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".

<table>
<thead>
<tr>
<th>Component</th>
<th>Explanation</th>
</tr>
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<tbody>
<tr>
<td>6</td>
<td>The chromosome number.</td>
</tr>
<tr>
<td>p</td>
<td>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).</td>
</tr>
<tr>
<td>21.3</td>
<td>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.</td>
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</table>
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

- AML
- MDS
- ALL
- CLL
- HES
- MM
- CML
<table>
<thead>
<tr>
<th>Acronym</th>
<th>Description</th>
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<tbody>
<tr>
<td>CML</td>
<td>Chronic Myelogenous Leukemia</td>
</tr>
<tr>
<td>AML</td>
<td>Acute Myeloid Leukemia</td>
</tr>
<tr>
<td>MDS</td>
<td>Myelodysplastic Syndrome</td>
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</tr>
<tr>
<td>HES</td>
<td>Hyper Eosinophilic Syndrome</td>
</tr>
<tr>
<td>MM</td>
<td>Multiple Myeloma</td>
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</table>
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.
- Fast Comprehensive.
- Markers for MRD
- Response to treatment
- Disease Progression
- Prognosis
- Classification
- Diagnosis
MOLECULAR CHARACTERIZATION OF CANCERS

We will discuss two methods:

- Conventional Karyotyping
- Fluorescence In-situ Hybridization
CONVENTIONAL KARYOTYPING

1. **Blood or marrow**
2. **Long-term culture** (Chapters 2, 4, 5, 6)
   - **MITOTIC INHIBITOR** (Chapter 2) (colchicine, velban)
   - **Enzymatic dispersal**
3. **Short-term culture** (Chapters 2, 3, 5, 7)
4. **Place in groups on karyotype** (Chapter 10)
5. **Cut out chromosomes**
6. **Photograph desired cells** (Chapter 9)
   - **Count & analyze metaphases on microscope** (Chapters 9, 10)
7. **Stain slide** (Chapter 6, 12)
8. **Drop cell suspension onto slides & allow to dry** (Chapter 2)
9. **Add fixative, resuspend** (Chapter 2)
10. **Wash with fixative 2 to 3 times. Resuspend cells**
11. **Add hypotonic solution**
12. **Centrifuge cells & remove hypotonic**
13. **Resuspend solution**
14. **Centrifuge cells & remove growth medium:**
    - **Resuspend**
15. **(Chapter 2)**
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**

First barrier filter
Selects excitation

Second barrier filter
Selects signal From background

Arc lamp

**objective lens**

Epi-illumination separates light source, Fluorescence signal

dichroic mirror

**spe**
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 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**

- Reciprocal BCR-ABL fusion
- BCR-ABL on ph chrom.
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)
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:

<table>
<thead>
<tr>
<th>Philadelphia Chromosome &quot;positive&quot;</th>
<th>Philadelphia Chromosome &quot;negative&quot;</th>
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<tbody>
<tr>
<td><strong>Chronic myelogenous leukemia</strong> (CML)</td>
<td><strong>Polycythemia vera</strong> (PV)</td>
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<tr>
<td></td>
<td><strong>Essential thrombocytosis</strong> (ET)</td>
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<td></td>
<td><strong>Myelofibrosis</strong> (MF)</td>
</tr>
</tbody>
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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

Diagram showing a chromosomal translocation involving chromosomes 15 and 17. The translocation results in the formation of a derivative chromosome (der(15)) and (der(17)). Following the translocation, a fusion event occurs between PML and RARα genes.
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**

- **Her2 testing IHC**
  - **0 or 1+**
    - Her-2 negative trastuzumab therapy not indicated
  - **2+**
    - Reflex to FISH recommended
  - **3+**
    - Her2 positive Eligibility for trastuzumab therapy

- **FISH**
  - **< 1.8**
  - **1.8-2.2**
  - **> 2.2**