RESEARCH ARTICLE

Editorial Process: Submission:07/01/2025 Acceptance:07/13/2026 Published:07/25/2026

Development and Validation of a One-Step Multiplex RT-qPCR Assay for BCR-ABL1 Transcript Detection and Monitoring in Moroccan Chronic Myeloid Leukemia Patients

Abdenacer Benchrifa[1,2,3], Basma Albanyahyati[1,2,3], Mounyr Balouiri[1], Hicham El Hadi[1], Youssef Bakri[2,3], Kamal Doghmi[4], Abdeladim Moumen[1]*

Abstract

Objective: Chronic myeloid leukemia (CML) is driven by the BCR-ABL1 fusion gene. Molecular monitoring of this transcript is essential for patient management. However, the high cost of imported assays limits access to regular monitoring in Morocco. **Methods:** We developed and validated a cost-effective one-step multiplex RT-qPCR assay that simultaneously detects BCR-ABL1 and ABL1 transcripts in peripheral blood mononuclear cells. Samples from 63 Moroccan CML patients were analyzed and compared with the GeneXpert system. **Result:** The assay showed efficiencies above 95% and $R^2 \geq 0.99$ for both targets. BCR-ABL1/ABL1 ratios correlated strongly with GeneXpert ($r = 0.93$, $p < 0.001$) and showed 86% overall agreement. **Conclusion:** This locally developed assay is a reliable and affordable alternative for routine BCR-ABL1 detection and monitoring, improving access to molecular testing in resource-limited settings.

Keywords: Chronic myeloid leukemia- BCR-ABL1- RT-qPCR- molecular monitoring- cost-effective assay

Asian Pac J Cancer Prev, **27 (7)**, 2481-2489

Introduction

Chronic myeloid leukemia (CML) is a myeloproliferative neoplasm disorder characterized by the presence of the Philadelphia chromosome (Ph) [1, 2], resulting from a balanced reciprocal translocation between the long arms of chromosomes 9 and 22, t(9;22)(q34;q11) [3]. This genetic aberration leads to the formation of the BCR-ABL1 fusion gene, BCR (breakpoint cluster region) located in chromosome 22, and c-ABL1 proto-oncogene (cellular Abelson) located in chromosome 9 [4, 5]. The BCR-ABL1 fusion gene encodes a constitutively active non-receptor tyrosine kinase, which is indeed the causative molecular aberration of CML [6, 7], making it a key diagnostic and monitoring molecular marker of the disease [8].

The annual incidence of CML varies from 0.6 to 2.8 cases per 100,000 individuals worldwide [9] and is estimated to be 0.6 cases per 100,000 inhabitants in the Casablanca region of Morocco [10]. CML is clinically divided into three phases: the chronic phase (CP), the accelerated phase (AP), and the blast phase (BP) [11, 12]. Unlike AP and BP, where myeloid cells lose their capacity to differentiate and mature, in CP-CML, myeloid cells maintain normal maturation, leading to asymptomatic patients [13]. About 95% of cases of CML are diagnosed in the chronic phase. Without treatment or in cases of therapy failure, CML advances to the accelerated and then the blastic phase within 3-5 years, which is typically resistant to treatment [14, 15].

The introduction of tyrosine kinase inhibitors (TKIs), particularly imatinib mesylate (STI571), has dramatically changed CML management and has become the first-line therapy for CML patients in the chronic phase over interferon-α and cytarabine, transforming the disease from fatal to a manageable chronic condition, where most patients remain in remission and require long-term follow-up and monitoring [16-18]. Second-generation TKIs, such as dasatinib, nilotinib, and bosutinib, have been approved for second-line therapy of CML patients intolerant of or in whom imatinib treatment failed [19-21]. Third-generation TKIs, ponatinib, olverembatinib, and asciminib offer better efficacy, safety, and fewer side effects than currently available frontline ATP-competitive

*[1]Diagnostic Kits and Medical Device Development Center, Moroccan Foundation for Advanced Science, Innovation and Research (MAScIR), University Mohammed VI Polytechnic (UM6P), Ben Guerir, Morocco. [2]Laboratory of Human Pathologies Biology, Department of Biology, Faculty of Sciences, Morocco. [3]Genomic Center of Human Pathologies, Faculty of Medicine and Pharmacy, University Mohammed V, Rabat, Morocco. [4]Department of Clinical Hematology, Military Hospital of Instruction Mohamed V, Rabat, Morocco. *For Correspondence: abdeladim.moumen@um6p.ma*

tyrosine kinase inhibitors (TKIs), especially against BCR-ABL1 T315I mutation, which confers a high degree of resistance to imatinib and all second-generation (2G) TKIs [22-24].

In Morocco, the standard approach for CML diagnosis is bone marrow cytogenetics, fluorescence in situ hybridization (FISH), which is invasive, labor- intensive, time-consuming, expensive, and incapable of accurately monitoring minimal residual disease (MRD) [25, 26], and also RT-qPCR where the imported GeneXpert is the most used one and not available in all laboratories because of its high price. The NCCN and the European LeukemiaNet recommend RT-qPCR to be performed even after a major molecular response MMR (MR3.0 or better) is achieved, which most Moroccan CML patients cannot afford [27, 28]. Therefore, an affordable, accurate, and reliable alternative test is needed. Developing the assay locally will not only increase the testing frequency and the accessibility of molecular monitoring, but it will also reduce the dependency on expensive imported kits and the reliance on their availability in the market.

Thus, in this study, we developed a cost-effective single-tube one-step multiplex RT-qPCR assay capable of simultaneously detecting BCR-ABL1 and ABL1 transcripts in peripheral blood mononuclear cells (PBMCs) of CML patients. Using ABL1 as an internal reference gene allows for normalization and ensures accuracy of BCR-ABL1 quantification. The Assay's performance was validated using 63 patients recruited from Military hospitals in Rabat, Meknes, and Marrakech, and the results were compared to the GeneXpert kit.

Successful development, validation, and implementation of this assay will make routine molecular diagnostic and monitoring more accessible to Moroccan patients, allowing early detection of treatment resistance and a timely therapy adjustment, as well as reducing the burden on both patients and the healthcare system.

Materials and Methods

Patient recruitment and sample collection

Fresh, prospectively collected EDTA whole blood specimens from 63 patients with CML at various stages of disease (following initial diagnosis, with or without prior exposure to tyrosine kinase inhibitor therapy or other CML treatment) were used in this study. The patients were recruited from Military hospitals in Rabat, Meknes, and Marrakech, Morocco. Informed consent was obtained from all participants prior to the study. Peripheral blood samples were collected from each patient, and peripheral blood mononuclear cells (PBMCs) were isolated.

Peripheral blood mononuclear cells (PBMCs) isolation

Peripheral blood mononuclear cells (PBMCs) were isolated from the obtained whole blood samples using density gradient centrifugation, as previously described by Boyom (1968) with minor modifications [29]. Briefly, 5 mL of fresh blood from each patient collected in anti-coagulant tubes containing ethylenediaminetetraacetic acid (EDTA) was prepared by mixing an equal volume of blood with phosphate-buffered saline (PBS) in sterile 50 mL centrifuge tubes. The diluted samples were layered onto a density gradient separation medium (Ficoll-Paque) and centrifuged at 400 x g for 30 minutes at room temperature. After discarding the upper plasma layer, the interface layer containing PBMCs was gently collected in a sterile 50 mL tube and washed twice with PBS. The isolated PBMCs were then ready for RNA extraction.

RNA extraction

Total RNA was extracted from the isolated PBMCs using the RNeasy Mini Kit (Qiagen, Hilden, Germany). The RNA extraction was performed according to the manufacturer's protocol, and the RNA concentration and purity were measured using a NanoDrop spectrophotometer (Thermo Scientific, Waltham, MA, USA). The extracted RNA samples were either tested immediately or stored at -80 °C until use.

Standard plasmid preparation

A plasmid containing both BCR-ABL1 and ABL1 sequences was used to generate a standard curve for the quantification of BCR-ABL1 transcripts. Standards were prepared by cloning PCR products using the cDNA of BCR-ABL1 (e14a2) and normal ABL1 as templates, as we have previously described [25]. The generated recombinant plasmid DNA was isolated and purified using the QIAprep Spin Miniprep Kit (Qiagen). The plasmid concentration was determined using NanoDrop 2000 (Thermo Scientific), and the copy number was calculated using the molecular weight of the plasmid. Serial dilutions ranging from 10^6 to 10 copies per reaction were prepared for standard curve generation.

One-Step Multiplex RT-qPCR Assay

Primers and probes were designed using Primer3Plus software (https://www.primer3plus.com/) [30]. For BCR-ABL1, primers were designed to amplify both e13a2 and e14a2 variants since more than 95% of CML cases correspond to the Major breakpoint (M-bcr) with 62 % e14a2, and 38% e13a2, previously known as b3a2, and b2a2, respectively [31-33]. ABL1 primers targeted a sequence present in both isoforms a and b of ABL1 transcripts, disrupted during the BCR-ABL1 fusion gene formation.

Probes were synthesized by GenScript (GenScript USA Inc., Piscataway, NJ, USA), with BCR-ABL1 probes labeled with 6-carboxyfluorescein (FAM) and ABL1 probes labeled with 2'-chloro-7'-phenyl-1,4-dichloro-6-carboxyfluorescein (VIC), as well as Black Hole Quencher-1 (BHQ1) as a quencher for both probes. The primer design also excluded the amplification of genomic DNA.

RT-qPCR assay was performed using the QuantStudio 6 Flex Real-Time PCR System with TaqMan Fast Virus 1-Step Master Mix (Applied Biosystems, Thermo Fisher Scientific Inc., Waltham, Massachusetts, USA). Each 10 µL reaction contained 2.5 µL Master Mix, 1 µL oligo mix (300 nM each primer, 200 nM each probe), and 6.5 µL RNA sample or standard. These concentrations were optimized to ensure maximum efficiency and sensitivity. The 6-log series of standards were processed with every

RT-qPCR.

The thermal profile consisted of an initial reverse transcription step at 50 °C for 5 minutes, followed by denaturation at 95 °C for 20 seconds, and 40 cycles of amplification (95 °C for 3 seconds, 60 °C for 30 seconds). This one-step protocol was chosen to minimize handling steps and reduce the risk of contamination [34].

Quantification and Data Analysis

RT-qPCR amplification curves were analyzed using QuantStudio Real-Time PCR software. The threshold cycle (Ct) values, which represent the cycle at which the fluorescence signal crosses the threshold, were obtained for both BCR-ABL1 and ABL1 transcripts. The standard curve generated from the known concentrations of the plasmid was used to calculate the absolute copy numbers of BCR-ABL1 transcripts in each sample. The results were normalized to the ABL1 transcript level, and the BCR-ABL1/ABL1 ratio was calculated. The conversion to the international scale (IS) has been performed using a conversion factor which corresponds to 1.2 as previously shown [47].

Comparison with GeneXpert

To validate the performance of the developed assay, all samples were also tested using the GeneXpert BCR-ABL Ultra kit (Cepheid, Sunnyvale, CA, USA) according to the manufacturer's instructions in their corresponding laboratories [35]. BCR-ABL1/ABL1 ratios obtained by the assay were compared with those obtained with GeneXpert. The results were statistically analyzed to evaluate the correlation between the two tests.

Statistical Analysis

To compare the assay with the GeneXpert assay, Passing-Bablok regression analysis was performed to determine the proportional and constant bias between the two assays [36], and Pearson's correlation coefficient was calculated to assess the strength of their relationship [37]. Furthermore, Bland-Altman analysis was conducted to evaluate the agreement across the measurement range. The 95% limits of agreement were calculated as the mean difference ±1.96 standard deviations of the differences [38].

Results

Patient characteristics

A total of 63 CML patients, with or without imatinib treatment, were included in this study. The patient cohort represented various stages of CML treatment response, allowing for a comprehensive evaluation of the assay's performance across a wide range of BCR-ABL1 transcript levels.

Standard curve performance

The standard curve was constructed to allow absolute quantification of BCR-ABL1 and ABL1 transcripts. In addition, the standard curve also allows us to determine reaction efficiency and the coefficient of determination R^2, key indicators of assay performance and reliability. The criteria for a reliable standard curve are to have an efficacy greater than 95% and an R^2 value exceeding 0.98 [39, 40]. Standard curves for both BCR-ABL1 and ABL1 showed excellent linearity over the six serial dilutions (10 to 10^6 copies), with an amplification efficiency of 97.48% for BCR-ABL1 and 95.7% for ABL1 (Figure 1). R^2 values were equal to 0.99 for both BCR-ABL1 and ABL1. These results indicate a high amplification efficiency and robust accuracy of the developed assay.

Comparison of BCR-ABL1 quantification between the developed assay and the GeneXpert system

The distribution of patients across different BCR-

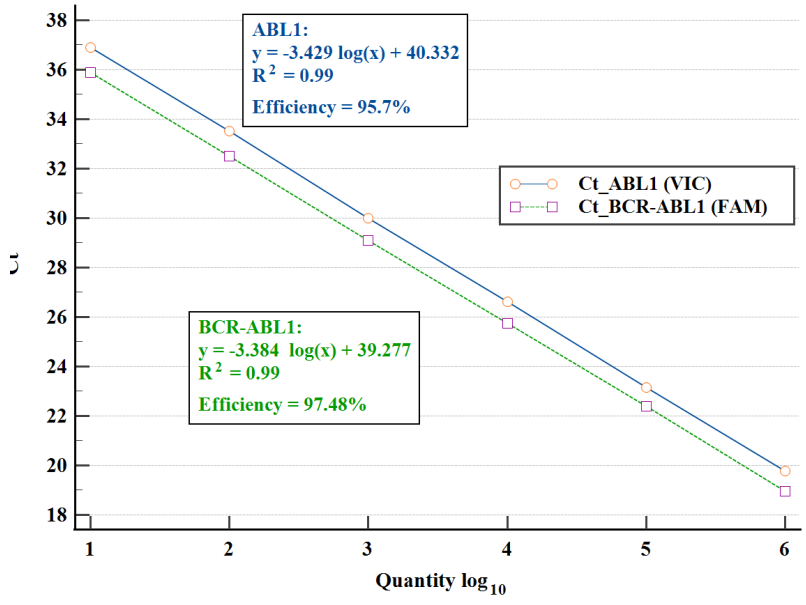

Figure 1. Standard Curves for BCR-ABL1 and ABL1 Quantification. The Ct versus BCR-ABL1 copy number (green-dotted line) and Ct versus ABL1 copy number (blue line) obtained by the assay are shown. Each data point represents the mean of the results in duplicate. The efficiency and R^2 values are included in the chart.

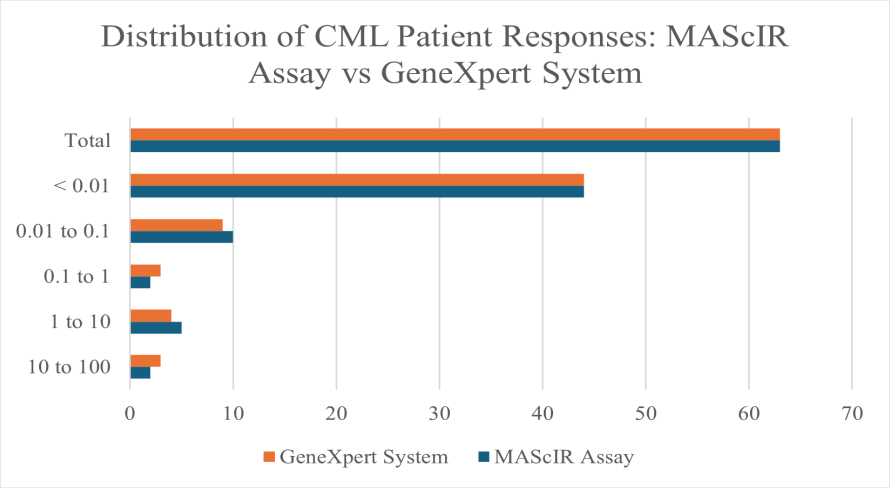

Chart 1. Comparison of CML Patient Response Distribution between the MAScIR Assay and the GeneXpert System

ABL1 ratio ranges, as determined by both the developed assay and GeneXpert assay, is presented in Table 1 and Chart 1. The developed assay (MAScIR assay) identified 2 patients in the diagnostic stage, 5 in complete hematologic response (CHR), 2 in complete cytogenetic response (CCyR), 10 in major molecular response (MMR), and 44 in deep molecular response (DMR). In comparison, GeneXpert detected 3 patients in the diagnostic stage, 4 in CHR, 3 in CCyR, 9 in MMR, and 44 in DMR. The overall agreement between the two tests was 86%.

A strong positive correlation was observed between the BCR-ABL1/ABL1 ratios obtained from the developed assay and the GeneXpert assay, with a Pearson correlation coefficient of ($r = 0.93$, $p < 0.001$). This high correlation indicates a strong linear association between the two methods across the entire range of BCR-ABL1 transcript levels.

To further assess the relationship and potential bias between the two methods, Passing-Bablock regression analysis was performed. The regression yielded a slope of (1.026) and an intercept of (-0.00686), suggesting minimal proportional and constant bias, respectively (Figure 2). These findings indicate that the MAScIR assay produces results that are in close alignment with the GeneXpert assay over the entire measurement range.

The agreement between the two methods was also evaluated using the Bland-Altman method [38]. The analysis showed a small mean bias of (-0.08), indicating minimal systematic differences between the two methods. The 95% limits of agreement (-1.33 to 1.18) showed that most differences fell within an acceptable range, with only 4 samples lying outside the limits (Figure 3). The relatively uniform distribution of differences across the range supports the consistency and reliability of the MAScIR assay.

Discussion

Timely and accurate measurement of BCR-ABL1 transcript levels is crucial for early detection of treatment failure and orienting therapy [12]. Standardization of RT-qPCR results reporting allowed it to become the gold standard for treatment monitoring and assessment of the risk of relapse in chronic myeloid leukemia patients [41, 42]. In this study, we developed and validated a cost-effective one-step multiplex RT-qPCR assay for the quantification of BCR-ABL1 transcripts in CML patients. The test showed high analytical performance and a very strong correlation with the GeneXpert assay, an automated cartridge-based detection system widely adopted in low-throughput laboratories [43, 44].

A very high Pearson correlation coefficient (PCC) was found ($r = 0.93$, $p < 0.001$), with a high agreement of 86% between the developed assay and GeneXpert across different response categories. Passing-Bablock regression analysis further confirmed the close agreement between the two methods, revealing a slope close to unity (1.026) and a minimal intercept (-0.00686), suggesting negligible proportional and constant bias. The Bland-Altman analysis supported these findings, showing a

Table 1. Comparison of CML Clinical Samples between the MAScIR Assay and the GeneXpert Assay. This table compares the classification of patient samples used in this study as determined by the developed assay (MAScIR assay) and the GeneXpert system. The overall agreement between the two methods was 86%.

Ratio	Results	MAScIR Assay	GeneXpert System
10 to 100	Diagnosis	2	3
1 to 10	Complete Hematological Response (CHR)	5	4
0.1 to 1	Complete Cytogenetic Response (CCyR)	2	3
0.01 to 0.1	Major Molecular Response (MMR)	10	9
< 0.01	Deep Molecular Response (DMR)	44	44
Total		63	63

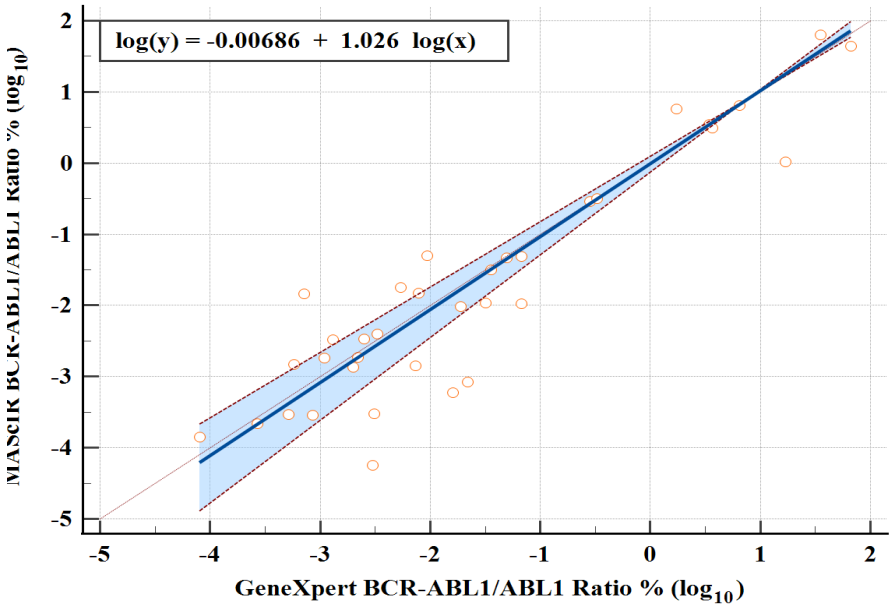

Figure 2. Passing-Bablock Regression Analysis Plot. The scatter plot shows the log-transformed BCR-ABL1/ABL1 ratios obtained from the MAScIR assay (y-axis) versus the GeneXpert assay (x-axis). The blue line represents the Passing-Bablock regression line, with the shaded area indicating the 95% confidence interval. This analysis assesses the agreement and systematic bias between the two methods.

small mean bias (-0.08) and relatively narrow 95% limits of agreement, with most samples falling within this range, highlighting minimal systematic differences between the assays. Moreover, the standard curve for both transcripts, BCR-ABL1 and ABL1, showed consistently a reaction efficacy (> 95%) and strong linearity ($R^2 \geq 0.99$) under the experimental conditions, demonstrating the efficiency and sensitivity of the assay at detecting very low transcript levels. This is very important in the context of making treatment decisions at low levels of deep molecular response to access eligibility for a treatment-free remission (TFR) attempt [27, 45].

The one-step multiplex format of the assay allows simultaneous detection of both BCR-ABL1 and ABL1 transcripts in a one-step single tube reaction, offering several advantages over simplex or two-step methods. Combining reverse transcription and qPCR in a single tube reduces the risk of sample contamination and sample-to-sample variation [34]. It also enhances assay reproducibility, simplifies the workflow, and minimizes both the required sample volume and reagent consumption. Consequently, this reduces hands-on time,

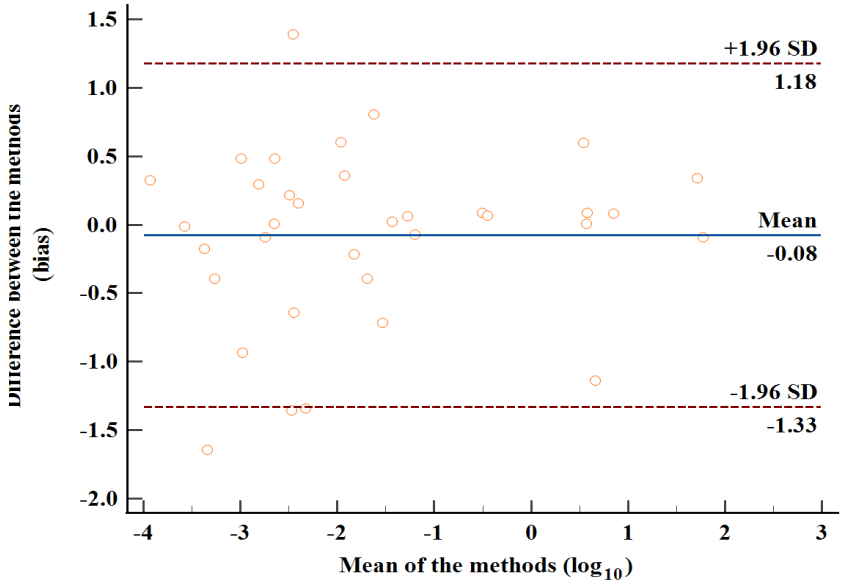

Figure 3. Bland-Altman agreement Plot. The y-axis represents the log of the differences between BCR-ABL1 values obtained from the MAScIR assay and GeneXpert assay, while the x-axis shows the log-transformed means of the two methods. The central solid line indicates the mean bias, and the dashed lines represent the 95% limits of agreement (mean ± 1.96 SD).

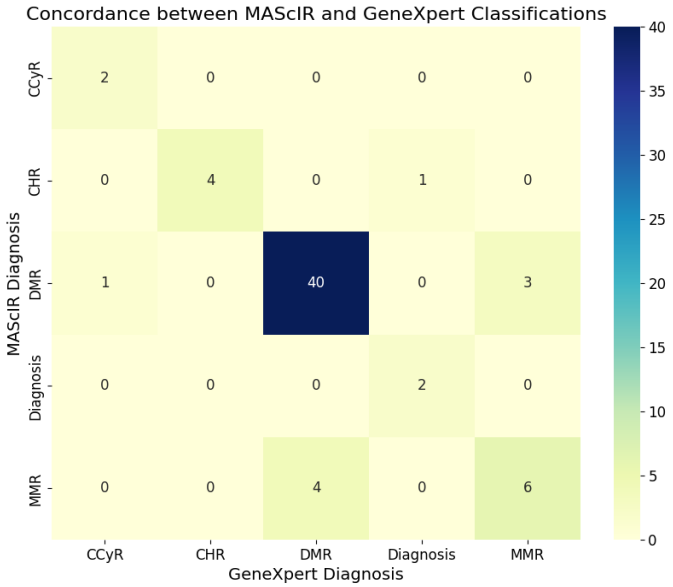

Figure 4. Concordance between the MAScIR assay and the GeneXpert assay across different diagnostic stages (treatment response).

decreases overall turnaround time, and diminishes the potential for experimental errors, as well as the test price, while maintaining high analytical sensitivity.

The use of ABL1 as an internal control ensures the quality of the extracted RNA and helps normalize for variations in sample quality and quantity, which is essential for accurate quantification of BCR-ABL1 transcripts [46]. Multiplexing also ensures a more accurate normalization of BCR-ABL1 to ABL1 as both targets are amplified under the same reaction conditions.

Indeed, one can argue that amplification competition can occur between the two target genes when multiplexing, especially when one of them is found at very low levels (BCR-ABL1). The high agreement found between the developed assay and GeneXpert assay in patients with deep molecular response ($\leq 0.01\%$; $\geq$ 4-log reduction from IRIS baseline) (Figure 4) confirms the non-competition between BCR-ABL1 and ABL1 in the reaction. These results align with previous studies that validated the performance of similar assays for BCR-ABL1 quantification in various patient cohorts [25, 47].

One of the key advantages of this assay is its cost-effectiveness. Although a detailed economic analysis was beyond the scope of this study, preliminary estimates suggest that this method could reduce the cost of BCR-ABL1 testing by approximately 60-70% compared to the GeneXpert system. This cost reduction is primarily due to lower reagent costs and the use of standard RT-qPCR equipment. In the context of Morocco, where the annual incidence of CML is estimated at 0.6 cases per 100,000 in the Casablanca region [10], and where many patients struggle to afford regular molecular monitoring, this cost reduction could have a huge impact on patient care and outcomes, aligning with global and national efforts to make healthcare more affordable and accessible.

The development of this assay addresses a critical need in CML management in Morocco and potentially other resource-limited settings. Currently, the standard approach for CML diagnosis in the country relies on bone marrow cytogenetics, FISH, and RT-qPCR using imported kits like GeneXpert. These methods are often invasive, labor-intensive, time-consuming, and expensive, making them inaccessible for many patients, especially in low-income regions [25]. This assay, being both cost-effective and reliable, has the potential to increase the frequency and accessibility of molecular monitoring for CML patients in Morocco, as well as in patients in low and middle-income countries (LMICs), aligning with the NCCN and European LeukemiaNet recommendations for regular BCR-ABL1 transcript monitoring [27, 28].

The use of peripheral blood in the assay, rather than bone marrow aspirates, is another significant advantage. This less invasive approach not only reduces patient discomfort but also simplifies the sample collection process, potentially enabling more frequent monitoring and improving patient compliance.

Despite these promising results, this study has some limitations that should be addressed in future research. First, while the patient cohort (n = 63) provided a good representation of various CML treatment stages, a larger sample size would further strengthen the validation of this assay. Second, longitudinal studies would be valuable to assess the assay's performance in tracking individual patients' responses to treatment over time. Additionally, while this assay showed an excellent correlation with the GeneXpert system, further studies comparing it with other established methods, such as digital PCR, could provide additional insight into its performance characteristics.

Furthermore, this assay currently detects only e13a2 and e14a2 transcripts, while it covers more than 95% of CML cases, it does not detect less common transcript variants such as e1a2, e6a2, e8a2, e19a2, e13a3, and e14a3, which collectively represent about 2-4% of patients [27]. While this limitation is shared by many commercially available assays, including this version of the GeneXpert assay we compared the test with, future iterations of the

assay could potentially be expanded to include these rare variants.

It is important to note that this assay, like other RT-qPCR methods, cannot differentiate between Ph+ CML patients and Ph+ acute lymphoblastic leukemia (ALL) patients, which represent about 25%, and in which, unlike CML, p210 is less frequent [48, 49].

While this assay is not designed to detect specific treatment resistance mutations, such as T315I and E255K in the ABL1 kinase domain [50], it can indirectly monitor treatment resistance by tracking the persistence or increase of BCR-ABL1 transcript levels. This information can guide treatment decisions, particularly in determining whether to switch to second or third-generation tyrosine kinase inhibitors (TKIs) or consider alternative therapies [51-53].

Looking ahead, the successful implementation of this assay in clinical practice could have far-reaching implications for CML management in Morocco and potentially other developing countries in Africa. Making routine molecular diagnostics and monitoring more accessible could enable earlier detection of treatment resistance and more timely therapy adjustments. This, in turn, could lead to improved patient outcomes and potentially reduce healthcare costs associated with managing advanced disease stages.

Future research directions could include the adaptation of this assay for the detection of other clinically relevant BCR-ABL1 variants or resistance mutations. Additionally, exploring the potential for automating parts of the workflow could further reduce costs and increase throughput, making the assay even more suitable for high-volume testing in resource-limited settings.

In conclusion, we developed a reliable, locally produced assay for BCR-ABL1 transcript detection and monitoring in Moroccan CML patients. This assay has the potential to significantly improve the accessibility of molecular monitoring, reduce the financial burden on patients and healthcare systems, and ultimately contribute to better management of CML in Morocco and similar LMICs. Further studies and clinical implementation will be crucial to fully realize the potential impact of this assay on CML patient care.

Author Contribution Statement

AB carried out most of the experiments. BA, MB, and HE helped in performing experiments. AB, and HE participated in the design of the study, performed the statistical analysis and helped draft the manuscript. YB participated in the design of the study and was involved with revising the manuscript critically. KD provided and managed the patients' samples and revised the manuscript critically. AM conceived, designed, coordinated the study and drafted the paper. All authors read and approved the final manuscript..

Acknowledgements

General

The authors would like to thank all the members of the Medical Biotechnology Center at the Moroccan Foundation for Advanced Science, Innovation, and Research (MAScIR) for their help in performing this research. We are grateful to the hematology departments of the Military Hospitals in Rabat, Meknes, and Marrakech for their cooperation in patient recruitment and sample collection.

Funding Statement

This work was funded by both the Moroccan foundation for advanced science, innovation and research (MAScIR) and the UM6P.

Ethical Declaration

This study was conducted retrospectively. All samples had been collected previously for routine diagnostic purposes and were fully anonymized before being provided to the research team. No identifiable personal data were accessed.

Conflict of Interest

The authors declare that they have no competing interests.

References

1. Nowell PC, Hungerford DA. Chromosome studies on normal and leukemic human leukocytes. J Natl Cancer Inst. 1960;25(1):85-109.
2. Nowell PC. The minute chromosome (Ph[1]) in chronic granulocytic leukemia. Blut. 1962;8:65-6. https://doi.org/10.1007/BF01630378
3. Rowley JD. A new consistent chromosomal abnormality in chronic myelogenous leukaemia identified by quinacrine fluorescence and Giemsa staining. Nature. 1973;243(5405):290-3. https://doi.org/10.1038/243290a0
4. Heisterkamp N, Stam K, Groffen J, de Klein A, Grosveld G. Structural organization of the bcr gene and its role in the Ph′ translocation. Nature. 1985;315(6022):758-61. https://doi.org/10.1038/315758a0
5. de Klein A, van Kessel AG, Grosveld G, Bartram CR, Hagemeijer A, Bootsma D, et al. A cellular oncogene is translocated to the Philadelphia chromosome in chronic myelocytic leukaemia. Nature. 1982;300(5894):765-7. https://doi.org/10.1038/300765a0
6. Daley GQ, Van Etten RA, Baltimore D. Induction of chronic myelogenous leukemia in mice by the P210bcr/abl gene of the Philadelphia chromosome. Science. 1990;247(4944):824-30. https://doi.org/10.1126/science.2406902
7. Druker BJ, Talpaz M, Resta DJ, Peng B, Buchdunger E, Ford JM, et al. Efficacy and safety of a specific inhibitor of the BCR-ABL tyrosine kinase in chronic myeloid leukemia. N Engl J Med. 2001;344(14):1031-7. https://doi.org/10.1056/NEJM200104053441401
8. Hughes T, Deininger M, Hochhaus A, Branford S, Radich J, Kaeda J, et al. Monitoring CML patients responding to treatment with tyrosine kinase inhibitors: review and recommendations for harmonizing current methodology for detecting BCR-ABL transcripts and kinase domain mutations and for expressing results. Blood. 2006;108(1):28-37. https://doi.org/10.1182/blood-2006-01-0092
9. Rohrbacher M, Hasford J. Epidemiology and etiology of chronic myeloid leukemia. In: Wiernik PH, Dutcher JP, Gertz MA, editors. Neoplastic Diseases of the Blood. Cham: Springer; 2018. p. 9-17. https://doi.org/10.1007/978-3-319-

64263-5_2
10. Benchikh S, Charlène SSG, Bousfiha A, Razoki L, Aboulfaraj J, Zarouf L, et al. Cytogenetic and epidemiological profile of chronic myeloid leukemia in Morocco. Ann Hematol. 2024;103(8):2765-74. https://doi.org/10.1007/s00277-024-05747-3
11. Arber DA, Orazi A, Hasserjian R, Thiele J, Borowitz MJ, Le Beau MM, et al. The 2016 revision to the World Health Organization classification of myeloid neoplasms and acute leukemia. Blood. 2016;127(20):2391-405. https://doi.org/10.1182/blood-2016-03-643544
12. Baccarani M, Deininger MW, Rosti G, Hochhaus A, Soverini S, Apperley JF, et al. European LeukemiaNet recommendations for the management of chronic myeloid leukemia: 2013. Blood. 2013;122(6):872-84. https://doi.org/10.1182/blood-2013-05-501569
13. Sawyers CL. Chronic myeloid leukemia. N Engl J Med. 1999;340(17):1330-40. https://doi.org/10.1056/NEJM199904293401706
14. Faderl S, Talpaz M, Estrov Z, Kantarjian HM. Chronic myelogenous leukemia: biology and therapy. Ann Intern Med. 1999;131(3):207-19. https://doi.org/10.7326/0003-4819-131-3-199908030-00008
15. Jabbour E, Kantarjian H. Chronic myeloid leukemia: 2018 update on diagnosis, therapy and monitoring. Am J Hematol. 2018;93(3):442-59. https://doi.org/10.1002/ajh.25011
16. Cohen MH, Williams G, Johnson JR, Duan J, Gobburu J, Rahman A, et al. Approval summary for imatinib mesylate capsules in the treatment of chronic myelogenous leukemia. Clin Cancer Res. 2002;8(5):935-42.
17. Deininger M, O'Brien SG, Guilhot F, Goldman JM, Hochhaus A, Hughes TP, et al. International randomized study of interferon vs STI571 (IRIS) 8-year follow up: sustained survival and low risk for progression or events in patients with newly diagnosed chronic myeloid leukemia in chronic phase (CML-CP) treated with imatinib. Blood. 2009;114(22):1126. https://doi.org/10.1182/blood.V114.22.1126.1126
18. Hochhaus A, Larson RA, Guilhot F, Radich JP, Branford S, Hughes TP, et al. Long-term outcomes of imatinib treatment for chronic myeloid leukemia. N Engl J Med. 2017;376(10):917-27. https://doi.org/10.1056/NEJMoa1609324
19. Cortes JE, Gambacorti-Passerini C, Deininger MW, Mauro MJ, Chuah C, Kim DW, et al. Bosutinib versus imatinib for newly diagnosed chronic myeloid leukemia: results from the randomized BFORE trial. J Clin Oncol. 2018;36(3):231-7. https://doi.org/10.1200/JCO.2017.74.7162
20. Gambacorti-Passerini C, Aroldi A, Cordani N, Piazza R. Chronic myeloid leukemia: Second-line drugs of choice. Am J Hematol. 2016;91(1):67-75. https://doi.org/10.1002/ajh.24247
21. Khoury HJ, Cortes JE, Kantarjian HM, Gambacorti-Passerini C, Baccarani M, Kim DW, et al. Bosutinib is active in chronic phase chronic myeloid leukemia after imatinib and dasatinib and/or nilotinib therapy failure. Blood. 2012;119(15):3403-12. https://doi.org/10.1182/blood-2011-11-390120
22. Cortes J, Lang F. Third-line therapy for chronic myeloid leukemia: current status and future directions. J Hematol Oncol. 2021;14(1):44. https://doi.org/10.1186/s13045-021-01055-9
23. Jabbour E, Koller PB, Oehler VG, Jamy OH, Mukherjee S, Hunter AM, et al. Olverembatinib (HQP1351) overcomes ponatinib resistance in patients with heavily pretreated/refractory chronic myeloid leukemia (CML) and Philadelphia chromosome-positive acute lymphoblastic leukemia (Ph+ ALL). Blood. 2022;140(Suppl 1):200-2. https://doi.org/10.1182/blood-2022-162387
24. Kantarjian HM, Chifotides HT, Haddad FG, Short NJ, Loghavi S, Jabbour E. Ponatinib-review of historical development, current status, and future research. Am J Hematol. 2024;99(8):1576-85. https://doi.org/10.1002/ajh.27355
25. Moumen A, Dehbi H, Kottwitz D, El Amrani M, Bouchoutrouch N, El Hadi H, et al. Quantitative real-time polymerase chain reaction as an efficient molecular tool for detecting minimal residual disease in Moroccan chronic myeloid leukemia patients. Genet Mol Res. 2015;14(1):1044-55. https://doi.org/10.4238/2015.February.6.8
26. Radich JP. How I monitor residual disease in chronic myeloid leukemia. Blood. 2009;114(16):3376-81. https://doi.org/10.1182/blood-2009-02-163485
27. Hochhaus A, Baccarani M, Silver RT, Schiffer C, Apperley JF, Cervantes F, et al. European LeukemiaNet 2020 recommendations for treating chronic myeloid leukemia. Leukemia. 2020;34(4):966-84. https://doi.org/10.1038/s41375-020-0776-2
28. Shah NP, Bhatia R, Altman JK, Amaya M, Begna KH, Berman E, et al. Chronic myeloid leukemia, version 2.2024, NCCN clinical practice guidelines in oncology. J Natl Compr Canc Netw. 2024;22(1):43-69. https://doi.org/10.6004/jnccn.2024.0007
29. Böyum A. Isolation of mononuclear cells and granulocytes from human blood. Isolation of mononuclear cells by one centrifugation, and of granulocytes by combining centrifugation and sedimentation at 1 g. Scand J Clin Lab Invest Suppl. 1968;97:77-89.
30. Untergasser A, Cutcutache I, Koressaar T, Ye J, Faircloth BC, Remm M, et al. Primer3--new capabilities and interfaces. Nucleic Acids Res. 2012;40(15):e115. https://doi.org/10.1093/nar/gks596
31. Baccarani M, Castagnetti F, Gugliotta G, Rosti G, Soverini S, Albeer A, et al. The proportion of different BCR-ABL1 transcript types in chronic myeloid leukemia. An international overview. Leukemia. 2019;33(5):1173-83. https://doi.org/10.1038/s41375-018-0341-4
32. Bennour A, Ouahchi I, Achour B, Zaier M, Youssef YB, Khelif A, et al. Analysis of the clinico-hematological relevance of the breakpoint location within M-BCR in chronic myeloid leukemia. Med Oncol. 2013;30(1):348. https://doi.org/10.1007/s12032-012-0348-z
33. Bouramtane A, El Aoufi E, Ahakoud M, Elmakhzen B, Lazraq A, Bouguenouch L, et al. P047: Correlation between clinico-biological elements and the type of BCR-ABl transcripts in chronic myeloid leukemia. Genet Med Open. 2023;1(1):100066. https://doi.org/10.1016/j.gimo.2023.100066
34. Wacker MJ, Godard MP. Analysis of one-step and two-step real-time RT-PCR using SuperScript III. J Biomol Tech. 2005;16(3):266-71.
35. Cepheid. Xpert® BCR-ABL Ultra assay [Internet]. Sunnyvale (CA): Cepheid; c2025 [cited 2024 Aug 5]. Available from: https://www.cepheid.com/en-US/tests/oncology-human-genetics/xpert-bcr-abl-ultra.html
36. Passing H, Bablok W. A new biometrical procedure for testing the equality of measurements from two different analytical methods. Application of linear regression procedures for method comparison studies in clinical chemistry, Part I. J Clin Chem Clin Biochem. 1983;21(11):709-20. https://doi.org/10.1515/cclm.1983.21.11.709
37. Mukaka MM. Statistics corner: A guide to appropriate use of correlation coefficient in medical research. Malawi Med J. 2012;24(3):69-71.
38. Bland JM, Altman DG. Statistical methods for assessing

agreement between two methods of clinical measurement. Lancet. 1986;1(8476):307-10.
39. Bustin SA, Benes V, Garson JA, Hellemans J, Huggett J, Kubista M, et al. The MIQE guidelines: minimum information for publication of quantitative real-time PCR experiments. Clin Chem. 2009;55(4):611-22. https://doi.org/10.1373/clinchem.2008.112797
40. Livak KJ, Schmittgen TD. Analysis of relative gene expression data using real-time quantitative PCR and the 2− ΔΔCT method. Methods. 2001;25(4):402-8. https://doi.org/10.1006/meth.2001.1262
41. Cross NCP, White HE, Colomer D, Ehrencrona H, Foroni L, Gottardi E, et al. Laboratory recommendations for scoring deep molecular responses following treatment for chronic myeloid leukemia. Leukemia. 2015;29(5):999-1003. https://doi.org/10.1038/leu.2015.29
42. White HE, Matejtschuk P, Rigsby P, Gabert J, Lin F, Wang YL, et al. Establishment of the first World Health Organization international genetic reference panel for quantitation of BCR-ABL mRNA. Blood. 2010;116(22):e111-7. https://doi.org/10.1182/blood-2010-06-291641
43. Raja S, Ching J, Xi L, Hughes SJ, Chang R, Wong W, et al. Technology for automated, rapid, and quantitative PCR or reverse transcription-PCR clinical testing. Clin Chem. 2005;51(5):882-90. https://doi.org/10.1373/clinchem.2004.046474
44. Winn-Deen ES, Helton B, Van Atta R, Wong W, Peralta J, Wang J, et al. Development of an integrated assay for detection of BCR-ABL RNA. Clin Chem. 2007;53(9):1593-600. https://doi.org/10.1373/clinchem.2007.085472
45. Saussele S, Richter J, Guilhot J, Gruber FX, Hjorth-Hansen H, Almeida A, et al. Discontinuation of tyrosine kinase inhibitor therapy in chronic myeloid leukaemia (EURO-SKI): a prespecified interim analysis of a prospective, multicentre, non-randomised, trial. Lancet Oncol. 2018;19(6):747-57. https://doi.org/10.1016/S1470-2045(18)30192-X
46. Wang YL, Lee JW, Cesarman E, Jin DK, Csernus B. Molecular monitoring of chronic myelogenous leukemia: identification of the most suitable internal control gene for real-time quantification of BCR-ABL transcripts. J Mol Diagn. 2006;8(2):231-9. https://doi.org/10.2353/jmoldx.2006.040404
47. Kottwitz D, El Hadi H, El Amrani M, Cabezas S, Dehbi H, Nadifi S, et al. Evaluation of a novel multiplex RT-qPCR assay for the quantification of leukemia-associated BCR-ABL1 translocation. Int J Hematol. 2015;102(3):335-41. https://doi.org/10.1007/s12185-015-1839-4
48. El Fakih R, Jabbour E, Ravandi F, Hassanein M, Anjum F, Ahmed S, et al. Current paradigms in the management of Philadelphia chromosome positive acute lymphoblastic leukemia in adults. Am J Hematol. 2018;93(2):286-95. https://doi.org/10.1002/ajh.24926
49. Moorman AV, Harrison CJ, Buck GAN, Richards SM, Secker-Walker LM, Martineau M, et al. Karyotype is an independent prognostic factor in adult acute lymphoblastic leukemia (ALL): analysis of cytogenetic data from patients treated on the Medical Research Council (MRC) UKALLXII/Eastern Cooperative Oncology Group (ECOG) 2993 trial. Blood. 2007;109(8):3189-97. https://doi.org/10.1182/blood-2006-10-051912
50. Soverini S, Hochhaus A, Nicolini FE, Gruber F, Lange T, Saglio G, et al. BCR-ABL kinase domain mutation analysis in chronic myeloid leukemia patients treated with tyrosine kinase inhibitors: recommendations from an expert panel on behalf of European LeukemiaNet. Blood. 2011;118(5):1208-15. https://doi.org/10.1182/blood-2010-12-326405
51. Branford S, Kim DDH, Apperley JF, Eide CA, Mustjoki S, Ong ST, et al. Laying the foundation for genomically-based risk assessment in chronic myeloid leukemia. Leukemia. 2019;33(8):1835-50. https://doi.org/10.1038/s41375-019-0512-y
52. García-Gutiérrez V, Hernández-Boluda JC. Current Treatment Options for Chronic Myeloid Leukemia Patients Failing Second-Generation Tyrosine Kinase Inhibitors. J Clin Med. 2020;9(7):2251. https://doi.org/10.3390/jcm9072251
53. Senapati J, Sasaki K, Issa GC, Lipton JH, Radich JP, Jabbour E, et al. Management of chronic myeloid leukemia in 2023 - common ground and common sense. Blood Cancer J. 2023;13(1):1-12. https://doi.org/10.1038/s41408-023-00823-9