BCR-ABL1 POSITIVE ACUTE LYMPHOBLASTIC LEUKAEMIA IN GHANAIAN PATIENTS

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THIS THESIS IS SUBMITTED TO THE UNIVERSITY OF GHANA, LEGON IN PARTIAL FULFILMENT OF THE REQUIREMENT FOR THE AWARD OF MPHIL HAEMATOLOGY DEGREE

DEPARTMENT OF HAEMATOLOGY

SCHOOL OF BIOMEDICAL AND ALLIED HEALTH SCIENCES

COLLEGE OF HEALTH SCIENCES

JULY 2018
DECLARATION

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DEDICATION

I dedicate this project to Almighty God for being my source of strength and wisdom and also to my dear mother, Vida Obeng Addai for her inspiration and support. I also dedicate this work to my dear wife, Rebecca Abora and my lovely daughter, Vida Obeng Addai Ofori.
ACKNOWLEDGEMENT

I would like to express my profound gratitude to Professor Susan Crocker, Department of Pathology and Molecular Medicine at Queens University, Canada. Professor Crocker made this work possible by arranging for me to visit her laboratory at Queens to carry out the benchwork and reviewed this work. I would also like to thank Brooke Ring-Snetsinger of Queens Laboratory for Molecular Pathology (QLMP) for her patience and the time she devoted to train me on the fluorescent in-situ hybridisation technique. I am also grateful to Shakeel Virk, Andy Zhang and all the other staff and students of QLMP for their assistance.

I wish to give special thanks to my supervisors Dr. Amma Anima Benneh and Dr. Edeghonghon Olayemi of Haematology Department (University of Ghana) for their supervision and counseling which has enabled me complete this work. I would also like to thank the other lecturers in the Department (Prof. J. K. Acquaye and Dr. Yvonne Dei-Adomako) for their advice and encouragement. To Francisco Torto and all the administrative and technical staff in the Haematology department who assisted me in various ways, I thank you. Many thanks to the staff of the Central laboratory (Haematology Special) and Haematology Day care unit of the Korle Bu Teaching Hospital.

I express my appreciation to Dr. Tom Ndanu (School of Medicine and Dentistry, University of Ghana) for his assistance in the statistical aspect of the work. Francis Krampa, Alice Charwudzi and all those who assisted me in diverse ways, I duly acknowledge your efforts.

Finally, many thanks to Brosaman Company Ltd and Achimota Mile 7 Church of Christ who supported me with funds to be able to carry out this work.
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ABBREVIATIONS

ABL1 – Abelson murine leukaemia viral oncogene homolog 1

AKT – Ak strain transforming

ALL - Acute lymphoblastic leukaemia

ATCC® - American Type Culture Collection

BCR - Breakpoint cluster region

BCR-ABL1 - Breakpoint cluster region - Abelson murine leukaemia viral oncogene homolog 1

CDKN2A – Cyclin-dependent kinase inhibitor 2A gene

DAPI - 4,6 – Diamidino-2- Phenylindole, Dihydrochloride)

CDKN2B - Cyclin-dependent kinase inhibitor 2B gene

DNA – Deoxyribonucleic acid

E2A – Transcription factor *E2-alpha* gene

EBFI - Early B-cell factor 1 gene

FISH – Fluorescence in situ hybridisation

GST T1- Glutathione S transferase theta 1 gene

ETV6-RUNX1 -ETS-variant 6 -runt-related transcription factor 1 gene

Hb - Haemoglobin

IL-7R – Interleukin-7 receptor
**IKZF1** - Ikaros family zinc finger 1 gene

**JAK** – Janus kinase

**KBTH** - Korle Bu Teaching Hospital

**MAPK** - Mitogen-activated protein kinase

**MD** - Monroe Dunaway

**mTor** – Mammalian target of rapamycin

**MTR – 5** – methyl tetrahydrofolate-homocysteine methyltransferase gene

**MW** - Mann-Whitney

**MLL-AF4** - Mixed-lineage leukaemia -ALL-1 fused gene on chromosome 4

**PAX5** - Paired box 5 gene

**PBS** – Phosphate buffered saline

**PI3** – Phosphatidylinositol -4,5- bisphosphate 3 - kinase

**Plt** – Platelet

**RAS** - Rat sarcoma

**SSC** – Saline-sodium citrate

**STAT** – Signal transducer and activator of transcription proteins

**WBC** - White blood cell
ABSTRACT

Background: Acute lymphoblastic leukaemia (ALL) is the accumulation of lymphoblasts in the bone marrow as a result of malignant transformation resulting in proliferation of immature lymphoid progenitors or lymphoblasts. It results from perturbation of several genetic loci. The chimeric BCR-ABL1 gene fusion is one of such genetic alterations. Even though the presence of BCR-ABL1 has been associated with poor prognosis, the incorporation of tyrosine kinase inhibitors in treatment protocols has been shown to be of enormous benefit. In Ghana as well as most African countries, research work on its prevalence and clinical associations is limited.

Aim: To determine the frequency and associated laboratory and clinical features of the chimeric BCR-ABL1 gene in patients diagnosed with acute lymphoblastic leukaemia at the Department of Haematology, Korle Bu Teaching Hospital (KBTH).

Methods: This is retrospective cross-sectional study. Methanol-fixed archived bone marrow aspirate slides of patients diagnosed with ALL at the Department of Haematology, KBTH were retrieved. Data on clinical features (signs) and haematological parameters was obtained from the patients’ medical records. The presence of the chimeric BCR-ABL1 fusion gene was determined on the bone marrow aspirate slides by fluorescent in situ hybridization (FISH).

Results: A total of 17 cases were studied of which 13 (76.5%) were males and 4 (23.5%) were females. The ages of the participants ranged from 15 to 67 years ((mean = 31.5 years, SD = 16.9 years). A frequency of 29.4% was obtained for the BCR-ABL1 fusion gene. Of the clinical features studied, lymphadenopathy was present in 7 (40%) of study cases whereas splenomegaly and hepatomegaly were present in 4 (23.5%) and 5 (35.7%) respectively. No significant association was established between BCR-ABL1 positivity and these clinical features. All
subjects had severe to moderate anaemia with haemoglobin concentration ranging from 3.7 to 8.7g/dL. The mean haemoglobin concentration for \textit{BCR-ABL1} positive cases was higher than that of the negative cases (7.26 versus 6.62 g/dL respectively), however, statistical significance was not reached (P = 0.506). The mean white blood cell count, bone marrow blast percentages and platelet counts were lower in \textit{BCR-ABL1} positive cases than in the negative cases (36.83, 73.00 and 54.60 ×10⁹/L versus 73.53, 82.18 and 74.33×10⁹/L respectively) although no significant association was established between these haematological parameters and \textit{BCR-ABL1} positivity (P = 0.879, 0.721 and 0.506 respectively). Also, there was no statistically significant difference in clinical outcome between the \textit{BCR-ABL1} positive and negative cases.

\textbf{Conclusion:} The \textit{BCR-ABL1} fusion gene is present in nearly one third of adult acute lymphoblastic leukaemia cases seen in this study and has no significant association with the clinical features and haematological parameters of the disease. A larger study will be needed to make a decision with regard to the modification of treatment regimen for adult \textit{BCR-ABL1} positive ALL.
CHAPTER ONE

INTRODUCTION

1.1 Background

Acute lymphoblastic leukaemia (ALL) is the accumulation of lymphoblasts in the bone marrow as a result of malignant transformation resulting in proliferation of immature lymphoid progenitors or lymphoblasts (Ahmed, Dawson, Smith, & Wood, 2006). It arises from several kinds of genetic alterations which affect haemopoietic stem cells, early progenitor cells or genes that regulate the growth and differentiation of lymphoid cells (Nagarajan, 2010; Rose, 2013). Among these mutations is the ETV6-RUNXI and hyperdiploidy which are generally associated with good prognosis and hypodiploidy, MLL-AF4 and the BCR-ABL1 which are associated with poor prognosis (Mrozek, Harper, & Aplan, 2009). The BCR-ABL1 fusion gene results from translocations involving chromosomes 9 and 22 and gives rise to BCR-ABL1 positive ALL (Fainstein et al., 1987). Even though this mutation has been associated with poor prognosis, the addition of tyrosine kinase inhibitors such as imatinib, nilotinib or dasatinib to treatment protocols has been shown to produce improved haematologic and cytogenetic remission rates (Ottmann et al., 2007). This study is aimed at obtaining the frequency and associated clinical features of the BCR-ABL1 fusion mutation among patients diagnosed with acute lymphoblastic leukaemia at the Korle Bu Teaching Hospital (KBTH).

Acute lymphoblastic leukaemia has an estimated global incidence of 1 to 4.75 per 100,000 people (Redaelli, Laskin, Stephens, Botteman, & Pashos, 2005). Acute Lymphoblastic leukaemia accounts for about 20% of leukaemias in adults and 80% of childhood acute leukaemias making it the most common leukaemia in children. (Jabbour, O'Brien, Konopleva, & Kantarjian, 2015).
It has been shown to have the highest frequency of nearly 34% among all leukaemias diagnosed in the Korle Bu Teaching Hospital in Ghana (Ekem & Dei-Adomako, 2015). A frequency of 16.9% was obtained in a study carried out in Nigeria (Damulak, Egesie, Jatau, Ogbenna, & Adediran, 2017). Furthermore, the findings of a study conducted at the Komfo Anokye Teaching Hospital in Ghana revealed that ALL accounted for 10% of childhood cancers, the second most common after Burkitt’s Lymphoma (Painstil et al., 2015).

The chimeric $BCR$-$ABL1$ gene mutation occurs at varying frequencies in ALL in the range of 1-5% and 11-29% in pediatric and adult cases respectively (Mrozek et al., 2009). The prevalence of $BCR$-$ABL1$ gene fusion in adult ALL cases from a multicenter study involving five cancer groups which includes the Cancer and Leukaemia group B and the MD Anderson Cancer Center is 20% whereas 15% was obtained in a population-based study of adult ALL cases in the United Kingdom (Anthony V Moorman, Chilton, et al., 2010; Roberts et al., 2015). In a study conducted in the South-western area of the Cape Province of South Africa, 9% of the patients diagnosed with ALL were blacks whereas 43% and 48% were of mixed ancestry (coloured) and white respectively (Jacobs, 1985). To date, no further studies have investigated the incidence of $BCR$-$ABL1$ fusion in this population and many other regions in Africa resulting in a paucity of information.

The cumulative mortality of 5-year survivors of childhood ALL at 25 years after diagnosis has been shown to be 13% in a cohort study conducted in United States and Canada. (Mody et al., 2008). The overall 5-year survival of newly diagnosed cases of adult ALL is 38% whereas 7% was observed for relapsed cases (Fielding et al., 2007).
Early allogenic bone marrow transplantation has been associated with improved overall survival in adult ALL cases particularly those associated with poor prognosis including those having the $BCR-ABL1$ gene fusion ($75\%$ 6-year overall survival compared with $41\%$ for delayed autologous stem cell transplantations) (Hunault et al., 2004). Furthermore, the incorporation of tyrosine kinase inhibitors to therapeutic protocols has been associated with improved survival in $BCR-ABL1$ positive ALL (Brissot et al., 2015; Fielding et al., 2014).

1.2 Problem statement

Acute lymphoblastic leukaemia is the accumulation of lymphoblasts in the bone marrow secondary to mutations in lymphoid stem cells (Ahmed et al., 2006; Hoffbrand & Moss, 2015). The chimeric $BCR-ABL1$ fusion gene which is the molecular equivalent of the Philadelphia chromosome results from translocation of the $ABL$ cellular oncogene on chromosome 9 to the $BCR$ gene on chromosome 22 (Mullighan, 2012). This results in the synthesis of either a 210kD or 190kD protein with enhanced tyrosine kinase activity compared with the normal 145kD protein (Hoffman et al., 2012; Kumar, Abbas, Fausto, & Aster, 2009).

The $BCR-ABL1$ fusion gene has been implicated as poor prognostic indicator in adult as well as childhood ALL as it has been associated with decreased overall and event free survival rates (Fletcher et al., 1991; Pullarkat, Slovak, Kopecky, Forman, & Appelbaum, 2008). Its frequency varies across different populations. Research on its frequency and clinical associations in Africa is limited and none has been carried out in Ghana. Since the prevalence of this mutant gene in ALL and its association with clinical features is unknown in Ghana, patients diagnosed with ALL are not routinely screened for its presence as it is costly. Treatment regimen is therefore not
adjusted for those who may be positive for the mutation even though tyrosine kinase inhibitors (TKIs) may be of enormous benefit to such patients and are available in Ghana.

Acute lymphoblastic leukaemia has an estimated global incidence of 1 to 4.75 per 100,000 people (Redaelli et al., 2005). The chimeric BCR-ABL1 gene mutation occurs at varying frequencies in ALL in the range of 1-5% and 11-29% in pediatric and adult cases respectively (Mrozek et al., 2009).

In a study conducted in the South-western area of the Cape Province of South Africa, 9% of the patients diagnosed with ALL were blacks whereas 43% and 48% were of mixed ancestry and white respectively (Jacobs, 1985). To date, no further studies have investigated the incidence of BCR-ABL1 fusion in this population and many other regions in Africa. Therefore, the prevalence and the associated clinical signs and haematological parameters of this genetic alteration in patients diagnosed with acute lymphoblastic leukaemia in Ghana are not known.

The overall 5-year survival of newly diagnosed cases of adult ALL is 38% whereas 7% was observed for relapsed cases (Fielding et al., 2007). The incorporation of tyrosine kinase inhibitors to therapeutic protocols has been associated with improved survival in BCR-ABL1 positive ALL (Brissot et al., 2015; Fielding et al., 2014).

1.3 Justification

Optimum outcome is achieved if treatment modalities in ALL is tailored according to the cytogenetic and/or molecular genetic abnormalities present since they are associated with different prognosis (Cario et al., 2005; Anthony V Moorman, Ensor, et al., 2010; Slovak et al., 2000). This study will provide information on the prevalence as well as associated clinical features and haematological parameters of the BCR-ABL1 fusion mutation among patients
diagnosed with ALL at the Department of Haematology, KBTH. The findings of this study may serve as the basis for informed decision making with regards to screening for $BCR-ABL1$ gene as part of the panel of tests for ALL patients and the establishment of the proper treatment protocol for the $BCR-ABL1$ positive cases thereby improving patient outcomes.

1.4 Aim

To determine the frequency and the associated laboratory and clinical features of the chimeric $BCR-ABL1$ gene in patients diagnosed with Acute lymphoblastic leukaemia at the Department of Haematology, Korle Bu Teaching Hospital (KBTH).

1.5 Specific Objectives

1. To determine the frequency of the chimeric $BCR-ABL1$ gene fusion in adult ALL patients seen at the Haematology Department, Korle Bu Teaching Hospital (KBTH).

2. To determine the association between BCR – ABL1 positivity and clinical features of adult ALL patients.

3. To determine the association between BCR – ABL1 positivity and haematological parameters (white blood cell counts, platelet counts, haemoglobin concentrations and bone marrow blast percentage) of adult ALL patients.

4. To determine association between $BCR-ABL1$ positivity and treatment outcome of adult ALL patients.
CHAPTER TWO
LITERATURE REVIEW

2.1 Definitions

Acute lymphoblastic leukaemia (ALL) is the accumulation of lymphoblasts in the bone marrow secondary to mutations in lymphoid stem cells (Ahmed et al., 2006; Hoffbrand & Moss, 2015). It is characterized by the presence of more than 20% leukaemic blasts in the bone marrow at clinical presentation although a lesser percentage is also definitive if specific leukaemic cytogenetic or molecular genetic abnormalities are present (Hoffbrand & Moss, 2015).

Among the genetic mutations implicated in the development of ALL is the chimeric \textit{BCR-ABL1} fusion gene which is the molecular equivalent of the Philadelphia chromosome which results from translocation of the ABL cellular oncogene on chromosome 9 to the BCR gene on chromosome 22 (Shtivelman, Lifshitz, Gale, & Canaani, 1985). This results in the synthesis of either a 190kD or 210kD protein with enhanced tyrosine kinase activity compared with the normal 145kD protein (Hoffman et al., 2012; Kumar et al., 2009). The p190 is the more common in ALL than the p210. The frequency of the p190 is in the region 50 to 70% and 80% for adult and pediatric cases respectively may occur simultaneously with the p210 in up to 19% of \textit{BCR-ABL1} positive ALL (Hoffman et al., 2013). The incidence of the \textit{BCR-ABL1} gene has been shown to vary across different ethnic groups in a study conducted in childhood ALL (Ariffin et al., 2007).
2.2 Classification

Acute lymphoblastic leukaemia may be either of a B-cell or a T-cell lineage. About 85% of the cases are B cell with equal incidence in both sexes whereas the remaining 15% has male predominance and originate from the T cell. (Hoffbrand & Moss, 2015) The table below shows the detailed immunophenotypic classification. The determination of the immunophenotype usually by flow cytometry aids in the establishment of diagnosis, monitoring response to therapy and prognostic assessment (Alves et al., 2012).

*Table 1: IMMUNOPHENOTYPIC CLASSIFICATION OF ACUTE LYMPHOBLASTIC LEUKAEMIA*

<table>
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<tr>
<th>Early Pre-B</th>
<th>Pre-B</th>
<th>Mature B</th>
<th>Pro-T</th>
<th>Pre-T</th>
<th>Cortical T</th>
<th>Mature T</th>
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<td>+</td>
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Some myeloid associated cluster of differentiation (CD) antigens are aberrantly expressed in ALL especially in $BCR-ABL1$ positive cases. These markers which include CD13, CD33, CD14 and CD15 are good indicators of the presence of leukaemic blasts and minimal residual disease although they have no prognostic importance (Stefan Faderl et al., 2010).

Acute lymphoblastic leukaemia may be classified as $BCR-ABL1$ positive or negative (the molecular counterpart of Philadelphia positive or negative ALL) based on the results of $BCR-ABL1$ testing whether positive or negative respectively and this is of prognostic significance (Nashed, Rao, & Gulley, 2003; Terwilliger & Abdul-Hay, 2017).

### 2.3 Epidemiology

Acute Lymphoblastic leukaemia occurs in both pediatric and adult populations with peak incidence at the age of 2 to 5 years (Hiroto Inaba, Mel Greaves, & Charles G. Mullighan, 2013; Levy, 2010). The second peak incidence occurs in adults over 50 years (Lysaght et al., 2013). It has an estimated global incidence of 1 to 4.75 per 100,000 people (Redaelli et al., 2005). It has been shown to have the highest frequency of nearly 34% among all leukaemias diagnosed in the Korle Bu Teaching Hospital in Ghana (Ekem & Dei-Adomako, 2015). About 60,000 new cases are recorded annually in the United States of America with a male to female ratio of 1.3:1 (Hiroto Inaba et al., 2013).

The chimeric $BCR-ABL1$ gene mutation occurs at varying frequencies in ALL in the range of 1-5% and 11-29% in pediatric and adult cases respectively (Mrozek et al., 2009). A frequency of 42% to 44% beyond age 44 has been reported in Germany (T. Burmeister et al., 2008). It was detected in 22% of the 18 cases of childhood ALL that were studied in Sudan (Siddiqui et al.,
2010). Also, 12.5\% of the 40 cases of childhood and adult ALL cases in a study conducted in Nigeria were $BCR-ABL1$ positive (Ajuba et al., 2016). A prevalence of 28.3\% was recorded in one study in India (Chopra et al., 2015).

In a study carried out in Saudi Arabia, none of the 16 cases of adult ALL investigated was positive for the $BCR-ABL1$ fusion gene (El-Sissy, El-Mashari, Bassuni, & EL-SWAAYED, 2006).

In one study, no significant association was found to exist between $BCR-ABL1$ positivity and age as well as gender (Hamid & Bokharai, 2017).

The two main types of the $BCR-ABL1$ fusion transcripts (p190 and p210) occur in ALL but the p190 is more prevalent (Cimino et al., 2006; Gleißner et al., 2002). In a study of 56 adult ALL cases in the United States of America by the Cancer and Leukaemia Group B (CALGB), the p190 variant accounted for 77\% of the cases whereas the p210 was detected in the remaining 23\% (Westbrook et al., 1992).

### 2.4 Aetiology

The acute lymphoblastic leukaemias arise from genetic mutations in the haemopoietic cells or early progenitor cells (Rose, 2013). The mutations result in malignant transformations of these cells by three mechanisms which include the following:

I) Enhanced rate of self-renewal and proliferation;

II) Impaired apoptosis resulting in survival advantage and

III) arrest of cellular differentiation (Hoffbrand & Moss, 2015).
These events result in overproduction of lymphoblasts which accumulate in the bone marrow and replace the normal cells as well as spill over into blood and infiltrate organs (Rose, 2013).

Also, mutations in the genes that regulate the growth and differentiation of lymphoid cells resulting in abnormal production of their respective proteins have also been implicated in the pathogenesis of acute lymphoblastic leukaemia (Nagarajan, 2010).

2.5 Cytogenetics and Molecular genetics

Some of the genetic alterations found in ALL include altered chromosome number (hyer diploidy and hypodiploidy), translocations which include t(12,21), t(4,11) and t(9,22) which give rise to ETV6-RUNX1, MLL-AF4 and the BCR-ABL1 fusion genes respectively (Mrozek et al., 2009). In order for ALL to be manifested, these chromosomal abnormalities often act in tandem with some other genetic lesions which include deletions or silencing of CDKN2A gene, alterations in the PAX5 gene and deletional changes affecting the E2A, EBF1 and IKZF family genes (Pui, Robison, & Look, 2008). Of these, PAX5 is the most common mutated gene and occurs in nearly 32% of cases (Mullighan et al., 2007).

IKZF1 gene deletion mutations have been shown to play major contributory role in the development of BCR-ABL1 positive acute lymphoblastic leukaemia (I. Iacobucci et al., 2009). Also, PAX5, CDKN2A/ARF and CDKN2B deletions have been associated with BCR-ABL1 positive ALL (Iacobucci et al., 2011).

The exact causes of the genetic mutation in most cases of ALL are not well elucidated; however, certain inherited and environment factors have been implicated in the aetiogenesis of these
events as they have been associated with increased risk of development of ALL and are discussed below (Chang et al., 2007).

In a higher proportion of cases, the disease arises spontaneously in otherwise healthy individuals without any predisposing factors (Terwilliger & Abdul-Hay, 2017)

### 2.6 Risk Factors

The risk factors are both inherited and environmental and include the following:

#### 2.6.1 Inherited factors

The inherited genetic disorders that increases the predisposition to the development of ALL are Down’s syndrome, Klinefelter’s syndrome, Fanconi’s anaemia, Bloom’s syndrome and ataxia-telangiectasia (Chang et al., 2007; Pui et al., 2008). These risk factors are associated with only a minority of cases (less than 5%) except in children with Down’s syndrome in which the risk is increased to about 40-fold below age five (H. Inaba, M. Greaves, & C. G. Mullighan, 2013; Paul, Kantarjian, & Jabbour, 2016).

Even though ALL in Down’s syndrome (trisomy 21) has not been associated with any cytogenetic abnormality, the over-expression of proto-oncogenes due to the extra 21st chromosome may account for the leukaemic transformations (Chang et al., 2007; Harris, 2015).

Fanconi’s anaemia, Bloom’s syndrome and ataxia-telangiectasia are associated with increased chromosomal instability which increases the risk of developing ALL (Chang et al., 2007).

The initiating mutation events (first hit) in childhood ALL occur in utero or early infancy during which lymphocyte expansion and recombinase activity are climactic whereas the promotional
mutation (second hit) which occurs afterwards and result in clinical manifestation of ALL is as a result of exaggerated response of the immune system to exogenous antigens resulting in over-proliferation of lymphoid cells (Bope & Kellerman, 2011).

2.62 Environmental risk factors

Exposure to certain drugs such as ALLN (N-acetyl-Leu-Leu-Norleu-al), alkylating agents and topoisomerase II inhibitors such as etoposide and epipodophyllotoxins have been associated with increased risk of ALL development (Appelbaum, Forman, Negrin, & Blume, 2011).

Chemicals such as benzene, pesticides, automobile exhaust, parental alcohol consumption and cigarette smoking have been proposed as etiologic risk factors and are being investigated (Estey, Faderl, & Kantarjian, 2007).

Also, in utero and post-natal exposure to radiation from atomic bombs (atomic bomb survivors of Hiroshima and Nagasaki), nuclear explosions and medical treatment has been implicated in the aetiology of ALL (Cullings, 2014; Weiner & Cairo, 2002). Even though diagnostic X-rays have not been associated with ALL development, intrauterine foetal exposures have been linked with increased risk of ALL in childhood (Vokes & Golomb, 2011). Low-frequency electromagnetic field has not been shown to pose any risk (Weiner & Cairo, 2002).

Even though no specific infectious agent has been implicated in the aetiogenesis of ALL, the second hit mutations resulting from abnormal immune response to infectious agents in infants who were not exposed to common infections in the first years of life has been linked with increased risk of ALL (Hayat, 2013; Pui et al., 2008).
Increased birth weight and advanced maternal age are have also been suggested as risk factors but with little evidence (Weiner & Cairo, 2002). Neonatal vitamin K administration and increased maternal intake of diets rich in nitrites have also been proposed (Pui & Evans, 1998). Even though an association was found between maternal exposure to second-hand tobacco smoking during pregnancy as well as childhood exposures and ALL, none was observed for maternal smoking and paternal smoking during pregnancy (Farioli et al., 2014).

2.7 Pathogenesis

The exact pathogenetic mechanism by which ALL occurs is not known as only a minority of cases have been directly linked to risk factors. Among the proposed mechanisms include a pathological exaggerated immune response after exposure of non-immune individuals with prenatal leukaemia cell lines to common environmental pathogens; influenza viruses have been implicated in childhood ALL (Greaves, 2018; Kroll, Draper, Stiller, & Murphy, 2006).

Polymorphism and alterations of genes involved in some metabolic and cell-signalling pathways are believed to play a role in ALL pathogenesis. The polymorphic expression of the GST T1 null variant of the glutathione S- transferase family of genes has been associated with increased development of adult ALL (Rollinson et al., 2000). This most likely results from DNA damage arising from decreased detoxification of carcinogens and removal of reactive oxygen species which are key functions of the gene (Singh & Michael, 2009).

The MTR 2756GG variant of the methionine synthase gene (MTR 2756 A>G) involved in the transfer of single carbon atoms in DNA methylation has been associated with the development of acute lymphoblastic leukaemia (Lightfoot et al., 2010).
Genetic alterations resulting in enhanced kinase signalling such as those involving the PI3K/AKT/mTOR, IL-7R/JAK/STAT and RAS/MAPK pathways have been associated with the leukaemogenesis of T-ALL (Bongiovanni, Saccomani, & Piovan, 2017).

2.8 Clinical features and laboratory findings

Clinical features include anaemia, thrombocytopenia and neutropenia which is as a result of bone marrow failure, as well as organ infiltration which manifests as lymphadenopathy, moderate splenomegaly, hepatomegaly and meningeal syndrome (Ferri, 2015). Mediastinal mass also occurs in ALL but has been associated with T-lineage rather than B-lineage ALL (Rossi et al., 1993). Constitutional symptoms such as fever, weight loss and night sweats as well as easy bruising, dyspnoea, fatigue and infection emanating from decreased blood cell counts are the most prevalent symptoms in ALL (Terwilliger & Abdul-Hay, 2017).

In a study conducted in Egypt, lymphadenopathy, splenomegaly and hepatomegaly occurred at frequencies of 58, 58 and 54% respectively in adult ALL (Elbossaty, 2017). However, a combined frequency of 40% was obtained in another study of Adult ALL in Netherlands (Daenen et al., 1998).

BCR-ABL1 positive ALL cases have been linked with higher white cell count and haemoglobin levels as compared to BCR-ABL1 negative cases. There is a similar incidence of splenomegaly, lymphadenopathy, mediastinal mass and hepatomegaly (Gleißner et al., 2002; Westbrook et al., 1992). However, no statistically significant difference was established between haemoglobin and white blood cell counts of BCR-ABL1 positive and negative cases (Westbrook et al., 1992). Also, lower platelet counts (P=0.07) and higher peripheral blood blast counts (P=0.02) have
been linked with the *BCR-ABL1* positive ALL compared with the negative cases (Pullarkat et al., 2008).

### 2.9 Laboratory Diagnosis

The initial laboratory tests to be carried out include complete blood count and examination of Romanowsky stained peripheral blood and bone marrow aspirate slides for the presence of increased numbers of lymphoblasts exceeding 20% of nucleated cells (Theml & Diem, 2011). The lineage of the lymphoblasts as well as stage of maturation is confirmed by immunophenotyping usually by flow cytometric detection for the expression of cluster of differentiation marker antigens. The use of cytochemical stains such as periodic acid Schiff reagent may be used to different ALL from acute myeloid leukaemia (Sun, 2012).

The next stage of diagnosis involves karyotyping in which chromosomes are examined for gross genetic changes such as deletions and translocations which include the t(9,22) (Ludwig & Thiel, 2012). The determination of the specific genetic lesions involved in these gross chromosomal changes such as the *BCR-ABL1* gene for t(9,22) may be carried out using molecular cytogenetic tests such as fluorescent in situ hybridisation or molecular genetic tests such as next generation sequencing, microarray analysis and polymerase chain reaction-based techniques (Leonard, 2016).

Typical fluorescent in situ hybridisation signal patterns for detection of *BCR-ABL1* fusion gene include 2 fusion signals, 1 red and 1 green, whereas one fusion signal is observed in deletions involving both the BCR and ABL genes; normal signals appear as 2 red and 2 green dots (Jain et al., 2012).
2.10 Treatment

The treatment of ALL is achieved with chemotherapy involving mainly 4 stages namely induction, consolidation, central nervous system prophylaxis and maintenance therapy (S. Faderl & Kantarjian, 2011). The table below shows the various stages and the drugs that may be used.

Table 2: DRUGS USED IN THE TREATMENT OF ACUTE LYMPHOBLASTIC LEUKAEMIA

<table>
<thead>
<tr>
<th>Remission Induction</th>
<th>Consolidation</th>
<th>CNS Prophylaxis</th>
<th>Maintenance</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>vincristine</td>
<td>Daunorubicin</td>
<td>Intrathecal</td>
<td>Mercaptopurine</td>
<td>(Hoffbrand &amp; Moss, 2015).</td>
</tr>
<tr>
<td>anthracycline</td>
<td>Cytarabine</td>
<td>methotrexate</td>
<td>Vincristine</td>
<td>(Florin, Ludwig, Aronson, &amp; Werner, 2011)</td>
</tr>
<tr>
<td>asparaginase and</td>
<td>Vincristine</td>
<td></td>
<td>methotrexate</td>
<td></td>
</tr>
<tr>
<td>cyclophosphamide</td>
<td>Etoposide</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>(optional)</td>
<td>mitoxantrone</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

The drugs in the table above are used in various combinations and cycles with age, gender and leucocyte counts at the onset of the disease being key determinants of treatment modalities. Treatment may last between 2 and 3 years and the intensity of the regimen, duration and whether or not stem cell transplantation will be carried out depends on the specific subtype and the risk of relapse (Kaye & Kaye, 2004). High intensity regimen are used for pediatric and young adults as well as high risk factors such as presenting white blood cell counts exceeding \(50 \times 10^9/\text{uL}\) whereas low doses are used for older adults (Rabin & Poplack, 2011).

The addition of tyrosine kinase inhibitors such as imatinib to treatment protocols in \(BCR-ABL1\) positive ALL has been associated with increased overall and disease-free survival rates (Bassan et al., 2010; Schultz et al., 2009).
2.11 Prognosis

The prognosis of ALL is poorer in adults than children and adolescents with 5-year survival rates of 40% and 90% respectively (Hunger et al., 2012; Pulte et al., 2014).

Even though *BCR-ABL1* positive and negative cases of ALL have been shown to have similar remission rates, more early relapses have been associated with *BCR-ABL1* positive ALL (Westbrook et al., 1992). Secondly, in a Cancer and Leukaemia Group B study in the United States of America, the median survival duration in *BCR-ABL1* positive and negative ALL was 11.2 and 21.8 months respectively but was statistically insignificant (p-value 0.26) (Westbrook et al., 1992). In a multicenter study conducted in Germany, the 3-year survival probability in *BCR-ABL1* positive versus negative ALL was 0.19 (+/-0.04SE) and 0.55(+/-0.04SE) respectively (p=0.0001) (Gleißner et al., 2002). Also, the *BCR-ABL1* positive ALL has been significantly associated with decreased overall and 5-year event-free survival compared to negative cases (A. V. Moorman et al., 2007). However, in an Italian based study, the expression of the *PAX5* wild-type without *IKZF1* deletion in *BCR-ABL1* positive ALL has been associated with good prognosis (prolonged disease-free survival and low incidence of relapse) compared with cases expressing normal *PAX5* and *IKZF1* deletion (Ilaria Iacobucci et al., 2009).

In a study conducted in Nigeria, no association was found between *BCR-ABL1* positivity and white cell count (p = 0.416) (Ajuba et al., 2016) even though higher WBC count is associated with ALL and considered a poor prognostic factor (Advani & Lazarus, 2010; S. Faderl & Kantarjian, 2011). In a south African based study of a heterogenous ethnic pediatric population, no significant statistical association was found between 5-year event free survival rate and clinical features (hepatomegaly, splenomegaly and mediastinal mass) as well as
immunophenotype (p = 0.9, 0.38, 0.41 and 0.87 respectively) (Wessels, Hesseling, Buurman, Oud, & Nel, 1997).

2.12 Monitoring

The detection of BCR-ABL1 rearrangement has been proven to provide significant guidance in the diagnosis, prognosis, monitoring of response to treatment and drug resistance in acute lymphoblastic leukaemia (Jiang et al., 2016). The detection of BCR-ABL1 transcripts by the polymerase chain reaction technique has been proven to be useful in the detection of minimal residual disease in Philadelphia-positive ALL as it aids in the monitoring of the effect of treatment and detection of relapse (Miyamura et al., 1992).
CHAPTER 3

METHODOLOGY

3.1 Study design

This is a retrospective cross-sectional study in which methanol-fixed archived bone marrow slides of patients diagnosed with ALL were used.

3.2 Study site

The study was carried out at the Department of Haematology, Korle Bu Teaching Hospital. The hospital is the third largest hospital in Africa and the leading referral centre in Ghana (https://kbth.gov.gh/korle-bu-trust-fund.html, 2016). The Department of Haematology provides laboratory and clinical services for patients with various haematological disorders from all over Ghana as well as neighbouring West African countries. About 4800 patients are seen at the department each year. In the hospital, an average 28 cases of ALL are diagnosed each year with 12 of them being adults.

The bench work of the project was carried out by the investigator at the Queen’s Laboratory for Molecular Pathology at Queen’s University in Canada where training in the fluorescent-in situ hybridisation technique was acquired and the bench work carried out within a three-month period. This trip was necessitated by the fact that the FISH procedure could hardly be carried out in Ghana due to unavailability of necessary equipment and technical expertise.
3.3 Study Population

The population consists of diagnosed cases of ALL at the Department of Haematology, KBTH from January 2013 to MAY 2017.

3.4 Inclusion criteria

1. Study cases must be patients ≥15 years old.
2. Cases should have been morphologically diagnosed as ALL.
3. The unstained bone marrow aspirate slides of cases should be available.

3.5 Exclusion criteria

1. Cases whose archived bone marrow aspirate slides and/or laboratory report at diagnosis were not available were excluded.
2. Cases with incomplete data from folders were excluded.

3.6 Sample size determination

The sample size was determined using the Cochran’s sample size formula which is given by the following equation:

\[ n = \frac{Z^2 \cdot p(1-p)}{d^2} \]

Where

\[ n = \text{minimum sample size} \]
\[ Z \text{ is } Z \text{ score } (Z=1.96 \text{ for confidence level of } 95\%) \]

Prevalence of BCR-ABL1 fusion gene in ALL = 11 – 29%....... (Mrozek et al., 2009)
Using the minimum value of the prevalence range ⇒ \( p = \) expected proportion= 11%

\[ d = \text{accepted margin of error} = 5\% \]

\[
\text{Therefore } n = \frac{1.96 \times 1.96 \times 0.11 (1 - 0.11)}{0.05 \times 0.05} = 150.43 \]

But the study is retrospective cross-sectional (January 2013 to December 2015) with a finite population made up of a total of 37 cases.

This finite population is corrected using the formula below:

\( n' = n \times \frac{N}{n + (N - 1)} \) .................................................(Naing, Winn, & Rusli, 2006)

where \( n' = \) corrected sample size for a finite population

\[ N = \text{finite population size} = 37 \]

\[ n = \text{sample size without taking the finite population correction into consideration} = 151 \]

\[ \therefore n' = 151 \times \frac{35}{151 + (37 - 1)} = 28.6 \approx 30. \]

However, only 25 of the 37 cases seen in the study period had archived bone marrow aspirate slides available. Therefore, even though 30 is obtained from the sample size calculation above, the sample size used for the study was 25. Hence 25 bone marrow aspirate slides were selected for the study.

3.7 Selection of samples and data collection

Unstained bone marrow aspirate slides of study cases (adults ALL) were retrieved from storage using laboratory numbers obtained from the Haematology laboratory log book. The storage
comprised of all bone marrow aspirate slides of all haematological disorders prepared from January 2013 to May 2017.

The data abstraction form shown on appendix A was used to obtain the following information from the patients’ medical records:

i) Clinical features present at presentation (i.e. hepatomegaly, splenomegaly, lymphadenopathy and/or presence of mediastinal mass)

ii) Laboratory variables were obtained from FBC which was performed at the time bone marrow aspirate was taken for diagnosis (i.e. haemoglobin concentration, white cell and platelet counts). The blast percentages were obtained from bone marrow aspirate smears.

3.8 Materials and Methods

The fluorescence in-situ hybridisation (FISH) technique was used for the detection of the BCR-ABL1 fusion gene in the unstained archived bone marrow aspirate slides.

Equipment and apparatus used include the following:

1. ThermoBrite Denaturation/Hybridisation System (Abbot Molecular)

2. Fluorescent Microscope (OLYMPUS BX61)

3. Computer with imaging software - GENASIS FISHView and Case Data Manager (CDM) (from Applied Spectral Imaging)

4. Phase Contrast Microscope (Zeiss Axio Lab. A1)

5. Water bath

6. Microcentrifuge
7. Vortex Mixer
8. Digital Thermometer
9. Micropipettes

The reagents used are listed in appendix C.

3.9 Procedure

The fluorescent in-situ hybridisation technique was performed by the investigator using the protocol of the Queens Laboratory for Molecular Pathology (Queens University, Canada): procedure as described below:

Preparation of Positive and Negative Control Smears

A positive control smear was prepared from a cell culture of a commercially prepared $BCR-ABL1$ positive cell line [K-562 (ATCC® CCL -243)]. A peripheral blood smear prepared from a $BCR-ABL1$ negative anonymised subject which was provided by the Queens Laboratory for Molecular Pathology was used as a negative control slide. Both slides were fixed in methanol for 3 minutes.

The FISH procedure was carried out on study cases and control slides as per the protocol of the Queens laboratory for Molecular pathology which is as follows:
DNA Unmasking

The slides were immersed in methanol for 1 minute followed by incubation in 2× SSC at room temperature for 5 minutes.

The slides were then incubated in 0.2N HCl for 5 minutes. 500ul of pepsin was added to the prewarmed 49.5ml of 0.01N HCl in water bath at 37°C. The resulting mixture was mixed well.

The slides were taken from the 0.2N HCl solution and excess liquid remove with paper towel and immediately immersed into the pepsin/HCl solution incubating at 37°C for 7 to 15 minutes.

The Slides were washed in ddH₂O for 10 minutes followed by fixation in 1% formaldehyde for 5 minutes, immersion in 1% phosphate buffered saline for 5 minutes and sequential dehydration in 70%, 85% and 100% ethanol for 2 minutes in each solution.

The slides were observed under phase contrast microscope for digestion progress (Unfinished digestion is signified by the appearance of white shiny cells with undefined nuclei whereas cells with clear blue nuclei shows complete digestion). If digestion is not finished, the slides were re-immersed in pepsin/HCl solution for a longer period and the subsequent steps followed until the phase contrast microscope shows a finished digestion.

Denaturation and Hybridization

The slides were air dried completely prior to the addition of the probe in the hybridisation steps which is described in the following paragraphs:
The ThermoBrite Denaturation/Hybridisation system was turned on and the program set for the following parameters:

- Denaturation time: 2 mins;
- Denaturation temperature: 73°C;
- Hybridisation Time: 24 hrs;
- Hybridisation temperature: 37°C.

The DNA probe (Vysis LSI BCR/ABL DC/DF translocation probe), Vysis LSI/WCP hybridisation buffer and the purified water were removed from storage and the reagents allowed to reach room temperature. The BCR/ABL DNA probe and the hybridisation buffer were vortexed for 2 to 3 seconds followed by centrifugation for 2 to 3 seconds.

Seven microlitres (7μL) of the hybridisation buffer, 2μL of purified water and 1μL of the BCR/ABL DNA probe were transferred into a microcentrifuge. The mixture was vortexed and centrifuged for 2 to 3 seconds each.

A micropipette was used to apply 10μL of the probe mixture to the target area of each slide. A cover slip was immediately applied without introducing bubbles. The coverslip was sealed using a syringe filled with rubber cement.

Two ThermoBrite humidity cards saturated with distilled water were inserted into the slot positions in the unit lid of the ThermoBrite Hybridisation/Denaturation system.

The slides were placed on the heating surface of the ThermoBrite Hybridisation/Denaturation system when prompted and was ensured that, the frosted edge of the slide hanged over the heating surface, lay flat and properly aligned into the marked positions in the slide locator. The ThermoBrite lid was closed and the program was started for denaturation and hybridisation to occur overnight.
Post-Hybridisation Wash

The post-hybridisation wash was carried out according to the following procedure:

The room was darkened and the coverslip removed from the slide by peeling off rubber cement.

The slides were incubated in 2× SSC/0.3% IgePal solution (180uL IgePal in 60ml 2× SSC) at 73°C for 2 minutes. The temperature was increased by 0.5°C for each slide if the slides were more than one. The slides were washed in 2× SSC for 5 minutes and air-dried in upright position under foil cap.

Counterstaining

Counterstaining was carried out by the application of 10uL DAPI to the middle of each slide. Coverslip was applied and air bubbles pushed out. They were stored in the dark at -20°C until fluorescent microscopy was carried out in the following step.

Fluorescent Microscopy

The fluorescent microscopy room was darkened and immersion oil added to the slides. They were then observed under the fluorescent microscope using the spectrum orange, spectrum green and the dual filter which allows the visualisation of ABL1, BCR and BCR/ABL1 gene respectively. A total of 100 interphase nuclei were scored for each slide. Images of the slides were captured using the imaging software GENASIS FISH View and processed using Case Data Manger (CDM). Representative images have been shown in section 4.2.
3.10 Data Handling

Study cases were assigned unique identification numbers. It is these identification numbers that were used to label the respective bone marrow aspirate slides and also for subsequent data processing.

The data was stored on a password protected computer. The names of subjects were not used in the data but were however kept in a different file. Only the Investigator and his supervisors had access to data obtained from the study.

3.11 Statistical analysis

The data was entered into Microsoft Excel and exported to Statistical Package for the Social Sciences (SPSS) for analysis. Data was expressed using summary and descriptive statistics such as frequency, percentages and median as appropriate and presented in tabular form. Chi Square, Fisher exact test and non-parametric test (Mann-Whitney U-test) were used to assess the association between categorical factors.
CHAPTER 4

RESULTS

A total of 25 bone marrow aspirate slides were selected for the study. However, FISH was successfully carried out on 17 as 8 slides were not suitable to be used for the test. Four of the slides upon examination under the microscope had structures suspected to be fungal hyphae covering the entire slide thus they could not be used for analysis. The remainder could not withstand experiment procedures as the glass slides used to prepare the bone marrow aspirate smears were not electrostatically charged type as such the cells were lost during the experimental procedure. Therefore, only slides from 17 patients were available for analysis as shown in figure 1.
Figure 1: FLOW DIAGRAM FOR THE SELECTION OF BONE MARROW ASPIRATE SLIDES FOR THE STUDY
4.1 Fluorescence In Situ Hybridization results and frequency of the BCR-ABL1 gene in samples.

The table below shows the results of detection of the presence of BCR-ABL1 fusion gene on unstained bone marrow aspirate slides of study cases by fluorescent in situ hybridization.

*Table 3: FISH RESULTS FOR BCR-ABL1 FUSION GENE*

<table>
<thead>
<tr>
<th>BCR-ABL1 gene results</th>
<th>Frequency</th>
<th>Percent</th>
</tr>
</thead>
<tbody>
<tr>
<td>Negative</td>
<td>12</td>
<td>70.6</td>
</tr>
<tr>
<td>Positive</td>
<td>5</td>
<td>29.4</td>
</tr>
<tr>
<td>Total</td>
<td>17</td>
<td>100.0</td>
</tr>
</tbody>
</table>

From the table above, it can be seen that, 29.4% (5 cases) were positive for the BCR-ABL1 gene. Therefore, the frequency of the BCR-ABL1 fusion gene for the adult Acute Lymphoblastic Leukaemia samples tested in this study was 29.4%.

The cut-off point for positivity was 1% and 15% for double fusion and single fusion respectively. Of the 5 positive cases, 4 were double fusion and one had single fusion.

The percentage scores for positive cases of the gene were 46%, 37%, 9% and 8% for double fusion and 20% for single fusion.
4.2 Selected FISH Images

The figure below shows a fluorescent photomicrograph of the negative control slide. No fusion signals are present.

Figure 2: Negative Control
The figure below shows the fluorescent photomicrograph of a study participant with no fusion signals present (negative case)

*Figure 3: BCR-ABL1 fusion Negative Case*
The figure below shows the fluorescent photomicrograph the \textit{BCR-ABL1} positive control smear. Double fusion signals which appear yellow are seen in the lower left and topmost nuclei.

\textit{Figure 4: Positive Control}
The figure below shows a fluorescent photomicrograph of a \textit{BCR-ABL1} positive case. Double fusion signals (either yellow dots or orange and green dots in juxtaposition) are seen in the two nuclei in the middle.

\textit{Figure 5:BCR-ABL1 fusion Positive Case}
In the figure below, a single fusion signal (yellow) is seen in the larger cell.

![Image](image-url)

*Figure 6: BCR-ABL1 fusion case showing a single fusion signal*

**KEY TO INTERPRETATION OF IMAGES**

*BCR-GENE* – Green signals (dots)

*ABL GENE* – Orange signals (dots)

*BCR-ABL1 FUSION GENE* – Orange and green signals in juxtaposition or yellow signal are seen for *BCR-ABL1* positive cases. Double fusion cases have 2 signals whereas 1 signal is seen single fusion cases). BCR-ABL negative cases show separated green and orange signals but no fusion signals).
4.3 Demographics

Table 4: BCR-ABL1 FUSION GENE AND SEX

<table>
<thead>
<tr>
<th>SEX</th>
<th>Frequency</th>
<th>BCR-ABL1 RESULTS</th>
<th>P-value (Fisher exact test)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Negative</td>
<td>Positive</td>
</tr>
<tr>
<td>female</td>
<td>3</td>
<td>1</td>
<td>4</td>
</tr>
<tr>
<td></td>
<td>75.0%</td>
<td>25.0%</td>
<td>100.0%</td>
</tr>
<tr>
<td>male</td>
<td>9</td>
<td>4</td>
<td>13</td>
</tr>
<tr>
<td></td>
<td>69.2%</td>
<td>30.8%</td>
<td>100.0%</td>
</tr>
<tr>
<td>Total</td>
<td>12</td>
<td>5</td>
<td>17</td>
</tr>
<tr>
<td></td>
<td>70.6%</td>
<td>29.4%</td>
<td>100.0%</td>
</tr>
</tbody>
</table>

In the study 76.5% (13) of the samples were drawn from males and 23.5% (4) were from females. From the table above, the proportion of BCR-ABL1 positive cases was lower compared to BCR-ABL1 negative cases (25.0% versus 75.0% within the female category and 30.8% versus 69.2% within the male category). From the Chi-Square test, there is a likelihood of no significant association between BCR-ABL1 positivity and sex of study cases. (p-value = 0.67).
**4.4 Age**

Participants in the study were categorized into two groups; adolescent and young adult group (AYA group) and older adult group with the age ranges from 15 to 39 years and 40 years and above respectively based on the guidelines of the National Comprehensive Cancer Network (NCCN), U.S.A. The corresponding mean ages were 22.2 (+/-7.7) years and 53.8 (+/-10.0) years respectively.

The ages of participants ranged from 15 years to 67 years (mean = 31.5 +/- 16.9 years). Table 5 shows the proportion of each group.

*Table 5: BCR-ABL1 FUSION GENE AND AGE*

<table>
<thead>
<tr>
<th>Age Group</th>
<th>Frequency</th>
<th>% within AYA group</th>
<th>Frequency</th>
<th>% within Older Adult group</th>
<th>Total</th>
<th>P-value (Fisher exact test)</th>
</tr>
</thead>
<tbody>
<tr>
<td>AYA</td>
<td>7</td>
<td>58.3%</td>
<td>5</td>
<td>41.7%</td>
<td>12</td>
<td>0.245</td>
</tr>
<tr>
<td>Older Adults</td>
<td>5</td>
<td>100.0%</td>
<td>0</td>
<td>0.0%</td>
<td>5</td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>12</td>
<td>70.6%</td>
<td>5</td>
<td>29.4%</td>
<td>17</td>
<td></td>
</tr>
</tbody>
</table>
In the table above, BCR-ABL1 positive cases constitute a lower proportion than BCR-ABL1 negative cases within each age group (41.7% versus 58.3% in the AYA group and 0.0% versus 100.0% in the Older Adult group. The p-value is = 0.245, hence there is no significant association between the BCR-ABL1 gene positivity and age groups (adolescent and young adult group and older adults. There were 12 cases (70.6%) in the AYA group and 5 cases (29.4%) in the Older Adult group.

4.5 Descriptive and Inferential Statistics of Clinical features and BCR-ABL1 gene

Table 6 show the frequencies of clinical features and test of association with the BCR-ABL1 gene. Cases which showed the presence of a clinical features are indicated by ‘Yes’ and ‘No’ if otherwise.
Table 6: BCR-ABL1 FUSION GENE AND CLINICAL FEATURES

<table>
<thead>
<tr>
<th>Clinical Feature</th>
<th>BCR-ABL1 RESULTS</th>
<th>Total</th>
<th>P-value (Fisher exact test)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Negative</td>
<td>Positive</td>
<td></td>
</tr>
<tr>
<td>LYMPHADENOPATHY</td>
<td>No</td>
<td>6</td>
<td>4</td>
</tr>
<tr>
<td></td>
<td>Yes</td>
<td>6</td>
<td>1</td>
</tr>
<tr>
<td></td>
<td></td>
<td>60%</td>
<td>40%</td>
</tr>
<tr>
<td></td>
<td></td>
<td>85.7%</td>
<td>14.3%</td>
</tr>
<tr>
<td>SPLENOMEGALY</td>
<td>No</td>
<td>9</td>
<td>4</td>
</tr>
<tr>
<td></td>
<td>Yes</td>
<td>3</td>
<td>1</td>
</tr>
<tr>
<td></td>
<td></td>
<td>69.2%</td>
<td>30.8%</td>
</tr>
<tr>
<td></td>
<td></td>
<td>75.0%</td>
<td>25.0%</td>
</tr>
<tr>
<td>HEPATOMEGALY</td>
<td>No</td>
<td>8</td>
<td>4</td>
</tr>
<tr>
<td></td>
<td>Yes</td>
<td>4</td>
<td>1</td>
</tr>
<tr>
<td></td>
<td></td>
<td>66.7%</td>
<td>33.3%</td>
</tr>
<tr>
<td></td>
<td></td>
<td>80.0%</td>
<td>20.0%</td>
</tr>
<tr>
<td></td>
<td>Total</td>
<td>12</td>
<td>5</td>
</tr>
<tr>
<td></td>
<td></td>
<td>70.6%</td>
<td>29.4%</td>
</tr>
</tbody>
</table>

From the Chi-Square analysis above, there is a likelihood of no significant association between BCR-ABL1 gene positivity and lymphadenopathy, splenomegaly and hepatomegaly of study cases. (P-values = > 0.05).
### 4.6 Descriptive and Inferential Statistics of Haematological parameters

**Table 7: BCR-ABL1 FUSION GENE AND HAEMATOLOGICAL PARAMETERS**

<table>
<thead>
<tr>
<th>BCR-ABL1 RESULTS</th>
<th>N</th>
<th>Mean</th>
<th>Std. Deviation</th>
<th>P-value MW-U- test</th>
</tr>
</thead>
<tbody>
<tr>
<td>WBC Count</td>
<td>12</td>
<td>73.53×10⁹/L</td>
<td>138.87×10⁹/L</td>
<td>0.879</td>
</tr>
<tr>
<td></td>
<td>5</td>
<td>36.83×10⁹/L</td>
<td>39.21×10⁹/L</td>
<td></td>
</tr>
<tr>
<td>Hb Concentration</td>
<td>12</td>
<td>6.63g/dL</td>
<td>1.82g/dL</td>
<td>0.506</td>
</tr>
<tr>
<td></td>
<td>5</td>
<td>7.26g/dL</td>
<td>1.34g/dL</td>
<td></td>
</tr>
<tr>
<td>BLAST PERCENTAGE</td>
<td>11</td>
<td>82.18%</td>
<td>28.16</td>
<td>0.851</td>
</tr>
<tr>
<td></td>
<td>4</td>
<td>73.00%</td>
<td>29.72</td>
<td></td>
</tr>
<tr>
<td>PLATELET Count</td>
<td>12</td>
<td>74.33×10⁹/L</td>
<td>59.86×10⁹/L</td>
<td>0.721</td>
</tr>
<tr>
<td></td>
<td>5</td>
<td>54.60×10⁹/L</td>
<td>37.75×10⁹/L</td>
<td></td>
</tr>
</tbody>
</table>

From the table above, the mean haemoglobin concentration for BCR-ABL1 positive cases was higher than that for the negative cases, whereas the mean white blood cell count, bone marrow blast percentages and platelet counts were lower in BCR-ABL1 positive cases than in the negative cases. However, the Mann-Whitney test p-values are >0.05, hence no significant association exist between BCR-ABL1 gene positivity and these parameters.
4.7 Descriptive and Inferential Statistics of BCR-ABL1 gene and Clinical Outcome

CLINICAL OUTCOME

Table 8: BCR-ABL1 FUSION GENE AND CLINICAL OUTCOME

<table>
<thead>
<tr>
<th>CLINICAL OUTCOME</th>
<th>FISH RESULTS</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Negative</td>
</tr>
<tr>
<td>Mortality</td>
<td>Frequency</td>
</tr>
<tr>
<td></td>
<td>3</td>
</tr>
<tr>
<td>% within Mortality</td>
<td>75.0%</td>
</tr>
<tr>
<td>Default</td>
<td>Frequency</td>
</tr>
<tr>
<td></td>
<td>2</td>
</tr>
<tr>
<td>% within Default</td>
<td>66.7%</td>
</tr>
<tr>
<td>Undetermined</td>
<td>Frequency</td>
</tr>
<tr>
<td></td>
<td>7</td>
</tr>
<tr>
<td>% within Undetermined</td>
<td>70.0%</td>
</tr>
<tr>
<td>Total</td>
<td>Frequency</td>
</tr>
<tr>
<td></td>
<td>12</td>
</tr>
<tr>
<td>% within CLINICAL OUTCOME</td>
<td>70.6%</td>
</tr>
</tbody>
</table>

On the basis of inability to determine the clinical outcome 13 out of 17 study samples (undertermined =10, default = 3), no inferential assumption will be draw for clinical outcome.
4.8 Features of the *BCR-ABL1* Positive Cases

The table below features of the *BCR-ABL1* positive cases in this study.

*Table 9: FEATURES OF BCR-ABL1 POSITIVE CASES*

<table>
<thead>
<tr>
<th></th>
<th>CASE 1</th>
<th>CASE 2</th>
<th>CASE 3</th>
<th>CASE 4</th>
<th>CASE 5</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>BCR-ABL1 results</strong></td>
<td>Positive</td>
<td>Positive</td>
<td>Positive</td>
<td>Positive</td>
<td>Positive</td>
</tr>
<tr>
<td><strong>Age</strong></td>
<td>16</td>
<td>24</td>
<td>36</td>
<td>16</td>
<td>15</td>
</tr>
<tr>
<td><strong>Sex</strong></td>
<td>Female</td>
<td>Male</td>
<td>Male</td>
<td>Male</td>
<td>Male</td>
</tr>
<tr>
<td><strong>Blast percentage</strong></td>
<td>36</td>
<td>95</td>
<td>-</td>
<td>66</td>
<td>99</td>
</tr>
<tr>
<td><strong>WBC count</strong></td>
<td>1.60</td>
<td>88.4</td>
<td>67.04</td>
<td>24.76</td>
<td>2.33</td>
</tr>
<tr>
<td><strong>Platelet count</strong></td>
<td>12</td>
<td>109</td>
<td>27</td>
<td>67</td>
<td>58</td>
</tr>
<tr>
<td><strong>Haemoglobin concentration</strong></td>
<td>5.8</td>
<td>8.5</td>
<td>8.5</td>
<td>7.6</td>
<td>5.9</td>
</tr>
<tr>
<td><strong>Lymphadenopathy</strong></td>
<td>No</td>
<td>No</td>
<td>No</td>
<td>No</td>
<td>Yes</td>
</tr>
<tr>
<td><strong>Splenomegaly</strong></td>
<td>No</td>
<td>No</td>
<td>No</td>
<td>Yes</td>
<td>No</td>
</tr>
<tr>
<td><strong>Hepatomegaly</strong></td>
<td>No</td>
<td>No</td>
<td>No</td>
<td>Yes</td>
<td>No</td>
</tr>
<tr>
<td><strong>Treatment outcome</strong></td>
<td>Undetermined</td>
<td>Undetermined</td>
<td>Defaulted</td>
<td>Undetermined</td>
<td>Death</td>
</tr>
<tr>
<td><strong>Fusion pattern</strong></td>
<td>Double</td>
<td>Double</td>
<td>double</td>
<td>double</td>
<td>single</td>
</tr>
<tr>
<td><strong>Percentage of fusion signals</strong></td>
<td>37</td>
<td>8</td>
<td>46</td>
<td>9</td>
<td>20</td>
</tr>
</tbody>
</table>
CHAPTER 5
DISCUSSION AND CONCLUSION

The results of this study show that the BCR-ABL1 gene fusion is present in nearly one-third of adult cases of ALL diagnosed in the Haematology Department of the Korle Bu Teaching Hospital which were tested. Additionally, the findings suggested no statistically significant association between positivity for this chimeric fusion gene and clinical features, haematological parameters.

5.1 Frequency

The findings of this study show that, the BCR-ABL1 fusion gene is present in nearly one third (29.4%) of adults diagnosed with acute lymphoblastic leukaemia at the Korle Bu Teaching Hospital which were tested.

The frequency of 29.4% in this study is consistent with the prevalence rates 28.3% in the study conducted by Chopra et al. in India and the 11-29% Mrozek et al. in a review of adult ALL studies in USA, UK and France. However, it is higher than the 12.5% value obtained by Ajuba et al. in Nigeria. The wide difference in frequency between this study and that of Ajuba et al. (both conducted in Sub-Saharan Africa) could arise from the fact that the cases considered in this study were adults whereas the subjects for Ajuba et al. were both children and adults. The frequency of the BCR-ABL gene in ALL is age dependent being higher in adults than children as shown by Mrozek et al. The frequency in this study is in contrast to the study in Saudi Arabia in which none of the 16 adult ALL cases was positive for the BCR-ABL fusion gene El-Sissy ET (El-Sissy et al., 2006). Ethnic difference may possibly account for the disparities as Ariffin et al.
showed variation in frequency between ethnic groups in their study in Singapore involving Indians, Malays and Chinese.

5.2 Age and Gender

There was no significant association between BCR-ABL1 positivity and the age groups of cases tested in this study (AYA and older adult). However, all the BCR-ABL1 positive cases belonged to the adolescent and young adult (AYA) group. This contrast the report of increased frequency of BCR-ABL1 with increasing age reaching 42% to 44% beyond age 44 in Germany (Thomas Burmeister et al., 2008). The exact reasons for the occurrence of the fusion gene in only the AYA group is not clear. However, the decreased representation of the older adult group in this study cannot be ruled out. Ghana has a young population with 58.5% aged 0 to 24 years and only 11% beyond age 50 years. This contrasts the population structure of advanced countries such as U.S.A, Germany and Japan where the population is relatively old with respective proportions of 33.0%, 24.7% and 22.5% in the 0 to 24 years age group whereas 34.2%, 40.3% and 45% are beyond age 50 years (Nations, 2017). The lower proportions old age population in Ghana which possibly reflected in this study can be attributed to the fact that there is relatively low life expectancy at birth (61.3 years) in Ghana compared to that of the U.S.A, Germany and Japan which is 78.9, 80.8 and 83.6 years respectively (United-Nations., 2017).

Similarly, this study suggests no significant association between BCR-ABL1 positivity and sex although 4 out of 5 cases of the BCR-ABL1 positive cases were males. This may be explained by the low proportion of females (23.5%) in this study. These findings of no significant association between BCR-ABL1 positivity and age as well as gender in study cases confirms the study of Hamid and Bokharaei in Iran.
5.3 Clinical Features

Secondly, the findings of this study suggest no significant association between *BCR-ABL1* positivity and clinical features (lymphadenopathy, splenomegaly and, hepatomegaly) of study cases.

Similarly, Westbrook et al. in a Cancer and Leukaemia Group B study in USA found no statistically significant association between *BCR-ABL1* positivity and these clinical features.

Studies involving clinical features and *BCR-ABL1* positive ALL from Africa (especially West Africa) are virtually not available.

Of the clinical features considered in this study, Lymphadenopathy was present 41.2% of all the cases in this study of which 14.3% were *BCR-ABL1* positive whereas 23.5% of the cases studied showed splenomegaly of which one quarter was *BCR-ABL1* positive. While hepatomegaly evident in 35.7% of study cases with exactly one fifth showing *BCR-ABL1* positivity, no incident of mediastinal mass was reported in this study. The frequencies of the organomegalies are lower than what has been reported in the study by Elbossaty et al. in Egypt in which lymphadenopathy, splenomegaly and hepatomegaly occurred in 58, 58 and 54% of adult ALL patients. However, it is consistent with the findings in Netherlands by Daenen et al. in which organomegalies (lymphadenopathy, splenomegaly and hepatomegaly) were less prevalent with a combined frequency of 40% in adult ALL.

5.4 Haematological Parameters

The mean white blood cell count, bone marrow blast percentages and platelet counts were lower in *BCR-ABL1* positive cases than negative (36.83, 73.00 and 54.60 versus 73.53, 82.18 and 74.33
respectively). Contrastingly, the mean haemoglobin concentration for *BCR-ABL1* positive cases was higher than in negative cases (7.26 versus 6.62 respectively). However, no significant association was established between these mean values of these haematological parameters and *BCR-ABL1* positivity (P = 0.879, 0.721, 0.506 and 0.851 for WBC counts, platelets counts, haemoglobin concentration and blast percentage respectively).

The results of this study confirm the findings of Ajuba et al. in Nigeria in which no statistically significant association was observed between *BCR-ABL1* positivity and white blood cell count, platelet count and haemoglobin concentration (P = 0.187, 0.658 and 0.303 respectively).

The findings of no statistically significant difference white blood cell count and haemoglobin concentration in this study is in contrast to the findings in the study by Gleißner et al. in Germany in which *BCR-ABL1* positive cases showed statistically significantly higher WBC count and haemoglobin concentration than *BCR-ABL1* negative cases. However, this study found no statistically significant difference in platelet counts and blast percentage in *BCR-ABL1* positive and negative cases as was reported by Gleißner et al.

All the cases in this study had severe to moderate anaemia with haemoglobin concentration ranging from 3.7 to 8.7g/dL. Also, with the exception of one, all cases in the study (approximately 94%) showed thrombocytopenia. These observed frequencies and degree of anaemia and thrombocytopenia is higher compared to developed countries based on studies conducted in Denmark and Italy (Chiaretti et al., 2013; Toft, Schmiegelow, Klausen, & Birgens, 2012). This may result from delayed presentation of the patients in this study to the Haematology clinic and hence late diagnosis and treatment thus allowing leukaemia cells
sufficient time to suppress normal erythropoiesis. This challenge is common in developing
countries such as Ghana due to inadequate number of Haematology centers.

5.5 Clinical Outcome

The clinical outcome of most of the cases which were studied could not be determined. This
arose from the fact that a total of 13 (76.5%) of study cases who constituted the default and
undetermined categories ceased clinic attendance based on financial constraint or unknown
reasons respectively.

5.6 Limitations of the Study

Inadequate filing system (for slides and folders), unavailability of corresponding clinical and
laboratory data from folders and laboratory and reduced number of good quality bone marrow
aspirate slides prevented one from obtaining the calculated sample size of 30; seventeen cases
were thus studied.

Secondly, the overall survival of majority study participants could not be determined due to
truncated clinic attendance.

5.7 Conclusion

With a frequency of 29.4%, the \textit{BCR-ABL1} fusion gene is an important molecular genetic lesion
in adult ALL cases in this study. There was no significant association between \textit{BCR-ABL1}
positivity and clinical features as well as haematological parameters of cases which were studied.
As no information of the \textit{BCR-ABL1} gene fusion in acute lymphoblastic leukaemia is available in Ghana due to the fact that no research has been carried out on it, this study thus provides an initial information on its presence, and association with clinical features and treatment outcome for stakeholders involved in the diagnosis and treatment of acute lymphoblastic leukaemia to make an informed decision with regard to the need for a bigger study.

The \textit{BCR-ABL1} fusion gene is thus expressed in adult acute lymphoblastic leukaemia cases seen in the country and has no significant association with the clinical features and haematological parameters of the disease the cases which were studied. A larger study will be needed to make a determination concerning the modification of treatment regimen for adult \textit{BCR-ABL1} positive ALL.

\textbf{5.8 Recommendation}

The investigator of this study suggests a larger multicenter prospective study in Ghana on \textit{BCR-ABL1} positive acute lymphoblastic leukaemia involving the characterization of the associated molecular signatures such as the \textit{IKZF1} and the \textit{PAX} genes so as to throw more light the expression of the disease in Ghanaian patients.
REFERENCES


doi:10.1038/s41568-018-0015-6


https://www.nature.com/articles/nature05690#supplementary-information


APPENDIX A

DATA ABSTRACTION FORM (Pages 21-22)

Personal Information

PATIENT UNIQUE ID NUMBER:

AGE: SEX: DATE OF DIAGNOSIS:

Clinical Feature

“A” will be circled for each of the clinical sign numbered 1-4 below if they are present and “B” if not indicated. Any others sign must be written in the space provide in front of number 5 below.

1. Lymphadenopathy - A. YES B. NO
2. Mediastinal mass - A. YES B. NO
3. Splenomegaly - A. YES B. NO
4. Hepatomegaly - A. YES B. NO
5. Other signs - ..........................................................
Clinical Outcome

Date of diagnosis (A):

Last date seen at clinic (B):

Outcome of treatment: A. remission B. death C. default D. failure

E. others: .........................................................

Survival duration = duration between A and B=

Haematological Parameters

PERIPHERAL BLOOD:

White cell count -

Platelet count -

Haemoglobin concentration –

BONE MARROW:

Blast percentage:
FISH results for *BCR-ABL1* fusion gene:

“A” or “B” will be circled below if bone marrow aspirate slide is *BCR-ABL1* positive or negative respectively.

A. POSITIVE  B. NEGATIVE

Percentage score (If FISH results above is positive) - .................%
APPENDIX B

TABLES OF RESULTS OF WBC COUNTS, PLATELET COUNTS, HAEMOGLOBIN CONCENTRATION AND BLAST PERCENTAGES

Table 1: WBC RESULTS

<table>
<thead>
<tr>
<th>WBC (×10^9/L)</th>
<th>Frequency</th>
<th>Percent</th>
<th>Valid Percent</th>
<th>Cumulative Percent</th>
</tr>
</thead>
<tbody>
<tr>
<td>Valid .95</td>
<td>1</td>
<td>5.9</td>
<td>5.9</td>
<td>5.9</td>
</tr>
<tr>
<td>1.60</td>
<td>1</td>
<td>5.9</td>
<td>5.9</td>
<td>11.8</td>
</tr>
<tr>
<td>1.61</td>
<td>1</td>
<td>5.9</td>
<td>5.9</td>
<td>17.6</td>
</tr>
<tr>
<td>2.33</td>
<td>1</td>
<td>5.9</td>
<td>5.9</td>
<td>23.5</td>
</tr>
<tr>
<td>6.41</td>
<td>2</td>
<td>11.8</td>
<td>11.8</td>
<td>35.3</td>
</tr>
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<td>7.20</td>
<td>1</td>
<td>5.9</td>
<td>5.9</td>
<td>41.2</td>
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<td>9.60</td>
<td>1</td>
<td>5.9</td>
<td>5.9</td>
<td>47.1</td>
</tr>
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<td>16.43</td>
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<td>5.9</td>
<td>5.9</td>
<td>52.9</td>
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<td>24.76</td>
<td>1</td>
<td>5.9</td>
<td>5.9</td>
<td>58.8</td>
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<td>38.58</td>
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<td>5.9</td>
<td>5.9</td>
<td>64.7</td>
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<td>42.50</td>
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<td>67.04</td>
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<td>5.9</td>
<td>5.9</td>
<td>76.5</td>
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<tr>
<td>88.40</td>
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<td>5.9</td>
<td>5.9</td>
<td>82.4</td>
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<td>1</td>
<td>5.9</td>
<td>5.9</td>
<td>88.2</td>
</tr>
<tr>
<td>160.70</td>
<td>1</td>
<td>5.9</td>
<td>5.9</td>
<td>94.1</td>
</tr>
<tr>
<td>485.91</td>
<td>1</td>
<td>5.9</td>
<td>5.9</td>
<td>100.0</td>
</tr>
<tr>
<td>Total</td>
<td>17</td>
<td>100.0</td>
<td>100.0</td>
<td></td>
</tr>
</tbody>
</table>
**TABLE 2: PLATELET COUNTS**

<table>
<thead>
<tr>
<th>PLT (×10⁹/L)</th>
<th>Frequency</th>
<th>Percent</th>
<th>Valid Percent</th>
<th>Cumulative Percent</th>
</tr>
</thead>
<tbody>
<tr>
<td>Valid 1.0</td>
<td>1</td>
<td>5.9</td>
<td>5.9</td>
<td>5.9</td>
</tr>
<tr>
<td>8.0</td>
<td>1</td>
<td>5.9</td>
<td>5.9</td>
<td>11.8</td>
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<td>9.0</td>
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<td>17.6</td>
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<td>12.0</td>
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<td>5.9</td>
<td>5.9</td>
<td>23.5</td>
</tr>
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<td>5.9</td>
<td>29.4</td>
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<td>29.0</td>
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<td>5.9</td>
<td>5.9</td>
<td>35.3</td>
</tr>
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<td>49.0</td>
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<td>5.9</td>
<td>5.9</td>
<td>41.2</td>
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</tr>
<tr>
<td>65.0</td>
<td>1</td>
<td>5.9</td>
<td>5.9</td>
<td>58.8</td>
</tr>
<tr>
<td>67.0</td>
<td>1</td>
<td>5.9</td>
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Table 3: HAEMOGLOBIN CONCENTRATIONS

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### TABLE 4: BLAST PERCENTAGE

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**Missing System**
- Frequency: 2
- Percent: 11.8

**Total**
- Frequency: 17
- Percent: 100.0

N.B. The blast percentage for two participants were not available.
APPENDIX C

The reagents for FISH procedure are listed below:

1. Vysis LSI BCR/ABL, dual colour, dual fusion translocation probe set which comprised:
   a. Vysis LSI BCR/ABL, dual colour, dual fusion translocation probe
   b. Vysis LSI /WCP hybridisation buffer

2. DAPI counterstain

3. Methanol

4. Ethanol

5. 2× Saline – Sodium Citrate (SSC) for molecular biology

6. 1×Phosphate buffered saline

7. 1% Formaldehyde

8. IgePal (Octyl phenyl-polyethylene glycol) for molecular biology

9. Rubber cement (ELMER’S)

10. 0.01N HCL

11. 0.2N HCL

12. Pepsin

13. Double distilled water (ddH₂O)
APPENDIX D

UNIVERSITY OF GHANA
COLLEGE OF HEALTH SCIENCES
ETHICAL AND PROTOCOL REVIEW COMMITTEE

Ref. No.: .......................... ..........................


Mr. Victor Obeng Ofori
Department of Haematology
School of Biomedical and Allied Health Sciences
University of Ghana
Kwame-Nkrumah

ETHICAL CLEARANCE

Protocol Identification Number: CHS-ET/M.5 - P 4.2/2016-2017

The Ethical and Protocol Review Committee of the College of Health Sciences on 2nd January, 2017 unaniusly approved your research proposal.

TITLE OF PROTOCOL: “A study of Adult BCR-ABL1 Positive Acute Lymphoblastic Leukemia seen at the Korle-Bu Teaching Hospital”

PRINCIPAL INVESTIGATOR: Mr. Victor Obeng Ofori

This approval requires that you submit six monthly review reports of the protocol to the Committee and a final full review to the Ethical and Protocol Review Committee at the completion of the study. The Committee may observe, or cause to be observed, procedures and records of the study during and after implementation.

Please note that any significant modification of this project must be submitted to the committee for review and approval before its implementation.

You are required to report all adverse events related to this study to the Ethical and Protocol Review Committee within seven (7) days verbally and fourteen (14) days in writing.

As part of the review process, it is the Committee’s duty to review the ethical aspects of any manuscript that may be produced from this study. You will therefore be required to submit the Committee with any manuscript for publication.

This ethical clearance is valid till 31st December, 2017.

Please always quote the protocol identification number in all future correspondence in relation to this protocol.

Signed: ........................................

PROFESSOR ANDREW A. ADJEI
CHAIRPERSON, ETHICAL AND PROTOCOL REVIEW COMMITTEE

cc: Provost, CHS
Dean, SBHS
Head of Department