CHAPTER – I

REVIEW OF LITERATURE
Leukaemia, the malignant state of leucocytes in blood has been discovered as back as 1838 by Richard Bright of Guy’s hospital. It took several years to ascertain the definitive explanation to such a diseased state, as, various overlapping components were found to mask the actual status under the event. Investigations pertaining to the mechanisms of initiation of leukaemia revealed a number of facts relating to the uncontrolled proliferation of leucocytes with a typical pathological feature and clinical symptoms involvement of the cellular dysfunction was thought to be of gene-locus oriented problems or sometimes a somatic expressions bearing definite chromosomal heterogeneity. [Luscher & Eisenmer 1990 (1), Golay et al 1996 (2)]. Apart from a large number of etiological factors like radiation, chemical carcinogen and residual bone marrow disorder, a number of transcription factor have also been implicated a development of leukaemia, the different oncogene like c-myc (Wolf et al 1997) (3) c-myb (Henrikson et al, 1996) (4), P53 (Mashal et al, 1990) (5), (Nakai et al, 1995) (6), C-Jun (Bohmal et al, 1987) (7) (Molliendo et al, 1993) (8), abl-bcr (Macdonald & Ford, 1996) (9), Homeobox gene (Lawrence et al, 1995) (10), bcl1 & bcl2 (Schuuring, 1995) (11) and few more were held responsible for induction of leukaemia in individual. Such defects were reflected in chromosomal abnormalities, eg, dislocation, fremsifting, abparation etc.

A large array of research investigation are still in progress with a target to identify the exact cause(s) of leukaemia inductions of which immunological characteristics were found to occupy the key mechanisms under the state of leukaemia. The characteristic feature of leukaemia cells were studied in detail with respect to their surface architectural
conformity, distribution of antigen receptors together with other immune marks serving as distinguishing components. Many such findings finally furnished that immunological status of the leukaemic subjects can play a vital role in individuals.

In early days of this Century it was extremely difficult to separate leucocytes from other formed elements of the blood, particularly red cells, and procedures employed for separation were inclined to cause cell damage of uncertain extent. The simplest and most widely used procedure was agglutination in vitro, a method long employed in experimental studies of the action of strong anti-leucocytic hetero-agglutinins, are reviewed by Cajano and Maurea (1950) (12). By this means, Moeschin and Wagner (1952) (13) demonstrated the presence of leucocyte agglutinins in these serum of patients with pyramidon-induced agranulocytosis. Lysis and phagocytosis of leucocytes in the presence of anti-leucocytic serum had been used to observe and assess the extent of antigen-antibody reactions [Finch, Ross and Ebach, 1953 (14); Miescher, 1953 (15); Robineaux, 1954 (16), Bessis, 1955 (17)]. Sensitization of leucocytes by antibodies had also been demonstrated by an antiglobulin test [Steffen and Schindler, 1955 (18); Van Loghem, der Heart and Dorstel, 1957 (19)], and by complements fixation [Milgrom et al, 1957 (20)], while specific precipitation of leucocytic antigens by appropriate antisera had been carried out using modifications of the ring-test and two dimensional gel methods of Qudin (1946) (21) and Quchterlony (1948) (22). In 1957 Seligman (23) had used also an immuno-electrophoretic technique for separating precipitation reactions involving different leucocytic antigens.
By the use of these multiplicity of technical methods, the antigenic structure of leucocytes has been increasingly revealed in recent years. At least ten specific nuclear or cytoplasmic antigens have been distinguished in normal leucocytes by precipitation in gel [Seligmann, Grabar and Bernard, 1955 (24)]. The possibility that leucocytes might be separable into groups with differing antigenic constitution, similar to the erythrocytes subdivisions, has been explored. Dausset, in 1954 (25), Payne in 1957 (a & b) (26,27) have reported about the presence of surface antigens of leucocytes and these surface antigens appear to be of great importance in stimulating isoantibody formation in patients. Dausset et al in 1957 (28) have further supported the presence of an antigen "Mac" in about 60 per cent of blood samples taken at random in France. It is upon this background of rapidly growing knowledge of normal leucocyte antigenicity that studies of leukaemic cell antigens must be viewed. No evidence of antigen specificity peculiar to leukaemic cells was found. The complement fixation reaction was used by Maculla (1947) (29) to compare the antigenic structure of various normal and tumour tissues from the mouse. Tumour cells were found to possess antigens in common with normal cells, but to have additional components in some cases. Specific antileukaemic cell antibodies were not, however, detected. An antibody or group of antibodies specific for the cells or an induced leukaemia in DBA/2 mice was obtained by Thompson (1955) (30). Antisera prepared in rabbits gave complement fixing reactions with both normal and leukaemic lymphocytic antigens, but absorption with normal lymphocytic antigen left an antibody residue reacting only with leukaemic tissue. In vivo cases, anti-leukaemic sera did not afford protection to other DBA/2 mice against transplanted tumour growth. There is, however, considerable indirect evidence of an abnormal immunological state in leukaemia, since the plasma in many
cases manifests activity of an "autoimmune" kind against erythrocytes and sometimes against leucocytes. [Ansdre et al 1954 (31)]. Evidence of Erythrocyte sensitization by abnormal "automimmune" gamma globulins in lymphocytic leukaemia has frequently been reported; the phenomenon is substantially confined to lymphocytic forms of the disease and is rarely, if ever, encountered in leukaemias of other cell types, [Davis; 1944 (32), Stats et al 1947 (33); Rossenthal et al 1955 (34). Rossenthal et all (1955) (34) have concluded that this might be due to disruption of lymphoid tissue in leukaemia, resulting in the elaboration of abnormal gammaglobulins immunologically against erythrocytes. Pirofsky (1956) (35) have reported that actively haemolytic material appears to be present in leucocytes from different varieties of leukaemia and is not confined to leukaemic lymphocytes; it is present also in cells of some patients who manifest no obvious haemolytic process.

The protein deletion hypothesis arose from observations that metabolites of carcinogenic aminoazo dyes combined with proteins in affected tissues in vivo [Miller and Miller, 1952 1953(36, 37)]. Further evidence suggested that protein-dye combination was an essential preliminary to later carcinogenesis, and that the tumour cells finally produced were deficient in those proteins which initially combined with the dye [Sorof and Cohen, 1951 (38)]. A similar deletion of proteins and enzymes might be brought about by physical and viral agencies. The later development of tumours in tissues so affected would be a consequence of deletion of proteins or enzymes having essential activities in regulating cell growth and development, but this supposition so far lacks experimental support. Green et al 1957 (39) have reported, that the final state of antigenic loss postulated to exist in the developed neoplastic cell would enable the cell to react immunologically with
antibody formation to the challenge of the specific tissue antigens it had lost.

Smith (1972) (40) has reported that the tumour bearing host is perfused continuously with tumour membrane proteins, fragments, and even whole cells. This antigenic barrage initially engages specific recognition receptors, leading to proliferation of lymphoid cell subsets, secretion of antibodies, and generation of cytotoxic cells may also express, in their membrane, structures of direct or indirect viral origin. Any of the so-called 'self-components' - those associated with embryonic development, organ or tissue specific differentiation structures, structures present at specified phases of the cell cycle or structures novel because they represent degraded membrane products-may be immunogenic to the lymphoreticular system (Boyse, 1970) (41).

Haywood and Mackham have shown in 1971 (42) that inverse correlation between the detectability of H-2 specificites on tumour cell membranes and tumour immunogenicity. They have found that the stimulating capacity of soluble tumour antigens has roughly an inverse relationship to the immunogenicity of the tumour from which it was obtained.

Smith in (1972) (40) has further discussed with tumour associated tumour antigen or TATA that chemically induced tumours have a unique transplantation antigen, whereas virus-induced tumours crossreact regardless of the organ system. So different studies of different workers concluded that leukaemic cells may have additional or different antigens or may lack some important antigens normally present. The leukaemic cell may perhaps be sufficiently foreign in structure to stimulate
antibody formation, and may even itself elaborate globulins with antibody activity against constituents of erythrocytes or other cells.

Recently in the year of 1990, Van Camp et al (43) and pellat deceunyek et al in 1995 (44) have observed in the case of multiple myeloma that a high expression of CD44, CD29, CD49d (VLA-4) and CD54 has been in normal and myelomatous plasma cells, whereas CD56 and CD58 (LFA-3) are expressed at higher levels on malignant plasma cells rather than normal plasma cells.

**The Immune Mechanism and Leukaemia:**

From the above studies of different workers, it has been found that the antigens which are the different oncogenes product responsible for the development of leukaemia through the modulation of cell subsets, but experimental evidence is still lacking on too many fundamental issues for any firm conclusions to be drawn.

The successful maintenance of living cells in artificial culture media outside the body, under conditions of controlled environment, would provide most valuable instrument for fundamental research on cell characteristics. The first attempts to grow bone-marrow cells in artificial culture were reported by Carrel and Burrows (1910) (45) and observed the outgrowth of spindle shaped cells after 3 day's incubation. Awrorow and Timofejewsky (1914) (46) used the plasma clot method to study in vitro the behaviour of leucocytes from the peripheral blood of patients with granulocytic leukaemia. In culture emigration of polymorphs and a few myelocytes was observed, while less mature cells were thought to be transformed into "polyblasts" or macrophages, with increased amount of cytoplasm, often vacuolated and containing phagocytosed materials.
Fibroblast-like cells were also prominent, and these were considered to arise from myeloblasts. Transformation of myeloblasts to myelocytes was not established, but some evidence of a myelocyte to metamyelocyte differentiation was noted. Timofejewsky and Benewolenskaja (1927, 1929) (47,48) concluded that macrophages and fibroblast like cells could arise from the lymphocytes and lymphoblasts of chronic lymphocytic leukaemia or from haemocytoblasts or myeloblasts of acute and chronic granulocytic leukaemia, but that the primitive nucleolated cells of acute myeloblastic leukaemia were also able to differentiable *in vitro* into neutrophil, eosinophil, and basophil myelocytes, which could subsequently from polymorphonuclear leucocytes. Timofejewsky (1929) (48) drew particular attention to his observation that the myeloblasts of acute leukaemia, though apparently unable to differentiate *in vivo*, could do so *in vitro*, and suggested that leukaemia should not be regarded as a malignant-neoplasm, since the cells had not entirely lost their capacity to mature normally. Hirschfeld (1927) (49) confirm the rapid degeneration of mature cells and the transformation of more primitive ones of each leucocytic series into spindleshaped cells and sometimes into macrophages. In this work and in later studies of lymphocytic leukaemia in cell culture (Hirschfeld and Klee-Ravidowiez, 1928), (50) the lymphocytes showed little sign of transformation, and the macrophages and fibroblast like cells were considered to arise from monocytes or immature cells. Veratti (1928), (51) Silberberg and Voit (1931), (52) Pierce (1932) (53) and Wallbach in 1936 (54) gave very comparable results about status of haematopoietic cells in leukaemia. They showed that Fibroblast like cells and phagocytic macrophages almost invariably came to dominate the cultures within few days, but proliferation of granulocytes was frequently noted. On the question of maturation and differentiation of precursor cells, whether leukaemic or not, opinions
were divided, some observers believing that limited maturation of myeloid elements took place and others that it did not. Conclusions had, of course, to be inferred from changes in relative proportions of cells in successive sections of materials cultured for varying periods of time, since direct observation of cells during division and possible maturation was very difficult and it was impossible to estimate the absolute numbers of the various cell stages present.

In contrast to these unsuccessful attempts to obtain persistant growth of human leukaemic cells on solid media, De Bruyn et al in 1949 (55) were able to maintain the malignant lymphoblasts from a transplantable mouse lymphosarcoma. In 1952, De bruyn and Gey (56) observed that murine malignant cells are mostly round lymphoblast like cells, larger than lymphocytes, containing a large nucleus with few nucleoli, devoid of peroxidase activity or phagocytic power and having rapid motility with the characteristic "hand mirror" appearance shown by lymphocytes and lymphoblasts during movement, due to the nucleus being situated anteriorly and a short tail of cytoplasm trailing posteriorly, mesenchymal fibroblastic cells and large phagocytic macrophages which were not always abundant and usually disappeared within a short time. Intraperitoneal or subcutaneous inoculation of susceptible mice with small numbers of isolated lymphoblast like cells readily produced fetal lymphosarcoma, but actively growing mesenchymal cells failed to induce tumour formation when injected.

Despite the success of these several techniques in providing established strains of leukaemia or lymphosarcomatious cells from mice, Fieschi and Astaldi (1946) (57) attempted to grow normal and pathological bonemarrow cells from man in a solid medium consisting of
coagulated human or chicken plasma with embryo extract, surrounded by a liquid phase of human serum and Tyrode's solution. Culture of marrow from acute stem cell leukaemia showed a different picture, the primitive cells disappearing after 5 to 6 days and being replaced by histocytes cells rather than fibroblasts.

Fieschi, Cambiaggi and Sacchetto (1954) (58) again confirmed the difference in behaviour between the cells of acute leukaemias and those of chronic leukaemias and found that survival of acute leukaemic cells was longer than that of chronic leukaemia cells, relatively few fibroblasts appeared and even as late as 16 days after the cultures were set up cells were clearly surviving with blastic characteristics. The authors concluded that there must exist a clear genetic or metabolic difference between acute leukaemic cells and analogous cells from normal or chronic leukaemic marrow including the primitive cells present in acute phases in chronic leukaemias.

Osgood and Brooke (1955) (59) reported that peripheral blood cells of monocytic leukaemia were predominantly like reticulum cells, although many fibroblast like cells had earlier been present. Occasional giant cells were also present, and some lymphocytic and erythroblastic forms were seen. After 60 days of culture cells from chronic myeloid leukaemia showed some rather distorted granulocytic precursors, but also many fibroblast like "monocytes". All these cells are clearly considerably different from haemic cells normally seen in blood or bone marrow.

Berman and his associates in 1955 (60) and Stulberg et al. in 1956 (61) showed that the first phase myeloid cells were still identifiable and possessed some mitotic activity but their numbers declined steadily.
Large, round, monocytoid or histocytic cells predominated in the second phase, many becoming attached to the glass. Finally, palisades and networks of spindle-shaped cells assembling fibroblasts and capable of indefinite propagation spread widely over the whole culture field. Among these cells there later appeared, on a few, occasion, certain persistent strains of epithelial polygonal cells, arranged in mosaics, not unlike the Hela cells derived by Gey et al from a carcinoma of the cervix (Gey et al., 1952) (62).

The most important results of all these studies have been the absence of any sharp or consistent difference between the behaviour of leukaemic and normal leucocytes in culture, whether in their initial capacity for differentiation, their early mitotic activity, their tendency to die out, their overgrowth by histiocytic or fibroblast like elements in solid media or on glass surfaces, or in their eventual transformation into established "altered" cells.

**Secondary Infections and Related Mortality:**

In managing patients with haematological neoplasms, the accurate diagnosis and treatment of infection can be as important as antitumour therapy. In patients with acute leukaemia or in some cases of chronic leukaemia, neutropenia is almost invariable and may result from either the disease itself or the treatment administered.

D.K. Boggs (1960) (63) and H.S. Kaplan in 1980 (64) have shown that the presence of frank, shaking chills is diagnostically helpful, because they are much more common with fever of infection than with neoplastic fever. M. Schdev et al. in 1973 (65) and in 1984 Barnes et al. showed that perirectal infections occur in 8% of patients with acute
leukaemia and may go undetected unless the perianal area is examined for evidence of swelling tenderness or erythema. Ikard (1981) (66) and Doki et al (1979) (67) showed that abdominal tenderness is always alarming, because necrotizing enterocolities is a serious consideration. Pennington et al in 1977 (68) have discussed in Am. J. Med. that in all instances of unexplained fever, a radiograph of the chest should be obtained, even if physical examination or the history fails to indicate pneumonia, because an early infiltrate may be relatively asymptomatic. Kaplan (1980) (64) reported that the septicemias in the absence of a demonstrable portal of entry is common, and in neutropenic patients urinary tract infection may occur without the expected increase in leukocytes in the urinary sediment. In the patient with septicemia, the only clue may be the sudden development of general sign of toxicity. The patient who suddenly appears ill is likely to have septicemia. Kaplan (1980) (63) also reported that the cause of neoplastic fever is unknown. The presence of fever correlates with the presence of active disease, but it does not reflect the severity of disease with any accuracy.

Stamm (1975) (69) also showed that the genito-urinary tract can also become a source of invasive infection if urethral catheters are introduced and left in sites bacteria and gram(-ve) septicemia are then significantly increased. Multiple defects in host defence mechanisms may increase the susceptibility to infection in the leukaemic patient, cellular or humoral defects may occur single or in combination along with reaches in physical barriers which normally provide protection against infecting organisms. Indeed, the patient with acute leukaemia represents, par excellence, such a compromised host. Hersh et al (1965) (70) and Levine et al (1974) (71) have described separately that the period between 1955 and 1963 the mortality rate from infection
alone in patients with acute leukaemia was 38%, whereas in the subsequent period between 1965 and 1971 it had increased to 69% in patients with haematological malignancies including acute leukaemia. The difference in mortality in the two series can be primarily accounted for by the decline proportion of deaths associated with haemorrhage.

Chang et al (1976) (72) also showed that infection alone is responsible for some 70% of deaths from acute leukaemia, while haemorrhage, as a primary cause, accounted for another 15%.

**Neutropenia and Neutrophil Dysfunction:**

Neutropenia is probably the most important factor responsible for the increased frequency of infectious complications in the leukaemic patients.

Body et al. (1966) (73) have demonstrated an inverse relationship between the number of circulating neutrophils and the risk of infection which rises sharply as the neutrophil count drops to below 500/ml and is a highest when the count is below 100/ml. In a study of 52 patients with acute leukaemia, Bodey showed that there were 43 episodes of major infection per 1000 days when the neutrophil count was less than 100/ml, compared to only four episodes per 1000 days when the count was greater than 1000/ml.

On the other hand, Gregory et al (1972) (74) and Holland et al (75) showed separately that leukaemic patients may also have qualitative neutrophil defects such as impairment of bone-marrow mobilization, chemotaxis phagocytosis and intracellular killing. Pickering et al. (1975) (76) have reported that in patients with acute lymphoblastic leukaemia
some functional impairment of neutrophils was noted even when remission has been achieved.

In another study of Thompson and Williams (1974) (77) the frequency of infection in children with ALL increased as the impairment in bactericidal function of neutrophils became more severe.

**Lymphopenia and Lymphocyte Dysfunction:**

In the book "Leukaemia" edited by A. Whittaker (1987) (78) described the role of lymphocytes in providing immunity against infections. The complex cooperative functions of several classes of lymphocytes and their interactions with monocytes are still poorly understood. He further described that cell mediated immunity is frequently impaired in patients who receiving radio or chemotherapeutic and steroid treatments. Such patients are at risk of developing infections by intracellular parasites such as protozoa or mycobacteria and viruses of which Herpes simplex, zoster and cytomegalovirus are the commonest.

**Monocytopenia:**

Steward and Bodey (1981) (79) have reported that the patients with hairy cell leukaemia in addition to being neutropenic commonly have monocytopenia. This may therefore explain their susceptibility to develop not only pyogenic but also mycobacterial and other opportunistic infections.

**Humoral Immune Defects:**
The humoral immune response, through the action of antibodies and complement plays an essential role against infection though in case of leukaemia they are impaired due to radio or chemotherapy.

Natvig & Kunkel (1973) (80) showed that the immunoglobulins mainly IgG and IgM are particularly important since they not only promote phagocytosis through opsonization but can also fix complement and lead to direct bacterial lysis. In addition Waldman and Ganguly (1976) (81) have reported that, the deficiency of IgA may facilitate invasion of organisms through mucosal surfaces which may be already compromised. As a consequence of hypogammaglobulinaemia, these patients are particularly prone to repeated infections by encapsulated gram-positive organisms, such as Streptococcus pneumonia. Nedelkova and his associates (1981) (82) showed that the complement activity is reduced during chemotherapy and also in those with relapsed leukaemia. Another study of Cignello (1978) (83) described that the deficiencies of rare isolated complements, for example 'c', are known to have increased susceptibility to infections. But remain within normal range in patients in remission from acute leukaemia.

Radiotherapy:

The efficacy of x-rays in the treatment of certain forms of leukaemia and allied diseases was demonstrated early in this century (Busey, 1902, (84) Senn, 1903 (85) and radiation therapy provided the chief means of control in chronic leukaemias. Acute leukaemias failed to respond and were sometimes exacerbated by x-ray treatment.

Satisfactory clinical and haematological remissions in chronic leukaemias have also been brought about by internal irradiation with
radioactive phosphorus (Lawrence, Scott—Tuttle, 1939) (86), but reduction of splenomegaly and lymphadenopathy is less well achieved by external irradiation (Reinhard et al, 1946) (87). An advantage of radioactive isotope treatment is the freedom from symptoms of radiation sickness, but, nevertheless, external irradiation has generally been preferred.

In a series of detailed reports, Heineke (1903, 1904a and b) (88, 89, 90) described the destructive effects of radiation on cells of the thymus, spleen, lymph glands and bone marrow in the intact animal. Nuclear fragmentation and pycnosis were apparent within a few hours after exposure and reached a maximum at about 12 hours, the cells involved being lymphoblasts and lymphocytes and, less conspicuously, all other precursor cells. Thymus, spleen and lymph glands shrink greatly as a result of the destruction of lymphatic tissue, and a progressive depopulation of the bone marrow was observed, terminating in complete aplasia after 5 or 6 days, when sublethal doses were used, a phase of hypocellularity was followed by regenerative activity after the sixth day with return to normal in 2 to 3 weeks. These fundamental studies were subsequently confirmed by many workers. Among a number of excellent review articles and monographs covering this field of research are those of Selling and Osgood (1938) (91), Waren and Dunlap (1942), (92) Debstad (1943), (93) Bloom (1948) (94) and Ellinger (1957) (95).

The studies of Osgood and Bracher (1939) (96), of Osgood (1942) (97), and of Gunz (1949) (98) showed that x-ray doses within the therapeutic range acted in vitro principally by inhibiting mitosis in haemopoietic cells. Indirect action by substances released in the medium could not be clearly demonstrated, and mature and resting
blood cells were radioresistant. The rapid fall in the peripheral leucocyte count found so often after local or whole body exposure to thereapeutic doses has, of course, long been thought to be secondary to some action other than direct leucocyte destruction [Lacassagne and Lavedan, 1924, (99) Jolly, 1925(100)].

The sensitivity of leukaemic cells in vitro to direct radiation in therapeutic doses has been established and it has also been shown that cells derived from an irradiated leukaemic patient may have diminished proliferative ability in culture (Gunz, 1949) (98). Leukaemic cells appear to be more radio-sensitive than normal cells of comparable maturity (Osgood, 1940), (101). While among the leukaemic leucocytes the last mature appear most sensitive, in agreement with Bergonic and Fribondeau's law (Piney and Riach, 1932) (102). The clinical and haematological response of irradiated leukaemic patients provides evidence of the importance of indirect effects. Good remissions in chronic myeloid leukaemia can be brought about by irradiation of the spleen alone, and both the peripheral leucocyte count and the bone marrow differential cytology return towards normal (Parsons et al, 1954) (103).

Studies of Klineberger and Zoeppritz (1906) (104), of Grawitz (1904) (105), of Caps and Smith (1943) (106) of Zacheri (1926) (107) and Burnes and Furth (1943) (108) showed that irradiation of the enlarged spleen, or spray irradiation of a large part of the skeleton, exposes a considerable bulk of sensitive leukaemic tissue to mitotic damage, including both the leucopoietic sites themselves and the peripheral blood cells of varying stages of maturity passing through irradiated areas throughout the time of exposure.
Selye (1946) (109) emphasized that many of the metabolic changes observed after radiation were similar to those following other injurious stimuli, such as traumatic shock, burns, and exposure to cold or excessive heat. In each case hyperglycaemia, rise in blood ketones, fall in blood cholesterol, increase in blood non-protein nitrogen and changes in acid base balance occurred. Selye regarded these changes as attributable to a non-specific stimulation of the pituitary-adrenal cortical stress mechanism probably contribute to the overall response.

**Chemotherapy:** A few patients die in the chronic phase of chronic myeloid leukaemia (CML) as it is relatively easy to control the clinical and haematological manifestations of the disease. Therapy is directed primarily to reducing the proliferating mass of granulocytes and platelets. The use of splenic irradiation was the only modality available for many years, until it was replaced by chemotherapy. The oral alkylating agents L-phenylalanine mustard, busulfan, cyclophosphamide, and chlorambucil, are all effective in reducing the leucocyte and platelets count.

Busulfan is the most effective because of its effects on haemopoietic stem cells. Remissions achieved with this agent could be prolonged for many months without the need for continuing the medication. The objectives of leukaemia treatment are related to the type of leukaemia. From a therapeutic perspective, the leukaemias are best divided into acute and chronic. In acute myeloid leukaemia (AML) and acute lymphoblastic leukaemia (ALL), the therapeutic goal is to completely eradicate the leukaemia cells and restore normal haematopoiesis. These objectives are best accomplished by the administration of relatively high-doses of antileukaemic drugs followed
by the cyclic re-administration of chemotherapy with or without treatment of the central nervous system to prevent leukaemia relapse.

The principles of therapy of the chronic leukaemias, chronic lymphoid leukaemia (CLL), chronic myeloid leukaemia (CML), and hairy cell leukaemia are somewhat different. The treatment of CML and CLL generally involves relatively low-dose chemotherapy. Recent efforts to cure CML with intensive chemotherapy, radiation and bone marrow transplantation therapy. Hairy cell leukaemia is usually treated by splenectomy above.

**Drug Used to Treat Leukaemia: Alkylating Agents:**

Alkylating agents are widely used drugs that substitute alkyl groups with hydrogen atom of certain organic compounds. Although the precise mechanism of action is still under investigation crosslinking of DNA remains the most important cytotoxic action.

Chabner et al (1977) (110), Price et al (1969) (111) Calabresi and Parks (1980) (112) have described differently that this cross-linking interferes with DNA replication and transcription. Hill and Baserga (113) have reported that alkylating agents are referred to as radiomimetic. Alkylating agents are cell cycle phase non-specific. From the several works of different workers, it has been established that there are five major classes of alkylating agents nitrogen mustard derivatives, alkyl sulfonates, ethylenimine derivatives, triazene derivatives and nitrosoureas.

Skipper et al (1972) (114) have shown that the nitrogen mustard derivatives have similar antitumour effects; resistance to one agent
usually indicates resistance to the others. Nitrogen mustard derivatives
include mechlorethamine (nitrogen mustard), cyclophosphamide,
chlorambucil are commonly used in the treatment of CLL either in a
daily or pulse scheduling established by Calabresi & Parks (1980) (112). 
Another studies of Brock (1976) (115) reported that chlorambucil is
generally well tolerate but adverse effects include bone marrow and
immune suppression, gastrointestinal symptoms, hepatotoxicity, and,
rarely, a poorly defined 'wasting' syndrome.

Melphalan is a phenylalanine derivative of nitrogen mustard with a
toxicity similar to that of other alkylating agents. Melphalan is frequently
used in multiple myeloma, usually in combination with prednisone.

Calabresi & Parks (1980) (112) have reported that Busulfan, which
is an alkyl sulfonate has an effect on granulocyte series and hence it is
most commonly used to treat CML. Rose (1975) (116) described that
toxicity of this drug arises when it is prolonged used. Adverse effects of
this drug may include hyper pigmentation, pulmonary fibrosis, cataracts
and gynaecomastia. Toxicity of this drug causes nausea and vomiting,
alopecia, haemorrhagic cystitis, urinary bladder fibrosis, which has led to
carcinoma of the bladder. Other complications include sterility,
immunosuppression, fetal damage, delayed carcinogenesis, cardiac
damage at very high doses and pulmonary fibrosis. Cyclophosphamide
may be used in the therapy of CLL and the leukaemic phase of poorly
differentiated lymphoma. Calabresi & Parks (1980) (112) also described
about another chemotherapeutic drug like Dacarbazine which is an
imidazol carboxamide derivative and requires biotransformation to
cytotoxic form. Adverse effects include severe anorexia, nausea, modest
bone marrow suppression, flu-like symptoms, alopecia and hepatic
dysfunction. Dacarbazine is occasionally used in the treatment of the leukaemic phase of non-Hodgkin lymphoma. In the seventh drug seminar (1976) (117) different workers have suggested that the nitrosoureas group of drugs undergo extensive biotransformation in vivo, have multiple mechanisms of action in addition to alkylation, and because of this they are generally not cross-resistant with other alkylating agents. Due to its lipid solubility it is permeable to blood-brain barrier. The nitrosoureas are lipophilic alkylating agents which include lomustine (CCNU), carmustine (BCNU) and semastine (methyl-CCNU), used for the treatment of lymphoma and myeloma but only rarely in acute and chronic leukaemia. Anthracyclines are natural products of the solid fungus streptomyces. Waring (1970) (118) has demonstrated that these drugs inhibit DNA replication by intercalation of base pairs. The two most commonly used anthracyclines in leukaemia are daunorubicin and its 14-hydroxyl derivative, doxorubicin. These agents are cell-cycle phase nonspecific and both are given by the intravenous route in high-dose pulse schedules. Bachur et al. (1977) (119) and Benjamin et al (1977) (120) showed that administration of doxorubicin through infusion reduce cardiotoxicity. Blum & Carter (1974) (121) have shown that anthracyclines have adverse reactions include severe bone marrow suppression, alopecia, stomatitis, vomiting and cardiac failure. Besides these, they have other adverse effects like renal failure, hyperpigmentation, skin rashes and oncholysis with epidermolysis.

**Plant Alkaloids:** The most commonly used plant alkaloids are the vinca alkaloids, vincristine and vinblastine, which are extracted from the periwinkle plant, Calabresi and Sparks (1980) (112) have reported that these drugs differ only in methyl (vinblastine) or formyl (vincristine) side chains, biological activities are markedly different. Vinca alkaloids are
cell-cycle phase-specific and have anti-tumour effect by binding to cellular microtubular protein, leading to mitotic arrest (Calabresi & Parks, 1980) (112). Rosenthal & Kaufman (1974) (122) showed that vincristine, given intravenously, and its main adverse effect is bone marrow suppression. Other toxicities include gastrointestinal symptoms, alopecia, malaise, weakness, and, rarely neurological problems. It is mainly used in treatment of non Hodgkin & Hodgkin lymphoma and has no major role in the treatment of leukaemia.

**Epipodophyllotoxins:** Etoposide and teniposide are semisynthetic derivative podophyllotoxin. Mathe et al. (1974) (123), Vegelzang et al. (1982) (124) Issel & Crooke (1979) (125) reported separately that the teniposide is a derivative of podophyllin, an alcohol extract of the plant Podophyllum peltatum. These drugs are phase specific agents active in G-phase, Etoposide has clear activity in non-Hodgkin lymphoma and AML and some reports suggest that etoposide is most active in the acute monocytic subtypes of AML (M4 & M5). Adverse reactions reported by Rivera et al (1980) (126) and Dahl et al (1982) (127) of the epipodophyllotoxins include myelosuppression, alopecia, stomatitis, nausea and vomiting. Fever, chills, peripheral neuropathy and radiation recall are less common.

**Antimetabolites:** Antimetabolites are analogues of normal compounds required for cell function and replication. Clinhe & Haskell (1980) (128) have described that antimetabolites can effect cells in several ways: i) interaction with enzymes causing damage to cells by substitution into intracellular molecules ii) Competition with normal metabolite for the catalytic site of enzymes, (iii) Competition with a normal metabolite that acts as an enzyme regulatory site to alter the catalytic rate of enzyme.
Commonly used antimetabolites are methotrexate, 5-fluorouracil, cytosine arabinoside, 6-marcaptopurine and 6-thioguamine. Hill & Baserga (1975) (113) have reported that all of these drugs are cell cycle phase-specific except 5-fluorouracil.

**Hydroxyurea:** Lewis & Wright (1974) (129) reported that hydroxyurea inhibits ribonucleoside diphosphate reductase, an enzyme essential for DNA synthesis and is cytotoxic to cells in S phase. Schwartz and Canellos (1975) (130) have seen the adverse effect of hydroxyurea including bone marrow suppression, gastrointestinal symptoms and occasional skin reactions (alopecia, hyperpigmentation) etc. Hydroxyurea is used to treat CML for which some clinicians prefer it to busulfan. It is also used in treatment of acute leukaemia (AML).

**L-Asparaginase:** Broome (1961) (131) has reported that L-asparaginase is an enzyme whose postulated mechanism of action in man relates to its ability to destroy extracellular supplies of L-asparagine. Another studies of Haskell & Canellos (1969) (132) showed that this results in death of cells lacking enzymes necessary to synthesize L-asparaginase which is generally considered to be phase nonspecific but it may also block cells in G, or S phase. Haskell et al. (1969) (133) have reported about the adverse effects of L-asparaginase that are, allergic reactions, depression of clotting factors, liver toxicity, central nervous system dysfunction, nausea, vomiting, hyperglycaemia pancreatites and immunosuppression. Yab et al (1981) (134), Amadori et al (1980) (135) and Capizz et al (1984)(136) showed that L-Asparaginase is used to treat ALL and it has also been combined with high-dose methotrexate with Ara-c as a form of rescue.
Adrenocorticosteroids: Review works of Fauci et al (1976) (137) & Melby (1977) (138) suggest that adrenocorticosteroids have short duration of action and relative weak potency but dexamethasone is more potent and has a longer duration of action. They also have found that adverse effects which occur with prolonged treatment include myopathies, osteoporosis, aseptic nurosis of bone, gastrointestinal toxicity, pancreatitis, central nervous system complications (pseudotumor ceribri), glaucoma, cataracts, hypertension, obesity, hyperlipidaemia, immune-suppression, hypothalamic pituitary-adrenal axis. Adrenocorticosteroids are commonly used to treat ALL and CLL. Sometimes it is used in combination therapy of AML.

Immunopotentiation: For the past twenty years the control of the immune response has been virtually equated to immune suppression because the great goal of applied immunology has been the transplantation of tissues between individuals.

With the discovery of tumour immunity—that even autochthonous tumours may excite a cell-mediated immune reaction directed against themselves [Foly, 1953 (139); Prehn & Main, 1957 (140); Old et al, 1962 (141); Klein et al, 1960 (142); Habel, 1961 (143); Sjogren et al, 1961 (144)] the focal point of immunological control has changed from immunosuppression to immunopotentiation, and correspondingly the great prize of applied immunology has become the prevention and control of malignant growth. The work of Turk and Pulter (1972a, b) (145,146) and Turn et al (1972) (147) makes it increasingly clear that the potentiation of cell-mediated immunity should be accompanied by inhibition of the humoral antibody response, which is also tumour specific. It is possible that adjuvants act at different time points during
the cellular division and differentiation pathways leading to antibody production. They might be thought of as substances which supply the necessary second stimulus which prevents a cell which is in the process of binding monomeric antigen becoming paralysed or killed (Bretscher 1972) (148). This effect might be mediated through stimulation of the division of antigen-sensitive cells (Dresser 1970) (149). In addition, adjuvants potentiate the immune response, possibly also by stimulating cell division but among cells some hours after specific commitment. It is not impossible that the two effects are the results of a non-specific 'insult' to the cell membrane [Munder et al, 1969 (150); Fischer et al 1970 (151)]. Dresser & Phillips (1973) (152) have concluded that at least some adjuvants can be either T or B-cell oriented or both. Furthermore a comparatively simple assay stem has been described where by a measurement of the relative effect of adjuvants on the M and the far more T-dependent γG classes, at both high and low concentrations of antigen, allows an assessment of the degree of T-and B-orientation of the adjuvant to be made.

The effects of killed corynebacterium parvum activity on T-cell have been studied by Howard et al (1973) (153). They have found that T-cell activities are inhibited (1) Graft versus host reaction (2) Mixed lymphocyte reactivity, and (3) PHA responsiveness of spleen or blood lymphocytes (but not lymph node cells). Removal of macrophages from the spleen cells of C-parvum treated donours restores full responsiveness to PHA. Conversely, these cells (or their class adherent fraction) will inhibit normal cells. Splenic B cells in C parvum treated mice react normally to the mitogen lilopolysaccharide. A strong adjuvant effect is demonstrable on the IgM response to higher immunizing doses of the T-independent antigen type 3 pneumococcal polysaccharide (SIII). By
contrast, Bordetella pertussis exerts only an inhibitory influence on the response to SIII. The reactivity of spleen cells to SIII is transiently but potently suppressed when they are transferred into mice irradiated with 900 R and pretreated C. parvum. Frost & Lance (1972) (154) have found that C. parvum is one of the strongest inducers of 'lymphocyte trapping' which their evidence implies is a macrophage mediated effect. This mechanism would facilitate extended contact between antigen-charged phagocytes and lymphoid cells and could provide a plausible explanation for the ability of C. parvum to stimulate a primary response to otherwise, sub-immunogenic doses of sheep and rat erythrocytes. Halpern et al (1973) (155) have shown that C. parvum appears to act at different cellular levels of the immune reaction: (1) it stimulates macrophages increasing their phagocytic activity and intracellular killing power towards ingested bacteria; (2) it stimulates the activity of T and B lymphocytes, increasing antibody synthesis against thymus-dependent or thymus independent antigens, and potentiating delayed hypersensitivity and lymphocytes cytotoxic activity. Evidence is provided by Halpern et al (1966) (156) that the cytotoxic property of lymphocytes obtained from C. parvum treated animals towards tumour cells is greatly increased. The cytotoxic action of lymphocytes tumour-bearing animals against their own tumour cells is potentiated but also that of lymphocytes from normal animals. The promising results obtained with C. parvum in human cancers stress the interest of this group of immunopotentiators in general and of C. parvum in particular.

Mathe et al (1971) (157) has shown that BCG (Bacillus calmette Guerin) considered to be immunity adjuvants are in fact, in some conditions, systemic immunostimulators, but may, in other conditions, be immunodepressants, not only in the Jerne test, but in the GVH
reaction. It also showed that, while their effect is nil or favourable as far as immunoprophylaxis of L-1210 leukaemia is concerned, it may be nil, favourable towards the ICIGCI, solid tumour and the Lewis solid tumour. The study of Bluming et al (1972) (158), who treated melanoma patients with two preparations of BCG and obtained positive immune stimulation and favourable results only with a preparation from the Pasteur Institute. A medical Research Council trial on acute lymphoid leukaemia conducted with BCG alone gave a negative result (Medical Research Council 1971) (159), the trial conducted by Powles et al (1973) (160) on acute myeloid leukaemia using BCG and leukaemic cells gave a positive result which confirmed Mathes et al (1968) (161) experiment on acute lymphoid leukaemia. Intravenous and subcutaneous administration of BCG effect on T and B lymphocytes respectively, on the killer and the helper T cell and on macrophages, in the different anatomical sites such as lymph nodes, spleen and bone marrow, of these, the latter organ may be the most important for eradicating, by active immunotherapy, 'the last tumours cell' in leukaemia.

Trebichavsk'y et al (1998) (162) used Bacillus firmus for immunopotentiatio and they have found that production of lymphocytes are increased in B. firmus challenged rats that induce antitumour immunity against Yoshida Sarcoma. They have also found B firmus induced IFN-γ synthesis in human blood lymphocytes whereas TNF-production by stimulated human peripheral blood mononuclear (PBMC) was lower than that PBMC stimulated with some other bacterial immunomodulators cells containing TGF-b or IL-8 were not found in human PBMC stimulated, with B. firmus.
**Immunotherapy:** Patients with AML and ALL have large burdens of leukaemia cells at the time of diagnosis, usually exceeding $10^{10}$. Because only a small portion of these cells are actively dividing, cell cycle-specific drug such as Ara-c and thioguanine, have a low probability of complete leukaemia cell kill. Cell cycle non-specific drugs such as daunorubicin and alkylating agents can theoretically overcome this limitation since they kill both dividing and non-dividing cells. Currently available drug have little or no therapeutic margin and thus substantial host toxicity limits their potential of complete eradication of leukaemia.

On the other hand, the immune system has the potential to eradicate residual leukaemia in man is no longer in doubt. This statement can be confidently made largely because of results from allogenic bone marrow transplantation, in which the presence of the incoming donor immune system generates a readily detectable graft versus leukaemia (GVL) effect. This activity has been shown in three ways [Barrett et al 1989 (163), Bortin et al 1979 (164), International Bone marrow transplant Registry 1989 (165), Horowitz et al (1990) (166), Apperley et al 1988 (167), Weiden et al 1979, 1981 (168,169), Ferrara & Deeg 1991 (170), Weisdorf et al 1987 (171) showed that first, patients who suffered from graft versus host disease (GVHD) are less likely to relapse than those who do not have this complication. Secondly, Horowitz et al in 1990 (166) & Goldman et al 1988 (172) have proven that patients receiving T-lymphocyte depleted bone marrow transplant-in whom the risk of GVHD is very low-may have a higher risk of relapse, and finally from the experiment of Horowitz et al (1990) (166) & from International Bone marrow registry (165) it has been found that recipients of syngeneic allograft have a higher risk of relapse than recipients of HLA-identical sibling grafts. From these results it has been
established that the incoming graft may contribute to the eradication of residual leukaemia.

Cheever et al (1986) (173) have shown through a number of models that MHC restricted antigen specific T-lymphocytes can protect animals against transplantable leukaemias. Another studies of Sosman et al (1990) (174) showed that antidiiotypic antibody responses can be generated against these unique tumour epitopes, they rarely succeed in eradicating the malignancy, since subclones are generated which either express Ig of modified idotype or No Ig at all. Although relatively little is known about antigen processing and presentation by leukaemic cells, data from Sosman et al (1989) (175) suggest that leukaemia specific antigens appear on leukemia cell surfaces. They described the generation of CD3+, CD4+ T cell clones reacting with allogenic ALL cells which do not react with remission lymphocytes from the same patient, implying that these clones recognise an antigen expressed on leukaemic but not on normal cells. There is less doubt that, if mutant proteins are presented by leukaemic cells, they can be distinguished from the wild type products by antigen specific T-cells. Jung & Schluesner (1991) (176) synthesized a ras-derived peptide in which Valine was substituted for glycine at residue 12 a substitution associated with transforming ability in the intact protein. They were able to generate an MHC restricted CD4+ T-cell line which proliferated only in response to mutant peptide and had no response to stimulation with wildtype peptide. Mutant specific responses may also be generated in vivo [Peace et al (1991) (177)] used a similar ras peptide but now with arginine substituted at residue 12. to immunize C57BL/6 mice. Again MHC restricted CD4+ specific T-cells were generated against a range of acute and chronic leukaemias and imply it as leukaemia specific vaccines to the patients.
Karre et al (1980) (178) have shown that antigen specific recognition of leukaemia blast cells genuinely exists, an alternative mechanism of anti-leukaemia effects or function may be relevant. Herberman & Ortaldo (1981) (179) have reported that Natural Killer (NK) cells are MHC unrestricted effector cells which can inhibit clonogenic leukaemia growth in vitro and which are particularly numerous and active [activated Killer (AK) cells] for the first few weeks following both autologous and allogenic bone marrow transplant (BMT) [Karre et al 1980 (178), Brenner et al 1986 (180)]. On the other hand studies of Rosenberg et al (1988) (181), Anderson et al (1988) (182), Gottlieb et al (1989a) (183), Smith (1988) (184) have shown that administration of cytokines such as interleukin-2 (IL-2) is also able to generate AK cells for the killing of leukaemia cells. It has now been demonstrated that both CD3- and CD3+ AK cells may in fact selectively kill leukaemic blasts despite their back of a conventionally functioning MHC restricted antigen specific CD3 receptor. Fisch et al (1990a, b) (185,186) were able to generate CD3 T-cell lines using Vy9 and V82 T-cell receptor proteins which could recognize ligands homologous to heat shock superantigens on the surface of Daudi, Burkitt lymphoma line cross-reactive antisera to GroEL-a protein from Escherichia coli (E.coli) homologous to mycobacterial heat shock proteins-blocked the proliferative response to these lines, but antibody to MHC products had no effect. In other words, proliferation was antigen specific (to the heat shock superantigens), but was also MHC unrestricted. If other leukaemia/lymphoma cells express qualitatively or even quantitatively different superantigens to their normal equivalents,
then the CD3 MHC unrestricted AK cells appearing after BMT or during cytokine infusion could contribute leukaemia specific killing.

Davignon et al (1981) (187), Simmons et al (1988) (188), Timonen (1990) (189) have demonstrated that in order to kill their target cells effectively, CD3-AK lymphocytes must first bind to them, an effect achieved by a variety of cell adhesion molecules. (CAM) Oblakowski et al (1991) (190) showed that normal and malignant CD3 myeloid progenitor cells may express different patterns of CAM. Normal CD34 cells express LFA-3 (CD58) the ligand for LFA-2 (CD-2) but no detectable ICAM-1 (CD54), a ligand for a second AK cell adhesion molecule LFA-1 (CD11a/18). Killing of normal progenitors by CD3 -AK cells is, therefore, largely dependent on the interaction of CD2-CD58 ligand-receptor pathway and monoclonal antibodies to CD58 block cytotoxicity. By contrast, CD34 myeloid blasts express large quantities of the ligands CD54 and CD58, so that binding of effectors may occur through both the CD11a/18-CD54 and CD2-CD58 pathway. As a consequence, killing of malignant blast is not prevented by monoclonal antibody to the CD58 ligand alone. The authors postulate that because of in vivo competition of erythrocytes which express high levels of CD58 AK cells will preferentially bind to and therefore, preferentially kill malignant blasts. Ciccone et al (1990a, b) (191,192) further showed that the other target cell surface molecules recognized by NK cells, in the pattern of their expression which permit selective recognition and killing.

In 1969 (193) Mathe and co-workers reported a benefit of immunotherapy in patients with ALL. Patients who achieved a remission following chemotherapy were treated with BCG, allogenic leukaemia cells, or both. Patients who received immunotherapy had substantial
prolongation in the first-remission compared with controls receiving chemotherapy alone. Medical Research Council (1971) (159) Heyn et al (1975) (194), Andrien & Eur, org for Research on Treatment of Cancer Hemopathies Working Party (1978) (195) had and Poplack et al (1978) (196) had taken attempts to confirm these results in large controlled prospective trials in patients with ALL had been unsuccessful. Similar trials have been carried out for AML, Foon et al (1983) (197) analysed 29 trials in which 1491 patients with AML received various forms of immunotherapy, including BCG, common MER of BCG, and coryebacterium parvum Some patient also received allogenic or autologous leukaemia cells. Only four of the 24 trials reported a significant prolongation of remission duration. Pooled data from the 24 studies were analysed but not statistically significant difference in remission median duration was found. Based on these data, it appeared unlikely that immunotherapy prolongs remissions in AML. Clearly these conclusions only apply to immunotherapy as currently conceived, and new approaches may be successful.

Rosenberg et al (1988) (181), Gottlieb et al (1988a (183), b (198]), Blaise et al (1990) (199) have show that at present IL-2 and perhaps α-interferon are the only cytokines to be used clinically as antileukaemic immunomodulators. Farrar et al (1982) (200) have reported that in animal and human preclinical models, IL-2 increases antigen specific cytotoxic effector function and also induces and enhances MHC unrestricted NK/AK function. Heslop et al (1989a, b) (201,202) have demonstrated that IL-2 also induces release of a number of other cytokines including TNF (Tumour necrosis factor) and γ-Interferon which themselves may have an antileukaemic effect. α-Interferon probable produces its therapeutic effects by antiproliferative mechanisms (Heslop
et al 1990) (203) but may also recruit AK function or increase the sensitivity of leukaemic cells to immune effector mechanisms.

**Interleukin-2 (IL-2):** Most studies of IL-2 in patients with minimal residual leukaemia have administered the drug to the patients themselves, although efforts have also been made to use the cytokine to treat narrow *ex-vivo* as a form of immunological purging [Charak et al 1991 (204), Gambacorti-Passerivi et al 1991 (205)]. Gottlieb et al (1989b) (198) have shown that the well-tolerated doses of IL-2 produced a high level of immune modulation, characterized by increase numbers of CD56+ CD16+ or CD8+, AK+ cells, Gottlieb et al (1989a) (182) have also reported that in general IL-2 was well tolerated when given as 5 day courses repeated twice, at doses between 200-800 mg/m^2/day. Another studies of Gottlieb et al (1989b) (198) showed that perhaps, there was substantial increase in the number and activity of cells able to inhibit the clonogenic a growth of leukaemic blasts. Leukaemic colony and cluster formation was inhibited by up to 95%. It was also possible to augment production of the antileukaemic/antiviral cytokines TNF and γ-interferon (Heslop et al 1989b). Serum levels of γ-interferon rose sharply detected, CD16 and CD3 lymphocytes cultured from these patients showed a great increased production of TNF *in vitro* if they were obtained during IL-2 infusion.

Heslop et al (1989b) (202) also showed that one residual concern about IL-2 infusion was that the cytokines induced, particularly TNF and γ-interferon, would not only inhibit the growth of malignant myeloid progenitor cells but would also damage the engrafting normal progenitor cells: although these cytokines may be preferentially cytotoxic to malignant-cells, their selectivity is relative, not absolute. Further studies
of Heslop et al (1991) (206) about IL-2 infusion showed that IL-3 and GM-CSF could both be detected in circulating lymphoid cells as transcripts and as proteins; there was also a rise in serum GM-CSF during infusion. On the other hand Barrett et al (1989) (161) have reported that despite production of IL-3, however platelet levels fell during IL-2 infusion, perhaps due to increased consumption mediated by the effects of TNF on vascular endothelium. Subsequent studies of IL-2 infusion in AML have produced comparable safety and immunologic efficiency data [Blaise et al 1990 (199), Soiffer et al 1992 (207), MacDonal et al 1991 (208), Foa et al 1991b (209), Olive et al 1991 (210), Lotzova et al 1991 (211), Higuchi et al 1991 (212)]. More recently, it has been shown by the Soiffer et al 1992 that subcutaneous low-dose was well tolerated when given above or after high-dose IL-2 infusion (Higuchi et al 1991) (212). In addition, Soiffer et al (1992) (207) showed that therapy with low-dose IL-2 above was well tolerated after allogenic BMT, and did not induce GVHD. Low-dose, Longterm administration (3 months) of IL-2 may, therefore, allow the potentially beneficial effects of IL-2 to be produced and maintained with minimal adverse effects. Smith (1997) (213) has described then the administration of IL-2 that augment the function of the immune system can be accomplished safely and without toxicity, provided a rational approach is used. Very recently vectors are used as cytokines applicators for cancer Herpes Simplex Viral amplicon vectors (HSV) therapy are efficient gene transfer vehicles and may overcome many limitations of prior gene transfer methods. Angeliea et al in 1998 (214) have reported that the interleukin-2 (IL-2) and B-galactosidase genes (lac) inserted into an HSV amplicon vector and tested in a subcutaneous squamous cell carcinoma of lung origin to determine the efficiency of in vivo gene transfer and utility of such direct gene transfer approach in cancer therapy. They have found that direct
intratumoural administration of HSV amplicon vectors result in efficient transfer of cytokine genes and have antitumour efficacy. HSV vectors are, therefore, potentially useful agents in such in vivo gene therapy strategies and simplify cytokine antitumour gene therapy strategies.

**Interleukin 6 (IL-6):** Interleukin 6 has proved to be one of the most pleiotropic of all of the cytokines. This cytokine is produced by many cell types in response to variety of signals, including IL-1 stimulation (Kishimoto 1989) (215). In fact, IL-6 has proved to be the direct effector molecule of several activities previously associated with IL-1. Interleukin 6 is now recognized to be an important regulator of immunoglobulin production, of T-cell growth and development, of the acute-phase response and of various components of the haemopoietic system (Kishimoto 1989) (215). Within the haemopoietic system, IL-6 has been implicated in the regulation of development of very early cells (IKebuchi et al 1987) (216) and of megakaryocyte progenitors (Ishibashi et al 1989) (217). In addition, its interactions with various leukaemic cell lines and normal cells under certain culture condition implicate it as a regulator in later stages of neutrophil and possible monocyte development [Shabo et al 1988 (218); Caracciolo et al, 1989 (219)].

The role of IL-6 in neutrophil development is somewhat less clear. Although IL-6 yields some neutrophil and macrophage colonies with murine target cells, in the human system these growth promoting properties have not been observed with primary preparation of human bone marrow (Wong et al, 1988) (220). However, that IL-6 may play a role has been suggested through some experiments with progenitor cells from peripheral blood. Peripheral blood preparations contain measurable
levels of erythroid and early myeloid progenitors but few, if any, late myeloid progenitors (Caracciolo et al. 1989) (219).

The regulation of cell growth and development within the megakaryocytic lineage is still poorly understood. A variety of regulators of growth and development have been described but, thus far, these molecules have not been purified and their genes molecularly cloned of the known growth factors, IL-3 and, to a lesser extent, GM-CSF, were recognized as being capable of supporting megakaryocyte colony formation (Mazur et al. 1987, 1988) (221,222). More recently, IL-6 and IL-7 have been found to affect megakaryocyte development. IL-7 (Williams et al., 1990) (223) to act predominantly as a differentiation promoting agent while IL-6 (Ishibashi et al. 1989) (224) by itself, at least with cultures of murine bone marrow cells, yields megakaryocytic colonies. Interleukin-6 also enhances the yield of megakaryocytic colonies supported by IL-3. Similar effects of IL-6 have not yet been reported in the human system.

Granulocyte-Macrophage Colony Stimulating Factor (GM-CSF):

Continuous intravenous administration of GM-CSF to normal primates (macaques) elicits a rapid and dramatic increase in total white blood cell count beginning within 1-2 days following the start of the infusion (Donahue et al. 1986) (225). This elevated white cell count is maintained as long as the GM-CSF administration continues and the white cell count returns to normal within a few days of terminating the infusion. The elevation in total blood cells results from increased numbers of neutrophils, eosinophils, monocytes and lymphocytes. Although GM-CSF has been reported to enhance IL-2 dependent T-cell proliferation (Santoli et al., 1988) (226), the effect on lymphocytes was unexpected and many result from production of other cytokines by GM-CSF stimulated
The effects of GM-CSF on reticulocyte and platelet counts have in general been less clear; some animals have shown responses in these lineages while others have not. Recent studies with GM-CSF have been designed to test its effects on neutropenias associated with cancer chemotherapy (Antman et al 1988) (227), on reconstitution of haemopoiesis following autologous bone marrow transplantation (Brandt et al 1988) (228) and as a stimulator of haemopoiesis in myelodysplastic syndrome or aplastic anaemia patients (Antin et al, 1988) (229). Granulocyte-macrophage colony-stimulating factor infused after chemotherapy has been found to shorten both the magnitude and the duration of the chemically induced neutropenia but an effect on the incidence of infection in these patients has not been clearly established. In bone marrow transplantation, GM-CSF administered during the recovery period appears to shorten the duration of neutropenia and lower the incidence of infection when compared with historical control groups. GM-CSF administration to myelodysplastic patients typically elicits increases in leucocyte counts but also, in some patients, has resulted in an increase in the proportion of blast cells in circulation whether or not this represents a real acceleration in the progression of the disease remains to be searched (Ganser et al, 1989) (230). GM-CSF has therapeutic potentiality but longer term studies are still required to demonstrate effects on patient survival and quality of life.

**Interleukin-3 (IL-3):** Administration of human IL-3 to non-human primates yielded substantially different results from those achieved with GM-CSF in the same model (Donahue et al 1988) (231). A 7-day infusion of IL-3 was found to elicit a slow increase in leucocyte count that peaks 2-3 days following termination of the infusion. The peak white counts observed were typically 15000-25000 or approximately 2-3 fold over
baseline values. Analysis of differential cell counts revealed that the cells responsible for the elevation were very similar to those observed with GM-CSF; neutrophils, monocytes, lymphocytes and eosinophils. In addition, IL-3 elicit significant numbers of basophils, especially late in the infusion.

Analysis of the effects of IL-3 on haemopoiesis in myelosuppressed primates indicates that this cytokine may also prove beneficial in the treatment of chemically induced cytopenias. Primates given IL-3 after myelosuppressive dose of cyclophosphamide experienced nadir neutrophil counts of 1000 or greater compared with untreated controls whose neutrophil counts dipped below 400 and did not recover to 500 for 11 days (Gillio et al 1988) (232). The potent stimulation of very early haemopoietic cells by IL-3 may overcome many of the problems of intense myelosuppressive therapy for cancer, especially in combination with one or more of the late-acting colony stimulating factors.

**Interferon (IFN):** The biological activity can be induced by IFN-1 without a requirement for binding to the cell surface receptor. Fidler et al (1985) (233) demonstrated that mouse macrophages could be activated for tumour cell Killing by either recombinant MuIFN-γ or HuLFN-γ when the interferon was encapsulated with muramyl dipeptide into liposomes. Induction of la expression on mouse macrophages has also been demonstrated after microinjection of HuIFN- (Smith et al 1990) (234). Compelling evidence for a role of intracellular IFN-γ was provided by Sanceau et al (1987) (235). In these experiments induction of antiviral activity in mouse cells was accomplished after transfecting the cells with a cDNA for HuIFN-γ that lacked the single peptide, thus cell is unknown, preventing secretion of IFN-γ to the exterior of the cell.
collectively, these studies not only abrogated the requirement for species-specific ligand binding by the IFN-γ receptor, but also provided evidence to suggest that internalization of IFN-γ is important in the induction of some of its biological activities. Evidence provided by Farrar et al (1991) (236) has shown that internalization of IFN-γ is not sufficient for the induction of class-I MHC expression clearly the kind of IFN used and the cell type studied are likely to be important in understanding the multifunctional roles of the IFNs.

Recent studies show that some inhibitory factors, such as transforming growth factor βI (TGF-βI) 16-19 and tumour necrosis factor (TNF) 20, have been shown to suppress haematopoietic cell growth by down-regulating SCF receptors and IFN-γ has been shown to down-regulate IL-6, IL-4 & Macrophage mannose receptors, the effect of IFN-γ on growth factor receptor expression of erythroid progenitor cells is unknown. It has been shown by Tan et al (1988) (237) through their excellent experiment that interferon gamma inducing factor (IGIF) can elicit T-cell dependent antitumour immunity associated with IFN-γ induction. They have also found that IGIF-transfected Renca and K1735 tumour cells can be rejected in vivo. The IGIF antitumour effect was abrogated in mice that were sublethally irradiated or depleted of both CD4+ and CD8+ T cells but not in mice depleted of either subpopulation above.

Drexler et al (1997) (238) have described that interferon-gamma(IFN-γ) is a pleiotropic cytokine involved in the regulation of various phases of immune and inflammatory responses. It also has antiviral and anti-proliferative activity; this effect was specifically neutralized by an anti IFN-γ monoclonal antibody. But it has been shown by them
that IFN-γ acted as a survival factor suppressing apoptosis. IFN-γ can induce myeloid leukaemia cells to proliferate and can modulate this proliferative response to other cytokines. Therefore, IFN-γ may be a pathologically relevant ligand for leukaemic cell proliferation in vivo. In physiological settings, IFN-γ might be a bifunctional regulator of haemopoietic cell proliferation, depending on other differential co-signals from the micro-environment. It has found that in CML continuous administration of IFN can promote granuloma formation in sarcoidosis by activating T-cell and macrophages.

Due to some adverse effects of single used cytokines, scientists concentrated their knowledge into combination therapy.

In 1996, Toren et al (239) have found that patients treated with combined IL-2 and interferon alpha (IFN-α) increase soluble interleukin 6 receptor (IL-6R) levels that potentiate the activity of IL-6 which may display antitumour activity. They have also shown that IL-6R levels also significantly increased following donor lymphokine activated Killer (LAK) cells, given in addition to IL-2 treatment. Increased levels of SIL-6R were observed in BMT patients treated with immunotherapy.

In 1997, Khar et al (240) have demonstrated that positive correlation between levels of IL-2, IFN-α, IL-12 and TNF- and onset of tumour rejection.

However, levels of IL-4 and IL-10 did not show a significant correlation with the tumour regression on profile. They have also suggested that cytokines may play an important role in keeping tumour infiltrated lymphocytes (TIL) in an activated state so that they could participate in killing tumour cells either directly by cellular interactions
or indirectly by producing additional cytokines such as TNF-α. So, application of combined cytokines may be useful in accomplishing complete tumour regression.

In 1997, Lasek et al (241) have found that the combined treatment with recombinant IL-12 and TNF would produce anti-tumour effects through activation of natural killer and T-cells. Potentiation of anti-tumour effects, which was observed in IL-12/TNF-α based immunotherapy could result from at least three different mechanisms, partly related to stimulation of IFN-γ and INF-α production in treated mice(a) direct cytostatic/cytotoxic effects on tumour cells, (b) induction of antitumour activity of macrophages, and (c) inhibition of blood vessel formation in the tumour. They demonstrate that combination immunotherapy with IL-12 and TNF-α use of poly(lactic acid) micro-spheres to deliver cytokines to the tumour environment could provide a safer and simpler alternative to gene therapy protocols in the treatment of cancer.

In 1998, Gol et al (242) have reported that the application of F-CSF partially prevented the suppression of bone marrow myelopoiesis in IL-12 treated mice. The augmented antitumour activity of combined IL-12/G-CSF immunotherapy could result from the enhanced stimulation of macrophage NO production and cytotoxicity. The simultaneous administration of IL-12 and G-CSF partially prevented suppression of bone marrow myelopoiesis in IL-12 treated mice. Moreover treatment with these cytokines also results in potentiated antitumour effects in a murine melanoma model.

In 1998, Vitale et al (243) have found in acute myeloid leukaemia (AML) patient that primary AML blasts were resistant to IL-2 activated
PBMC (peripheral blood mononuclear cell) effectors generated from normal individuals, allogenic and autologous patients in five, six and eight of the 10AML-samples tested, respectively, IL-12 above proved in effective in generating antileukaemic activity. But IL-2 resistant blasts may be lysed be a low-dose combination of IL-2/IL-12 feasible studies with this cytokine combination are worthy of exploration in vivo.

Interleukin-15 (IL-15) is a potent T-cell stimulating factor which has recently been used for pre-clinical in vivo immunotherapy. In 1997, Korholz et al (243) have shown that exogenously administered IL-10 significantly reduced the IL-15 and IL-2 mediated IFN-γ and TNF-production, whereas T-cell proliferation and CD 25 expression were not affected. The inhibitory effects of exogenously administered IL-10 on T-cell effects cytokine production appeared indirect, and are likely secondary to decreased IL-12 production by accessory cells. Inhibition of endogenous IL-10 binding to the IL-10 receptor significantly increased IFN-γ and TNF-α release from T-cells. These data suggest that endogenous IL-10 can regulate activated T-cell production of IFN-γ and TNF-α via a paracrine negative feedback loop. The observations of this study could be of relevance for the therapeutic use of IL-15 in vivo.

Zagozdzon et al (1998) (245) have reported that the combination of chemotherapeutics with appropriate cytokines are more beneficial than chemotherapeutics given alone. They have found that doxorubicin augments IL-12 stimulated production of interferon-gamma in vivo into macrophages depletant or irradiant mice. Their observations demonstrating potentiation of the antileukaemic effects of the IL-12 and doxorubicin combined use of the two agents could be beneficial in leukaemia therapy.
Very recently Fallarino et al (1999) (246) used cell and cytokine for cancer therapy. They administered splenic dendritic cells (SDC) with interleukin-12 into mice, found only 10-20% CTL function. CTLs were detected both in the spleen and in the peripheral blood. Immunization with irradiated, protein pulsed peripheral blood mononuclear cell (PBMC) plus rIL-12 resulted in protection against challenge with tumours expressing the specific antigen in all mice. The case by which human PBMC can be prepared provides a straight forward vaccination approach to be used in clinical trials of peptide based immunization in melanoma.

Hopeful thinking about therapeutic remissions of leukaemia is still under intense investigations especially with respect to immunological manoeuvres in the patient concerned. Many of the important attempts to establish these procedures depend on ethical clearance with prior successful trials in animal system. Experimental animal models for leukaemia and BRMs as therapeutic components are expected to open new horizon in the field of immunotherapy of leukaemia and related haematological malignancies.