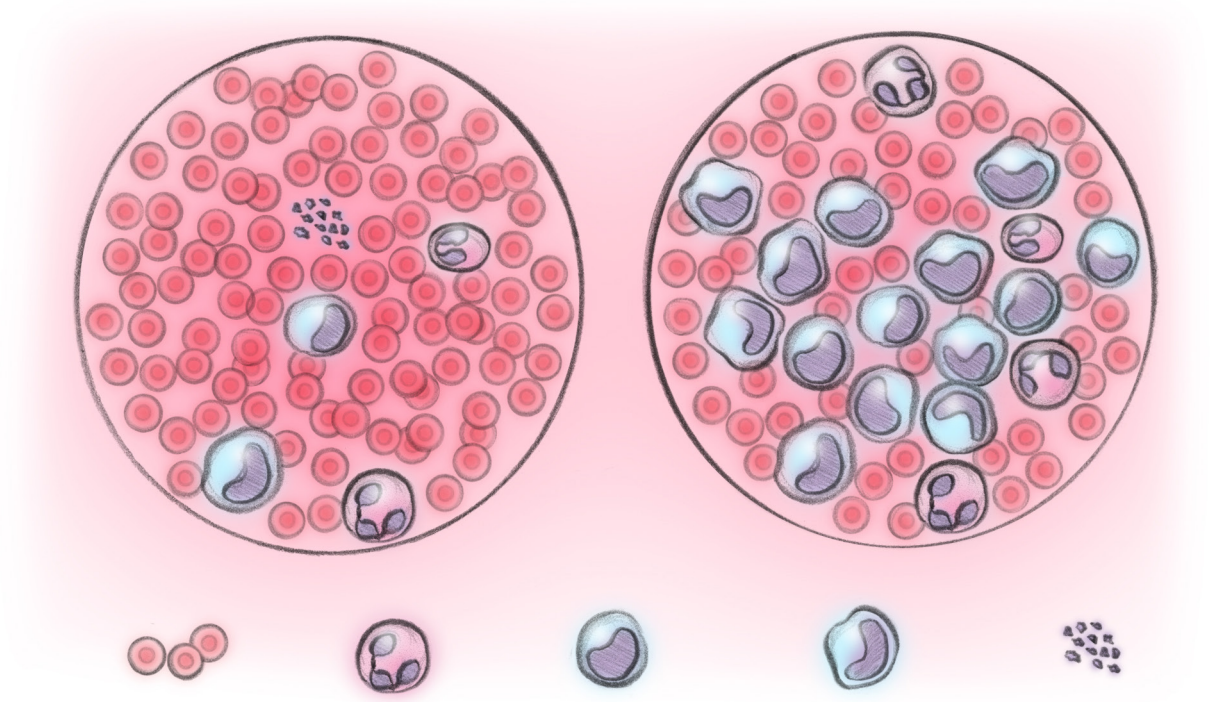
Genomic testing in CLL



Picture: A simplified image of blood cells

Leukaemia Care
YOUR Blood Cancer Charity

Genomic testing in CLL

Over the last decade, there has been increasing understanding of the genetic changes within CLL cells, meaning that genomic testing is an important part of managing the disease. In this toolkit, we look at what genomic testing is, why it's important, and what the changes mean for you.

Why is molecular testing important?

Over the years, several changes in individual genes or chromosomal changes have been identified that are important in CLL (these are covered below). Testing of chromosomal (cytogenetic testing) and/or testing for changes in genes (DNA sequencing) enables clinicians to identify whether these changes are present within the cancer cells.

Genomic changes in the CLL cells can help to determine:

1. Prognosis - How your disease is likely to behave in the future

And/or

2. Response to treatment - Which type of treatment your disease is likely to respond well to

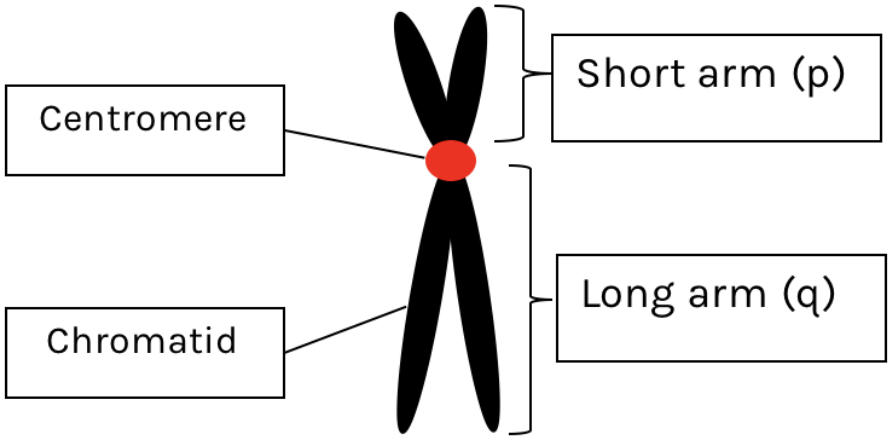
Newer, targeted therapies are also being introduced that are appropriate for the treatment of CLL with specific mutations or chromosomal changes. It is, therefore, increasingly important for patients to undergo genomic testing in order for clinicians to identify appropriate treatments.

What is cytogenetic testing?

'Cytogenetics' refers to the study of chromosomes.

Chromosomes are made from 2 chromatids joined at the centromere. This creates a short arm (p) and a long arm (q) of each chromosome.

Basic structure of a chromosome



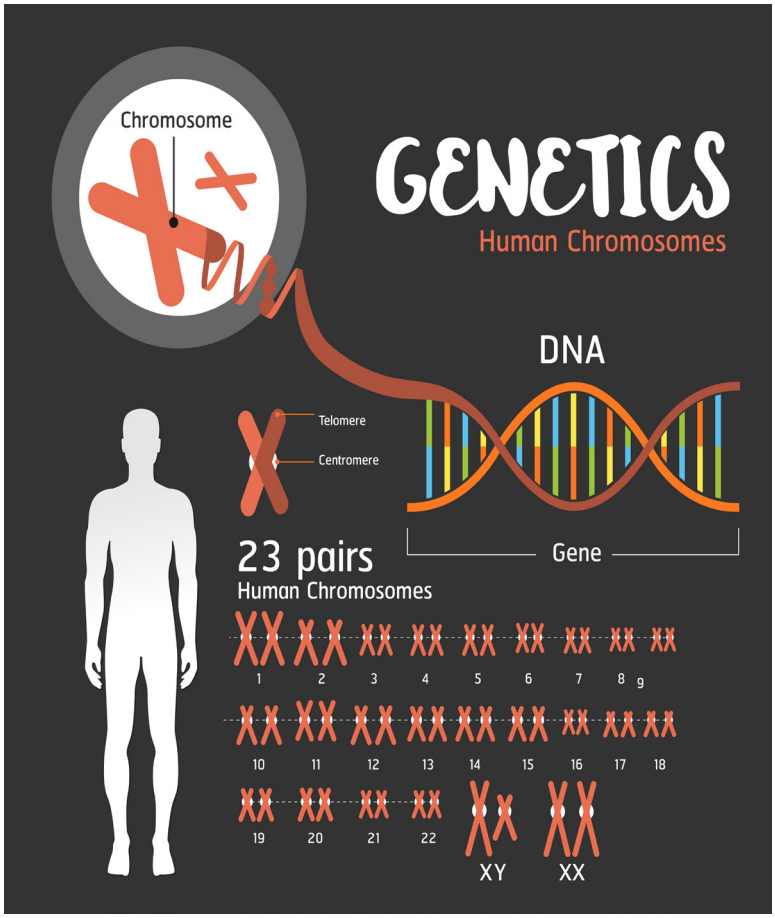
Within every human cell there are 22 pairs of chromosomes, plus 2 sex determining chromosomes (X and Y for males, X and X for females). One of each pair is inherited from each of your parents.

Chromosomes are made up of genes (sections of DNA that code for proteins). The proteins formed from genes control how our body functions and determines how we look.

For example, one gene encodes the protein that determines our eye colour. There are different versions of this gene, called alleles, allowing for different eye colours.

There are genes that produce proteins that help to prevent cancer (tumour-suppressor genes) and those that drive cancer (oncogenes).

Genomic testing involves analysing a patient's leukaemia cells, to determine whether there are chromosome changes, or gene mutations, known to affect prognosis or response to treatment.



What techniques are used for genomic testing in CLL?

FISH (Fluorescent in-situ hybridisation)

In the clinical setting, FISH is the test used for detecting changes in the structure and number of chromosomes in the CLL cells. This is how it works:

In FISH, a unique probe is produced that is complimentary (the same as) a certain region of DNA that researchers wish to detect. This could be a section of the chromosome, or a specific gene.

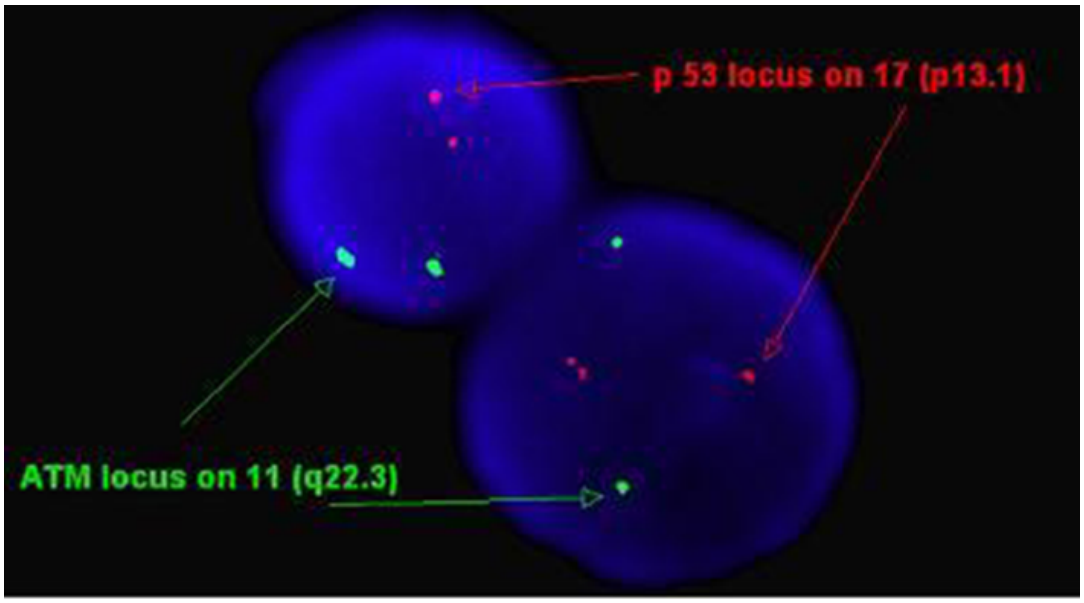
Attached to the probe are fluorescent markers that can be detected under a microscope.

The probe binds to, or attaches to, the complimentary single strand of the DNA

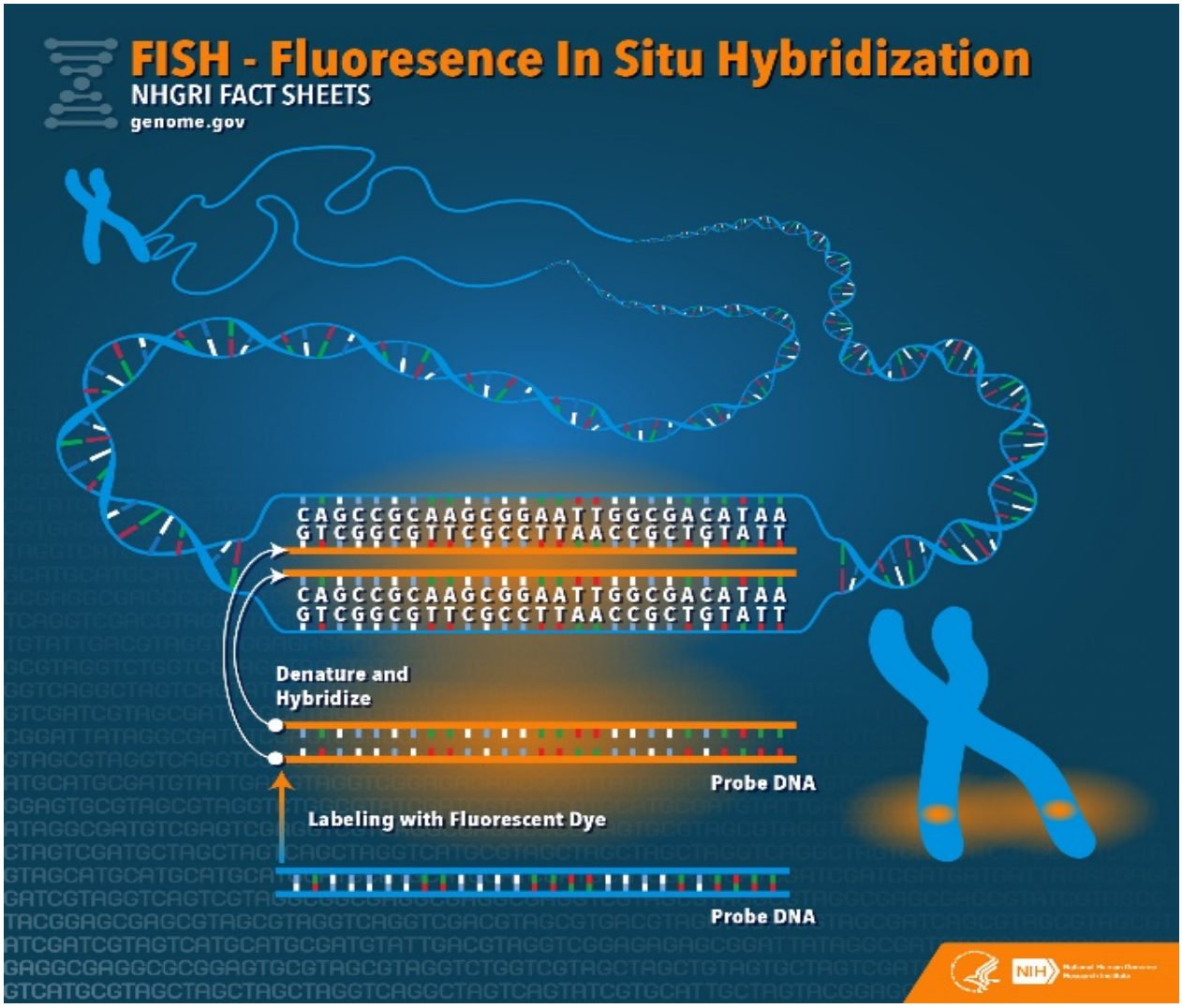
Under a fluorescent microscope, the probe will shine a bright fluorescent light.

A trained clinical scientist counts the number of probes within each CLL cell to detect extra copies of chromosomes or loss of a specific part of a chromosome.

This image shows two cells tested for 11q deletion and 17p deletion by FISH.



Two green dots per cell indicate two copies of the long arm of chromosome 11 are found in each cell. Two red dots per cell indicate that two copies of the short arm of chromosome 17 are found in each cell. This is a normal result. A clinical scientist will usually examine > 200 cells under the fluorescent microscope to determine the FISH result for each patient.



Can DNA changes in CLL cells be inherited from parents to children?

Some conditions are caused by inheriting a change in DNA or chromosomes. For example, Down syndrome is caused by inheriting an extra copy of chromosome 21. Other diseases, e.g. cystic fibrosis, are caused by inheriting two faulty copies of a gene. In inherited genetic disorders, the abnormal chromosome or gene is found in every cell of the person's body.

In cancer, we are usually not talking about changes in genes inherited from a person's father or mother. Cancer cells have damaged or broken chromosomes and genes. In some cases, the cancer cell has extra copies of an entire chromosome. In other cases, part of a chromosome is missing or deleted. In other cases, a specific gene has acquired an abnormal change affecting it's ability to do its job.

Taken together, all these changes are called somatic mutations. Somatic mutations are only found in the cancer cells, not in the normal healthy cells of a person with cancer. It is possible for a cancer cell to have more than one type of somatic mutation.

We know that somatic mutations can be caused by exposure to ultraviolet light (e.g. skin cancers) or radiation. However, for most people with CLL, it is not known where the somatic mutations in the CLL cell comes from.

Somatic mutations, or changes in the cancer cell cannot be passed on to your children.

What are the known CLL genomic abnormalities?

The important genomic changes identified in CLL are shown below:

TP53 disruption (deletion of 17p or TP53 mutation)

The TP53 gene lies on the short arm (P) of chromosome 17 or chr17p for short.

In cancer cells, the function of the TP53 gene may be broken or disrupted in two ways.

1) The chromosome itself may be lost or deleted, known as deletion of 17p or del(17p) for short. This is detectable by FISH. For more on FISH, refer to page four.

2) Chromosome 17 may look normal on the FISH test, but the TP53 gene itself may acquire a mutation. Mutations in TP53 are detected by DNA sequencing.

Either a deletion of the 17p (containing TP53) or a mutation in TP53 gene results in disruption to the function of TP53 (see box for more information on how TP53 works). That is why it is recommended to test for both deletion by FISH and mutation by DNA sequencing before treatment is started.

A deletion or mutation in TP53 is found in the CLL cells of 5-10% of patients starting their first CLL treatment. A deletion or mutation in TP53 is found in up to 30% of CLL patients who's disease has recurred after chemotherapy.

CLL with a deletion or mutation in TP53 is less likely to respond to conventional chemotherapy treatment. Or if there is a response to chemotherapy initially, the duration of disease control is likely to be short for CLL with deletion or mutation in TP53.

CLL doctors do not recommend chemotherapy for treating CLL with deletion or mutation in TP53 but use newer oral targeted therapies.

What does the TP53 gene do?

TP53, found on chromosome 17 is a key tumour suppressor gene. TP53 gene makes P53 protein. The P53 protein is responsible for stopping cell division, allowing for DNA damage repair to take place. If the DNA is beyond repair, then P53 triggers cell death (apoptosis).

It is this activity that makes the P53 protein so important for treatment with chemotherapy. Traditional chemotherapy treatments damage the tumour cell DNA and need working P53 to trigger death of the cancer cell.

When TP53 doesn't make P53 protein (either because the gene is deleted or the gene is mutated) the leukaemia cells are more resistant to chemotherapy.

11q deletion

11q deletion means that a section of the long arm of chromosome 11 is missing.

This chromosomal abnormality is identified by FISH in between 10-32% of CLL patients. It is found more commonly in younger men and CLL when the lymph glands are very large.

11q deletion and the ATM gene

Within the 11q region is the ATM gene (ataxia telangiectasia mutated gene). The gene product is a serine/threonine protein kinase, that helps to activate proteinsinvolved in DNA damage repair, for example P53. Many of these proteins are tumour suppressors. Therefore, when ATM is deleted, the response to DNA damage is altered and the tumour suppressor activity of these proteins is affected.

Drugs that target the DNA damage response are currently being tested in clinical trials for cancer with ATM deletion.

Older clinical trials of chemotherapy found that 11q deletion was associated with a shorter period of remission

13q deletion

This means that a section on the long arm of chromosome 13 is missing. 13q deletion is present in around 14-40% of CLL cases and is associated with good prognosis, if it is the only cytogenetic change present.

Not all clinical laboratories test for deletion in 13q, as detecting a deletion in in 13q does not change the management of CLL.

Possible ways in which loss of 13q affects the CLL cell

One of the genes that is commonly deleted in this region is the DLEU2 gene – aptly named 'deleted in leukaemia number 2'. The DLEU2 gene makes proteins which regulate (switch off or on) other genes. One gene controlled by DLEU2 is the BCL2 gene. The BCL2 gene makes a protein, which helps cells survive. When the long arm of chromosome 13 is deleted, one copy of the DLEU2 gene is lost, so less DLEU2 protein is made. This leads to an increase in BCL2 protein, helping CLL cells to grow and survive.

When DLEU2 is deleted, however, there is an increase in the BCL-2 protein. This protein helps the CLL cells to survive, by preventing the activity of proteins that promote cell death (apoptosis).

Trisomy 12 (+12)

'Trisomy' means that there are three copies of a chromosome, instead of two. In this case, there are three copies of chromosome 12.

Trisomy 12 is present in around 20% of CLL cases. Trisomy 12 has and it is little impact on response to treatment or duration of remission after treatment..

There is little known about how trisomy 12 is implicated in CLL. Some studies have demonstrated higher CD20 (the target for monoclonal antibody therapies like rituximab) on the surface of CLL cells with trisomy 12.

Tell me more about trisomy 12

The NOTCH1 gene makes the NOTCH1 protein, which turns on other genes to control cell growth, cell division and cell death. NOTCH1 protein is part of a group of proteins called transcription factors. In a healthy cell, the NOTCH1 protein is destroyed when it is no longer needed. In a cancer cell with a mutation in the NOTCH1 gene, the NOTCH1 protein is not destroyed and continues to play a role in cell growth

In newly diagnosed CLL patients, the NOTCH1 mutation is associated with a shorter time to first treatment.

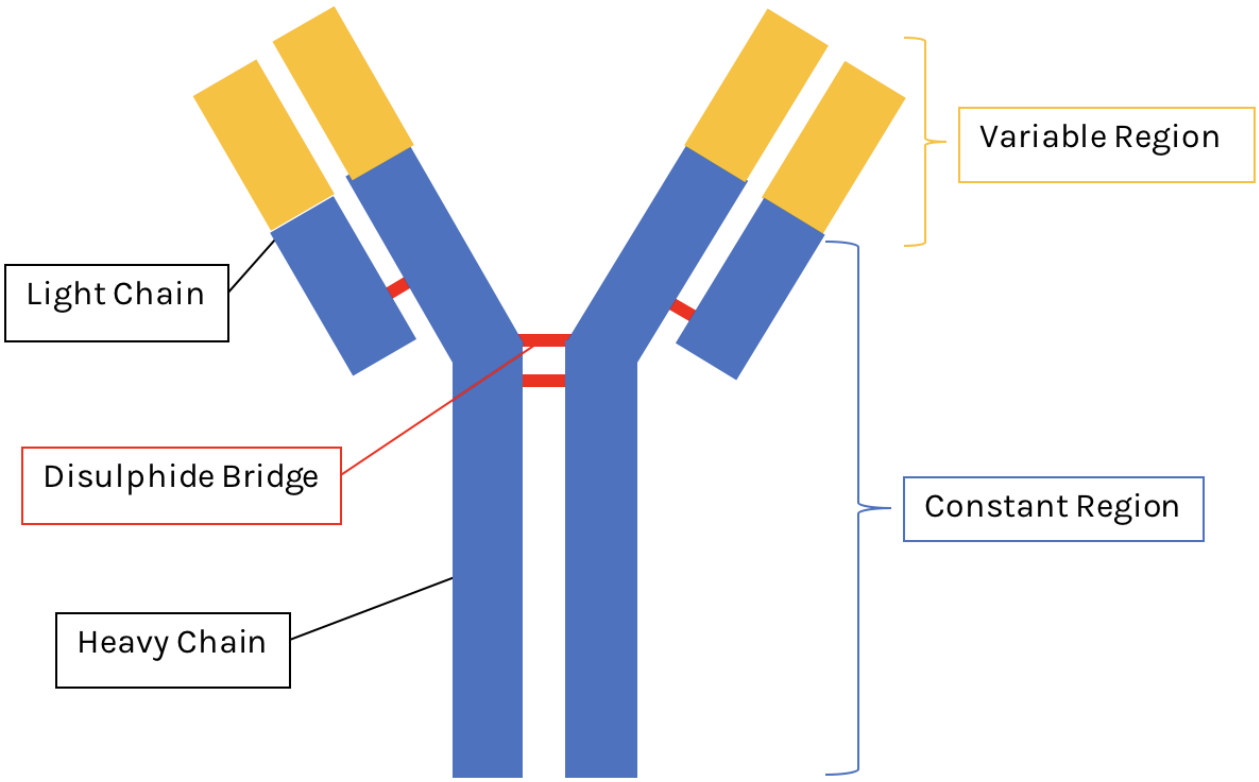
CLL patients are not currently routinely tested for NOTCH1 mutations, unless they are involved in research or clinical trials.

IgHv (Immunoglobulin Heavy-chain variable region) gene mutation status

Immunoglobulins are also known as antibodies. Antibodies recognise antigens on the surface of foreign or harmful cells and help to start an immune response against these cells. This is part of the body's natural immune response.

Antibodies are Y shaped proteins.

The bottom part of the protein will always stay the same structure (the constant region), whereas the upper arms of the protein are variable regions that can differ slightly in structure. This allows different antibodies to attach to different shaped antigens.



The different sections of the protein are coded for by different genes and as the name suggests, the IgHv gene encodes for the variable region of the heavy chain (in yellow).

In CLL, the IgHv gene exists in two forms. It may be mutated or unmutated. CLL with mutated IgHv gene and CLL with unmutated IgHv gene behaves slightly differently. Unlike the genes discussed above, mutated IgHv gene is generally associated with favourable outcomes.

In newly diagnosed CLL, patients with mutated IgHv gene tend to have a longer period of time on watch and wait before their first treatment is needed compared to CLL patients with unmutated IGHV gene. IgHv testing (in combination with other factors) can be helpful to estimate the time before treatment is likely to be needed in a newly diagnosed CLL patient.

After chemotherapy and monoclonal antibody treatment, CLL with unmutated IgHv genes tend to have shorter duration of remission than CLL with mutated IgHv. For CLL patients preparing for treatment, IgHv testing (in combination with other factors) can be helpful to estimating how long their CLL will remain controlled after chemotherapy.

Finding out more about how changes in DNA and chromosomes affect cancer: The 100,000 Genomes Project – Genomics England

CLL patients are one cohort of NHS patients that are eligible to be part of the 100,000 Genomes Project.

Researchers are looking at the whole genomes (all the genes and DNA within a cell) of patients to identify any currently unknown genomic changes that are implicated in disease. The 100,000 Genomes Project compares the DNA from healthy cells (which is the DNA inherited from parents) with the DNA from cancer cells (which have acquired "somatic mutations").

By comparing the DNA sequence from the tumour with the DNA sequence from the healthy cells, the 100,000 Genomes Project helps to uncover the exact genomic changes responsible for an individual's cancer

You can find out more about the project on the Genomics England website: https://www.genomicsengland.co.uk/the-100000-genomes-project/

Further questions:

If you have any further questions about genomic testing in CLL then you can contact our Campaigns and Advocacy team. They are available Monday to Friday from 9:00am – 5:30pm. If you would like to speak to them, you can:

Call our office line on 01905 755977

Send them an email at advocacy@leukaemiacare.org.uk

You can also call the Helpline, free of charge on 08088 010 444. Available weekdays 9am – 10pm and weekends 9am – 12:30pm. The team will pass your enquiry onto the Campaigns and Advocacy team.

Please note that our Campaigns and Advocacy team are unable to provide:

Detailed medical advice or recommendations

Legal advice

Advocacy for a course of action which is contrary to the aims and objectives of Leukaemia Care

Thank you to Dr. Niamh Appleby, Haematologist & Health Education England/Genomics England Genomics Medicine Fellow at Oxford