



Leukaemia and its classification.

Historical perspective - future work

Historical Review

- White blood cells were first reported around 1750 and lymphocytes described in 1780.
- The first reported work in relationship to leukaemia was reported by **Velpeau in 1827** following autopsy were the patient was reported to have a large spleen and the blood was thick.
- **Bennett** a Scottish pathologist in 1845 reported the first case of what would have been CGL.
- 6 Weeks later **Virchow** described a case at autopsy that would be CLL.
- The term leukaemia was first coined by **Virchow in 1847** meaning white blood
- He identified that the excess white cell were not due to an infection but rather by the tissue that produces the white blood cells.
- In **1857 Nikolaus Friedreich** he first described acute leukaemia
- In 1868 it was seen that changes in the bone marrow was established as the link between the source of blood and bone marrow.
- **Neumann** stated in 1872 that leukaemia was a disease of the bone marrow.

Paul Ehrlich developed a stain for blood cells in 1877
30 years after leukaemia was first described.



Bennett 1812 - 1875



Virchow 1821 - 1902

Treatment

- Lissauer in 1865 used 1% Arsenic Trioxide in the treatment of leukaemia.
- In 1895 X-rays brought a new treatment for leukaemia.
- Blood transfusions were used in the treatment of leukaemia in 1873 and they stated that
- 'only human blood should be used'

- Karl Landsteiner discovered the ABO blood groups in 1900

- The treatment of cancers and leukaemia remained very much incurable and unscientific until after World War Two- late 1940's

1960 - 1970

- The haemopoietic stem cell was discovered in 1960.
- During the 60's major developments occurred in chemotherapy and radiotherapy and the idea of combination chemotherapy was born.
- Cytogenetic analysis was used as a tool to identify remission. This was the beginning of new biological understanding of leukaemia.
- During this period was the development in standardisation of microscopic morphology, cytochemistry and its development into immunocytochemistry and cytogenetics.
- The phase contrast microscope and the electron microscope in 1950 and 60's had had little impact on developments in our understanding of leukaemia.
- The discovery of the Philadelphia chromosome as a reciprocal translocation between 2 chromosomes in 1973 had a major impact on the future drive in the biology of leukaemia.
- The 1970 also introduced other translocations t(8;14) Burkitts and t(15;17) in AML

1980 - 1990

- The developments of and in molecular biology in the 1980 lead to the development of individual probes for chromosomes and fluorochromes which saw the development of FISH.
- Comparative genomic hybridisation CGH allowed the identification of gain or loss of DNA.
- Molecular biology continued to progress in the 1990 up to the present time with the development of PCR , nucleoside sequencing, discovery of restriction endonucleases and recombinant DNA production.
- From this technology oncogenes were identified, cell regulatory and apoptosis genes and proteins that influence the cell were identified.
- DNA chip analysis and proteomics continue to developing.

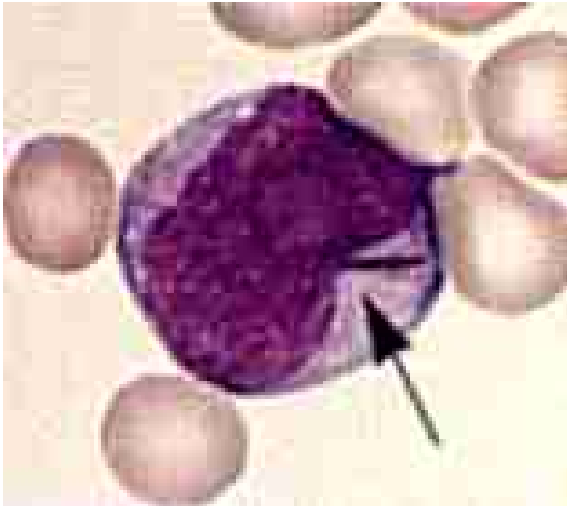
The need for change.

- In 1975 the FAB group was established with the aim to regularly review the morphology of the acute leukaemia's.
- The classification was based on morphology, cellularity, blast numbers and cytochemistry.
- With the continued development in the technology the FAB classification was not able to accommodate the new findings at the molecular level, both developments in flow cytometry and oncogene identification.
- The present WHO classification was presented in 1997/2001 it differs from the FAB classification in that it takes into account all the information not only morphology

WHO Classification of AML

- 1. Acute myeloid leukaemia with recurrent genetic abnormalities
 - About 30% of patients will have genetic abnormalities
- 2. Acute myeloid leukaemia with multilineage dysplasia.
 - Morphological evidence of multilineage dysplasia, at least cells of 2 or more myeloid lineages.
- 3. Acute myeloid leukaemia and myelodysplastic syndromes which is therapy related
 - Disorder usually appears 4 – 7 years after exposure to the mutagenic agent
- 4. All others with AML
 - This contains those that do not satisfy the other criteria.

AML: Immunophenotype



CD34

CD13

CD33

Myeloperoxidase

WHO classification of ALL

B cell neoplasms

- Precursors B cell neoplasm - B cell lymphoblastic leukaemia
- and mature peripheral B cell neoplasms – B cell CLL
- Burkitt cell leukaemia, Plasma cell Myeloma.

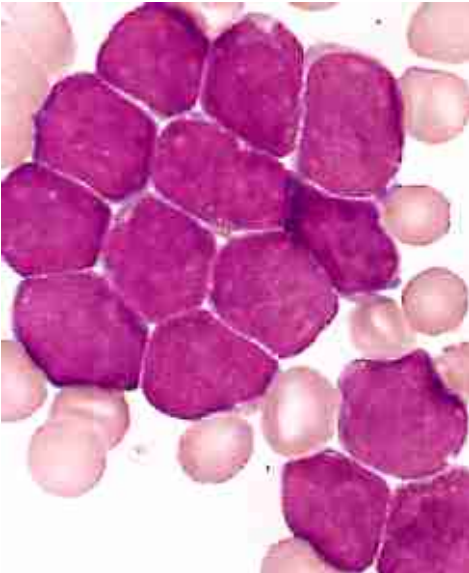
- T and NK cell neoplasms

 - T lymphoblastic leukaemia

 - Mature T and NK cell neoplasms T cell CLL, T cell granular CLL.

- Hodgkins lymphoma

Acute lymphoblastic leukaemia



Immunophenotype:

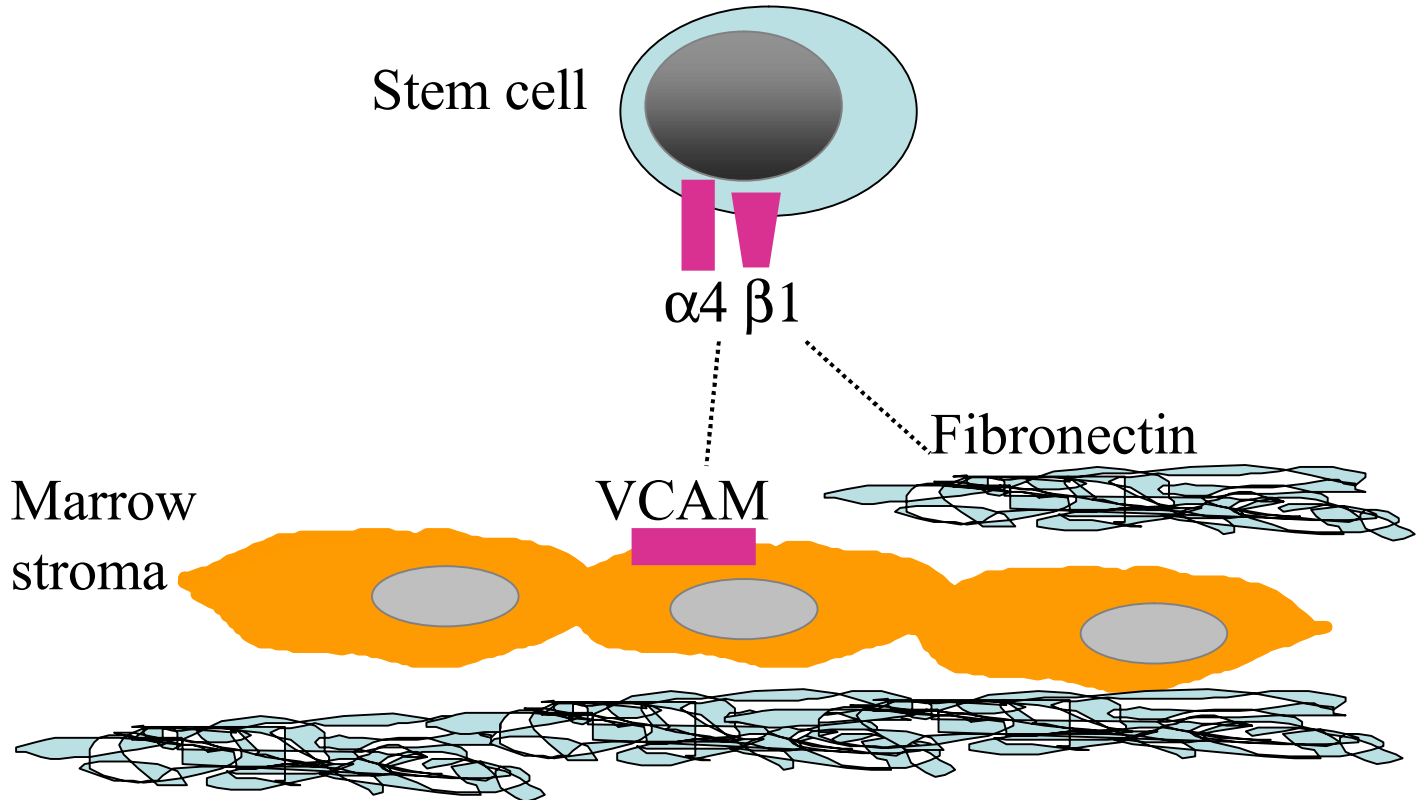
CD10	“Common” Ag
CD19	B-cell marker
CD34	Stem cell Ag
TdT	Nuclear enzyme

Cytoplasmic CD3 and surface CD3
HLA - DR

Production of normal cells – what goes wrong??

- Cellular production is a tightly controlled process.
- This involves strict regulation of stem cell renewal, lineage commitment and differentiation.
- This is achieved by a number of polypeptide growth factors, cytokines.
- These growth factors activate receptors expressed on the cell membrane by binding to them.
- The binding results in signal transduction, causing activation of nuclear transcription factors, this effects gene expression and consequently cell survival, proliferation and differentiation.
- Disruption of any one of these processes to the stem cell pool results in a haematological malignancy.

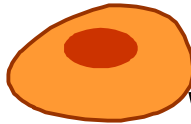
Stem cell niches in marrow



Stem cell hierarchy



**Totipotent stem cells
(fertilised egg)**



Pluripotent stem cells



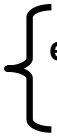
**Multipotent stem cells
(adults and embryo)**



**Haematopoietic
stem cells**

**Stem cells for nerves,
skin, muscle, etc**

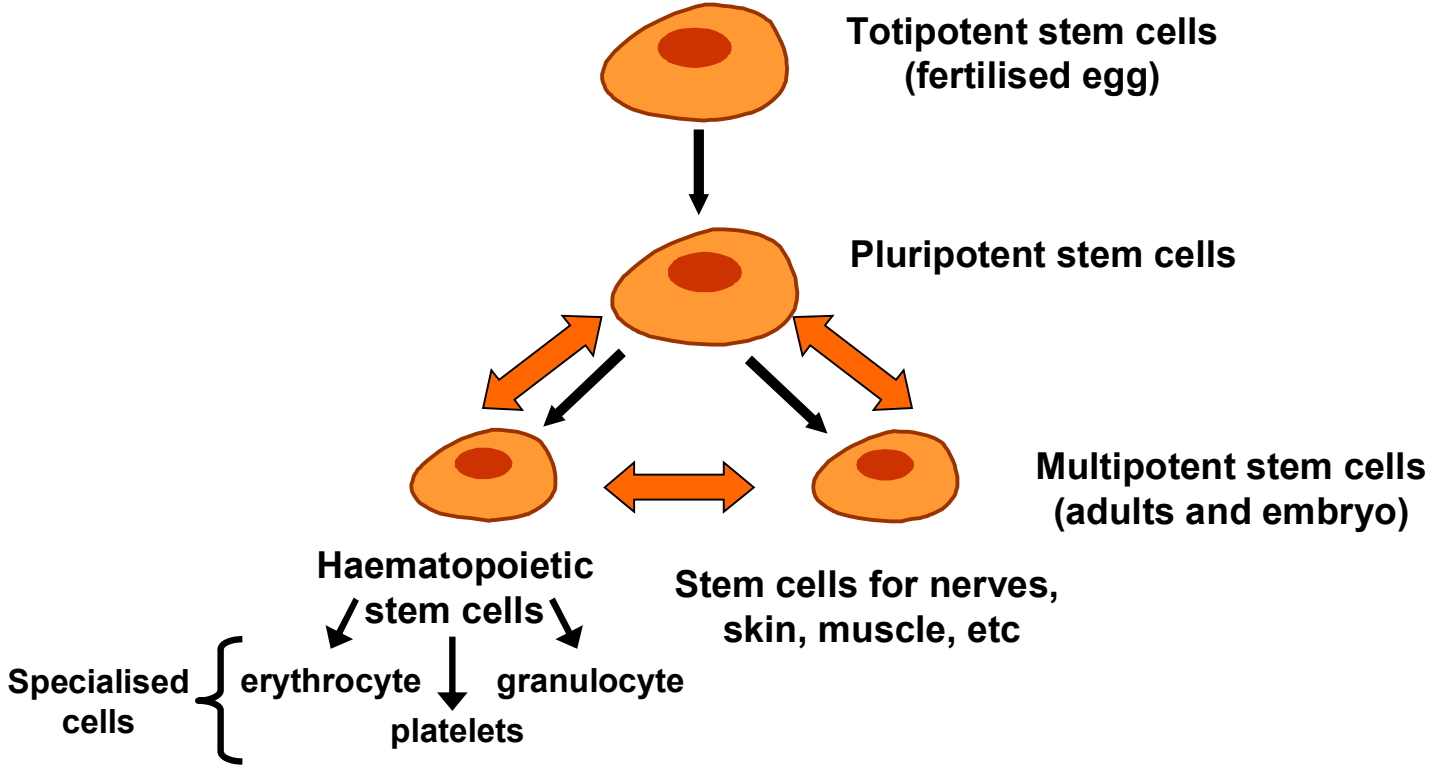
**Specialised
cells**



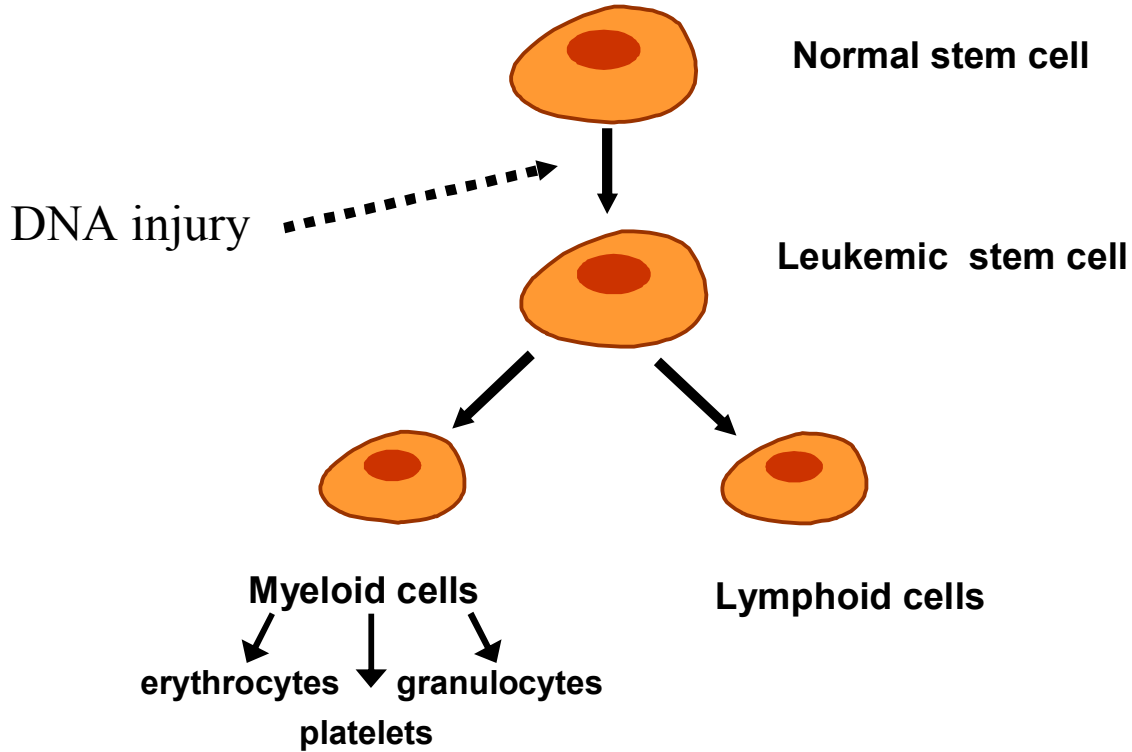
erythrocyte

platelets

granulocyte



Leukaemogenesis



Leukemia: Aetiology

- Unknown

- Chemicals/toxins

- Radiation

- Viruses

Secondary

Possible Pathogenic Processes, why it needs to be taken on board.

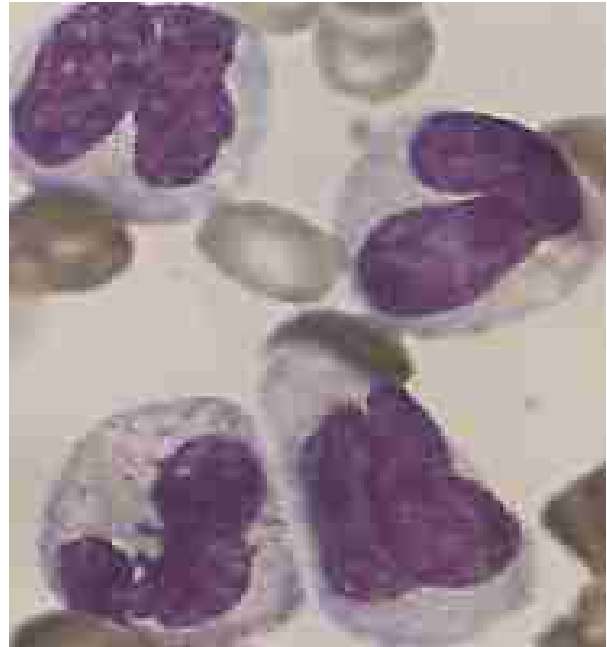
- Genes involved in the pathogenesis of leukaemia are thought to act by two general mechanisms.
- The first involves the generation of a novel gene, the oncogene. The protein product of the oncogene is normally involved in cellular control ie abnormal proliferation, differentiation and survival and induces the malignant characteristics to the cell.
- The second mechanism involves the inactivation of the tumour suppressor genes.
- Following years of research it has been identified that these changes have not developed as a change in one gene but due to a change in a number of genes, thus it is referred to as the 'multi hit theory'
- The cells that develop from the originally abnormal gene acquire additional mutations and eventually develops selective advantage so the abnormal clone develops and expands with the progressive genetic changes, clinical systems of the disorder become apparent.

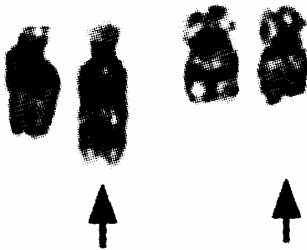
- What have we learned from the genetic changes that have occurred to the cell???

Acute promyelocytic leukemia t(15;17) M3

- Specific morphological variant
- Highly responsive to chemotherapy
combined with retinoic acid

Acute promyelocytic leukemia:





t(15;17)

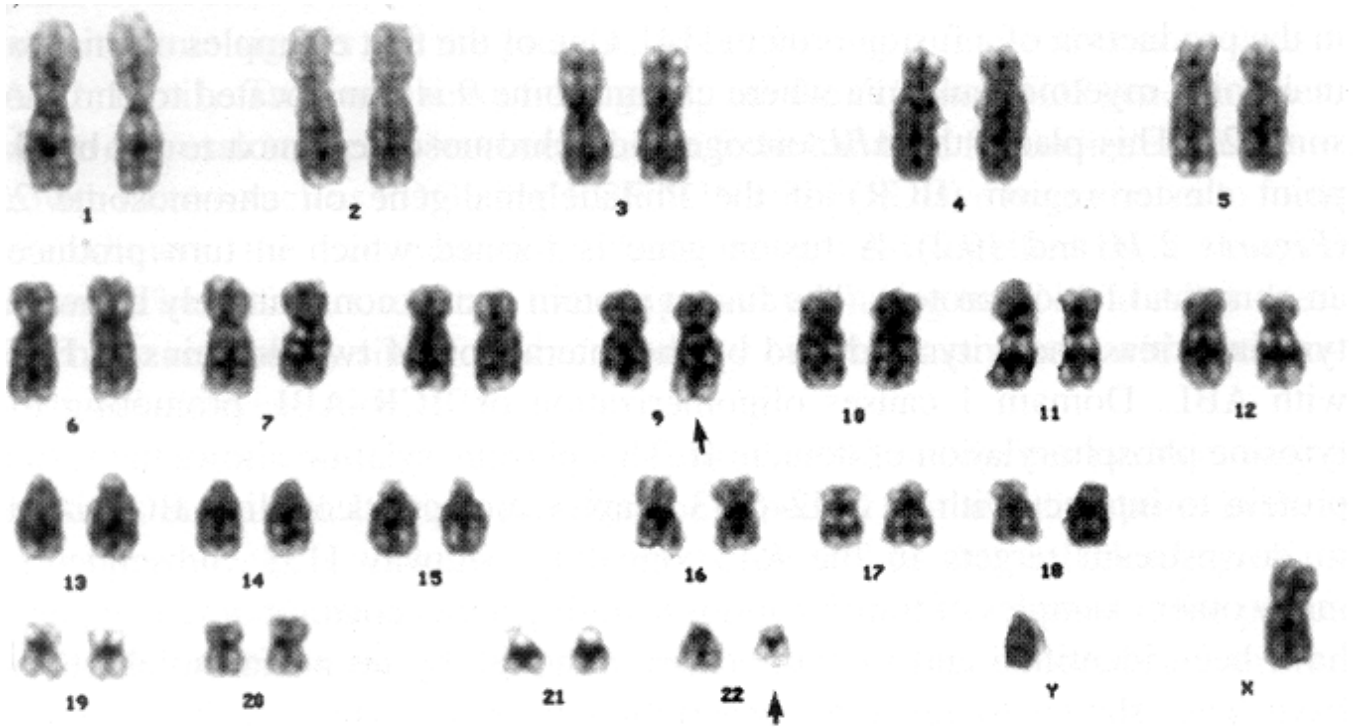
What does the translocation mean??

- It results in the rearrangement of the retinoic acid receptor alpha (RAR α), which is located on chromosome 17 and is fused to the PML gene on chromosome 15.
- PML gene is normally transcribed in haemopoietic cells
- The chimeric gene plays a role in the differentiation block that occurs in M3
- The treatment of M3 with ATRA is very successful.
- ATRA converts the PML-RAR α from a transcriptional repressor to a transcriptional activator, thus inducing terminal differentiation

What is the effect of the T(8;14) Burkitt Translocation mean.

- The recombination of *Ig* genes with c-MyC upregulates c-MyC expression.
- C-Myc is a nuclear transcription factor involved in cell proliferation and control of apoptosis.
- This results in uncontrolled expansion of the malignant B cell clone.

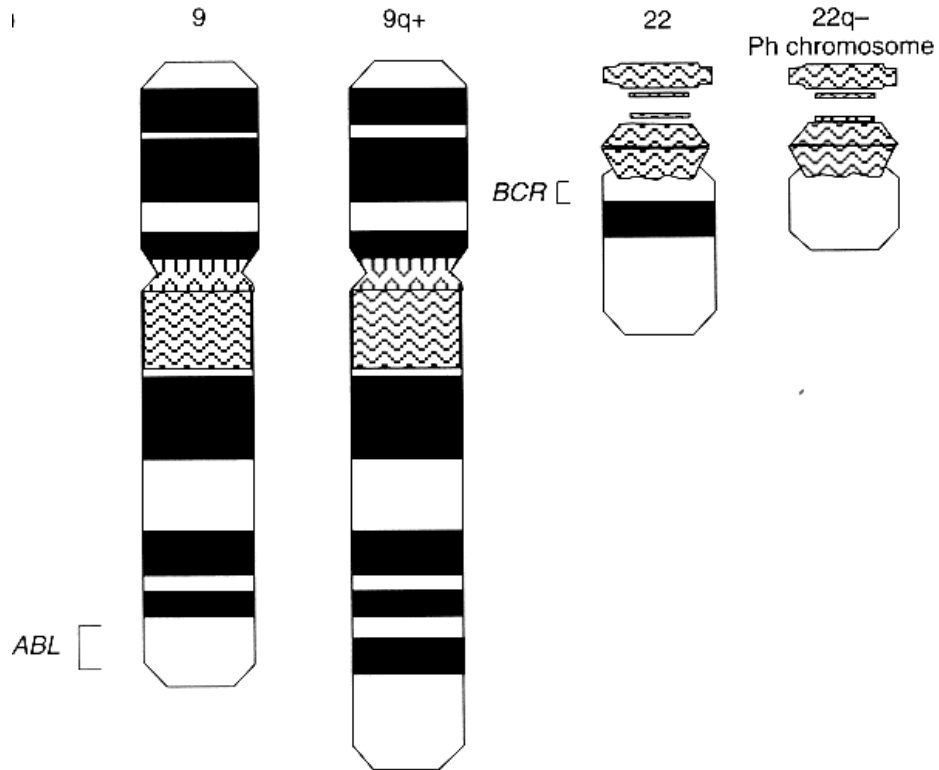
Philadelphia Chromosome

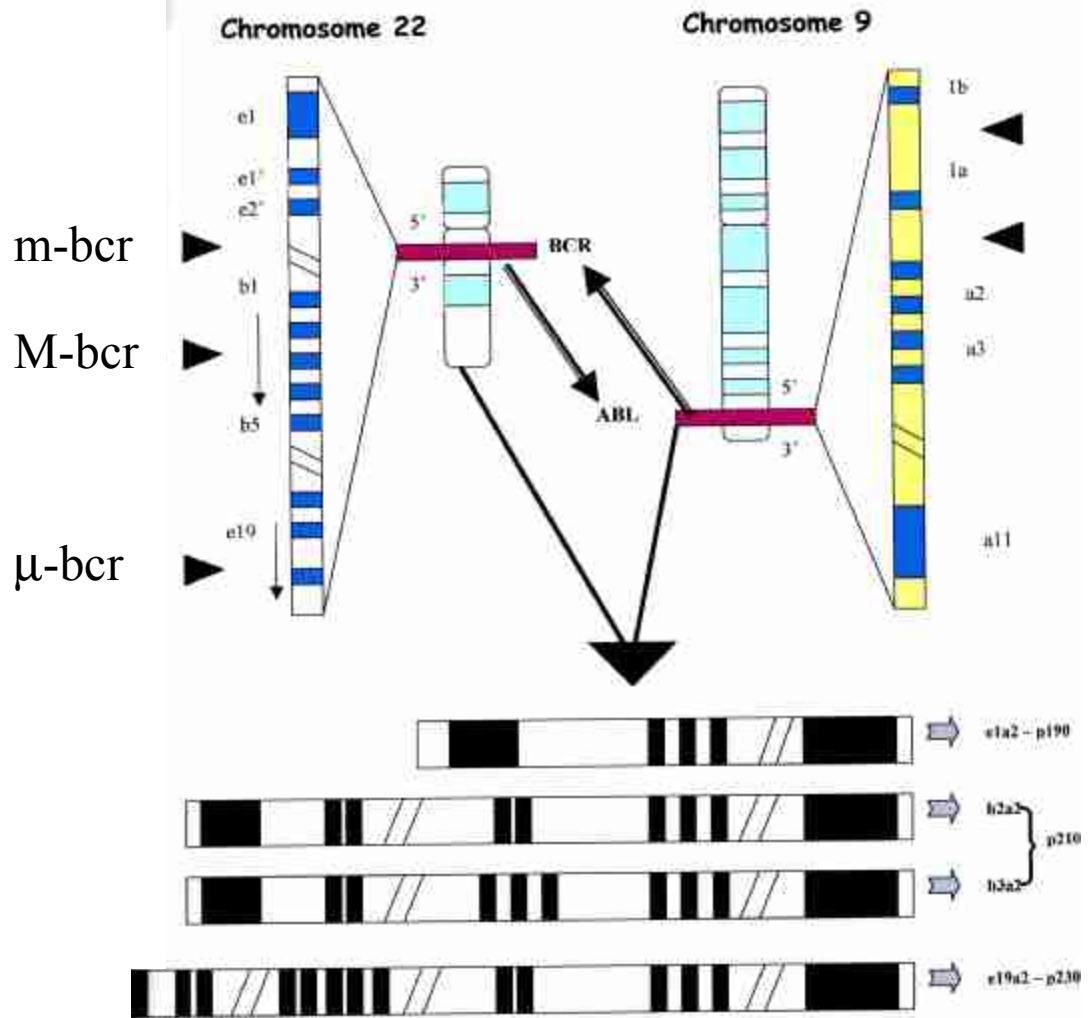


Chromosome rearrangements

- Generation of a tumourigenic fusion (bcr-abl)
 - Altered activity of tyrosine kinase

Philadelphia Chromosome





What is the effect of the BCR-ABL fusion on the cell.

- The translocation fusion results in a chimeric gene which codes for a oncoprotein which has raised thyrosine kinase activity .
- The increased activity induces increased growth of the leukaemic clone.
- The BCR-ABL gene may impede programme cell death

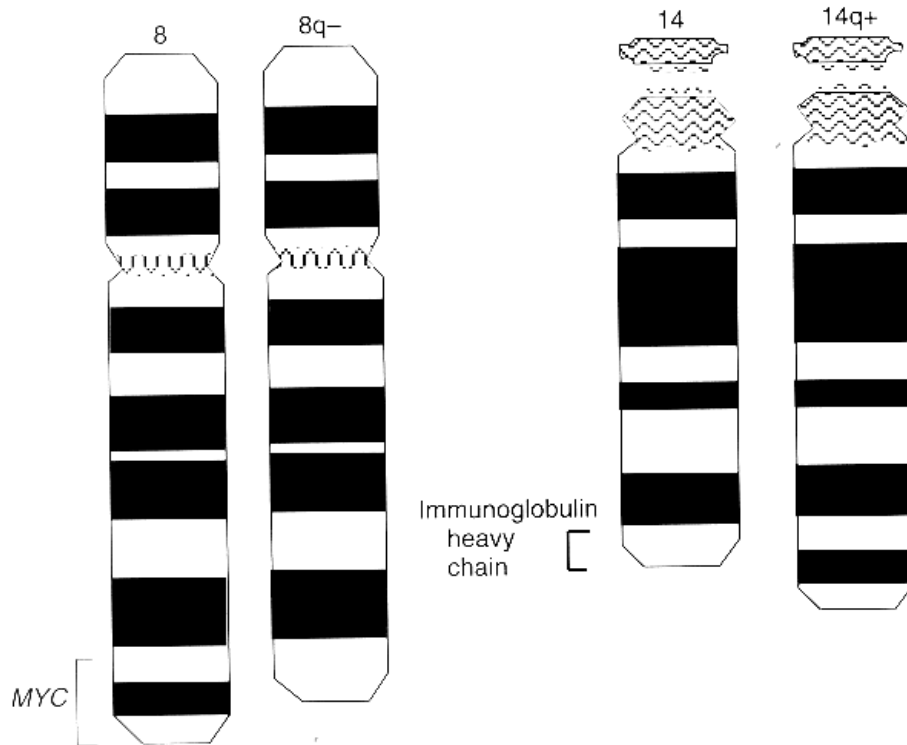
Chromosome rearrangements

- Activation of gene expression (MyC)
- t(8-14). 80% T(2;8) 6% t(8;22) 16%
 - Myc moved to IgH locus

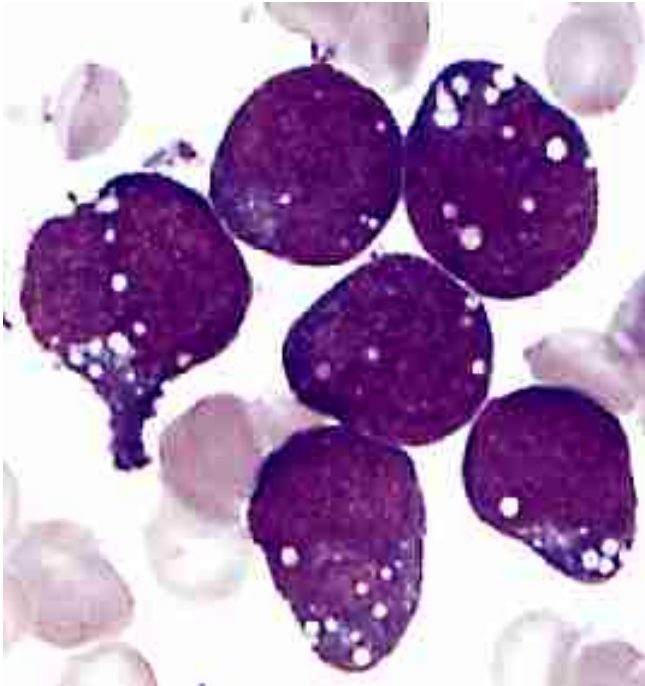
t(8;14) Burkitt's Lymphoma



t (8;14) Burkitt's Lymphoma



“Burkitt-type” B-ALL



- 1-2% of ALL
- B-lineage:
expresses surface Ig
- EBV-related
- Translocations involve
c-myc
- Morphology very closely related to
translocation ie Genotype reflects
Phenotype

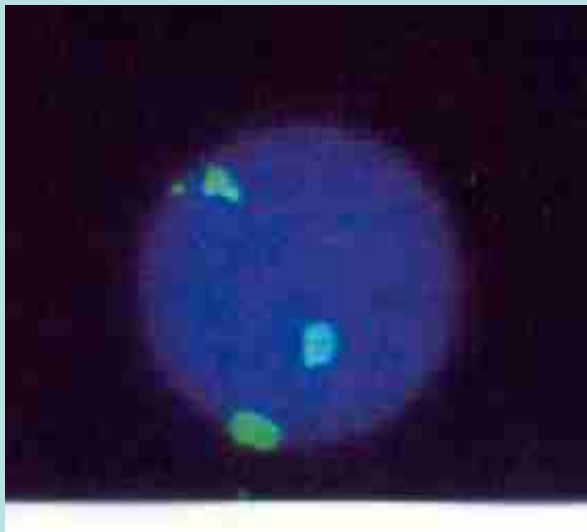
Leukemia diagnosis. Role of the laboratory

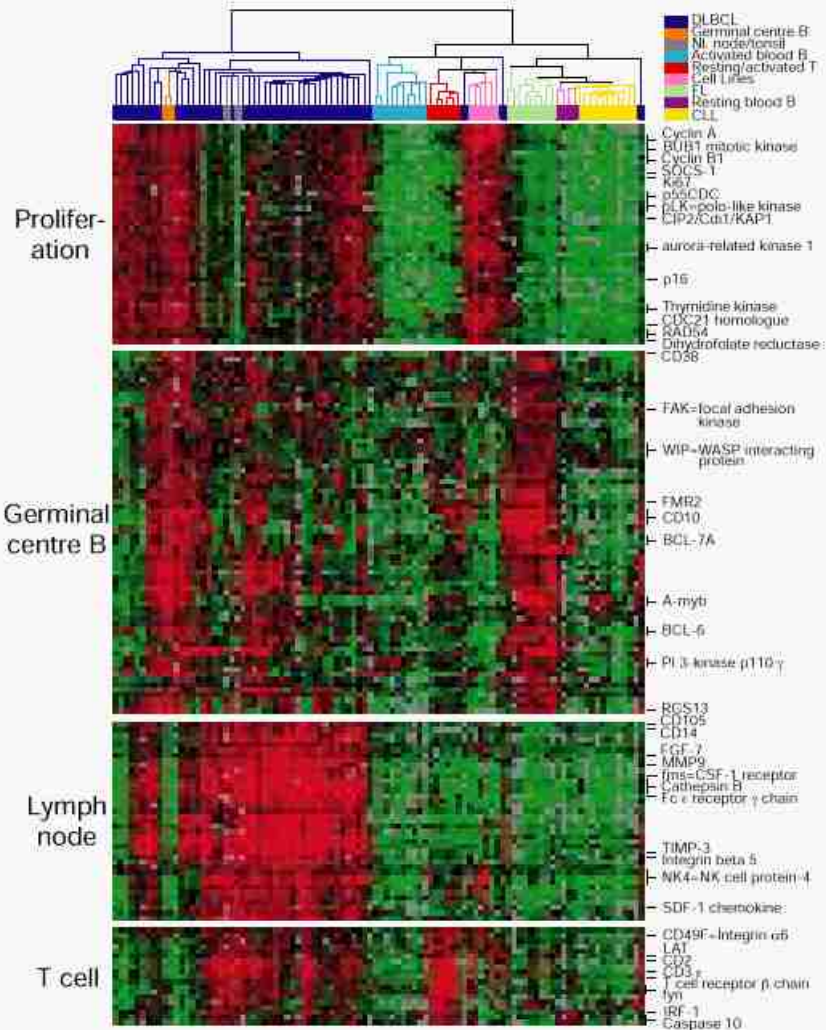
- Usually follows the clinical presentation of the patient
- FBC - Essential in all patients
- Bone Marrow Morphology -- Essential in all patients.
- Cytochemistry – Selective.
- Immunophenotype – indicated in all patients in whom the leukaemia is not obviously myeloid. Can help monitor MRD
- Cytogenetics – Essential on all patients. Bone marrow sample.
- Molecular genetics – Selective. If a marker is available. Can help to monitor MRD.

Where do you see the Laboratory Technology from here?

- Morphology.
- Continued development in Flow Cytometry, both cytoplasmic and surface receptors
- Fluorophore –FISH
- Real Time PCR.
- DNA chip microarrays.
- Examination of protein products.
- Understand the dynamics of the disease process.
 - 1. Greater understanding of the signalling pathway components.
 - 2. Greater understanding of the regulatory pathways in the cell.

Interphase FISH





Microarray expression profile in leukemia and lymphoma

Future directions in clinical treatment

- Immune manipulation
- Using residual disease quantification to “tailor” therapy
- “Mini-BMT”/ induction of graft-versus-leukemia effect
- Gene transfer/manipulation
- Alternative pathways to apoptosis

WHY CANCER MOLECULAR DIAGNOSTICS?

- **Improves accuracy of diagnosis, allowing stratification for good/poor risk patients.**
- **Allows early detection of molecular changes prior to development of clinical symptoms**
- **Detects early evidence of disease relapse allowing clinical intervention**
- **Increases understanding of how cancer develops, facilitating development of new drugs**

Requirements for molecular assay

- Relevance
- Accuracy/Specificity
- Sensitivity
- SOP
- Automation/Speed
- Yields results!

- Where to from here ??

- *I would like to thank IEQAS.*