CHAPTER 2

LITERATURE REVIEW

2.1 Overview of Acute Lymphoblastic Leukaemia

Acute lymphoblastic leukaemia (ALL) is the most common leukaemia in children but accounts for only 20% of adult acute leukaemias. (Copelan EA, 1995; Ward E, 2014) Normal lymphoid precursors will undergo somatic recombination at their immunoglobulin (Ig) or T-cell receptor (TCR) gene loci, and successful completion of VDJ recombination, with the resultant formation of a functional Ig or TCR, is required for the survival of lymphocyte precursors. Positive and negative selection steps ensure that only lymphocytes with Ig or TCRs that function appropriately in the context of an individual's immune microenvironment are allowed to proceed through the proliferation and differentiation steps. Uniform structural and numerical chromosomal abnormalities are frequently demonstrated in leukaemic lymphoblasts, including identical rearrangements of Ig or TCR genes, which are somatic in origin (Melissa Burns, 2013).

ALL is usually suspected when circulating lymphoblasts are detected in the peripheral blood with or without painless lymphadenopathy, in the setting of unexplained cytopenia. Patients may present with fatigue, recurrent infections, easy or spontaneous bleeding / bruising, constitutional symptoms, bone pain, hepatomegaly and/or splenomegaly (Hoffbrand AV, 2016; Kaunshansky K, 2015). Evaluation should include a complete blood count with differential parameters, examination of the peripheral smear, immunophenotype of peripheral blood or marrow, bone marrow examination and lumbar puncture to rule out central nervous stem (CNS) infiltration. (Hoffbrand

AV, 2016; Kaunshansky K, 2015) (Dr Hiroto Inaba, 2013, Volume 381, Issue 9881) The diagnosis of ALL requires at least 20% bone marrow lymphoblasts (JE, 2017). Marrow aspirate sample should also be analyzed using cytogenetic and molecular techniques to enable categorization according to the World Health Organization (WHO) classification (Swerdlow SH, 2017). The flow cytometry immunophenotyping will identify whether the leukaemia is of B-cell or T-cell lineage (Bain BJ, 2016; Swerdlow SH, 2017). ALL originates from precursor lymphoid cells consisting of a B-cell (80-85%) or T-cell (20-25%) derivation.

Unlike B-ALL in children, where the cure rate is up to 80-90% through intensive chemotherapy, adults with B-ALL usually suffer from relapse, especially in high-risk cases (Juliusson G, 2011). The survival rate is poor, approximating only 25 to 40% of adults with ALL (Hoelzer D, 2000). Several factors contribute to the less favorable prognosis for adults with ALL, including a higher rate of therapy- resistant cell immunophenotype, a lower rate of favorable genotypes and more frequent high-risk genetics, as well as a worse response to initial therapy (Hoelzer D, 2016; Hoelzer D, 1988). In addition, older adults also suffer from comorbidities associated with older age that impair the ability to tolerate intensive multi-agent chemotherapeutic regimens that have been used successfully in children. (Rowe JM, 2005). Treatment of ALL involves the use of a combination of chemotherapy agents administered in different phases; induction, consolidation, and maintenance regimens which includes CNS prophylaxis (Storring JM, 2009) Chemotherapy agents include the use of steroids, antracycline, vincristine, asparaginase, cyclophosphamide and methotrexate (Stock W, 2013). Treatment varies according to different protocol adopted such as BFM, GMALL,

UKALL, Hyper-CVAD, with pediatric-inspired protocols being more intensified (Chang JE, 2008; Kantarjian HM, 2000).

Allogeneic HSCT is a potentially curative therapeutic modality for adults with ALL. Allogeneic stem cell transplantation allows considerably higher doses of chemoradiotherapy to be administered, enabling a greater initial myeloablative and leukaemic cell killing effect (Juliusson G, 2011; Oliansky DM, 2012). However, the application of allo-HSCT is one of the most controversial areas of clinical practice in ALL, especially in the early stages of development, where transplant-related mortality could potentially offset the curative effect of allogeneic stem cell transplant (Socie G, 1999). Although the survival outcome of adult ALL who underwent allogeneic stem cell transplant has been widely reported, most of the data was derived from developed regions such as North America and Western European countries (Gupta V, 2013; Howlader N, 2015). Survival data and associated prognostic factors in multiethnic Asian adult ALL patients undergoing allo-HSCT are very scarce. This is especially true for B-ALL, which makes up approximately 75% to 80% of all adult ALL cases (Copelan EA, 1995).

2.2 *BCR-ABL1* in B-Acute Lymphoblastic Leukaemia

The clonal origin of leukaemia was first suggested by the identification of the Ph chromosome in CML. The Ph chromosome, discovered in Philadelphia, USA, in 1960 by Nowell and Hungerford, was the first clonal cytogenetic abnormality (Bain BJ, 2016). The result of this balanced translocation was a new fusion gene that encoded the translation of a tyrosine kinase with increased enzymatic activity. Translocation of the proto-oncogene tyrosine-protein kinase (ABL1) gene located on chromosome 9 to the

breakpoint cluster region (BCR) gene located on chromosome 22 resulted in genomic recombination between BCR, located on the long arm of chromosome 22, and ABL1 is located on the long arm of chromosome 9, resulting in their juxtaposition, which generates the *BCR-ABL1* fusion gene (Zhi-Jie Kang, 2016). The location of the genomic breakpoints of BCR and ABL1 is highly variable, but recombination usually involves fusion of intron 1, intron 13/14 or exon 19 of the 5'end of the BCR with a 140-kb region of the 3'end of the ABL1 gene between exons 1b and 2 (Figure 1). Known as p210*BCR-ABL1*, the fusion of BCR exon 13 and ABL1 exon 2 (e13a2) or e14a2 constitutes the major *BCR-ABL1* transcript (M-BCR, originally referred to as b2a2 and b3a2). Both transcripts result in a hybrid 210 kDa protein. p210 *BCR-ABL1* is most commonly detected in CML and occasionally in ALL or AML. p190*BCR-ABL1* (e1a2) constitutes the minor *BCR-ABL1* transcript (m-BCR), which encodes a hybrid 190-kDa protein. p190BCR-ABL is commonly detected in B-ALL and occasionally in AML but is rarely observed in CML. p230*BCR-ABL1* (e19a2), also known as the µ-*BCR-ABL1* transcript (µ-BCR), encodes a hybrid 230-kDa protein. p230*BCR-ABL1* is generated by fusion of almost the entire BCR gene with the ABL1 gene and is considered a molecular diagnostic marker for neutrophilic-chronic myeloid leukemia (CML-N). The three *BCR-ABL1* protein isoforms; p210, p190, and p230 interfere with normal downstream signalling pathways, causing enhanced cell proliferation, differentiation arrest, and resistance to cell death (Kurzrock R, 1988).

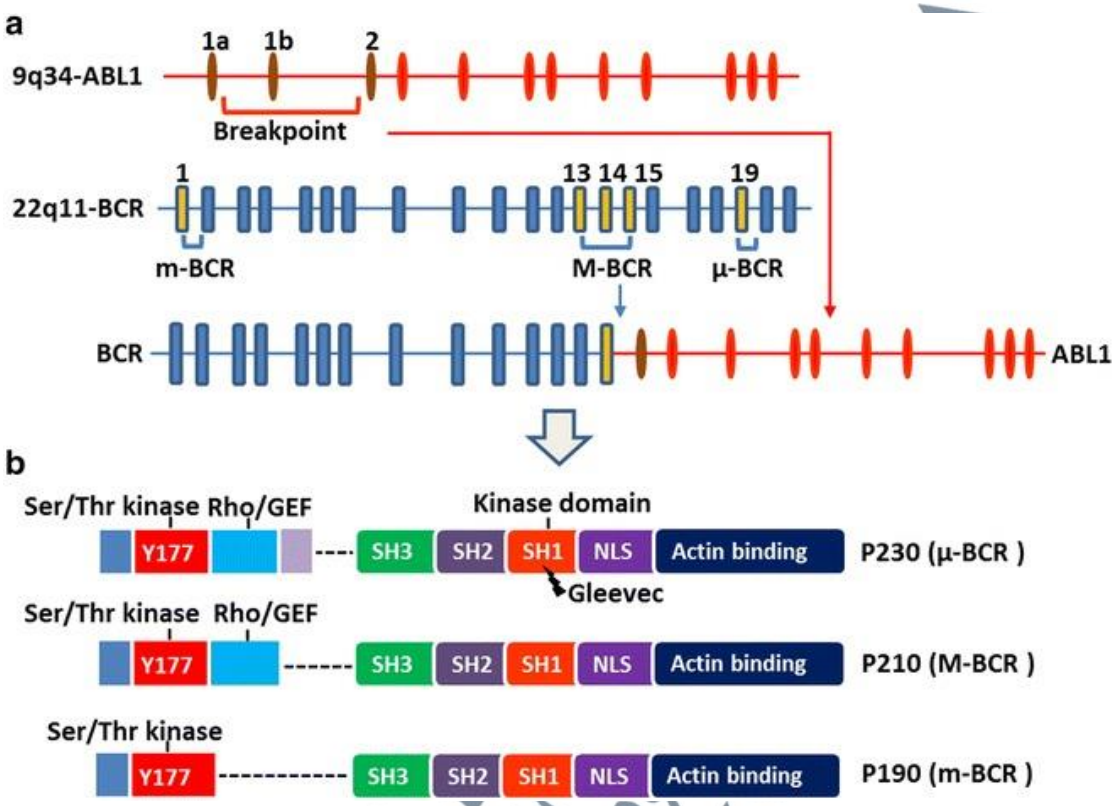

Figure 3: Schematic representation of the structure of the breakpoint cluster region (BCR) proto-oncogene tyrosine protein kinase (*ABL1*) gene and protein. (a) The breakpoints of the BCR and ABL1 genes resulting in the *BCR-ABL1* fusion gene. (b) The different breakpoints that generate the p230, p210, and p190 tyrosine kinase isoforms. (Adapted from: (Zhi-Jie Kang, 2016)

The *BCR-ABL1* protein contains several domains of both BCR and ABL1. BCR domains include an N-terminal coiled coil (CC) domain (amino acids 1–63), a Ser/Thr kinase domain containing a coupling site, also known as phosphorylated tyrosine 177 (Y177) for adaptor protein growth factor receptor bound protein 2 (GRB2), and a ras homolog gene family / guanine nucleotide exchange factors (Rho/GEF) kinase domain (amino acids 298–413) (Maru Y, 1991), whereas the domains from ABL1 include src homology (SH) (SH1 / SH2), a proline rich domain, and DNA- and actin-binding domains (Figure 1b). Although different transcripts encode different proteins, a common feature of all the hybrid proteins (p210/190/230) is the constitutively active

protein kinase activity compared to wild-type ABL1. Most Ph-positive ALL cases express the p190 BCRABL1 transcript. The p210 BCRABL1 transcript is detected in 30% of adult and 20% of childhood patients with Ph-positive ALL. The *BCR-ABL1* variant e3a2 (exon 3 of BCR and exon 2 of ABL1) can also be detected in cases of Ph-positive ALL, which is similar to ALL with the p190 BCRABL1 transcript.

B-ALL with *BCR-ABL1* is a subset of ALL that harbors the genetic alteration of the Ph chromosome and is known as a high-risk disease with a poor prognosis due to shorter duration of remission, higher resistance to standard chemotherapy, and shorter overall survival despite receiving stem cell transplantation (Adele K Fielding, 2009 May 7; Loghavi, 1 September 2015). TKIs targeting the *BCR-ABL1* gene are considered the most successful targeted therapy for Ph-positive leukemia. The Philadelphia chromosome is seen in more than 90% of patients with CML but also 20% of adult ALL, 5% or less of children with ALL and 2% or less of children with AML (Zhi-Jie Kang, 2016) Before the introduction of molecularly targeted therapy, Ph^+ ALL was associated with lower remission rates and a very poor prognosis, with a median survival time of only approximately 9 months (Simona Soverini, 2019) and less than 5% of adults being cured. With the introduction of therapy using TKIs targeted to the aberrant BCR-ABL protein, the outcome of Ph^+ ALL has improved substantially and has been the major treatment advance for adults with ALL in the past two decades. The combination of chemotherapy with TKI, in newly diagnosed Ph- positive ALL, imatinib has a remarkable efficacy, with a complete remission rate of 95% and a patient survival rate of 55% after 3 years.(Zhi-Jie Kang, 2016). The addition of TKI has significantly improved complete remission rate and has led to deeper molecular

responses, as the presence of the gene can be measured by quantitative polymerase chain reaction of the *BCR-ABL1* transcript. This has paved the way for success of ensuing SCT (Deborah A Thomas, 2004 Jun 15; Renato Bassan, 2010). However, post-transplantation relapse, which is a major sign of treatment failure, can still occur.

2.3 Minimal Residual Disease Monitoring in B-ALL with *BCR-ABL1*

The prognostic importance of MRD is used in direct therapeutic decisions, as well as in the determination of high-risk patients. Assessment of MRD assessment has become a standard part of disease monitoring. Prognostication based on response to treatment such as MRD detection has been identified as a discriminatory prognostic assessment to determine which patient should further receive stem cell transplant after chemotherapy (M.Rowe, 2 August 2010). Therefore, proper management of ALL requires a good prognosis assessment as a good risk stratification is important to help decide which patients should receive SCT to improve their clinical outcome.

Several studies have shown that patients with measurable MRD before transplantation will have poorer outcomes compared to those who had a CMR (Merav Bar, 2014 Mar 23). A research group in Italy investigating the prognostic value of the MRD level found that out of 65 patients who underwent HCT, MRD negativity at the time of conditioning was associated with a significant benefit in terms of risk of relapse at 5 years, with a relapse incidence of 8% compared to 39% for patients with MRD positivity ($P = 0.007$) (Federico Lussana, November 01, 2016). Another study looking at 85 patients in the United States found that at 3 months, the achievement of CMR vs. the response less than CMR was associated with longer median overall survival (OS) (127 vs 38 months, respectively; $P = 0.009$) and relapse-free survival (RFS) (126 vs 18 months,

respectively; *P* = 0.007) (Nicholas J. Short, July 28, 2016). Minimal residual disease assessment may vary between chemotherapy regimens with combination of Tyrosine Kinase Inhibitors (TKI) due to their varying potencies and different kinetics of leukemic burden reduction. Designing prospective trials using MRD-based risk stratification to guide the treatment decision for B- ALL with patients with *BCR-ABL1* may elucidate the optimal post remission treatment of these patients.

2.4 Management of B-ALL with *BCR-ABL1*

Treatment of ALL traditionally involves conventional cytotoxic chemotherapy administered in three phases consisting of induction therapy, consolidation therapy and maintenance therapy(Shilpa Paul, November 2016). The goal of these therapies is to help patients achieve complete remission without relapse of the disease. Stem cell transplantation is usually considered in patients who have relapsed after receiving induction therapy or in high-risk patients defined as those with Ph-positive ALL, high white blood cell count, cytogenetic status [t(9;22), t(4;11), −7, +8], CD20 positivity and hypodiploidy(Samer K. Khaled, 2012 Mar; 24(2)). ALL is lethal if left untreated. Despite the fact that recent medical improvements in the treatment of ALL have resulted in improved child survival rates of up to 90%,(Stephen P. Hunger, October 15, 2015), the survival in adults diagnosed with ALL remains poor, with cure rates (otherwise defined as long-term remission) of only 20-40%. (Elias Jabbour, August 1, 2015)

The primary goal of therapy in B-ALL with *BCR-ABL1* is to induce a complete hematologic response (CHR). This means that there is sufficient regenerated haematopoiesis with the following cell counts in peripheral blood: granulocytes

≥1.5x10^9/L, Platelet ≥100x10^9/L. Once a CHR is achieved, an optimal and durable response to MRD is the next major clinical goal, as well as an important determinant of long-term survival. It has been well established that residual disease monitoring in CML has set the stage for routine use of real-time quantitative PCR (qPCR) for response monitoring in Ph-positive ALL. Consolidated CHR patients face the next most important step toward achieving a 'cure', that is, an allogeneic stem cell transplantation (SCT). The chemotherapy regime for B-ALL with *BCR-ABL1* is shown in Table 1.

Table 1: Chemotherapy regimen for B-ALL with *BCR-ABL1* in Hospital Ampang

Chemotherapy	**Regime**
Induction	Modified GMALL 07/2003 protocol (vincristine, daunorubicin, dexamethasone, L-asparaginase, methotrexate, rituximab, GCSF*).
Consolidation	Hyper-CVAD A/B (dexamethasone, vindesine, methotrexate, folinic acid, AraC, VP16, GCSF*, rituximab), the CLAEG regimen(Cladribine, VP16, AraC, GCSF, methotrexate and dexamethasone) and the FLAG-IDA regime (Fludarabine, AraC, Idarubicin, GCSF, methotrexate, dexamethasone). * Patients received a total of 3 to 4 cycles of consolidation chemotherapy before transplantation.
Prophylaxis for central nervous system	At least 4 doses of intrathecal chemotherapy for each patient.
All ALL patients with *BCR-ABL1* were treated with TKI, mainly imatinib, as induction and/or consolidation therapy.	

An allogeneic SCT has long been the only means to reach a cure in a number of Ph+ ALL patients. By improving the rate and duration of the CHR, TKI-based treatments have allowed haematologists to transplant more patients into the first CHR, from both related and unrelated HLA-matched donors. In the TKI era, an allogeneic SCT can be performed in 45–80% of patients with CHR, representing a major contribution to an overall survival of 35-55% between 2 and 5 years, and up to 60–70% among allografted patients (Table 2). These data established the current standard treatment paradigm for adult Ph+ ALL, consisting of a TKI-based induction/consolidation followed by an allograft, however, with a number of caveats. The limitations of allogeneic SCT typically concern patients transplanted in MRD positive status and MRD negativity. Which group of patients is at high risk of post-transplantation relapse remains a good question for evaluation before transplant workup and also further management required. For these reasons, all patients require careful pre- and post-transplantation MRD monitoring for timely reinstitution of TKI therapy or other interventions. For patients older than 50 to 55 years, is allogeneic SCT the best option for them? An increased risk of transplant-related mortality (TRM) remains a major challenge among this group.

Table 2: Long-term results of TKI-based clinical trials for adult Ph+ (patients in complete hematologic remission), with an emphasis on allogeneic stem cell transplantation.

Study (author, ref.)	No. of patients	TKI-based therapy	Treatment outcome (%)						
			General	SCT			No SCT		
				No.	Outcome	TRM	No.	Outcome	*P* (vs. SCT)
Pfeifer (2010)	247	IM/CT	40–50 (OS, 4 years)	180	57 (OS, 3 years) 72 (DFS)	21–26	–	14 (OS, 3 years)	NR
Bassan (2010)	53	IM/CT	38 (OS, 5 years) 39 (DFS)	34	46 (DFS, 5 years)	17	19	30 (OS, 2 years)	0.019 (DFS)
Ribera (2012)	56	IM/CT	37–63 (EFS, 2 years)	32	NR	31	24	NR	NR
Thyagu (2012)	30	IM/CT	53 (OS, 3 years) 50 (EFS)	16	56 (OS, 3 years) 70 (EFS)	37.5	14	50 (OS, 3 years) 45 (EFS, 3 years)	0.34 (OS) 0.51 (DFS)
Fielding (2014)	161	IM/CT	38 (OS, 4 years) 50 (DFS) 33 (EFS)	93	52 (OS, 4 years) 72 (DFS) 49 (EFS)	NR	44	19 (OS, 4 years) 14 (DFS) 14 (EFS)	NR
Chalandon (2015)	254	IM/CT	45.6 (OS, 5 years) 37.1 (EFS)	148	56.7 (OS, 5 years) 48.3 (DFS)	25.8	106	35 (OS, 5 years) 28 (DFS)	0.02 (OS) 0.03 (DFS)
Daver (2015)	39	IM/CT	43 (OS. 5 years) 43 (DFS)	16	63 (DFS, 5 years)	NR	23	43 (DFS, 5 years)	0.52 (DFS)
Lim (2015)	82	IM/CT	39(OS, 5 years) 33 (DFS)	56	53 (OS, 5 years) 43 (DFS)	30	26	NR	NR
Chiaretti (2016)	47	IM/CT	48.8 (OS, 5 years) 45.5 (DFS, 5 years)	23	NR	13	24	NR	0.03 (OS)
Wang (2018)	136	IM/CT	69.2 (OS, 4 years) 61 (DFS)	77	82.6 (OS, 4 years) 71.3 (DFS)	10	56	45.6 (OS-4 years) 43.9 (DFS)	< 0.00 1 (OS, DFS)
Chiaretti (2015)	60	DAS/CT	58 (OS, 3 years) 49 (DFS, 3 years)	NR	NR	NR	NR	NR	NR
Ravandi (2016)	83	DAS/CT	69 (OS, 3 years) 62 (DFS) 55 (EFS)	41	76 (DFS, 3 years)	0	53*	56 (OS, 3 years) 51 (DFS)	0.037 (OS) 0.038 (DFS)
Kim (2015)	82	NIL/CT	72 (OS, 2 years)	57	78 (DFS, 2 years)	19	25	49 (DFS, 2 years)	0.045 (DFS)

Study (author, ref.)	No. of patients	TKI-based therapy	Treatment outcome (%)						
			General	SCT			No SCT		
				No.	Outcome	TRM	No.	Outcome	*P* (vs. SCT)
Ottmann (2018)	68	NIL/CT	47 (OS, 4 years) 42 (EFS)	24	61 (OS, 4 years)	25	44	39 (OS, 4 years)	NS
Jabbour (2018)	76	PON/CT	71 (OS, 5 years) 83 (DFS, 3 years) 67 (EFS)	15	70 (OS, 3 years)	20	61	87 (OS, 3 years)	0.32 (OS)

*Including eight patients with no protocol SCT

Abbreviations OS, overall survival; DFS, disease-free survival; EFS, event-free survival, shown is a long-term estimate at 3+ years (length of follow-up); CT, chemotherapy; IM, Imatinib; TRM, transplant-related mortality; NR, not reported; NS, not significant.

Adapted from: (Simona Soverini, 2019)

The main open questions about TKI-based induction therapy revolve around the issue of whether associated chemotherapy is necessary. If it is necessary, then, in what form? If treatment selection is possible outside of a clinical trial and regardless of local regulations, is there a better TKI to use? The issue of associated chemotherapy requires a careful analysis of clinical trial results, especially in older patients who are at increased risk of early death by infections and bleeding after intensive chemotherapy (Simona Soverini, 2019). This consideration led the Gruppo Italiano Malattie Ematologiche dell'Adulto (GIMEMA) group to test whether a chemotherapy-free schedule, with TKI monotherapy plus corticosteroids, could be as effective, but less toxic than combined intensive induction treatment. The first trial, conducted in the most

critical setting for elderly Ph+ ALL, reached a notable 100% CHR rate in a group of patients with a median age of 69 years. A comparable small randomized trial carried out by the German Multicenter Study Group for Adult ALL (GMALL) provided similar results (no induction deaths) with a low incidence of resistance (4%) (Simona Soverini, 2019) Subsequent GIMEMA studies confirmed the value of monotherapy with imatinib, dasatinib, or imatinib alternating with nilotinib or ponatinib (CHR 95–100%), with only occasional induction of resistance or death and a good toxicity profile in all age groups, including unfit patients (Simona Soverini, 2019)

A related point of concern is the management of patients who exhibit high-risk characteristics and / or are unable to undergo a subsequent allotransplantation. For these cases, intensive chemotherapy/TKI associations may still be recommended. The question of which TKI to choose is a difficult one due to the comparably high CHR rates reported for all regimens, including simple imatinib monotherapy. However, many of the patients who exhibited resistance to TKI in these studies were found to have *BCR-ABL1* kinase domain mutations. In addition, we should consider the effects of induction and early consolidation therapy with different TKIs and chemotherapy combinations on the next most important step after CHR: the achievement of a complete or major molecular response (CMR), which is an MRD negative CHR.

2.5 Gap Analysis

The decision for allogeneic HCT has always been based on conventional ALL risk factors (e.g. white cell count, Philadelphia chromosome positive, CR>1). Recently, the monitoring of MRD and various genetic markers has been incorporated into the decision-making prior to allogeneic HCT. In a resource-limited setting such as

Malaysia, we are unable to stratify our adult patients precisely due to the inability to cover all adverse molecular markers and perform vigorous MRD monitoring, hence with the availability of a matched sibling donor, allo-HSCT is performed in both standard and high-risk patients. Allogeneic HCT is still considered a relatively affordable procedure with acceptable treatment-related mortality as opposed to the financial burden imposed by various novel investigational tools and immunotherapies