 doi:10.20944/preprints202008.0199.v1

*Article*

# Clinicopathological Variables and Outcome in Chronic Phase Chronic Myeloid Leukemia Associated with BCR-ABL1 Transcript Type and Body Weight

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**Abstract: Introduction:** Chronic myeloid leukemia (CML) is a myeloproliferative neoplasm characterized by the dysregulated production and uncontrolled proliferation of mature and maturing granulocytes with fairly normal differentiation. The hallmark of CML is *BCR-ABL1* (breakpoint cluster region gene-Abelson murine leukemia viral oncogene homolog 1) on Philadelphia chromosome, which is the result of a reciprocal translocation between the long arms of chromosomes 9 and 22 (t[9;22][q34;q11]). With rare exceptions, breaks in chromosome 22 localize to one of three BCRs and determine the portions of BCR retained in the BCR-ABL1 fusion mRNA and protein. In contrast, the chromosome 9 breaks can occur over a large genetic region, 5′ of ABL1 exon Ib, 3′ of ABL1 exon Ia, or most commonly between the two alternative first ABL1 exons. In an overwhelming majority of CML patients, the break occurs in the major BCR (M-BCR), generating e13a2 or e14a2 fusion mRNAs and a $p210^{BCR-ABL}$ fusion protein. P230 BCR-ABL, the largest of the fusion proteins, corresponds to a break in the micro BCR (μ-BCR), an e19a2 fusion mRNA, and is associated with neutrophilic predominance and possibly less aggressive disease. Molecular monitoring of *BCR-ABL1* transcript levels following treatment with tyrosine kinase inhibitors (TKIs) is central to the effective clinical management of patients with CML. *BCR-ABL1* transcripts measured at standardized time points is used to define responses at key milestones in treatment allowing early signs of poor adherence or resistance to treatment to be detected and allow for early, effective clinical intervention. **Objective:** The aim of this study is to evaluate response to treatment with standard dose TKI in obese and non-obese CML patients together with *BCR-ABL1* transcript type. **Methodology:** A retrospective analysis of clinicopathological variables and response to treatment was performed for 37 chronic phase CMLs to compare, obese vs normal weight, and *BCR-ABL1* transcript type determined at diagnosis. Patients' management and response assessment was done based on ELN 2013 guidelines. Response to treatment was assessed using RT-qPCR analysis of blood calculated on the International Scale (IS). Various statistical methods used, all Statistical

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analyses were done using statistical packages SPSS 22.0 (SPSS Inc. Chicago, IL) and Epi-info (Centers for Disease Control and Prevention, Atlanta, GA) software. **Results:** The study cohort included 26 males (70.3%) and 11 females (29.7%) with mean age at diagnosis 36.8 years. 59.5% (n=22) expressed an e14a2 transcript, and 40.5% (n=15) an e13a2 transcript, most patients were started on imatinib, then switched either due to toxicity or failure. Median follow-up was 18 months for both transcript types.WBC, platelet counts, spleen size and Sokal scores at diagnosis, both median and Inter-quartile range (IQR) were observed to be higher in e14a2 compared to e13a2 transcript group, and, lower in obese patients compared patients with normal weight. At one year, patients with e13a2 transcript had higher percentage of CCyR (or better) 60% (95% CI 36.6, 80.3%) compared to e14a2 group 46.7% (95% CI 24.8, 69.9%), however this difference was statistically insignificant (odds ratio =1.71, 95% CI 0.40, 7.29; P=0.464). Overall, there was higher and faster achievement of CCyR and MMR in patients with e13a2 transcript compared to e14a2 transcript, and in the obese vs normal-weight patients. Patients in e13a2 group and obesity group had a lower rate of treatment failure and fewer indications to switch TKI. Of note MMR was observed to be significantly higher in patients of African origin (n=10) compared to patients with Asian ethnicity (50% vs 16%; P=0.038), which could be reflect differences in disease biology. **Conclusion**: In the patient cohort studied an e14a2 *BCR-ABL1* transcript type / normal body weight was associated with an inferior outcome when compared to e13a2 transcript / obesity groups

**Keywords:** chronic myeloid leukemia; BCR-ABL1 transcript; obesity

## 1. Introduction

Chronic myeloid leukemia (CML, also known as chronic myelocytic, chronic myelogenous, or chronic granulocytic leukemia) is a myeloproliferative neoplasm characterized by the dysregulated production and uncontrolled proliferation of mature and maturing granulocytes with fairly normal differentiation. It accounts for approximately 15 to 20 percent of leukemias in adults [1]. It has an annual incidence of 1 to 2 cases per 100,000, with a slight male predominance [2]. The median age at presentation in western countries is approximately 50 years for patients enrolled on clinical studies, but the actual median age from cancer registry data may be 10 years older. Exposure to ionizing radiation is the only known risk factor [3].

The hallmark of CML is BCR-ABL1 (breakpoint cluster region gene-Abelson murine leukemia viral oncogene homolog 1) on Philadelphia chromosome, which is the result of a reciprocal translocation between the long arms of chromosomes 9 and 22 (t[9;22][q34;q11]) [4].

With rare exceptions, breaks in chromosome 22 localize to one of three BCRs and determine the portions of BCR retained in the BCR-ABL1 fusion mRNA and protein. In contrast, the chromosome 9 breaks can occur over a large genetic region, 5′ of ABL1 exon Ib, 3′ of ABL1 exon Ia, or most commonly between the two alternative first ABL1 exons. Rare exceptions aside, splicing consistently leads to fusion mRNAs that encompass ABL1 exons 2 to 11 [5]. Breakpoints in the minor BCR (m-BCR) give rise to an e1a2 fusion mRNA and p190BCR-ABL, which is found in two-thirds of Ph+ve acute lymphoblastic leukemia (ALL) cases. The very rare p190BCR-ABL positive CML is associated with monocytosis and exhibits a more aggressive clinical course [6]. In an overwhelming majority of CML patients, the break occurs in the major BCR (M-BCR), generating e13a2 or e14a2 fusion mRNAs (formerly referred to as b2a2 and b3a2) and a p210BCR-ABL fusion protein. p230BCR-ABL, the largest of the fusion proteins, corresponds to a break in the micro BCR (μ-BCR), an e19a2 fusion mRNA, and is associated with neutrophilic predominance and possibly less aggressive disease [7].

Obesity is defined as abnormal or excessive fat accumulation that presents a risk to health. A crude population measure of obesity is the body mass index (BMI), a person's weight (in kilograms) divided by the square of his or her height (in meters). According to current WHO classification, a person with a BMI of 18.50 - 24.99 is considered normal, whereas a person with a BMI of 30 or more is generally considered obese, with further subclassification as follows: BMI of 30.00 - 34.99 is obese

 doi:10.20944/preprints202008.0199.v1

class I, BMI of 35.00 - 39.99 is obese class II, BMI of ≥40.00 is obese class III [8]. It has been reported that general adiposity in adulthood and early adulthood, and greater height may increase the risk of almost all types of lympho-hematopoietic cancers [9], while a study done in MD Anderson found that obesity and adult weight gain are independent risk factors for CML [10].

Molecular monitoring of BCR-ABL1 transcript levels following treatment with tyrosine kinase inhibitors (TKIs) is central to the effective clinical management of patients with CML. BCR-ABL1 transcripts measured at standardized time points is used to define responses at key milestones in treatment allowing early signs of poor adherence or resistance to treatment to be detected and allow for early, effective clinical intervention.

## 2. Methodology

We conducted a retrospective analysis of the files of 37 patients being treated in our center for CML in chronic phase (CMP-CP) with known BCR-ABL1 breakpoints, patients' management and response assessment was done based on ELN 2013 guidelines. Analysis is done based on two main groups, obese vs normal BMI, and then based on BCR-ABL1 transcripts: e13a2 vs e14a2.

Single step end-point reverse transcription PCR (RT-PCR) is used to exclude or detect and characterize BCR-ABL1 fusions in diagnostic samples. A multiplex PCR assay is used and includes primers for an internal control fragment that enables cDNA quality to be assessed. The test can detect e19a2, e13a2 (previously b2a2), e13a3 (b2a3), e14a2 (b3a2), e14a3 (b3a3), e1a2, and e1a3 variants which account for >99% of leukemia patients with a t(9;22) BCR-ABL1 rearrangement. The primers used may not detect some very rare BCR-ABL1 fusions. It is essential to characterize the variant as this determines the method and means of future monitoring. For patients with e1a2, e13a2, e14a2 fusions, disease levels can be monitored by real-time quantitative PCR (RQ-PCR). Other rare variants are monitored using non-quantitative RT-PCR.

Descriptive statistics were used to summarize demographic, anthropometric, hematological, and clinico-pathological characteristics of the patients. The normally distributed data and results were reported with mean and standard deviation (SD); the remaining results were reported with median and range. Categorical data were summarized using frequencies and percentages. Preliminary statistical analyses were conducted to examine the distribution of the data variables using the Kolmogorov–Smirnov test. Associations between two or more qualitative variables were assessed using Chi-square ($\chi 2$) test, Fisher Exact or Yates corrected Chi-square tests as appropriate. Quantitative data and outcome measures between the two independent groups were analyzed using unpaired t test (or Mann Whitney U test for skewed data). Survival functions were estimated with the Kaplan–Meier survival curve method followed by Log rank test. For response parameters (MMR and CCyR), cumulative incidences were calculated and comparisons between cumulative incidences were performed by the Gray test. Pictorial presentations of the key results were made using statistical graphs Box plots and Bar diagrams. All P values presented were two-tailed, and P values <0.05 was considered as statistically significant. All Statistical analyses were done using statistical packages SPSS 22.0 (SPSS Inc. Chicago, IL) and Epi-info (Centers for Disease Control and Prevention, Atlanta, GA) software.

## 3. Results

In our present study, we attempted to evaluate the transcript distribution across the various demographics, anthropometric, hematological and clinico-pathological characteristics. Patients included 26 males (70.3%) and 11 females (29.7%) with mean age at diagnosis 36.8±13.1 years (median, 33; range 21 to 57 years). Box plots depict distribution of WBC, platelet counts, spleen size and sokal scores at diagnosis across both transcripts e13a2 and e14a2 and it clearly indicates that for all these four parameters both median and Inter-quartile range (IQR) were observed to be higher in e14a2 compared to e13a2 transcript group (Figure 3). Furthermore, Figure 4 revealed that WBC, platelet counts, spleen size measured at diagnosis had lower median and IQR values in obese patients compared patients with normal weight groups.

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The characteristics (demographics, anthropometric, hematological and clinico-pathological) of the patients and their association with transcript types and obesity are summarized in Table 2. Twenty-two patients (59.5%; 95% CI 43.5, 73.7%) expressed e14a2, 15 (40.5%; 95% CI 26.4, 56.5) expressed e13a2 transcripts. Patients with e14a2 transcript had significantly higher platelets (median, 432 ×109/L; range, 86 to 895 ×109/L) compared with e13a2 (median, 248 ×109/L; range 119 to 578 ×109/L; P=0.008). Similar was the case with the WBC count, however this difference was found to be statistically insignificant (P=0.548). Demographic and anthropometric measures (age, gender and BMI), CBC (WBC, hemoglobin, blast %) and patients treated with the different TKI modalities were comparable and showed insignificant (P>0.05) differences between e14a2 and e13a2 transcripts. Both mean spleen size (20.4±5.6 vs 17.1±3.5; P=0.111) and sokal scores (0.89±0.26 vs 0.79±0.24; P=0.340) measured at diagnosis were observed to be higher in patients with e14a2 compared to e13a2 patients, however this difference didn't reach to statistical significance (P>0.05). The distribution of patients and risk stratifications by Sokal scores on diagnosis showed the percentage of patients with low risk was found to be higher among e14a2 patients (60%) compared to e13a2 (28.6%) group. Similar was the case with the fibrosis in bone marrow, however these differences were found to be statistically insignificant (P>0.05) as shown in Table 2.

- **Patient outcomes, cytogenetic and molecular responses by transcripts type e13a2 and e14a2:**

The median follow-up was 18 months (range 6 to 134.4 months) and 18 months (range 10.8 to 156 months) in e14a2 and e13a2 patients, respectively (P=0.790, computed using non-parametric Mann Whitney U test). Only one patient died in this cohort of patients and it was in e14a2 transcript group. Patients with e13a2 transcript showed an observational difference in the cumulative incidence (CI) of CCyR compared to patients with e14a2, however this difference noted to be statistically insignificant (P>0.05). At one year, patients with e13a2 transcript had higher percentage of CCyR of 60% (95% CI 36.6, 80.3%) compared to e14a2 group 46.7% (95% CI 24.8, 69.9%), however this difference was statistically insignificant (odds ratio =1.71, 95% CI 0.40, 7.29; P=0.464). There was a substantial difference in median time to CCyR when e13a2 (24 months, 95% CI 0, 176.6 months) was compared to e14a2 (81.6 months, 95% CI 12.5, 35.5 months; Log rank P-value=0.305), while CI of CCyR after five years of treatment was 82.3% versus 38.8% (P-value<0.05) respectively as shown in Figure 2.B. Similarly, the CI of MMR after 18 months was found to be higher among patients with e13a2 compared to e14a2 group (25% vs. 16.9%), the difference between e13a2 and e14a2 was statistically insignificant (P-value=0.816) (Figure 2.A). These findings suggest a better molecular and cytogenetic response rate in the e13a2 transcript group as compared to e14a2.

- **Association between demographic, anthropometric, hematological, and clinico-pathological characteristics of the patients with MMR and CCyR:**

As indicated by Box plots shown in Figure 5, median and IQR values for WBC and spleen size was found to be lower among patients who achieved MMR compared to non-MMR group. Whereas both platelet counts and sokal scores had higher values of median and IQR in MMR compared to non-MMR groups. Similar trend observed when compared these hematological parameters between CCyR and non-CCYR groups (Figure 6). Interestingly, the percentage of MMR was significantly higher in obese patients compared to patients who had normal weight (41.2% vs 11.1%; P=0.042). The MMR proportions were observed to be significantly higher in African patients compared to patients with Asian ethnicity (50% vs 16%; P=0.038). Moreover, absence of dysplasia in both CBC and bone marrow and lower fibrosis in bone marrow both had positively associated with MMR, however these differences were statistically insignificant (P>0.05). No statistically significant difference was observed in mean/median age and hematological parameters between MMR and non-MMR groups (P>0.05) as shown in Figure 5 and Figure 7. Very similar association trends were observed when compares such parameters and patients' characteristics with CCyR as indicated in Figure 6 and 7.

## 4. Discussion

Several studies have been done to find out the prognostic significance of the BCR-ABL1 transcripts, we will review here most of the studies comparing the common breakpoints with e14a2 (b3a2) vs e13a2 (b2a2) transcripts with regards to prognosis.

Tefferi A et al in 1990 [11] conducted a study on 62 patients with CML-CP, 39 patients with 5′ breakpoints (zones 1-3) and 23 patients with 3′ breakpoints (zones 4 and 5), they found no correlation between the clinical phase of the disease at last follow up and breakpoint distributions. Presenting clinical features, chronic phase duration, and the rates of lymphoblastic transformation were similar among the subgroups.

Shepherd P et al in 1995 [12] conducted analysis of 219 patients with of Ph+ve CML and 15 Ph-ve, BCR+ve CML, 119 cases have had RNA analysis performed to determine the type of BCR/ABL transcript and have also been analyzed in a similar way. Presenting features at diagnosis including age, sex, white-cell count and platelet count showed no significant difference for those with 5' and 3' breakpoints and those with either b2a2 or b3a2 BCR/ABL transcripts. No correlation was found either for genomic breakpoint site or BCR/ABL RNA transcript in terms of duration of chronic phase or survival. When stratified by randomized therapy, either interferon-alpha or standard chemotherapy, no difference was noted in relation to genomic breakpoint site or BCR/ABL transcript. Cytogenetic response was not related to the molecular findings.

Prejzner W in 2002 [13] conducted a study on 71 patients with CML-CP, 61 of them with known BCR-ABL1 transcripts. He reported no correlation between the clinical course, prognostic index, or survival was observed in patients with 5' (b2a2) and 3' (b3a2) breakpoints. The patients with b3a2 transcript experienced longer survival than the patients expressing b2a2 transcript. However, no significant differences were observed in the duration of the chronic phase between the two groups.

de Lemos JA et al in 2005 [14] conducted an observational cohort study on 22 patients with CML treated with imatinib and were followed for six months during treatment. There was a significant difference in the levels of the two major transcripts of BCR-ABL, b2a2 and b3a2 ($P = 0.0347$), indicating that b2a2 may be more sensitive to imatinib. They hypothesized that patients who express the b2a2 transcript may have a better prognosis.

Vega-Ruiz A et al in 2007 [15] published an abstract analysis of 480 pts with CML-CP treated with imatinib, 251 receiving imatinib as frontline therapy and 229 after interferon (IFN) failure. They concluded that patients with the b3a2 transcript have a better molecular response to imatinib that those with b2a2 (significantly higher probability of achieving a major molecular remission (MMR) and complete molecular remission (CMR; ie, undetectable transcript levels)), there was a trend for an improved transformation-free survival for pts with b3a2 compared to those with b2a2 (4-yr rates 98% vs 93%, respectively, p=0.08) and event-free survival (94% vs 87%, p=0.37). Although fewer pts co-expressed both transcript types, they behaved like the pts with b3a2.

Lucas CM et al in 2009 [16] conducted a study involving 78 patients with CML-CP, age > 16, treated with imatinib 400 mg daily. 71 of them had either e13a2 or e14a2 transcripts. They reported that patients expressing the e14a2 transcript type have a higher rate and more rapid complete cytogenetic responses (54%) than e13a2-expressing patients (25%), which may be due to higher BCR-ABL tyrosine kinase activity. e14a2 patients had a higher event-free survival rate in the first 12 months of treatment, although overall survival did not differ significantly between the patients with the two types of transcript. The pre-treatment pCrKL/CrKL ratio (a surrogate marker of BCR-ABL tyrosine kinase activity) was higher in patients with e13a2 transcripts than in those with e14a2 (p=0.017).

Hanfstein B et al in 2014 [17] conducted a study on a total of 1105 newly diagnosed imatinib-treated patients were analyzed according to transcript type at diagnosis (e13a2, n=451; e14a2, n=496; e13a2+e14a2, n=158). No differences regarding age, sex, or Euro risk score were observed. A significant difference was found between e13a2 and e14a2 when comparing white blood cells (88 vs. 65 × 10(9)/L, respectively; P<0.001) and platelets (296 vs. 430 × 10(9)/L, respectively; P<0.001) at diagnosis, indicating a distinct disease phenotype. Cumulative molecular response was inferior in e13a2 patients (P=0.002 for major molecular response; P<0.001 for MR4) but no difference was observed with regard to cytogenetic response and overall survival. They concluded that no risk prediction can be made according to e13a2 versus e14a2 BCR-ABL1 transcript type at diagnosis.

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Jain P et al in 2016 [18] conducted a study involving 481 patients with chronic phase CML expressing various BCR-ABL transcripts. Two hundred patients expressed e13a2 (42%), 196 (41%) expressed e14a2, and 85 (18%) expressed both transcripts. They concluded that compared to e13a2 transcripts, patients with e14a2 (alone or with coexpressed e13a2) achieved earlier and deeper responses, predicted for optimal European Leukemia Net (ELN) responses (at 3, 6, and 12 months) and predicted for longer event-free and transformation-free survival.

Lin HX et al in 2016 [19] conducted a retrospective analysis of 166 patients who have been treated with imatinib for up to 10 years. They concluded that both gender and BCR-ABL transcript, but not age, were significantly associated with the molecular response. Men with b2a2 represent a less favorable group in their response to imatinib treatment and may need alternative therapy regimen and closer monitoring.

Castagnetti F et al in 2017 [20] analyzed 559 patients enrolled in 3 prospective studies, treated with imatinib, and followed for at least 5 years. 52% of the patients had a e14a2 transcript, 37% a e13a2 transcript, 11% co-expressed both transcripts and 1% had other rare transcripts. The complete cytogenetic response rates were comparable in e14a2 and e13a2 patients. The median time to MR3.0 (6 and 12 months) and MR4.0 (41 and 61 months) was significantly shorter for e14a2 patients compared to e13a2 patients, with a higher cumulative probability of MR3.0 (88% and 83%, P < .001) and MR4.0 (67% and 52%, P = .001). The 7-year overall survival (90% and 83%, P = .017), progression-free survival (89% and 81%, P = .005) and failure-free survival (71% and 54%, P < .001) were significantly better in patients with e14a2 transcript.

Azad NA et al in 2018 [21] conducted a study involving 42 cases of CML treated with imatinib, they had either e13a2 (b2a2) or e14a2 (b3a2) transcripts, they concluded that there is no overall prognostic implication of either the e13a2 or the e14a2 transcript type across the spectrum of indicated clinical parameters evaluated. Even the overall survival analysis of the two transcript types revealed no prognostic association whatsoever.

D'Adda M et al in 2019 [22] conducted a study involving 173 patients with CML, 67 (38.7%) had the e13a2 transcript, and 106 (61.3%) had the e14a2 transcript. Complete cytogenetic and major molecular remissions were not affected, whereas the achievement of both a DMR (P = .008) and an sDMR (P = .004) was favored significantly in patients who had the e14a2 transcript. They concluded that e13a2 transcript hinders the achievement of deep responses and the possibility of stopping TKI treatment in patients with CML

Sazawal S et al in 2019 [23] conducted a study involving 400 patients with CML-CP, b3a2 transcript was observed in 288 (72%) followed by b2a2 in 104 (26%) and hybrid fusion transcript (b3a2 + b2a2) was seen in 8 (2%) cases. They concluded that MMR was significantly higher in patients with b3a2 transcript as compared to patients with b2a2.

Greenfield G et al in 2019 [24] conducted a study on 69 CML patients with an e13a2 or e14a2 transcript. The e13a2 group was on average significantly younger (45.0 years v 54.5 years), had a higher average white cell count (189.8 × 109/l v 92.40 × 109/l) and lower platelet count (308 × 109/l v 644 × 109/l) in comparison to the e14a2 group suggesting that these are distinct biological entities. They concluded that patients with an e13a2 transcript demonstrate an inferior molecular response to imatinib.

The following table summarizes these studies.

| No. | Reference | No. of patients with known breakpoint/transcript | Conclusion |
|---|---|---|---|
| 1. | Tefferi A et al, 1990 [11] | 62 | No difference |
| 2. | Shepherd P et al, 1995 [12] | 119 | No difference |
| 3. | Prejzner W, 2002 [13] | 61 | No significant difference |

 doi:10.20944/preprints202008.0199.v1

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|---|---|---|---|
| 4. | de Lemos JA et al, 2005 [14] | 22 | e13a2 (b2a2) better |
| 5. | Vega-Ruiz A et al, 2007 [15] | 480 | e14a2 (b3a2) better |
| 6. | Lucas CM et al, 2009 [16] | 71 | e14a2 better response, no difference in overall survival, pCrKL/CrKL ratio higher in e13a2 |
| 7. | Hanfstein B et al, 2014 [17] | 1105 | Significant difference in WBC and Plts, molecular response better in e14a2, no difference in cytogenetic response and overall survival |
| 8. | Jain P et al, 2016 [18] | 481 | e14a2 better |
| 9. | Lin HX et al, 2016 [19] | 166 | Male worse, e13a2 (b2a2) worse, male with b2a2 much worse |
| 10. | Castagnetti F et al, 2017 [20] | 559 | e14a2 better |
| 11. | Azad NA et al, 2018 [21] | 42 | No difference |
| 12. | D'Adda M et al, 2019 [22] | 173 | No difference in CCyR and MMR, e14a2 better in DMR |
| 13. | Sazawal S et al, 2019 [23] | 400 | e14a2 better |
| 14. | Greenfield G et al, 2019 [24] | 69 | e14a2 better |
| 15. | Our study | 37 | e13a2 better, obese better |

From this table it is obvious that several studies reported no difference in outcome according to BCR-ABL1 transcript, 2 of them were relatively old - before the introduction of TKIs [11, 12] or shortly after that [13], in addition to 1 recent study [21]. In addition, the study by Hanfstein B et al [17] which has by far the largest cohort of patients reported no difference in cytogenetic response and overall survival despite that patients with e14a2 had better molecular response. And the study by D'Adda M et al [22] which reported no difference in CCyR and MMR between the 2 groups, but e14a2 better in DMR and this group achieved better treatment free remission compared with e13a2.

7 of these studies [15, 16, 18, 19, 20, 23, 24] reported better response in e14a2 group. This might be due to higher BCR-ABL-1 tyrosine kinase activity (pCrKL/CrKL ratio) in patients with e13a2 transcripts than in those with e14a2 [16], which may be due to the structural difference between both transcripts, due to the insertion of a 25 amino acid segment coded by the b3 exon in b3a2. In total, structural differences are found between the two proteins in five $\alpha$-helices ($\alpha$25, $\alpha$', $\alpha$26, $\alpha$27 and $\alpha$29) and nine β-strands (β12, β13, β15, β', β17, β30, β'', β34 and β35). These differing structural elements are present in the SH3, SH2, SH1 and DNA-binding domains which can result in different roles played by the two isoforms in mediating signal transduction during the course of CML. [25]

One study [14] reported better outcome with e13a2 like our study, but number of patients were low and duration of follow up was short.

It is worth mentioning that in our cohort there were significant differences in baseline characteristics and Sokal scores which most likely have affected the outcome with advantage for the e13a2 group, in most of the previously mentioned studies there were no differences in baseline characteristics and Sokal scores between different groups. Our cohort had the opposite WBCs and Plts compared to the cohort included in Hanfstein B et al [17] study.

We recognize the limitation of our study being retrospective with some missing data and a small cohort, but it is unique in that it incorporated detailed clinico-pathological characteristics and compared the outcome in obese and normal BMI and different transcripts. Despite other authors

described obesity as an independent risk factor for CML [10] our is the first to study a possible association between obesity and outcome in CML patients. This pilot study lay the basis for others that should include a larger cohort of patients in order to confirm the preliminary findings and gain further insights on the role of obesity and breakpoint regions on the outcome of CML and justify further studies on the molecular basis of the phenomenon.

## 5. Conclusion

In the patient-cohort studied an e14a2 BCR-ABL1 transcript type / normal body weight was associated with an inferior outcome when compared to e13a2 transcript / obesity groups

**Author Contributions:** "Conceptualization, Yassin M A; methodology, Abdulla M AJ, Yassin M A; software, Chandra P; formal analysis, Abdulla M AJ, Chandra P, Yassin M A; data curation, Abdulla M AJ, Yassin M A; writing—original draft preparation, Abdulla M AJ, Chandra P, El Akiki S; writing—review and editing, Abdulla M AJ, Sorio C, Tomasello L, Boni Ch, Yassin M A.; visualization, Abdulla M AJ, Chandra P; supervision, Yassin M A. All authors have read and agreed to the published version of the manuscript.

**Funding:** Qatar National Library

**Acknowledgments:** European Leukemia Net

**Conflicts of Interest:** The authors declare no conflict of interest.

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