Original Article

# The Predicting Effect of ASXL1 Mutation on BCR-ABL Transcript Types and Responses to Tyrosine Kinase Inhibitors in Patients with Chronic Myeloid Leukemia



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## ABSTRACT

**Objective:** In order to support clinical management, we sought to show how ASXL1 mutations affect prognosis as a bio-marker for various management responses and illness progress.
**Study Design:** Cross-sectional study
**Place and Duration of Study:** This study was conducted at the Al-Diwaniyah Teaching Hospital and the Department of Pharmacology & Therapeutics, College of Medicine, University of Al-Qadisiyah, Iraq from 1st July 2024 to 31st January 2025.
**Methods:** A total of 51 patients with CML were enrolled. DNA was extracted from all patients for amplification of theASXL1 gene using specific primers and detection of the 1934 (c.1934dupG) mutation by sequencing.
**Result:** Only 2 were found to have this type of mutation, as revealed by sequencing of the amplified gene.
**Conclusion:** ASXL1 mutations might be promising prognostic bio-markers to regulate each patient's finest TKIs and avoid CML progression.
**Key Words**: Leukemia, ASXL1 gene mutation, PCR, Sequencing, TKIs, Resistance

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## INTRODUCTION

Leukemia is a cancer of blood cells, considered via the abnormal proliferation of abnormal white blood cells. It is the greatest mutual blood cancer kind in the United States. There are four main types of leukemia based on cell lineage: B-ALL, T-ALL, AML and CML.[1] The Philadelphia (Ph) chromosome (derivative chromosome 22), The first disease-specific chromosomal abnormality linked to a cancer, chronic myeloid leukemia, was caused by a translocation between chromosomes 9 and 22[2] and this translocation functions as a target for the tyrosine kinase inhibitors (TKIs).[3] Typically, the ABL1 gene's breakpoint lies between exons a1 and a2. The most frequent BCR-ABL rearrangements caused by these breakpoints are e14a2 (b3a2) and e13a2 (b2a2), which code for the 210-kDa protein p210. Together transcripts may coexpress in certain patients.[4] Multiple hematological neoplasms have mutations in the ASXL1 (further sex combs-like 1) gene. The most prevalent genetic modification is c. 1934dupG, p. gly646fs. We looked into how various people's proofreading skills affected things.

Polymerase DNA on ASXL1, another popular technique for ASXL1 genotyping is conventional Sanger sequencing, which is used to identify the 1934dupG somatic mutation.[5] When ASXL1 mutations are found during the chronic phase of CML, they are linked to worse outcomes.[6] For newly diagnosed CML, imatinib has been approved by the Food and Drug Administration as a first-line treatment. After 21 years of clinical use, imatinib, the first inhibitor of the BCR-ABL1 tyrosine kinase, has shown a better safety profile than anticipated.[7] A variety of mechanisms of resistance to TKI treatment have been documented, including kinase-domain mutations, BCR-ABL over expression and abnormal drug transporter expression, as well as some that are BCR-ABL-independent, like mutations in the ASXL1 gene.[8] ASXL1 productions a character in epigenetic regulation in the body. Concluded unknown resistance mechanisms, the ASXL1 mutation in the CML-CP sick in this case statement provided resistance to TKI. Despite the lack of a clear molecular mechanism for TKI resistance in ASXL1 mutations in CML, epigenetic modulation is a likely way for the disease to progress.[9]

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# METHODS

This cross-sectional study was conducted at Department of Pharmacology and Therapeutics, College of Medicine, University of Al-Qadisiyah, Iraq, and Al-Diwaniyah Teaching Hospital both, for the period from 1st July 2024 to 31st January 2025. A total of 51 sick with CML were enrolled. A specialist care giving physician/hematologist diagnosed and recruited all candidates' patients. The study involved a total of 51 patients, comprising 28 men and 23 women, whose ages extended as of 20 to 70 years. Each individual was facing the challenges posed by chronic myeloid leukemia and was actively receiving treatment with tyrosine kinase inhibitors. These patients represented a diverse group, each with their unique journey through the complexities of their illness, united by their battle against this persistent form of cancer.

The Philadelphia chromosome has a long-established presence, definitively indicates chronic myeloid leukemia in individuals aged 12 and older, underscoring the necessity for prompt diagnosis and intervention and every patient with CML on TKI therapy were included. Pregnancy and TKI contraindication were excluded. Completely sick comprised in the research stood taking oral TKI. Two ml of blood samples were collected using EDTA tubes, transported cooled to the laboratory, and subjected directly to molecular processing.

Conventional PCR was used to amplify the genome using a particular program that was optimized following the completion of a gradient for the gene. Next, we ran the PCR result on a 1 percent agarose gel to confirm it. An automated Sanger sequencing (SeqStudio) analyzer was then used to sequence the samples, and the results were examined for discrepancies.

Rendering to the manufacturers' instructions for the DNA extraction kit (Geneaid, Taiwan), DNA stayed removed as of blood testers, and the purification and concentration were examined using the Nanodrop System (Thermo Scientific, UK). Targeting the ASXL1 gene, a set of primers [(F: F:5́-GGACCCTCGCAGACATTAAA-3́) and R:(5́CACCACCATCACCACTGCT-3́)] stayed intended for the current research founded on NCBI-GenBank. Prepare the PCR reaction tubes to a ending size of 50 µl by the GoTaq Green Master Mixture Kit (Promega, USA). For the PCR reaction, the following circumstances of the Thermal Cycler system (BioRad, USA) were observed: 1 cycle for early denaturation (95°C/5 minutes); 35 cycles for denaturation (95°C/30 seconds), annealing (60°C / 30 seconds), and extension (72°C/30 seconds); and 1 cycle for final extension (72°C/7 minutes). The PCR yields stood electrophoresed in a 2% agarose gel marked with ethidium bromide at 120V for 45 minutes, and the outcomes stood examined by the gel documentation system (Syngene, Thailand) at approximately 174 bp. The primers were conducted by conventional PCR technique (means using primers specific for the ASXL1 gene (partial region) that appeared with the band with size= 174 bp), then conducting Sanger sequencing technique by Macrogen, South Korea. The data was entered and analyzed through SPSS-26.

| Primer | 5'-----3' |
|---|---|
| Forward | GGACCCTCGCAGACATTAAA |
| Reverse | CACCACCATCACCACTGCT |

# RESULTS

In the study, added sex combs-like 1 (ASXL-1) gene mutation rate in patients with CML was evaluated and the rate was 2 cases out of 51 cases, i.e. it accounted to approximately 3.9%. Also transcript types of BCR-ABL1 were also evaluated and the rates were as following: b2a2 (11.8 %), b2a3 (21.6 %), b3a3 (78.4 %), b3a2 (23.5 %) and e1a2 (17.6%) [Table 1].

**Table No.1: Rate of ASXL-1 mutations in patients with CML**

| Mutation | Positive | | Negative | |
|---|---|---|---|---|
| | No. | % | No. | % |
| ASXL-1 | 2 | 3.9 | 49 | 96.1 |

**Table No.2: The associations of ASXL-1 to BCR-ABL1 transcript types**

| Transcript type | | Positive (2 cases) | | Negative (49 cases) | | P value | Interpretation |
|---|---|---|---|---|---|---|---|
| | | No. | % | No. | % | | |
| b2a2 | Positive | 0 | 0.0 | 6 | 12.2 | 1.000 | Not significant |
| | Negative | 2 | 100.0 | 43 | 87.8 | | |
| b2a3 | Positive | 1 | 50.0 | 10 | 20.4 | 0.388 | Not significant |
| | Negative | 1 | 50.0 | 39 | 79.6 | | |
| b3a3 | Positive | 1 | 50.0 | 39 | 79.6 | 0.388 | Not significant |
| | Negative | 1 | 50.0 | 10 | 20.4 | | |
| b3a2 | Positive | 0 | 0.0 | 12 | 24.5 | 1.000 | Not significant |
| | Negative | 2 | 100.0 | 37 | 75.5 | | |
| e1a2 | Positive | 1 | 50.0 | 8 | 16.3 | 0.325 | Not significant |
| | Negative | 1 | 50.0 | 41 | 83.7 | | |

Evaluation of ASXL-1 mutations has been carried out, and results were not significant (p> 0.05) [Table 2].

The effect of ASXL-1 mutation on the response of CML patients to TKI was not significant (p> 0.05), as shown in Table 3.8. Effects of b2a2, b2a3, b3a3, b3a2, and e1a2 transcripts on the response of CML patients to TKI were not significant (p>0.05) [Table 3].

**Table No.3: Effect of ASXL-1 mutation on response of CML patients to TKI**

| Parameter | ASXL-1 | | P value |
|---|---|---|---|
| | Positive | Negative | |
| WBC | 12.25 | 8.54 (5.00) | 0.173 |
| HB | 11.95 | 12.00 (2.00) | 0.696 |
| PLT | 229.50 | 220.00 (103.00) | 0.734 |
| Molecular | 3.81 | 0.05 (4.54) | 0.593 |

# DISCUSSION

Numerous myeloid malignancies, comprising 11 percent of MDS, 43 percent of CMML, 8 percent of MPN, 20 percent of AML, and 12 to 5 percent of CML, have been linked to mutations distressing exon 12 of the ASXL1 gene. Boultwood et al were the first to describe ASXL1 mutations. and found to be a novel molecular occurrence in CML. A more comprehensive document is required to elucidate the correlation between these mutations and their predictive properties in hematological tumors, as there are differing views on the subject.[10] Sick with ASXL1 mutations differed significantly from those without mutations in terms of the risk link of ASXL1 mutations with illnessprogress (P<0.007). Numerous studies also indicate that patients with CML who have ASXL1 mutations have a poorer prognosis and worse outcomes.[11]

The most prevalent change in CP-CML was found to be an ASXL1 mutation, which was found in 12% of patients tested at diagnosis and 14% of all evaluable patients. Significantly lower EFS and FFS were linked to these mutations.[4] Previously, ASXL1 mutations were found to be the sole gene mutated in both adult and pediatric CML, suggesting that these mutations may occasionally play a significant role. Since a poorer reaction to nilotinib management was linked to the existence of an ASXL1 mutation at analysis, the training existing here offers new indication for an opposing reaction to TKI management in CML sick transport mutant ASXL1.[5]

In the present study, peripheral blood testers stayed composed as of 51 patients analyzed with chronic myeloid leukemia. The samples were transported to the laboratory under refrigerated conditions to preserve their integrity. Genomic DNA stayed first extracted from the blood samples, followed by amplification using the polymerase chain reaction (PCR) technique. The amplified DNA products were then subjected to agarose gel electrophoresis to assess the quality and integrity of the PCR products. Subsequently, DNA sequencing was performed by Sanger sequencing of ASXL1 to identify potential genetic mutations. Sequencing analysis revealed that among the 51 CML patients, only two individuals harbored a mutation in the ASXL1 gene (c.1934dupG), resulting in a Frame shift. One of the mutations was identified as a result of a deletion, and the other as a result of a TG substitution. Even though the distressed sick from whom follow-up testers stayed examined had their mutations cleared, CML sick with an ASXL1 mutation at analysis experience slower treatment responses. This implies that leukemic cells' susceptibility to TKIs and, in turn, their response to therapy are influenced by clonal progress with ASXL1 mutations in addition to BCR-ABL1. More research is needed to determine the molecular mechanism underlying this phenomenon.[12,13] Neutrophilic dysplasia brought on by mutant asxl1 may increase the risk of myeloid cancer progression and cause chronic inflammation.[14,15] The study found an ASXL-1 gene mutation rate of 3.9% (2 out of 51 cases) in CML patients. This rate is on the lower end compared to other studies, which report rates from 7.6% to 24% at diagnosis. The variation in reported rates could be due to several factors, including differences in patient groups, geographic locations, disease stages at diagnosis, and the sensitivity of the detection methods used. Although the frequency observed in this study is relatively low, ASXL-1 mutations are generally considered important in CML because they are linked to disease progression and prognosis. The study found that the influence of ASXL-1 mutation on CML patients' response to TKI stayed not statistically important (p>0.05). Similarly, the effects of b2a2, b2a3, b3a3, b3a2, and e1a2 transcripts on TKI response also showed no significance (p > 0.05). These outcomes designate that, in this study, neither ASXL-1 mutation status nor BCR-ABL1 transcript type alone could predict TKI response. This lack of significance, especially regarding ASXL-1 mutations, contrasts with a growing body of research linking ASXL-1 to poor prognosis and TKI resistance in CML. Other studies consistently report worse molecular responses, higher progression risk, and TKI resistance in CML patients with ASXL-1 mutations, which suggests that the small number of ASXL-1 positive cases in this study (n=2) may be a major limitation.

# CONCLUSION

Small fraction of CML patients carries the ASXL-1 mutation which has no impact on response to TKI. Significant proportion of CML patients carries single or multiple BCR-ABL transcript types that have been proved to have no significant impact of on response to TKI.

**Author's Contribution:**

| | |
|---|---|
| Concept & Design or acquisition of analysis or interpretation of data: | Rabab Ajmi Askar, Bassim I. Mohammad |
| Drafting or Revising Critically: | Rabab Ajmi Askar, Hussein. A Sahib |
| Final Approval of version: | All the above authors |
| Agreement to accountable for all aspects of work: | All the above authors |

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