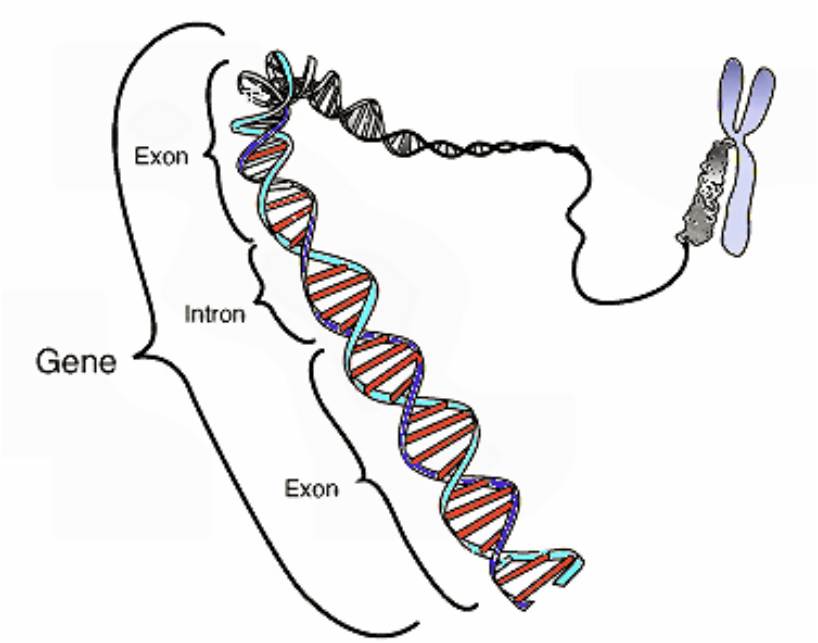
Molecular and genetic testing for leukemia

What are chromosomes?

- A chromosome is an organized structure of DNA and protein found in cells. It is a single piece of coiled DNA containing many **genes**, regulatory elements and other nucleotide sequences
- Chromosomes in humans can be divided into two types: autosomes and sex chromosomes
- Human cells have 23 pairs of chromosomes (22 pairs of autosomes and one pair of sex chromosomes), giving a total of **46 per cell**
- . Certain genetic traits are linked to a person's sex and are passed on through the sex chromosomes. The autosomes contain the rest of the genetic hereditary information

What are genes?

- Gene is the name given to some stretches of DNA and RNA that code for a polypeptide or for an RNA chain that has a function in the organism



This diagram shows a gene in relation to the double helix structure of DNA and to a chromosome (right). The chromosome is X-shaped because it is dividing. This diagram labels a region of only 50 or so bases as a gene. In reality, most genes are hundreds of times larger

What Is a locus?

- In genetics, a locus (plural loci) is the specific location of a gene or DNA sequence on a chromosome

Nomenclature

The chromosomal locus of a gene might be written "6p21.3".

Component	Explanation
6	The chromosome number.
p	The position is on the chromosome's short arm (*p* for *petit* in French); *q* indicates the long arm (chosen as next letter in alphabet after p).
21.3	The numbers that follow the letter represent the position on the arm: region 2, band 1, sub-band 3. The bands are visible under a microscope when the chromosome is suitably stained. Each of the bands is numbered, beginning with 1 for the band nearest the centromere. Sub-bands and sub-sub-bands are visible at higher resolution.

Nomenclature

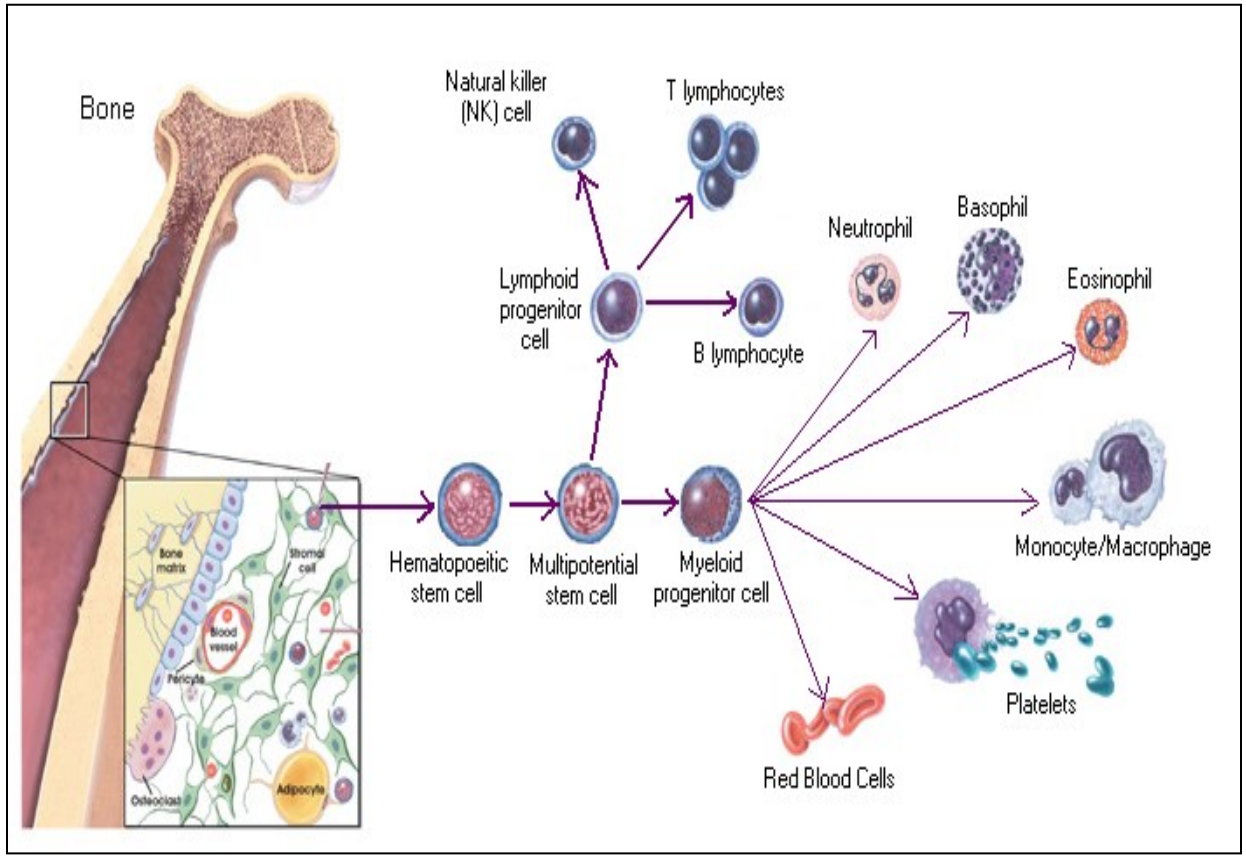
- A range of locales is specified in a similar way. For example, the locus of gene OCA1 may be written "11q1.4-q2.1", meaning it is on the long arm of chromosome 11, somewhere in the range from sub-band 4 of band 1, and sub-band 1 of band 2.
- The ends of a chromosome are labeled *"pter"* and *"qter"*, and so *"2qter"* refers to the telomere of the long arm of chromosome 2.

What is karyotyping?

- A karyotype is the number and appearance of chromosomes in the nucleus
- Karyotypes describe the number of chromosomes, and what they look like under a light microscope. Attention is paid to their length, the position of the centromeres, banding pattern, any differences between the sex chromosomes, and any other physical characteristics
- The preparation and study of karyotypes is part of **cytogenetics**

What is leukemia?

- Leukemia is cancer of the body's blood-forming tissues, including the bone marrow and the lymphatic system.
- Many types of leukemia exist. Some forms of leukemia are more common in children. Other forms of leukemia occur mostly in adults
- Leukemia usually starts in the white blood cells. In people with leukemia, the bone marrow produces abnormal white blood cells, which don't function properly

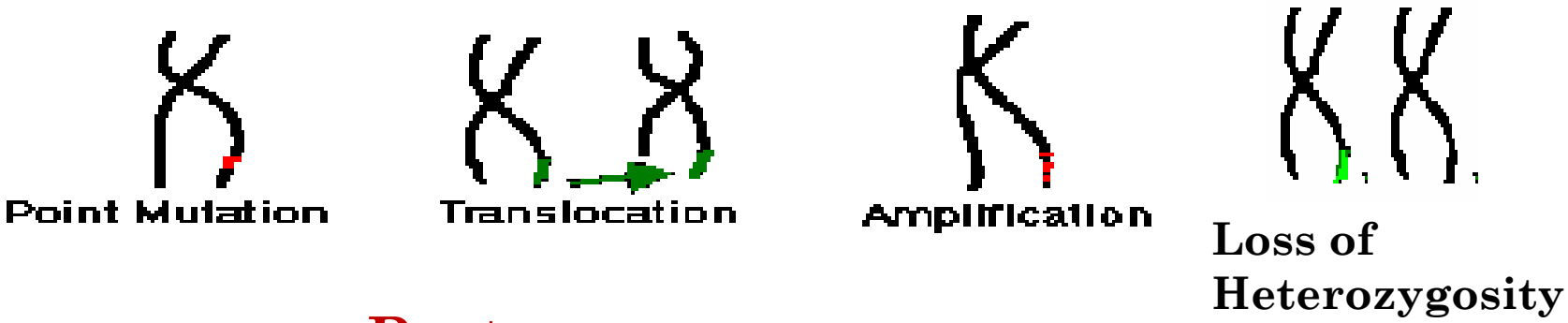


Haematological Malignancies

- CML
- AML
- MDS
- ALL
- CLL
- HES
- MM

CML	Chronic Myelogenous Leukemia
AML	Acute Myeloid Leukemia
MDS	Myelodysplastic Syndrome
ALL	Acute Lymphocytic Leukemia
CLL	Chronic Lymnphocytic Leukemia
HES	Hyper Eosinophilic Syndrome
MM	Multiple Myeloma

Different types of gene alterations in cancer



Proto oncogenes are identified by gain of function.

Cell proliferation ie. function as growth factors, growth factor receptors, regulators of replication & transcription & signaling

Tumor suppressor genes are identified by loss of function.

Key regulators of cell proliferation, differentiation, and development.

Role of genetic testing in cancers

Targeted Therapy.

Markers for MRD

Response to treatment

Disease Progression

Prognosis

Classification

Diagnosis

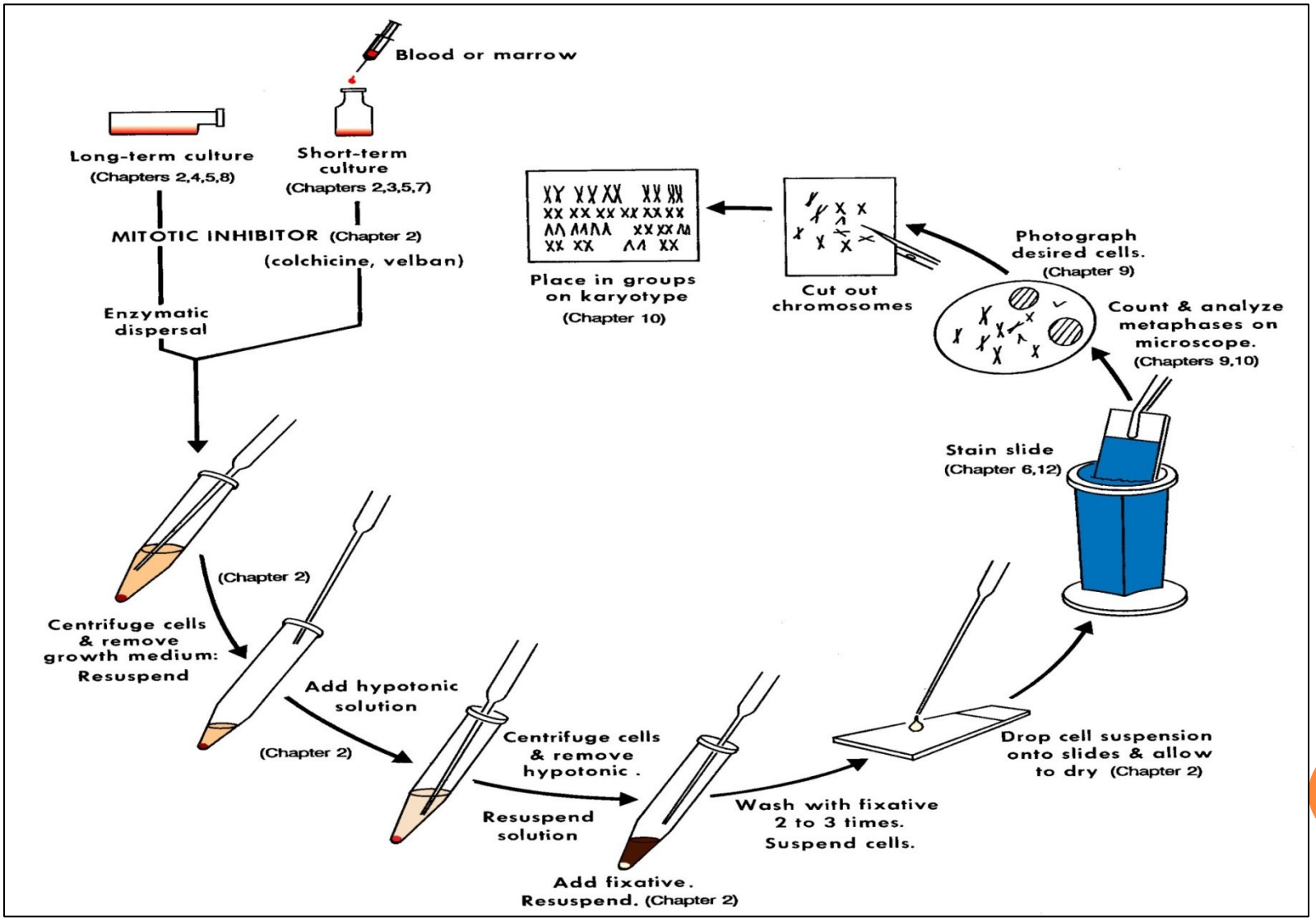
Fast Comprehensive.

Molecular characterization of cancers

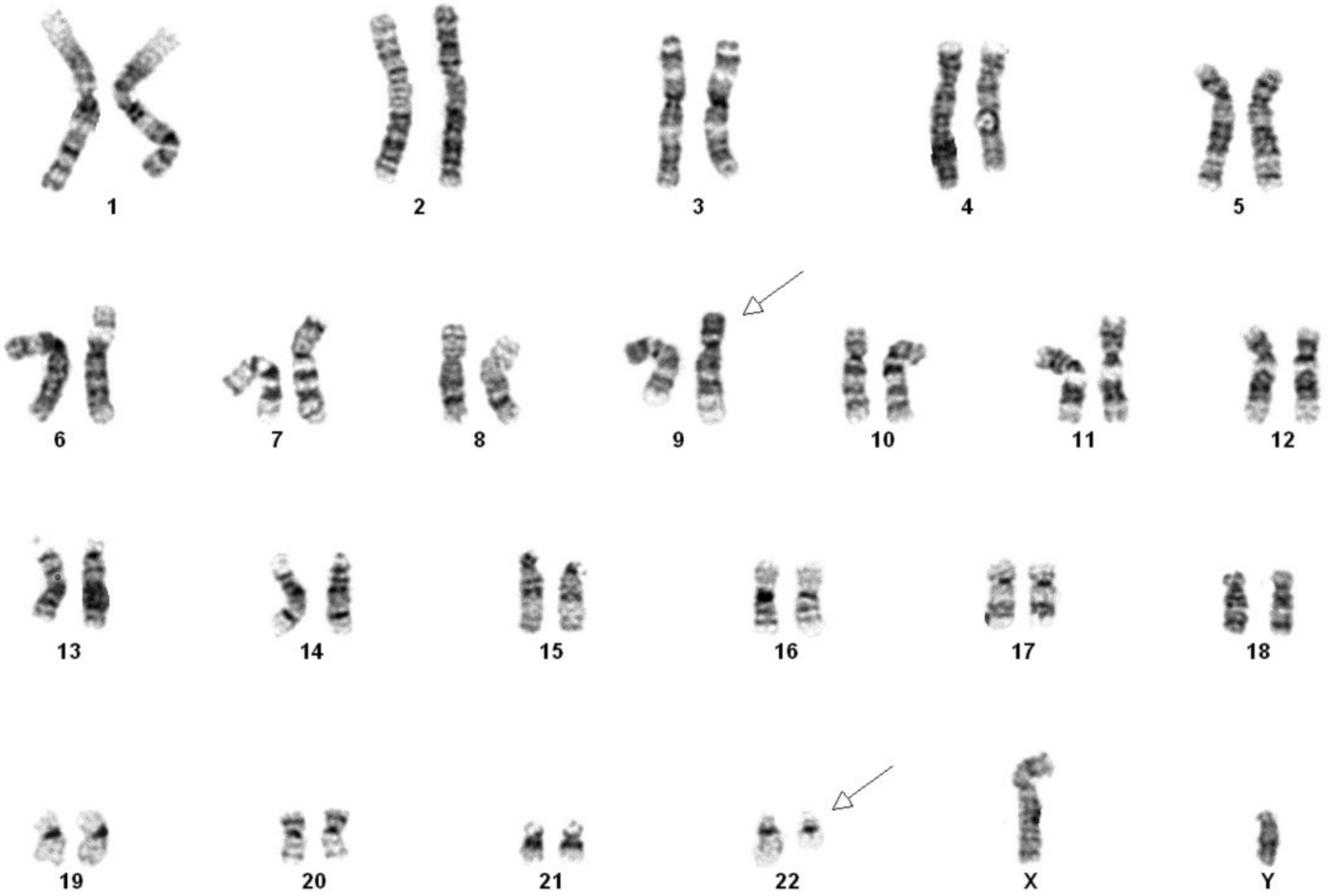
We will discuss two methods:

- Conventional Karyotyping
- Fluorescence In-situ Hybridization

Conventional karyotyping



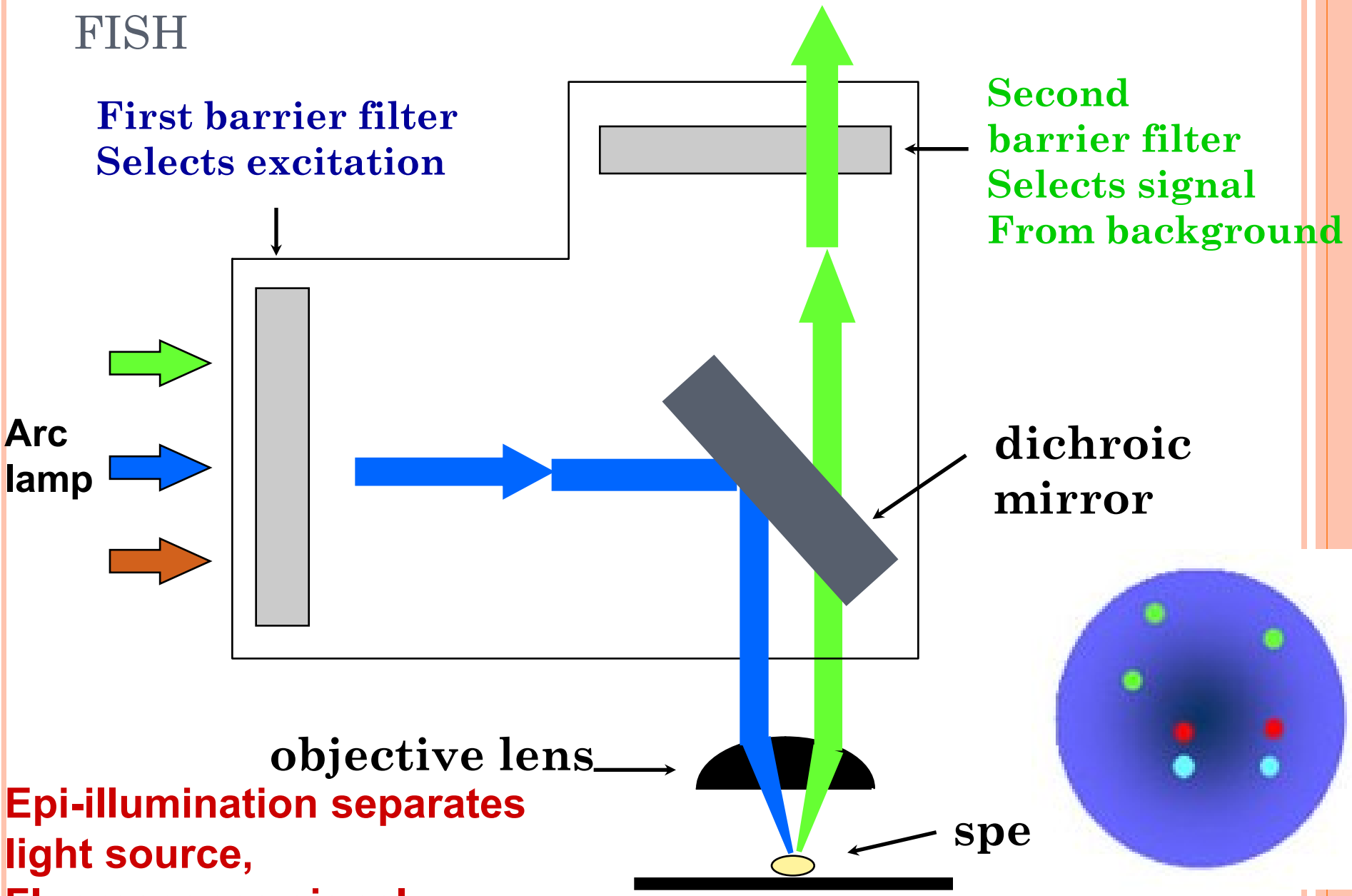
Example of conventional karyotype (c/o CML)



Limitations of conventional karyotyping

- Suboptimal chromosome morphology
- Lack of dividing neoplastic cells
- Preferential growth of normal cells in culture.

Fluorescence in-situ hybridization - FISH



Fluorescence in-situ hybridization - FISH

Advantages

- It has a rapid turnaround time,
- Detects small numbers of abnormal cells
- Performed on non-dividing (interphase) cells.
- FISH can detect cryptic or subtle rearrangements that might be difficult to detect by routine karyotyping.

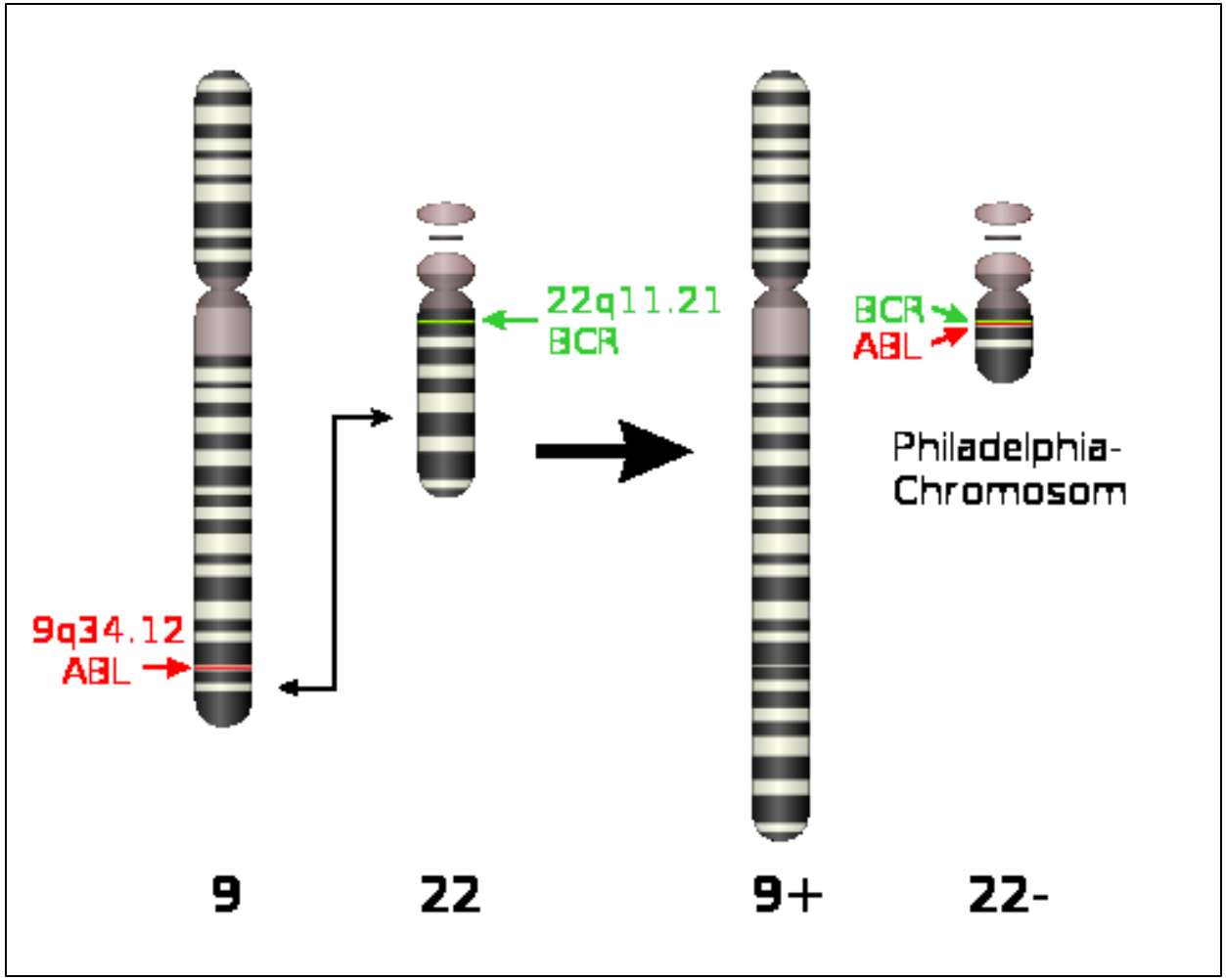
Chronic Myelogenous Leukemia

A form of leukemia characterized by the increased and unregulated growth of predominantly myeloid cells in the bone marrow and the accumulation of these cells in the blood. CML is a clonal bone marrow stem cell disorder in which proliferation of mature granulocytes (neutrophils, eosinophils, and basophils) and their precursors is the main finding

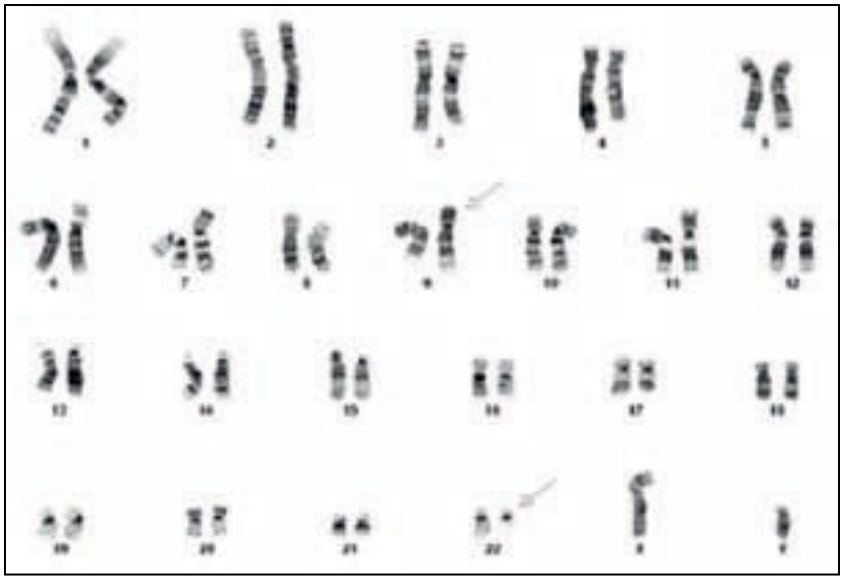
Philadelphia chromosome

- Philadelphia chromosome is the name given to a genetic alteration in which:
  - There is a reciprocal translocation between chromosomes 9 and 22
  - Is depicted as **t(9;22)(q34;q11.2)**
  - A fusion gene is created by juxtapositioning the ABL1 gene on chromosome 9 (region q34) to a part of the BCR ("breakpoint cluster region") gene on chromosome 22 (region q11)
  - Creating an elongated chromosome 9 (*der 9*), and a truncated chromosome 22 (*the Philadelphia chromosome)*
  - The oncogenic **BCR-ABL** gene fusion is located on the shorter derivative 22 chromosome

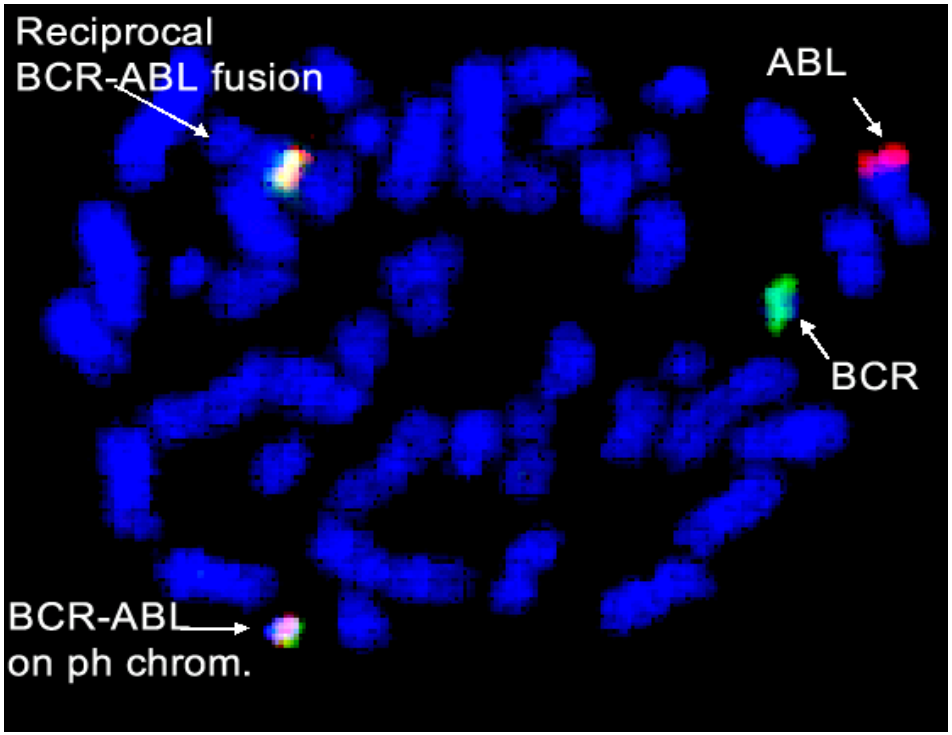
Schematic diagram of BCR/ABL fusion

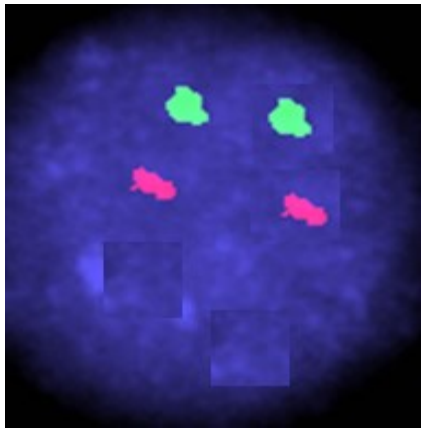


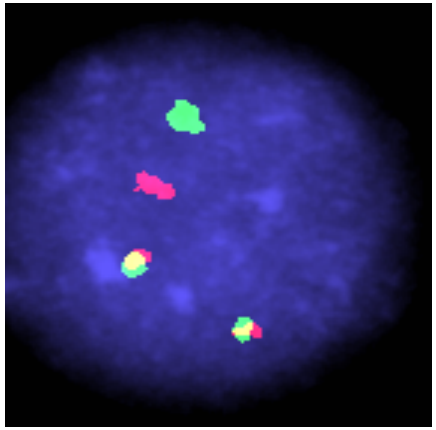
Conventional karyotype in CML



FISH IN CML







Clinical significance

- The presence of this translocation is a highly sensitive test for CML, since **95% of people with CML** have this abnormality
- However, the presence of the Philadelphia (Ph) chromosome is not sufficiently specific to diagnose CML, since it is also found in acute lymphoblastic leukemia (ALL, 25–30% in adult and 2–10% in pediatric cases) and occasionally in acute myelogenous leukemia (AML)
- **Imatinib (Glivec by Novartis)** is a BCR-ABL tyrosine kinase inhibitor that inhibits proliferation of BCR-ABL-expressing hematopoietic cells

Myelo-proliferative disorders (MPD)

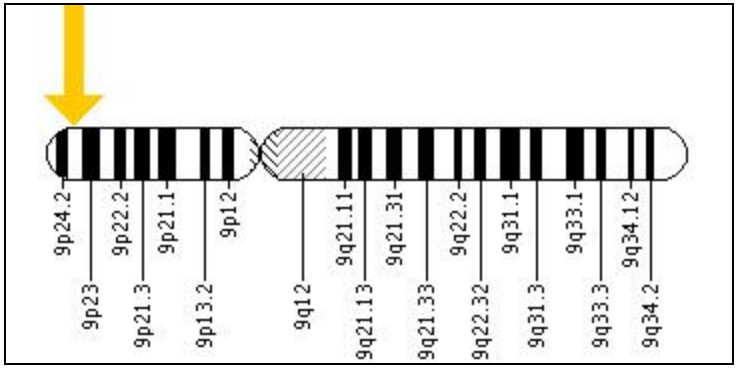
CLASSIFICATION

- The myeloproliferative diseases (MPDs) or myeloproliferative neoplasms (MPNs) are a group of diseases of the bone marrow in which excess cells are produced
- There are four main myeloproliferative diseases, which can be further categorized by the presence of the Philadelphia chromosome

Philadelphia Chromosome "positive"	Philadelphia Chromosome "negative"
•Chronic myelogenous leukemia (CML)	•Polycythemia vera (PV) •Essential thrombocytosis (ET) •Myelofibrosis (MF)

JAK2 MUTATION

- Stands for ‘Janus Kinase 2’
- The *JAK2* gene provides instructions for making a protein that promotes the growth and division (proliferation) of cells
- The JAK2 protein is especially important for controlling the production of blood cells from hematopoietic stem cells
- The JAK2 gene is located on the short (p) arm of chromosome 9 at position 24



Clinical significance of JAK2

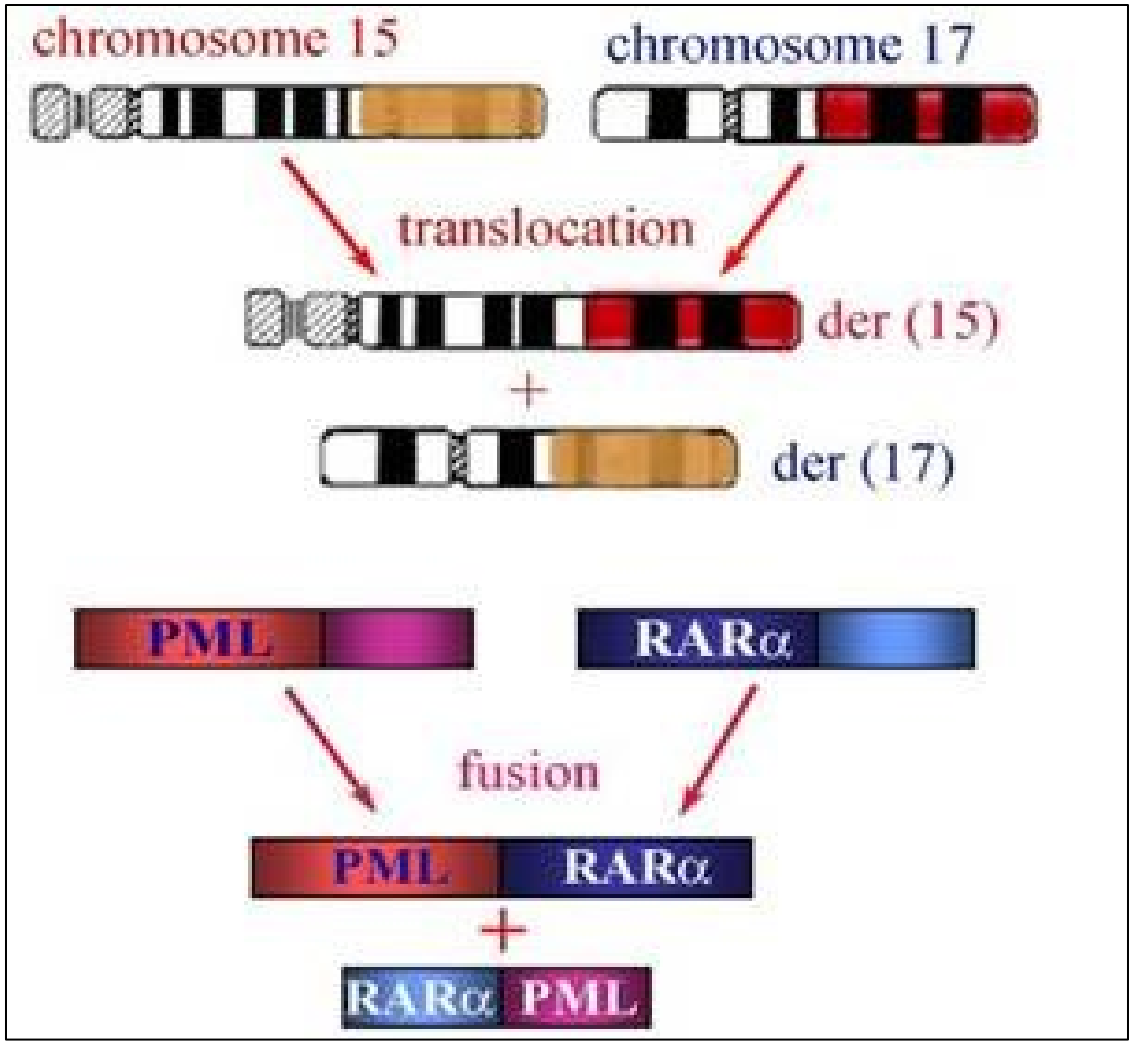
- The most common mutation (written as Val617Phe or V617F) replaces the protein building block (amino acid) valine with the amino acid phenylalanine at position 617 in the protein
- The V617F mutation is found in approximately 96 percent of people with polycythemia vera
- *JAK2* gene mutations result in the production of a constitutively activated JAK2 protein, which seems to improve the survival of the cell and increase production of blood cells

Acute myeloid leukemia (aml)

PML/RARA

- Acute Promyelocytic Leukemia (APL) is an aggressive subtype of AML with distinct morphology and clinical presentation
- APL is characterized by the reciprocal translocation t(15;17)(q22;q21) resulting in fusion of PML and RARA genes
- Based on the presence of RARA rearrangement chemotherapy has been adopted resulting in complete remission in 80% of the cases

PML/RARA


chromosome 15
chromosome 17
translocation
der (15)
+
der (17)
PML
RARα
fusion
PML
RARα
+
RARα
PML

Breast cancer

HER-2 NEU

- HER2 is encoded by ERBB2, a known proto-oncogene located at the long arm of human chromosome 17 (17q12)
- HER2 (Human Epidermal Growth Factor Receptor 2) also known as Neu, ErbB-2, is a protein that in humans is encoded by the ERBB2 gene. HER2 is a member of the epidermal growth factor receptor (EGFR/ErbB) family
- **Amplification or over-expression** of this gene has been shown to play an important role in the pathogenesis and progression of certain aggressive types of breast cancer

Clinical significance

HER-2 amplification associated with

- Invasive breast cancers
- Aggressive tumor
- Reduced survival rates

HER-2 INTERPRETATION