CHAPTER I

INTRODUCTION

Background and Rationale

Acute lymphoblastic leukemia (ALL) is the most common malignancy in children. It accounts for one-fourth of all childhood cancers and approximately 75% of all cases of childhood leukemia.[1] ALL is a malignant neoplasm of lymphocytes characterized by the accumulation of immature blood cells in the bone marrow. These abnormal cells are arrested in the lymphoblast stage of the normal maturation pathway. Aberrations in the proliferation and differentiation of these cells are common, and normal hematopoiesis is suppressed. Patients have failed to produce normal blood cells, leading to anemia, infection, and bleeding.[2] Moreover, leukemic cells can infiltrate many organs, thus causing enlargement and dysfunction. Symptoms result from varying degrees of anemia, neutropenia and thrombocytopenia.[3]

ALL is a heterogeneous disease with distinct biological and prognostic groups. Diagnosis relies on traditional cytomorphological and immunohistochemical evaluation of the leukemic blasts.[4] ALL developes as a consequence of malignant transformation of a single abnormal progenitor cell that has the capability to expand by indefinite self-renewal. In pediatric ALL, there is evidence that these events occur in committed lymphoid precursors. It appears that they may occur earlier because there is evidence of mutation in multiple cell lineages.[5] The events that lead to the process of malignant transformation are complex and multifactorial. It has been proposed that ALL results from spontaneous mutations that may occur in lymphoid cell of B or T-cell lineage or in their precursor cells.[6] There is emerging evidence that the causative mutations may occur years before the presence of clinical leukemia. During normal lymphoid development, lymphocyte precursor may be at a higher risk for spontaneous mutation because of the intrinsically regulated mutagenic activity occurring during the process of gene rearrangement and the high rate of proliferation in these cells. Many of the

described molecular mutations bear evidence of immunoglobulin VDJ and T-cell receptor (TCR) recombinase activity. The rearrangement of immunoglobulin and TCR genes has also been studied as a marker of clonality in ALL of pre-B lineage.[7] In most cases, identical patterns of immunoglobulin and TCR gene rearrangement are observed in leukemia obtained at diagnosis and relapse. However, the leukemia cells share at least one identical immunoglobulin gene rearrangement implying a common clonal origin.[8]

Leukemia transformation is unlikely to be the result of a single event but rather the culmination of multiple processes involving complex interactions. The causes of leukemia include genetic[9], physical[10], chemical[11] and infectious causes[12]. Like all human cancer, ALL occurs as a result of specific genetic changes.[5,13] Cytogenetic abnormalities have been detected in more than 60% of ALL, including hyperdiploidy, translocations, inversions and deletions.[14-16] The most important aberration is chromosomal translocation, which creates a fusion gene. Distinct translocations lead to the activation of proto-oncogene products or creation of tumor-specific fusion proteins. Chromosomal translocations in the human acute leukemias rearrange the regulatory and coding regions of a variety of transcription factor genes. The resultant protein products can interfere with regulatory cascades that control the growth, differentiation, and survival of normal blood-cell precursors.[5,13,14] They likely act in concert with other classes of genetic lesions in the multi-step pathways that culminate in leukemic transformation.[14] The most frequent targets of chromosomal translocations in the acute leukemias are genes that encode transcription factors, emphasizing the critical role of regulatory proteins in the control of blood cell development.[17]

Transcription factors are proteins that bind to regulatory regions and help control gene expression. The transcription of each gene is controlled by the regulatory region of DNA, relatively near the site where transcription begins. The regulatory proteins that turn genes on are known as transcriptional activators or gene activator proteins. This mode of gene regulation is called positive control, as in negative control by a transcriptional repressor. The transcription factors can act as either activators or repressors depending

on the exact placement of the DNA sequence they recognize in relation to the promoter of the target genes. For example, if the binding site for the protein overlaps the promoter, and interferes with the polymerase binding site, such a factor acts as a repressor.[18]

The most common gene translocation in childhood ALL is between the *TEL* gene on chromosome 12p21 and the *AML1* gene on chromosome 21q22 (TEL-AML1 translocation). The TEL-AML1 fusion gene will encode TEL-AML1 fusion protein and function as a transcription factor, like other gene translocations. The TEL-AML1 fusion protein or TEL-AML1 transcription factor can bind to DNA specific sequence and can control gene expression.[19] Several groups of investigators have reported that ALL patients with TEL-AML1 fusion gene had a good prognosis .[20,21] The mechanism that underlies this observation is unknown because the TEL-AML1 fusion protein can act as a transcription factor and can bind to various target genes. The structure of TEL-AML1 suggests that it retains the ability to bind to the AML1 DNA target sequence.[22] AML1 is recognized by the DNA sequence 5'-TGTGGT-3' in the transcriptional regulatory region.[23] So, the target genes of TEL-AML1 transcription factor also should also have the DNA sequence 5'-TGTGGT-3' on the regulatory region. A number of genes have this region, including *Interleukin-3 (IL-3), T-cell receptor gamma (TCRγ), Complement receptor type 1 (CR1), Protein kinase C (PKC), Recombination activating gene 1 (RAG1)*[24].

Although there have been reports about TEL-AML1 from several countries, the frequency of TEL-AML1 translocation in Thai children with ALL is unknown. This study aims to determine the prevalence of TEL-AML1 among Thai children with ALL, and to investigate the role of TEL-AML1 fusion protein functions as a transcription factor that controls the expression of various target genes.

Research questions

1. What is the prevalence of TEL-AML1 among Thai children population ?
2. Do TEL-AML1 positive ALL and TEL-AML1 negative ALL have different profiles of target gene expression ?

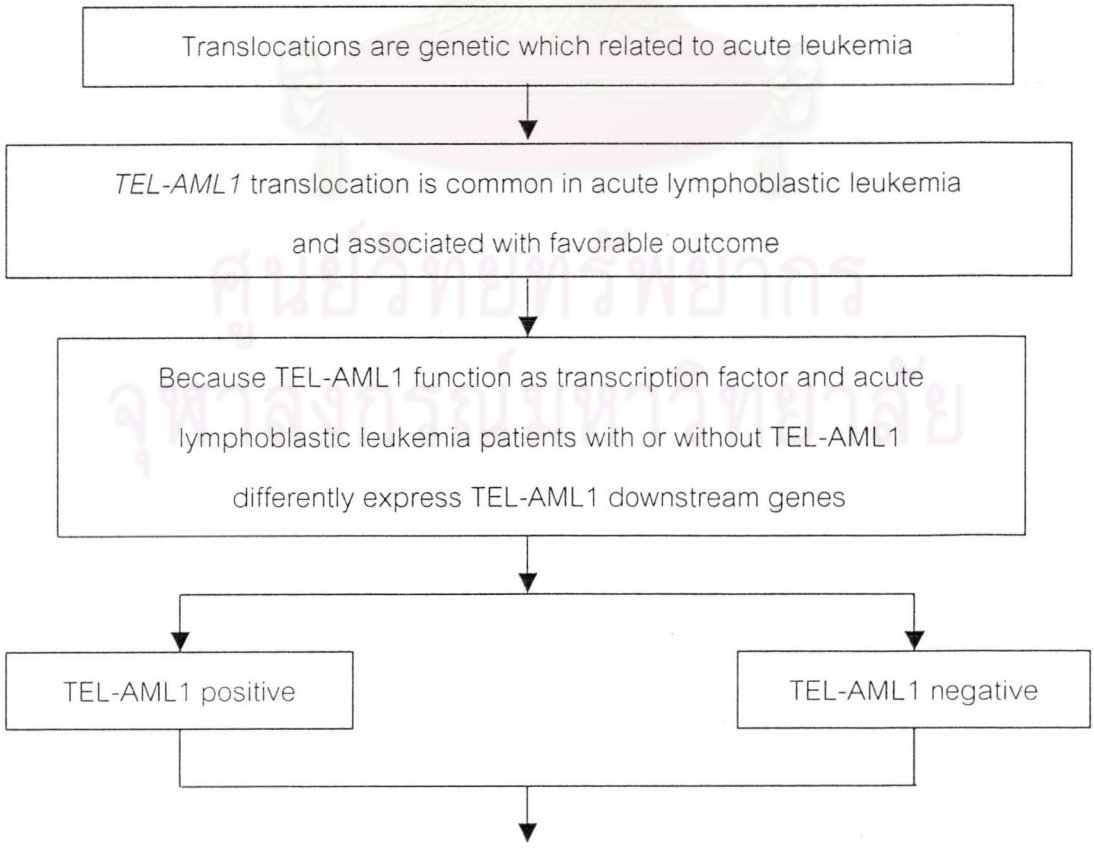
Objectives

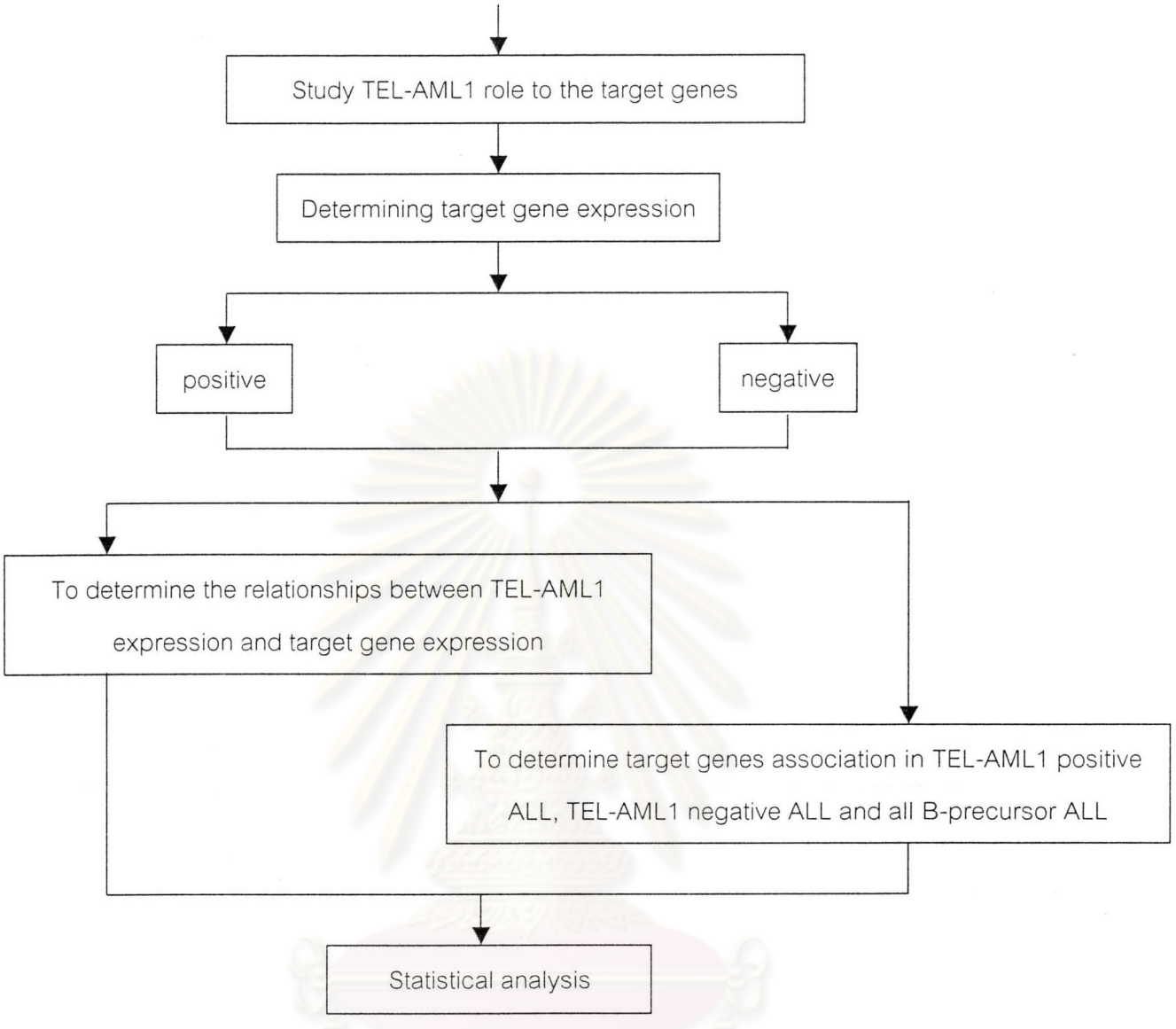
1. To determine the prevalence of TEL-AML1 among Thai children population.
2. To study the role of TEL-AML1 fusion protein in target gene expression.

Hypotheses

1. Childhood ALL patients in Thailand have the same prevalence of TEL-AML1 as other countries.
2. Target gene expression in cancer cells in TEL-AML1 positive ALL and TEL-AML1 negative ALL, are different.

Conceptual framework





Operation definition

Translocation is a chromosome alteration in which a whole chromosome or segment of a chromosome becomes attached to, or interchanged with, another whole chromosome or segment, the resulting hybrid segregating together at meiosis.

Expected benefit

The prevalence of TEL-AML1 among Thai children population is unknown. This knowledge would be a direct benefit. Furthermore, when target gene expression is

understood, the relationship between TEL-AML1 and target gene expression can be elucidated. Because TEL-AML1 is a significant prognostic factor, we can demonstrate a relation between TEL-AML1 and prognosis.

Research methodology

1. Sample collection

   Childhood patients with acute leukemia who were diagnosed by a hematologist at King Chulalongkorn Memorial Hospital and the Children's Hospital, were the subjects of the study.

2. Process of the study
   - Bone marrow collection
   - Immunophenotyping
   - Immunomagnetic isolation
   - RNA extraction
   - cDNA synthesis
   - Polymerase chain reaction (PCR)
   - Agarose gel electrophoresis
3. Data collection and analysis