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THE SIGNIFICANCE OF THE BCR::ABL1 BIOMARKER IN THE DIAGNOSIS AND TREATMENT MONITORING OF CHRONIC MYELOID LEUKEMIA: A NARRATIVE REVIEW

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ABSTRACT

Chronic myeloid leukemia is defined by the presence of the BCR::ABL1 fusion, which plays a central role in disease pathogenesis, diagnosis, treatment selection, and molecular monitoring. This narrative review aimed to summarize the current clinical significance of BCR::ABL1, with particular emphasis on diagnostic methods, assessment of response to tyrosine kinase inhibitors, mechanisms of resistance, and treatment-free remission. A literature search was conducted using PubMed and Google Scholar, focusing primarily on English-language guidelines, review articles, and clinically relevant studies published between 2015 and 2025, while also including earlier landmark publications. The review shows that cytogenetic analysis, fluorescence in situ hybridization, and reverse-transcription polymerase chain reaction provide complementary diagnostic information. Serial RT-qPCR measurement of BCR::ABL1 on the International Scale remains the standard method for assessing the depth and kinetics of molecular response. Interpretation should consider previous results, treatment adherence, dose modifications, assay variability, and the overall clinical context. Detection of kinase-domain mutations, particularly T315I, may explain treatment resistance and guide subsequent TKI selection. Targeted next-generation sequencing and digital PCR may improve the detection of resistant subclones and low-level residual disease, but their findings should not be used as isolated treatment criteria. Overall, accurate and standardized BCR::ABL1 monitoring remains essential for individualized CML management, while further research is needed to validate additional prognostic markers and improve prediction of treatment-free remission.

KEYWORDS

Chronic Myeloid Leukemia, BCR::ABL1, Molecular Monitoring, RT-qPCR, Tyrosine Kinase Inhibitors, Treatment-Free Remission

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Introduction

Chronic myeloid leukemia (CML) is a clonal myeloproliferative neoplasm arising from a transformed hematopoietic stem cell (Jabbour & Kantarjian, 2024; Khoury et al., 2022) and characterized by the expansion and accumulation of myeloid cells in the bone marrow and peripheral blood (Arber et al., 2022). It accounts for approximately 15% of newly diagnosed leukemias in adults (Jabbour & Kantarjian, 2024).

A defining feature of CML is the presence of the Philadelphia chromosome, which results from a reciprocal translocation involving the long arms of chromosomes 9 and 22 (Jabbour & Kantarjian, 2024; Rowley, 1973). This rearrangement leads to the fusion of the BCR and ABL1 genes and the formation of the BCR::ABL1 fusion gene (Cross et al., 2023). The protein encoded by this gene has constitutive tyrosine kinase activity, which promotes the proliferation of myeloid cells and enhances the survival of the leukemic clone (Arber et al., 2022; Deininger et al., 2000).

Because of its direct involvement in the pathogenesis of the disease, BCR::ABL1 is regarded as the principal biomarker of CML (Jabbour & Kantarjian, 2024). Its detection is essential for diagnosis, while quantitative assessment of the transcript level makes it possible to monitor the effectiveness of tyrosine kinase inhibitor therapy (Cross et al., 2023). Serial measurements are also used to determine the depth of molecular response, identify an inadequate response or emerging resistance, and support decisions regarding the continuation or modification of treatment (Apperley et al., 2025; Branford, 2016; Press et al., 2013). Consequently, monitoring of this biomarker should remain an integral part of the long-term care of every patient with CML (Apperley et al., 2025; Branford, 2016).

The development of molecular diagnostics has profoundly changed modern hematology., as contemporary methods allow not only for the precise diagnosis of hematologic malignancies, but also for prognostic assessment, monitoring of treatment response, and early detection of therapeutic resistance (Arber

et al., 2022; Cross et al., 2023). The identification of fusion genes and clinically relevant mutations makes it possible to define the molecular subtype of a disease more accurately and, in selected cases, to choose a treatment directed against a specific molecular alteration (Alikian et al., 2017; Apperley et al., 2025).

In CML, quantitative reverse-transcription polymerase chain reaction, or RT-qPCR, is of particular importance, as it enables both the detection of the BCR::ABL1 transcript and the quantitative assessment of its level in the examined sample (Alikian et al., 2017; Cross et al., 2023; Press et al., 2013; White et al., 2022). Fluorescence in situ hybridization, or FISH, also has a complementary role because it allows chromosomal rearrangements to be identified directly within cells (Cross et al., 2023).

The aim of this narrative review is to present current knowledge regarding the significance of BCR::ABL1 in the diagnosis of chronic myeloid leukemia, with particular emphasis on its role in monitoring treatment response and on the use of molecular findings in clinical decision-making.

Methodology

This study was designed as a narrative review aimed at analyzing the current state of knowledge concerning the importance of BCR::ABL1 in the diagnosis and treatment monitoring of chronic myeloid leukemia. The SANRA scale was consulted during the preparation of this narrative review to support a clear statement of the review aim, a transparent description of the literature search, appropriate referencing, and a balanced presentation of the available evidence (Baethge et al., 2019)

The literature search was conducted using the PubMed and Google Scholar databases. Relevant publications were identified with keywords and combinations of terms including chronic myeloid leukemia, CML, BCR::ABL1, Philadelphia chromosome, molecular diagnostics, molecular monitoring, RT-qPCR, tyrosine kinase inhibitors, and treatment-free remission.

The review primarily included English-language publications released between 2015 and 2025. Particular attention was paid to current clinical guidelines, review articles, and studies of substantial clinical relevance. Earlier landmark publications that contributed significantly to the development of molecular diagnostics and CML treatment were also considered.

Recommendations issued by international scientific organizations, particularly the European LeukemiaNet, were included alongside publications addressing the molecular biology of CML, methods used to detect BCR::ABL1, molecular response monitoring, and the clinical application of successive generations of tyrosine kinase inhibitors (Jabbour & Kantarjian, 2024). Conference abstracts without an available full text, publications of limited scientific value, and articles not directly related to the subject of the review were excluded.

The selected publications were subjected to a qualitative analysis, which focused on the current applications of BCR::ABL1 in the diagnosis of CML, the monitoring of treatment response, and the selection or modification of therapeutic strategies.

Results

Pathogenesis of CML and Formation of the BCR::ABL1 Fusion Gene

A characteristic feature of chronic myeloid leukemia is the presence of the Philadelphia chromosome. It is formed through a reciprocal translocation, involving the exchange of genetic material between the long arms of chromosomes 9 and 22, which is conventionally described as t(9;22)(q34;q11) (Cross et al., 2023; Deininger et al., 2000; Khoury et al., 2022).

As a consequence of this translocation, a fragment of the ABL1 gene located on chromosome 9 becomes fused with a fragment of the BCR gene located on chromosome 22 (Cross et al., 2023). This rearrangement creates the BCR::ABL1 fusion gene, which encodes an abnormal protein with constitutive, continuously active tyrosine kinase activity (Cross et al., 2023; Deininger et al., 2000; Jabbour & Kantarjian, 2024).

Under physiological conditions, tyrosine kinases participate in the regulation of cell growth, differentiation, and survival, and their activity is subject to strict control. In CML, however, the BCR::ABL1 kinase remains persistently active, leading to the stimulation of several intracellular signaling pathways, including RAS/MAPK, PI3K/AKT, and JAK/STAT (Deininger et al., 2000; Jabbour & Kantarjian, 2024).

Activation of these pathways results in excessive proliferation of hematopoietic cells, inhibition of apoptosis, impaired adhesion of cells to the bone marrow stroma, and increased genomic instability (Deininger

et al., 2000). Together, these mechanisms contribute not only to the development of CML, but also to its subsequent progression (Khoury et al., 2022).

The BCR::ABL1 fusion gene is considered the molecular hallmark of CML and is detected in the vast majority of patients (Press et al., 2013). Because of its central role in disease pathogenesis, it is not only the key diagnostic biomarker, but also the principal target of molecularly directed treatment with tyrosine kinase inhibitors (Apperley et al., 2025; Hochhaus et al., 2017; O'Brien et al., 2003). Monitoring the level of its transcript allows clinicians to evaluate treatment effectiveness and identify treatment failure before overt clinical progression occurs (Apperley et al., 2025; Press et al., 2013).

It is important to distinguish between the Philadelphia chromosome, which is a cytogenetically detectable abnormality, and the BCR::ABL1 fusion gene that results from the underlying rearrangement (Apperley et al., 2025; Cross et al., 2023). Although the classical t(9;22) is identified in most patients, variant translocations may occur, and approximately 1–5% of patients harbor cryptic BCR::ABL1 fusions that are not visible by conventional chromosome banding analysis (Apperley et al., 2025). Accordingly, the diagnosis of CML depends on the detection or inference of BCR::ABL1 using complementary cytogenetic and molecular methods, including chromosome banding analysis, FISH, and RT-PCR (Apperley et al., 2025; Cross et al., 2023)..

BCR::ABL1 as a Diagnostic Biomarker

The detection of the BCR::ABL1 fusion gene is fundamental to the diagnosis of chronic myeloid leukemia (Khoury et al., 2022). Confirmation of the disease requires the demonstration of the t(9;22)(q34;q11.2) translocation, the Philadelphia chromosome, the BCR::ABL1 fusion gene, or its transcript (Cross et al., 2023). When BCR::ABL1 is not identified despite clinical and hematologic findings suggestive of CML, the possibility of an atypical transcript or a cryptic rearrangement should first be excluded using complementary molecular and cytogenetic methods (Apperley et al., 2025; Cross et al., 2023). If BCR::ABL1 remains undetectable, another myeloid neoplasm should be considered (Arber et al., 2022; Cross et al., 2023).

Three main groups of methods are used in the diagnosis of CML: conventional cytogenetic analysis, fluorescence in situ hybridization, and polymerase chain reaction-based molecular techniques (Cross et al., 2023). These methods provide distinct and complementary information and should not be regarded as fully interchangeable, as each has specific diagnostic capabilities and limitations (Cross et al., 2023; Rack et al., 2019).

According to European LeukemiaNet recommendations, the initial diagnostic evaluation should include hematologic assessment, conventional cytogenetic analysis of bone marrow, and RT-PCR testing performed on peripheral blood or bone marrow (Apperley et al., 2025). This approach allows clinicians to determine the disease phase, detect variant translocations and additional chromosomal abnormalities, and identify the exact BCR::ABL1 transcript type (Cross et al., 2023). These findings are essential for subsequent molecular monitoring and therapeutic decision-making (Apperley et al., 2025; Cross et al., 2023).

Conventional Cytogenetic Analysis

Conventional cytogenetic analysis, also known as chromosome banding analysis, involves the examination of the karyotype in metaphase cells. The test is preferably performed using material obtained by bone marrow aspiration, although a karyotype may sometimes be obtained from peripheral blood at diagnosis (Cross et al., 2023).

This method enables the Philadelphia chromosome to be identified and the proportion of Ph-positive metaphases to be determined (Cross et al., 2023). It can also detect additional chromosomal abnormalities, some of which are associated with an increased risk of disease progression and adverse clinical outcomes (Arber et al., 2022; Cross et al., 2023).

The principal advantage of conventional cytogenetic analysis is that it provides an assessment of the entire karyotype rather than examining only the BCR::ABL1 rearrangement. It may therefore reveal additional abnormalities such as a second Philadelphia chromosome, trisomy 8, isochromosome 17q, or trisomy 19 (Arber et al., 2022; Cross et al., 2023).

The limitations of conventional cytogenetic analysis include its dependence on obtaining an adequate number of metaphase cells and its inability to detect cytogenetically cryptic BCR::ABL1 rearrangements. Approximately 1–5% of patients have a cryptic BCR::ABL1 fusion without visible involvement of chromosomes 9 or 22; consequently, the karyotype may appear normal despite the presence of CML (Cross et al., 2023).

Fluorescence In Situ Hybridization

Fluorescence in situ hybridization, or FISH, uses fluorescently labeled probes that bind to defined regions of the BCR and ABL1 genes. A characteristic arrangement of the fluorescent signals indicates the presence of the BCR::ABL1 rearrangement (Cross et al., 2023).

FISH may be performed on metaphase cells or interphase nuclei and, unlike conventional chromosome banding analysis, does not require the examination of actively dividing cells (Cross et al., 2023). Interphase FISH is commonly performed using peripheral blood leukocytes and may provide an alternative diagnostic approach when bone marrow material is unavailable or insufficient (Cross et al., 2023; Jabbour & Kantarjian, 2024; Press et al., 2013).

The advantages of FISH include a relatively short turnaround time and the ability to detect most BCR::ABL1 rearrangements, including cryptic rearrangements that may not be apparent by conventional chromosome banding analysis (Apperley et al., 2025; Cross et al., 2023). Metaphase FISH may also help confirm the location of BCR::ABL1 in patients with variant translocations or cryptic rearrangements (Cross et al., 2023).

FISH is nevertheless a targeted method and assesses only the genomic regions recognized by the probes used. It does not provide a comprehensive evaluation of the karyotype and generally does not detect additional chromosomal abnormalities unrelated to the BCR::ABL1 rearrangement (Apperley et al., 2025; Cross et al., 2023). Moreover, FISH cannot determine the BCR::ABL1 transcript type and has insufficient sensitivity for routine monitoring of deep molecular response, for which RT-qPCR or RT-dPCR should be used (Apperley et al., 2025; Cross et al., 2023; Press et al., 2013).

RT-PCR and RT-qPCR

Molecular methods based on reverse transcription allow the RNA transcript of BCR::ABL1 to be detected. During the first stage of the procedure, RNA is converted into complementary DNA, which is subsequently amplified (Cross et al., 2023).

Qualitative RT-PCR confirms the presence of the transcript and identifies its type. The most common transcripts are e13a2 and e14a2, both of which encode the p210 protein (Baccarani et al., 2019; Zenebe et al., 2023). A small proportion of patients have atypical variants, including e1a2, e19a2, e13a3, or e14a3 (Baccarani et al., 2019; Cross et al., 2023; Kearney et al., 2020; Zenebe et al., 2023).

Identification of the transcript type before treatment begins is clinically important because it makes it possible to select an appropriate assay for subsequent disease monitoring (Cross et al., 2023; Zenebe et al., 2023). Diagnostic assays restricted to e13a2 and e14a2 may fail to detect atypical fusions; therefore, a negative standard RT-PCR result in a patient with positive cytogenetic or FISH findings should prompt testing with a broader multiplex assay, followed by sequencing when necessary (Cross et al., 2023; Kearney et al., 2020; Zenebe et al., 2023). Quantitative RT-qPCR determines the number of BCR::ABL1 transcript copies relative to a reference gene, most commonly ABL1, GUSB, or BCR (Cross et al., 2012; Zenebe et al., 2023). It is currently the principal method used to monitor molecular response during treatment with tyrosine kinase inhibitors (Alikian et al., 2017; Press et al., 2013; White et al., 2022).

The main advantages of RT-qPCR include high analytical sensitivity, quantitative assessment of disease burden, and the ability to use peripheral blood for serial monitoring (Alikian et al., 2017; Cross et al., 2023; Press et al., 2013; Zenebe et al., 2023). Serial measurements allow the kinetics and depth of molecular response to be evaluated and may identify loss of response or molecular relapse before overt hematologic progression becomes apparent (Cross et al., 2023; Press et al., 2013; White et al., 2022).

The result may, however, be affected by RNA quality, the collection and storage conditions of the sample, the reference gene used, and the degree of laboratory standardization (Cross et al., 2023). Reliable monitoring therefore requires validated methods, appropriate quality control, and proper calibration of reported results (Cross et al., 2023; White et al., 2022).

None of the methods used in the diagnosis and monitoring of CML provides all the required information when applied alone. Conventional chromosome banding analysis enables assessment of the complete karyotype and the detection of additional chromosomal abnormalities, whereas FISH provides targeted cellular detection of the BCR::ABL1 rearrangement. Qualitative RT-PCR identifies the BCR::ABL1 transcript type, which is required for the selection of an appropriate monitoring assay (Cross et al., 2023). Quantitative RT-qPCR, in turn, remains the principal method for the long-term assessment of molecular response to tyrosine kinase inhibitor therapy (Alikian et al., 2017; Cross et al., 2023; Press et al., 2013; White et al., 2022)..

The Significance of BCR::ABL1 in Treatment Monitoring

The Role of Tyrosine Kinase Inhibitors

The introduction of tyrosine kinase inhibitors into the treatment of CML fundamentally altered the clinical course of the disease (Apperley et al., 2025; Press et al., 2013). These agents inhibit the activity of the BCR::ABL1 kinase and thereby suppress the intracellular signals responsible for the proliferation and survival of leukemic cells (Hochhaus et al., 2017; O'Brien et al., 2003).

Established first-line TKI options for patients with newly diagnosed chronic-phase CML include imatinib, dasatinib, nilotinib, and bosutinib (Apperley et al., 2025; Jabbour & Kantarjian, 2024). Asciminib has also become available as a first-line treatment option in some jurisdictions following the results of the ASC4FIRST trial, which demonstrated superior molecular efficacy and a favorable safety profile compared with investigator-selected TKIs (Apperley et al., 2025; Hochhaus et al., 2024). In later treatment lines, asciminib and ponatinib are important therapeutic options for patients with resistant or intolerant disease, with ponatinib having particular relevance in the presence of the BCR::ABL1 T315I mutation (Apperley et al., 2025; Cortes et al., 2021; Cortes et al., 2018). The selection of an individual TKI should take into account the disease phase, clinical risk, treatment goals, comorbidities, previous response or intolerance, the adverse-effect profiles of the available agents, and the presence of mutations affecting sensitivity to individual drugs (Apperley et al., 2025; Cortes et al., 2021; Cortes et al., 2018; Jabbour & Kantarjian, 2024).

Imatinib is a first-generation inhibitor and was the first targeted agent to produce a major improvement in treatment outcomes for patients with CML (Hochhaus et al., 2017). Second-generation inhibitors, including dasatinib, nilotinib, and bosutinib, may lead to a faster molecular response, although they differ considerably in their toxicity profiles (Brummendorf et al., 2022; Cortes et al., 2016; Kantarjian et al., 2021). Dasatinib may cause pleural effusion (Cortes et al., 2016), nilotinib is associated with metabolic complications (Apperley et al., 2025) and cardiovascular events (Kantarjian et al., 2021), while bosutinib may result in diarrhea and liver dysfunction (Brummendorf et al., 2022). Ponatinib remains an important therapeutic option in resistant disease, particularly in the presence of the T315I mutation, although its use requires careful assessment and management of cardiovascular risk (Cortes et al., 2021; Cortes et al., 2018).

The effectiveness of TKI therapy is evaluated primarily through regular measurement of the BCR::ABL1 transcript level in peripheral blood leukocytes (Cross et al., 2023; Press et al., 2013). A decrease in this level reflects a reduction in the size of the leukemic clone, whereas sequential measurements provide information about the kinetics of response and may reveal loss of treatment efficacy or emerging resistance at an early stage (Apperley et al., 2025; Cross et al., 2023).

Results obtained after 3, 6, and 12 months of therapy are particularly important (Apperley et al., 2025; Hughes et al., 2010). At each of these time points, the response is assessed to determine whether treatment is progressing as expected and whether the existing regimen should be continued or modified (Hughes et al., 2010).

Monitoring the BCR::ABL1 Transcript Level

Quantitative RT-qPCR is the principal method used to assess BCR::ABL1 transcript levels and monitor molecular response during TKI therapy (Branford, 2016; Cross et al., 2023; Press et al., 2013; White et al., 2022). Molecular monitoring should be performed at least every three months until major molecular response has been achieved and confirmed. Thereafter, testing at four- to six-month intervals is appropriate in patients who maintain a stable MMR or deeper molecular response (Apperley et al., 2025).

More frequent monitoring is recommended when BCR::ABL1 transcript levels fluctuate or rise, when the kinetics of response are unclear, and when treatment toxicity or intolerance results in dose interruption or reduction (Apperley et al., 2025). Frequent molecular assessment is also required when evaluating eligibility for TKI discontinuation and during follow-up after treatment has been stopped (Apperley et al., 2025; Cross et al., 2023).

The International Scale

BCR::ABL1 results should be reported as a percentage on the International Scale (IS), which was developed to harmonize molecular monitoring and improve the comparability of results generated by different laboratories (Branford, 2016; Cross et al., 2023; Cross et al., 2012; Press et al., 2013; White et al., 2022). Meaningful interlaboratory comparison requires the use of an assay that has been appropriately calibrated to the IS, either through a validated laboratory-specific conversion factor or a system traceable to World Health Organization reference material (Cross et al., 2023; White et al., 2022).

A value of 100% BCR::ABL1IS corresponds to the standardized baseline established in the IRIS study and should not be interpreted as an individual patient's pretreatment transcript level (Cross et al., 2023; Press et al., 2013; White et al., 2022). Molecular response levels are expressed as logarithmic reductions relative to this standardized baseline; MR4 corresponds to BCR::ABL1IS ≤0.01%, whereas MR4.5 corresponds to BCR::ABL1IS ≤0.0032% (Cross et al., 2023; Cross et al., 2012; White et al., 2022). Accurate classification of deep molecular response depends on assay sensitivity, sample quality, reference-gene transcript numbers, and analytical variability, particularly when results lie close to the thresholds defining MR4 and MR4.5 (Branford, 2016; Cross et al., 2023; White et al., 2022).

Levels of Molecular Response

The molecular response level is determined from the ratio of BCR::ABL1 transcripts to transcripts of a reference gene, most commonly ABL1 or GUSB, and the result is expressed as a percentage on the International Scale (Cross et al., 2023; White et al., 2022).

A BCR::ABL1IS value of 1% or lower is broadly regarded as a molecular surrogate for complete cytogenetic response (Cross et al., 2023; Press et al., 2013). A level of 0.1% or lower defines major molecular response, also referred to as MR3 (Apperley et al., 2025; Cross et al., 2023; Cross et al., 2012; White et al., 2022). Achieving and maintaining MMR is associated with favorable long-term outcomes and a low risk of disease progression (Apperley et al., 2025; Cross et al., 2023). Loss of a previously achieved response, or an unexpected increase in BCR::ABL1 transcript levels, should prompt clinical reassessment, including evaluation of treatment adherence, dose interruptions, drug interactions, and possible resistance (Apperley et al., 2025; Cross et al., 2023; Ibrahim et al., 2011; Marin et al., 2010). A BCR::ABL1IS value of 0.01% or lower corresponds to MR4, representing a reduction of at least four logarithms from the standardized baseline, whereas a value of 0.0032% or lower defines MR4.5. MR4 or a deeper response is described as a deep molecular response (Apperley et al., 2025; Cross et al., 2023; Cross et al., 2012; White et al., 2022).

An undetectable BCR::ABL1 transcript should not automatically be interpreted as a complete absence of disease (Cross et al., 2023). The significance of such a result depends on the quality of the sample, the number of reference gene copies, and the sensitivity of the assay used (Press et al., 2013; White et al., 2022).

Clinical Application of Molecular Response Monitoring

Serial monitoring of BCR::ABL1 transcript levels allows clinicians to determine whether molecular response is developing as expected. A BCR::ABL1IS level of 10% or lower at three months is generally defined as an early molecular response, while MMR remains an important long-term therapeutic objective (Apperley et al., 2025; Press et al., 2013). Molecular results obtained at six and twelve months also have prognostic significance; in the IRIS analysis, BCR::ABL1IS levels above 10% at six months and above 1% at twelve months were associated with inferior long-term outcomes (Hughes et al., 2010).

A decision to change treatment should not be based solely on a single BCR::ABL1 measurement. Previous results, the kinetics of molecular response, dose interruptions or reductions, treatment adherence, tolerability, comorbidities, and the potential benefits and risks of switching to another inhibitor should all be considered (Apperley et al., 2025). Treatment adherence should be specifically assessed when the response is inadequate or previously achieved response is lost, as poor adherence has been associated with failure to achieve molecular response and with loss of cytogenetic response during imatinib therapy (Ibrahim et al., 2011; Marin et al., 2010).

Regular molecular monitoring supports individualized treatment by confirming therapeutic efficacy and identifying inadequate response, molecular relapse, or possible resistance (Apperley et al., 2025; Press et al., 2013). It also enables patients with a sustained deep molecular response to be identified as potential candidates for a carefully supervised attempt at TKI discontinuation (Apperley et al., 2025; Etienne et al., 2017; Mahon

et al., 2024; Radich et al., 2021; Ross et al., 2013; Shah et al., 2023). The DESTINY study additionally investigated gradual dose de-escalation and treatment discontinuation in patients with stable molecular responses, including individuals who had maintained MMR without achieving MR4 (Clark et al., 2019).

BCR::ABL1 Mutations and Treatment Resistance

Despite the high efficacy of tyrosine kinase inhibitors, treatment resistance develops in a proportion of patients (Apperley et al., 2025; Cross et al., 2023). Mutations within the BCR::ABL1 kinase domain represent one of the best-characterized mechanisms of TKI resistance and may contribute to an inadequate response or loss of a previously achieved response (Soverini et al., 2019; Soverini et al., 2011).

BCR::ABL1 kinase domain mutations may directly interfere with inhibitor binding or alter the conformation of the kinase, thereby reducing its sensitivity to individual TKIs (Soverini et al., 2019; Soverini et al., 2011). Under the selective pressure of treatment, cells carrying a resistant mutation may gain a relative survival and proliferative advantage. Low-level resistant subclones may consequently expand and become dominant, particularly when treatment is continued with a TKI against which the mutation confers reduced sensitivity (Soverini et al., 2020).

Individual mutations are associated with distinct patterns of TKI sensitivity. The Y253H, E255K/V, and F359V/C/I mutations are associated with reduced sensitivity to nilotinib, whereas V299L and F317L/V/I/C are associated with reduced sensitivity to dasatinib (Soverini et al., 2011). Identification of a clinically relevant mutation can therefore support the selection of a TKI that is more likely to retain activity against the altered BCR::ABL1 protein (Soverini et al., 2019; Soverini et al., 2020; Soverini et al., 2011).

The T315I mutation, commonly referred to as the “gatekeeper” mutation, is of particular clinical importance. It results from the substitution of threonine by isoleucine at position 315 of the ABL1 kinase domain and markedly reduces the binding of ATP-competitive inhibitors. Consequently, T315I confers resistance to imatinib and all second-generation TKIs (Apperley et al., 2025; Jabbour & Kantarjian, 2024; Soverini et al., 2011). Ponatinib retains activity against BCR::ABL1T315I and is supported by clinical evidence from the OPTIC and PACE trials (Apperley et al., 2025; Cortes et al., 2021; Cortes et al., 2018; Jabbour & Kantarjian, 2024). Asciminib, which binds allosterically to the ABL1 myristoyl pocket rather than to the ATP-binding site, is also an effective treatment option for T315I-mutated chronic-phase CML after previous TKI therapy (Apperley et al., 2025; Cortes et al., 2024).

Routine BCR::ABL1 kinase-domain mutation testing is not recommended at diagnosis before the initiation of TKI therapy (Apperley et al., 2025; Cross et al., 2023; Soverini et al., 2019). Mutation analysis is performed primarily when therapy fails, a previously achieved response is lost, or the decline in BCR::ABL1 does not follow the expected pattern (Soverini et al., 2019; Soverini et al., 2011).

Targeted next-generation sequencing is currently the preferred method for detecting kinase-domain mutations (Apperley et al., 2025; Cross et al., 2023). The recommended input material is RNA isolated from peripheral blood or bone marrow leukocytes, which is reverse-transcribed into cDNA to permit selective analysis of the kinase domain derived from the BCR::ABL1 fusion transcript (Apperley et al., 2025; Cross et al., 2023; Soverini et al., 2019). When NGS is not available, Sanger sequencing remains an acceptable alternative (Apperley et al., 2025; Cross et al., 2023).

The result of mutation testing should always be interpreted together with the BCR::ABL1 level and its changes over time (Soverini et al., 2019). The presence of a variant does not necessarily prove that it is the direct cause of treatment failure; nevertheless, the detection of a mutation with an established association with resistance provides a strong rationale for changing the TKI (Apperley et al., 2025; Cross et al., 2023; Soverini et al., 2019; Soverini et al., 2020).

Future Perspectives in the Diagnosis and Monitoring of CML

Advances in molecular diagnostics have made the assessment of treatment response in CML more precise and clinically informative (Alikian et al., 2017; Cross et al., 2023). The increasing sensitivity of next-generation sequencing may allow emerging BCR::ABL1 kinase-domain mutations and resistant subclones to be detected earlier than with conventional sequencing methods (Cross et al., 2023; Soverini et al., 2019). At the same time, improved molecular monitoring enables the depth and stability of response to be evaluated more accurately and supports the careful selection of patients who may be considered for TKI discontinuation (Alikian et al., 2017; Apperley et al., 2025; Kockerols et al., 2020). The principal areas of ongoing development include next-generation sequencing, digital PCR and other highly sensitive methods for residual-disease

assessment, and strategies aimed at achieving and maintaining treatment-free remission (Alikian et al., 2017; Kockerols et al., 2020).

Next-Generation Sequencing

Next-generation sequencing allows many molecules to be analyzed simultaneously and makes it possible to detect small populations of cells carrying mutations associated with TKI resistance (Soverini et al., 2019; Soverini et al., 2020). Such mutations may be identified before they become detectable by conventional Sanger sequencing or develop into dominant leukemic clones (Soverini et al., 2019; Soverini et al., 2020).

Appropriately designed NGS assays can also distinguish compound mutations occurring within the same BCR::ABL1 molecule from mutations present in separate leukemic clones (Cross et al., 2023; Soverini et al., 2019). Targeted NGS is therefore currently the preferred method for BCR::ABL1 kinase-domain mutation analysis when a validated assay is available (Apperley et al., 2025; Cross et al., 2023). Broader NGS panels can identify alterations in genes that are not directly related to BCR::ABL1, including ASXL1, RUNX1, TP53, and BCOR (Cross et al., 2023). Such abnormalities may contribute to the assessment of progression risk, although their full prognostic value requires further investigation and validation (Apperley et al., 2025; Cross et al., 2023).

Future integration of sensitive BCR::ABL1 mutation testing, broader genomic profiling, and highly sensitive quantitative methods such as digital PCR may provide a more comprehensive assessment of clonal architecture and residual disease (Alikian et al., 2017; Kockerols et al., 2020). Whether such integrated approaches can reliably predict treatment failure and improve therapeutic decision-making remains to be established in prospective clinical studies (Apperley et al., 2025; Cross et al., 2023).

Treatment-Free Remission

Treatment-free remission, or TFR, refers to the maintenance of major molecular response or a deeper molecular response after the planned discontinuation of tyrosine kinase inhibitor therapy. It has become an established long-term treatment goal for appropriately selected patients with CML (Apperley et al., 2025; Mahon et al., 2024).

An attempt to discontinue therapy may be considered only in carefully selected patients who remain in the chronic phase, have maintained a stable deep molecular response, and have reliable access to frequent, high-quality BCR::ABL1 monitoring (Etienne et al., 2017; Ross et al., 2013). Eligibility criteria differ between clinical trials and guidelines. Most discontinuation studies required at least three years of TKI therapy and a sustained MR4 or deeper response for at least one year, although some studies applied more stringent requirements (Etienne et al., 2017; Mahon et al., 2024; Radich et al., 2021; Ross et al., 2013; Shah et al., 2023). The DESTINY trial used a distinct strategy involving TKI dose de-escalation before discontinuation and also included patients with stable MMR who had not achieved MR4 (Clark et al., 2019). Across conventional TKI discontinuation studies, approximately 40–50% of appropriately selected patients maintain molecular response without treatment over longer-term follow-up (Mahon et al., 2024; Radich et al., 2021; Ross et al., 2013; Shah et al., 2023). The depth of residual disease measured by digital PCR may provide additional prognostic information regarding the likelihood of maintaining TFR, although it is not currently sufficient as a stand-alone criterion for treatment discontinuation (Kockerols, Valk, Dulucq, et al., 2024).

The risk of molecular recurrence is highest during the first months after TKI discontinuation, with more than 80% of losses of MMR occurring within the first 6–8 months. Particularly frequent BCR::ABL1 monitoring is therefore required during this early period (Apperley et al., 2025; Etienne et al., 2017; Mahon et al., 2024; Radich et al., 2021; Ross et al., 2013; Shah et al., 2023). Loss of MMR is the generally accepted indication for restarting TKI therapy and usually does not require confirmation in a second sample (Apperley et al., 2025; Mahon et al., 2024; Radich et al., 2021; Shah et al., 2023). Approximately 90–95% of patients who experience molecular recurrence regain their previous molecular response after treatment is restarted (Radich et al., 2021; Shah et al., 2023).

Current research is focused on identifying factors that may more accurately predict successful treatment-free remission. Longer duration of TKI therapy and longer maintenance of deep molecular response before discontinuation have been associated with a greater likelihood of sustained TFR (Clark et al., 2019; Mahon et al., 2024; Radich et al., 2021; Ross et al., 2013; Shah et al., 2023). Other potential prognostic factors include the BCR::ABL1 transcript type (Apperley et al., 2025; Baccarani et al., 2019; Mahon et al., 2024), the depth of residual disease measured using highly sensitive methods such as digital PCR (Apperley et al., 2025; Kockerols, Valk, Dulucq, et al., 2024; Kockerols et al., 2025; Kockerols, Valk, Janssen, et al., 2024), and

features of the immune response, including changes in natural killer-cell and T-cell populations (Apperley et al., 2025; Clark et al., 2019).

The development of NGS, digital PCR, and other sensitive molecular techniques may provide additional information for clinical decision-making, although their applications should be distinguished. NGS is primarily useful for detecting resistant subclones and characterizing the molecular architecture of CML (Alikian et al., 2017; Cross et al., 2023), whereas digital PCR may improve the quantification of deep molecular response and refine the prediction of TFR success (Apperley et al., 2025; Kockerols, Valk, Dulucq, et al., 2024; Kockerols et al., 2025; Kockerols, Valk, Janssen, et al., 2024; Kockerols et al., 2020). Digital PCR also provides improved detection of low-level BCR::ABL1 during deep molecular response and after treatment discontinuation (Smitalova et al., 2021). However, these techniques should currently complement, rather than replace, established clinical and molecular criteria for TKI discontinuation (Apperley et al., 2025; Kockerols, Valk, Dulucq, et al., 2024; Kockerols et al., 2025; Kockerols, Valk, Janssen, et al., 2024; Smitalova et al., 2021).

Discussion

BCR::ABL1 is the defining molecular biomarker of chronic myeloid leukemia, and its detection is fundamental to confirming the diagnosis (Arber et al., 2022; Cross et al., 2023; Jabbour & Kantarjian, 2024; Khoury et al., 2022). Quantitative measurement of BCR::ABL1 transcript levels is subsequently used to assess the effectiveness and depth of response to tyrosine kinase inhibitor therapy (Apperley et al., 2025; Cross et al., 2023; Press et al., 2013). Serial monitoring may also identify an inadequate response, loss of a previously achieved response, or a pattern suggesting emerging resistance, thereby supporting decisions regarding additional testing and modification of treatment (Apperley et al., 2025; Cross et al., 2023; Jabbour & Kantarjian, 2024). BCR::ABL1 monitoring therefore extends from diagnosis throughout the course of TKI therapy and, in appropriately selected patients, continues after treatment discontinuation to detect molecular recurrence during treatment-free remission (Apperley et al., 2025; Cross et al., 2023). This broad clinical applicability makes BCR::ABL1 an unusual example of a biomarker used across multiple stages of disease management.

It should be emphasized that none of the laboratory methods used in the diagnosis and monitoring of CML independently provides all the information required for a comprehensive assessment of the disease (Cross et al., 2023). Conventional chromosome banding analysis enables evaluation of the entire karyotype and detection of additional chromosomal abnormalities, although it depends on the availability of metaphase cells and cannot identify cytogenetically cryptic BCR::ABL1 rearrangements (Cross et al., 2023). FISH provides targeted detection of the BCR::ABL1 rearrangement, including cryptic abnormalities that may not be apparent by conventional karyotyping. However, it does not provide a comprehensive assessment of the karyotype, generally does not detect additional chromosomal abnormalities, and cannot determine the BCR::ABL1 transcript type (Cross et al., 2023). Qualitative RT-PCR allows the BCR::ABL1 transcript type to be identified, which is particularly important because assays restricted to common variants may fail to detect atypical transcripts (Cross et al., 2023; Zenebe et al., 2023). Quantitative RT-qPCR, in turn, is the principal method for long-term monitoring of molecular response during TKI therapy (Alikian et al., 2017; Cross et al., 2023; Press et al., 2013). Its reliability depends on sample quality, RNA extraction and cDNA synthesis, assay performance, quality-control procedures, and appropriate standardization of reported results (Alikian et al., 2017; Cross et al., 2023). The methods used in CML should therefore be regarded as complementary rather than competing approaches, with each providing distinct diagnostic or monitoring information (Cross et al., 2023).

Serial measurement of BCR::ABL1 transcript levels by RT-qPCR plays a central role in monitoring the efficacy of TKI therapy, assessing the depth and kinetics of molecular response, and identifying patients whose response is not progressing as expected (Apperley et al., 2025; Cross et al., 2023; Hughes et al., 2010; Press et al., 2013; White et al., 2022). A decision to change treatment should not normally be based on a single BCR::ABL1 measurement. Results should be interpreted in relation to previous measurements, the direction and rate of change, the maintenance or loss of molecular response, and the patient's actual exposure to treatment (Apperley et al., 2025). A persistent or otherwise unexplained rise in BCR::ABL1 transcript levels may indicate emerging resistance and should prompt further evaluation. Before resistance is assumed, however, potential explanations such as treatment interruption, dose reduction, poor adherence, toxicity- or intolerance-related changes in dosing, and analytical variability of the RT-qPCR assay should be considered (Apperley et al., 2025; Ibrahim et al., 2011; Marin et al., 2010; White et al., 2022). Particular caution is required when changes are small or occur close to clinically relevant decision thresholds, because assay variability may contribute to differences between consecutive measurements (White et al., 2022). Molecular results should

therefore be assessed together with treatment history, adherence, dosing, adverse effects, other laboratory findings, and the overall clinical picture (Apperley et al., 2025; Cross et al., 2023; Ibrahim et al., 2011; Marin et al., 2010; White et al., 2022).

Testing for mutations within the BCR::ABL1 kinase domain is an important addition to the diagnostic work-up in cases of treatment failure (Apperley et al., 2025; Soverini et al., 2011). The identification of a specific mutation may explain reduced sensitivity to the current inhibitor and help guide the selection of subsequent therapy (Apperley et al., 2025; Cross et al., 2023; Soverini et al., 2019). The T315I mutation is of particular clinical importance because it confers resistance to imatinib and all second-generation ATP-competitive TKIs; ponatinib and asciminib retain clinically relevant activity against T315I-mutated disease (Cortes et al., 2021; Cortes et al., 2018; Cortes et al., 2024; Soverini et al., 2019). Mutation testing should, however, not be interpreted in isolation. The result should be assessed together with the BCR::ABL1IS level and its kinetics, the type and level of the detected mutation, previous treatment, and the overall laboratory and clinical picture (Apperley et al., 2025; Cross et al., 2023; Soverini et al., 2019). Low-level findings are not equivalent: a known mutation that confers resistance to the ongoing TKI may undergo selective expansion and be clinically actionable, whereas a low-level nonrecurrent variant or a variant of uncertain significance may represent an artefact or a bystander clone and should generally be confirmed in an independent sample (Cross et al., 2023; Soverini et al., 2020).

Emerging molecular technologies may improve the biological and prognostic characterization of chronic myeloid leukemia. Targeted next-generation sequencing of the BCR::ABL1 kinase domain can detect low-level resistant subclones that may not be identified by conventional sequencing. Broader genomic panels can also reveal additional cancer-associated variants; however, in chronic-phase CML, the prognostic and therapeutic significance of many such alterations remains uncertain, and their routine assessment is currently restricted mainly to investigational settings (Alikian et al., 2017; Apperley et al., 2025; Cross et al., 2023). More sensitive quantitative methods, particularly RT-dPCR or ddPCR, can improve the assessment of very low levels of residual BCR::ABL1 and may contribute to risk stratification before an attempt at TKI discontinuation (Apperley et al., 2025; Cross et al., 2023; Kockerols, Valk, Dulucq, et al., 2024; Kockerols et al., 2025; Kockerols, Valk, Janssen, et al., 2024; Kockerols et al., 2020; Smitalova et al., 2021). Nevertheless, no molecular biomarker currently has sufficient predictive accuracy to serve as a stand-alone criterion for treatment-free remission. The probability of sustained TFR is determined by a combination of factors, most consistently the total duration of TKI therapy and the duration of sustained deep molecular response. The depth of molecular response at discontinuation and the BCR::ABL1 transcript type, particularly the presence of e14a2, may provide additional prognostic information (Apperley et al., 2025; Baccarani et al., 2019; Etienne et al., 2017; Kockerols, Valk, Dulucq, et al., 2024; Kockerols et al., 2025; Kockerols, Valk, Janssen, et al., 2024; Kockerols et al., 2020; Mahon et al., 2024; Radich et al., 2021; Shah et al., 2023). The mechanisms that permit continued control of residual disease after TKI withdrawal remain incompletely understood. Immune-mediated control is considered a plausible contributor but remains under investigation and is not currently a routinely validated criterion for selecting patients for treatment discontinuation (Apperley et al., 2025; Ross et al., 2013). Decisions concerning a TFR attempt should therefore integrate treatment duration, the depth and stability of molecular response, transcript type, the quality and sensitivity of molecular testing, the patient's clinical history, and the feasibility of frequent molecular monitoring rather than rely on a single laboratory marker (Apperley et al., 2025; Clark et al., 2019; Cross et al., 2023; Etienne et al., 2017; Kockerols, Valk, Dulucq, et al., 2024; Kockerols et al., 2025; Kockerols, Valk, Janssen, et al., 2024; Kockerols et al., 2020; Mahon et al., 2024; Radich et al., 2021; Ross et al., 2013; Shah et al., 2023; Smitalova et al., 2021).

A major future challenge is not merely to increase the analytical sensitivity of molecular laboratory methods, but to establish the clinical significance and actionability of the abnormalities they detect. Identification of very low-level residual disease or a rare genetic variant may provide potentially relevant information, but it should not automatically lead to a change in treatment when its prognostic significance, biological role, or relationship to drug resistance has not been validated. Clinical interpretation should therefore take into account the type and level of the abnormality, the kinetics of BCR::ABL1, the treatment context, and the possibility of analytical artefacts or biologically unrelated variants (Alikian et al., 2017; Apperley et al., 2025; Cross et al., 2023).

Further studies should define clinically validated thresholds and decision rules for low-level findings, clarify which additional genetic abnormalities are independently prognostic or therapeutically actionable, and develop multivariable models capable of improving the prediction of treatment response, emerging resistance, and sustained treatment-free remission (Alikian et al., 2017; Apperley et al., 2025; Cross et al., 2023;

Kockerols et al., 2020). Parallel efforts should focus on harmonizing pre-analytical procedures, assay validation, limits of detection and blank, reporting on the International Scale, and the interpretation of low-positive or undetectable results. More sensitive techniques such as digital PCR may improve the precision of residual disease assessment and contribute to risk stratification, particularly before TKI discontinuation, but they should complement rather than replace established clinical and molecular criteria (Apperley et al., 2025; Cross et al., 2023; Kockerols et al., 2020).

Conclusions

The BCR::ABL1 fusion is the defining molecular abnormality of chronic myeloid leukemia and remains the central biomarker for diagnosis, treatment monitoring, and therapeutic decision-making. Identification of the transcript type before treatment and regular quantitative assessment of BCR::ABL1 during therapy are essential for evaluating response, recognizing treatment failure or possible resistance, and guiding timely modifications of treatment. Although RT-qPCR remains the standard method for molecular monitoring, targeted NGS and digital PCR may further improve the detection of resistant clones and the assessment of very low-level residual disease. However, increasingly sensitive results must always be interpreted in the clinical context and should not be used as isolated criteria for changing therapy or attempting TKI discontinuation. Further progress will depend not only on improving analytical methods, but also on validating additional prognostic and predictive markers that enable more precise treatment individualization and more reliable selection of patients for treatment-free remission.

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