1

Introduction to Blood Cancer

The concept of blood cancer as a defined disease has only recently been recognised, as has the viewpoint of blood as an organ. This latter notion follows from the definition of an organ as a collection of diverse cells and tissues that together have a particular function. Diseases of the blood are common, and malignancy of the individual components of the blood has long been described and brings considerable population morbidity and mortality.

The objective of this chapter is to provide a basic but sound introduction to blood cancer that will inform chapters to come, as described in the preface, in four sections. An exploration of blood cancer is impossible without an understanding of the physiology of blood, and this is where we shall start, in Section 1.1. Key subsections will focus on the different types of blood cells, how they arise (haemopoiesis) and the importance of a small number of organs that have important roles in haematology. We then move, in Section 1.2, to focus on the role of the blood science laboratory, whilst Section 1.3 describes a historical perspective of the blood cancer, and then introduces leukaemia, lymphoma, myeloma and other forms of blood cancer. Section 1.4 focusses on key components of the molecular pathology of cancer, therefore forming a bridge to Chapter 2, where laboratory methods are examined.

Learning Outcomes

After studying this chapter, you should confidently be able to…

- Explain the key features of blood
- List common blood tests for blood cancer
- Describe simple features of the leading blood cancers
- Comment upon the molecular pathology of cancer

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1.1 Fundamental Aspects of Blood

Blood has two components – the cells (red blood cells, white blood cells and platelets) and the liquid in which they circulate – the plasma. The latter is water in which are dissolved various ions, atoms and molecules, many of which directly impact on the physiology of the blood cells. An example is the interactions between certain blood cells (platelets) and certain molecules (such as fibrinogen) in haemostasis, most commonly resulting in the formation of a thrombus, or clot.

It may be argued that blood can be found in two different compartments – within the vascular system of arteries, capillaries and veins (the circulation), but also within the organs of the body. Furthermore, the distribution of

various blood cells within the skin, lymph nodes and spleen varies: the makeup of the circulating white blood cells differs markedly from those in the bone marrow. As the reasons for this are fundamental to blood and blood cancer, this demands an explanation.

1.1.1 Haemopoiesis

In the healthy adult, three types of blood cells are generated in the bone marrow by the process of haemopoiesis. All are derived from a series of haemopoietic stem cells, which in the embryo are found first in the aorta-gonad-mesonephros and yolk sac as haemocytoblasts (which also give rise to endothelial cells). By two months, these would have completed their migration to the liver and spleen, where they will reside for months 2–7, and then move on to the bone marrow, principally of the sternum and long bones, by months 5–9. The mature haemopoietic stem cells, of which an adult has some 50,000, are headed by pluripotent stem cells, themselves the product of haemangioblasts, and are supported by an architectural matrix of blood vessels, endothelial cells, stromal cells, osteoblasts, adipocytes, macrophages, fibroblasts and other cells. These cells come together to form a haemopoietic niche.

Pluripotent stem cells can self-perpetuate but also produce two lineage-specific unipotent stem cells – those of the myeloid lineage and those of the lymphoid lineage. The former is often described as the granulocyte–erythrocyte–monocyte–megakaryocyte (GEMM) common myeloid progenitor, while the latter is the common lymphoid precursor, and both may be prefixed by CFU – colony forming unit – reflecting their activity in vitro (Figure 1.1). The direction concerning which lineage the stem cells take is governed by certain genes, which input from a number of hormones and cytokines, the finer details of which will follow.

The Myeloid Lineage

The process of myelopoiesis begins with CFU–GEMM in the lower left of Figure 1.1, which gives rise to two unipotent stem cells: those of the erythrocyte and megakaryocyte lineage (CFU–EMk) and those of the granulocyte and monocyte lineage (CFU–GMo). The former, in turn, produces a stem cell for erythrocytes (of which 10^{11} are produced daily), and hence for erythropoiesis, and another for megakaryocytes, and so platelets, the process of thrombopoiesis. Similarly, CFU–GMo generates a dedicated stem cell for the three granulocytes (G: neutrophils, eosinophils and basophils, named for their cytoplasmic granules, important in antimicrobial defence), and for monocytes (M).

This developmental process is under the control of certain genes, variably acting at different stages, but it also requires stimulation of the cells by growth factors: both are required to act in concert to move the cell on to the

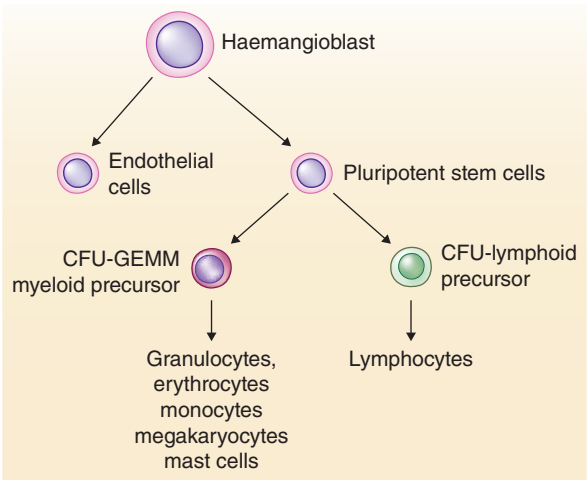
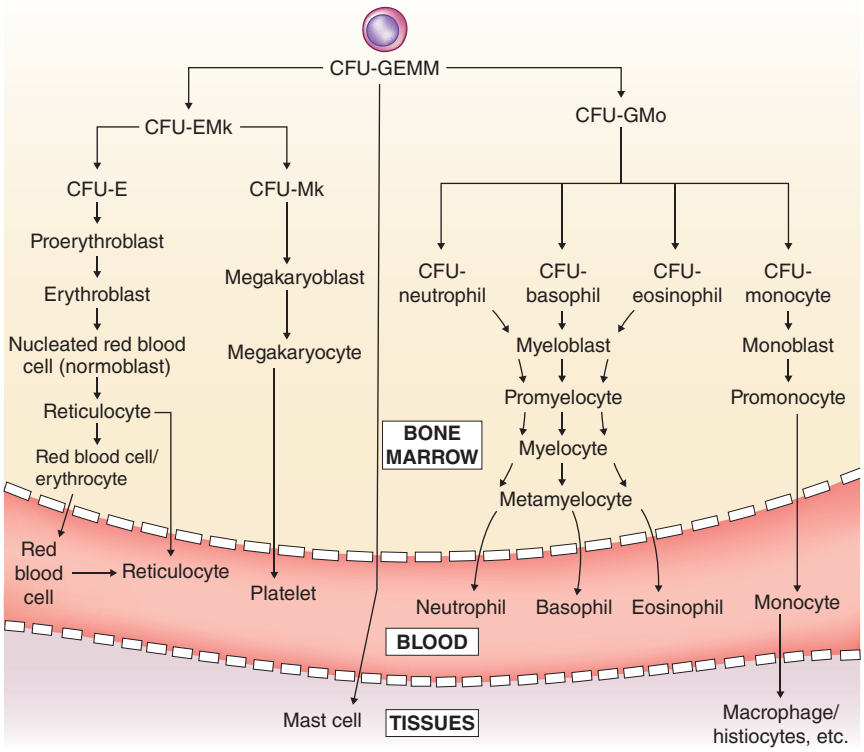


Figure 1.1 Early stages of haemopoiesis.

Figure 1.2 Myelopoiesis.



next stage. Failure to do so can lead to disease, such as blood cancer. Erythropoiesis and thrombopoiesis require the growth factors erythropoietin and thrombopoietin, respectively. Each developmental stage following the CFU stem cells is a series of blast cells, which in this respect are cells of intermediate differentiation status found only in the bone marrow (Figure 1.2):

- In the erythroid lineage, erythroblast (or normoblast) produces the nucleated red blood cell, reticulocyte (which may occasionally appear in the blood) and the mature red blood cell.
- The platelet lineage involves the megakaryoblast producing megakaryocytes, that in turn produce mature platelets.
- In monocyte pathway, the monoblast produces mature monocytes, some of which become macrophages, histiocytes, dendritic cells, and other cells in the tissues.
- The myeloid lineage has one CFU for each granulocyte (in the process of granulocytopoiesis), hence each has a myeloblast, a promyelocyte, a myelocyte, a metamyelocyte and, therefore, the mature neutrophil, eosinophil and basophil that enter the blood.

Monocytes may leave the blood and transform into macrophages, which in turn may undergo additional transformation in the tissues into cells such as dendritic cells, Kupffer cells and Langerhans cells, some of which may be described as histiocytes. Mast cells arise directly from the myeloid stem cell, passing through the blood to the tissues.

The Lymphoid Lineage

In contrast to the myeloid lineage, the lymphoid lineage has only one CFU, and this produces a lymphoblast. However, the precise nature of the subsequent cell, a prolymphocyte, is not completely understood, as its position

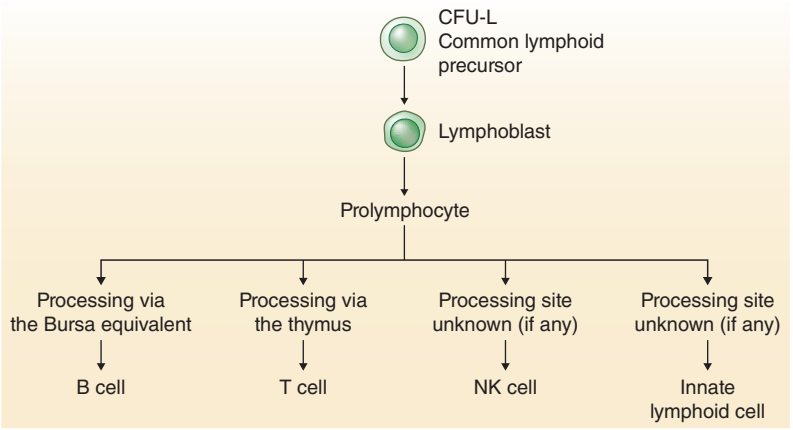


Figure 1.3 Lymphopoiesis.

in the development of four mature lymphocytes (B cells, T cells, NK [natural killer] cells and innate lymphoid cells [ILCs]) is unclear. Nevertheless, what is clear is the requirement for some immature lymphocytes (or, perhaps, prolymphocytes) to mature in the thymus, and therefore become T cells, which have a defined immunological function.

Similarly, other immature lymphocytes are required to mature into B cells, which also have precise immunological functions. The exact location of this maturation is unknown, but it may be a lymph node or the bone marrow itself. In birds, this organ is the Bursa of Fabricius (hence B cells), the mammalian equivalent of which has not been identified. NK cells are a heterologous group of lymphocytes, some of which may be described as 'large granular lymphocytes', with a number of functions, whilst ILCs migrate to the skin and elsewhere where they have sentinel duties.

As with myelopoiesis, lymphopoiesis is also the product of a carefully orchestrated series of genes and growth factors, with some of the latter being interleukins (ILs): B cells require IL4, T cells require IL2, IL4 and IL10 and NK cells require IL1 and IL15, whilst all need the action of IL7. Figure 1.3 summarises lymphopoiesis.

1.1.2 The Function of Blood Cells

The primary function of red blood cells is the carriage of oxygen from the lungs to the tissues, a secondary function being transport of a small amount of carbon dioxide as bicarbonate ions. Platelets are needed to ensure haemostasis – the balance between thrombosis and haemorrhage. An important component of the latter is the coagulation pathway, composed of numerous plasma proteins synthesised by the liver.

The function of white blood cells is complex. They defend us from a large number of micropathogens, by the processes of immunology and inflammation, with each type of cells doing so in a different manner. A simplified summary of their functions is as follows:

- Amongst the functions of neutrophils is the ability to attack and destroy bacteria, viruses and fungi by phagocytosis, although they are also important in the repair following damage, the release of and response to cytokines and the removal of debris following injury.
- Each of the lymphocyte groups has a different function: aided by some T cells and modified macrophages (as antigen-presenting cells), B cells transform into plasma cells and produce antibodies. Other T cells are active in destroying cells infected with a virus, and some are regulators of the immune system. NK cells are capable of seeking out and destroying a variety of targets, whilst tissue ILCs perform a number of functions, many of which overlap with those of the other lymphocytes.

- The immunological activity of monocytes is in the secretion of cytokines, and in phagocytosis. They continue to perform this antimicrobial function when they move into the tissues as macrophages. A subgroup, such as dendritic cells, is capable of cooperating with T and B cells in antibody production, while others provide sentinel roles.
- Eosinophils have general 'housekeeping' antibacterial duties but are also important in allergic (hypersensitivity) reactions (such as asthma) and defence from viruses and helminth parasitic worms such as filariasis.
- Basophils have much in common with eosinophils, but are more focussed on hypersensitivity, where their combination with certain antibodies can cause severe clinical reactions including asthma, anaphylaxis and conjunctivitis, conditions whose effects can be life-threatening.
- Tissue-based mast cells also provide help in the defence from micropathogens, coordinating other cellular processes by the release of various inflammatory mediators.

1.1.3 The Organs of Blood

The importance of bone marrow as the site of haemopoiesis has been describe above. Other organs of interest to haematologists include the liver, thymus, spleen, and lymph nodes. The importance of the thymus is demonstrated in many cases of DiGeorge syndrome, where most parts or all of this organ are missing, and consequently, the individual lacks T lymphocytes, leading to immunodeficiency (a weak or absent immune system). The liver is home to Kupffer cells, modified macrophages that perform immunosurveillant roles, but may also be the site of antibody production. The spleen may be regarded as a storage organ for neutrophils and red blood cells and is also a site of antibody production. As we will discuss, both organs can be infiltrated by the blood cancer cells (and also by other malignant cells).

Despite potential roles for the liver and spleen, the leading organs for antibody production are lymph nodes. T cells, B cells and modified macrophages (as dendritic antigen-presenting cells) come together to produce the five classes of antibodies – IgG, IgM, IgA, IgE and IgD. Scores of these small bodies are connected to each other by a second circulatory system, the lymphatics, which carries the lymphocytes in lymph, a fluid with plasma-like properties. An important feature of this system is that potential pathogens can be collected from the tissues and other organs and passed along the chain of lymph nodes so that defences can be mobilised. Just as the liver and spleen can be affected by blood cancer, so too can the lymph nodes. Figure 1.4 shows the anatomy of a lymph node.

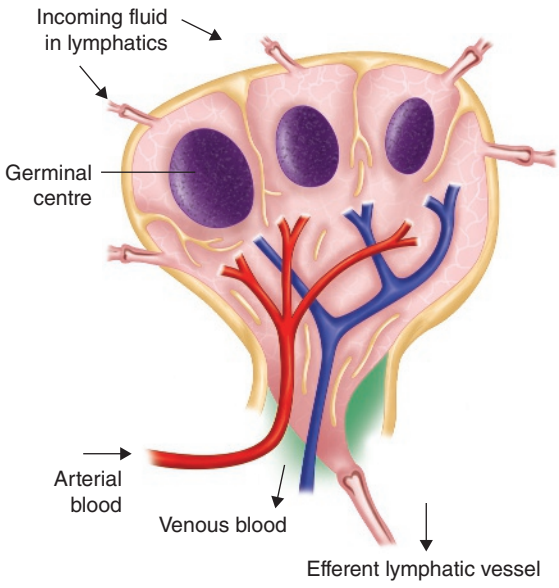


Figure 1.4 Anatomy of the activated lymph node. Lymphatic vessels carry lymph fluid (possibly loaded with pathogens and cytokines) from the tissues to the lymph node. A germinal centre is primarily the focus of the activity of T helper cells and antigen-presenting cells in promoting antibody production. Each node is fed by an arterial blood vessel, whilst vein carries effluent blood back to the circulation. A lymphatic vessel may carry cells and lymph fluid to the next node in the chain. *Source*: Blann and Ahmed, 2014/with permission of John Wiley & Sons.

1.2 Tools for Investigating Blood Cancer

Two important specialities provide crucial information regarding blood cancer. Hospital imaging departments provide services such as ultrasound, X-ray, computer tomography, positron emission tomography and magnetic resonance imaging, most often regarding the investigation of a lymphoma or a myeloma. These are discussed in Section 6.2.1. The second, blood science, is more frequently called upon in all forms of blood cancer and warrants additional discussion. Whilst haematology, biochemistry and immunology laboratories may be separate departments in many hospitals, in others they are combined into a single blood science laboratory. Although there may well be crossover in the roles of each department, in that in one hospital a certain test may be performed in one laboratory, whereas in another hospital it is performed in a different laboratory, it is nonetheless convenient to consider each separately.

1.2.1 Haematology

The greater part of the work of this laboratory is the full blood count (FBC) and the blood film, both of which can be performed on complex analysers, although in some cases a blood film may need to be viewed by light microscopy.

Full Blood Count

This crucial test provides, from a single blood sample (in a vacutainer that has the anticoagulant EDTA), a minimum of six metrics of the red blood cell population, a further six of the white blood cells, and the number of platelet some analysers provide additional results such as the red blood cell distribution width and the proportion of red blood cells with low mass of haemoglobin (and therefore hypochromic). In most cases, the results are presented as the number of particular cells and are sent out to the requesting health care professional along with a local reference range. Table 1.1 shows an example of a normal FBC, a leading feature of which

Table 1.1 A normal FBC.

Analyte	Result	Unit	Reference range
Haemoglobin	145	g/L	Male: 133–167 Female: 118–148
Red blood cell count	4.6	10^{12}/L	Male: 4.3–5.7 Female: 3.9–5
Haematocrit	0.44	L/L	Male: 0.35–0.53 Female: 0.33–0.47
Mean cell haemoglobin	32	pg	26–33
Mean cell haemoglobin concentration	332	pg/L	330–370
Mean cell volume	95	fL	77–98
Reticulocytes	30	10^9/L	25–125
White blood cells	5.8	10^9/L	4–10
Lymphocytes	1.8	10^9/L	2–7
Neutrophils	3.4	10^9/L	1–3
Monocytes	0.5	10^9/L	0.2–1
Eosinophils	0.1	10^9/L	0.02–0.5
Basophils	0.03	10^9/L	0.01–0.1
Blasts/Atypical cells	0	10^9/L	~0.01
Platelets	241	10^9/L	140–400

are differences in certain indices between the sexes – several metrics are lower in women than in men, and a reference range. This result is from a man, so all indices are within the reference range. In some settings, an erythrocyte sedimentation rate (ESR; reference range <10 mm/h) is also part of the FBC and may be measured in a separate sample.

The Blood Film

In most cases, a drop of blood is placed on a glass slide, drawn out, dried, stained and the cells' morphology, and other features, are determined by an analyser or by light microscopy. Figures 1.5–1.9 show examples of the five white blood cells and representative red blood cells. Key features are that the nucleus of the neutrophils is irregular, with several lobes, whereas that of the lymphocyte is round and occupies perhaps 90% of the cell. The nucleus of the monocyte is slightly indented and occupies perhaps 75% of the cell, whilst that of the eosinophil is bi-lobed and the cytoplasm has red/pink granules. The nucleus of the basophil is almost completely obscured by black/purple granules. These morphological features are important aspects of malignancies of white blood cells.

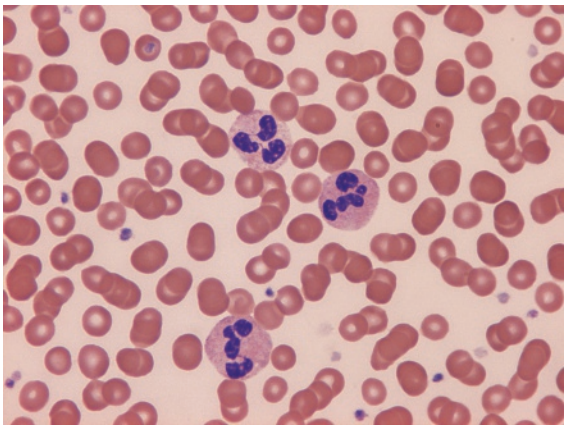


Figure 1.5 Neutrophil leukocytes. *Source*: Courtesy of D. Bloxham, Cambridge Hospitals NHS Trust.

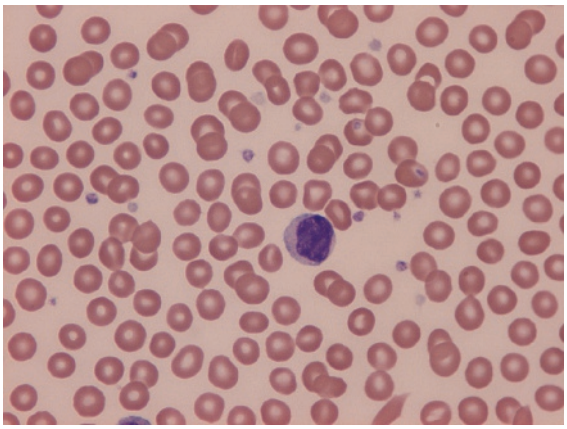


Figure 1.6 A lymphocyte. *Source*: Courtesy of D. Bloxham, Cambridge Hospitals NHS Trust.

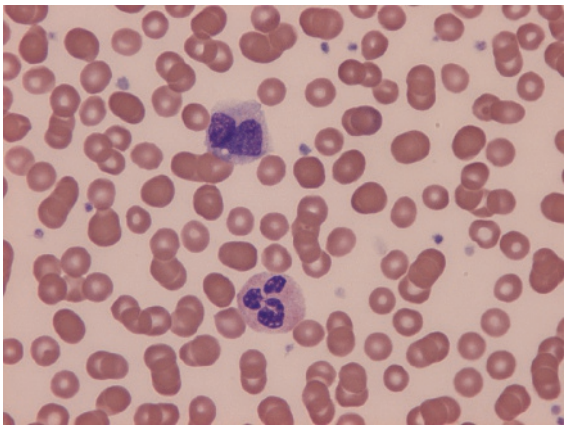


Figure 1.7 A monocyte and a neutrophil. *Source*: Courtesy of D. Bloxham, Cambridge Hospitals NHS Trust.

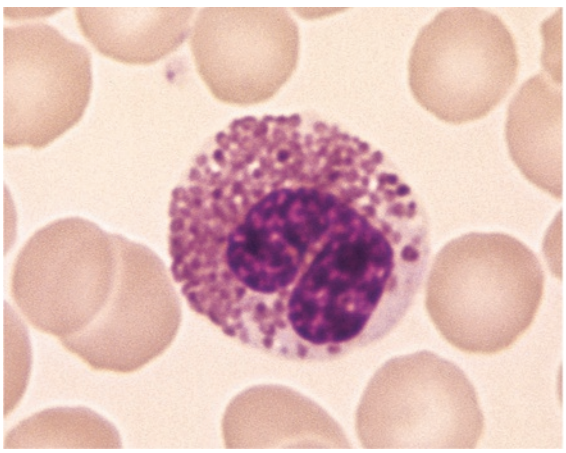


Figure 1.8 An eosinophil. *Source*: Hoffbrand et al., 2024/ with permission of John Wiley & Sons.

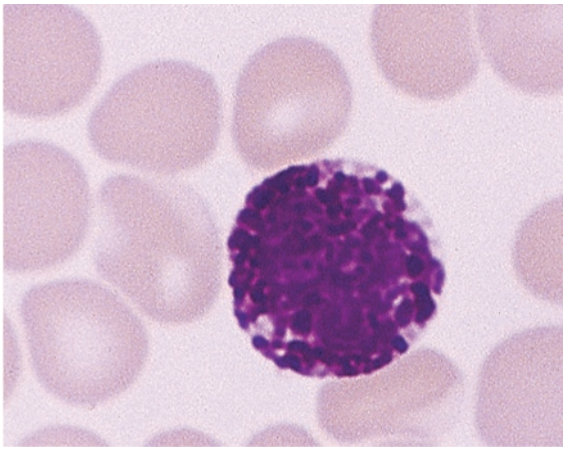


Figure 1.9 A basophil. *Source*: Hoffbrand et al., 2024/with permission of John Wiley & Sons.

1.2.2 Biochemistry

This discipline differs from haematology in that it generally calls for a sample of serum. The leading tests are urea and electrolytes (U&Es), which tell us of renal function, which may impact the red blood cell production. Liver function tests (LFTs) provide information on the state of this organ, crucial in several haematological diseases, with the panel being:

- alanine aminotransferase (AAT)
- aspartate aminotransferase (ALT)
- alkaline phosphatase
- bilirubin
- gamma glutamyl transferase (GGT)

For a complete picture, all five tests are required because of issues with sensitivity and specificity, with examples being that AAT and ALT may arise from muscle cells, alkaline phosphatase from bone. As the liver produces albumin, some also include this in the panel, as levels may fall in severe renal disease. Many practitioners focus on the levels of uric acid and the enzyme lactate dehydrogenase (LDH) in blood cancers (as in other diseases), with increased levels generally indicating cell disruption and/or damage.

A further important test is serum protein electrophoresis that, with a densitometer, can quantify certain proteins (Figure 1.10). It can also be used to determine proteins in the urine and has a place in the diagnosis and management of conditions such as renal failure and in myeloma and related conditions, discussed in Section 8.6. Further testing, immunoelectrophoresis, can detect with more precision the nature of an abnormal antibody protein, although this can also be achieved by methods that do not employ electrophoresis.

1.2.3 Immunology

This discipline can be divided into serology (assessing humoral immunity) and cellular. The former provides data on the different classes of immunoglobulins (IgG, IgM, IgA, IgD and IgE), the acute phase reactant CRP, β-2-microglobulin (β2M) and others. In some settings, the serum protein electrophoresis is conducted by immunologists. Tests of the functions of lymphocytes and neutrophils are rarely called for, but their enumeration is important.

A fluorescence flow cytometer (often better known as a FACS) can quantify the number of cells in a sample, once they have been labelled with monoclonal antibodies (mAbs) to a particular cell surface marker according

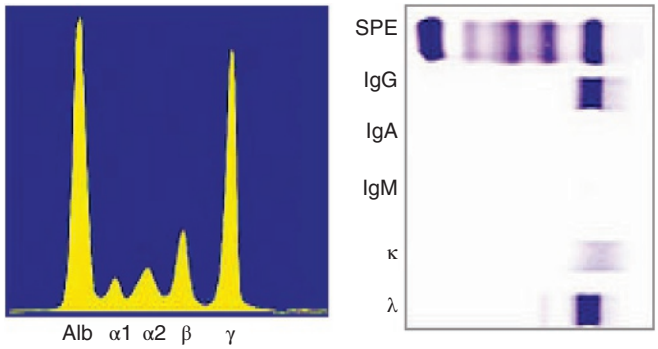


Figure 1.10 Protein electrophoresis. *Source*: Courtesy of Dr JA Katzmann.

Table 1.2 Selected CD markers.

CD molecule	Cell(s) marked
CD1	Antigen-presenting cells
CD2	T lymphocytes, NK cells
CD3	T lymphocytes
CD4	T helper cells, some monocytes and Macrophages, e.g. dendritic cells
CD7	T lymphocytes
CD8	T cytotoxic cells, some NK cells
CD10	B lymphocytes
CD11	Monocytes, macrophages and some B cells
CD14	Monocytes, dendritic cells
CD15	Granulocytes
CD19	B lymphocytes
CD20	B lymphocytes
CD22	B lymphocytes
CD24	Granulocytes, B cells
CD25	Regulator T cells
CD27	Plasma cells
CD33	Myeloid cells
CD34	Stem cells
CD45	All white blood cells
CD56	NK cells, some T cells
CD117	Myeloid cells, stem cells

to a 'cluster of differentiation' (CD) system. A selection of CD markers and the cells on which they may be found is presented in Table 1.2. Many of these are present in panels to determine the nature of a particular blood cancer, principally leukaemia. The determination of further details of malignant cells is described as immunophenotyping.

1.2.4 Histology

Often defined as the study of the microscopic structure of tissues, in blood cancer it examines the structure and components of the bone marrow (as an aspirate or a trephine sample) and of lymph nodes with stains such as haematoxylin and eosin (Figure 1.11).

Tissues and blood films may also be stained with special stains (histochemistry), but also for the presence of specific antigens using mAbs. These can be linked to an enzyme that ultimately provides a coloured product, a technique described as immunocytochemistry (Figure 1.12), and is therefore a sister technique to the immunophenotyping of circulating leukocytes. These histological techniques are of great importance in the lymphomas.

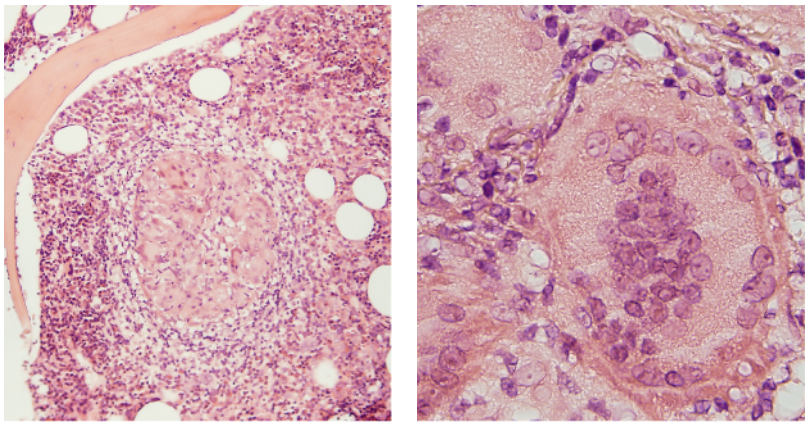


Figure 1.11 Routine histology. Low- and high-power images of a case of sarcoidosis, showing well-demarked granulomas within a sample of bone marrow stained with haematoxylin and eosin.

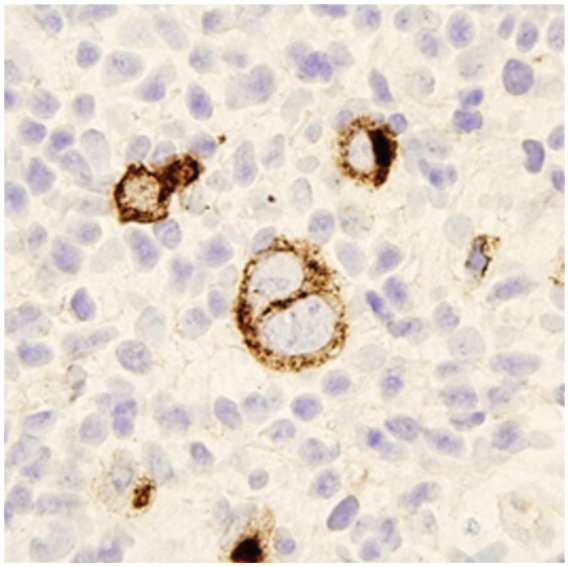


Figure 1.12 Immunohistochemistry. Cells identified with an mAb to CD30.

1.3 A Historical Perspective of Blood Cancer

The scientific/clinical origins of three leading forms of blood cancer can be traced back to the early 19th century, although considerable progress was made during the Victorian era. Early surgeons were faced with a debilitating bone disease, often with deformity and fractures, with the investigations often finding a mass of red pulp – the bone marrow. Adopting *myelos* from the Greek for bone marrow gives myeloid. In parallel, other observations, often in infections and inflammatory diseases, noted enlargement of discrete masses, later attributed to lymph nodes, from which we get lymphoma. For decades, these two pathological conditions remained separate until the weight of evidence brought them together into the haematology we recognise today, as shown in Figure 1.2. What follows is a summary of major events of this period, concluding some 50 years ago with the birth of molecular genetics, and thus, molecular pathology.

1.3.1 Leukaemia

This was the first blood cancer to be described in detail. Kampen (2012) has meticulously collated the works of pioneering clinical scientists, such as Bennett and Virchow, with the latter describing 'white blood', and subsequently leukaemia, in the mid-19th century. Numerous case reports of this period also noted splenomegaly (a large spleen) and hepatomegaly (a large liver), and the occasional enlarged lymph node (lymphadenopathy), in addition to pus-like exudate. Although 'granular cells' and 'globules' had been described using light microscopy of unstained cells, the classification of the precise cell type of the malignancy began in earnest following the development of dyes to enable the recognition of leukocyte subsets. A leading step forward was taken in 1870 when the disease was linked to abnormalities in the bone marrow.

As the 20th century developed, different forms of the disease were described, such as leucocythaemia, leukanaemia and lymphocythaemia, and in 1908, Longcope and Donhauser described large lymphocytes in an acute lymphocytic leukaemia that may (in today's setting) be large granular lymphocytes/NK/T cells. In 1906, Gluzinski and Reichestein described what we would today recognise as primary plasma cell leukaemia, whilst in 1917, di Guglielmo reported a case of what we now describe as erythroleukemia. In 1920, Tuohy reported a case of splenomegaly with polymorphonuclear neutrophil hyperleukocytosis, interpretable today as chronic neutrophil leukaemia.

By mid-century, lymphocytic, myeloid, monocytic, eosinophilic and megakaryocytic leukaemia had been described in detail. In 1958, Bouroncle and co-workers reported 26 cases of leukemic reticuloendotheliosis, with their photographs clearly showing cells we would today recognise as hairy cells, a term coined in 1966, and so hairy cell leukaemia. Although Joachim described basophil cell leukaemia in 1906, several cases were most likely a variant of a granulocytic leukaemia, whilst Kyle and Pease published two cases of basophilic leukaemia in 1966. However, the leading advance in this area was the report by Rowley in 1960 of the Philadelphia chromosome, fully discussed in Section 3.4. Also of importance was Osgood's description, in 1969, of the bi-lineage myelomonocytic leukaemia, which was to have important repercussion for our understanding of myeloid malignancies.

The next phase of the unravelling of leukocyte biology focussed on lymphocyte subsets, with the recognition of B cells and T cells. Data from Froland in 1972 pointed to the binding of sheep erythrocytes to lymphocytes to be a probable marker of T cells, whilst others defined T cells by the presence of terminal deoxynucleotidyl transferase. In 1973, Dickler et al. and Seligmann et al. provided evidence of a T cell leukaemia, whilst the principal marker of B cells was the presence of surface membrane immunoglobulin, used to diagnose B cell leukaemia. The development of mAbs in 1975 enabled the precise identification of B and T cells, and of the third lymphocyte subset, NK cells, regarded in many cases as the large granular lymphocytes previously noted, and NK leukaemias were subsequently reported. These cells, and their malignant counterparts, are defined by the presence of particular CD markers in Table 1.2.

1.3.2 Lymphoma

In 1832, Hodgkin published his now classic paper on seven cases of enlarged lymph nodes, in many instances accompanied by an enlarged spleen, which he considered to have some cause other than an infection such as tuberculosis. In 1865, Virchow described a tumour of the lymph nodes as a lymphosarcoma, descriptions of a lymphoma appearing regularly in the literature from 1880, with referral to Hodgkin lymphoma at the turn of the century. The defining cytological feature was described by Reed and by Sternberg, in Baltimore (1902) and Vienna (1898), respectively, of the multinucleate cell that now bears their name. During the 1960s, cytogenetics was used to demonstrate numerical and structural chromosomal abnormalities in malignant lymphoma, whilst a major review in the *British Medical Journal* referred not to Hodgkin lymphoma but to Hodgkin disease. Proof of the lymphocyte as the root of the malignancy was ultimately made in the 1970s by immunological methods, and in the 1980s using cytogenetics.

In the decades that followed, clinical science identified several forms of lymphoma in which Reed–Sternberg cells could not be identified, leading to the umbrella grouping of non-Hodgkin lymphoma (NHL) in 1974, many based on anatomy and location of particular lymph nodes. Subtypes of NHLs recognised in the 1940s included follicular lymphoblastoma, zonal lymphoma in the 1970s, and diffuse large B cell lymphoma in the 1980s. The dominant cell type in the majority of these cases was B lymphocytes: T cell lymphomas were recognised as a distinct entity in the 1970s. The diverse cellular nature of these tumours prompted significant difficulties in classification until international consensus documents were published, one of the first being in 1994, subsequently adopted by the World Health Organization (WHO), with regular updates till 2022.

An important member of the NHL group was reported by Burkitt and co-workers in 1961 as a tumour present in the children in East Africa and is worthy of further discussion. In 1965, Epstein and co-workers isolated a virus from transformed cell lines from the patients with this disease, subsequently proven to have aetiological causal link by transforming normal leukocytes with the Epstein–Barr virus (EBV) into lymphoblastoid cells resembling those of Burkitt lymphoma cells, and noting chromosomal abnormalities. However, EBV is globally widespread, and as such often fails to act as an oncovirus implying a second factor to be required for a malignancy, with malaria being a leading contender, another being immunodeficiency, such as is due to HIV. This principle is important as it is reminiscent of, and is likely to support, Knudson's 1971 two-hit theory of carcinogenesis.

1.3.3 Myeloma

In 1844, Solly described cases of bone deformity, and six years later, MacIntyre reported a 'Case of Mollities and Fragilitas Ossium, accompanied with urine strongly charged with animal matter'. It is very likely that today's diagnosis would be malignant myeloma, a further contribution being from Henry Bence Jones, with his referral to *albuminous* urine, published in 1848. The site of production of Bence–Jones protein (BJP, as it was becoming known) was suggested by Weber in 1898 to be the bone marrow, whilst Wright Wilson, and Bramwell and Patton, concluded, in the 1920s, that BJP was a globulin. In 1937, Tiselius laid the foundations of protein electrophoresis, and in 1939, Longsworth and co-workers reported the electrophoresis of serum and plasma from various diseases, including myeloma. This breakthrough was followed shortly afterwards by the works of Moore, Gutman and co-workers who reported paired analysis of urine and serum showing increased levels of gammaglobulins in both samples.

These crucial techniques set the scene for later findings and the diagnosis of myeloma and related conditions to the present day. One such was the report in 1944 by Waldenström of a macroglobulinaemia that now bears his name, and the study of paraproteinaemia developed by others. We now recognise that these paraproteins, of whatever nature, are leading contributors to the increased ESR, considered by Ainley, and by Harboe, in the 1950s, to be an important feature of these diseases.

Further advances were of direct immunoelectrophoresis and immunofixation, made by Ouchterlony, Grabar and Williams in 1953, and used by Korngold and Lipari in 1956 to report of two classes of BJP: kappa and lambda,

subsequently developed by Edelman and co-workers. Later analyses in the decades that followed demonstrated various subtypes of the hypergammaglobulinaemia according to immunoglobulin group, which later heralded diagnoses such as heavy chain disease, with the recognition that these conditions can progress to myeloma. This was only made possible by advances in protein chemistry, such as that of Alper and Hohnson in 1969, that allowed the development of antisera to each of the gammaglobulin heavy chains. During the 1970s, Kyle and co-workers noted a proportion of cases with an abnormal gammaglobulin profile, but did not have a myeloma, describing these as a monoclonal gammopathy of undetermined significance (MGUS), with the subsequent description of smouldering multiple myeloma (SMM), being the presence of a myeloma with a gammopathy not deemed sufficiently pathological as to warrant treatment.

Whilst protein chemistry advanced during the Victorian era and 20th century, the same cannot be said for the cell biology of the disease. Although Waldeyer coined 'plasma cell' in 1875, an extensive review by Bayrd in 1948 discussed opposing and confusing views as to the nature of the cell responsible for the disease. However, he does describe a cell phenotype we would most likely recognise today as a plasma cell, with its extensive blue cytoplasm. In his 1952 review, Lumb described various forms of myeloma, and also plasma cells as a tumour, in the blood, and as a leukaemia. Hallek et al. (1998) subsequently proposed a model for a stepwise progression of the disease from physiology, via MGUS, to SMM, intra-medullary and extra-medullary myeloma, and plasma cell leukaemia.

1.3.4 Other Blood Cancers

Classification of the relatively rare conditions that follow was understandably difficult until a sufficient mass of knowledge had accumulated by the middle of the last century, bringing the use of the umbrella term *myeloproliferative syndromes*, and later *myeloproliferative neoplasms* (neoplasm = new, abnormal growth). Further details of molecular pathology of these conditions are discussed in Chapters 3–5.

Polycythaemia Vera

Reports of polycythaemia from the early 20th century focussed on cyanosis, splenomegaly and an increased number of red blood cells and so haemoglobin, with some reporting an increased number of white blood cells. The precise aetiology remained elusive, although familial or hereditary types were described, as were numerous secondary causes, such as congenital heart disease, emphysema and chronic hypoxic states, and the presence of myelosclerosis noted. However, Marson and Meynell, in 1949, described marrow hyperactivity of the myeloid and erythroid cells with a marked increase in megakaryocytes, named polycythaemia vera (PV), to distinguish it from secondary forms, and in 1951, Dameshek pointed out that polycythaemia is often a pancytosis, with increases in all three major lineages, and so may, in certain cases, be an atypical leukaemia with a leuko-erythroblastic blood picture.

Despite the common use of radioactive phosphorus, links with myeloid metaplasia and leukaemia (which, in one study, developed in 8% of patients), PV was not classified as a myeloproliferative disease until the 1960s, with the presence of chromosomal abnormalities noted in 1970. As the century developed, so did the recognition that PV can be a multi-lineage disease, with increases in the numbers of white blood cells and platelets. In the 1960s, the role of erythropoietin (the leading erythropoietic growth factor, a product of the kidney) was described, whilst in the 1970s, complex tissue culture investigation showed increased erythroid progenitor activity in PV but not in secondary disease. The molecular basis of Chuvash polycythaemia was described in the 1990s, and that of PV in the early 2000s.

Essential Thrombocythaemia

Literature from the 1930s and 1940s referred to several cases of high platelet count, often with hepato- and splenomegaly, essential thrombocythaemia (ET) being reported in the 1960s, with many cases accompanied by haemorrhage. In the early 1980s, two groups provided evidence that the lesion was clonal at the level of a multipotent

stem cell, whilst others reported cases of the transformation of ET into myelofibrosis (MF), and to myeloid leukaemia. Primary thrombocythaemia was classified as a myeloproliferative disorder in 1986, and in 1991, ET was purported to be due to the impaired regulation of megakaryocyte progenitors, whilst reports of the molecular basis appeared in the late 1990s.

Myelofibrosis

As the name implies, the aetiology of MF is fibrosis of the bone marrow, described in two case reports from 1937. A key aspect of diagnosis is the demonstration of fibres of reticulin and/or other connective tissue components in a bone marrow sample. A further feature is the presence of extra-medullary erythropoiesis or haemopoiesis, most often in the spleen, followed by the liver. As with many other myeloid diseases, MF became classified as primary or secondary, and the possibility of transformation to leukaemia acknowledged. Classification systems of MF were published in 1980s and 1990s, with the WHO guidelines following to the present. In 1978, a case terminating in AML reported duplication of part of the long arm of chromosome 6, whilst genetic analysis of idiopathic (i.e. primary) MF may be traced to a report in 1987, which also included cases of PV and ET, and role of growth factors contributing to the aetiology of MF acknowledged in 1992.

Myelodysplasia

A literature search on this topic is confounded by the use of the term in an orthopaedic bone and joint setting, with one of the first uses in haematology being in 1978 by Broxmeyer and Moore, and although there was no clear definition, it refers generally to disordered haemopoiesis and a pre-leukaemic state. A year later, a case of myelodysplasia (MDS) terminating in acute leukaemia was reported, with MDS being described in the early 1980s. By the end of the decade, it was linked to an accompanying anaemia, such as refractory, with ring sideroblasts, or with excess blasts, and cytogenetic abnormalities noted. Subsequent research noted that around 50% of cases were clonal, and by 2002, MDS was part of the WHO classification of the myeloid cancers.

1.3.5 The Epidemiology of Blood Cancer

The key sources for the epidemiology of blood cancers are online publications of the WHO and of the UK's Office for National Statistics (ONS). The various forms of all types of diseases follow the system of the International Classification of Diseases, also available online.

Data from the WHO

The leading source of information is the WHO's International Agency for Research on Cancer, also described as the Global Cancer Observatory, which reported a 2022 incidence of 19,976,499 cases of cancer and 9,743,832 deaths. These important statistics therefore give an indication of the likelihood of death from cancer, while dividing mortality by incidence gives 0.488, i.e. slightly less than a 50% chance, once diagnosed. Data from other sources differ slightly: from the European Commission, these are 3,483,065 and 1,640,724, and so a marginally better likelihood of 0.471. The WHO collects and publishes data only on leukaemia, Hodgkin lymphoma, NHL and multiple myeloma: data on the other blood cancers are not reported. The incidence and prevalence of these conditions globally and in the UK from 2022 are shown in Table 1.3, showing that the global likelihood of mortality from blood cancers is some 9% higher than from all cancers, but in the UK this figure, around 14% lower, is therefore markedly better.

Globally, the incidence of all blood cancers relative to all cancers is in the order of 6.6%, with mortality being around 7.2%. In the UK, these figures are 7.8 and 8.2%, respectively, so in both cases, these cancers bring an above-average risk of mortality. Both globally and in the UK, NHL has the highest incidence, whereas globally, leukaemia has the highest mortality rate, and in the UK, this position is held by NHL. Differences in both sets of metrics may be at least partly explained by the population frequency of particular diseases and by the effects of the health care systems.

Table 1.3 Incidence of and mortality from blood cancers.

	All blood cancers	Leukaemia	Non-Hodgkin lymphoma	Hodgkin lymphoma	Multiple myeloma
Global					
Incidence	1,311,104	487,294	553,389	82,469	187,952
	(1)	(0.372)	(0.422)	(0.063)	(0.143)
Mortality	700,205	305,405	250,679	22,733	121,388
	(1)	(0.436)	(0.358)	(0.032)	(0.073)
Likelihood of mortality	0.534	0.627	0.453	0.276	0.646
UK					
Incidence	35,576	10,755	16,047	2,270	6,504
	(1)	(0.302)	(0.451)	(0.064)	(0.183)
Mortality	14,934	5,335	5,671	310	3,618
	(1)	(0.357)	(0.380)	(0.021)	(0.242)
Likelihood of mortality	0.420	0.496	0.353	0.136	0.556
England and Wales					
Mortality	12,193	4,368	4,555	290	2,774
	(1)	(0.358)	(0.373)	(0.024)	(0.227)

Note: Data denote the number of persons in each category or the likelihood of mortality. Data in parenthesis are proportions relative to all blood cancers.

Data from the ONS

This source focusses on England and Wales and describes blood cancer as 'malignant neoplasms of lymphoid, haemopoietic and other tissues'. Some of this data for 2023 is shown in Table 1.3 and broadly mirrors the WHO data on the UK. However, the ONS also reported 47 deaths due to other specified types of T/NK cell lymphoma, 105 deaths due to malignant immunoproliferative diseases and 54 deaths due to other forms of blood cancer. Overall, blood cancer accounted for 12,193 deaths, which constituted 8.2% of all cancer deaths (of which there were 148,705), hence exceeding those of the prostate (11,072, 7.4%) and breast (9,973, 6.7%), but less than the deaths from colorectal cancer (15,946, 10.7%) and of the bronchus and lung (27,808, 18.7%).

1.4 The Molecular Pathology of Cancer

An understanding of the clinical aspects of blood cancer would be incomplete without a basic understanding of the cytogenetic and molecular genetic aspects of the disease, not merely because in numerous cases these abnormalities are linked to management.

1.4.1 Carcinogenesis

Introduction

Almost all the cells in the body at some point have a need to reproduce (by mitosis), and most develop a soluble product by transcription and translation, with these key components of physiology generally working well.

However, should this process become faulty, a cell may transform into one with a malignant potential. The process by which a normal gene becomes an abnormal gene is the primary feature of carcinogenesis, perhaps by the action of a carcinogen such as a toxin or by the effects of ionising radiation, which can damage the DNA.

Normal and Abnormal Genes

It has long been established that many of us, perhaps all of us, harbour abnormal genes, often in all the cells of our body, and these genes are described as somatic, which are normally dormant, and so with limited oncological potential. Cancer may arise when these abnormal proto-oncogenes are somehow 'awakened' by a carcinogen or by the action of another gene, to become fully active oncogenes. Alternatively, one single apparently healthy cell can acquire a mutation in its DNA due to the action of a carcinogen, and become malignant. This system of proto-oncogenes and oncogenes is a fully established oncological process in many diverse cancers in various organs and organ systems. There are several routes by which abnormal genes lead to cancer, some of which are discussed in the following.

Signal Transduction

Mitosis is highly regulated, with the cell receiving information from the exterior which then passes on to the nucleus for processing, which may lead to replication. Transcription also follows from the instructions received at the cell membrane, and results in the generation of messenger RNA and so a protein product. Errors in either of these processes can lead to cancer. The physiology of this process is as follows:

- Step 1 in the process is the external factor, which may be a physical interaction with another cell, or a soluble ligand, such as insulin, with its receptor at the cell surface (in this case, the insulin receptor). Once the two have interacted, a conformational change in the receptor occurs, which is detected by soluble components of the cytoplasm, including enzymes.
- Step 2 is that the message is transferred to the nucleus by a host of cytoplasmic second messengers, with the process being described as signal transduction. In many cases, this process consists of a cascade of molecules that are serially activated and then activate another, in many cases by the addition of a phosphate group, hence phosphorylation.
- Step 3 involves the terminal members of the signal transduction pathway entering the nucleus (perhaps by a receptor in the nuclear membrane) to interact with the DNA via transcription factors and therefore promoting or preventing replication or transcription.

Should any of these steps be at fault, cancer may arise. In step 1, this may be due to the altered receptor product of an abnormal gene (i.e. an oncogene) that does not need to be activated by binding its ligand, and therefore becoming permanently activated. Similarly, genes coding for second messengers may transform, and therefore produce a mutant molecule that is constitutively activated, independently of the first step. The same principle applies to many other cellular processes. Figure 1.13 show key concepts of the signal transduction pathway.

The signal is passed from the receptor, via second messengers, to the nucleus. The importance of the kinase enzyme will be explained in the following sections, such as in the development of a chronic myeloid leukaemia (Section 3.4) and its treatment (Section 3.4.3).

Tumour Suppressors

Genes can cause cancer not only by their presence but also by their absence. Should the processes of replication and translation be defective, with the formation of altered, perhaps damaged DNA, it can be corrected with repair pathways. Similarly, should DNA be damaged by an external agent, it too can be repaired. The function of these genes is to code a product, often a transcription factor or direct gene regulator, that has a role in cell physiology

such as control of the cell cycle. Therefore, loss of these repairing and regulating systems can lead to the altered but active DNA remaining in the genome, and continuing to function in an abnormal manner, which may lead to cancer. Failure of tumour suppressors such as *TP53* are important features of many blood cancers, as we shall see in sections to come.

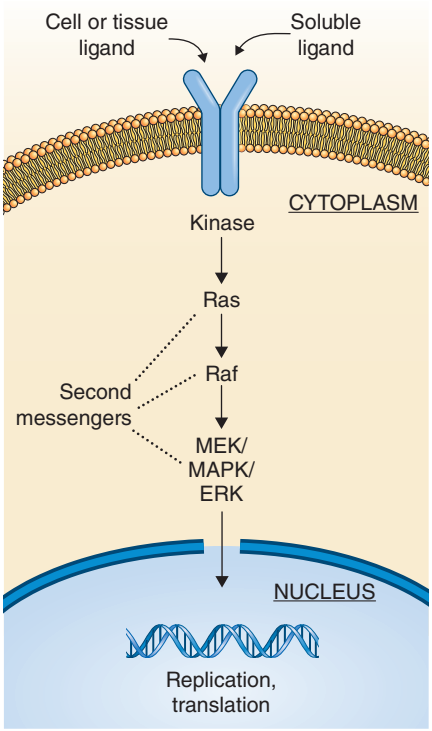


Figure 1.13 Signal transduction.

Apoptosis

Should the cell itself detect an abnormality, regardless of whether it may be transformative, the process of apoptosis can take steps that will result in the death of the cell, which involves the activation of the caspase family of enzymes. The initiating steps in certain apoptotic pathways are linked to second messengers, so that failure of this pathway, or of the generation of caspases by their own genes, may lead to the escape of the cell (which may have malignant potential) from apoptosis, leading to a possible cancer. Genes involved in this process include *BCL-2* and *BCL-6*.

Epigenetics

DNA is packed within chromatin by wrapping around the histone proteins, and as part of this packaging, some of the double helix is exposed. This histone/DNA complex can be modified by the enzymatic attachment of methyl and acetyl groups. Other enzymes, such as that coded for by *HDAC1*, remove these groups. Certain genes coding for these modifying enzymes can themselves become abnormal, thereby producing enzymes with different activities, some of which can lead to transformation.

1.4.2 Chromosomes

Physiology and Fine Structure

The many diseases linked to changes in chromosomes demands a revision of the biology of these crucial macromolecules which, as chromatin, are composed of DNA, packed together with protein histones. Sections of histones which do not take part directly in DNA processing may be covalently modified by the addition of chemical groups to certain amino acids such as cytosine and lysine, most often leading to methylation and acetylation. In many cases, the addition of these groups by transferases radically alters the function of the nearby genes, such as their silencing. Accordingly, these enzymes may be the target of pharmacotherapy.

The haploid genome comprises a little over 3,000 Mbp of nucleotides with a little under 20,000 protein-coding genes, but these data sum to only <2% of the genome. However, the remaining 98% (once described as 'junk' DNA) may not all be inert: there is much that codes for RNAs that do not translate, and there are also some 14,000 non-functioning pseudogenes. It is possible that some pseudogenes may be regulators, such a potential in driving erythropoiesis. The genome also contains around a million duplications of 300 base pair sequences known as *Alu* repeats. Mitochondrial DNA is a ring with 16,569 base pairs, consisting of 37 genes that code for transfer and ribosomal RNA, and components of the electron transport chain.

Chromosome integrity is assessed by cytogenetics. This calls for the ability to manipulate the cell and nucleus with chemicals in order to separate the individual chromosomes so that their number can be determined in the form

of a karyogram. Figure 1.14 shows a normal male karyogram of 46 chromosomes, where the karyotype is 46,XY, described as euploidy (eu = true, ploidy = normal chromosome number). The normal female karyotype is 46,XX.

Described by Flemming in 1882, the centromere lies between two arms, with the shorter arm being described as p (in French for small – *petit*) and the longer arm described as q (as it follows p in the alphabet). When the centromere is roughly in the middle of the chromosome, it is said to be metacentric; when slightly offset, it is submetacentric; and when markedly offset, it carries the descriptor acrocentric. The extreme ends of the chromosome are the telomeres which, alongside the centromere, do not code for a product, with the nucleotide sequence TTAGGG being repeated up to 800 times, providing protection from erosion and damage.

In the late 1960s and early 1970s, Caspersson and co-workers published on the variations in the density of uptake of a fluorochrome in the DNA within an isolated chromosome into bands of intense and light staining. Subsequent staining with dyes such as Giemsa further refined this process, enabling each chromosome to be identified according to its banding profile. Sections with a high component of cytosine and guanine, which are loosely packed, stain lighter, whilst dark staining G-bands are more likely to localise dense chromatin enriched for thymine and adenine. Others used fluorescent dyes such as propidium iodide and diamidino-2-phenylindole to reveal alterative banding patterns, which are numbered from the centromere to the telomere (Figure 1.15). In the years that followed, these bands also provided the opportunity to map the location of particular genes.

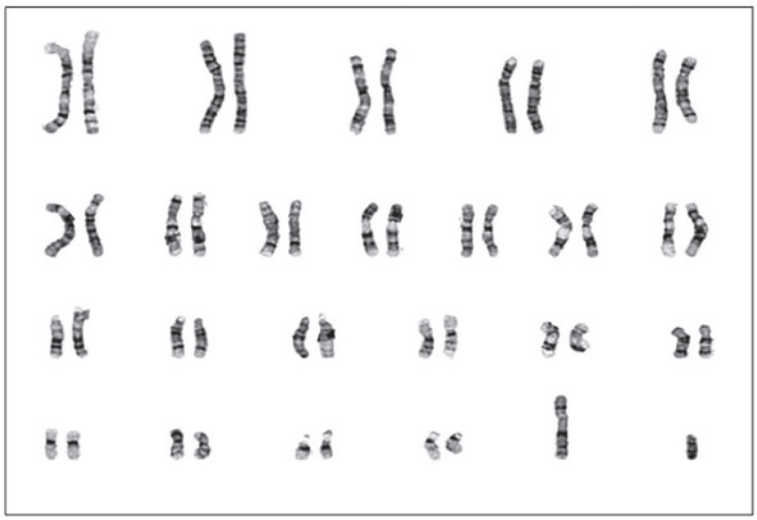


Figure 1.14 A male karyogram. *Source*: Blann, 2025/with permission of John Wiley & Sons.

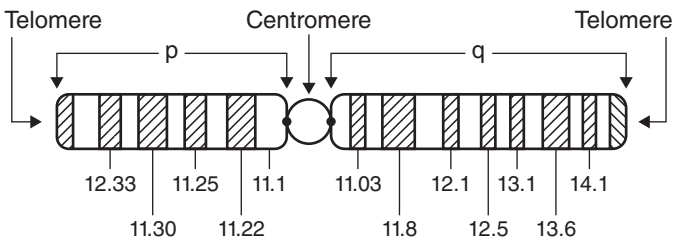


Figure 1.15 Structure and banding of a chromosome. The numbers refer to particular bands. The size and position of the bands are for illustrative purposes only. *Source*: Blann, 2025/with permission of John Wiley & Sons.

Table 1.4 Principal cases of aneuploidy.

Karyotype	Clinical descriptor	Frequency
46,XO	Turner syndrome	500/million female births
47,XY or 47,XX	Down syndrome (Trisomy 21)	1,150/million
47,XY or 47,XX	Edwards syndrome (Trisomy 18)	200/million
47,XY or 47,XX	Patau syndrome (Trisomy 13)	46–100/million
47,XXY	Klinefelter syndrome	1,000/million male births
47,XYY	Jacob syndrome	980/million male births
47,XXX	Triple X syndrome	1,000/million female births

Alterations in Chromosome Number

There are several instances where the chromosome number deviates from normal, and as such is described as aneuploidy (i.e. not euploidy). When the chromosome number exceeds euploid, hyperploidy is present (i.e. there are duplicated chromosomes, one being a trisomy), and when it is less than euploid, hypoploidy is present (i.e. a chromosome is missing, often described as a monosomy). Table 1.4 shows the principal cases of aneuploidy, with one hypoploidy, three trisomies and three with an additional sex chromosome.

Among the cases of hyperploidy, an increased risk of leukaemia (principally AML) in Down syndrome was recognised in the 1950s and later ascribed directly to the chromosomal lesion. Notably, the same trisomy is also linked to leukaemia in those free of Down syndrome. Whilst in many cases the abnormality is present in every cell in the body (i.e. it is germline), there are numerous examples where it is present only in certain cells (i.e. is sporadic, or somatic), in which case a mosaic is present. Examples include the cases of Turner syndrome, where some cells are XX, other XO, and Klinefelter syndrome, where both 46,XY and 46,XYY are present. Depending on the particular cells in which a mosaic is present, physiological and/or pathological changes may be present.

1.4.3 Intra-chromosomal Pathology

Further advances in cytogenetics and other techniques demonstrated a number of changes within and between chromosomes with a direct bearing on malignant transformation. Many of these changes occur at susceptible positions in the DNA, described as breakpoint regions (BCR, as in the lesion of the Philadelphia chromosome). Other descriptors include the umbrella term gene rearrangement, which is present in all of the genetic lesions that follow. These generally occur during mitosis, with errors at the metaphase plate, and can be illustrated using words and letters, with a specific abbreviation as defined by the International System for Human Cytogenetic Nomenclature (ISCN).

Translocation

This common and important change involves two chromosomes which swap sections of their DNA and may bring together two genes that would never normally meet, which can have considerable pathological significance. An illustration of this is XXXPATHOXXX on one chromosome being placed next to XXXLOGICALXXX on another, so forming XXXPATHOLOGICALXXX. As we shall see in several chapters to come, translocations are frequently found in all forms of blood cancer, and in many other malignancies.

Deletion

This is loss of a segment of a chromosome, and so any number of genes, depending on its size, but there may also be deletion of a single nucleotide. There are many examples of how a fully functioning XXXMOSTCRUCIALXXXX section becomes XXXMOSIALXXX, and so inactive, with pathophysiological consequences, especially in cancer.

Duplication/Amplification

In this abnormality, a section of a chromosome is replicated, generally within the same arm, and occasionally the copy may be continuous with the original section. This may lead to an increase in its protein product, such as a mitosis regulator or a cell-surface receptor. Pseudogenes may also be regarded as duplicates, but of protein-coding genes that have loss-of-function mutations of microdeletions. Together, short insertions and deletions may be described as indels, of which there are predicted to be some 400,000–700,000 in each genome.

Inversion

This occurs when the left-to-right (or vice versa) orientation of a section of a chromosome is reversed, so the order of genes gets disrupted, such as XXXXFUNCTIONINGXXXX becoming XXXFUNNOITCINGXXXX, often described as a nonsense gene. The inversion may be within only one of the chromosomal arms or include the centromere, described as paracentric or pericentric, respectively. This change may bring together genes that are normally far apart or that are simply inactivated: both may lead to a malignancy.

Insertion

This can be a section from another chromosome, or a section of DNA from a virus, and may be as small as a single nucleotide, in which case a frameshift will occur (as in many cases of cystic fibrosis). This may be illustrated by GER being placed into the middle of XXXDANOUSXXX. An example of this is the hepatitis B virus insertional mutagenesis causing hepatocellular carcinoma. However, the insertion of alien DNA into a functioning gene is likely to render it non-functional, with an example being the disruption of XXXWORKINGXXX by ABCBA, forming the nonsense gene XXXWORABCBAINGXXX. Insertions into tumour suppressor genes can render them inactive, and therefore disabling them from suppressing a potential malignant transformation.

Isochromosome

This is formed from one of the arms of a chromosome being present on either side of the centromere and so represents both a deletion and a duplication. Should this occur, an isochromosome will have two copies of the same p arm, or two copies of the same q arm.

Ring Chromosomes

This abnormality develops in the merger of two terminal ends, occasionally incorporating the teleomere(s), and so the DNA is neither gained nor lost. The shorthand r(6)(p23;q25) tells us that the break and rejoin position is between p23 and q25.

Single Nucleotide Polymorphisms

Research into the haemoglobinopathies was undoubtedly the first to demonstrate a direct link between an exact abnormality in a gene, the malformed protein product, and a relatively common clinical disease. These changes were described by different researchers as a point mutation, a single nucleotide substitution, a single nucleotide transition and a single nucleotide base mutation, which we now refer to as a single nucleotide polymorphism (SNP), although an alternative descriptor for an SNP is a single nucleotide variant (SNV). Each of us are estimated to have 3–4 million SNPs (5,000 restricted to the individual, i.e. 'private'). Despite this high frequency, not all are pathogenic as certain codons, such as AAA and AAG, both code for lysine, and are described as synonymous.

Table 1.5 summarises certain other shorthand notations in this section.

Table 1.5 Selected shorthand notations for chromosome variants.

Shorthand	Meaning	Example
t	Translocation	t(9:22)(q34;q11)
del	Deletion	del(7p) or −7p
inv	Inversion	inv(X)(p22.2;q13.1)
der	Derivative	der(21)
i	Isochromosome	i(12p)
r	Ring chromosome	r(6)(p23;q25)

1.4.4 Implications for Clinical Practice

As will be discussed in detail in the chapters to come, the first important *translocation* to be described was of the Philadelphia chromosome and its link with chronic myeloid leukaemia (CML). This class of abnormalities is present in many blood and other cancers.

Deletions too are common in many haematological conditions, and the blood cancers, and we have already discussed the deletion of an X chromosome in Turner syndrome, whilst monosomy of chromosome 7 has been observed in around one-third of cases of juvenile myelomonocytic leukaemia. A deletion in the q arm of chromosome 22, so described in ISCN terms as del(22q), may be present in DiGeorge syndrome, where complete or partial loss of a thymus leads to reduced or absent T lymphocytes. One of four deletions are present in 80% of patients with chronic lymphocytic leukaemia (CLL), with the most common (in 55%) being del(13q), whilst del(7p12.2) of *IKZF1*, coding for a transcription factor important in regulating the cell cycle, is present in around 15% of paediatric acute lymphocytic leukaemia (ALL) cases.

However, a leading form of deletion in oncology involves some or all of the nucleotide sequences of certain *tumour suppressors* described in Section 1.4.1. Loss-of-function SNPs (as may result in the substitution of a nucleotide in a crucial region) or deletions in key parts of these genes prevent their function in regulating and correcting abnormalities. In effect, therefore, these abnormalities in the DNA are not removed or corrected and so may lead to a cancer by the 'escape' of a malignantly transformed cell. This explains why deletion of 17p13.1 or 13q14.1 is common in cancer as these are the locations of *TP53* and *RB1*.

One of the more prominent forms of *duplication* in blood cancer is an internal tandem duplication in *FLT3* at 13q12.2 hence (FLT3-ITD), leading to a gain in function that bring a poor prognosis when present in an AML. Another tandem duplication, in *UBTF* at 17q21.31, coding for upstream binding transcription factor, is present in ~4% of paediatric AMLs. Copy number variation (CNV) of various sections of a chromosome is an important feature of many acute leukaemias. In a study of 99 ALLs, at least one CNV was present in 65% of cases, most being of *PAX5*, *CDKN2A* and *CDKN2B*, whilst a subset of T-ALL cases demonstrated selective amplification and overexpression of the oncogene *MYB*.

An example of an oncogenic *inversion* carcinogenesis is illustrated by some AMLs where a pericentric inversion in chromosome 16 reverses the section between p13 and q22, written in ISCN shorthand as inv(16)(p13;q22). This brings together *MYH11* at 16p13.5, coding the muscle myosin heavy chain, with *CBFB*/*PEBP2B* at 16q22, coding for the transcription factor core-binding factor beta subunit, a fusion that causes a block in myeloid differentiation, and so maturation arrest, causing leukaemia.

Cryptic *insertion* of *MYC*, an oncogene present in various cancers, has been described in some cases of Burkitt lymphoma. The non-translocation cryptic insertion of *BCR* next to *ABL1* at 9q34.12 or, conversely, of *ABL1* next to *BCR* at 22q11.23, both generate a CML. Whilst FLT3-ITD is of undoubted importance, some consider certain

tandem duplications to be an insertion. Although, as described above, the leading pathogenic aspects of *KMT2A* are duplications and translocations, it may become inserted into or adjacent to other genes such as *AFF3* at 2q11.2, coding a nuclear transcriptional activator, with carcinogenic consequences. Should any particular genetic lesion have a size greater than 50 base pairs, it may be described as a structural variation.

Despite the above, it has been suggested that SNPs are the leading pathogenic abnormality in cancer and in blood cancer. One source suggests that of the almost 85 million SNPs, only 167,000 have links to disease, a selection of which is as follows:

- SNPs that inhibit the function of *PAX5* (at 9p13.2, coding a transcription factor) increase the likelihood of B cell ALL.
- Pre-B cell ALL and Waldenström's macroglobulinaemia are linked to *TP53* SNPs at codons 276 (GCC to CCC, Ala to Pro) and 237 (T insert, Asn to stop), respectively.
- A substitution SNP in *N-RAS*, such as GGT to GAT (Gly to Asp), is found in 7 of 20 cases of juvenile chronic myelogenous leukaemia.
- Loss-of-function SNPs in tumour suppressor *CDKN2* are present (and are probably causative) in adult T cell leukaemia.
- Increased expression of an SNP in *IL10*, coding the immunosuppressive cytokine IL10, can be present in Hodgkin lymphoma and reactive benign follicular lymphoid hyperplasia.

SNPs are also relevant in management. Common forms of cytotoxic chemotherapy include 5-fluorouracil (5-FU) and capecitabine (which is metabolised to 5-FU), which are detoxified principally by hepatic dihydropyrimidine dehydrogenase. Levels of this enzyme are controlled by *DPYD* (located at 1p21.3) such that low activity leads to slow removal of the drugs, and so a longer half-life. This greater cytotoxic effect has potentially fatal consequences for the patient, such as liver and renal failure. Accordingly, any patient starting these drugs will need to have their *DPYD* status determined, so that the dose of the drug can be adjusted accordingly and toxic effects minimised. Similarly, *TPMT* at 6p22.3 codes for thiopurine methyltransferase, which adds a methyl group to thiopurine drugs such as azathioprine, 6-mercaptopurine and 6-thioguanine. Variants of this gene produce different levels of the active enzyme, and so influence levels of the particular anti-metabolite, high levels of which can also cause undesirable effects such as bone marrow suppression. This meeting of genetics and pharmacology may be described as pharmacogenetics.

The discovery and recognition of non-coding RNAs (ncRNAs) can be traced back to the concept of junk DNA that transcribes to RNA, but which fails to translate, including ribosomal and transfer RNAs. However, the genome also contains some 14,000 variants of long non-coding RNAs (hence lncRNAs), 5,000 small ncRNAs, and 2,000 very small micro-RNAs (miRNAs), many of which have regulatory roles, abnormalities in which have been shown to be linked to pathology and cancers.

CLL was one of the first diseases to be linked to abnormalities in the miRNAs, where del(13q) is associated with a reduction in miR-15a and miR-16-1 in 66% of patients. A possible implication for carcinogenesis arises as these molecules would normally act to reduce the levels of the antiapoptotic protein Bcl-2, coded for by *BCL-2* at 18q21.33, thereby promoting apoptosis. Therefore, their absence fails to modulate Bcl-2, hence reducing the likelihood of an abnormal cell moving into apoptosis, which would therefore survive and potentially become malignant. A second target of the miRNAs is *ROR1* at 1p31.3, coding a type 1 trans-membrane protein with tyrosine kinase activity, expressed on the surface of CLL cells and with onco-embryonic activity and effects on proliferation. A further example is miR15a targeting *c-Myb* and hence influencing the development of CFU–E, whilst miR-451 targets *GATA1*, abnormalities that have been implicated in erythroid blood cancers, and often in other myeloid malignancies.

Whilst the studies of ncRNAs provide fascinating insights into gene regulation and carcinogenesis, they have not, as yet, entered routine practice in blood cancer, but may do so in the coming decades.

1.5 Chapter Summary

This introductory chapter has set the scene for the remainder of this book. Section 1.1 described key features of haemopoiesis, arising in the bone marrow and forming various lineages for each of the different types of blood cells. This was followed by an explanation of the function of these cells, and the various organs of relevance to blood cancer. The blood science laboratory (Section 1.2) provides important routine information on the constituents of the blood, and how these are relevant to the pathophysiology of these cancers. Other important tools are histology, and its sub-analyses, especially valuable in examining samples of bone marrow and soft tissues such as lymph nodes.

The historical perspective in Section 1.3 summarised the development of blood cancer in each of the major groups of diseases, and provided an epidemiological comparison of the frequency and mortality with other diseases, and of the diseases that make up blood cancer. Section 1.4 introduced the molecular biology of cancer, and then summarised how leading abnormalities in chromosomes and in genes are linked directly to a malignant transformation. This final section also provided a bridge to Chapter 2, where details of laboratory methods in molecular pathology are discussed.

References

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