CHAPTER 1

INTRODUCTION

1.1 Introduction

B-Acute Lymphoblastic Leukaemia (B-ALL) is a haematological malignancy in which the bone marrow produces neoplastic lymphoblasts that are committed to the B-cell lineage. The complexity and spectrum of haematological malignancies highlight the importance of clinicopathological correlation with the availability of ancillary studies (Loghavi et al., 2015). Approximately 20-30% of adult ALLs harbour the Philadelphia chromosome, which produces the *BCR-ABL1* fusion gene that represents a significant prognostic and survival impact to patients (Swerdlow et al., 2016). About half of cases produce the p210 fusion protein, which is the characteristic transcript of *BCR-ABL1* detected in cases of chronic myeloid leukaemia, while the remainder produces the p190 transcript. The incidence of ALL with *BCR-ABL1* increases with age and is identified in up to 50% of all ALL cases diagnosed in patients over the age of 50 years old (Fielding et al., 2009; Thomas et al., 2004). This genetic alteration confers a poor prognosis that delineates shorter remission and survival with higher rates of resistance to standard chemotherapy (Faderl et al., 1998; Moorman et al., 2007; Preti et al., 1994). The presence of this fusion gene serves as a unique molecular signature, becoming an effective monitoring tool for measurable residual disease (MRD) monitoring to identify patients who would likely benefit from stem cell transplant (Brüggemann et al., 2006; Dhédin et al., 2015; Gökbuget et al., 2012). Several studies have suggested that detection of *BCR-ABL1* transcripts by quantitative real-time polymerase chain reaction (qRT-PCR) is associated with an increased risk of relapse, while deeper molecular

responses have been associated with improved outcomes (Nashed et al., 2003; Short et al., 2016). However, the effectiveness of MRD monitoring in B-ALL with *BCR-ABL1* is not well defined. (Fielding, 2015; Short et al., 2016) The impact of achieving a complete molecular response (CMR) in B-ALL with *BCR-ABL1 is* also undefined. Therefore, a time-point analysis for the quantitation of the *BCR-ABL1* transcript could reveal the prognostic impact of achieving CMR in these patients. Therefore, this study aimed to retrospectively investigate the prognostic impact of *BCR-ABL1* molecular monitoring of *BCR-ABL1* at different time points on the survival of adult patients with B-ALL with *BCR-ABL1* from 2006 to 2018 in a major transplant centre in Malaysia.

1.2 Background of the Study

Acute Lymphoblastic Leukaemia (ALL) is a malignant transformation and proliferation of lymphoid progenitor cells in the bone marrow, blood, and extramedullary sites. (team, October 17, 2018) B-Acute Lymphoblastic Leukaemia or B-Lymphoblastic Leukemia (B-ALL) is a haematological malignancy in which the bone marrow produces neoplastic lymphoblasts that are committed to the B-cell lineage. The most recent World Health Organization (WHO) classification has added two new entities, one of which includes *BCR-ABL1*–like B-ALL with translocations involving tyrosine kinases or cytokine receptors. (Loghavi et al., 2015) These clonal lymphoblasts harbour a unique translocation between the BCR located on chromosome 22 and the *ABL1* oncogene on chromosome 9, also known as t(9;22) or the Philadelphia chromosome (Ph). The t(9;22) translocation results from the fusion of BCR at 22q11.2 and the cytoplasmic tyrosine kinase gene ABL1 at 9q34.1, thus producing a *BCR-ABL1* fusion protein.

This fusion protein is the characteristic molecular marker of another haematological malignancy known as Chronic Myeloid Leukaemia (CML). Although B-ALL accounts for a large proportion of childhood leukaemias, B-ALL with *BCR-ABL1* is more common in adults than in children, accounting for about 25% for the former and 2-4% for the latter. (WHO Classification of Tumours of Haematopoietic and Lymphoid Tissues, 2017 revised edition) About half of all cases produce the p210 fusion protein that is the characteristic transcript of *BCR-ABL1*-positive CML, and the remainder produce the p190 transcript. The incidence of Ph-positive ALL increases with age and occurs in up to 50% of ALL diagnosed in patients over the age of 50 years (Pui CH, 2006 Jan 12; Secker-Walker LM, 1991, Mar). Patients harbouring this translocation have an increased risk of central nervous system involvement and a more aggressive clinical course compared to Ph-negative ALL.

The gold standard therapy in CML is a tyrosine kinase inhibitor (TKI) that has been shown to be effective in eradicating the *BCR-ABL1* gene (Simona Soverini, 2019). However, the added presence of the *BCR-ABL1* gene in ALL is regarded a poor prognostic marker. According to the WHO, B-ALL with *BCR-ABL1* has been considered to have the worst prognosis of the major cytogenetic subtypes of ALL (WHO Classification of Tumours of Haematopoietic and Lymphoid Tissues, 2017 revised edition). Its higher frequency in adult ALL explains in part the relatively poor outcome of adults with ALL. However, TKI therapy has been shown to have a significant favourable outcome (WHO Classification of Tumours of Haematopoietic and Lymphoid Tissues, 2017 Revised Edition). The ultimate goal of treatment in B-ALL with *BCR-ABL1* is the elimination of the neoplastic clone, which can only be

achieved by replacing the diseased bone marrow with normal healthy donor stem cells. Unfortunately, not all patients will be suitable to undergo this procedure. The use of TKI alone in this context is insufficient to achieve a sustainable response due to the presence of other oncogenic events in the haematopoeitic stem cells that drive the proliferation of neoplastic lymphoblasts. Therefore, standard chemotherapy for ALL is used with adjuvant TKI to enhance the eradication of the neoplastic clone. Response to treatment in this context is also termed complete remission (CR), where peripheral counts would normalise indicating bone marrow regeneration. The bone marrow would show normocellularity with adequate presence of all haemapoietic elements and blast cells ≤5%. However, according to the European Leukaemia Network (LeukaemiaNet), which is the largest international consortium for leukaemia research, the revised definition of CR in ALL includes measurable residual disease negativity.

The assessment of measurable residual disease (MRD) has emerged as a prognostic tool for patients with ALL. In patients with B-ALL with *BCR-ABL1*, MRD evaluation is important in the treatment of B-ALL with *BCR-ABL1* to identify high-risk patients who would benefit from MRD-directed therapy. Haematologists use MRD-directed therapy to inform their decision as to intensify or de-escalate leukaemia treatment in these patients. The evaluation of MRD can also identify patients who are more likely to benefit from stem cell transplantation (SCT) when patients enter complete remission for the first time (CR1). There are many methods for MRD detection, however, specific for B-ALL with *BCR-ABL1*, detection and quantitation of the *BCR-ABL1* fusion gene transcript by quantitative PCR (qPCR) is the most important tool. A cutoff point of *BCR-ABL1* detected at 0.01% or 10^4 of nucleated cell from a bone marrow sample is

used to stratify the risk of the patients. The *BCR-ABL1* transcripts detected below this limit are termed a complete molecular response (CMR). However, the impact of achieving CMR with B-ALL with *BCR-ABL1* remains undefined. Therefore, a time-point analysis of the quantitation of the *BCR-ABL1* transcript could reveal the prognostic impact of achieving CMR in these patients towards the outcome of treatment.

1.3 Problem Statement

There is a need to better define the epidemiology and therapeutic outcomes of adult patients with B-ALL who undergo allogeneic haemopoietic stem cell transplantation (HSCT) in Malaysia. This is especially relevant in a resource-limited setting such as Malaysia, where the impact of allogeneic stem cell transplant should be assessed to fine-tune the management of adult B-ALL in this part of the world. The number of patients with haematological malignancy that require chemotherapy and stem cell transplants is increasing. According to the 2012-2016 Malaysian National Cancer Registry report, lymphoma and leukaemia are the fourth and sixth most common cancers among Malaysian adults with an incidence rate of 5.1% and 3.7%, respectively. With Malaysia striving to achieve the status of a developed country, the number of haematology cancers is expected to increase and thus become a health burden for the country. The number of patients who require SCT as a lifesaving treatment has also increased. According to communication with the head of the Stem Cell Unit at Hospital Ampang, in 2017, 16% of referral cases were unable to be accepted due to insufficient beds and manpower. The number of cases on the waiting list for stem cell transplantation is also increasing yearly. More than half of these cases are delayed by more than 3 months, in which time the disease relapses and poor outcomes are likely to

occur. Therefore, to improve the survival assessment of patients with B-ALL with *BCR-ABL1*, it is necessary to propose a method or algorithm that would objectively and accurately prognosticate patients based on their molecular status.

1.4 Research Questions

a. Does residual disease measurement have a prognostic impact in achieving a complete molecular response on adult B-lymphoblastic leukaemia patients?

b. Does *BCR-ABL1* level have an impact on adult B-Lymphoblastic Leukaemia patients with *BCR-ABL1*?

c. Which time point of *BCR-ABL1* level has the biggest impact on outcome on adult B-Lymphoblastic Leukaemia patients with *BCR-ABL1*?

d. Does *BCR-ABL1* level have an impact on adult B-Lymphoblastic Leukaemia with *BCR-ABL1* post allogeneic transplantation outcome?

1.5 Objectives of the Study

1.5.1 General Objective

To investigate the prognostic impact of measurable residual disease in achieving a complete molecular response among adult B-Lymphoblastic Leukaemia patients with *BCR-ABL1*.

1.5.2 Specific Objectives

a. To determine the *BCR-ABL1* level at different time-points during the course of treatment:

i. Level of *BCR-ABL1* quatitation post induction, Time-Point 1 (TP1).

ii. Level of *BCR-ABL1* quantitaiton at week 16, post consolidation , Time-Point 2 (TP2).

iii. Level of *BCR-ABL1* at the end of chemotherapy for patient with no transplant option or level of *BCR-ABL1* pre allogeneic stem cell transplantation, Time-Point 3 (TP3).

b. To determine the overall survival of patients based on their *BCR-ABL1* level at different time-points during the course of treatment.

c. To compare the overall survival of patients between those who had achieved major molecular response (MMR) and those who did not achieve MMR prior to stem cell transplantation.

1.6 Significance of The Study

It is well documented that early detection of MRD for ALL and prompt treatment are cornerstones for pre-transplant workup. Cases of B-ALL with *BCR-ABL1* can be detected by pre-emptive molecular testing so that targeted therapy with TKI and early stem cell transplantation can convert this disease from an incurable to a potentially curable disease. However, the impact of CMR on the survival of adult B-ALL with *BCR-ABL1* and related treatment outcomes in Malaysia is still unknown. Given the usefulness of qPCR to quantify the *BCR-ABL1* transcript, the data provided after each round of chemotherapy could elucidate the effect of each phase of treatment. The goal of each qPCR is to provide information for the optimal management plan. Therefore, a time point analysis for MRD monitoring at 3 time points: time point one (TP1) – post induction; second time point (TP2) – consolidation phase at week16; three time points (TP3) – end of chemotherapy or pre transplant would provide this information.

1.7 Scope of the Study

This is a retrospective study that investigates the participation of adult patients who have been diagnosed with B-ALL with *BCR-ABL1*, which confers additional risk. It is an observation of 12 years from 2006-2018, involving only a single-centre analysis. However, Hospital Ampang is the largest government referral centre for haemopoietic stem cell transplantation for haematological malignancies and, therefore, would represent the majority of transplanted B-ALL with *BCR-ABL1*. Figure 1 shows the increasing number of HSCT procedures in Hospital Ampang over the span of 20 years. The haematology services were first established in 1986 at Kuala Lumpur General Hospital. In 2006, the entire Department of Haematology moved to Hospital Ampang, which was designated as the national referral centre for Haematology consisting of both clinical haematology and advanced laboratory services with well-equipped facilities. Therefore, data prior to 2006 were not collected. To investigate the prognostic impact of complete molecular remission with their treatment outcomes. The data collected were secondary data and, as such, may be incomplete or missing. However, this is minimized by cross-checking with the hardcopy results available at Makmal Rujukan Klinikal Hematologi (MRKH), Hospital Ampang, which is the only government laboratory that performs and records the quantification of molecular levels of *BCR-ABL1*.

1.8 Research Conceptual Framework

According to the WHO, ALL with *BCR-ABL1* confers a high risk of relapse and a poor prognosis. Access to the tyrosine kinase inhibitor, which is specifically targeted for *BCR-ABL1* as part of the treatment strategy, has changed the risk stratification for ALL

patients who harbour this fusion gene. As *BCR-ABL1* is routinely quantified for the monitoring of its myeloproliferative counterpart, incorporation of its quantification by real-time polymerase chain reaction could be a reliable parameter for the monitoring of MRD for ALL with *BCR-ABL1*. Whether this has replaced conventional risk factors in deciding whether patients would need a transplant or not is yet to be determined. The impact of CMR on the survival of adult B-ALL with *BCR-ABL1* and related treatment outcomes in Malaysia is still unknown. This can be answered by retrospectively analysing specific data points of molecular monitoring (Figure 2).

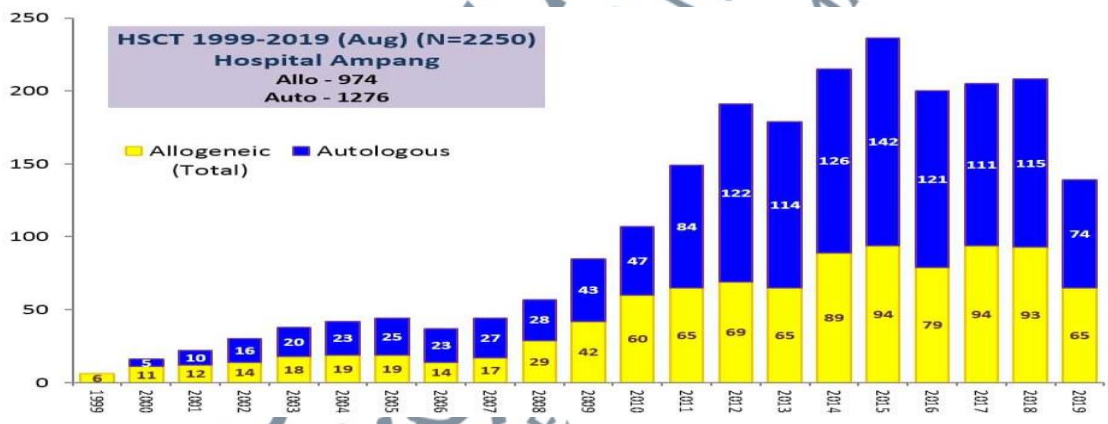

Figure 1: Number of stem cell transplants performed from 1999 to 2019.

(Personal communication and figure provided by the Head of the Stem Cell Transplant Unit in the Hospital Ampang)

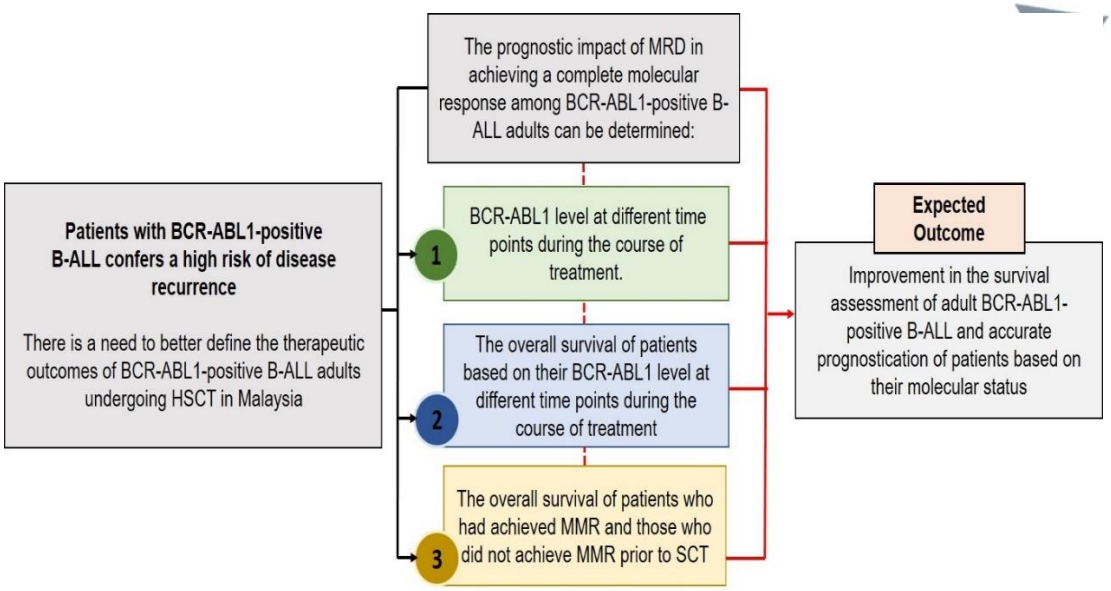

Figure 2: Conceptual framework of the research

1.9 Operational Definitions

1.9.1 *BCR-ABL1* Molecular Monitoring

BCR-ABL1 monitoring was performed in bone marrow mononuclear cells. RNA was extracted and quality was assessed using a Nanodrop spectrometer before proceeding to real-time quantitative PCR (RT-QPCR). The number of *BCR-ABL1* copies was quantified using RT-QPCR. The results were expressed as the ratio of the *BCR-ABL1* copy number to the ABL1 copy number on the International Scale (IS). The kit used was MolecularDiagnostic for major type p210 and Ipsogen minor type p190. The p210 assay was carried out using RotorGene 6000 and p190 by light cycle (LC) 1.5. These two assays are enrolled in the RCPA and UKNEQAS external quality program.

1.9.2 Complete Haematological Response (CHR)

The complete hematologic response (CHR) for B-ALL is defined as the normocellular bone marrow with 0% blasts or ≤5% blasts, ≥15% erythropoiesis, ≥25% granulopoiesis

and normal megakaryopoiesis, absence of blasts in peripheral blood and distant organs free of leukaemic cells.

1.9.3 Complete Molecular Response (CMR)

The complete molecular response (CMR) was defined as the absence of detectable *BCR-ABL1* transcript with a sensitivity of 0.01%.

1.9.4 Major Molecular Response (MMR)

The major molecular response (MMR) was described as *BCR-ABL1*: ABL1 ratio <0.1% on the International Scale (IS) for p210 *BCR-ABL1* or a 3-log reduction in transcripts for p190 *BCR-ABL1*, but not meeting the CMR criteria.

1.9.5 First Complete Remission (CR1)

CR1 refers to the remission achieved after the start of the induction phase of treatment, which means the first cycle of chemotherapy. CR >1 refers to the remission status achieved after the installation of intensification chemotherapy beyond the induction phase.

1.9.6 Engraftment

To examine post-transplantation results, graft is defined as the first of 3 consecutive days with an absolute neutrophil count higher than 0.5 x 10^9 /L (sustained >20 x 10^9 /L platelets and hemoglobin >80 g/L, free of transfusion requirements) (Enric Carreras, 2019). Donor engraftment was determined by FISH for sex chromosomes (for

mismatched HSCT of the same sex) or short tandem repeat for same-sex HSCT, performed on bone marrow samples every 3-4 months. Mixed chimerism was detected by the above-mentioned molecular methods.

1.9.7 Disease Relapse

Disease relapse was defined hematologically based on morphology with recurrence of >5% blasts in a bone marrow aspirate detected after remission status or by the presence of extramedullary disease or molecularly by a ratio of *BCR-ABL1* to ABL1 >0.1% on the IS for two consecutive analyzes.

1.9.8 Disease-free Survival (DFS)

Disease-free survival (DFS) was calculated from day 0 to any type of relapse or death while in remission.

1.9.9 Overall Survival (OS)

Overall survival (OS) was calculated from the time of initiation of treatment to death (with patients alive at the time of the last follow-up being administratively censored)