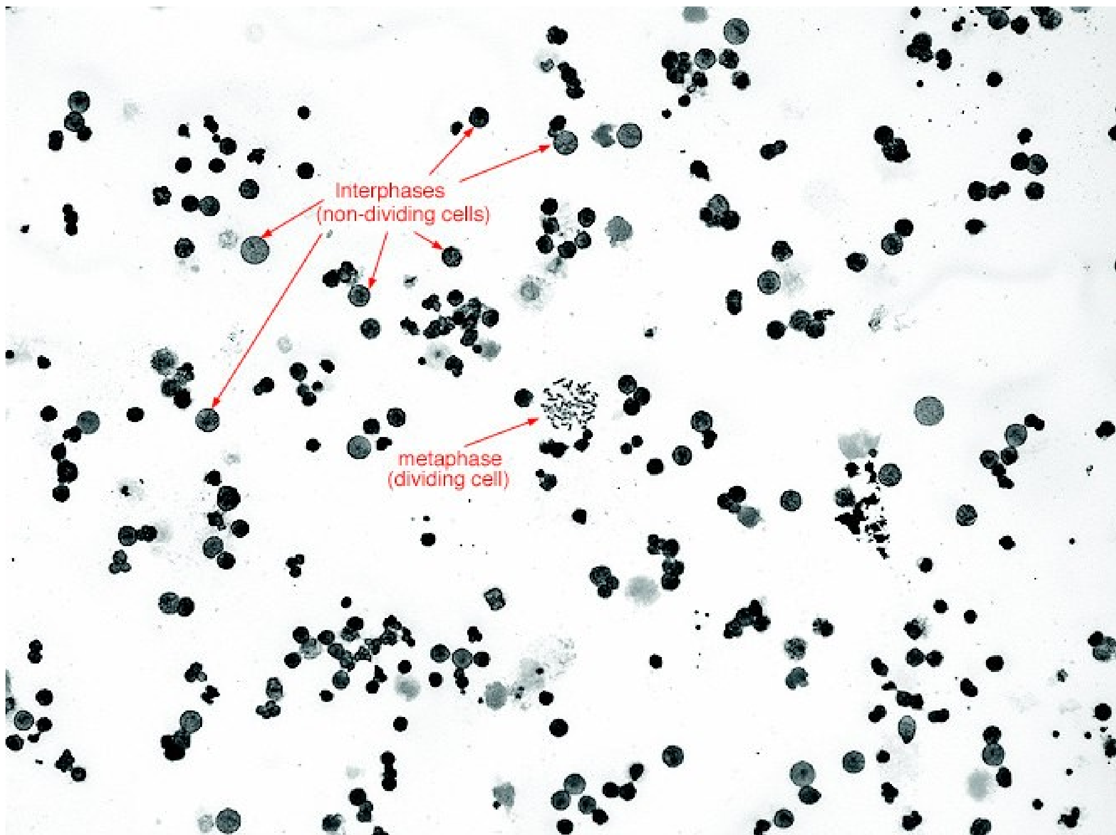
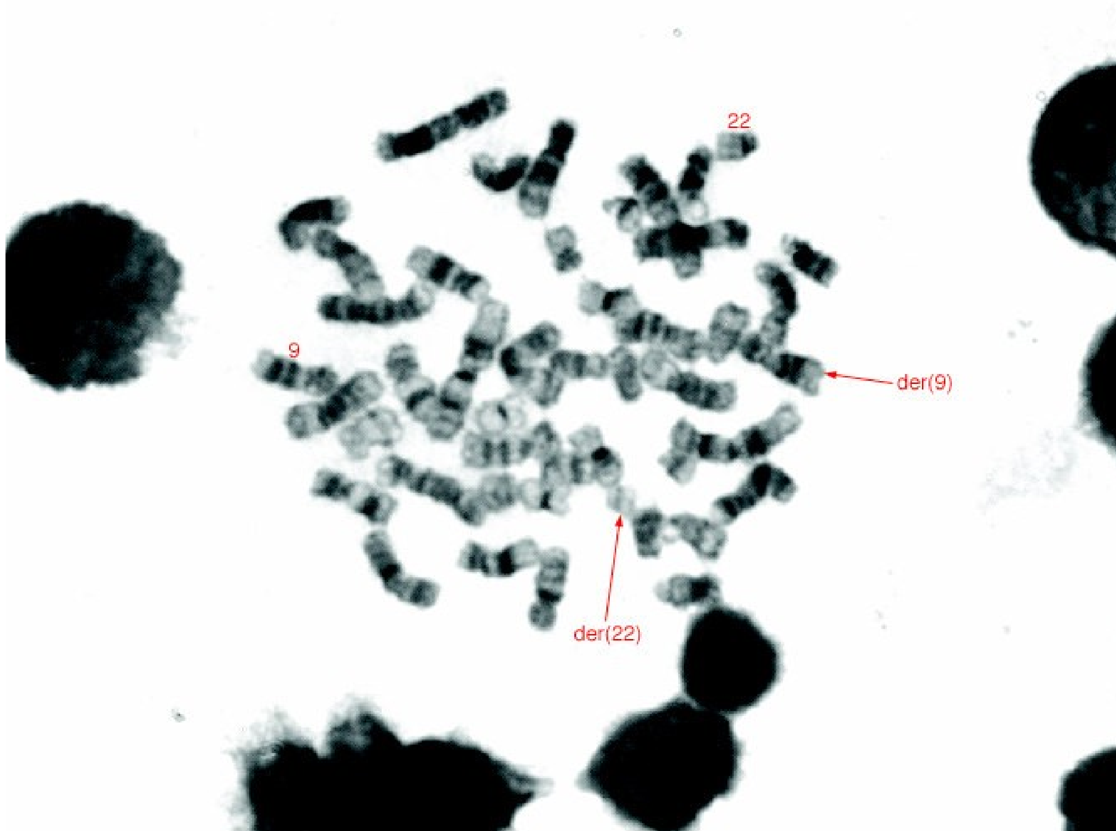
CYTOGENETICS SERVICE

CYTOGENETICS AND FLUORESCENT IN SITU HYBRIDISATION (FISH) AT KING'S

Cytogenetic analysis entails the analysis of chromosomes, which we observe in dividing cells. During disease, the chromosomes can become changed and rearranged, and the changes are associated with varying disease types. Some are very specific such as the translocation t(15;17) in acute promyelocytic leukaemia and others more general, such as trisomy 8 (extra chromosomes 8) in myeloid disorders. The figure below shows a low power magnification field with many interphases (non-dividing cells) and one metaphase (a dividing cell).



Metaphase cells are analysed using high power magnification as observed in the next picture (below). Chromosomes are individually recognised by a technique known as G banding, akin to a bar code, a cytogeneticist needs to learn all the banding patterns on the chromosomes to establish normality or abnormality. The picture below shows the t(9;22) translocation more commonly known as the Philadelphia translocation in chronic myeloid leukaemia.

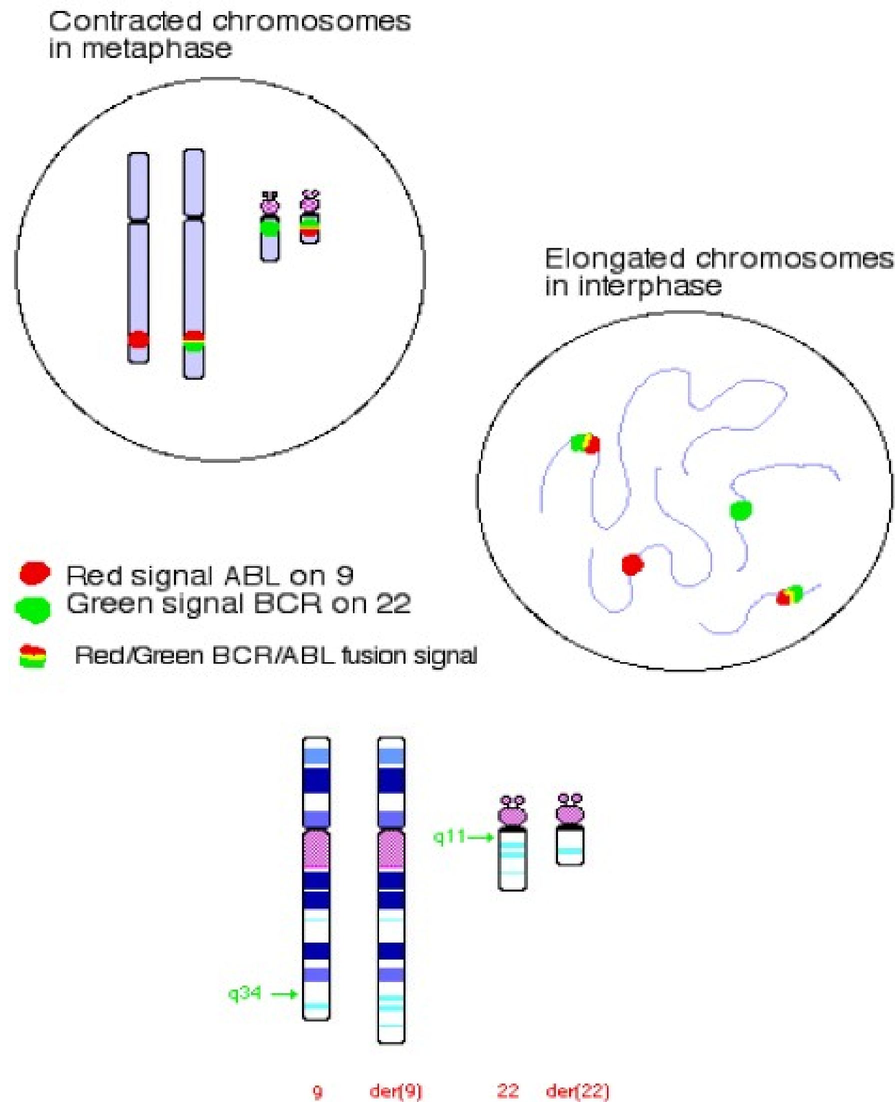


There are many ways chromosomes can become abnormal, such as the above translocation, they can also lose material in deletions. Whole chromosomes can be lost, as in monosomy, and inversions can occur when there are two breaks in the same chromosome and the chromosomal material flips vertically, giving a different banding pattern to the chromosome. The different abnormalities can indicate a good, intermediate or poor prognosis.

As one can see from the first picture, we are limited to small numbers of dividing cells for analysis.

Fluorescent in-situ hybridisation (FISH) however, allows us to observe certain types of chromosome abnormality in non dividing cells, and thus supplement analysis in cases where large numbers of cells need to be assessed. The metaphase (dividing cell) shown here demonstrates the abnormality found in patients with chronic myeloid leukaemia (CML), the translocation t(9;22). Fluorescently labelled probes for certain chromosome regions are commercially available for this abnormality and many others. Using a charged coupled device for image capture (the FISH system)

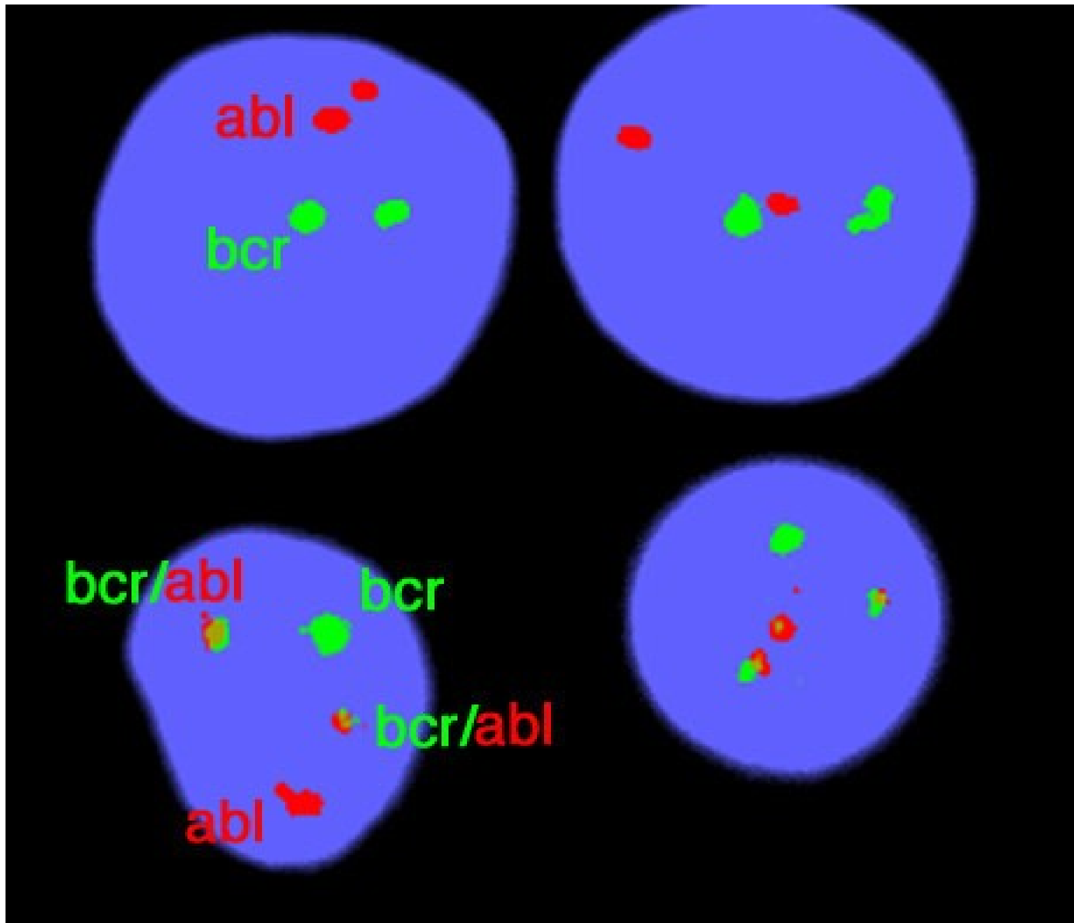
we can observe the minute areas of chromosome where genes have been disrupted during the process of chromosome aberration. The metaphase above shows what we call derivative chromosomes, where two normal chromosomes exchange material giving rise to two abnormal chromosomes. A diagram of this abnormality is shown below.



FISH probes show the fusion of two genes, abl (on chromosome 9) and bcr (on chromosome 22). The fusion of these genes gives rise to an abnormal protein which causes problems in the life of the cell, and ultimately leads to disease.

When patients are being monitored during treatment, it is important to establish how many normal and abnormal cells are present, and looking at large numbers of cells is helpful in establishng the patient's status. Below is an image showing normal and abnormal interphase cells (non-dividing cell) using FISH techniques employing a bcr/abl probe. The fluorescent signals show up as dots indicating the presence of the gene for which the probe has been made. In this instance, there are

normal and abnormal cells present, the normal cells have two red signals (abl gene on chromosome 9) and two green signals (bcr gene on chromosome 22). In the abnormal cells, the signal pattern is one red signal, (abl gene), one green signal (bcr gene) and two fused red/yellow/green fusion signal (bcr/abl on the derivative chromosome 9 and derivative 22). This would indicate the presence of the t(9;22) translocation.



Chromosomes can also be 'painted' with probes which light up the entire chromosome. There are 24 colour fish techniques (which a new system would enable us to utilise), that can indicate very subtle and cryptic chromosome rearrangements which may not be detected using conventional techniques. The detection of abnormality at diagnosis is important in subsequent follow up studies in order to assess disease status in the patient.

Multi colour techniques would be very useful in defining complex karyotypes such as the one shown in the picture below where some chromosomes (markers) could not be identified.

KingsPath
Clinical Diagnostic Pathology Services
King's College Hospital NHS
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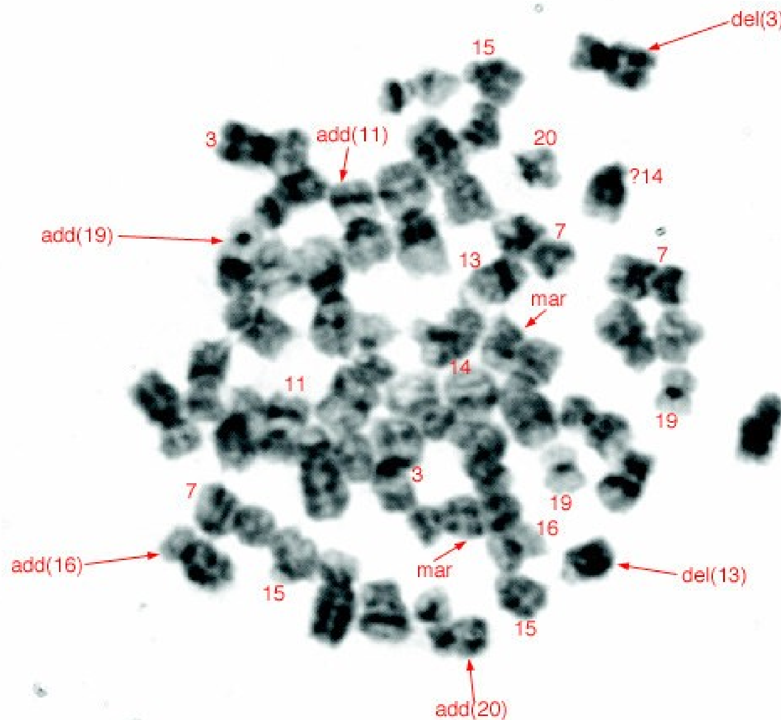

There are many problems that can be encountered while trying to obtain a cytogenetic result from bone marrow aspirate or blood. Most samples fail because of a low white cell count leading to insufficient cells for the test. Occasionally, samples which have a very high white cell count (such as CML) also fail because of loss of viability during transportation to the laboratory. The cell will continue to divide in the transport medium and sometimes, they divide so quickly that they 'commit suicide' in transit, leaving the laboratory with a tube full of dead cells.

Even if there are enough cells, failure still occurs if there are no dividing cells. Cytogenetic analysis is then impossible. However, if the patient has a known abnormality, which has a commercially available probe, FISH can be employed in these instances.

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