CHAPTER I
INTRODUCTION

1.1 Statement of problems

Cancer is one of the major public health problems for many countries around the world. According to the World Cancer Report, malignant tumors were responsible for 7.6 million (13%) of the nearly 58 million deaths worldwide from all causes in the year 2005 [1]. About 5.3 million men and 4.7 million women developed a malignant tumor. Moreover, cancer rates could further increase by 50% from 10 million new cases in the year 2000 to 15 million in the year 2020 [2]. The American Cancer Society (ACS) reported that cancer is the second leading cause of death in the United States (US). Nearly half of all men and a little over one-third of all women in the US will develop cancer during their lifetimes. In addition, about 1.4 million new cancer cases are expected to be diagnosed in 2006 [3]. According to a report from the Bureau of Health Policy and Planning, Ministry of Public Health of Thailand, cancer is the leading cause of death. In 2004, the cancer death rate was 81.3 per 100,000 population. The cancer death rate has been gradually increasing year by year, from 58.6 in 1999 to 81.3 in 2004 [4]. Moreover, cancer was a major cause of death in Chiang Mai province in the years 2002 and 2003 [5].

Leukemia is a group of diseases involving the blood-forming organs, and is characterized by uncontrolled increase of white blood cells. Approximately 35,000 new cases of leukemia will be diagnosed in United States in 2006 [6]. Furthermore, leukemia is one of the top ten leading causes of cancer death in Thailand. In the year 2004, the leukemia death rate per 100,000 population was 3.0. The leukemia death rate has been gradually increasing year by year, from 2.5 in 2000 to 3.0 in 2004 [7]. Development of leukemia has been linked to certain environmental and genetic risk factors, including exposure to radiation, toxic chemicals, chemotherapeutic agents, viral and other microorganisms, genetic disorders associated with chromosomal instability, cigarette smoking, and other environmental factors. These factors lead to genetic changes in leukemic cells that in turn affect the functions of other genes, including tumor suppressor genes and oncogenes. Some cases of mutation in

oncogenes have provided useful molecular markers for monitoring the course of the disease during treatment. The detection of overexpression in specific oncogenes or tumor suppressor genes provides information that is useful in the diagnosis of leukemia and prognosis of the disease. The overexpression of Wilms' tumor (WT1) protein in leukemia is a good example.

The *Wilms' tumor1 (WT1)* gene is one of the genes involved in tumorigenesis. The *WT1* gene was first described by Max Wilms in 1899. It is located on the human chromosome 11p113. [8, 9]. In normal tissue, WT1 is restricted to kidney, testis, ovary, spleen, hematopoietic precursors, and the meseothelial cell lining of visceral organs [10]. *WT1* expression has been detected in the fetal spleen, liver, and thymus, tissues in which hemopoiesis takes place during embryonic development. In addition, WT1 transcripts have been detected in adult bone marrow, lymph nodes, and peripheral blood. These data suggest that WT1 may play a role in the development of erythroid, myeloid, and lymphoid cells during both the fetal and the adult stages. Previous studies demonstrated that the *WT1* gene is overexpressed in many types of cancer. It has been described as a tumor suppressor gene responsible for Wilms' tumor or nephroblastoma and other cancers [11-14]. However, recent studies have shown that *WT1* acts as an oncogene in many types of malignant tumors especially leukemia [15]. It plays an important role in the progression of disease and prognosis of human malignancies [16, 17]. Significant levels of *WT1* gene expression have been found in leukemic samples; the average levels are approximately 1,000 to 100,000 times higher than in normal bone marrow and peripheral blood, respectively [16].

The *WT1* gene product is a 48-52 kDa nuclear protein that has four zinc fingers and acts as a transcriptional activator or repressor, depending on the cellular or chromosomal context [18, 19]. The four major isoforms of WT1 protein identified to date result from two alternative splices in its transcript. WT1 protein plays an important role in the development of normal cells and tissues. It may influence the proliferation and differentiation of blood cells by differential regulation of the genes for *TGFβ*, *C-Myc*, *C-Myp*, *Bcl2*, and the retinoic acid receptor [20]. Low levels of WT1 protein expression have been found in normal blood cells. In contrast, the overexpression of WT1 protein has been found in leukemic blood cells. The expression of WT1 in leukemic cells is involved in cell proliferation, differentiation,

and apoptosis [20, 21]. Moreover, the expressions of the WT1 gene and its product have been used as biological markers for diagnosis and evaluation of the prognosis of leukemia and minimal residual disease (MRD) [16, 22]. All of these findings supported that *WT1* gene expression may influence in leukemogenesis.

To date, the rapid growth of advanced technologies and new discoveries in the diagnosis and treatment of leukemia have made surviving from leukemia more likely than in the past. Chemotherapy is one of the methods used most frequently in the treatment of leukemia. Chemotherapy works by destroying leukemic cells, hence stopping them from growing or multiplying. However, some normal cells are destroyed as well by this method of treatment. Due to the wide range of biological activities and lack of toxicity in animal models, natural remedies have been used as alternative treatments for leukemia and other malignant tumors.

Turmeric (*Curcuma longa* Linn) is one of the most popular herbs used for medical treatment, due to its wide variety of medicinal properties, such as anti-inflammation, anti bacterial infection, anti fungal, anti-oxidation, anticarcinogen, antimutagen, and anticancer properties. The active constituents of turmeric are known as curcuminoids, yellow pigmented substances isolated from the rhizome of turmeric. Curcuminoids constitutes about 3-5% of the composition of turmeric. This active extract can be subdivided into three distinct components: curcumin or pure curcumin (curcumin I), demethoxycurcumin (curcumin II), and bisdemethoxycurcumin (curcumin III). Curcuminoids are a group of diet-derived agents that are being clinically evaluated as chemopreventive agents for major cancer targets, including the breast, prostate, lung, stomach, duodenal, and colon cancers, as well as leukemias [23, 24]. Curcuminoids, especially pure curcumin, have strong anticancer agent and anti-tumor progression properties [25-27].

Duvoix, *et al*. [28] found that pure curcumin could induce cell death in two leukemic cell lines: K562 and Jurkat cells. Kuo, *et al*. [29] demonstrated that the dietary component of pure curcumin induces apoptosis in human leukemia HL-60 cells at the very low concentration of 3.5 μg/mL. Recently, Anuchapreeda [30] found that all three curcuminoids exhibited excellent cytotoxic activity on leukemic cell lines: HL-60, U937, and K562. Moreover, the inhibitory effects of pure curcumin were

associated with a decrease in the levels of *WT1* gene expression in patient leukemic cell and K562 cell lines [31, 32].

In this study, the effects of turmeric curcuminoids on *WT1* gene expression in leukemic cell lines were examined. Turmeric curcuminoids included commercial grade curcuminoid mixtures (Sigma-Aldrich), in-house curcuminoid mixtures, pure curcumin, demethoxycurcumin, and bisdemethoxycurcumin. Each compound was evaluated for its effect on *WT1* gene expression in four types of leukemic cell lines: K562, U937, HL-60, and Molt4.

1.2 Literature review

1.2.1 Cancer

Cancer is a generic term for a group of more than 100 diseases that can affect any part of the body. Other terms used are malignant tumor and neoplasm. Such diseases are characterized by uncontrolled, abnormal cell division leading to growth of abnormal tissue.

Hippocrates, the ancient Greek physician, is credited with being the first to recognize the difference between benign and malignant tumors. Benign tumors are not cancer. They often can be removed and, in most cases, they do not recur. Cells in benign tumors do not spread to other parts of the body. As a result, benign tumors are usually not considered life-threatening, though they should certainly be monitored and treated correctly. In contrast, malignant tumor or cancer cells can invade and destroy the tissue around them. Moreover, cancer cells can invade nearby tissues and spread through the bloodstream and lymphatic system to other parts of the body, causing death from cancer. This process is called metastasis. One of the most important things required for metastasis is the growth of a new network of blood vessels. This process is called angiogenesis. Normally, angiogenesis is necessary for the repair or regeneration of tissue during wound healing. Tumor angiogenesis, by contrast, is the growth of a network of blood vessels that (a) penetrates into cancerous growths, (b) removes waste products, and (c) supplies them with nutrients and oxygen. This process starts with cancerous tumor cells releasing molecules that send signals to activate genes in the host tissue that make proteins to encourage growth of new blood vessels.

Cancer can be divided into four types according to the type of tissue where it originates. The first, Carcinomas, are the most common types of cancer. They start in cells that cover external and internal body surfaces. Lung, breast, and colon cancer are the most frequent cancers of this type. The second type, sarcomas, start in the supporting tissues of the body such as bone, cartilage, fat, connective tissue, and muscle. Rhabdomyosarcoma (a tumor arising from muscle), Ewing's sarcoma (a tumor that typically arise from the bone), and osteosarcoma (a tumor that arises from bone) comprise the majority of sarcomas seen in children and young adults. The third type, lymphomas, begin in the lymph nodes and tissues of the reticuloendothelial (RE) system. Lymphomas can be divided into two major categories, Hodgkin's lymphoma

and non-Hodgkin's lymphoma. The latter, leukemias are cancers of the immature blood cells that grow in the bone marrow; such cells can accumulate the large numbers in bloodstream.

1.2.1.1 Genes involved in human cancers [33]

The multistep process that results in the development of cancer is the consequence of genetic changes in the cell. Genes that participate in this neoplastic cell transformation fall into three broad categories, proto-oncogenes and oncogenes, tumor suppressor genes, and DNA repair genes.

1.2.1.1.1 Proto-oncogenes and oncogenes

Oncogenes are mutated forms of normal cellular genes called proto-oncogenes. Proto-oncogenes are genes which code for proteins that regulate normal cellular growth processes such as proliferation, differentiation, and programmed cell death, and disruption of their normal function has profound consequences for the cell. Proto-oncogenes can be classified into five major groups based on the biochemical and functional properties of their protein products (Table 1). These groups are growth factors, growth factor receptors, signal transducers, transcription factors, and programmed cell death regulators. Oncogenes act in an autosomal dominant fashion to promote tumor development through a gain-of-function mechanism. To date, more than 100 different oncogenes have been discovered, with the majority identified in experimental animal models of cancer. However, only a relatively small number have been implicated in the initiation and progression of human cancers. The activation of oncogenes involves genetic changes to the cellular proto-oncogene either by mutation, gene amplification or chromosomal rearrangement.

Table 1. Some examples of proto-oncogenes and their products [33]

Proto-oncogene	Function of protein product
1. Growth factor	
sis	Platelet-derived growth factor
int-2	Growth factor
2. Growth factor receptors	
erb B	Tyrosine kinase/EGF receptor
erb B-2	Tyrosine kinase
kit	Tyrosine kinase
fms	Tyrosine kinase/CSF1 receptor
met, trk, ret, ros, sea	Tyrosine kinase
mas	Angiotensin receptor
3. Signal transduction	
H-ras, K-ras, N-ras	Binds GDP/GTP
src, abl, fes, fgr, lck, yes	Tyrosine kinase
raf, pim, mos	Serine/threonine kinase
gsp,gip	G protein
4. Transcription factors	
c-myc, L-myc, N-myc	DNA binding
fos	DNA binding/AP-1 complex with *jun*
jun	DNA binding/AP-1 complex with *fos*
erb A	DNA binding/T3 receptor
Ets, rel, ski, myb	DNA binding
5. Programmed cell death regulation	
bcl-2	Membrane protein/apoptosis

1.2.1.1.2 Tumor suppressor genes

Tumor suppressor genes are genes that reduce the probability that a cell in a multicellular organism will turn into a tumor cell and prevent cancer cell growth. Most tumor suppressor genes function directly in cell growth regulation pathways, and thus inactivating mutations lead to a selective growth advantage for the affected cells. The mechanism of action for the products of tumor suppressor genes is diverse and not fully understood. Conceptually, the products of tumor suppressor genes can be thought of as functioning to receive and process growth inhibitory signals from their surroundings. The tumor suppressor gene products work to suppress cell proliferation through the regulation of signal transduction and nuclear transcription. A mutation or deletion of such a gene leads to altered cellular morphology, loss of normal intracellular signaling pathways, and loss of normal intercellular interactions, all of which are features of neoplastic cells. A list of known or putative tumor-suppressor genes is given in Table 2.

1.2.1.1.3 DNA repair genes

These genes recognize and repair damaged DNA. They were first implicated as a cause of human disease by the study of rare syndromes with autosomal recessive inheritance, for example xeroderma pigmentosum, Bloom syndrome, and ataxia telangiectasia. More recently, DNA repair defects have also been implicated in cancer predisposition syndromes with an autosomal dominant mode of inheritance. The best example is the hereditary non-polyposis colorectal cancer syndrome (HNPCC), in which germ line mutations in any of at least four DNA repair genes, *hMSH2, hMLH1, hPMS1*, and *hPMS2*, have been identified [34-36]. However, few studies on the correlation between DNA repair genes and cancer development have been published.

Table 2. Some tumor-suppressor genes and their related cancer [37]

Gene	Inherited cancer	Sporadic cancer
Rb1	Retinoblastoma	Retinoblastoma, sarcomas, bladder, breast, esophageal, and lung carcinomas
p53	Li-Fraumeni cancer family syndrome	Bladder, breast, colorectal, esophageal, liver, lung, and ovarian carcinomas, brain tumors, sarcomas, lymphomas, and leukemias
APC	Familial adenomatous polyposis	Colorectal, stomach, and pancreatic carcinomas
WT1	Wilms' tumor	Wilms' tumor
WT2	Weidemann-Beckwith syndrome	Renal rhabdoid tumors, embryonal rhabdomyosarcoma
WT3	Wilms tumor	-
NF1	Neurofibromatosis type1	Colon carcinoma and astrocytoma
NF2	Neurofibromatosis type1	Schwannoma and meningioma
VHL	von Hippel-Lindau syndrome	Renal cell carcinomas
MEN1	Multiple endocrine neoplasia type1 (MEN1)	Endocrine tumors such as pancreatic adenomas
nm23	-	Melanoma, breast, colorectal, prostate, meningioma, others
MTS1	Melanoma	Melanoma, brain tumors, leukemias, sarcomas, bladder, breast, kidney, lung, and ovarian carcinomas

1.2.1.2 Causes of cancer

Cancer is now clearly understood to be a disease of abnormal gene function. Thus, factors that can cause gene defects may be causative factors for cancer development. Many causative factors have been identified for cancer, but heredity and environmental factors such as chemical agents, radiation, and viruses appear to be the most important factors.

1.2.1.2.1 Heredity

A brief survey of the common cancers reveals that most forms exhibit a significant correlation in familial incidence, suggesting some genetic contribution to susceptibility. Heredity is thus seen to operate in several ways in the causation of cancer. Some cancer may be induced by a single Mendelian gene. More commonly, multiple genes may affect the malignant transformation of particular cell types or of the systemic response to transformed cells. Finally, some latent tumor-producing viruses maybe maternally transmitted. Hereditary cancers are estimated to account for only 5-10% of all cancers. One of the most common of these is HNPCC. Other examples of hereditary cancers are listed in Table 3.

1.2.1.2.2 Chemical agents

The notion that human cancer might be caused by chemicals began in 1775. Pott noted the unusually high incidence of scrotal cancer among chimney sweeps exposed to chimney soot [38]. According to the International Agency for Research on Cancer (IARC) Monographs Database, more than 850 compounds were evaluated as carcinogens. Some information from this database is given in Table 4. Most chemicals that have been identified as carcinogens are by-products of industrial processes. Some such chemicals require metabolic activation, while others act directly as carcinogens.

Chemicals can contribute to tumor formation in at least three ways: (a) as genotoxins, (b) as co-carcinogens, and (c) as tumor promoters. By itself, Genotoxin can initiate the process of carcinogenesis by causing a mutation. In contrast, a co-carcinogen is a substance that by itself dose not causes a tumor to form, but rather enhances the potency of a genotoxin when they are present together. A tumor promoter is a substance that by itself does not cause a tumor, but enhances tumor formation

Table 3. Some examples of inherited cancer syndromes [39]

Syndrome	Primary tumor	Chromosomal location	Cloned gene
Dominant inheritance			
Familial retinoblastoma	Retinoblastoma	13q14.3	*Rb1*
Li-Fraumeni syndrome	Sarcomas, Breast cancer	17p13.1	*p53*
Familial adenomatous polyposis (FAP)	Colorectal cancer	5q21	*APC*
HNPCC	Colorectal cancer	2p16 3p21 2q32 7q22	*hMSH2* *hMLH1* *hPMS1* *hPMS2*
Neurofibromatosis type1	Neurofibromas	17q11.2	*NF1*
Neurofibromatosis type2	Acoustic neuromas meningiomas	22q12.2	*NF2*
Wilms tumor	Wilms tumor	11p13	*WT1*
Familial breast cancer 1	Breast cancer	17q21	*BRCA1*
Familial breast cancer 2	Breast cancer	13q12	*BRCA2*
Hereditary papillary renal cancer (HPRC)	Renal cancer (papillary type)	7q31	*c-met*
Familial melanoma	Melanoma	9q21 12q13 Others?	$p16^{INK4A}$ *cdk4*
MEN1	Pancreatic Islet cell cancer	11q13	*MEN1*
MEN2	Medullary thyroid cancer	10q11.2	*c-ret*
Recessive inheritance			
Ataxia telangiectasia	Lymphoma	11q22	*ATM*
Bloom syndrome	Solid tumors	15q26.1	*BLM*
Xeroderma pigmentosum	Skin cancer	Multiple complementation groups	*XPB, XPA*
Fanconi anemia	Acute myelogenous leukemia	9q22.3 16q24.3 Two others?	*FACC* *FACA*

Table 4. Some well-known chemical carcinogens from the IARC Monograph Databases [40]

Chemical carcinogen
Category 1[a]
- TCDD (dioxin)
- 4-aminobiphenyl
- Benzidine
- Sulfur mustard
- Nickle, chromium, cadmium , and arsenic
- Asbestos
- Vinyl chloride
- Tobacco smoke and tobacco products
Category 2A
- 2,3,7,8-tetrachlorodibenzo-p-dioxin benzo[a]pyrene
- Benzo[a]anthracene
- Formaldehyde
- Cisplatin
Category 2B
- Manynitrosamines
- Benzo[b]fluoranthene
- Cobalt
- Alfatoxin M1
- Ceramic fibers
- Glasswool
- Hydrazine
Category 3
- Acrylic fibers
- Hydrogen peroxide
- Ethylene

[a] Categorization by group based on the following criteria of evaluation for agents as stated in the Preamble to the IARC Monographs. Category 1, the agent is carcinogenic to humans. Category 2A, the agent is probably carcinogenic to humans. Category 2B, the agent is possibly carcinogenic to humans. Category 3, the agent is not classifiable as to its carcinogenicity to humans.

when it is given repeatedly after exposure to a genotoxin. However, it has been estimated that chemical pollution accounts for less than 1% of human cancers [41].

1.2.1.2.3 Radiation

The carcinogenic risks of radiation exposure in people come from many sources, including occupational exposure (e.g., radiologists and uranium miners), therapeutic exposures, and accidental exposures. However, most information has been derived from studies of the atom bomb survivors in Hiroshima and Nagasaki, who were exposed in 1945. Moreover, the best available information is from studies of exposure during medical x-ray examinations, particularly of pregnant women, which resulted in fetal exposure to irradiation. Radiation risk is defined as an increase in the number of cancer deaths over that expected for an unirradiated population. It has been observed to increase the incidence of skin cancer, leukemia, lung cancer, and other types of cancer [42-44].

Ionizing radiation from radioisotopes also causes cancer. Leukemia among radium handlers was reported about the same time as leukemia among persons exposed to x-rays. The ingestion of small amounts of radium led to its deposition in bone and induced sarcoma of bone [45]. Not only ionizing radiations can cause cancer, but also nonionizing radiation in form the ultraviolet (UV) light also cause cancer. During the early part of the 20^{th} century, sunlight was suspected as a cause of human cancer [46]. Ionizing and UV radiation can both give rise to tumors, but different DNA lesions probably initiate the process of carcinogenesis. For ionizing radiation, the critical damage probably leads to instability of the DNA and increased probability of errors in DNA replication occurring in subsequent cell cycles. This may lead to changes in expression or activation of oncogenes or inactivation of tumor suppressor genes. Carcinogenesis induced by UV light requires multiple exposures. Tumor induction increases with total dose and has wavelength dependence similar to that for sunburn and for induction of pyrimidine dimers in DNA.

1.2.1.2.4 Oncoviruses

Within the last three decades, it has become increasingly clear that viruses play an important role in the development of a significant percentage of human cancers. At

present, it is recognized that viral infections are linked to at least 15% of all malignant tumors in humans [47]. One cause of mutation, insertional mutagenesis, occurs when cells are infected by viral genes. This brings the cellular oncogenes under the influence of viral promoters or regulators that cause mutations by adding new DNA rather than altering existing DNA.

Cancer-associated viruses are found in several virus families and encompass both DNA and RNA viruses [Table 5]. Some animal studies have indicated that viruses may be involved in carcinogenesis [48, 49]. There is already some evidence linking the Epstein-Barr virus (EBV), one of the most common of all viruses, to two types of cancer, a carcinoma and lymphoma [50]. Another DNA virus group, the herpes virus, has been shown to have oncogenic potential in many types of cancers [51]. The human papilloma virus (HPV) is the cause of cervical carcinoma. Among the RNA viruses and retroviruses, evidence is accumulating that links these viruses to breast cancer, leukemias, and osteosarcomas [52-54].

Two cancer theories propose the involvement of oncoviruses. One infers that exogenous viral infection occurs and that reverse transcriptase is required to intergrate the viral genome into the host genome. The other considers that oncogenes, some of which may represent proviruses, are inherited through endogenous genes that are acquired at birth and require only minimal mutation for expression.

However, many people can be infected with a cancer-causing virus yet never develop cancer. For example, many women have a high risk HPV infection, but never develop cervical cancer.

1.2.1.2.5 Other environmental factors

Other environmental factors which are involved in cancer development are dietary, tobacco smoking, alcohol drinking and some of chemotherapeutic drugs. Dietary factors are estimated to account for approximately 30% of cancers in western countries and up to 20% in developing countries. In the western world, many people eat too many animal fats and not enough fresh fruit and vegetables. This type of diet is known to increase the risk of cancer. Some food additives, such as saccharin, may be a carcinogens [55]. In addition, pickled foods may increase the risk of stomach cancer and esophageal cancer [56]. Moreover, frying and baking foods at high temperatures

can create chemicals called heterocyclic amines and polycyclic aromatic hydrocarbons. These may increase the risk of some types of cancer, such as stomach cancer and colon cancer.

Table 5. Human oncoviruses and their associated human cancers [57]

Viruses	Virus family	Associated human cancers
DNA viruses		
- HPV	Papovaviridae	Anogenital, skin and oral cancer
- Hepatitis B virus (HBV)	Hepadnaviridae	Hepatocellular carcinoma
- EBV	Herpesviridae	Nasopharyngeal carcinoma, Lymphomas
- Human herpesvirus 8 (HHV8)	Herpesviridae	Kaposi's sarcoma
RNA viruses		
- Hepatitis C virus (HCV)	Flaviviridae	Hepatocellular carcinoma, Body-cavity lymphoma
- Human T cell lymphotropic virus-1 (HTLV-1)	Retroviridae	Adult T-cell leukemia

Tobacco smoking accounts for at least 30% of all cancer deaths. It is a major cause of cancers of the lung, larynx, oral cavity, pharynx, and esophagus [58-60]. About 87% of lung cancer deaths are caused by smoking. In addition, smoking is a contributing factor in the development of cancers of the bladder, pancreas, liver, uterine cervix, kidney, colon and rectum, stomach, breast, and some leukemias [60-64]. Furthermore, the smoke from cigarettes has a harmful health effect on those exposed to the smoke. To date, more than 4,000 individual compounds have been identified in tobacco and tobacco smoke. Among these are more than 60 compounds that are known carcinogens.

According to a report form the American Institute for Cancer Research, alcohol increases the risk of cancer of the mouth, pharynx, larynx, and esophagus. The risk of

upper respiratory tract cancer is greatly increased if drinkers also smoke. In addition, alcohol also increases the risk of liver cancer [65] and probably increases the risk for colon, rectal, and breast cancer [66, 67]. The sensitive tissues of upper respiratory tract are directly exposed to alcohol in beverages, and thus may be damaged to the point where cancer may develop.

1.2.2 Leukemia

Leukemia was first recognized by the German pathologist Rudolf Virchow and John Bennett in 1845 [68, 69]. It is a group of acute or chronic malignant diseases that involves the blood-forming organs, characterized by an abnormal proliferation, differentiation, and excessive production of white blood cells and their precursors in the bone marrow, with or without a corresponding increase of those in the circulating blood. This results in decreased production and function of normal blood cells. Leukemia can spread to the lymph nodes, spleen, liver, central nervous system, and other organs. From studies on laboratory animals, four factors have been proposed as playing a role in causation of leukemia (Table 6).

Table 6. Factors proposed to play a role in leukemogenesis [70]

Factors	Examples
Hereditary abnormalities or genetic susceptibility	Down's syndrome, Fanconi anemia, Kleinfelter syndrome, Bloom's syndrome, Wiskott-Aldrich syndrome, Blackfan-Diamond syndrome
Somatic mutation	Radiation, Chemicals, Drugs
Viral infection	Retroviruses (HTLV-1)
Immunologic disorders	Wiskott-Aldrich syndrome, Ataxia telangiectasia Bruton's type X-linked agammaglobulinemia, Immunosuppressive therapy

1.2.2.1 Classification of leukemia

Leukemia is divided into four categories, according to the degree of cell differentiation (acute or chronic), and according to predominant type of cell involved (myelogenous or lymphocytic). Acute leukemia is characterized by the presence of at least 30% of blasts, which are immature and functionless white blood cells. Large quantities of blasts generally overgrow the bone marrow, leaving very little space for normal bone marrow cells. This form of the disease generally requires immediate treatment. Chronic leukemia is characterized by a large and uncontrolled growth of more mature white blood cells. These types of leukemias tend not to progress as rapidly, and treatment is often milder than that for acute leukemias. Thus, the four types of leukemia are acute myelogenous leukemia (AML), chronic myelogenous leukemia (CML), acute lymphoblasttic leukemia (ALL), and chronic lymphocytic leukemia (CLL).

In 1976, a group of investigators formed the French-American-British (FAB) cooperative group, with the purpose of establishing a classification system for acute leukemia that would have clinical and investigational relevance [71]. The initial intent of the FAB group was to classify AML and ALL into subcategories on the basis of morphological and cytochemical characteristics. Since then the classification has undergone several modifications which, together with cytogenetics, immunological markers, and *in-vitro* cell culture findings, may provide a more accurate prognostic, therapeutic, and investigational subclassification of the acute leukemias [72-75]. According to this classification, AML was classified into 8 subcategories (M0 to M7), while ALL was classified into 3 subcategories, L1 to L3.

In 1986, the First Morphologic, Immunologic and Cytogenetic (MIC) Cooperative Study Group published the criteria for classification of ALL on the basis of morphological, immunological, and cytogenetic findings [76]. Then, the second MIC workshop published the criteria for classification of AML [77-78]. After the fourth MIC meeting in 1989, the criteria for classification of CLL were published [79].

In the years 1999 to 2001, the World Health Organization (WHO) classification, the most recent classification for tumors of hematopoietic and lymphoid tissues, was proposed [80-82]. In an attempt to define biological homogenous entities

that have clinical relevance, morphological, immunophenotypic, genetic and clinical features were incorporated into the classification of leukemias.

Some important information about leukemia classification is provided in the following section.

1.2.2.1.1 Acute myelogenous leukemia (AML)

AML is a clonal malignant disease of hemopoietic tissue that is characterized by the proliferation of abnormal blast cells, principally in the marrow, and impaired production of normal blood cells. AML involves a variety of cell types, including myeloblasts, promyelocytes, monoblasts, promonocytes, erythroblasts, and megakaryoblasts. AML is also referred to as acute nonlymphoid leukemia (ANLL), acute myeloid leukemia, acute granulocytic leukemia, acute myeloblastic leukemia, and acute myelocytic leukemia. Similar to the other neoplastic processes, AML is considered as a monoclonal disorder. Leukemogenesis appears to be a complex process. It includes at least two major steps: (a) development of a clone of abnormal precursor cells due to structural alterations or mutation of certain genes, and (b) progression to acute leukemia by further genetic changes involving one or more oncogenes and/or suppressor genes. AML occur primarily in adults and in infants younger than 1 year. It accounts for only 15 to 20% of the leukemias in children. There appears to be an increase in incidence of AML in adults after 50 years of age.

AML begins in a single somatic hematopoietic progenitor that transforms to a cell incapable of normal differentiation. Many of these cells no longer possess the normal property of apoptosis, thus resulting in a cell with a prolonged life span and unrestricted clonal proliferation. Because the transformed cell lacks normal regulatory and growth constraints, it has a favorable competitive advantage at the expense of normal hematopoietic cells. The result is the accumulation of abnormal cells with qualitative defects. A major cause of morbidity and mortality is the deficiency of normal functioning mature hematopoietic cells rather than the presence of numerous malignant cells.

Splenomegaly from leukemic infiltration further contributes to pancytopenia by sequestering and destroying circulating erythrocytes and platelets. As the disease

progresses, there are increasing signs and symptoms resulting from anemia, thrombocytopenia, and neutopenia. Moreover, leukemic cells may infiltrate other tissues, causing many significant complications including central nervous system (CNS) involvement, pulmonary dysfunction and skin infiltration.

Classification:

According to the FAB classification, the original proposal has been revised several times and the updated version includes 8 major groups (M0 to M7) with subtypes for four of them (Table 7). The classification criteria are based on morphological and cytochemical features. In addition, immunophenotyping is necessary for some of the categories [74, 75]. The FAB classification of AML is a lineage-based morphological classification that categorizes cases according to the degree of maturation of the leukemic cells and their lineage differentiation.

The MIC classification established 10 subtypes of AML which are characterized by unique cytogenetic, morphological, and immunological criteria (Table 8). To date, the last update of AML classification is the WHO classification. A preview of the WHO classification was published recently [83]. The newly revised WHO classification of AML is shown in Table 9. These classifications address the problems of an exclusively lineage-based or an exclusively cytogenetic/molecular classification by combining the best features of both. The result is a classification that enhances clinical and diagnostic utility and retains usability.

Although three major classifications of AML were widely used, the FAB classification was selected as an example to explain the criteria for classifying AML in this section.

A) Acute myeloid leukemia with minimal differentiation (M0)

In 1991, the FAB group published a proposal to designate AML with minimal differentiation as AML-M0 [75]. Since then, contributions from various authors have supported a better definition of this form of leukemia [84-86]. AML-M0 accounts for approximately 5% of AML cases [87-89]. It is concentrated in children younger than 3 years old and adults older than 60 years of age [90]. AML-M0 is considered an aggressive disease characterized by a poor prognosis, low remission rate and early

Table 7. The FAB classification of AML

Type	Key morphology
AML-M0	Myeloblasts >90% of the nonerythroid BM cells; no Auer rods; <3% MPO+
AML-M1	Myeloblasts >90% of the nonerythroid BM cells; rare Auer rods; >3% MPO+
AML-M2	Myeloblasts >30% but <90% of the nonerythroid BM cells; frequent Auer rods
AML-M3 Subtypes: Hypergranular Microgranular	30% or more promyelocytes
AML-M4 Subtype: M4Eo	Immature monocytes comprising 20-80% of the nonerythroid BM cells Eosinophils with basophilic granules
AML-M5 Subtypes: M5a M5b	 Monoblasts comprising >80% of the nonerythroid BM cells Mixture of monoblasts and more mature monocytic cells
AML-M6 Subtypes: Acute pure erythroid leukemia Acute erythroleukemia	 50% or more erythroid precursors; increased erythroblasts, marked dysplasia 50% or more erythroid precursors; 30% or more myeloblasts in the nonerythroid BM cells
AML-M7	30% or more megakaryoblasts

Table 8. The MIC classification of AML [77]

Karyotypic change	Frequency* (%)	Morphology (FAB)	Suggested MIC nomenclature
t(8;21) (q22;q22)	12	M2	M2/t(8;21)
t(15;17) (q22;q12)	10	M3, M3v	M3/t(15;17)
t/del/(11) (q23)	6	M5a (M5b, M4)	M5a/t(11q)
inv/del(16) (q22)	5	M4Eo	M4Eo/inv(16)
t(9;22) (q34;q11)	3	M1 (M2)	M1/t(9;22)
t(6;9) (p21-22;q34)	1	M2 or M4 with basophilia	M2/t(6;9)
inv(3) (q21q26)	1	M1 (M2, M4, M7) with thrombocytosis	M1/inv(3)
t(8;16) (p11;p13)	< 0.1	M5b with phagocytosis	M5b/t(8;16)
t/del (12) (p11-13)	< 0.1	M2 with basophilia	M2 Baso/t(12p)
+4	< 0.1	M4 (M2)	M4/+4

***** Percentage incidence among AML patients with abnormal karyotypes calculated on the basis of the information in Mitelman F [91].

Table 9. The WHO classification of AML [92]

Type	Type
AML with recurrent cytogenetic translocations - AML with t(8;21) (q22;q22) - Acute PML with t(15;17) (q22;q21) - Variant acute PML with t(v;17) - AML with abnormal bone marrow eosinophils inv(16) (p13;q22) or t(16;16) (p13;q22) - AML with 11q23 abnormalities	**AML with multilineage dysplasia** - With prior myelodysplastic syndrome (MDS) - Without prior myelodysplastic syndrome (MDS)
AML and myelodysplastic syndrome, therapy related - Alkalating agent related - Topoisomerase II inhibitor related - Other types	**AML not otherwise categorized** - AML minimally differentiation - AML without maturation - AML with maturation - Acute myelomonocytic leukemia - Acute monocytic leukemia - Acute erythroid leukemia - Acute megakaryocytic leukemia - Acute basophilic leukemia - Acute panmyelosis with myelofibrosis

relapse. The morphological features of blast cells in AML-M0 appear quite heterogenous but a uniform finding is agranular cytoplasm, absence of Auer rods and lack of cytochemical markers of myeloid differentiation. In most cases, the blast cells are large, with round or oval nuclei, and clear or moderately basophilic cytoplasm. The nuclear/cytoplasmic (N/C) ratio is variable. However, there are cases in which blasts may be small or pleomorphic, with a variable degree of basophilic cytoplasm and high N/C ratio. In some cases a proportion of the blast population can be vacuolated and monocytoid shaped. Less than 3% of blast cells are positive for myeloperoxidase (MPO) and Sudan Black B (SBB). The peripheral white blood cell count may be low.

Flow cytometric analysis shows variable expression of CD13 and CD33. Moreover, the stem cell marker CD7, CD34, HLA-DR, and TdT are frequently expressed.

B) Acute myeloblastic leukemia without maturation (AML-M1)

AML-M1 accounts for approximately 20% of the AMLs. It is characterized by a lack of myeloid maturation and the presence of 90% or more myeloblasts in the nonerythroid component of the bone marrow. At least 3% of the blast cells are MPO and/or SBB positive. Auer rods are infrequent. AML-M1 blasts are usually positive for CD13, CD33, and HLA-DR, and may express CD34. AML cells in a small proportion of cases are TdT positive. For cytogenetic studies, the most frequent cytogenetic abnormalities association with AML-M1 are t(9;22) and t(11;19).

C) Acute myelogenous leukemia with maturation (AML-M2)

AML-M2 accounts for approximately 30% of the AMLs. It shows evidence of partial maturation of myeloblasts. In addition to myeloblasts, promyelocytes, myelocytes, and more mature myeloid cells are also present. Auer rods may be present not only in myeloblasts but in more mature cells. Myeloblasts are strongly MPO and SBB positive and often express HLA-DR, CD13, CD15, and CD33. Expression of CD34 and CD117 is less frequent. Non-specific esterases (NSE) stain is negative.

The most frequent cytogenetic abnormalities associated with AML-M2 are t(8;21), observed in about 20% of the cases. These cases often show eosinophilia and CD34 positive blasts. A small proportion of the AML-M2 cases demonstrate t(6;9), often associated with bone marrow basophilia.

D) Acute promyelocytic leukemia (AML-M3 or APL)

Promyelocytes are the predominant immature cells in AML-M3. Myeloblasts are also increased, but not to the extent of promyelocytes. AML-M3 is divided into two major morphologic subtypes: hypergranular and microgranular (hypogranular). The hypergranular variant is characterized by the presence of atypical promyelocytes heavily loaded with azurophilic granules. Auer rods are often present and may appear in bundles in some of the promyelocytes (faggot cells). In the microgranular variant, promyelocytes contain fewer and finer azurophilic granules. The nuclei are often

lobulated, folded or convoluted, displaying a monocyte-like morphology. Auer rods are often present.

The leukemia cells in AMLM3 are strongly MPO and SBB positive. A small proportion of cases may also demonstrate NSE reactivity. Moreover, CD13, CD33, CD11, and CD15 antigens are often express but HLA-DR and CD14 are negative.

The cytogenetic abnormality associated with AML-M3 is t(15;17), which has been observed in over 90% of the cases. This translocation puts two genes, *PML* and retinoic acid receptor-α (*RAR-α*), together with a hybrid protein product that appears to block the myeloid differentiation process. Rare cases of AML-M3 are associated with t(11;17).

E) Acute myelomonocytic leukemia (AML-M4 or AMML)

In AMML, myeloblasts, monoblasts, and promonocytes are the predominant immature cells.Therefore, there is considerable pleomorphism in cell size, nuclear shape, and the amount of cytoplasm. The proportion of the monocytic component in the leukemic population ranges from more than 20% to less than 80%. Auer rods may be present but are infrequent. Patients with AMML often demonstrate an absolute peripheral blood monocytosis with the presence of immature forms.

Immunophenotypic studies reveal expression of a combination of granulocytic and monocytic markers such as CD11c, CD13, CD14, CD15, CD33, CD64, CD68, and HLA-DR. The leukemic cells in some cases may also express CD2, CD4 or TdT.

The most frequent cytogenetic findings in AMML are t(6;9) and 11q23 abnormalities. Approximately 15% to 30% of the cases are associated with atypical eosinophilia and abnormality of chromosome 16, (16q22). The eosinophils in this subtype (AML-M4Eo) contain a mixture of eosinophilic or basophilic granules. The M4Eo subtype has a more favorable clinical outcome, but has a higher frequency of CNS involvement.

F) Acute monoblastic leukemia (AML-M5 or AMoL)

AMoL accounts for about 10% of all AML cases. It is divided into two subtypes: M5a and M5b.

M5a demonstrates minimal morphological evidence of monocytic differentiation. Monoblasts account for 80% or more of the leukemic cells. They have a variable amount of gray-blue or deep blue cytoplasm, often round or oval nuclei and a single or a few very prominent nuclei.

M5b displays partial monocytic differentiation with a mixture of monoblasts, promyelocytes and more mature monocytic cells. Promonocytes are characterized by abundant pale blue cytoplasm, scattered azurophilic granules, folded or lobulated nuclei, and finely dispersed chromatin. Nucleoli are usually prominent. Auer rods are detected in a small proportion of the M5b subtype.

Leukemic cells are usually strongly positive for NSE and lysozyme and express all or some of the monocytic-associated markers such as CD14, CD64, and CD68. They are often positive for CD4, CD11c, CD33, and HLA-DR, and may also express CD34. Abnormalities of chromosome 11 (11q23) and trisomy 8 are the most frequent cytogenetic findings.

G) Acute erythroid leukemia (AML-M6)

Most AML-M6 cases are preceded by a refractory anemia. Diagnosis of AML-M6 is established based on the following bone marrow findings: (a) a reversed myeloid: erythroid (M: E) ratio (more than 50% of the bone marrow cells are erythroid); and (b) 30% or more of the nonerythroid marrow cells are myeloblasts. The erythroid lineage shows marked dysplasia, such as the presence of bi-or multinucleated erythroblasts, giant forms and cells with nuclear fragments. Megaloblastic erythropoiesis is common. An increased number of megakaryoblasts may be present. Blood smears often show abnormal red blood cell (RBC) morphology and marked anisopoikilocytosis. The *de novo* AML-M6 accounts for about 5% of all AML cases.

The erythroid precursors express glycophorin A, transferrin receptor (CD71), hemoglobin and spectrin, and may show chunk-like cytoplasmic PAS positivity. Myeloblast are MPO and SBB positive and express CD13, CD33, HLA-DR, and sometimes CD34. Cytogenetic studies may demonstrate structural abnormalities in chromosome 3, 5, and 7, or trisomy 8.

H) Acute megakaryoblastic leukemic (AML-M7)

The cell population in AML-M7 is predominantly megakaryoblasts. They are pleomorphic and vary in size, amount of cytoplasm, chromatin density, and the number of nuclei. Bone marrow fibrosis, as a consequence unsuccessful marrow aspiration, is one of the characteristic features of AML-M7 observed in over 70% of the cases. Megakaryoblasts often appear in clusters trapped within the fibrotic tissue. AML-M7 comprises about 5% of all AML cases but is probably the most common type of AML associated with Down's syndrome.

Megakaryoblasts and immature megakaryocytes express CD41, CD42, and CD61, and are positive for factor VIII. They do not express MPO and SBB but demonstrate platelet peroxidase (PPD) activity by immunoelectron microscopy and may show dot-like NSE positivity.

Trisomy 21 and t(1;22) have been reported in *de novo* AML-M7. Moreover, trisomy 8 and structural abnormalities of chromosomes 5 and 7 have been found in therapy-related cases.

1.2.2.1.2 Acute lymphoblastic leukemia (ALL)

ALL is a malignant disorder resulting from a clonal proliferation and accumulation of progenitors that exhibit cell markers associated with the earliest stages of lymphoid maturation. The leukemia originates in the marrow, and the leukemic clone may exhibit features of either B-cell or T-cell commitment. ALL is the most common malignancy in children, representing nearly one third of all pediatric cancers.

In cases of ALL, a lymphoid progenitor cell becomes genetically altered and subsequently undergoes dysregulated proliferation and clonal expansion. In most cases, the pathophysiology of transformed lymphoid cells reflects the altered expression of genes whose products contribute to the normal development of B cells and T cells. Leukemic blasts have long been thought to represent the clonal expansion of hematopoietic progenitors blocked in differentiation at discrete stages of development. Recent data challenge this theory and suggest that leukemia arises from a stem cell that acquires features of differentiated cells. While this may appear to be a subtle difference, it is important because it implies the need to eradicate the leukemic stem cell, and not just the differentiated blasts, to achieve a cure. Nevertheless,

leukemic blasts provide large uniform populations for molecular and functional analyses.

ALL is generally thought to arise in the bone marrow, but leukemic blasts may be present systemically at the time of presentation, including in the bone marrow, thymus, liver, spleen, lymph nodes, testes, and the CNS.

Classification:

The FAB group has defined three groups of ALL (L1, L2, and L3), based on the morphology and heterogeneity of bone marrow lymphoblasts (Table 10). Cytological features used to evaluate blast morphology include cell size, nuclear chromatin, nuclear shape, nucleoli, amount of cytoplasm, amount of basophilia in cytoplasm, and cytoplasmic vacuolation. Up to 10% of the cells may vary from each specific feature within the group.

According to the 1st MIC classification report, ALL may be classified into two major categories; that include both B-cell and T-cell lineages. The classification is based on morphological, immunophenotypic , and cytogenetic characteristics.

For the WHO classification, there are three major categories of ALL, B-cell precursor, T-cell precursor, and Burkitt cell leukemia (Table 11). This classification is based on immunophenotypic characteristics. Within the B-cell precursor category there are several subtypes, identified by cytogenetic/molecular abnormalities. The major treatment and prognostic groups in childhood ALL are identified in this classification. In this section, the FAB classification is used as an example for ALL classification.

A) ALL-L1

This is the most common ALL found in children. It appears to have the best prognosis. The key to this subtype is homogeneity of lymphoblasts. Most cells within any one case are homogeneous in size. The lymphoblasts are predominantly small, up to twice the size of a small lymphocyte. The chromatin is usually finely dispersed but may appear more condensed in small cells. The chromatin pattern may vary from case to case but is homogeneous within cases. The nuclear shape is regular with occasional clefts or indentations. Nucleoli are not prominent and may be absent. The cytoplasm is reduced and only slightly or moderately basophilic.

Table 10. The FAB criteria for the subtypes of ALL [70]

Cytological features	L1	L2	L3
Cell size	Small cells predominate	Large, heterogeneous in size	Large and homogeneous
Nuclear chromatin	Homogeneous in any one case	Variable-heterogeneous in any one case	Finely stippled and homogeneous
Nuclear shape	Regular, occasional clefting or indentation	Irregular; clefting and indentation common	Regular-oval to round
Nucleoli	Not visible, or small and inconspicuous	One or more present, often large	Prominent; one or more vesicular
Amount of cytoplasm	Scantly	Variable; often moderately abundant	Moderately abundant
Basophilia of cytoplasm	Slight or moderate, rarely intense	Variable; deep in some	Vary deep
Cytoplasmic vacuolation	Variable	Variable	Often prominent

Table 11. The WHO classification of ALL [92]

Type of ALL
Precursor B-cell ALL Cytogenetic subtypes - t(9;22) (q34;q11) - t(v;11) (v;q23) - t(1;19) (q23;p13) - t(12;21) (p12;q22)
Precursor T-cell ALL
Burkitt cell leukemia

B) ALL-L2

This is the most frequent ALL found in adults. Occasionally, the cells have granular inclusions, making it difficult to distinguish L2 from AML-M2, if cytochemical stains are not performed. In contrast to granules in myeloid cells, the granules in lymphoblasts are peroxidase-negative and the cells are positive for TdT. In contrast to the blasts of L1, the blasts in L2 demonstrate a marked heterogeneity in any one case and between cases. There is a great deal of variability in cell size within each case, but the cells are generally two times the size of a small lymphocyte. The nucleus is irregular with clefting and indentations. The chromatin pattern in any one case is heterogeneous, varying from finely reticular to condensed. Nucleoli are always present but vary in size and number. Often they appear very large. The cytoplasm is abundant with variable basophilia.

C) ALL-L3

This is the rarest form of ALL. It occurs in both adults and children. The lymphoblasts are similar in appearance to those found in Burkitt's lymphoma. The blasts are homogeneous both within and between cases. The cells are large with abundant, intensely basophilic cytoplasm. There is prominent cytoplasmic vacuolization. Vacuoles also may be present in L1 and L2 lymphoblasts but it is much less intense than in L3. The nucleus is oval to round, with dense but finely stippled chromatin and one or more prominent nucleoli.

1.2.2.1.3 Chronic myelogenous leukemia (CML)

CML is a hemopoietic stem cell disease that is characterized by extreme blood granulocytosis, granulocytic immaturity, anemia, basophilia, thrombocytosis, and splenomegaly. There is an accumulation of malignant hematopoietic cells in the bone marrow, which may ultimately lead to bone marrow failure. Cytopenia may result in hemorrhage, infection, and organ compromise such as congestive heart failure from severe anemia. CML accounts for 7 to 15% of all leukemias in adults [93-94].

CML is characterized by three phases that occur during the course of the disease. Initially, there is a chronic phase that may last approximately 2 to 5 years. Then, with progression of the disease there is an accelerated phase that lasts 6 to 18

months. Finally, blast crisis phase develops, which appears similar to an aggressive acute leukemia with a survival of only about 3 to 6 months. The disease has a very high propensity to evolve into an accelerated, rapidly fatal phase resembling acute leukemia and it can develop to the acute or blast crisis phase. In blast crisis, abnormal immature white blood cells in bone marrow and peripheral blood are extremely high. This evolution leads to a disease analogous to acute leukemia. It is a phase of the disease that is very difficult to control, due to the complications of the disease such as infection, bleeding, kidney problems and fever. These complications lead to a decrease in the survival rate of the patients.

CML was the first malignancy to be linked to a clear genetic abnormality. It is characterized by a balanced reciprocal translocation between the long arms of chromosomes 9 and 22 [t(9;22)(q34;q11.2)] that occurs in about 90% to 95% of cases. This translocation leads to overtly foreshortened long arms of one of the chromosome pair number 22, referred to as the Philadelphia (Ph) chromosome [95, 96]. Moreover, this translocation results in the juxtaposition of the *c-abl* proto-oncogene from chromosome 9 with a portion of the *bcr* gene located on chromosome 22 thereby producing a novel *bcr/abl* fusion gene. The gene product results in an 8.5 kb mRNA transcript that generates a 210 kDa bcr/abl fusion protein having abnormal tyrosine kinase activity. Through phosphorylation this enzyme may activate different signal transduction pathways that may result in increased proliferation and decreased apoptosis of hematopoietic cells.

Many infectious, inflammatory, severe hemorrhagic, hemolytic, and malignant disorders can cause a leukemoid reaction indistinguishable from true CML. At times, the clinical findings may permit an accurate diagnosis, but in some cases differential diagnosis requires further investigation. In a leukemoid reaction, the leukocytosis is generally accompanied by a predominance of segmented neutrophils and bands on the blood smear. Myeloblasts and promyelocytes are prominent in CML while rarely present in a leukemoid reaction. In addition, levels of monocytes, eosinophils, and basophils are generally not elevated in a leukemoid reaction, whereas they are typically increased in CML. Other differential diagnostic tests, which can differentiate a leukemoid reaction from CML, are the leukocyte alkaline phosphatase (LAP) score

and the Philadelphia chromosome. LAP is an enzyme present in mature myeloid cells. Thus, the LAP score is typically increased in a leukemoid reaction but lower in CML.

Classification:

The classification of chronic myeloid leukemia is based on peripheral blood differential counts, cytological features in blood and bone marrow, cytogenetics and molecular genetics. Bone marrow findings are less useful in diagnosis than the peripheral blood features. Cytochemistry can be of some value if cytogenetic and molecular genetic analyses are not available, but otherwise is redundant. Immunophenotyping is of no value during the chronic phase of these diseases although it has a role in identifying the lineage of blasts during acute transformation.

The FAB classification group classified CML into nine categories, including CML with and without the Ph chromosome, atypical CML, chronic myelomonocytic leukemia (CMML), chronic neutrophilic leukemia, chronic eosinophilic leukemia, chronic basophilic leukemia, chronic mast cell leukemia or systemic mastocytosis, juvenile chronic myeloid leukemia, and other myelodysplastic/myeloproliferative disorders of childhood and CML following other myeloproliferative disorders.

Recently, the WHO, in conjunction with the Society for Hematopathology and the European Association of Hematopathology, published a new classification for hematopoietic and lymphoid neoplasms. In the WHO proposal, CML is defined specifically as a myeloproliferative disease that is characterized by the invariable presence of the Ph chromosome or the *bcr/abl* fusion gene. Although in most cases the diagnosis is easily made from morphological evaluation of the blood smear, confirmation by genetic studies is essential, particularly in view of the advent of therapy that targets the bcr/abl fusion protein [97]. The WHO committees relied upon the literature as well as upon the collective experience of the clinical advisory committee members to refine the criteria for accelerated and blast phase [98-101] that are outlined in Table12.

Table 12. Criteria for accelerated and blast crisis phases of CML [102]

CML, accelerated phase (AP) Diagnose if one or more of the following is present: - Blasts 10 to 19% of peripheral blood white cells or bone marrow cells - Peripheral blood basophils at least 20% - Persistent thrombocytopenia (<100 x 10^9/L) unrelated to therapy, or persistent thrombocytosis (>1000 x 10^9/L) unresponsive to therapy - Increasing spleen size and increasing WBC count unresponsive to therapy - Cytogenetic evidence of clonal evolution (ie, the appearance of an additional genetic abnormality that was not present in the initial specimen at the time of diagnosis of chronic phase CML) Megakaryocytic proliferation in sizable sheets and clusters, associated with marked reticulin or collagen fibrosis, and/or severe granulocytic dysplasia, should be considered as suggestive of CML-AP. These findings have not yet been analyzed in large clinical studies, however, so it is not clear if they are independent criteria for accelerated phase. They often occur simultaneously with one or more of the other features listed.
CML, blast crisis phase (BP) Diagnose if one or more of following is present: - Blasts 20% or more of peripheral blood white cells or bone marrow cells - Extramedullary blast proliferation - Large foci or clusters of blasts in bone marrow biopsy

1.2.2.1.4 Chronic lymphocytic leukemia (CLL)

CLL is a neoplastic disease characterized by the accumulation of small mature lymphocytes in bone marrow, blood, and lymphoid tissues. It is the most common adult leukemia in western societies and affects mainly elderly individuals [103]. About a third of patients are less than 60 years of age at diagnosis [104]. Generally, the neoplastic lymphocytes are of the B-cell lineage. However, less than 2 percent of cases represent a T-cell origin of neoplastic cells. CLL follows an extremely variable clinical course with overall survival times ranging form months to decades. Some patients have no or minimal signs and symptoms during their entire disease course and have a survival time similar to age-matched controls. Other patients experience rapidly deteriorating blood counts and organomegaly and suffer from symptoms at diagnosis or soon thereafter, necessitating therapy. Patients subsequently may develop cytopenia as a result of progressive bone marrow involvement or from autoimmune abnormalities. This may allow bacterial, fungal, and viral infections to occur due to hypogammaglobulinemia and T-cell dysfunction. Moreover, CLL patients may also develop either transformation to a large cell lymphoma (Richter's syndrome) or prolymphocytic leukemia.

In most case of CLL, the Bcl-2 protein is overexpressed [105]. This protein is produced from the *bcl-2* proto-oncogene and functions as an inhibitor to apoptotic cell death. Although the etiology of CLL is unknown, chromosomal abnormalities are present in approximately 80% of cases. Most common are deletions of chromosomes 13q14 and 11q22-23 as well as trisomy 12 [106].

Classification:

The CLL comprise a very heterogeneous group of neoplastic disorders. Until the publication of the FAB proposals, there was no uniform classification that considered morphological as well as immunological issues. Thirteen different subtypes of CLL were described with detailed morphological/immunological profiles for each disorder. The MIC proposals emphasized the importance of cytogenetics. Recently, the WHO proposals incorporated all of the above and added molecular genetics. The final result is a comprehensive diagnostic approach that should permit recognition of the vast majority of cases and permit appropriate therapeutic choices.

For the first classification of CLL, the FAB Cooperative Leukemia Study group employed flow cytometry to separate CLL into two broad categories: B-CLL and T-CLL [107]. It was recognized that certain cytomorphological features were so strongly associated with a specific diagnosis that markers were of minor interest. In addition, the significance of cytogenetic studies was beginning to impact on the recognition of basic molecular defects. For the B-CLL, nine different disease groupings were described, including typical B-CLL, prolymphocytic leukemia (B-PLL), mixed cell CLL (CLL-PLL), Hairy Cell leukemia (HCL), follicular lymphoma, mantle cell lymphoma, splenic lymphoma with villous lymphocytes (SLVL), plasma cell leukemia, and Waldenstrom's macroglobukinemia. For T-CLL, four types of the diseases were classified include typical T-CLL, T-PLL, adult T-cell leukemia lymphoma (ATLL), and Sézary's syndrome.

Concerning MIC classification, the fourth meeting focused on the classification of CLL. Conclusions from this meeting included: replacing the term "T-CLL" with "large granular lymphocyte leukemia: LGLL". A variety of clonal and nonclonal karyotypic changes were discussed, including trisomy 12, 13q aberrations, 14q+, and t(11; 14); in B-PLL and inv(2) in T-PLL.

In 1994, there appeared proposals from the International Lymphoma Study Group [108]. Referred to as the REAL classification, this system rapidly became universally accepted. However, it incorporated virtually all of the chronic B- and T-CLLs. One suspects that the rationale for this was the identical lymph node histology in CLL whenever this material was available and the common evolution of many of the non-Hodgkin lymphomas (NHL) to a leukemic terminal phase.

The WHO proposals follow the FAB chronic T-cell leukemia proposals, with two notable exceptions. First, Sézary's syndrome is considered under the cutaneous lymphomas, rather than as leukemia; even thought circulating cerebriform T cells are always present. Secondly, a relatively new disorder described by Chan, *et al*. [109] in the early 1990s is included, namely aggressive NK-cell lymphoma.

In addition, CLL is classified by two cytological staging systems, which are known as the Rai Classification [110] and Binet Staging [111], respectively. The clinical features in Rai and Binet staging are shown in table 13. The Rai Classification

separates chronic lymphocytic leukemia into low-, intermediate-, and high-risk categories, which correspond with stages 0, I & II, and III & IV, respectively.

- Rai Stage 0: patients are at low risk and have lymphocytosis, a high lymphocyte count defined as more than 15,000 lymphocytes per cubic millimeter
- Rai Stage I: patients are at intermediate risk and have lymphocytosis plus enlarged lymph nodes (lymphadenopathy).
- Rai Stage II: patients are also at intermediate risk but have lymphocytosis plus an enlarged liver (hepatomegaly) or enlarged spleen (splenomegaly), with or without lymphadenopathy.
- Rai Stage III: patients are at high risk and have lymphocytosis plus anemia, a low red blood cell count (hemoglobin < 11 g/dL), with or without lymphadenopathy, hepatomegaly, or splenomegaly.
- Rai Stage IV: patients are also at high risk but have lymphocytosis plus thrombocytopenia, a low number of blood platelets (< 100 x 10^3 cells/μL).

Binet Staging classifies CLL according to the number of lymphoid tissues that are involved (i.e., the spleen and the lymph nodes of the neck, groin, and underarms), as well as the presence of low red blood cell count (anemia) or low number of blood platelets (thrombocytopenia).

- Binet Stage A: patients have fewer than three areas of enlarged lymphoid tissue. Enlarged lymph nodes of the neck, underarms, and groin, as well as the spleen, are each considered "one group," whether unilateral (one-sided) or bilateral (on both sides).
- Binet Stage B: patients have more than three areas of enlarged lymphoid tissue
- Binet Stage C: patients have anemia plus thrombocytopenia (platelets <100 x 10^3 cells/μL).

Table 13. The clinical features in Rai and Binet staging

Stage	Lymphocytosis	Lymphadenophthy	Hepato or splenomegaly	Hemoglobin (g/dL)	Platelets (x 10^3/μL)
Rai					
0	Yes	No	No	> 11	> 100
I	Yes	Yes	No	> 11	> 100
II	Yes	Yes/No	Yes	> 11	> 100
III	Yes	Yes/No	Yes/No	< 11	> 100
IV	Yes	Yes/No	Yes/No	Any	< 100
Binet					
A	Yes	Yes/No (< 3 nodal groups positive)	Yes/No	> 10	> 100
B	Yes	Yes/No (< 3 nodal groups positive)	Yes/No	< 10	> 100
C	Yes	Yes/No	Yes/No	< 10	< 100

1.2.2.2 Causes of leukemia

Acute leukemia and other hematopoietic disorders result from a complex series of genetic events. Circumstances favoring these events are called risk factors. There are many risk factors that can cause leukemia, but most of them are poorly understood. Some risk factors, such as having a rare inherited disease, are out of a person's control. On the other hand, risk factors, such as smoking, chemical exposure, and radiation exposure, can possibly be avoided. Such risk factors fall into two broad groups: constitutional factors, which are present at conception, and acquired factors, which appear after conception (Table 14). Significant progress has been made recently in elucidating both inherited and environmental causes of hematopoietic neoplasia. In many cases, a common element can be found between acute leukemia and other disorders in the same patient. In some instances, the presence of acute leukemia is the first sign of an underlying genetic disorder.

This section is included to describe the risk factors in hematopoietic neoplasia, especially leukemia, and to suggest further appropriate studies in affected patients.

Table 14. Risk factors for hematopoietic neoplasia [112]

Risk factors
Constitutional risk factors - Population- based factors - Genetic factors - Chormosomal instability and bone marrow failure syndromes - Dysmorphic syndromes - Other constitutional hematopoietic disorders
Acquired risk factors - Environmental factors - Acquired hematopoietic disease and blood donation - Infectious and immune-related factors - Medical interventions - Pregnancy and birth-related factors - Other acquired risk factors

1.2.2.2.1 Constitutional risk factors

A) Population-based factors

Population-based factors predisposing one to hematopoietic neoplasia have been recognized, but in most cases, the underlying mechanisms remain obscure.

Gender is an important risk factors. Females are overrepresented among neonates and infants with acute leukemia, especially leukemia with *MLL* rearrangement [113]. Males predominate among all patients with acute leukemia [114]. Particular HLA variants predispose to or protect against hematologic malignancy [115-117]. Moreover, a familial disease may appear at a progressively earlier age and run an unusually rapid course, especially when acquired risk factors are superimposed [118].

B) Genetic factors

Constitutional autosomal anomalies are known to predispose to hematopoietic neoplasia. Interestingly, many resemble the genetic anomalies commonly seen as acquired anomalies in hematopoietic malignancy. Many karyotypic abnormalities are associated with hematopoietic malignancy, involving almost one-half of the somatic chromosomes and both sex chromosomes [119-121].

Inherited mutations and polymorphisms of several genes are known to confer an increased risk of hematopoietic malignancy. The involved genes include those encoding *N*-acetyl transferase, *AML1*, *NF*, *p53*, and others [122-124].

C) Chromosomal instability and bone marrow failure syndromes

These disorders are similar, often presenting with cytopenias and skeletal malformations and carrying an increased risk of acute leukemia, especially therapy-related leukemia. In some cases, the onset of acute leukemia is the first sign of an underlying constitutional disorder.

The chromosomal instability syndromes are characterized by an inability to synthesize and/or repair DNA correctly. Fanconi's anemia carries an increased risk of clonal myeloid and lymphoid disease, as well as therapy-related malignancy [125].

Bloom's syndrome, Griscelli's Syndrome, and Werner's syndrome are other rare chromosomal instability disorders with a propensity to develop acute leukemia.

The bone marrow failure syndromes are characterized by the early onset of cytopenias and an increased risk of acute leukemia. In some cases, the underlying genetic defect has been discovered. These disorders include congenital hypoplastic anemia, familial sideroblastic anemia, severe congenital neutropenia, and others.

D) Dysmorphic syndromes

Dysmorphism or congenital anomalies, which may be attributable to either constitutional or acquired factors, are associated with an increased incidence of hematopoietic malignancy.

Specific dysmorphic disorders reported with hematologic neoplasia include Adams-Oliver syndrome, cardiac anomalies, cleft lip and palate, Dubowitz's syndrome, familial microcephaly, Marfan's syndrome, Noonan's syndrome, and others.

E) Other constitutional hematopoietic disorders

Preexisting constitutional hematopoietic disorders are present in some patients with hematopoietic neoplasia. It is tempting to speculate that chronic stimulation of hematopoiesis is the unifying characteristic in these disorders. Possibly the constant demand for stem cell renewal creates an increased opportunity for the genetic aberrations that initiate clonal disease.

Hemoglobinopathies reported in patients with hematopoietic neoplasia include β-thalassemia, hemoglobin C, hemoglobin S, and others [126, 127].

Other hematopoietic disorders reported with hematologic neoplasia include glucose-6-phosphate dehydrogenase deficiency, hemophilia, hereditary spherocytosis, platelet storage pool deficientcy, and pyruvate kinase deficiency. Moreover, cystic fibrosis may also predispose patients to acute leukemia [128, 129].

1.2.2.2.2 Acquired risk factors

A) Environmental factors

The environment contains numerous risk factors for hematologic neoplasia. These act synergistically with constitutional risk factors to produce hematopoietic disease at an earlier age and higher frequency than might otherwise occur. Substances in the environment act directly on those who are exposed. Many of these substances are found in the workplace; others are found in the general environment. Those found in the workplace contribute to an increased risk of leukemia in some occupations.

Environmental agents and substances implicated in hematopoietic oncogenesis include arsenic, cadmium, tri-halomethanes, chloroform, zinc, asbestos and related minerals, benzene, paints, petroleum products, organic solvents, pesticides and other agricultural chemicals, radiation from solar and earth sources, nuclear reactors and weapons, therapeutic devices, ethanol, and tobacco.

B) Acquired hematopoietic diseases

Preexisting hematopoietic disease may be present years before the onset of hematopoietic neoplasia; in such cases, it likely acts as a risk factor. As in the case of constitutional hematopoietic disease, it is tempting to speculate that acquired hematopoietic diseases require an increased, long-term demand for hematopoietic stem cells and thus increase the chance of a genetic event leading to neoplasia. In some cases, increases in hematopoietic stem cells and clonal emergence caused by increased cell cycling have been documented. Hematopoietic disorders, which appear to carry increased risk of leukemia, include aplastic anemia, pure red cell aplasia, immune-mediated thrombocytopenia, and pernicious anemia.

C) Infectious and immune-related factors

Infectious and immune disorders have long been implicated in leukemogenesis. General evidence in support of this hypothesis comes from demonstration of increased risk with population mixing and exposure to siblings and clustering of leukemia cases in space and time.

Infectious agents associated with an increased risk of hematologic neoplasia include cytomegalovirus, Epstein-Barr virus [130, 131], dengue virus [132], hepatitis viruses [133], human herpesvirus 6 [134], human immunodeficiency virus (HIV) [135], human T lymphotropic virus type I [136], influenza virus [137], Parvovirus B19 [138], rubella virus, and vericella virus [139, 140].

Hypoimmune states are associated with an increased risk of leukemia. Such states include Kawasaki's disease [141], HIV infection, and tonsillectomy [142].

D) Medical interventions

Medical interventions have become an increasingly important cause of leukemia. Populations at especially high risk are patients with underlying constitutional risk factors and those treated for prior malignancies. Numerous therapies have been implicated in leukemogenesis.

Antibiotic and anti-inflammatory agents have occasionally been implicated in leukemogenesis [143]. In most cases, these drugs are no longer commonly used. Antineoplastic agents are well-known leukemogens and are especially potent when used in patients with constitutional risk factors and those with leukemia and lymphoma [144].

Granulocyte and granulocyte-monocyte colony-stimulating factors are associated with the onset and rapid progression of leukemia. Caution must be used in making a diagnosis of high-grade acute leukemia in patients on growth factors because clonal expansion may reverse upon drug withdrawal.

Other medical interventions involved in leukemogenesis are UV light therapy, radiotherapy, immunosuppressive agents and stem cell transplantation. For stem cell transplantation, it is the setting for increasing numbers of leukemia cases [145]. After transplantation, leukemia usually arises in host cells, but may also occur in donor cells [146]. Caution should be exercised in making the diagnosis of acute leukemia after stem cell transplantation because dysplastic changes and even clonal proliferations may regress over time.

E) Pregnancy and birth-related factors

Abundant evidence indicates that childhood leukemia arises *in utero*, likely from intrauterine exposure to environmental risk factors [147]. It is often difficult to separate intrauterine from other risk factors.

Risk factors related to pregnancy and birth may be parental or nonparental in origin. Parental risk factors for childhood leukemia include exposure to environmental carcinogens, maternal age younger than 21 or older than 40 years, and maternal consumption of dietary bioflavonoids [148]. Dietary bioflavonoids are naturally occurring DNA topoisomerase II inhibitors that cleave sites on the mixed lineage leukemia (*MLL*) gene; maternal ingestion may account for the high rate of *MLL* translocation seen in infant leukemia.

Nonparental risk factors include birth weight less than 2,500 unit or more than 4,000 g, delivery by cesarean section, multiple birth, and low neonatal serum thyroid-stimulating hormone level. Birth order affects the risk of childhood leukemia. The risk of acute lymphoblatic leukemia is highest for a firstborn child and decreases thereafter. In contrast, the risk of acute myeloid leukemia is lowest for a firstborn child and increase thereafter.

F) Other acquired risk factors

Age is directly correlated with the incidence of hematopoietic neoplasia, likely attributable to the combined effect of constitutional and acquired factors over time. Acute leukemia in the elderly resembles that seen in patients with therapy and toxin induced disease. In addition, increased body weight may be directly related to the risk of acute leukemia in adults, as it is in newborns [149].

Other acquired risk factors involved in leukemogenesis are connective tissue diseases, endocrine disorders, hormones and potent hematopoietic growth factors, inflammatory bowel disease, renal disorders, and other tumors.

1.2.2.3 Genes involved in leukemia

Approximately 50% of *de novo* acute leukemias have distinctive molecular abnormalities, most frequently chromosomal translocations. These translocations typically involve genes that are involved in transcription and differentiation. As a

result of the translocation, these genes are disrupted and the 5'-segment of one gene is joined to the 3'-end of a second gene to form a novel fusion gene, from which chimeric mRNA is transcribed and protein is translated. Inversions occur as well, which also create a fusion gene from which novel proteins are generated [150-152]. In contrast, many of the known translocations that occur in chronic leukemias and malignant lymphomas affect proto-oncogenes, which are involved in cell proliferation or survival, and an antigen-receptor gene. Under the influence of the antigen-receptor gene enhancers or promoters, the proto-oncogene is constitutively expressed, resulting in increased expression (overexpression) of the normal (nonmutated) protein. Other molecular mechanisms such as point mutations and gene deletions have been implicated in leukemogenesis [153]. Subsequent inactivation of the remaining normal copy by hypermethylation, point mutation, or deletion allows neoplastic transformation. Often more than one mechanism is involved, leading to the accumulation of genetic changes that culminate in leukemogenesis or subsequently contribute to disease progression.

There are many genes that are involved in leukemogenesis. As a result of the chromosomal translocation in leukemias, many types of fusion gene occur. *Promyelocytic leukemia-retinoic acid receptor α* (*PML-RARα*) and *bcr-abl* fusion gene are examples [154-156]. The *PML-RARα* fusion gene encodes a chimeric mRNA and protein, which plays a role in neoplastic transformation and inhibits myeloid differentiation. This fusion gene was detected in approximately 80-90% of APL cases at the time of initial diagnosis. Other examples of fusion gene are *aml1-eto* fusion gene and *NPM-RARα*. The *bcr-abl* fusion gene is located on chromosome 22 or Philadelphia chromosome. It plays a crucial role in the initiation of CML. Philadelphia chromosome, which can be found in 90-95% of cases of CML, is the cytogenetic hallmark of this disease. However, evidence of this translocation is also found in approximately one-third of cases of ALL and in <1% of AMLs

Additionally, gene matations have been identified in leukemias, involving either proto-oncogenes or tumor suppressor gene. Genes that are commonly affected in AMLs include the *c-ras* gene family, *Rb1*, and *p53*. The *c*-H-*ras*, *c*-K-*ras*, and *c*-N-*ras* proto-oncogenes encode homologous 21kDa proteins that are located at the inner surface of the plasma membrane. These proteins are involved in signal transduction

and cellular proliferation. Mutations of the *c-ras* genes represent one of the more frequent molecular abnormalities identified in AML and ALL [157, 158]. Abnormalities in *Rb1* gene have been identified in a subset of AMLs. In a study by Ahuja, *et al*. [159] using Southern blot analysis, 5/54 (9.3%) cases of AML had structural abnormalities of the *Rb1* locus, including 4/15 (26.7%) of AMLs with monocytic differentiation (FAB M4 and M5). For *p53* gene mutation, point mutations were identified in approximately 6% and 9% of AML and ALL cases, respectively [160, 161]. The presence of *p53* gene mutations also correlates with older patient age, the presence of myelodysplasia and poor prognosis of leukemias [162, 163]. The other example of gene involve in leukemia is *Wilms' tumor1* (*WT1*) gene. In some types of cancer, *WT1* gene was isolated as a tumor suppressor gene while an oncogenic activity was observed in leukemias [15, 16].

1.2.2.4 Leukemic cell lines [164]

In 1951, Gey, *et al*. [165] established the first continuously growing human cell line (HeLa) from a uterine cervix carcinoma. The HeLa cell line and other human cell lines subsequently established from various solid tumors adhere to the culture vessel growing in monolayers. Pulvertaft [166] established the first continuous human hematopoietic cell lines, a series of cell lines derived form Nigerian patients with Burkitt's lymphoma in a suspension-type cell culture. RAJI is the best known cell line of this panel. In suspension cultures, these cells are free-floating, singly or in clusters, in the nutrient medium. The first leukemia-derived cell line was thought to be RPMI 6140, established from an American patient with AML containing EBV particles in the cells.

In 1971, the hematopoietic cell lines MOLT-1, -2, -3, and -4 were established from the peripheral blood of a patient in relapse form ALL by Minowada, *et al*. [167]. These cells lacked surface and cytoplasmic immunoglobulins and EBV infection. The most distinctive characteristic of the MOLT-1, -2, -3, and -4 cells was their rosette-forming ability with sheep, goat, horse, and pig erythrocytes. Furthermore, K562, the first myeloid-erythroid cell line, was established from Ph' chromosome positive CML blast crisis patient [168-170]. After that, more than fifty leukemic cell lines were generated in order to establish an immortalizing cell for leukemia studies including

U937, HL-60 and JURKAT cells in 1976 and 1977, respectively [171-174]. Some characteristics of leukemic cell lines which were used in this study are provided in Table 15.

Table 15. Some characteristics of leukemic cell lines [164]

Characteristics	Molt4	K562	U937	HL-60
- Year of establishment	1971-1972	1970-1973	1974-1976	1976-1977
- Type of cell line	T-cell line	Erythroid cell line	Monocytic cell line	Promyelocytic cell line
- Original disease of patient	T-cell ALL	CML blast crisis	Histiocytic lymphoma	AML-M3, M2
- Patient data (age/sex)	19/M	53/F	37/M	35/F
- Culture medium	RPMI 1640 + 10% FBS	RPMI 1640 + 10% FBS	RPMI 1640 + 10% FBS	RPMI 1640 + 10% FBS
- Doubling times	40-72 h	24-30 h	30-40 h	25-40 h
- Properties in suspension cultures	Free-floating, singly or in clusters	Free-floating, singly	Free-floating, singly or in clusters	Free-floating, singly

1.2.3 *Wilms' tumor1* (*WT1*) gene and its product (WT1 protein)

The *Wilms, tumor1* (*WT*) gene is a gene responsible for a renal pediatric malignancy (Wilms' tumor or neophroblastoma) that was first described by Max Wilms in 1899. The expression pattern of the *WT1* gene indicates that the function of the gene is not restricted to the kidney but plays a role in the development and homeostasis of other tissues as well. The *WT1* gene is located on human chromosome 11p113. It spans about 50 kb, contains 10 exons, and specifies a 3-kb mRNA as shown in Figure 1 [8, 9]. *WT1* gene expression is restricted to a limited set of tissues, including gonads, uterus, kidney, and mesothelium, but is highest in the developing

kidney. It has been considered a tumor suppressor gene on the basis of findings [11, 175-178] such as intragenic deletions or mutations in Wilms' tumor, germline mutations in patients with leukemic predisposition syndromes and WT1-mediated

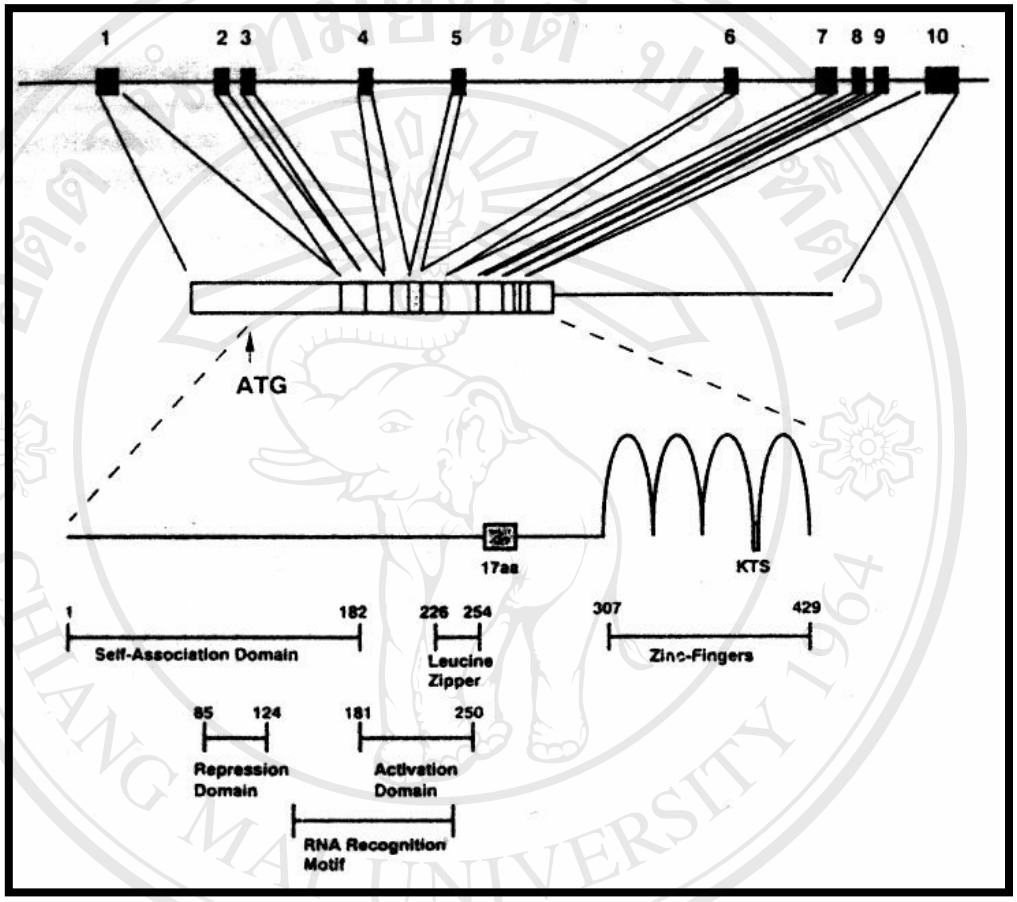

Figure 1. Schematic structure of *WT1* mRNA and gene products with various functional domains [20]. *WT1* spans about 50 kb, contains 10 exons and specifies a 3 kb mRNA. As a result of alternative RNA splicing, the gene encodes four different proteins.

growth suppression of Wilms' tumor cells expressing a WT1 splicing variant. Its product, WT1 protein, is a zinc-finger transcription factor which contains 429 amino acids (Figure 2). The predicted protein sequence provided the first clue to the biochemical function of WT1 [8]: it features four zinc fingers on the Kruppel C2-H2 class in the carboxyl-terminal part and a praline-glutamine-rich amino terminus,

suggesting a role of WT1 as a transcription factor. The four zinc fingers (amino acids 307-429), which share high homology with the zinc finger region of Egr-1 family members, form the DNA-binding domain [11]. Region 226-254 contains a partial heptad repeat of leucine residues, and this potential leucine zipper may mediate protein-protein interactions [179]. Besides binding to other proteins, the WT1 isoforms can also self-associate. The major domain required for this self-association has been mapped to the first 182 amino acids of WT1 [180, 181]. This region partially overlaps with residues 85-124 and 181-250, which can autonomously repress or activate transcription, respectively [182]. The activity of these domains, however, may be cell type dependent [179]. WT1 protein represses transcription of growth factor genes including platelet-derived growth factor (PDGF)-α chain, colony-stimulating factor (CSF)-1, insulin-like growth factor (IGF)-II, epidermal growth factor receptor (EGFTR), and other genes such as *RAR-α*, *c-myb*, *c-myc*, N-*myc*, and *bcl-2* [183-190]. On the other hand, the WT1 protein activates transcription of the retinoblastoma suppressor-associated protein 46 (RbAp46), *Dax-1*, and *bcl-2* genes [191-193].

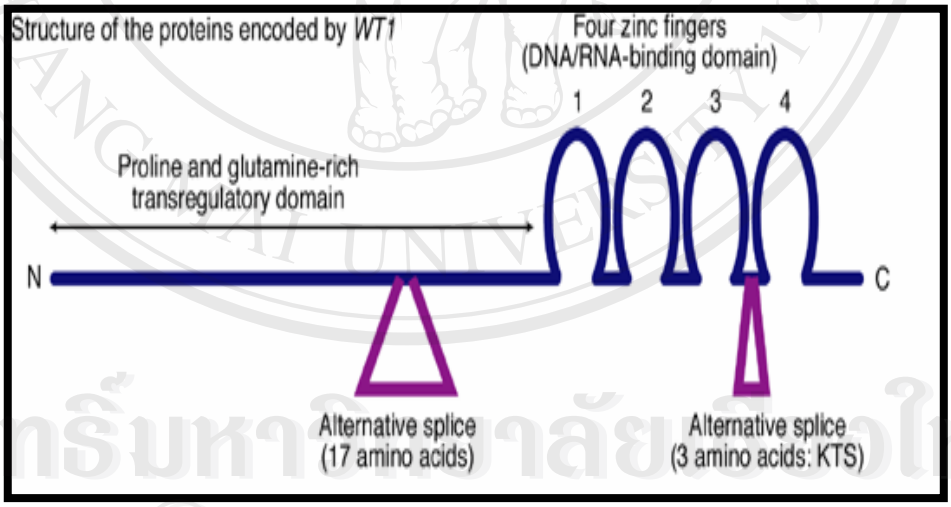

Figure 2. Structure of the WT1 protein [194]. The WT1 protein contains 429 amino acids. It has a proline- and glutamine-rich transregulatory domain at the N-terminal end and four zinc fingers in a C-terminal DNA/RNA-binding domain. There are two alternative splices (shown by purple triangles): one inserts 17 amino acids in the transregulatory domain and the other inserts three amino acids (KTS) between the third and fourth zinc fingers.

As a result of alternative RNA splicing [195], the gene encodes four different proteins, which have molecular weights of about 48-54 kDa [196]. One alternative splicing event results in either inclusion or exclusion of exon 5, which encodes a stretch of 17 amino acids (±17 aa) at the N-terminal of the four zinc fingers. The other alternative splicing event involves a splice acceptor site in exon 9, resulting in the presence or absence of a 3 amino acid insert [lysine-threonine-serine (±KTS)] between zinc fingers 3 and 4. The schematic representation of the WT1 protein isoforms is shown in Figure 3. The presence of the KTS insert in chickens, alligators, and marsupials, and the conservation of the sequence in the genomic DNA of zebrafish, underlines the fundamental importance of this alternative splice form in vertebrate development [197]. However, the 17 amino acid insertion is present only in mammals. In this thesis, WT1 (-/-), WT1 (+/-), WT1 (-/+), and WT1 (+/+) isoforms were used to refer to the WT1 splice variant which lacks both inserts, which only contains the 17 aa insert, which only contains the KTS insert, and which contains both inserts, respectively. Occasionally, WT1 (-KTS) and WT1 (+KTS) have been used to refer to the splice variants which lack the KTS insert and contain this insert, respectively. The alternative splicing of exon 5 does not affect any currently recognized functional domain in the WT1 protein. However, its presence or absence may nevertheless affect protein stability or function. Alternative splicing of this exon may therefore be of biological significance. The alternative splicing site in exon 9 is known to alter protein function.

Furthermore, the RNA editing [198] at nucleotide 839 of the WT1 mRNA results in the replacement of leucine 280 in WT1 proteins by proline. From this result, the *WT1* gene may thus produce eight different mRNA and protein isoforms. In addition to the eight WT1 protein isoforms generated through translation initiation at the initiator AUG of the eight mRNAs, larger and smaller WT1 isoforms have been identified (Figure 3) Translation initiation at an in-frame CUG codon upstream of the initiator AUG results in WT1 protein isoforms with molecular masses of 60-62 kDa [199]. Internal translation initiation at an in-frame AUG 127 codon downstream of the initiator AUG generates smaller WT1 isoforms with apparent molecular masses of 36-38 kDa [200]. Both the larger and the smaller WT1 isoforms could be detected in

different mammalian tissues. When these isoforms are included, 24 WT1 protein isoforms have been described to date.

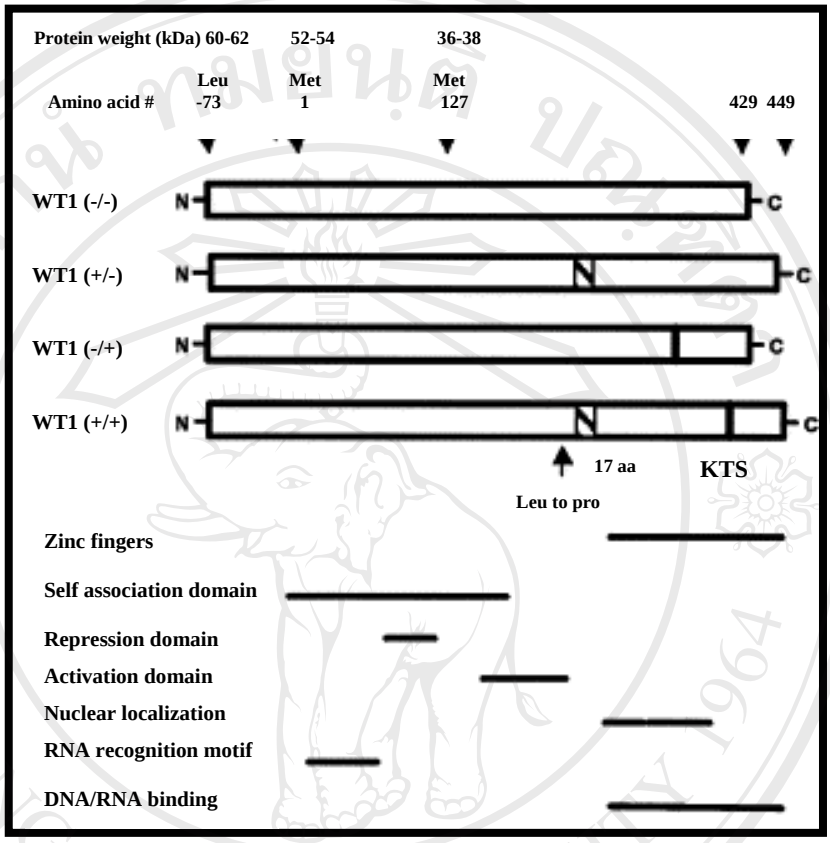

Figure 3. Schematic representation of the WT1 protein isoforms [202]. Insertion or exclusion of the two splice inserts (17 aa and KTS) generates the four main WT1 protein isoforms starting at the first initiator AUG (Met) of the WT1 mRNAs. Translation initiation at an upstream, in-frame CUG codon generates four larger WT1 proteins, while translation initiation at an in-frame AUG 127 codons downstream of the initiator AUG leads to four smaller WT1 proteins. Moreover, RNA editing at codon 280 raising the total number of WT1 protein to 24 isoforms.

The main four isoforms of WT1 protein have been shown to differ in their ability to bind the early growth response 1 (EGR-1) DNA consensus sequence. DNA binding was abolished by the insertion of the three amino acids (+KTS). In contrast, the presence or absence of 17 amino acids of exon 5 had no effect on the ability of WT1 to bind the EGR-1 consensus sequence [19]. Interestingly, the two +KTS WT1

transcripts predominate in tissue expression of WT1 [194]. Binding competition studies using these isolated DNA sequences suggested that the two +KTS WT1 isoforms may have different affinities for the same DNA target and may also be able to bind to different targets [201]. These data suggested that the +KTS and –KTS isoforms of WT1 protein regulate the expression of distinct subsets of genes.

1.2.3.1 Expression of *WT1* gene in normal and malignant tissues

In situ hybridization on sections of chicken, mouse, and human embryos showed that *WT1* is expressed during embryonic development in the urogenital system, the mesothelial cells lining the organs within the thoracic and abdominal cavities, the differentiating body wall musculature, the spleen, and pocket regions in the brain and the spinal cord [197, 203, 204]. These data imply that *wt1* plays a common role in the development of these structures.

In support of such a function, it was found that a *wt1*-null mutation in mice resulted in failure of kidney, gonad, and mesothelial development [205]. Furthermore, WT1 transcripts were detected in fetal kidney, spleen, and two leukemic cell lines by northern blot analysis [8, 206].

It has been reported that the *WT1* gene is expressed in a large percentage (75%) of ovarian tumors [207, 208], renal cell carcinomas [209], and mesotheliomas [210, 211]. In addition, *WT1* expression was found in cases of melanoma [212], desmoplastic round-cell tumor [213], and breast cancer [214]. *WT1* expression was examined in 34 solid tumor cell lines and detected in 3 of 4 gastric cancer cell lines, 5 of 5 colon cancer cell lines, 12 of 15 lung cancer cell lines, 2 of 4 breast cancer cell lines, 1 germ cell tumor cell line, 2 ovarian cancer cell lines, 1 uterine cancer cell line, 1 thyroid cancer cell line, and 1 hepatocellular carcinoma cell line [215]. Thus, 28 of 34 (82%) solid tumor cell lines examined expressed the *WT1* gene. Furthermore, fresh lung cancer specimens were examined for *WT1* expression. The *WT1* expression levels were approximately 10 to 1,000 times higher in fresh lung cancer specimens than in normal lung tissues. Menssen, *et al*. [216] also analyzed *WT1* expression in small cell and non-small cell lung cancer, colon cancer, glioblastoma, and their respective tumor cell lines. They found *WT1* expression in 5 of 8 glioblastomas, 5 of 11 lung cancers, and 5 of 15 colon cancer cell lines. However, *WT1* expression was detected in only 1

of 5 glioblastomas, 1 of 12 lung cancers, and none of colon cancer specimens. Harada, *et al*. [217] examined the expression of *WT*1 expression in testicular germ cell tumors by quantitative RT-PCR. It was found that WT1 mRNA was highly expressed in 6 of 9 (67%) high-stage tumors but in only 5 of 25 (20%) low-stage tumors. Thus, a significant correlation was observed between *WT1* expression level and tumor stage. These finding suggest that the progression of testicular germ cell tumors may be diagnosed by WT1 assay.

1.2.3.2 Expression of *WT1* gene in normal and malignant hemopoietic cells

WT1 expression has been detected in the fetal spleen, liver, and thymus, tissues in which hemopoiesis takes place during embryonic development. In addition, WT1 transcripts have been detected in adult bone marrow, lymph nodes, and peripheral blood. These data suggested that WT1 may play a role in the development of erythroid, myeloid, and lymphoid cells during both the fetal and the adult stages. Since *WT1* expression is mainly found in the more immature cells, it has been suggested that WT1 plays a role in early hemopoiesis [16, 218, 219].

The *WT1* gene was originally defined as a tumor suppressor gene. However, Sugiyama H [15] proposed that the *WT1* gene has two basic functional aspects, as a tumor suppressor gene and an oncogene. Especially in leukemia and various types of solid tumors, it performs an oncogenic rather than tumor suppressor gene function. These hypotheses originated from the following findings: (a) high levels of expression of wild-type *WT1* exist in both leukemic blast cells and various types of solid tumor cells; (b) there is a clear, inverse correlation between *WT1* expression levels and prognosis in acute leukemias; (c) increased *WT1* expression exists in relapse patients; (d) there is growth inhibition of leukemic cells and solid tumor cells after treatment with WT1 antisense oligomers; and (e) blocking of differentiation, but induction of proliferation in response to granulocyte colony-stimulating factor, exist in myeloid progenitor cells.

Inoue, *et al*. [16] provided new insights into the significance of *WT1* gene expression leukemias by quantifying the expression levels of *WT1* via quantitative reverse transcriptase polymerase chain reaction (RT-PCR). In all leukemic samples examined (45 AML, 22 ALL, 6 AMLL, 23 CML), significant levels of *WT1* gene

expression were found, and the average levels were approximately 1,000 to 100,000 times higher than in normal bone marrow and peripheral blood, respectively. Miwa, *et al*. and Miyagi, *et al*. [218, 219] examined *WT1* gene expression in leukemias using Northern blot analysis and detected expression of *WT1* gene in some cases of AML, ALL, and CML in accelerated phase or blast crisis. A clear correlation between *WT1* expression levels and the prognosis of leukemia has been observed [220]. In addition, Brieger, *et al*. [221] detected WT1 transcripts in 41 (79%) of 52 AML patients, whereas most of the 14 patients in complete remission studied showed a loss of *WT1* expression. Menssen, *et al*. [222] also found *WT1* gene expression in 53 (93%) of 57 AML patients, 12 (86%) of 14 pre-pre-B-ALL patients, 33 (80%) of 41 common ALL patients, and 23 (74%) of 31 T-ALL patients. Moreover, a clear correlation was observed between the minimal residual diseases (MRD) detected in the paired bone marrow and peripheral blood samples for various types of leukemias. These findings demonstrated that the WT1 mRNA is a novel tumor marker for leukemic blast cells and its expression level is a new diagnostic factor for leukemias.

WT1 gene expression has been detected in many erythroid, myeloid, and lymphoid cell lines such as K562, HL-60, and Molt4 [223-225]. Several of these cell lines have been used to investigate the function of WT1. Maurer, *et al*. [226] demonstrated that *WT1* was expressed in subset of CD34+ progenitors and rapidly downregulated after induction of myeloid differentiation. The differentiation of the HL-60 cells correlated with the downregulation of WT1 expression. A similar downregulation was observed when K562 cells were induced by sodium butyrate or tetra porbal acetic acid (TPA) to differentiate into erythroid cells or megakaryocytic cells, respectively [227]. Furthermore, the *WT1* antisense treated K562 cells stop growing and subsequently undergo apoptosis. The WT1 protein may influence the proliferation and differentiation of blood cells by differential regulation of the genes for TGFβ, C-Myc, C-Myp, Bcl2, and the retinoic acid receptor, as shown in Figure 4. All of these findings support the conclusion that *WT1* gene expression may influence the proliferation, differentiation, and apoptosis of human blood cells.

1.2.4 Turmeric curcuminoids

Curcuma longa Linn or turmeric (Fig 5) is a perennial herb widely cultivated in south and southeast tropical Asia. It is a member of the Zingiberaceae or ginger family. Turmeric is used for its flavor and attractive golden color. A robust herbaceous perennial, grows to a height of 1 meter, with a short stem and tufted leaves. Turmeric is usually propagated from fingers or small sections of rhizome. The rhizomes grow best in a hot, moist climate. Turmeric smells peppery and fresh with a hint of oranges and ginger. It tastes pungent, bitter, and musky. Its rhizome is extensively used for imparting color and flavor to food such as curry [228]. Because of its attractive gold color, turmeric is used as a traditional textile dye. Turmeric is used commercially in sauces and in processed food. It is often added to mustard blends. It became a very important spice to mankind when it was observed that the addition of turmeric powder in food preparation preserved its freshness and nutritive value.

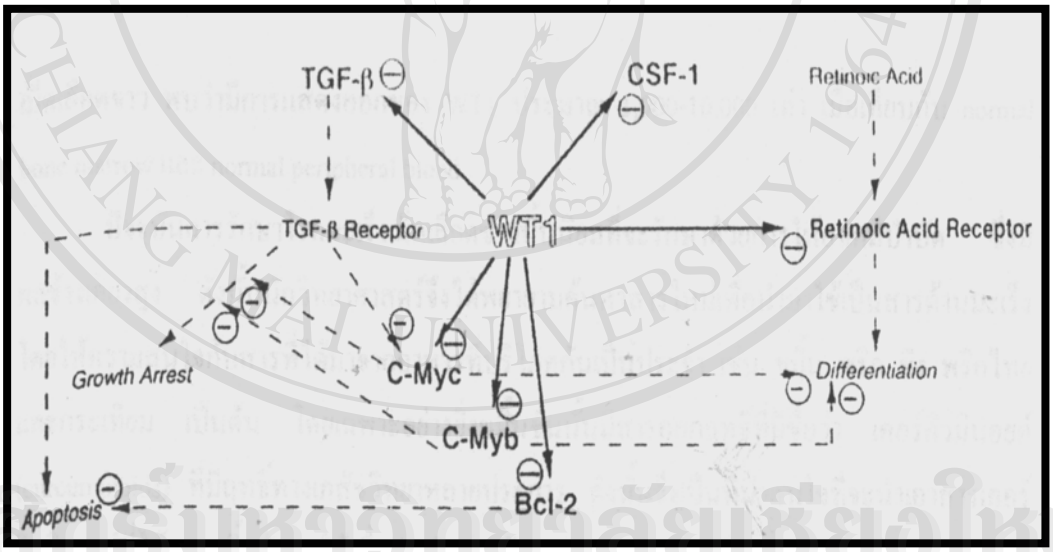

Figure 4. A proposed scheme for regulation by WT1 in hemopoietic cells [20]. WT1 may influence the proliferation and differentiation of blood cells by differential regulation of the gene for TGFβ, C-Myb, C-Myc, Bcl-2, and the retinoic acid receptor. TGFβ may induce growth arrest and apoptosis. Overexpression of C-myc or C-myb may result in increased apoptosis upon TGFβ stimulation and may inhibit growth arrest and differentiation. An overexpression of Bcl-2 inhibits apoptosis but dose not affect growth arrest.

Figure 5. A stemless rhizomatous of *Curcuma longa*. Leaves emerge directly from the underground rhizome with overlapping petioles 8-15 cm long or more. They have ellipse-shaped or elongated lance-shaped blades. The plant consists of large pale green, pouch like, curved bracts, each with two or more pale yellow or pale pink flowers. The rhizome is bright orange or yellow within and scented. After drying and homogenizing, the powder of turmeric is orange [229-231].

Turmeric has many common names in Thailand, such as Khamin (general), Khamin Kaeng, Khamin yok, Khamin hua (Chiang Mai), Khamin chan (central peninsula), Kheemin, Min (peninsula), Taa-yo (Karen-Kamphaengpet), and Sa-yo (Karen-Mae Hong Son) [232]. *Curcuma* includes more than 100 species and several varieties of rhizomatous herbs. Its rhizome has been found to be a rich source of beneficial phenolic compounds knows as the curcuminoids. Curcuminoids are the yellow coloring matter in turmeric. They constitutes about 3-5% of the composition of turmeric. Three main curcuminoids derivatives, curcumin or pure curcumin (curcumin I), demethoxycurcumin (curcumin II), and bisdemethoxycurcumin (curcumin III), have

been isolated from turmeric powder. The quantity of curcuminoids contained in the rhizome of the *Curcuma* species varies with the site and cultivation period, as shown in Table 16. It seems that *Curcuma longa* Linn (turmeric) has the highest concentration of curcuminoids as compared with other species. Other organic compounds found in turmeric are shown in Table 17.

Table 16. Curcuminoid contents in the rhizome of *Curcuma* species [233]

***Curcuma* species**	**Origin (year)[a]**	**Curcuminoids (%)[b]**			
		Total	**Cur**	**Dcur**	**Bdcur**
Curcuma longa Linn.	Nan-Chang (1965)	1.41	0.70	0.35	0.25
Curcuma longa Linn.	Cheng-du (1979)	3.83	2.03	1.12	0.82
Curcuma wenyujin	Che-Chiang (1979)	0.20	0.13	0.07	0.02
Curcuma longa Linn.	Kwang-Chou (1979)	1.28	0.63	0.40	0.52
Curcuma xanthorrhiza Roxb.	Kwang-Chou (1980)	2.10	1.43	0.86	0.12
Curcuma longa Linn.	Beijing (1980)	3.82	1.79	1.11	0.75
Curcuma sichuanesis	Si-Chuan (1980)	0.04	0.01	0.01	<0.01
Curcuma kwanginosa	Yun-nan (1980)	1.54	0.89	0.57	0.23
Curcuma aeruginosa Roxb.	Si-Chuan (1980)	0.04	0.01	0.01	<0.01
Curcuma elata Roxb.	Kwang-see (1980)	0.01	<0.01	<0.01	<0.01
Curcuma longa Linn.	Nan-ning (1981)	3.97	1.84	1.09	1.01

[a]Origin, the site of cultivation or collection; year, the time of sample collection.

[b]Total, total curcuminoid; Cur, curcumin; Dcur, demethoxycurcumin; Bdcur, bisdemethoxycurcumin.

Table 17. The organic material that is found in turmeric rhizomes [234]

Organic material	Percent content	Organic material	Percent content
Moisture	8-9%	Calcium	0.15%
Fat	5.1%	Iodine	0.01 g/100 g
Fixed oil	7.5-8.8%	Iron	47.5 mg/100 g
Volatile oil	4.4-9.2%	Lead	0.5 ppm
Protein	6.1-10.8%	Phosphorus	0.28%
Carbohydrate	69.4%	Potassium	2.5 g/100 g
Pure starch	29.6-40.1%	Sodium	0.01 g/100 g
Fiber	2.6-5.8%	Ascorbic acid	49.3 mg/100 g
Mineral matter	3.5%	Niacin	4.8 mg/100 g
Total ash	6.0-8.5%	Thiamine	0.09 mg/100 g
Soluble ash	4.7-6.1%	Nicotinic acid	2.3 mg%
Food energy	349.39 Cal/100 g	Riboflavin	0.19 mg/100 g

The use of turmeric became more widespread when it was found to act as a therapeutic agent for various illnesses. It is also used in home remedies in the treatment of cuts, wounds, bruises, sprains, and liver problems. The juice of the raw rhizomes added to ointments is applied to treat skin diseases. In paste form it is applied as a beauty mask. Moreover, activities as an anti-inflammatory [235], antioxidant [236], antibacterial agent [237], antifungal agent [238], antiviral agent [239], anti-mutagen [240], anticarcinogenic, and anticancer activities [241] have been reported.

The rhizomes contain: (a) 3 major pigments of curcuminoids; (b) 5'-methoxycurcumin, a natural antioxidant; (c) a new curcuminoid, cyclocurcumin, which was isolated from the nematocidally active fraction of turmeric; (d) two new natural phenolics, which possess anti-inflammatory and antioxidant activities; (e) two new pigments; (f) several sesquiterpenes, germacrone, tumerone, ar-(+)-,α-,β-turmerones, β-bisabolene, α-curcumene, zingiberene, β-sesquiphellandene, bisacurone, curcumenone, dehydrocurdione, procurcumadiol, bis-acumol, curcumenol, isoprocurcumenol, epiprocurcumenol, procurcumenol, zedoaronediol, curlone,

turmeronol A and turmeronol B have been identified from the rhizomes; (g) four new polysaccharides-ukonans having activity on the reticuloendothelial system; and (h) stigmasterol, β-sitosterol, cholesterol, and 2-hydroxymethyl anthraquinone.

The three major pigments can absorb visible light at wave lengths from 420-425 nm. The structures of the three main curcuminoids that have been isolated from turmeric powder are shown in Figure 6; their properties are as follows [242-244].

1. Pure curcumin or Bis-(feruloyl) methane or 1, 7-bis(4-hydroxy-3-methoxyphenyl)-1, 6-heptadiene- 3, 5- dione is occurs as 49% of total pigments. Its formula is $C_{21}H_{20}O_6$. Molecular weight is 368 Daltons. Its melting point is 183 ºC. Its color is yellow-orange. It is a little soluble in ether and quite soluble in ethanol, acetone propylene glycol, and benzene. It forms a brown-red color in base and a yellow in acid.
2. Demethoxycurcumin or Feruloyl-(p-hydroxycinnamoyl) methane occurs as 28.7% of the total pigments. Its formula is $C_{20}H_{18}O_5$. Its molecular weight and melting point are 338 Daltons and 168 ºC, respectively.
3. Bisdemethoxycurcumin or Bis(p-hydroxycinnamoyl) methane occurs as 22.3% of the total pigments. Its formula is $C_{19}H_{16}O_4$. Its molecular weight and melting point are 308 Daltons and 224 ºC, respectively.

Recently, turmeric extract products have been wildly used for health benefits. Most of these consist of approximately 2-8% of pure curcumin. GPO CURMIN® is one of the popular products in Thailand. It is the product from the Government Pharmaceutical Organization (GPO) of Thailand. Each capsule contains turmeric extract equivalent to 250 mg of curcuminoids. The other products are recognized by Thai consumers, but most of them do not shown information concerning their active ingredients. In laboratory research, turmeric powder is extracted and isolated by 95% ethanol and then precipitated by petroleum ether. Crude curcuminoid mixtures derived from this method contain 78% of pure curcumin, 16% of demethoxycurcumin, and 5% of bisdemethoxycurcumin [245]. Commercial grade curcuminoids (Sigma-Aldrich) are isolated from the powdered dry rhizome of *Curcuma longa* and contain approximately 77% pure curcumin, 17% demethoxycurcumin, and 3% bisdemethoxycurcumin.

The effects of curcuminoids have been reported in basic science publications, clinical trials, and previous reviews. The curcuminoids have been found to have a number of antioxidant activities, including scavenging of such reactive oxygen species as superoxide anions and hydrogen peroxide, inhibition of lipid peroxidation [246], and inhibition of the oxidation of low-density lipoprotein (LDL) [247]. Furthermore, the curcuminoids have demonstrated antiviral, anti-fungal, and immunomodulating effects, mostly *in vitro*. Pure curcumin has been described as having HIV-1-inhibiting effects *in vitro* [248-250]. Some of curcuminoids and turmeric extracts have exhibited *in vitro* anti-fungal activity, specifically against *Candida albicans, Candida kruseii*, and *Candida parapsilosis*. Moreover, pure curcumin inhibits Th1 cytokine profile in $CD4^+$ T cell by suppressing interleukin (IL)-12 production in macrophages [251].

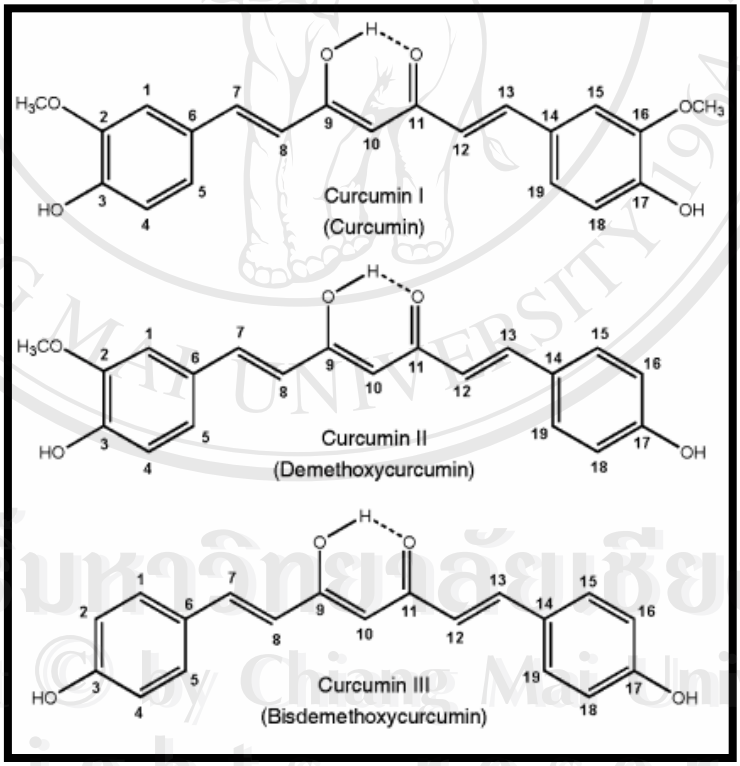

Figure 6. Chemical structure of pure curcumin, demethoxycurcumin, and bisdemethoxycurcumin [252].

Previous studies have shown that pure curcumin at 5 μM inhibited the lipopolysaccharide (LPS)-induced production of tumor necrosis factor-alpha (TNF-α) and IL-1 by a human monocytic macrophage cell line [253]. As a consequence, downstream events involving TNF-α and IL-1 are affected. For example, TNF-α induced expression of leukocyte adhesion proteins, such as intracellular adhesion molecule-1 (ICAM-1) and vascular cell adhesion molecule-1 (VCAM-1), is lowered in pure curcumin treated cells [254]. In addition, levels of certain cytokines also are affected by pure curcumin. Many genes that are implicated in the initiation of immune/inflammatory responses are regulated at the level of transcription by the nuclear transcription factor NF_KB. Several of the beneficial effects of pure curcumin are consistent with its ability to inhibit the activity of NF_KB [255-257]. Singh and Aggarwal [258] observed that pure curcumin inhibits NF_KB activation pathway after the convergence of various stimuli mediated by protein tyrosine kinase. However, pure curcumin is not a specific inhibitor of the signaling kinase in the NF_KB pathway, since it also inhibits the c-Jun N-terminal kinase (JNK) signaling pathway [259]. In addition, pure curcumin inhibits the up-regulation of matrix metalloproteinases (MMPs), probably because of its inhibitory potential on protein kinase C (PKC) [260].

Curcuminoids, especially pure curcumin, are apoptotic agents [29]. While a low concentration of pure curcumin is known to arrest cell proliferation in the G0-G1/G2/S phase, a high concentration of pure curcumin induces apoptosis in rat A7r5 cells [261, 262]. Several hallmarks of apoptosis, including DNA laddering, chromatin condensation and fragmentation, and an apoptosis specific cleavage of 28S and 18S ribosomal RNA, were observed after treatment of immortalized mouse embryo fibroblasts (NIH 3T3), erb B2 oncogene-transformed (NIH 3T3), mouse sarcoma (S180), human colon cancer cells (HT-29), human kidney cancer cells 293, and human hepatocellular carcinoma (Hep G2) cells with pure curcumin [263]. Moreover, curcumin-induced apoptosis in human basal cells was shown to be dependent on a p53-signaling pathway [264].

These are examples of biological actions of curcuminoids. Several other biological actions are summarized in Table 18.

Table 18. Biological actions of curcuminoids [265].

Protective biological effect observed	Cells/tissues/biological processes affected	Mediators	Mechanism (s)	Gene (s) identified to be influenced
Anti-inflammatory	Cells of the immune system	↓ TNFα, ↓ IL-1, ↓ IL-2, ↓ IFN-γ, ↓ ICAM-1, ↓ VCAM-1, ↓ E-Selectin	↓ Activation of IκB kinases, ↓ Dissociation of IκB complexed to NF-κB	↓NF-κB expression
Anti-inflammatory	Cells of the immune system, endothelial cells	↓ TXA_2, ↓ PGE_2, ↓ LTB_4, ↓ LTC_4 ↓ Availability of precursor-arachidonic acid	↓ COX-2 ↓ LOX ↓ Phospholipases, ↓ Δ^5, Δ^6 desaturase	?
Anti-inflammatory	Cell of the immune system	↓ Degradation of Collagen, ↓ Elastin ↓ Hyaluronic acid	↓ Collagenaes, ↓ Elastase ↓ Hyaluronidase ↓ Matrix metalloproteinase ↓ Macrophage migration inhibitory factor	↓Protein kinase C
Antioxidant	Most eukaryotic tissues, Lipid peroxidation	↓ Superoxide anions, ↓ Hydrogen peroxide, ↓ Nitric oxide, ↓ Oxidative stress	↑ SOD, ↑ Catalase, ↑ GSH Peroxidase, ↓ Nitric oxide synthase, ↑ Hemeoxygenase-1	?
Immune-stimulatory; Antithrombotic	Blood cells	?	↑ WBC count, ↓ TXA_2 ↓ Platelet aggregation, ↓ Platelet activating factor	?
Anti-carcinogenic	Hepatic, renal cells	↓ Metabolic activation ↑ Detoxification	↓ Cytochrome P450, ↓ Aryl hydrocarbon hydroxylase, Competes for AhR and CYP1A1 ↓ Phenolsulfatransferase ↑ GSH-S-transferase	?
Apoptotic agent Antimitotic agent	Cancer cells	↓ Cell proliferation	↑ DNA laddering, ↑ Hsp 70 ↑ Cleavage of 28S and 18S RNA, ↑ Shrinkage, ↑ Phosphatidyl Serine exposure ↑ Calcium depletion ↓ Membrane potential ↓ ATP synthesis	↓NF-κB
Anti-apoptotic	Most eukaryotic cells, inhibition of induced apoptosis	↓ DNA laddering	↓ AP-1 activity ↓ Caspase ↓ Janus kinases, ↓ PARP ↑ GSH, ↑ Thiols	?
Wound healing	Skin cells, tissue repair and remodeling	↓ Superoxide anions ↓ Lysosomal enzymes	↑ Granulation, ↑ TGF-β1 ↑ Extracellular matrix	↓MMP-1, ↓MMP-9 ↓HGF, ↓NF-κB
Antidiabetogenic	Renal cells, diabetes	↓ Lipid peroxidases, ↓ Cholesterol ↓ Urinary excretion of albumin, urea, Na^+, K^+ and Pi	↓ Antioxidant enzymes	?
Anti-Stressor	Hepatic and adrenal cells fibroblasts	↑ Hsp27, ↑ Hsp70 ↑ Acute phase proteins	?	?
Antiviral; Antibacterial; Antifungal agent	Microbes	↓ P^{24} antigen production	↓ HIV-1 integrase	?
Anti-cancer	Cancer cells	↓ Metastasis ↓ Tyrosine phosphorylation ↓ DNA adducts ↓ Angiogenesis	↓ Collagenase ↓ Tyrosine kinase ↓ Protein kinase C ↓ VEGF, ↓ bFGF	?
Antilithogenic	Endocrine tissue	↑ Bile flow	↑ Gall bladder contraction	?

Note: ? = Unknown

Anticarcinogenic effects of pure curcumin in animals, as indicated by its ability to inhibit both tumor initiation induced by benzo(a)pyrene and 7, 12-dimethybenz(a)anthracene and tumor promotion induced by phorbol esters [266, 267], is possibly due to suppression of PKC activity and nuclear oncogene expression [268]. In addition, the effect of pure curcumin, demethoxycurcumin, bisdemethoxycurcumin, and tetrahydrocurcumin on 12,0-tetradecanoylphorbol-13-acetate (TPA) induced tumor promotion suggests that pure pure curcumin and demethoxycurcumin have an equally potent inhibitory effect on TPA induced tumor promotion in DMBA-initiated mouse skin. Bisdemethoxycurcumin and tetrahydrocurcumin were less active [267, 269]. Hong, *et al*. [270] demonstrated the ability of pure curcumin to inhibit the growth and colony formation of several breast cancer cell lines. Interestingly, the IC_{50} of pure curcumin for several breast cancer cell lines is close to that of chemotherapeutic agents, such as 5-fluorouracil.

Curcuminoids are group of diet-derived agents that are being clinically evaluated as chemopreventive agents for major cancer targets, including the breast, prostate, lung, stomach, duodenal, colon, and leukemias [23, 24]. Limtrakul, *et al*. [271] exhibited the inhibitory effect of commercial grade curcuminoids (Sigma-Aldrich) on the proliferation of cancer cell lines such as mouse hepatoma (Hep1), human larynx cancer (Hep2), and human lung cancer (PC9 and PC14). In addition, pure curcumin induces apoptosis in human colon cancer cell lines and lung cancer cell lines (A549 and H1299) by inhibiting the release of apoptosis inducing factor and caspases [272, 273]. Moreover, pure curcumin seems to be able to modulate the *in vitro* expression and function of *Multidrug resistance1* (*MDR1*) gene and P-glycoprotien (P-gp) in multidrug-resistant human cervical carcinoma (KB-V1) cells [274, 275].

In leukemia research, Duvoix, *et al*. [28] found that pure curcumin displayed the ability to induce cell death in two leukemic cell lines: K562 and Jurkat cells. Curcumin I and III exhibited *in vitro* cytotoxicity against human chronic myeloid leukemia in a dose dependent manner [276]. Kuo, *et al*. [29] demonstrated that the dietary component pure curcumin induced apoptosis in human leukemia HL-60 cells at the very low concentration of 3.5 μg/mL. This finding was supported by the research of Banjerdpongchai and Wilairat [277]. Recently, Anuchapreeda, *et al*. [30]

demonstrated that all three curcuminoids exhibited excellent cytotoxic activity on leukemic cell lines, HL-60, U937, and K562 cell lines. The inhibitory concentrations at 50% (IC_{50}) were 7, 7 and 20 µg/mL, respectively. Moreover, the inhibitory effects of pure curcumin were associated with a decrease in the levels of *WT1* gene expression in patient leukemic cell and K562 cell lines [31, 32, 278, 279].

The safety of *Curcuma longa* and its derivatives has been studied in various animal models [280]. It is clear that turmeric is not toxic to animals even at high doses. A single feeding of a 30 percent turmeric diet to rats did not produce any toxic effects. In a 24 h acute toxicity study, mice were fed dosages of 0.5, 1.0, and 3.0 g/kg of turmeric extract daily. There was no increase in mortality rate when compared to the respective control. A 90-day feeding of turmeric extract resulted in no significant weight gain [281].

Due to its wide range of biological and pharmacological effects and lack of toxicity in animal models, curcuminoids were examined in this study to determine their effects on *WT1* gene expression in leukemic cell lines; K562, U937, HL-60, and Molt4.

1.3 Objectives

1. To study the effect of turmeric curcuminoids on *WT1* gene expression in leukemic cell lines
2. To study the effect of turmeric curcuminoids on WT1 protein level in leukemic cell lines