STUDY ON *BCR-ABL1* POSITIVE CHRONIC MYELOID LEUKAEMIA PATIENTS AND ITS ASSOCIATIONS WITH CLINICAL, LABORATORY, PROGNOSTIC PROFILE AND OUTCOME

DR NABIHAH BINTI MOHD SHAKRI

DISSERTATION SUBMITTED IN PARTIAL FULFILLMENT OF THE REQUIREMENTS FOR THE DEGREE OF MASTER OF PATHOLOGY (HAEMATOLOGY)

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LIST OF ABBREVIATIONS AND SYMBOLS

AP	Accelerated phase
ACA	Additional chromosomal abnormality
BP	Blastic phase
BCR-ABL1	Breakpoint cluster region-Abelson fusion gene
BM	Bone marrow
CML	Chronic myeloid leukaemia
CP	Chronic phase
CHR	Complete haematological response
ELN	European LeukemiaNet
EUTOS	European Treatment and Outcome Study
ELTS	EUTOS long-term survival
Hb	Haemoglobin
HRPZII	Hospital Raja Perempuan Zainab II
MPN	Myeloproliferative neoplasm
M-*BCR*	Major breakpoint cluster region
m-*BCR*	Minor breakpoint cluster region
PLT	Platelet
RT-PCR	Reverse transcription polymerase chain reaction
TFR	Treatment free remission
WBC	White blood cell
µ-*BCR*	Micro breakpoint cluster region

ABSTRAK

Objektif: Pengesanan molekular *BCR-ABL1* adalah satu ujian penting bagi pengesahan kes-kes 'chronic myeloid leukaemia' (CML). Kajian ini bertujuan untuk mengetahui pembahagian transkrip *BCR-ABL1* di kalangan pesakit CML dan menghubungkaitkan transkrip major dengan parameter klinikal, parameter makmal dan profil prognosis dan hasil penyakit.

Kaedah: 98 orang pesakit CML telah dikenal pasti daripada rekod Hematologi di HUSM dan HRPZII bermula Januari 2010 sehingga Disember 2021. Kajian dua belas tahun ini merupakan kajian keratan rentas yang dijalankan menggunakan data yang diestrak daripada rekod perubatan pesakit di pejabat rekod dan juga sistem pangakalan data pesakit. Keputusan molecular *BCR-ABL1* bagi pesakit CML diambil, dimana pengesanan gabungan gen ini dijalankan dengan menggunakan 'One-Step Multiplex RT-PCR'. Bagi pesakit yang menunjukkan keputusn positif major *BCR-ABL1*, data yang dikumpulkan adalah data demografik (umur, jantina, etnik), profil klinikal (gejala, ukuran limpa, fasa CML), data makmal (sel darah putih, hemoglobin, platelet, peratusan eosinofil dan basofil, sel 'blast' di dalam darah dan juga sum-sum tulang dan keputusan sitogenetik) dan juga profil prognosis dan hasil penyakit (Skor 'Sokal', Transformasi, reaksi hematologi penuh dan 'overall survival'). Hanya 72 orang pesakit yang dipilih untuk kajian '3-year overall survival', iaitu mereka yang menjalani rawatan susulan selama 3 tahun (didiagnos bermula 2010 sehungga 2018)

Keputusan: Daripada 98 orang pesakit, 56% mempunyai gabungan transkrip e14a2 dan 41% mempunyai transkrip e13a2. Dua pesakit selebihnya merekodkan e14a3, dan satu pesakit mengekspresi kedua-dua transkrip e13a2 dan e14a2. Dikalangan pesakit yang mempunyai

transkrip ‘Major’ *BCR-ABL1*, pesakit dengan transkrip e14a2 menunjukkan median umur yang lebih tua (p=0.025), manakala sel darah putih adalah lebih tinggi dikalangan pesakit dengan transkrip e13a2 (p=0.014). Di samping itu, pesakit yang membawa transkrip e13a2 menunjukan lebih ramai pesakit yang mengalami transformasi kepada fasa ‘blastic’ (p=0.038). Selain itu, kajian ini mendapati pesakit yang mengalami fasa ‘blastic’ CML (n=19) mempunyai median umur 26 tahun pada waktu transformasi. Manakala, median tempoh latensi merupakan sembilan bulan. Dikalangan mereka, majoriti jenis sel leukemia adalah mieloid (68.4%), diikuti limfositik sel B (26.3%) dan seterusnya ‘Mixed phenotype’ (5.3%).

Konklusi: Transkrip e14a2 merupakan transkrip gabungan yang lebih ketara dikalangan pesakit CML kami. Perbezaan ungkapan transkrip mungkin boleh mempengaruhi sesetegah parameter. Ini termasuk umur yang lebih tua didapati lebih ketara pada pesakit yang mempunyai transkrip e14a2. Manakala sel darah putih yang tinggi dan kadar transformasi lebih tinggi dikalangan pesakit yang membawa transkrip e13a2. Justeru, setiap pesakit yang dikhuatiri mengalami CML haruslah menjalani ujian pengesanan gabungan gen *BCR-ABL1* bersama identifikasi jenis transkrip dari awal kerana jenis transkrip boleh mempengaruhi fenotip penyakit mereka.

ABSTRACT

STUDY ON *BCR-ABL1* POSITIVE CHRONIC MYELOID LEUKAEMIA PATIENTS AND ITS ASSOCIATIONS WITH CLINICAL, LABORATORY, PROGNOSTIC PROFILE AND OUTCOME

Objectives: Molecular detection of the *BCR-ABL1* has been an important diagnostic marker in defining CML cases. This study aims to determine the distribution of *BCR-ABL1* fusion transcripts among our CML patients and the association of the major fusion transcripts with clinical, laboratory, prognostic profiles and outcome.

Methods: Ninety-eight (98) patients diagnosed as CML with *BCR-ABL1* detected using One-Step Multiplex RT-PCR were identified from Haematology records in HUSM and HRPZII from January 2010 until December 2021 and distribution of the different fusion transcripts were studied. This twelve-year cross-sectional study was conducted using data extracted from patients' medical records. In patients with positive *BCR-ABL1* expressing major fusion transcripts (95 patients), further data obtained were the demographic data (age, gender, and ethnicity), clinical presentation (presenting symptoms, spleen size, phase of the disease), laboratory data (white blood cell, haemoglobin, platelet, eosinophil and basophil count, blast count, bone marrow blast count, cytogenetic study), prognostic profile and outcome (Sokal score, complete haematological response, transformation and overall survival). Only 72 patients were included in the study of 3-year overall survival, whom had a follow-up of more than 3 years (diagnosed from 2010 until 2018).

Results: Out of 98 patients, 56% had e14a2, and 41% had e13a2 fusion transcripts. The remaining 2 patients had e14a3 while 1 patient co-expressed e13a2 and e14a2. Among

patients with major *BCR-ABL1* transcript type (e13a2 and e14a2), e14a2 patients showed older median age (p=0.025) while WBC count was significantly higher in patients with e13a2 fusion transcripts (p=0.014). In addition, there were significantly more patients with blastic transformation in e13a2 groups (p=0.038). Other parameters compared showed no significant associations with either group. The median age at transformation in patients with blastic transformation of CML (n=19) was 26 years old, with a median latency period of 12 months. The blast cell lineages were myeloid 68.4%, followed by B-lymphoid 26.3%, and mixed phenotypic 5.3%.

Conclusion: In our population, e14a2 was the more common *BCR-ABL1* fusion transcript type among CML patients. The difference in fusion transcripts expression might influence some parameters, including older age patients who were associated with e14a2 fusion transcript. Meanwhile, patients exhibiting e13a2 might be associated with higher WBC count at diagnosis, and more vulnerable to blastic transformation of CML. Hence, every suspected CML patient should be tested for *BCR-ABL1* gene fusion with the identification of the fusion transcript type, as these might influence their disease phenotype.

Keywords: CML, *BCR-ABL1*, fusion transcripts, prognosis, major breakpoint cluster region

CHAPTER 1

INTRODUCTION

Definition of CML

Chronic myeloid leukaemia is a type of myeloproliferative neoplasm (MPN) exhibiting dysregulated and uncontrolled proliferation of the granulocytic series with fairly normal maturation (Abdulla *et al.*, 2018). It is characterized by distinct chromosomal translocation t(9;22)(q34.1;q11.2), involving a fusion of the ABL proto-oncogene 1, non-receptor tyrosine kinase *(ABL1)* from chromosome 9q34 with the breakpoint cluster region *(BCR)* gene on chromosome 22q11.2. This rearrangement results in the Philadelphia chromosome. At the molecular level, the consequence of this translocation is the formation of a *BCR-ABL1* fusion oncogene, causing the proliferation of white blood cells. The natural history of untreated CML can progress from an initial indolent chronic phase (CP), into an accelerated phase (AP) and followed by blast phase (BP) (Swerdlow *et al.*, 2016). Using the targeted therapy with tyrosine kinase inhibitor (TKI), majority of people with chronic phase can be maintained and have a normal life expectancy. The ultimate goal of CML treatment is to achieve durable deep molecular response and discontinue medications for treatment-free remission (TFR) (Hochhaus *et al.*, 2020).

Epidemiology of CML

CML accounts for about 15% of adult leukaemia (Siegel *et al.*, 2017). The incidence of CML was 1-2 cases per 100 000 population annually, without any major geographic or ethnic differences (Swerdlow *et al.*, 2016). Males patients are more commonly affected with a male-to-female ratio of 1.3-2.6:1 (Kuan and Michael, 2018). The median age at presentation in Europe CML patients ranges between 60 to 65 years, but it is considerably lower in the Asia population which was 36 to 55 years old (Au *et al.*, 2009; Hochhaus *et al.*, 2017).

Genetic pathogenesis and fusion gene in CML

CML is constantly associated with the rearrangement of the long arms of chromosome 9 (*ABL1* gene) and 22 (*BCR* gene), resulting in the Philadelphia (Ph) chromosome, creating the fusion oncogene *BCR-ABL1* (Hochhaus *et al.*, 2017). This genetic event encodes a constitutively active tyrosine kinase that occurs in a haemopoietic progenitor. *BCR-ABL1* transformed cells exhibit uncontrolled cell proliferation, growth-factor independence, and anti-apoptotic, which are brought on by the activation of a complex network of signaling pathways (Quintás-Cardama and Cortes, 2009). The breakpoints of the *BCR* and *ABL1* genes occur within the introns of each gene and are unique to each individual. The *ABL1* breakpoints are almost invariably located in the introns between exons 1 and 2. In contrast, the site of *BCR* breakpoints in chromosome 22 can be variable, either in introns between exons 1-2, 13-15 or 19-20. These regions are known as the minor-*BCR* (m-*BCR*), major-*BCR(*M-*BCR*), and micro-*BCR* (μ-*BCR*) respectively (Yeung and Huges, 2016). Usually, the *BCR* gene breaks in an intron between exons 12-16 (M-*BCR*) (Swerdlow *et al.*, 2016). Together with the *ABL1* breakpoint, the resulting fusion transcripts are named as e13a2 and e14a2 transcripts (previously known as b2a2 and b3a2), which encode for a 210 kDa protein product (p210). These transcripts are the most commonly encountered fusion transcripts in CML (Jain *et al.*, 2016). In comparison, breakpoint at m-*BCR* produces the e1a2 transcript, which encodes the p190 BCR-ABL1 protein, commonly seen in Ph+ ALL. Other transcripts, including e13a3,e14a3, e6a2, e8a2, or e19a2 are less commonly reported (Hochhaus *et al.*, 2020).

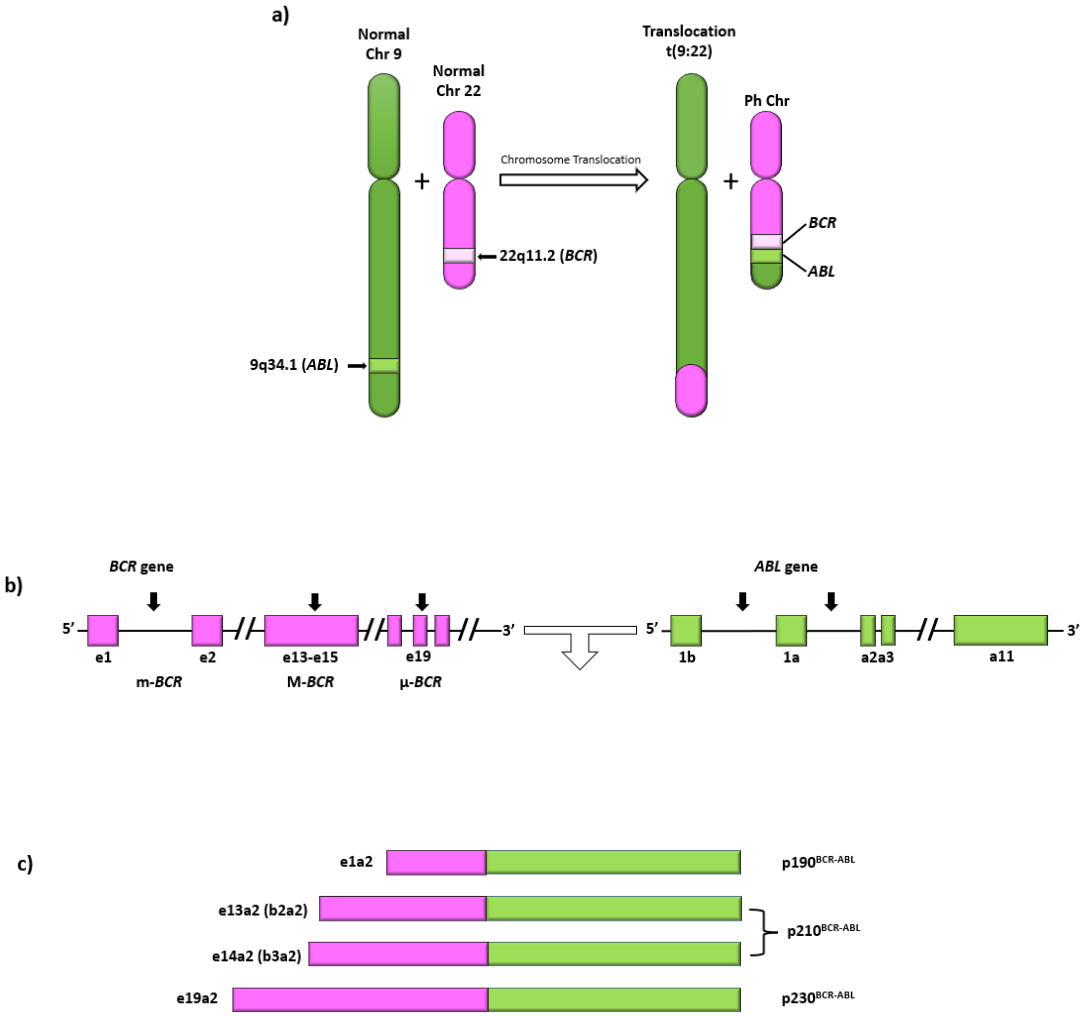


Figure 1: a) Schematic diagram of Philadelphia chromosome, b) Breakpoint locations between BCR and ABL1 genes (black arrow) c) Different fusion protein combinations yield different outcomes.

Clinical manifestation

Most of the CML patients presented in CP, while about 5% of cases are diagnosed in AP or BP (Hochhaus *et al.*, 2017). The clinical presentation at diagnosis varies among reported case series and also depends on which phase they are presented with. Nearly half of CML patients are asymptomatic and identified during routine medical examinations (Swerdlow *et al.*, 2016). On the other hand, the commonest presenting clinical features are left upper fullness or pain, fatigue, weight loss, and fever (Hochhaus *et al.*, 2017; Korubo *et al.*, 2013). Splenomegaly is the most consistent physical sign detected in 40-50% of cases (Hochhaus *et al.*, 2017). Disease progression from CP to BP typically occurs within 3-5 years after the diagnosis. However, with the advanced of treatment with TKI, such cases have reduced (Yeung and Huges, 2016). Cases of CML underwent transformation are distinguished by deteriorating performance status, constitutional symptoms, symptomatic cytopenia, an increase in white blood cell (WBC) count, and splenic enlargement (Swerdlow *et al.*, 2016).

Diagnosis

The diagnosis of CML is generally straightforward. Most cases can be diagnosed based on distinctive peripheral blood findings combine with the detection of the Ph chromosome and/or *BCR-ABL1* fusion gene (Swerdlow *et al.*, 2016). Nevertheless, a bone marrow aspirate should be obtained not only for full karyotype analysis, but the confirmation of the phase of the disease can be determined (Smith *et al.*, 2020). The typical findings in blood and bone marrow of CML in CP according to WHO are illustrated in Table 1.

Table 1. Haematological findings of CML in CP according to WHO, Revised 4th Edition, 2016
Peripheral blood Leukocytosis (12-1000x10^9 /L) Neutrophils in all stages of maturation (peaks in myelocytes and segmented neutrophils) No significant dysplasia of WBCs Blast cells typically <2% of the WBCs Absolute basophilia and eosinophilia Platelet count is either normal or increased **Bone marrow aspirate** Hypercellular with marked granulocytic proliferation with maturation that reflects the peripheral blood Blasts usually <5% of the marrow cells No significant dysplasia Decreased in erythroid precursors Megakaryocytes may be normal or slightly decreased Dwarf megakaryocytes Increased in eosinophils and basophils Pseudo-Gaucher cells are common **Bone marrow biopsy** Similar to bone marrow aspirate findings A thicker layer of immature granulocytes in the paratrabecular area Variable degree of reticulin fibrosis

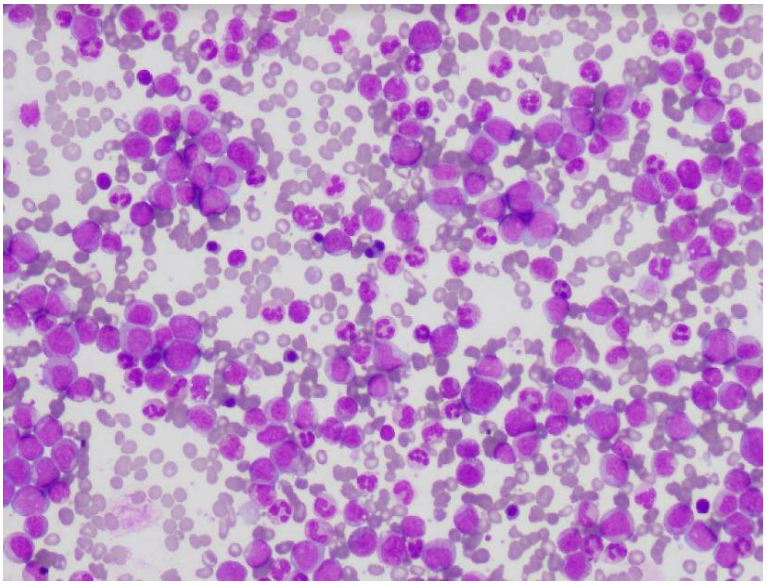
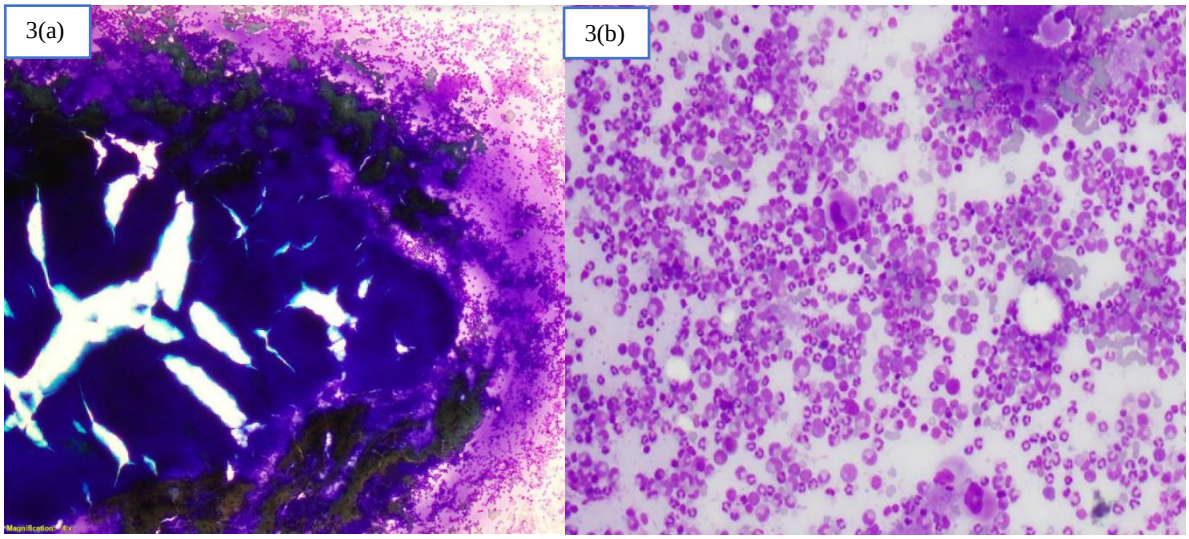


Figure 2: Peripheral blood of CML patient

Figure 3: Bone marrow aspirate of CML, BCR-ABL1-positive (chronic phase)



3(a) Bone marrow aspirate showed hypercellular fragments
3(b): There were granulocytic proliferation with peaks in the proportions of myelocytes and segmented neutrophils. No increase of blast cells seen

The clinical and morphological boundaries between CP, AP, and BP are not always sharp. However, recognition of the disease progression is important for the prognosis and treatment purpose (Swerdlow *et al.*, 2016). World Health Organization Classification of Tumours of Haematopoieitic and Lymphoid Tissues in 2016 and European LeukemiaNet (ELN) proposed their defining criteria for AP and BP which are compared in Table 2. The upcoming fifth edition of the WHO Classification of Haematolymphoid Tumors has been recently summarized in a review article where they have omitted the AP in the current classification. Whereas, the latest defining criteria of BP include: (1) ≥20% myeloid blasts in the blood or bone marrow; or (2) the presence of an extramedullary proliferation of blasts; or (3) the presence of increased lymphoblasts in peripheral blood or bone marrow (Khoury *et al.*, 2022).

Table 2. Defining criteria of AP and BP of CML according to WHO 2016 and ELN		
	WHO(Swerdlow *et al.*, 2016)	**ELN**(Hochhaus *et al.*, 2017; Smith *et al.*, 2020)
AP	Defined by the presence of one of the following: 1. Blood or marrow blasts 10-19% 2. Basophils in blood ≥20% 3. Persistent thrombocytopenia (<100 $x10^9$ /L) unrelated to therapy 4. Persistent or increasing splenomegaly, unresponsive to therapy 5. Persistent or increasing leukocytosis (>10$x10^9$ /L) unresponsive to therapy 6. Persistent thrombocytosis (>1000 $x10^9$ /L) unresponsive to therapy 7. Additional clonal chromosome abnormalities in Philadelphia (Ph) chromosome-positive (Ph+) cells at diagnosis that include 'major route' abnormalities 8. Any new clonal cytogenetic aberration during therapy	Defined by the presence of one of the following: 1. Blood or marrow blasts 15-29%, or blasts plus promyelocytes >30% (with blast <30%) 2. Basophils in blood ≥20% 3. Persistent thrombocytopenia (<100 $x10^9$ /L) unrelated to therapy 4. Clonal chromosome abnormalities in Ph+ cells
BP	Defined by presence one of the following 1. ≥20% blasts in the blood or bone marrow 2. Extramedullary blast proliferation 3. Large foci or clusters of blasts in bone marrow biopsy	Defined by presence one of the following 1. ≥30% blasts in the blood or bone marrow 2. Extramedullary blast proliferation

At diagnosis, 90-95% of cases of CML have the classical t(9;22)(q34.1;q11.2) that can be detected by conventional cytogenetic analysis. The remaining cases manifest as either variant translocation that involved a third or even a fourth chromosome in addition to chromosomes 9 and 22 (Variant Ph chromosome) or a cryptic translocation of 9q34.1 and 22q11.2 that only can be identified by fluorescence in situ hybridization (FISH) analysis and/or reverse transcriptase polymerase chain reaction (RT-PCR) (Ankathil *et al.*, 2020;

Swerdlow *et al.*, 2016). This aberrancy has been considered to carry similar outcome with the classical t(9;22) cases (Ankathil *et al.*, 2020). Meanwhile, a smaller proportion of CML patients may harbour additional chromosomal abnormalities (ACA) in Ph positive cells. This reflects the genetic instability of the leukaemic cells and raises concern for disease progression (Ankathil *et al.*, 2020; Hochhaus *et al.*, 2020a). High risk ACAs such as second Ph chromosome, trisomy 8, isochromosome 17q, trisomy 19, complex karyotype, 11q23 and abnormalities of 3q26.2 are considered validated risk factor for progression of the disease into BP (Yeung and Huges, 2016). A qualitative reverse transcriptase polymerase chain reaction (RT-PCR) on peripheral blood cells is mandatory to detect and identify the type of *BCR-ABL1* fusion transcripts. This is essential to guide the future management (Smith *et al.*, 2020). Majority (97-98%) of CML cases express either e13a2 or e14a2 fusions. Only a minority of cases carry atypical variants (Hochhaus *et al.*, 2017). Variable frequencies of these fusion transcripts in CML patients are observed around the world (de Almeida Filho *et al.*, 2019).

Prognosis and predictive factors

Three prognostic systems namely Sokal, Euro(Hasford) and European Treatment and Outcome Study(EUTOS) score are arithmetically derived scoring systems useful to estimate the survival risk at baseline, before starting of any treatment (Table 3) (Hochhaus *et al.*, 2020). These scoring systems were proposed in 1984, 1998, and 2011 respectively (Hasford *et al.*, 1998, 2011; Sokal *et al.*, 1984). Simple clinical evaluation (age, spleen size), as well as haematological data (platelet count, blood blasts, basophil and eosinophil count), are incorporated into the calculation and stratify the patients into low, intermediate, and high risk. Low-risk patients respond significantly better to TKIs than high-risk individuals

(Swerdlow *et al.*, 2016). The fourth scoring system, the EUTOS long-term survival (ELTS) score was later introduced in 2016 in order to distinguish three risk groups with significantly different possibilities of dying from CML and this is the current scoring system recommended by ELN (Markus Pfirrmann *et al.*, 2020). At-present, there are few online calculators available to calculate these scores. Nevertheless, in the current era of TKI therapy, the most important prognostic indicators are the response to treatment at the hematological, cytogenetic, and molecular level (Swerdlow *et al.*, 2016).

Table 3: Prognosis scoring system

Score	Calculation	Definition of risk groups
Sokal score(Sokal *et al.*, 1984)	Exp 0.0116 × (age in years - 43.4) + 0.0345 × (spleen - 7.51) + 0.1889 [(platelet count⁄700]^2 - 0.563) + 0.0887 × (blast cell counts - 2.10)]	Low-risk: <0.8 Intermediate-risk: 0.8-1.2 High-risk: >1.2
Euro/Hasford score(Hasford *et al.*, 1998)	4 (0.6666 × age [0 when age <50 years; 1,otherwise] +0.0420 × spleen size [cm below costal margin] + 0.0584 × blasts [%] + 0.0413 × eosinophils [%] + 0.2039 × basophils [0 when basophils <3% ;1, otherwise] + 1.0956 × platelet count [0 when platelets <1500 × 10^9/L; 1, otherwise]) × 1000.	Low-risk: ≤780 Intermediate-risk: >780 and ≤1480 High-risk: >1480
EUTOS score(Hasford *et al.*, 2011)	(0.0700 × basophils) + (0.0402 × spleen size)	Low-risk: ≤ 87 High-risk: > 87
ELTS score (M. Pfirrmann *et al.*, 2015) (Hochhaus *et al.*, 2020a)	0.0025 x (age/10) ^{3} + 0.0615 x spleen size below costal margin + 0.1052 x blasts in peripheral blood + 0.4104 x (platelet count/1000)$^{-0.5}$	Low-risk: ≤1.5680 Intermediate-risk: 1.5680-2.2185 High-risk: >2.2185

Treatment of CML

The treatment landscape in CML patients had evolved from chemotherapeutic drugs (hydroxyurea and busulfan) between 1959 and 1982 and later on with interferon-alpha or

allogeneic stem cell transplantation from the 1980s until 2000. In 1998, the era of tyrosine kinase started off with Imatinib being introduced as the first generation of BCR–ABL1 tyrosine kinase inhibitors (TKIs) followed by other TKIs (Goldman, 2010; Santos *et al.*, 2011). With the advent of TKIs, current treatment goals are to provide normal life expectancy in CML-CP, prevent disease progression, achieve treatment-free remission (TFR) after a stable deep molecular response, and prevent long-term organ toxicity while maintaining a good quality of life (Deininger *et al.*, 2020; Hochhaus *et al.*, 2020a).

For the initial treatment of CML, both the first and second-generation TKIs are preferred agents with the exception of cases diagnosed during pregnancy. The United States Food and Drug Administration (FDA) has currently approved four TKIs, namely imatinib, dasatinib, nilotinib, and bosutinib (Hochhaus *et al.*, 2020) described in Table 4 to be used in CML.

The selection of first line TKIs depends on few factors such as the phase of disease, age, comorbidities, cost, toxicity profile of TKI, possible drug interaction and patient preference (Deininger *et al.*, 2020; Ono, 2021) with Imatinib is the recommended first-line treatment for the majority of adults and children with CML presenting in CP (Smith *et al.*, 2020). Based on several studies, generic imatinib is the most cost-effective initial therapy option for CML in CP (Hochhaus *et al.*, 2020). In the majority of patients, there is no reason to choose another TKI over imatinib, which has a well-established safety profile and no reported life-threatening long-term adverse effects (Smith *et al.*, 2020). However, patients with intermediate or high risk Sokal or Euro score may be the candidates for second generation of TKIs as these agents are associated with lower risk of disease progression (Deininger *et al.*, 2020). Moreover, in younger patients, especially women

whose fertility is of concern and wish to get pregnant, they may benefit from second generation TKI as first line therapy (Smith *et al.*, 2020). This is due to the evidence of faster molecular response and greater rates of major molecular response (MMR) and deep molecular response (DMR) with second generation TKI. Such favorable responses may enable the discontinuation of TKI therapy in the future with the aim of TFR (Deininger *et al.*, 2020).

Table 4. First and second generation TKI inhibitors (Hochhaus *et al.*, 2020a)

Drugs (e.g.)	Imatinib (Glivec®, Novartis, or generic)	Dasatinib (Sprycel®, Bristol-Myers Squibb)	Nilotinib (Tasigna®, Novartis)	Bosutinib (Bosulif®, Pfizer)
TKI generation	1st	2nd	2nd	2nd
Uses	First line choice of treatment of CML	• Approved for first line treatment • Active against several imatinib-resistant BCR-ABL1 mutants e.g., Y253H, E255V/K, F359V/I/C	• Approved for first line treatment • Active against several imatinib-resistant BCR-ABL1 mutants e.g., F317L/V/I/C, T315A, V299L	• Approved for first line treatment • Active against several imatinib-resistant BCR-ABL1 mutants e.g. F317L/V/I/C, T315A, Y253H, E255V/K, F359V/I/C
Dosage	400mg OD -in CP, lower dose 300mg OD if usual dosage is not tolerated -in AP 400mg BD*	100mg OD -in AP: 70mg BD	300mg BD -as second line TKI: 400mg BD	400mg OD -as second line TKI: 500mg OD
Side effects	Early fluid retention, gastrointestinal symptoms	Pleuro-pulmonary toxicity (e.g., pleural effusion)	Cardiovascular events	Transient diarrhea and raised transaminases
Contraindication	No absolute contraindications	Previous or concomitant	Previous coronary heart	No absolute contraindications

		pleuro-pulmonary or pericardial diseases	disease, cerebrovascular accidents, or peripheral arterio-occlusive disease	

* In patients with progression to more advanced disease a change to second generation TKI (2GTKI) is recommended

Patients experiencing treatment failure, resistance, or intolerance as a result of treatment-related side effects are candidates for second-line treatment (Hochhaus *et al.*, 2020a). The introduction of new drugs should be accompanied by a BCR-ABL1 kinase domain mutational analysis, and the choice of second-line therapy should be guided based on the presence of BCR-ABL1 kinase domain mutation (Gambacorti-Passerini *et al.*, 2016). In addition to Table 4, Ponatinib, a third generation TKI is the only TKI active against T315I mutation (Yang *et al.*, 2022). Meanwhile, in the absence of BCR-ABL1 KD mutation, all second generation TKIs are effective, but the choice of treatment should be individualized depends on the age, comorbidities and toxicity of the first TKI used (Hochhaus *et al.*, 2020a).

In the advanced phase of CML (characterized by clinical deterioration, the emergence of high-risk ACA, failing hematopoiesis, and blast cell proliferation), patients should be considered for different treatment approach. In patients presented with de novo AP, they should be ideally treated with second generation TKI. Subsequently, in a case of suboptimal response, allogenic stem cell transplantation (alloSCT) should be considered (Smith *et al.*, 2020). In CML-BP, treatment consist of intensive combination chemotherapy with or without TKI in preparation for prompt alloSCT (Hochhaus *et al.*, 2020a). In the UK, FLAG-Ida regime (fludarabine, idarubicin, granulocyte-colony stimulating factor (GCSF) and high-dose cytarabine) with or without a TKI, has become the predominant approach, notably if

there is a substantial delay in arranging allo-SCT (Milojkovic *et al.*, 2012; Smith, *et al.*, 2020).

Treatment monitoring

The objectives of CML treatment include the restoration of blood counts to normal values, reduction and elimination of the Ph+ chromosome and reduction and elimination of *BCR-ABL1* gene expression. These can be ascertained by the measurement of haematologic, cytogenetic and molecular response respectively (Cortes *et al.*, 2011). Overall, quantification of molecular monitoring is by far the strongest predictor of outcome and by proper monitoring, development of treatment resistance can be foreseen (Smith, *et al.*, 2020).

Once patient is started on TKI, the initial monitoring will include the blood cell counts with differential cell counts every two weeks until a complete hematologic response (CHR) (Table 5) is achieved or, more frequently, if patients develop haematological adverse events. According to ELN standards, achieving CHR within 3 months of therapy initiation is the optimal haematological response (Hochhaus *et al.*, 2020a). Previously, it was recommended that cytogenetic response monitored at 3, 6 and 12 months until CcyR is achieved then every 12 months (Baccarani *et al.*, 2013). As per current ELN recommendations and BCSH guidelines, bone marrow aspirate, cytogenetic analysis or FISH are not routinely required to assess treatment response (Hochhaus *et al.*, 2020a; Smith, *et al.*, 2020). This is because cytogenetic response (refer Table 5) alone is not sensitive enough to determine response (Hochhaus *et al.*, 2020). It is measured by conventional cytogenetic analysis (CCA), which has a low limit of detection (1:20) and limited sensitivity (5%), detecting one abnormal metaphase out of the 20 metaphases analysed (Ankathil *et al.*, 2020). However, in some individuals, cytogenetic response still plays a beneficial role. This include to those with

atypical translocations, uncommon or atypical *BCR-ABL1* transcripts that cannot be identified by qPCR, therapy failure/resistance to exclude ACA, and progression to AP or BP (Hochhaus *et al.*, 2020).

Molecular monitoring of treatment response in CML is a more sensitive method and it is necessary to detect minimal residual disease for clinical management (Dominy *et al.*, 2020). It is by the detection of *BCR-ABL1* mRNA using real-time quantitative polymerase chain reaction (qPCR) from total peripheral blood leucocytes. This is the only method capable of monitoring responses after the patient has achieved CCyR, as *BCR-ABL1* transcripts often continue to be detectable after CCyR. In addition, it allows for molecular monitoring without bone marrow aspiration as there is a strong correlation between results from both sites (Deininger *et al.*, 2020). Other alternative includes the detection by digital PCR (Smith, *et al.*, 2020). Irrespective of method used, molecular response must be assessed according to the International Scale (IS) as the ratio of *BCR-ABL1* transcripts to *ABL1* transcripts, or to other internationally accepted control transcripts. It reported as *BCR-ABL1* % on a log scale, where 1%, 0.1%, 0.01%, 0.0032%, and 0.001% correspond to a decrease of 2, 3, 4, 4.5, and 5 logs, respectively, below the standardized baseline that was used in the IRIS study (Cross *et al.*, 2012). As per ELN recommendations, patients on TKI therapy should be monitored for molecular response at least every 3 months even after an MMR is achieved and confirmed (Hochhaus *et al.*, 2020a).

Assessment of specific degrees of disease reduction at specific time points during treatment with first line and second line TKIs can be assessed using qPCR (IS) by the evaluation of TKI response milestones at 3, 6, and 12 months (Refer table 6). The response can be either optimal, failure/resistance or warning. Thus, guiding the clinician whether the

treatment should be continued, changed or carefully considered for continuation or change, depending on patients´ characteristics, comorbidities and tolerance (Hochhaus *et al.*, 2020a).

In the era of potent and advent TKIs therapy, the current new treatment goal for patients with CML (CP) is the TFR in patients who achieved stable deep molecular response (DMR) which is 4-log reduction (transcript level of ≤ 0.01% or MR^4) or deeper (Hochhaus *et al.*, 2020). However, for the discontinuation of TKI, a number of conditions must be met after careful consideration (Hochhaus *et al.*, 2020). In keeping with the new treatment landmark that has significantly changed since last few years, several centers in Malaysia had incorporated TFR in treating their CML patients. A multicentre observational study involving major hospitals in Malaysia to observe the clinical practice to End TKI In CML (EnTIC) was carried out in 2021 until 2022. A total of 88 patient were studied, whom diagnosed with CML in chronic phase and received first line TKI (imatinib, nilotinib or dasatinib) for at least four years with sustained MR^4 (IS: 0.01%). Among them, 64.8% were able to remain in MMR after TKI cessation, demonstrating a clinically significant finding (Chong *et al.*, 2022).

Table 5. Definitions of heaematological, cytogenetic and molecular response based on ELN recommendation (Baccarani *et al.*, 2009, 2013; Hochhaus *et al.*, 2020)

Type of response	Definitions
Complete haematological response	• Complete normalization of FBC with WBC count < 10 x 10^9/L • Platelet count <450 x 10^9/L • No immature granulocytes, basophil <5% • No sign and symptoms of disease with disappearance of palpable splenomegaly
Cytogenetic response	Complete cytogentic response (CCyr): no Ph+ metaphases (<1 % *BCR-ABL1*-positive nuclei of at least 200 nuclei if by FISH)

	Partial cytogenetic response (PCyr)	1–35 % Ph+ metaphases
	Minor cytogenetic response (mCyr)	36–65 % Ph+ metaphases
	Minimal cytogenetic response (minCyr)	66–95 % Ph+ metaphases
	No cytogenetic response (noCyr)	>95 % Ph+ metaphases
Molecular response		
	Early molecular response (EMR)	*BCR-ABL1* transcripts level ≤10%% IS at 3 and 6 months
	Major molecular response (MMR) or MR^3	*BCR-ABL1* transcripts level ≤0.1% IS
	$^+MR^4$	*BCR-ABL1* transcript level ≤0.01% IS or undetectable disease in cDNA with >10,000 ABL1 transcripts
	$^+MR^{4.5}$	BCR-ABL1 transcript level ≤0.0032% IS or undetectable disease in cDNA with >32,000 ABL1 transcripts in the same volume of cDNA used to test for *BCR-ABL1*
	$^+MR^5$	BCR-ABL1 transcript level ≤0.001% IS with >100,000 ABL1 transcripts in the same volume of cDNA used to test for *BCR-ABL1*

$^+$Deep molecular response (DMR) refers to MR^4 or deeper

Table 6. Milestones for treating CML expressed as *BCR-ABL1* on the International Scale (IS) (Hochhaus *et al.*, 2020a)			
	Optimal	**Warning**	**Failure**
Baseline	NA	High-risk ACA High-risk ELTS score	NA
3 months	≤10%	>10%	>10% if confirmed within 1-3 months
6 months	≤1%	>1-10%	>10%
12 months	≤0.1%	>0.1-1%	>1%
Anytime	≤0.1%	>0.1-1% Loss of ≤0.1 (MMR)	>1%, resistance mutation, high risk ACA

For patients aiming at TFR, the optimal response (at any time) is BCR-ABL1 ≤ 0.01% (MR^4).

A change of treatment may be considered if MMR is not reached by 36–48 months.

Standard practice in Hospital USM and HRPZII

HRPZII and Hospital USM are located in the state of Kelantan, in the north-eastern region of peninsular Malaysia (Mohamad *et al.*, 2012). Both hospitals serve as tertiary referral centers. Comparable to elsewhere, the management of CML from the first evaluation, diagnosis, treatment, and monitoring, involves a coordinated effort involving primary care physicians, haematopathologists, and, most critically, hematologists/haemato-oncologist with expertise in CML (Soverini *et al.*, 2016).

In patients suggestive of CML by blood count and peripheral blood film, they are subjected to comprehensive initial workup which includes thorough history taking, physical examination, personal, family and financial situation and assessing risk profiles by commonly used prognostic scoring such as Sokal score or ELTS score. Qualitative molecular analysis of *BCR-ABL1* fusion gene with the identification of transcript type using peripheral blood is sent to confirm the diagnosis (Hochhaus *et al.*, 2017). This test is available in the Haematology laboratory in Hospital USM (Hassan *et al.*, 2008). While waiting for the result, standard clinical chemistry assay is sent as well as cardiac evaluation performed for baseline

evaluation before initiating TKI treatment (Hochhaus *et al.*, 2020). Bone marrow examination which consists of morphological assessment, flow cytometry, histology as well as cytogenetic study are mandatory to confirm the phase of disease as well as to evidence the presence of Ph chromosome as a part of diagnostic tool (Smith *et al.*, 2020). A short course of hydroxyurea typically will be given in symptomatic patients with leucocytosis and/or thrombocytosis until the diagnosis of CML has been confirmed by molecular or cytogenetic analysis (Hochhaus *et al.*, 2020).

Once the diagnosis is confirmed, discussion regarding the diagnosis, treatment options and the need for continuous monitoring are explained by the treating haematologist to the patients and their partner or family members. In HRPZII, once patients consent to treatment, they are registered under the Malaysian Patient Assistance Programme (MyPAP), whereby the government will purchase and provide Imatinib to patients for free of charge (Kuan and Michael, 2018). It is a public–private partnership between the Ministry of Health Malaysia (MOH) and the pharmaceutical company Novartis and is managed by DKSH Malaysia. This programme was started since 2007. It was a shift from the previous Glivec International Patient Assistance Programme (GIPAP) (Wan Puteh *et al.*, 2022). In contrast to the MOH hospital, where TKIs are more easily accessible, the Imatinib supply at Hospital USM is limited based on the annual hospital allocation. When the quota has been met and the new patient cannot afford to purchase Imatinib on their own, they are referred to HRPZII or another MOH facility for Imatinib under the MyPAP program. Under this program, imatinib and nilotinib, are provided for free to CML patients as the first and second-line treatment, listed in the National Drug Formulary (Lim *et al.*, 2017). The starting dose of imatinib in newly diagnosed CP-CML patient is 400mg daily as per FDA approved (Sacha,

2014). In addition, in some instances, TKIs other than Imatinib may be considered as first line treatment. Patients with a greater prognostic risk at the time of diagnosis may benefit from a second generation TKI as their initial therapy, as this reduces their risk of disease progression compared to Imatinib (Deininger *et al.*, 2020).

In terms of disease monitoring, as per ELN recommendations, initial monitoring includes two-weekly blood count until CHR is achieved (Hochhaus *et al.*, 2020). Patients are also closely monitored for their compliance with TKI and intolerance due to side effects during follow up. With the availability of qPCR for *BCR-ABL1* quantitation in some centers in Malaysia, quantification molecular monitoring of *BCR-ABL1* transcripts utilising peripheral blood has replaced marrow cells as the preferred monitoring method for our CML patients (Ting *et al.*, 2017). Both of these centers tried to comply to current ELN panel recommendation on treatment monitoring which must be performed at least every 3 months even after MMR is achieved and confirmed (Hochhaus *et al.*, 2020), however, this is not always the true scenario. Due to financial concerns, HRPZII only sends six monthly *BCR-ABL1* quantification for their patient. In HRPZII this test is currently out-sourced to laboratory in Hospital Tunku Azizah, Kuala Lumpur. Meanwhile, in Hospital USM, testing is dependent on the availability of drug company vouchers and the test is sent to Sunway Medical Center Molecular Diagnostic Lab.

Second generation TKIs, mainly Nilotinib 400mg BD or Dasatinib 100mg OD are considered for patients with AP. Other option includes Imatinib 400mg BD (Hochhaus *et al.*, 2020). Individualized treatment selection with consideration of any comorbidities is important. AlloSCT is typically considered in this group of patient, especially in patients with suboptimal response to second generation TKI (Smith *et al.*, 2020). On the other hand, CML-

BP patients are initially assessed for the blast lineage by flow cytometry and they are subjected to either AML or ALL intensive combination chemotherapy with or without a TKI accordingly (Hochhaus *et al.*, 2020). After initial disease control, all efforts are made to offer alloSCT to eligible patients. Patients who cannot tolerate intensive chemotherapy regimens are typically treated with a palliative strategy involving less intense therapy (Hochhaus *et al.*, 2020). As followed in HRPZII, patient with blast transformation will be subjected to intensive chemotherapy to achieve chronic phase, then will be maintained with selected choice of TKI namely dasatinib while waiting for alloSCT. Dasatinib had been known for its promising therapeutic potential in managing intracranial leukemic disease which are usually seen in lymphoid blast crisis of CML (Porkka *et al.*, 2008; Smith *et al.*, 2020).

BCR-ABL1 **fusion transcripts and its significance**

Around the world, variable frequencies of the fusion transcript in CML patients were reported. Overall, majority of CML cases (97-98%) exhibited e13a2 and e14a2 fusions. The remaining 2-3% expressed a variety of unusual fusions involving *ABL1* (a3) or other *BCR* exons (often e1, e6, e8, e19) (Smith, *et al.*, 2020). With regard to the major transcripts e13a2 and e14a2, many literatures suggested mixed opinions on their distribution. Few studies had reported higher e14a2 (de Almeida Filho *et al.*, 2019; Kagita *et al.*, 2018; Khazaal *et al.*, 2019) than e13a2 while others had shown the opposite (Osman *et al.*, 2010; Paz-Y-Miño *et al.*, 2002). Baccarani et al, systematically investigated a large cohort (45,000 patients) of CML patients in an international retrospective study involving 45 countries. They found e13a2 and e14a2 transcripts were detected in 38% and 62%, respectively. Atypical, rare

transcripts were found in 666 patients (1.93%) which include e1a2, e19a2, e13a3, e14a3, etc. (Baccarani *et al.*, 2019).

Many literatures also had investigated the influence of these divergent groups on several parameters such as demographic, clinical, hematological, prognosis and treatment outcome. However, their findings were variable (Al-Achkar *et al.*, 2016). This include findings of male and female were significantly associated with e14a2 and e13a2 fusion transcript, respectively (Khazaal *et al.*, 2019). Study in Tunisia had observed that e14a2 patients were found to be older than patients with e13a2 transcript (56.61 vs. 26.56 years) (Bennour *et al.*, 2013).

With regards to haematological findings, a study in Pakistan found that patients with e13a2 had higher TWC in comparison to e14a2 while higher platelet counts were observed in e14a2 group (Amin and Ahmed, 2021). The relationship of e14a2 fusion transcripts with increased platelet count was one of the most intriguing findings, with some study supporting this association and others contradicting it (Bennour *et al.*, 2013; Khazaal *et al.*, 2019). Meanwhile, other study discovered patients with e19a2 transcripts had typically pronounced neutrophilic maturation and/or thrombocytosis (Langabeer *et al.*, 2012; Swerdlow *et al.*, 2016).

Previous research had also compared the response of patients with these various fusion transcripts to TKI therapy. Few studies found that MMR was significantly higher in patients with e14a2 transcript as compared to patients with e13a2, supporting inferior outcome in the later (Jain *et al.*, 2016; Nachi *et al.*, 2020; Sazawal *et al.*, 2019). With regards to haematological and cytogenetic response, Kagita et al. found in their study that e14a2 might be related with a poor response and prognosis in CML patients treated with imatinib (Kagita *et al.*, 2018).

Rationale of the study

To date, there is contrasting data on the frequency of different types of *BCR-ABL1* fusion transcripts in CML patients worldwide, as well as its impact on the disease characteristics (de Almeida Filho *et al.*, 2019). It is well recognised that the detection and identification of the fusion gene at the time of the first diagnosis is significant, since it is subsequently utilised as a tool for determining the treatment's efficacy (Zhou *et al.*, 2020). However, little is known about whether these different fusion transcripts are connected with any parameter that could assist the clinician in risk-stratifying patients or predicting the course of the disease (de Almeida Filho *et al.*, 2019).

An international review had shown that distribution of *BCR-ABL1* transcript types is not the same worldwide with evidence of geographical variation (Baccarani *et al.*, 2019). To date, studies on this subject in the Southeast Asia region are rather sparse. In our neighboring country Thailand for an example, the distribution of fusion transcripts was 73% and 27% for e14a2 and e13a2 respectively and they found that these different groups had no prognostic significance (Auewarakul *et al.*, 2006). However, we think that is it essential to conduct this study since we have different ethnicity and population that might produce different outcomes. Furthermore, there is no representative study conducted on the association between types of fusion transcript genes and CML patients' clinical, laboratory parameters, prognosis and outcomes in Malaysia. Therefore, the aim of this study is to know the distribution of *BCR-ABL1* fusion transcripts in our local population and analyze their associations with clinical, laboratory, prognostic profiles and outcomes. By knowing the association, we hope to provide some insight into understanding the disease phenotype and perhaps contribute to better care of CML patients in deciding treatment options.

Dissertation Organization

The dissertation was arranged according to Format B (manuscript ready format) according to guideline by Postgraduate Office, School of Medical Sciences (2019). Chapter 2 contains the study protocol that had been submitted and obtained ethical approval from Jawatankuasa Etika Penyelidikan Manusia (JEPeM), Universiti Sains Malaysia. Chapter 3 is the manuscript entitled "Study on *BCR-ABL1*-positive Chronic Myeloid Leukaemia patients and its associations with clinical, laboratory, prognostic profile and outcome". The appendices (chapter 4) contain elaboration of methodology and additional references. The raw data is included in the attached CD.