CHAPTER 3

METHODOLOGY

2.1 Introduction

This is a retrospective observational study. Medical records for a 12-year period starting from 2006-2018 of selected patients from Hospital Ampang were reviewed and the relevant data extracted. Data were extracted on the day of diagnosis, during chemotherapy and after allogeneic stem cell transplantation.

2.2 Research Design

All patients diagnosed as B-ALL with *BCR-ABL1* were retrospectively analysed to determine the impact of the molecular response on overall survival (OS) and disease-free survival (DFS) after allogeneic transplantation. In summary, chemotherapy protocols were administered in three phases; induction, consolidation, and maintenance. The protocols used for the treatment of B-ALL with *BCR-ABL1* follow the Modified GMALL 07/2003 (Scherrer et al., 1994), BFM (Henze, 1981) and Hyper-CVAD (Garcia-Manero & Kantarjian, 2000) regimens which were administered according to the patient's clinical presentation. After the induction phase, patients would receive a total of 3-4 cycles of consolidation therapy prior to transplantation. Central nervous system prophylaxis was also instituted for all patients consisting of at least 4 doses of intrathecal chemotherapy). TKI was only made available after 2011 when our national health program could offer imatinib to B-ALL with *BCR-ABL1*. A bone marrow evaluation was performed at least 4 weeks before transplantation to assess remission status. This included an aspirate and trephine for morphological review, cytogenetics

and molecular studies. Because genetic identification-based risk stratification and MRD monitoring was not fully accessible in the past, our center offered SCT to all eligible ALL patients, even with standard risk, if a matched sibling donor was available. Allogeneic SCT patients received grafts from a sibling with identical human leukocyte antigen or an unrelated donor. For transplantation, HLA match was determined by allelic typing using a resolution of 4 digits per allele. A matched sibling donor was assigned to siblings that demonstrated a 10/10 loci match. If a family-related donor was unavailable, then a matched unrelated donor would be accepted with a 10/10, 9/10 or 8/10 loci match. If a suitable donor could not be identified by the above methods, a haploidentical match that demonstrates a 5-8/10 loci match from a family-related donor would be an option. Stem cell sources were peripheral blood, bone marrow, or unrelated cord blood, whichever was available.

2.3 Molecular Monitoring Strategy

Evaluations of MRD at three different time points (TP); post-induction (TP1), post-consolidation or week 16 (TP2) and end-of-treatment (TP3) were analyzed. *BCR-ABL1* copies were quantified using qRT-PCR as previously described (Arora & Press, 2017; Gabert et al., 2003; Mocellin et al., 2003; Yu et al., 2017). Any MRD positive sample outside the quantitative range was termed below the detection limit. A sample was defined as 'negative' when all replicates were negative with at least 1000 ABL1 copies detected. The MRD value of each follow-up sample was calculated as the logarithmic reduction with respect to the diagnostic value.

2.4 Study Location

The location of the study includes MRKH and Clinical Hematology, Department of Hematology, Hospital Ampang.

2.5 Population and Sampling

Patients diagnosed with B-ALL who received treatment between 2007 and 2017 were evaluated.

2.5.1 Inclusion Criteria

a. Patients above 12 years of age.

b. Patients diagnosed with B-Lymphoblastic Leukaemia with *BCR-ABL1*.

c. Diagnostic and follow-up RNA for patients with B-ALL with *BCR-ABL1* stored in Hospital Ampang Repository.

2.5.2 Exclusion Criteria

a. Patients with Non-B-Lymphoblastic Leukaemia with *BCR-ABL1*.

b. Patients with B-Lymphoblastic Leukaemia without *BCR-ABL1*.

c. Incomplete records of the molecular *BCR-ABL1* time-points.

2.6 Research Instruments

2.6.1 Hospital Information System (HIS)

A Hospital Information System (HIS) is a comprehensive and integrated information system that is designed to be an element of health information that is primarily focused on the hospital's administrative needs. In public hospitals, Malaysian government has

implemented three (3) types of HIS: Total Hospital Information System (THIS), Intermediate Hospital Information System (IHIS), and Basic Hospital Information System (BHIS).

The THIS was implemented at Hospital Ampang, which was led by the Information Technology Unit. Its system combines multiple modules, including the Laboratory Information System (LIS), PhIS (Pharmacy Services), Picture Archiving and Communication System (PACS), and RIS (Radiology Services). One of the most essential functions of this is to assist in the establishment of the medical record. It is a structured record of important data on a single patient that is transformed into a format that meets medicolegal and professional requirements. For our subjects, we extract EMR data from THIS, notably clinicopathological data.

2.6.2 Laboratory Results

Hospital Ampang uses a laboratory information system (LIS), which is a software-based solution with characteristics that support current laboratory operations. In this regard, all units under MRKH are linked to the LIS. However, there are times when the LIS is down or the system is offline. As a result, laboratory results are printed in a standard portable document format (PDF) that is readable by commonly used document reading applications (e.g., Adobe Reader or Microsoft XPS Viewer), and the printed results are stored in each unit under appropriate storage conditions that allow for reliable retrieval. Furthermore, the printed results are saved as a document file in a separate filling system in each unit, from which they may be downloaded using generally accessible transportable storage media such as a USB drive, DVD-ROM, or

portable Hard Disk. Any missing data from the system is double-checked with the printed results kept in MRKH

2.6.3 Data Collection

A total of 2,250 stem cell transplants were performed from 1999 to 2019, with 974 allogeneic stem cell transplantation and 1,276 autologous stem cell transplantation (Figure 1). For this study, all subjects who met the inclusion and exclusion criteria were recruited. Subjects who did not have molecular results for three time points were excluded from this study. Patients diagnosed from 2018 onward were not included because treatment would not have been completed at the time of study.

2.7 Statistical Analysis

Continuous variables were summarized using the median and range. Categorical variables were summarized using count (n) and proportion (%). Differences between subgroups were evaluated with the t test, ANOVA or Kruskal-Wallis test, depending on the sample size and distribution. The association between categorical variables was analyzed using the Chi-square test or Fisher's exact test. The probabilities of OS and DFS were calculated using the Kaplan-Meier method, and the differences between the subgroups were tested using the logarithmic rank test. The prognostic significance of the presenting and transplantation covariates was determined using the Cox proportional hazard regression model. Covariates were selected based on statistical significance in the univariate analysis, which included MRD status and treatment responses. A prognostic factor was considered statistically significant if the p-value <0.05, using the likelihood ratio test. Statistically significant patient characteristics

were included in the multivariate model. Statistical analyzes were performed with SPSS version 26 (SPSS Inc. Chicago, IL, USA). Survival analysis was performed with R version 3.6 and packages such as 'survival'.