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A Molecular Approach to the Diagnosis, Prognosis, and Treatment of *BCR-ABL1*-Positive Chronic Myeloid Leukaemia

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Review Article

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Abstract

BCR-ABL1 is a gene sequence expressed in an abnormal copy of chromosome 22 in people who are diagnosed with certain forms of leukaemia. Molecular monitoring with *BCR-ABL* real-time quantitative Polymerase chain reaction (RQ-PCR) for assessing treatment success and quantifying minimal residual disease (MRD) is a crucial determinant of practical therapeutic decision-making in chronic disease management. Chronic myelogenous leukaemia (CML) and several other leukaemia's, unlike other malignancies, are caused by a single, particular genetic mutation in one chromosome. This review article assesses CML, TKI response in targeted CML therapy, and data interpretation in molecular diagnosis. It highlights the purpose, application, and likely future of PCR-based molecular surveillance.

Keywords: *BCR/ABL*; Leukaemia; RQ-PCR; Molecular Diagnosis

Abbreviations: RQ-PCR: Real-Time Quantitative Polymerase Chain Reaction; MRD: Minimal Residual Disease; CML: Chronic Myelogenous Leukaemia; ALL: Acute Lymphocytic Leukaemia.

Introduction

Cancer is a group of diseases distinguished by the uncontrollable growth of aberrant cells capable of penetrating and harming healthy biological tissue while dividing uncontrollably. Cancer has a strong proclivity to spread throughout the tissues of the body. Cancer is the second largest cause of death in the globe. An estimated 1.24 million new instances of blood cancer are recorded each year, accounting for around 6% of all cancer cases. Hematologic malignancies, often known as blood tumours, affect blood cell growth and function. These tumours are most commonly seen in the bone marrow, which creates blood.

Chronic myeloid leukaemia (CML), a myeloproliferative neoplasm, affects 1-2 adults out of every 100,000. It is responsible for 15% of newly diagnosed adult leukaemia cases. Chronic myeloid leukaemia (CML), a myeloproliferative neoplasm, affects 1-2 out of every 100,000 adult individuals. It accounts for 15% of newly diagnosed adult leukaemia patients. In the United States, it is expected that 8450 new CML cases will be detected in 2020, with 1080 people dying as a result of the disease [1].

The breakpoint cluster region (*BCR*) is translocated as a result of a reciprocal translocation between chromosomes 9 and 22 (t (9; 22) (q34; q11), and the Abelson proto-oncogene (*ABL1*) is involved in the formation of chronic myeloid leukaemia (CML).This translocation results in the development of the *BCR-ABL1* oncogene, which codes for the chimeric *BCR-ABL1* protein, which activates tyrosine kinase constitutively [2].

This chromosomal translocation gives rise to a plethora of subtypes, the bulk of which are impacted by the distinct breakpoint positions. The *ABL1* gene's frequent site breakpoint is located in the area upstream of exon a2. Breakpoints downstream of exons e13 or e14, e1, and e19, as well as e13a2, e14a2, e1, and e19a2 that lead to fusion transcripts encoding p210, are the most common in the *BCR* gene [3] (Figure 1).

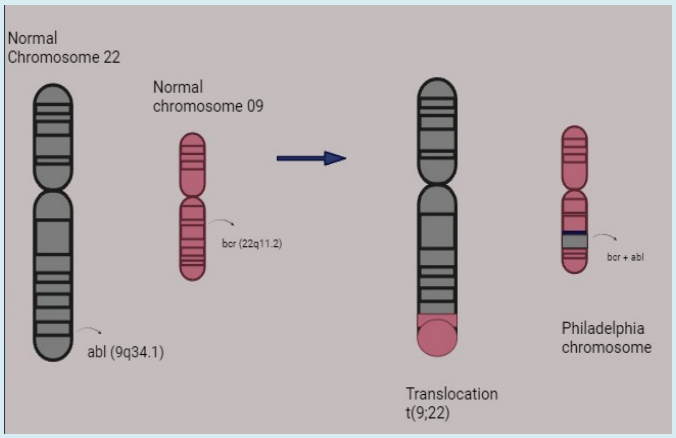

Figure 1: Shows the Chromosome 22 and 9 –wild and Philadelphia chromosome.

Role of Reverse Transcriptase (q-RT) PCR – *BCR/ABL* Diagnosis

The most sensitive and precise approach for detecting *BCR-ABL* currently available is the reverse transcriptase polymerase chain reaction (RTPCR). The level of disease burden for particular therapy can be determined by quantitative PCR for *BCR-ABL1* transcripts [4]. Reverse transcription is the first step in the quantitative PCR process. The assay searches for the more prevalent chimeric RNA transcripts generated by the fused genes rather than chromosomal DNA. The results were used to diagnose cases that were suspected and to evaluate molecular responses to therapy and recurrence [5] (Figure 2).

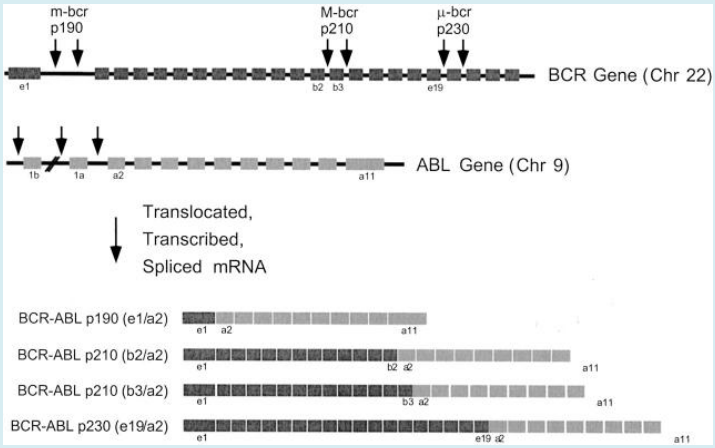

Figure 2: Diagrammatic representation of the *BCR* and *ABL* genes and the corresponding *BCR-ABL* fusion transcripts. Arrows designate three different breakpoint cluster regions in the BCR gene [6-8]. Breaks in the M-BCR are most commonly associated with CML, the m-BCR with ALL, and the µ-BCR with chronic neutrophil leukaemia. *ABL* breakpoints usually occur between exons 1a and 2, resulting in fusion transcripts containing the tyrosine kinase domain of the *ABL* gene and variable portions of the *BCR* gene [9].

Whole blood or bone marrow cell RNA extraction yields high-quality total RNA, which is then specifically transcribed into the *BCR/ABL* fusion gene proteins using target-specific probes and primers. A reporter fluorescent dye at the 5′ end and a quencher non-fluorescent dye at the 3′ end of an oligonucleotide Taqman probe hybridise to the template. The

reporter and quencher were separated during the extension phase of the PCR reaction utilising the Taq polymerase enzyme's endonuclease activity, allowing the monitoring PCR system to detect fluorescence. Every cycle of the PCR will be quantified by measuring the amplicons based on changes in the fluorescence signals; the final quantification is based on the cycle threshold (Ct) value [10-12].

According to the manufacturer's instructions, standard curve plots were created using established quantitative standards from 10 to 1 lakh copies or higher based on the standard IVD certified kit. Based on the linear relationship between the Ct value and the quantity of known template, a standard curve was created [13].

One damaged cell can be found out of 100,000 healthy cells using amplification assays. Because of its high sensitivity, RT-PCR is a good tool for determining if a patient has minimal residual disease after treatment. The entire *BCR-ABL1* quantification procedure, including sample RNA extraction, RNA quality, reverse transcription, PCR amplification efficiency, control gene selection, and result expression, definitely had an impact on the outcome.

BCR-ABL Gene & Protein

BCR-ABL results in juxtaposition of the 3′ sequences of *ABL* on chromosome 9 and the 5′ sequences of the truncated *BCR* on chromosome 22. Consequently, the *BCR/ABL* fusion gene forms on chromosome 22, which encodes *BCR-ABL1* oncoprotein, a constitutively active tyrosine kinase.

The constitutive tyrosine kinase activity of the chimeric *BCR-ABL1* oncoprotein deregulates the downstream signalling of many pathways, resulting in uncontrolled proliferation, arresting the differentiation of hematopoietic stem cells (HSC), and limiting apoptosis. Consequently, normal HSC transform into leukemic stem cells (LSC), which accumulate in the bone marrow (BM) and replace normal stem cells.

Three different *BCR-ABL* chimeric proteins emerged from the fusion of mRNA molecules of different lengths of the *ABL* gene with the *BCR* gene. The most common breakpoint in *BCR* occurs in intron 13 or intron 14, followed by exon 13 or 14 exons (M-BCR) fused to the *ABL1* gene at exon a2, which is referred to as e13a2 or e14a2. These fusions result in a *BCR-ABL1* protein with a molecular mass of 210 k5Da (p210 *BCR-ABL1*), present in 95% of CML cases [14]. The second alternative (<1%) is that the e19a2 fusion transcript produces a larger fusion protein with 230 kilo Dalton weight (p230 *BCR-ABL1*), a diagnostic marker for neutrophil-chronic myeloid leukaemia. The third alternative is the p190 *BCR-ABL1* protein resulting from the e1a2 fusion transcript, which is most common in B-cell ALL, less so in AML, and rarely present in CML (Figure 3) [15].

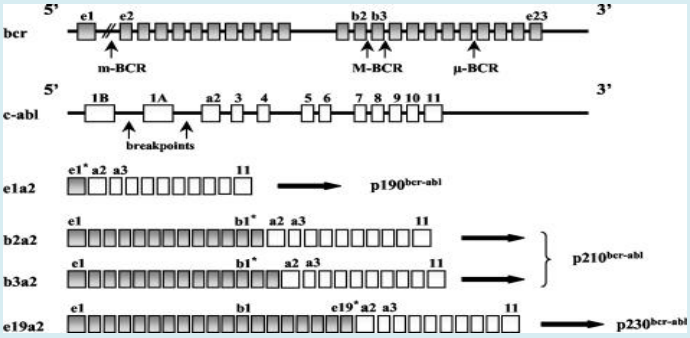

Figure 3: Molecular structure of BCR and c-ABL genes and their main translocated transcripts. Arrows indicate breakpoints in BCR and c-ABL. **Asterisks** show sense primers which are used for the detection of different transcripts.

The *BCR-ABL* oncoprotein possesses different domains from *BCR* and *ABL*. The BCR portion includes the NH2-terminal coil-coil (CC) domain (amino acid 1-63), an oligomerization domain, followed by a serine/threonine kinase domain-containing Tyr 177, a binding site for growth factor receptor-binding protein 2 (GRB2), and Ras homolog gene/guanine nucleotide exchange factor (Rho/GEF) kinase domain, a GTPase-activating protein that is activated when bound to GTP and turned off when bound to GDP, thus controlling the activation of Ras protein. The *ABL* domains include Src homology (SH) SH3, SH2, and SH1 (kinase) domains, followed by proline-rich domain and binding domain (BD), and facilitation of nuclear protein, actin, and DNA binding. CC domain and Y177 are required for efficient *ABL* kinase activation [16]. Tetramerization of *BCR-ABL* is essential for constitutive kinase activity. Targeting the CC domain deregulates the tetramerization of *BCR-ABL*, reduces kinase activity, and increases sensitivity to imatinib

(a tyrosine kinase inhibitor). Tyrosine kinase (TK) regulates cell cycle activity in a normal physiological state. Constitutive activation of TK interrupts the normal state of the cell cycle, resulting in inhibition of cell differentiation, uninterrupted activation of cell proliferation, and escape of cell apoptosis. Therefore, *BCR-ABL* deregulates several pathways [17-20].

The *ABL* protein shuttles between the nucleus and cytoplasm; however, the chimeric *BCR-ABL* oncoprotein stops this shuttle and retains the *ABL* protein in the cytoplasm, where it interacts with many different proteins that are involved in malignant transformation. In the *BCR-ABL* fusion, the N-terminal oligomerization domain of *BCR* activates *ABL* tyrosine kinase by blocking SH3 on the N-terminal of *ABL1*. Accordingly, the *BCR-ABL* oncoprotein activates diverse signalling pathways, including the RAS, JAK2/STAT, and PI3K-AKT-mTOR pathways. *BCR-ABL* oncoprotein directly activates the JAK2/STAT pathway and continuously enhances CML cell survival [21].

Clinical application of *BCR-ABL1* measurement

In CML, the *BCR-ABL1* fusion gene serves as both a diagnostic marker and a therapeutic target. Its measurement is primarily used to track patient reactions to therapy in order to assess therapeutic efficacy, and the transcript level offers an indicator of potential prognosis. At three months, a full haematological response is anticipated for the criteria of normalizing PB numbers and having no additional medullary involvement. A complete cytogenetic response (CCyR), which is strongly associated to a hopeful remission, is based on cytogenetic analysis of BM samples and the absence of Ph+ chromosomes (at least 20 cells in metaphase).

Imatinib can cause faster and substantial responses compared with interferon plus cytarabine and analysis of the long-term data monitoring proved the evidence of the predictive value of molecular responses. This indicated that patients who achieved CCyR or Major molecular response (MMR) had significantly lower progression rates. Cytogenetic and molecular responses at 6, 12 and 18 months are the three landmarks to indicate that patients have continuous event-free survival (EFS), accelerated phase/blast crisis (AP/BC) and overall survival, or they do not.

MMR is a milestone for monitoring prognostic significance in patients undergoing treatment. However, deep molecular responses and *BCR-ABL1* transcript levels below the MMR were achieved in some patients with the advent of new TKI therapy. The term MR4 (≥4 log reduction from IRIS baseline) is defined as either *BCR-ABL1* level of ≤0.01% *BCR-ABL1* (IS) or undetectable disease in cDNA with ≥10 000 *ABL1* transcripts; MR4.5 as ≤0.0032% *BCR-ABL1* (IS) or undetectable disease in cDNA with ≥32 000 *ABL1* transcripts, corresponding to the definition of MMR. Deep molecular responses depend on the sensitivity and ability of the testing laboratories, and achievements of deep responses correlate with better long-term prognosis and can also be regarded as an indication for discontinuation of treatment in CML [22].

Moreover, *BCR-ABL* kinase domain (KD) mutations are found in imatinib-resistant patients and may have increased *BCR-ABL1* levels. The incidence of KD mutations is related to treatment failure and a high risk of disease progression; therefore, so a study showed that KD mutation detection should be performed if there is a two-fold increase in *BCR-ABL1* levels.

Response of TKIs

The *BCR-ABL* fusion protein, a key leukemia factor, has been targeted with Imatinib (Gleevec) for treating CML. However, resistance has increased due to gene mutations or amplification, prompting the development of new tyrosine kinase inhibitors (TKIs) to combat this issue [23-25].

TKIs have dramatically changed CML treatment and prognosis. TKI target proliferating mature *BCR-ABL* cells, which are considered major drivers of CML. However, a minority of subclones with genetic aberrations and quiescent leukemic stem cells have not been eliminated by TKI. Molecular resistance was measured by quantitative PCR for *BCR-ABL1* and cytogenetic resistance (Ph+ persistence). These procedures are considered the main standards for monitoring patient response to therapy, predicting relapse, and guiding treatment decisions.

Binding of ATP to the tyrosine kinase active site of the *BCR-ABL* oncoprotein phosphorylates the tyrosine residue of the substrate, resulting in CML progression. TKI impede ATP-tyrosine kinase binding, thereby halting the constitutive tyrosine kinase activity of the *BCR-ABL1* oncoprotein and inhibiting CML progression. There are three types of TKI. Type I is an ATP competitive inhibitor, such as imatinib and nilotinib, which competes with ATP in the ATP-binding site of the kinase domain. Consequently, this prevents auto phosphorylation and substrate phosphorylation, thus inhibiting proliferation and inducing apoptosis in *BCR-ABL* cells. Type II is ATP competitive inhibitors like dasatinib, which bind to the ATP-binding site as well as the hydrophobic adjacent binding site which is accessible only when the kinase is in an inactive configuration. The third type is allosteric inhibition, which involves the binding of TKI to the allosteric-myristate binding pocket, induces a conformational change in the kinase domain, and renders the kinase-inactive [26].

The dominant mechanism of imatinib resistance is an acquired mutation in the kinase domain (KD) of *BCR-ABL* with greater frequency in patients with AP and BP CML than in patients with CP. These mutations reside in the phosphate-binding loop at positions M244, G250, Q252H, Y253H/F, E255K/V, gatekeeper (T315, F317), activation lobe (H396), SH2, and C-lobe (M351, F359) that ultimately impair TKI binding by affecting the essential residue for direct contact or by preventing the inactive conformation of the *ABL* kinase domain appropriate for imatinib binding. Nilotinib shares the same binding site as imatinib with significantly higher affinity that overcomes many imatinib-resistant mutations, except for Y253, E255, T315I, and F359 mutations.

Data Interpretation

Draws the ideal trajectory towards ideal results, as determined by real-time quantitative reverse transcription-polymerase chain reaction (RQ-PCR). Tyrosine kinase inhibitors (TKI) for *BCR-ABL* have improved the prognosis, leading to higher overall patient survival and slower rates of disease progression. By comparing changes in *BCR ABL1* transcript levels to a standard pre-treatment baseline level, set at 100% (IRIS Study, Imatinib treatment), the effectiveness of TKI therapy is assessed. A 3-log decrease in *BCR-ABL1* transcript levels from this reference baseline is regarded as 0.1% on the International scale (IS), which denotes a major molecular response (MMR) [27]. In comparison to individuals who did not achieve MMR, those who did had a lower risk of disease progression and a better long-term Progression-free Survival (PFS). Therefore, it is essential to keep an eye on *BCR*.

MMR The degree of the *BCR-ABL1* transcript level drop in peripheral blood serves as a measure of the molecular response. A qPCR test is employed to calculate the percentage of blood cells that carry the *BCR-ABL1* gene. The early molecular reaction of a patient to treatment is important for forecasting results and deciding on additional treatment options. The most accurate way to measure blood levels of *BCR-ABL1* is by the molecular response. When the *BCR-ABL1* level is 10% or less three and six months after the commencement of treatment, this is known as an early molecular response (EMR). The leukaemia cells have therefore been decreased by 90% or more, according to this. When the *BCR-ABL1* level is down to 0.1 percent, MMR.This indicates that 99.9% or more of the leukaemia cells have been eliminated. When the *BCR-ABL1* level drops to 0.01 percent or less, a deep molecular response (DMR) occurs [28].

The outcomes of qPCR tests are measured using the International Scale (IS), which is a standardized scale. The results of the qPCR test show how many cells carry the *BCR-ABL1* gene. It is employed to assess how well a course of treatment is working. *BCR-ABL1* at 100 percent is the established baseline according to the International Scale (IS). This baseline was created using data from the IRIS clinical study, which involved examining a sizable sample of pre-treatment patient samples and normalizing the mean patient results. The beginning of treatment is referred to as the "baseline". All CML patients follow the standardized International Scale baseline Figure 4.

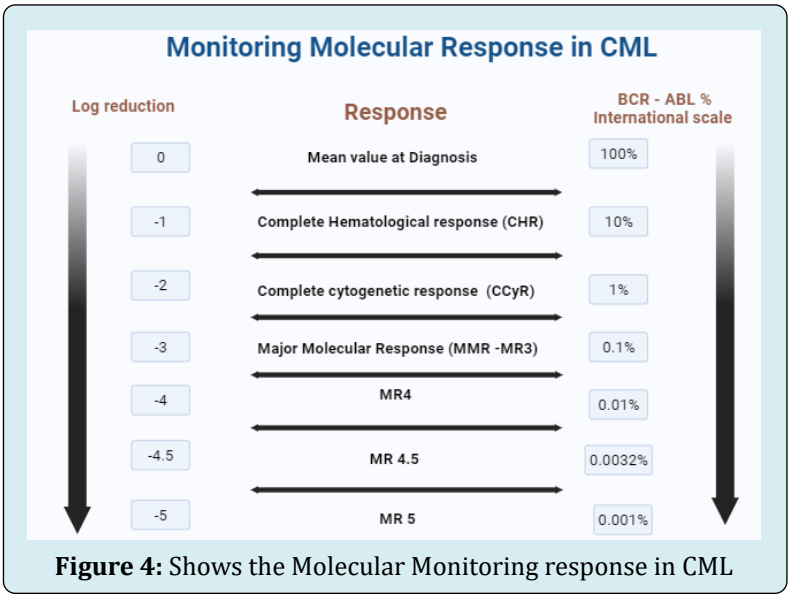

Figure 4: Shows the Molecular Monitoring response in CML

A log reduction is a measurement that shows a change in the *BCR-ABL1* level from the reference baseline. A 1-log decline indicates that the *BCR-ABL1* level has dropped to a level that is 10 times lower than the established baseline. The *BCR-ABL1* gene is present in 10% of cells, or 100 cells out of every 1000. When this reduction is attained within

3 to 6 months of starting treatment, it is equal to an early molecular response. The *BCR-ABL1* level has dropped to a level 100 times lower than the established baseline, or a 2-log decline. The *BCR-ABL1* gene is present in 1% of cells, or 1 out of every 100 cells.

The *BCR-ABL1* level has dropped to 1,000 times below the established baseline, or a 3-log decline. This indicates that the *BCR-ABL1* gene is present in 0.1 percent of cells (1 out of 31 for every 1,000 cells). Another name for it is "major molecular response (MMR)." A 4-log reduction indicates that the *BCR-ABL1* gene is present in 0.01% of cells, or 1 out of every 10,000 cells. 4.5-log reduction is known as a "complete molecular response (CMR)" or a "deep molecular response (DMR)." Doctors would call this "MR4.5." The *BCR-ABL1* gene is present in 0.0032% of cells, or 1 out of every 32,000 cells, according to a 4.5-log reduction. A deep molecular response can indicate the remission of a disease.

Under close physician monitoring, patients who develop and then maintain a profound molecular response for a significant amount of time may be considered candidates for stopping TKI therapy. The *BCR-ABL1* gene is present in 0.001% of cells, or 1 out of 100,000, according to a 5-log reduction. The patients have attained undetectable *BCR-ABL1* by achieving a 5-log decrease.

Summary

Cancer is a disease characterized by abnormal cell proliferation, causing harm to healthy tissue and causing death. Blood cancer accounts for 6% of all cancer cases, while chronic myeloid leukaemia affects 1-2 out of 100,000 adults and accounts for 15% of new cases. The *BCR* gene's breakpoint positions impact subtypes. The reverse transcriptase polymerase chain reaction (RT-PCR) is the most sensitive method for detecting *BCR-ABL*. It determines the disease burden for specific therapy by analysing chimeric RNA transcripts. The process involves RNA extraction, transcription, and fluorescence detection. RT-PCR is a reliable tool for determining residual disease after treatment, with the entire process impacting outcomes.

The *BCR-ABL1* fusion gene is a diagnostic marker and therapeutic target in CML, with a complete cytogenetic response (CCyR) and Major molecular response (MMR) indicating lower progression rates. Deep molecular responses correlate with better long-term prognosis and may indicate discontinuation of treatment. *BCR-ABL* kinase domain mutations in imatinib-resistant patients may increase *BCR-ABL1* levels, increasing the risk of disease progression.

Tyrosine kinase inhibitors (TKI) for *BCR-ABL* have improved prognosis, leading to higher patient survival and slower disease progression. A 3-log decrease in *BCR-ABL1* transcript levels is considered a major molecular response (MMR), with those who achieve MMR having a lower risk of disease progression and better Progression-free Survival (PFS). The International Scale (IS) measures the effectiveness of treatment, with *BCR-ABL1* at 100% being the established baseline. TKIs, including imatinib, nilotinib, dasatinib, and allosteric inhibition, impede ATP-tyrosine kinase binding, halting constitutive tyrosine kinase activity and inhibiting CML progression. These inhibitors are essential for monitoring patient response and treatment decisions. Further research and development of novel therapeutic agents are warranted to achieve better treatment outcome.

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