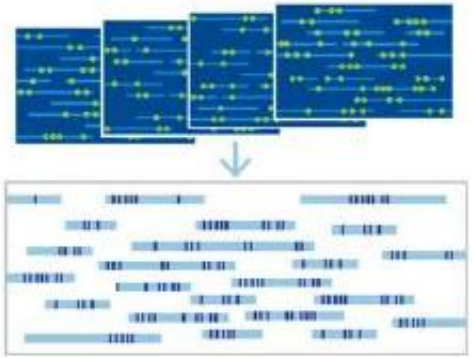


2.3.2 Optical Genome Mapping

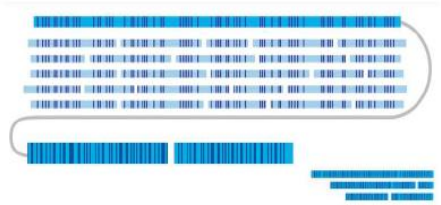
Raw Image Data:
Direct observation of DLS labels on long DNA molecules
(150 kb up to 3 Mb)



Algorithms convert the raw images into .bnx files
=> population of molecules



Algorithms align different molecules for constructing Consensus Genome Maps (.cmap files)



Cross mapping across a Reference

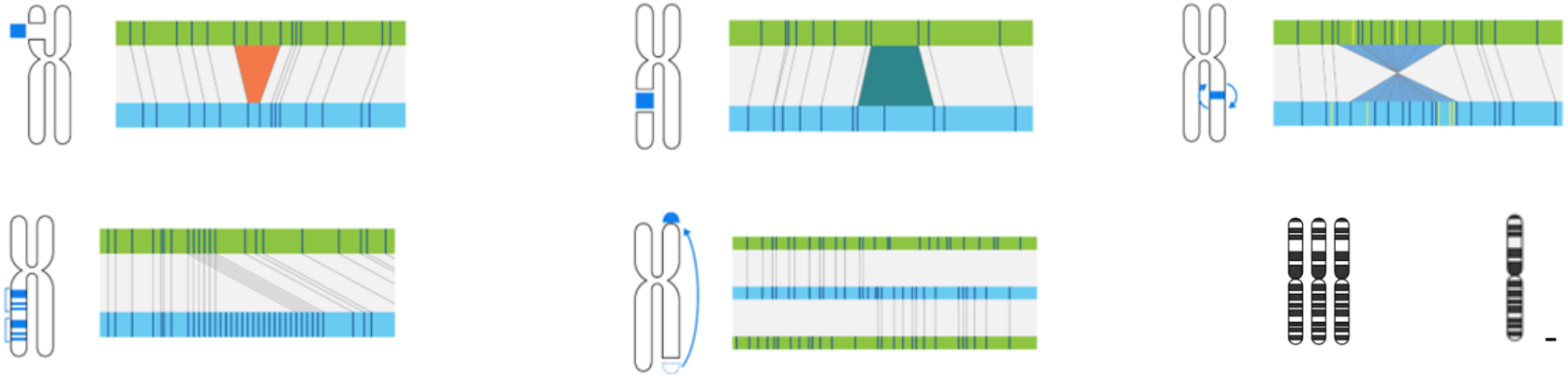
- CNA profile
- SA

insertion

2.3.2 Optical Genome Mapping

+ Can detect copy number and structural abnormalities genome-wide

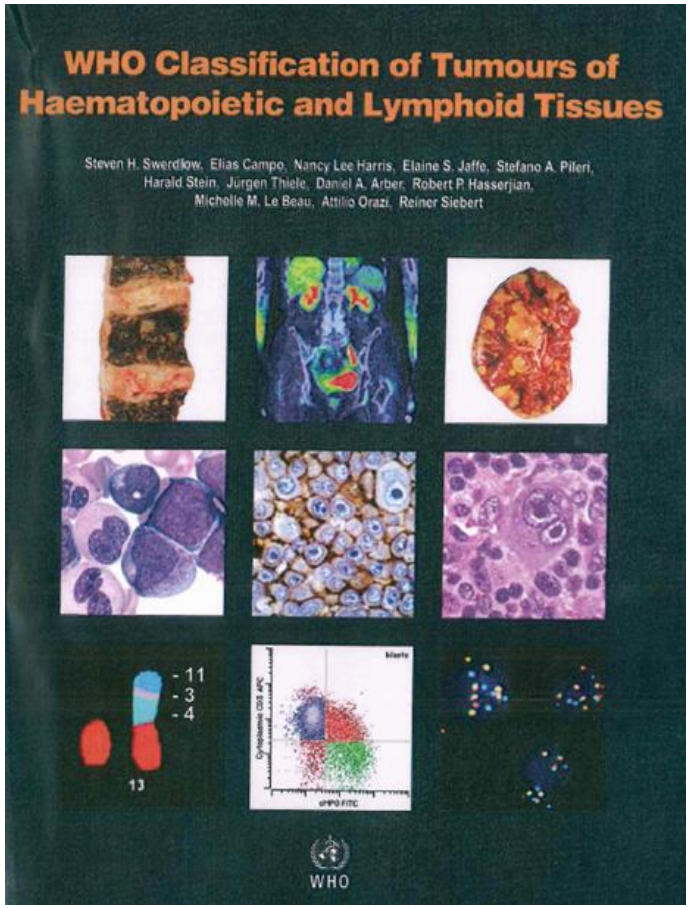
- Difficulties to detect atypical ploidy as well as aberrations in certain genomic regions



3.1 Cytogenetics: diagnostic value

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

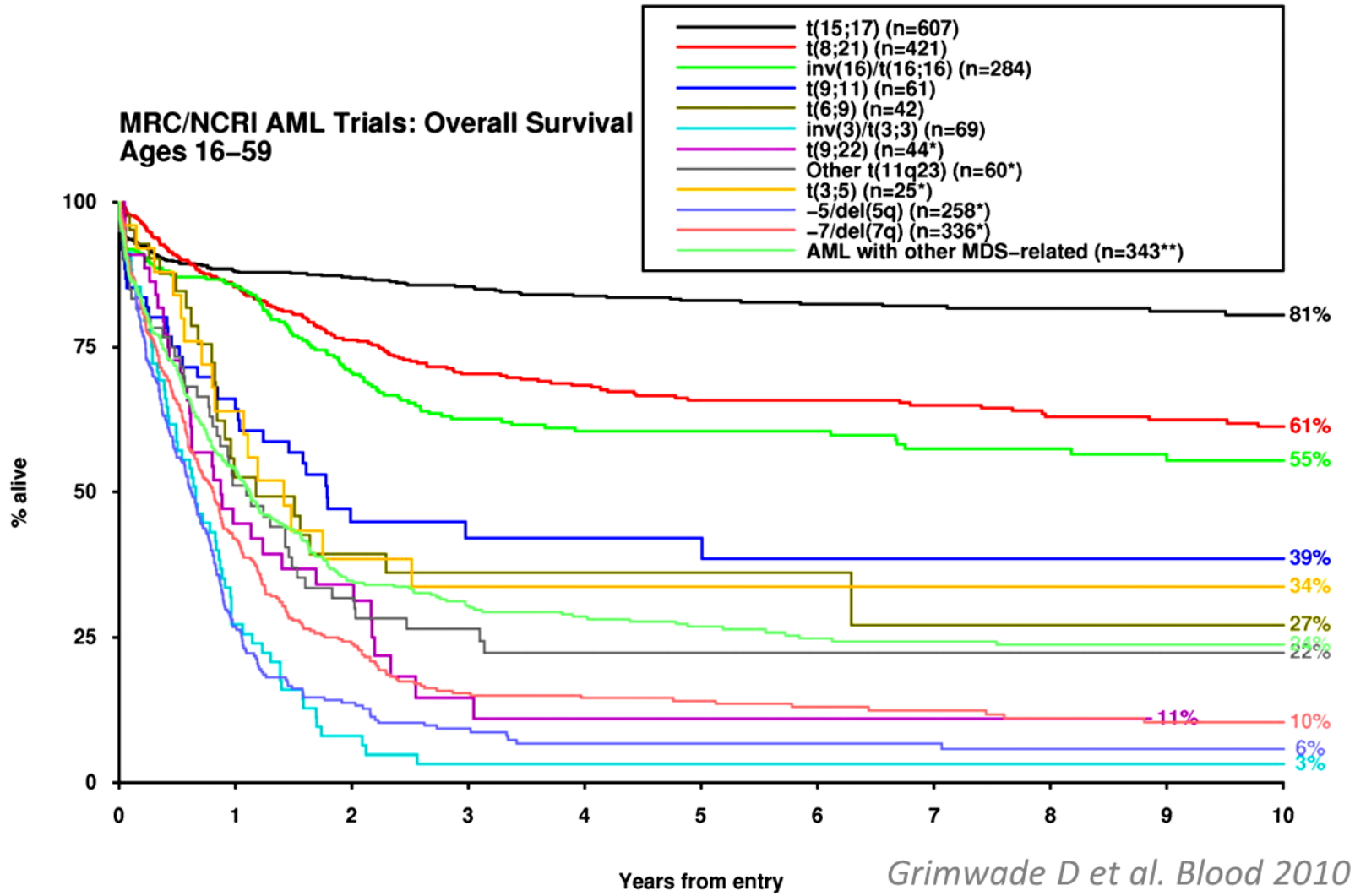
- Mandatory at diagnosis: acute leukemia, MPN (CML), MDS
- Mandatory in follow-up: CML, CLL before treatment
- Recommended at diagnosis: MM
- Useful at diagnosis: NHL



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

3.2 Cytogenetics: prognostic value

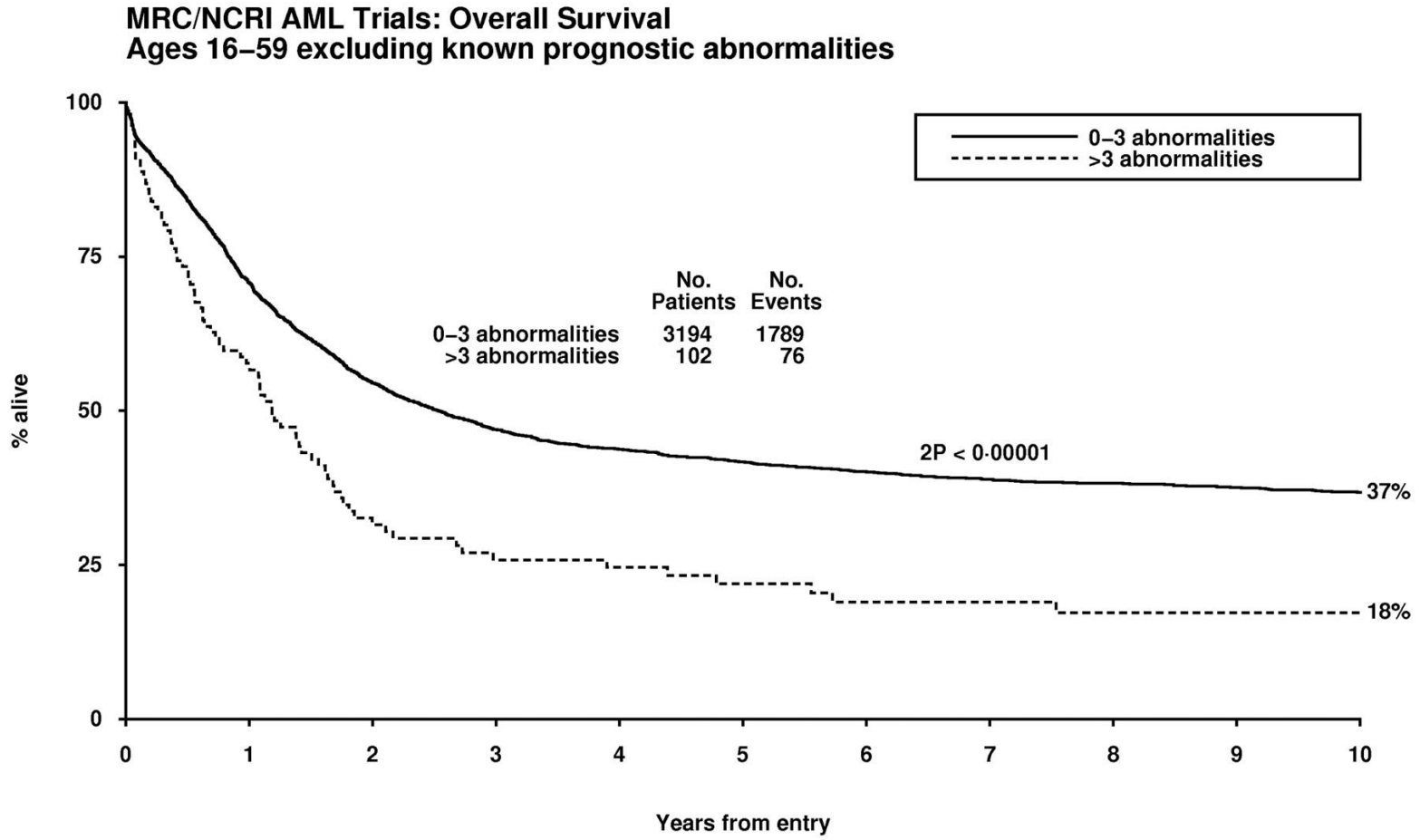
Example: prognostic value of the specific cytogenetic aberrations seen at diagnosis in AML.
Used to stratify patients into cytogenetic risk groups



Grimwade D et al. Blood 2010

3.2.1 Cytogenetics: prognostic value in AML

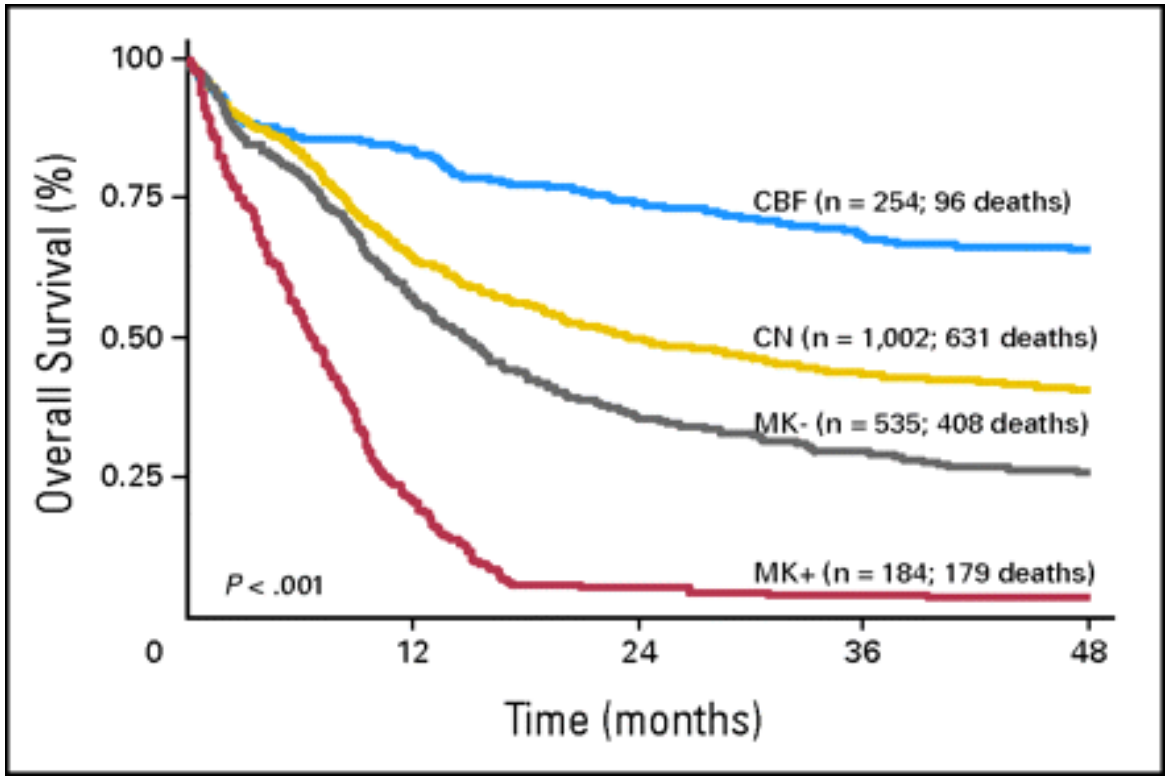
Impact of karyotype complexity on survival in AML



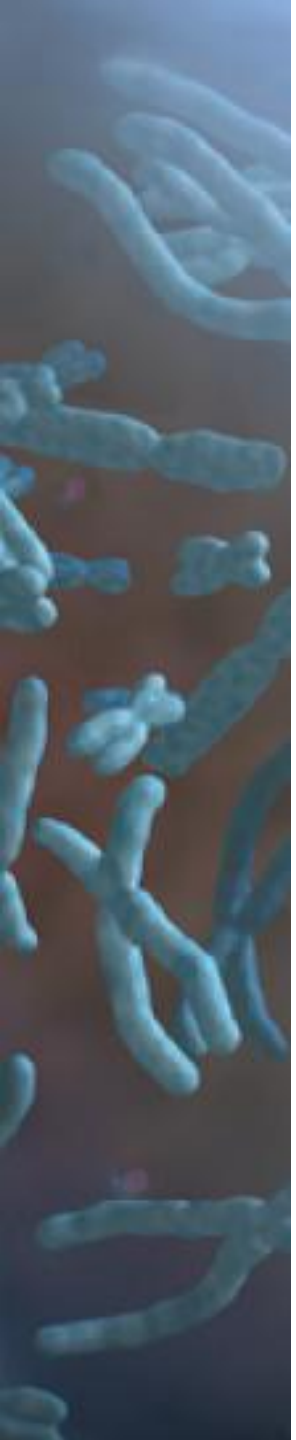
Grimwade D et al. Blood 2010

3.2.1 Cytogenetics: prognostic value in AML

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

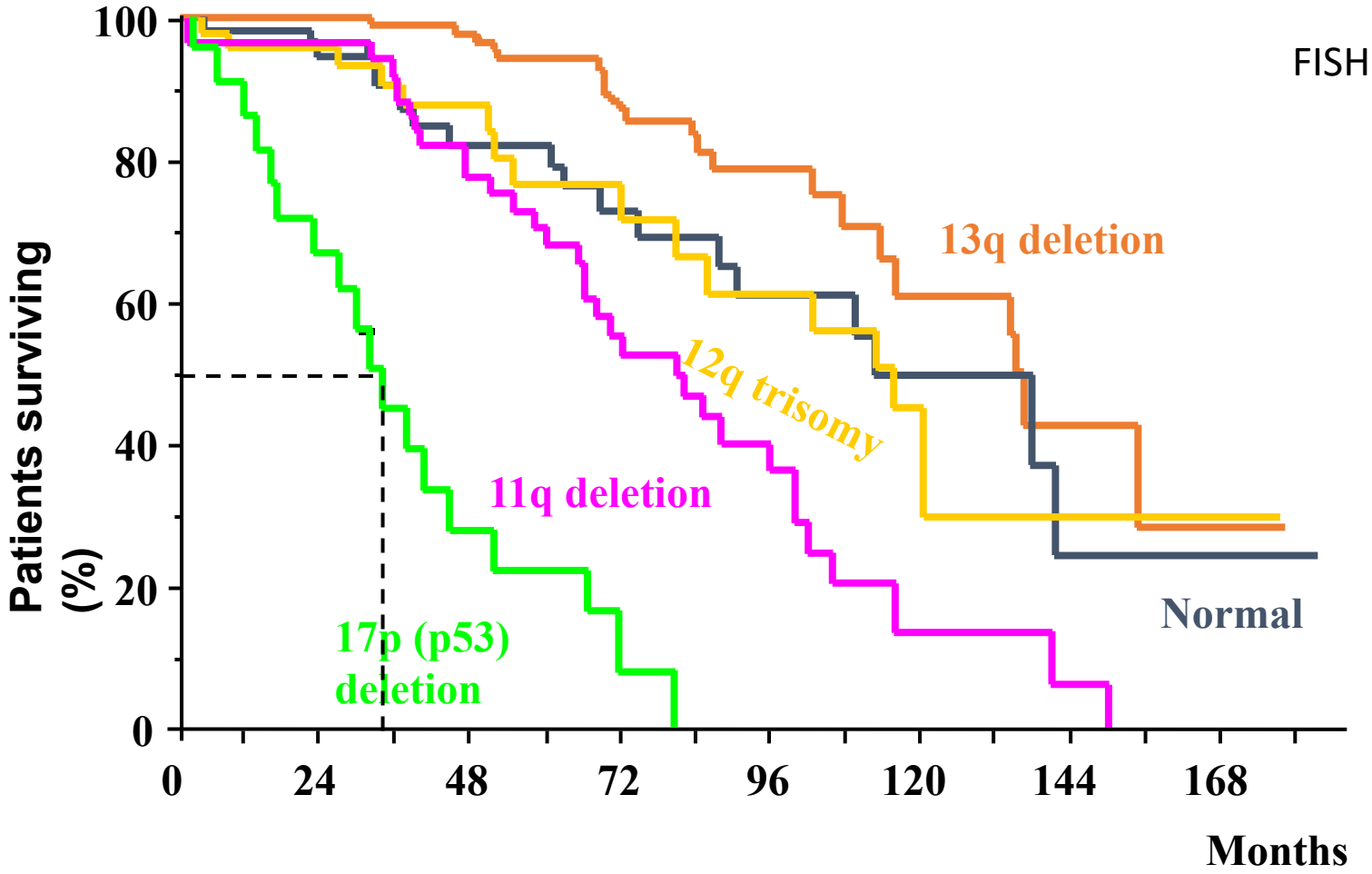


2022 ELN risk stratification by genetics at diagnosis

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.

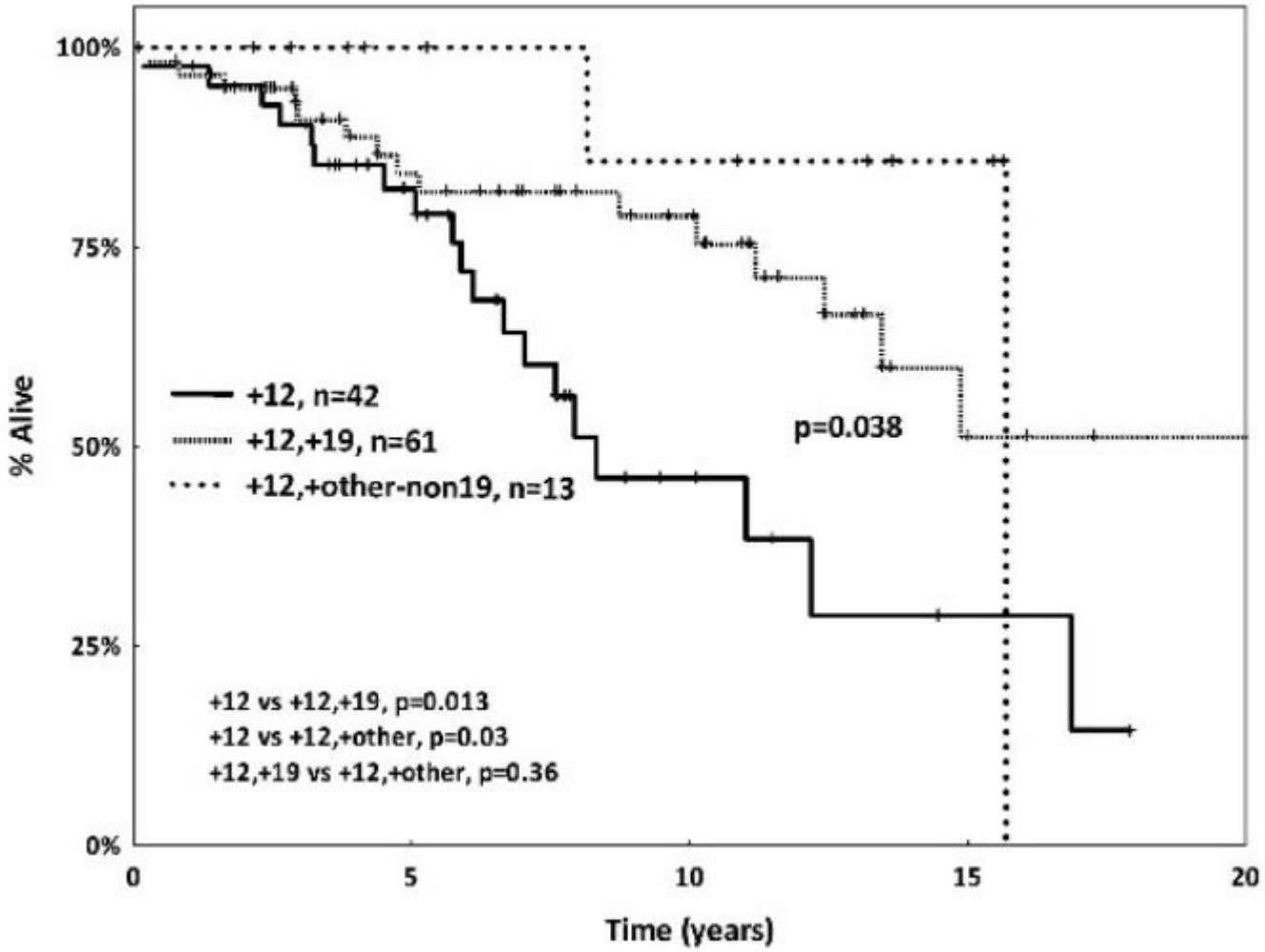
3.2.2 Cytogenetics: prognostic value in CLL



Döhner et al. N Engl J Med 2000

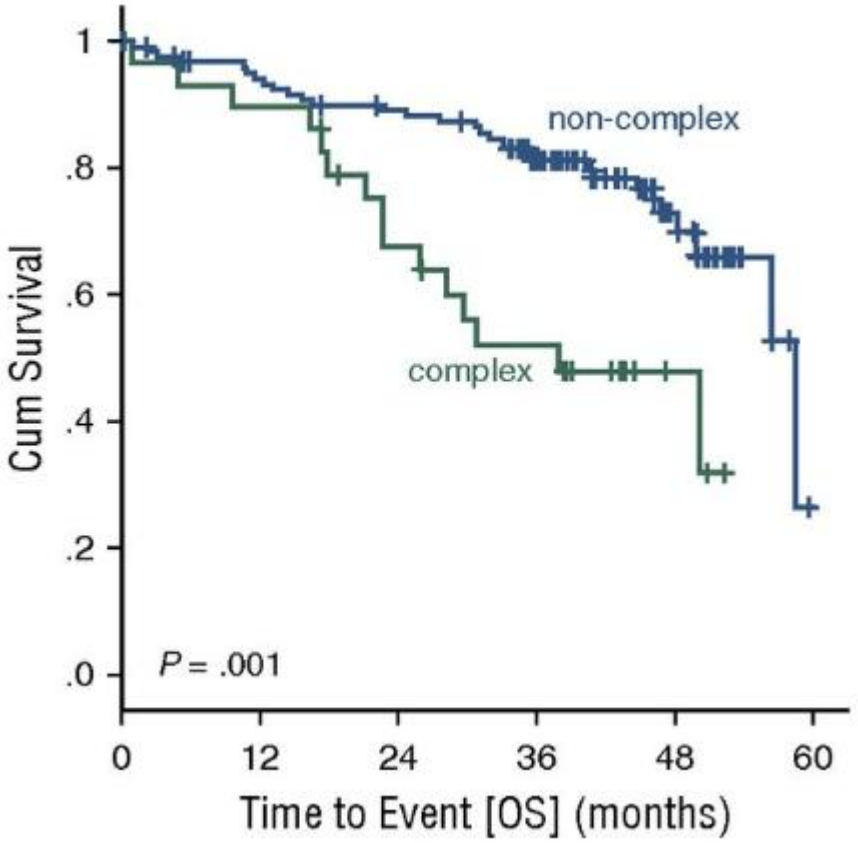
Landmark paper

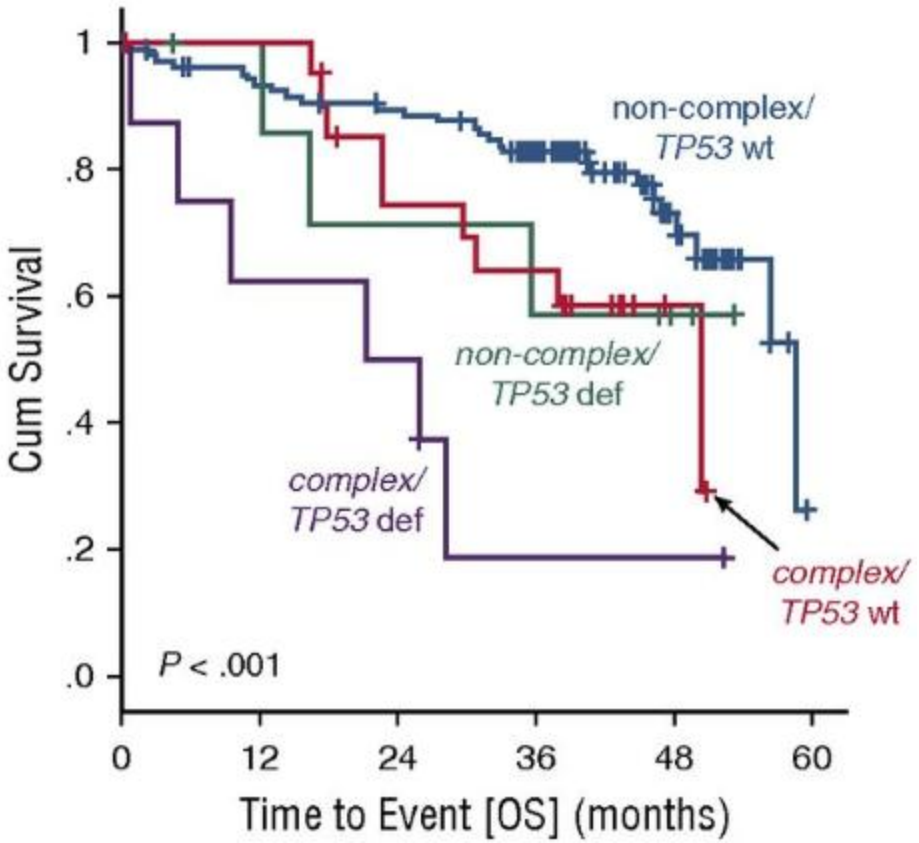
3.2.2 Cytogenetics: prognostic value in CLL



Baliakis P et al. Haematologica 2016.

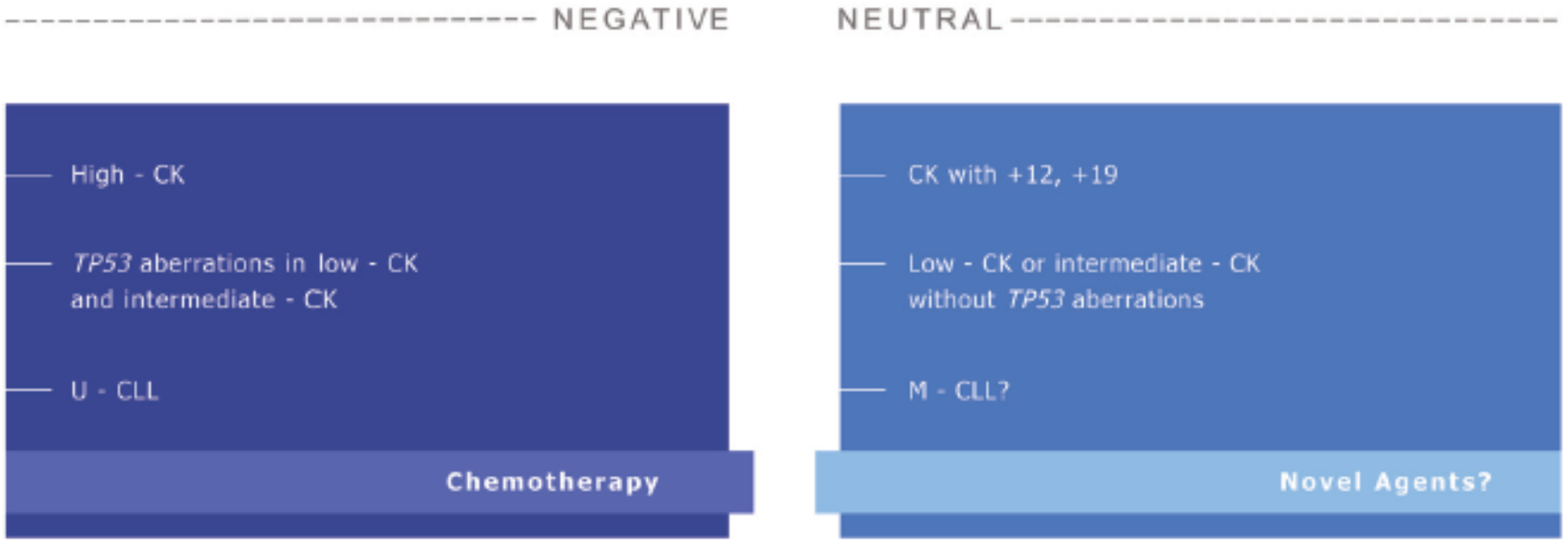
3.2.2 Cytogenetics: prognostic value in CLL





Herling CD et al. Blood 2016.

3.2.2 Cytogenetics: prognostic value in CLL

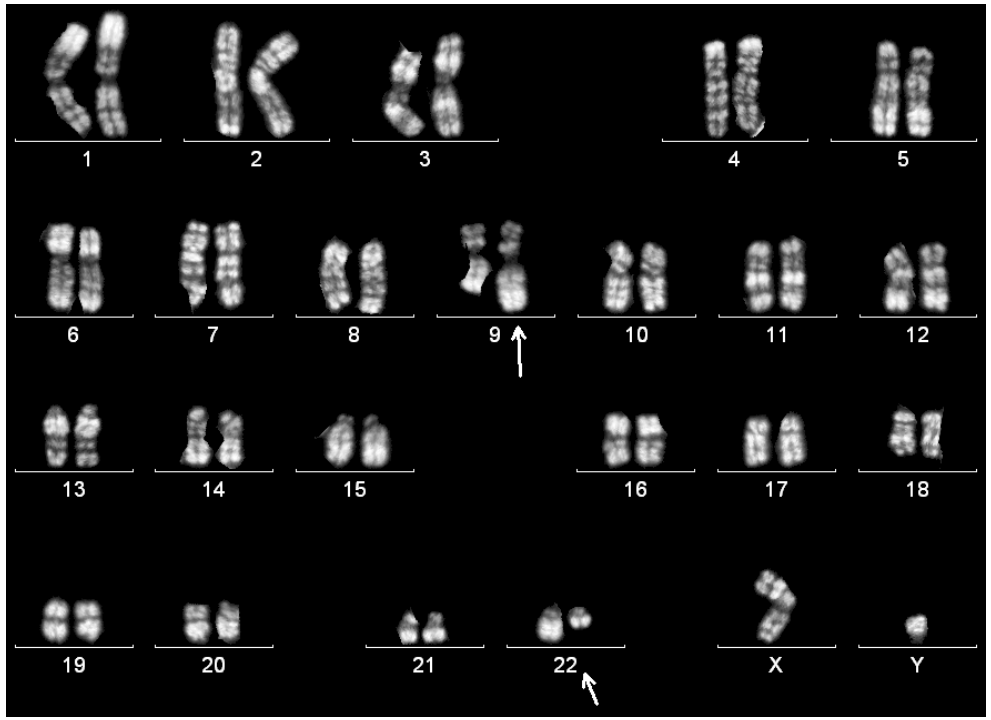


ERIC recommendations 2022

Baliakas et al. Hemasphere 2022

3.3 Cytogenetics: therapeutic value

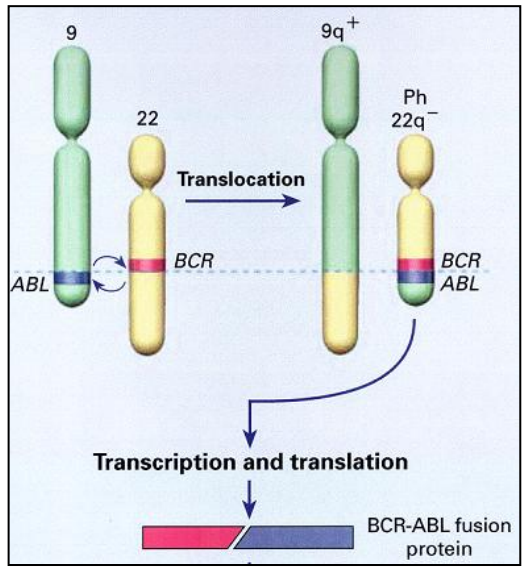
Chromosomal aberrations → **potential therapeutic target** / influence treatment choice



1960: Ph chromosome detection

1985: *BCR::ABL1* fusion protein

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



Constitutive activation of ABL1 TK leading to malignant transformation

3.3 Cytogenetics: therapeutic value

Chromosomal aberrations → potential therapeutic target / **influence treatment choice**

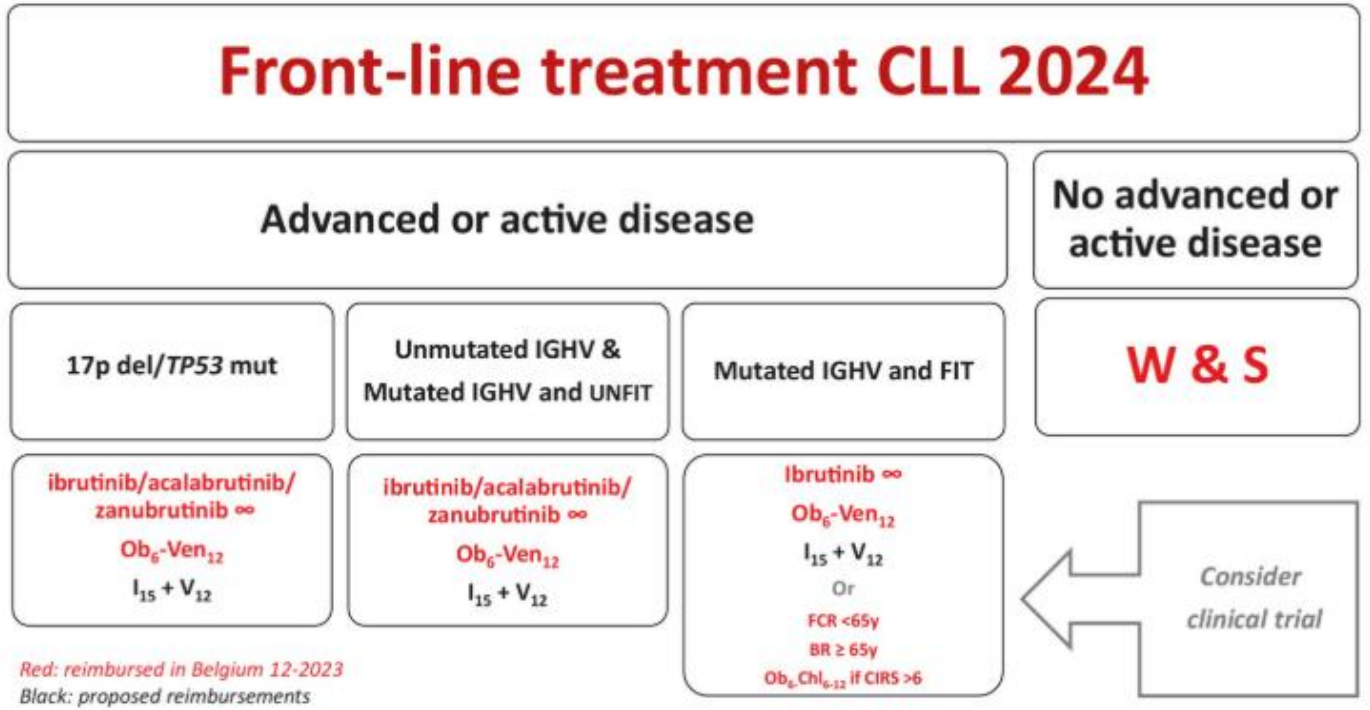
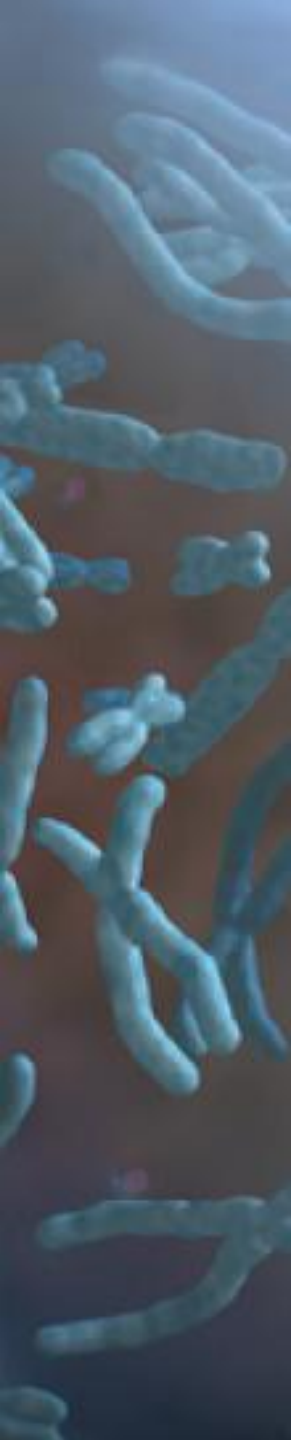


FIGURE 2. *Treatment algorithm TN CLL anno 2024 Belgium.*
B: bendamustine; Chl: chlorambucil; CIRS: cumulative illness rating scale; del: deletion; mut: mutation; I+V: ibrutinib + venetoclax; IGHV: immunoglobulin heavy chain variable region genes; R: rituximab; Ob: Obinutuzumab; Ven: venetoclax; W & S: waitand-see; ∞: continuously.

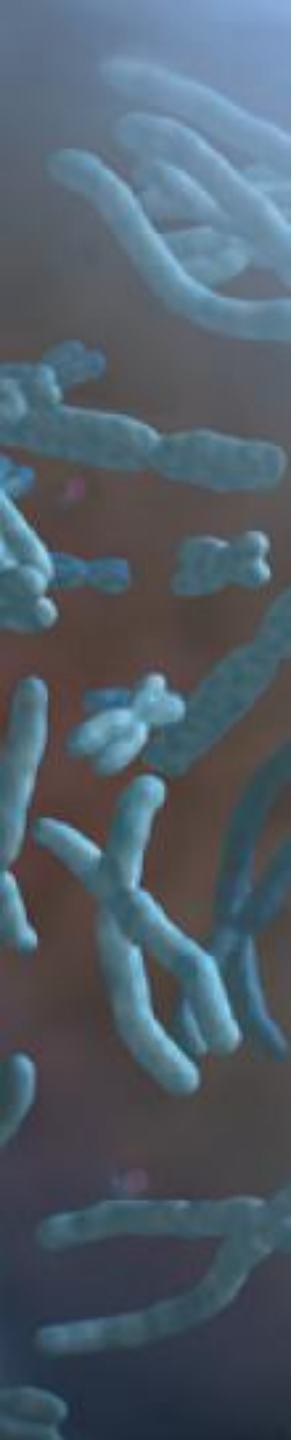
BHS guidelines, anno 2024. Janssens A *et al*, BJH 2023.



POLL - 3

Which of the following AML patients has (cytogenetically) the worst prognosis?

- 46,XX[20]
- 46,XX,del(5)(q13q31),add(8)(p22)[10]/46,XX[10]
- 47,XY,inv(16)(p13q22),+22[18]/46,XY[2]
- 45,XX,-10,del(20)(q11q13)[14]/46,XX[6]

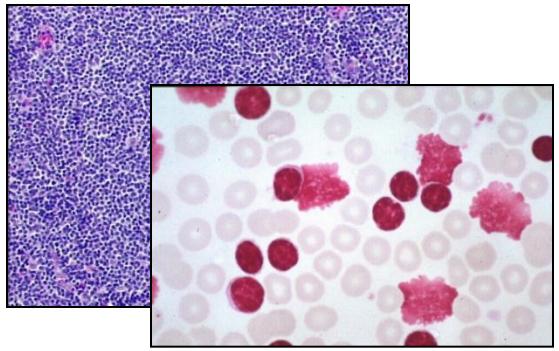


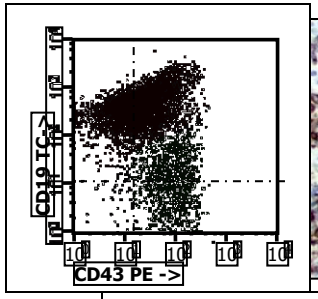
Take home messages

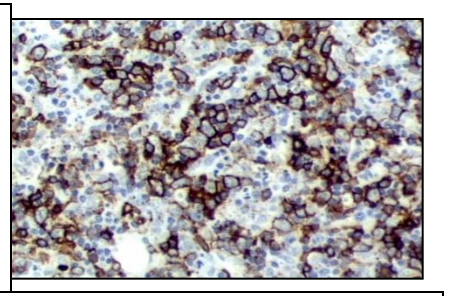
Cytogenetic analyses in hematological malignancies:

- Useful for diagnostic and prognostic purposes and mandatory in some disorders: CLL, AML, MDS,…
- Molecular cytogenetics: expanding but expensive tools

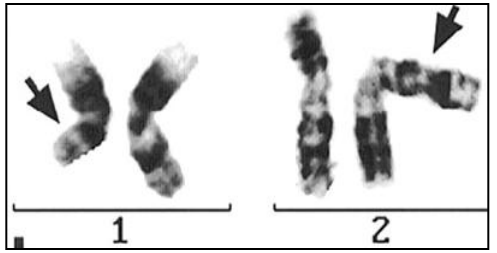
Cytogenetics
= part of **multidisciplinary** approach







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

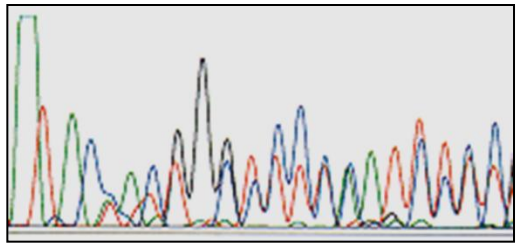


Cytogenetics

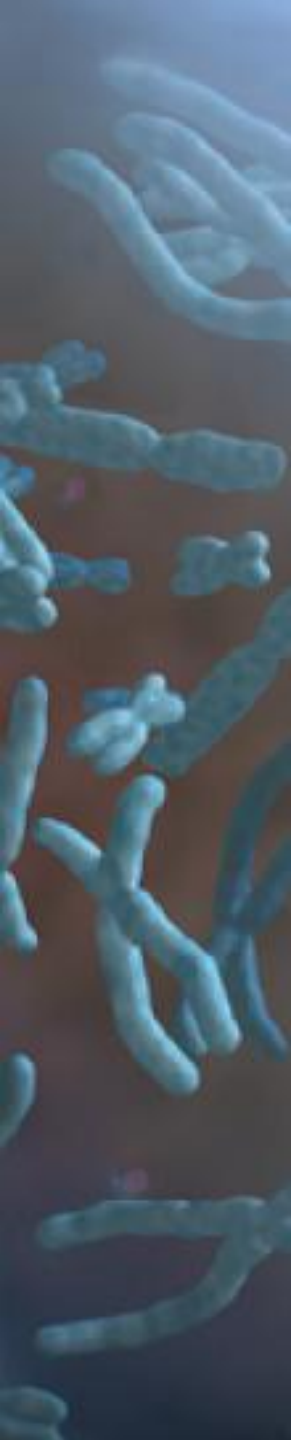
Clinics

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.

POLL - 1

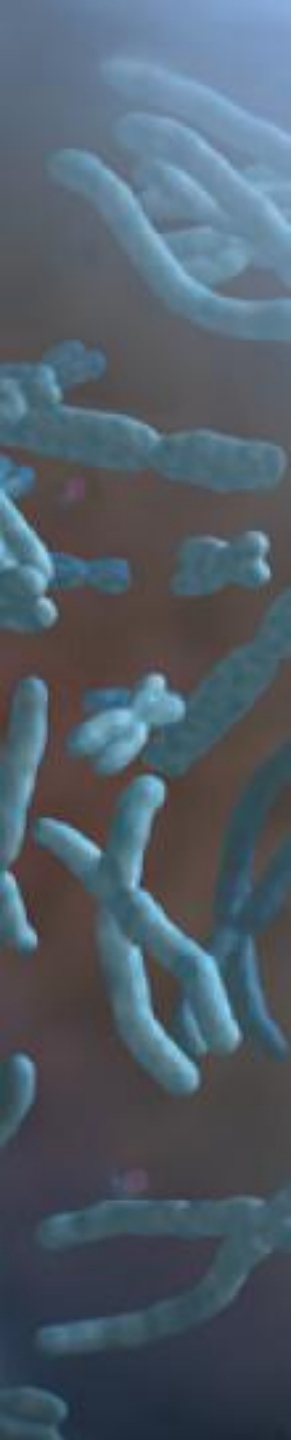
Acquired cytogenetics are used in the clinic to...

- establish a diagnosis of a hematopoietic malignancy
- determine the appropriate treatment strategy
- estimate prognosis
- provide a marker for follow-up of disease progression
- **all four options are true**

POLL - 2

Patient X is referred to the hospital for unexplained cytopenia. Bone marrow investigation reveals dysplasia in the myeloid lineage and megakaryocytes. Chromosome banding analysis reveals a deletion on the long arm of chromosome 5 in 6 mitoses and a monosomy 7 in another 2 mitoses. What is the correct karyotype annotation?

- 46,XY[12]/46,XY,del(5)(q13q31)[6]/45,XY,-7[2]
- 46,del(5)(q13q31),XY[6]/45,-7,XY[2]/46,XY[12]
- **46,XY,del(5)(q13q31)[6]/45,XY,-7[2]/46,XY[12]**
- 45,XY,-7[2]/46,XY,del(5)(q13q31)[6]/46,XY[12]
- 46,XY,del(5)(q13q31)[6]/46,XY,-7[2]/46,XY[12]



POLL - 3

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- **45,XX,-10,del(20)(q11q13)[14]/46,XX[6]**