SAS Journal of Medicine
Abbreviated Key Title: SAS J Med
ISSN 2454-5112
Journal homepage: https://saspublishers.com

OPEN ACCESS

Hematology

Contribution of Molecular Biology in the Diagnosis and Follow-Up of Chronic Myeloid Leukemia

H. Bennani[1*], A. El Ouarradi[1], H. Lazrek[1], H. Yahyaoui[2, 3], M. Chakour[2, 3]

[1]Hematology Laboratory, Mohamed VI University Hospital, Marrakech, Morocco
[2]Hematology Laboratory, Avicenne Military Hospital, Marrakech, Morocco
[3]Faculty of Medicine and Pharmacy, Marrakech, Morocco

DOI: 10.36347/sasjm.2023.v09i08.018 | **Received:** 19.07.2023 | **Accepted:** 26.08.2023 | **Published:** 31.08.2023

***Corresponding author:** H. Bennani
Hematology Laboratory, Mohamed VI University Hospital, Marrakech, Morocco

Abstract **Original Research Article**

Background: The advent of tyrosine kinase inhibitors and the evolution of molecular biology techniques have revolutionized the management and outcome of patients with chronic myeloid leukemia, it is currently considered a model of carcinogenesis and successful targeted therapy. ***Methods:*** Our study was retrospective, descriptive and observational, carried out within the hematology laboratory of the Avicenna Military Hospital in Marrakech, spread over a period of 6 years and included a total of 10 patients from the department of clinical hematology. The aim of our study was to highlight the impact of molecular biology, in particular the GeneXpert technique, on the diagnosis and monitoring of Chronic myeloid leukemia (CML), and to discuss afterwards the different techniques as well as their contribution. ***Results:*** Regarding the diagnostic data; all of our patients underwent a complete blood count, myelogram, and bone marrow karyotype. The complete blood count (CBC) showed leukocytosis in 100% of cases, thrombocytosis and anemia in 80% of cases. The myelogram showed a medullary blastosis with a rate <10% in 90% of the cases, thus classifying them in the chronic phase. At karyotype, all patients had the translocation (9; 22). Concerning molecular biology, quantification of the BCR-ABL transcript by GeneXpert was performed in 60% of patients, only 20% of cases benefited from both the quantitative study by GeneXpert and the qualitative study by multiplex PCR. During treatment, 80% of our patients were put on hydroxyurea pending confirmation of the diagnosis. Later on, all of our patients were treated with first-line imatinib, of which 3 (30%) had treatment failure requiring to be put on second-linenilotinib. Of these, 2 were treated with third-line dasatinib. GeneXpert was used for follow-up, a major molecular response (MMR) was achieved in 70% of the cases with an average delay of 9 months. The profiling of patients who obtained an MMR showed a male predominance (gender ratio M/F: 2.5) with a median age of 55 years. As for the 3 patients who failed to respond to TKI treatment, the type of transcript was identified in a single patient (b2a2 and b3a2), two other patients benefited from the search for the T315I mutation by direct sequencing which subsequently turned out to be negative. ***Conclusions:*** Based on the results of our study, we conclude that the sensitivity, standardization and automation of GeneXpert, provides us with a powerful tool to ensure the diagnosis of patients carrying major transcripts and subsequently assess the molecular response in order to identify more quickly patients in failure or at high risk of failure, thus allowing a better understanding of the prognosis and establishing an efficient therapeutic strategy. However, each laboratory must be aware of the necessity of multiplex RT-PCR technique during diagnosis, in order to be able to detect all the known BCR-ABL1 molecular rearrangements, and then enable an adequate molecular follow-up. The implementation of next generation sequencing (NGS) is also desirable in order to guide the choice of treatment through characterizing the mutational profile of the disease.

Keywords: Chronic myeloid leukemia – BCR–ABL molecular Biology–GeneXpert.

INTRODUCTION

Chronic myeloid leukemia (CML) is a hematologic malignancy belonging to the group of myeloproliferative neoplasia [1]. It is the first disease associated with a chromosomal abnormality: the Philadelphia chromosome (Ph1). The latter results from a reciprocal translocation t (22q; 9q) between the long arms of chromosomes 9 and 22, which leads to the formation of a Breakpoint Cluster Region-Abelson Murine Leukemia (BCR-ABL) fusion gene, followed by the translation of the BCR-ABL fusion protein with

an intense tyrosine kinase activity, which causes the leukemic transformation of the disease [2].

CML thus represents a model of oncogenesis. Identifying its molecular marker BCR-ABL has allowed the development of targeted therapy with tyrosine kinase inhibitors (TKIs) and the implementation of more efficient techniques for its diagnosis and follow-up [3, 4].

As a result, new proposals for patient management have been developed. Due to their low sensitivity, cytogenetic tests are no longer considered reference methods for the diagnosis and follow-up of CML, giving way to biomolecular tests performed on peripheral blood and with high sensitivity in the qualitative research and quantitative measurement of BCR-ABL1 transcripts [5].

Currently, the qualitative multiplex reverse transcription polymerase chain reaction (RT-PCR) is recommended for diagnosis in order to detect the different possible BCR- ABL1 rearrangements and to confirm the diagnosis. As for the real-time quantitative polymerase chain reaction (RQ-PCR), it quantifies in vitro the BCR-ABL1 fusion transcripts at diagnosis in order to serve as a reference for the evaluation of the response to treatment and allows, at follow-up, to define molecular response values common to all laboratories [3, 4].

Nevertheless, RQ-PCR has its drawbacks. Its laborious approach, the difficulties in standardizing results, and its cost have necessitated the use of automation. The GeneXpert technique has been introduced as a robust and reproducible alternative that allows a faster and less expensive quantitative detection with a sensitivity in the range necessary for clinical decisions [6].

Indeed, it is by using the GeneXpert test that it has become easier to verify the efficacy of the treatment, to highlight poor compliance or resistance to treatment. It is also easier to initiate a change of TKI and a search for the mechanism of resistance, particularly a search for a mutation in the tyrosine kinase domain of BCR- ABL using molecular biology techniques, in particular, next generation sequencing (NGS) [4].

However, in our country, access to these means of diagnosis and molecular monitoring of CML still needs to be improved. It is limited to a few hospitals, thus hindering the establishment of adequate management following the recommendations and exposing patients to the risk of progression of the disease to advanced phases, which are most often fatal.

The objective of our work is to highlight the contribution of molecular biology techniques, in particular the GeneXpert technique, in the diagnosis and follow-up of CML in order to elaborate recommendations adapted to our context, which will serve for the optimal application of molecular biology techniques and will allow not only the improvement of the strategy of management of these patients but also the reduction of the socio-economic burden of the disease.

MATERIALS AND METHODS

Our study concerned ten patients referred by the clinical hematology department to the hematology laboratory of the Avicenne military hospital (HMA) - Marrakech for their CML diagnosis and biological follow-up. The diagnosis was confirmed by molecular biology or cytogenetics, and the quantitative PCR technique of BCR-ABL transcript performed the follow-up. Patients with incomplete anamnestic and biological data and for whom follow-up was not based on molecular biology were excluded from our study.

We conducted a descriptive, observational, retrospective study over six years (between April 2015 and February 2021). Within the medical analysis laboratories of the HMA-Marrakech is the hematology laboratory composed of three units: cytology, hemostasis, and molecular biology. Our study took place in the molecular biology unit.

Clinical data were collected from medical records archived in the clinical hematology department, and biological data were collected from a computer database in the hematology laboratory. All the data were processed using a data processing sheet specifying each patient's socio-demographic, clinical, and biological aspects while focusing on information concerning the diagnosis and follow-up by molecular biology and their impact on management.

The GeneXpert BCR-ABL Ultra model GX-I automated system is used for sample analysis. Results are automatically interpreted and expressed on the International Scale (IS) by the GeneXpert software from the measured fluorescent signals and built-in calculation algorithms. The software allowed us to determine a threshold (modifiable) according to the basic fluorescence. This threshold defines cycles at threshold: cycle threshold (Ct). Ct's are the number of PCR cycles required to detect a fluorescent signal significantly higher than the baseline. A calibration curve with the Ct versus the amount of fluorescence is plotted to determine the amount of transcript present in the sample.

We performed statistical analysis of the data using Excel 2016 software. Text and tables were entered in Microsoft Word 2016, and graphs were entered in Excel 2016. The results were expressed as a percentage or as a mean and median according to the variables studied.

RESULTS

I-Demographics:

Of the ten patients studied, the sex ratio (M/F) was 2.3, seven were male, and three were female. The mean age was 59.2 years, with a standard deviation of 8.6 (59.2 ± 8.6) and extremes ranging from 46 to 74 years.

As for antecedents, 20% were chronic smokers, 10% had concomitant multifocal tuberculosis at diagnosis, and 20% had hypertension. Exposure to hydrocarbons and/or ionizing radiation was not reported in any of our patients. The most frequent mode of revelation was the anemic syndrome in 60% of patients. The physical signs observed in the patients in our series were characterized by the predominance of splenomegaly (SPM) in 50% of cases. Isolated mucocutaneous pallor was observed in 20% of patients.

II-Biological diagnosis:

The blood count performed at the time of diagnosis showed pathological results in 100% of the cases studied. The leukocyte count was the most disturbed parameter: hyperleukocytosis was observed in all patients with a mean of 166.9 G/L and extremes ranging from 57.9 to 270 G/L. Thrombocytosis was objectified in 8 patients (80%) with a mean of 482.2 G/L and extremes ranging from 468 to 1607 G/L. We noted the presence of normocytic normochromic anemia in 80% of patients, with a mean hemoglobin value of 10.9 g/dL and extremes ranging from 12 to 7 g/dL. All patients also had myeloma, as shown on the blood smear, with an average of 36%. Blood blasts were present in 90% of patients.

The myelogram showed the presence of bone marrow blasts in 90% of our patients. The bone marrow karyotype showed the presence of the t (9,22) translocation without additional cytogenetic abnormalities (ACA) in all patients.

At the time of diagnosis, the quantification of BCR-ABL1 fusion transcript by the GeneXpert test was performed in 80% of the patients whose results were positive, with a median BCR-ABL1/ABL1 ratio of 68% (Figure 1). The molecular study by multiplex RT-PCR was performed externally in 20% of the patients (2 patients), showing the co-expression of the two types of transcript e13a2 and e14a2 (b2a2 and b3a2) in all the samples analyzed, with the absence of other types of transcript, namely e19a2, and e1a2.

Among the ten patients included in our study, nine patients were in the chronic phase of the disease. Only one patient was in the accelerated phase at the time of diagnosis.

For the prognostic score, we classified the patients according to the European Treatment Outcome Study Long-Term Survival (ELTS), which allowed us to divide them into three groups:

- Low-risk group with a score ≤ 1.5680, comprising 4 patients;
- Intermediate risk group with a score > 1.5680 and ≤ 2.2185, comprising 3 patients;
- High-risk group with a score > 2.2185, comprising three patients.

Regarding treatment, all patients had received initial hospitalization with peripheral venous hyperhydration with 9% isotonic saline (500 mL/6h). Only one patient was transfused with two packed red blood cells following a poorly tolerated anemia. Three patients (30%) were on anticoagulant therapy with low molecular weight heparin at a dose of 0.4 mL/d. Eight of our patients (80%) were put on pre-phase hydroxyurea pending confirmation of the diagnosis at a dose of 2g/d for an average period of 6.75 days, with extremes ranging from 3 to 14 days. None of our patients underwent transplantation. Only one patient (10%) received anti-bacillary therapy (ERIPK4:4cp/d) following a concomitant diagnosis of pulmonary tuberculosis.

For targeted therapy, all patients in our study were initially started on imatinib 400mg/d for a mean duration of 18 months, with extremes ranging from 3 to 47 months. During treatment, 50% of patients experienced adverse events, including muscle cramps (10%), eyelid edema (10%), febrile neutropenia with myocarditis (10%), and drug-induced toxidermia in two patients. Symptomatic treatment with dose reduction was maintained in the majority of cases. The patient with myocarditis was hospitalized, and the treatment was stopped for one month. Only one patient had benefited from a therapeutic change.

Four patients (40%) were switched to a second-line TKI (nilotinib) at a dose of 400mg x2/d, one because of intolerance to imatinib and three because of treatment failure. The median duration of treatment with nilotinib was 18 months, ranging from 3 to 24 months. Following nilotinib failure, two patients were started on a third-line TKI (dasatinib) at a dose of 140 mg/d for a median duration of 34 months. We noted poor adherence in both patients following difficulties in the treatment supply. Reintroduction of nilotinib was initiated in one patient following the onset of adverse events on dasatinib, including thrombocytopenia and febrile neutropenia that were arrested at three months, with the occurrence of pleural effusion at 25 months. In our series, no patient had to stop treatment.

III- Molecular follow-up:

The follow-up of the response to TKI treatment was based on molecular biology. It was performed according to the criteria defined by the European Leukemia Network (ELN) recommendations

[4] with concomitant monitoring of hematological responses. It was performed for a mean duration of 3 years with extremes ranging from 1.5 to 6 years.

1. Results of BCR-ABL1 transcript quantification and kinetics:

The molecular evaluation was performed on all patients at different points of their molecular follow-up.

The two patients whose primary transcript could be identified were either:

- In optimal response to treatment (n=1) defined by a decrease in BCR-ABL1 ≤ 10% at three months, ≤ 1% at six months, and ≤ 0.1% at 12 months. In this patient, we also observed the achievement of a significant molecular response (MMR), which corresponds to a 3log (≤0.1%) reduction in BCR-ABL1 transcript level at six months, MMR4 at nine months, an MMR4.5 at 12 months, and an MMR5 at 15 months of treatment.
- In treatment failure (n=1), defined by a BCR-ABL1 level >10% at 3 and 6 months and >1% warranting recourse to second-line therapy under which we noted persistent failure.
- The eight patients whose transcript was not identified were either in the:
- Optimal treatment response (n=6) with the early molecular response (eMR) defined as BCR-ABL1 decrease ≤ 10% at three months, ≤ 1% at six months. We noted in all of these patients the achievement of an MMR, with subsequently:
    - o RM4 (n=2), which corresponds to more than 4 log (≤0.01% IS) reduction in BCR-ABL transcript level;
    - o RM4.5 (n=2), which corresponds to a reduction of more than 4.5 log (≤0.0032% IS) of the BCR-ABL transcript level;
    - o RM5 (n=2), which corresponds to a greater than 5 log (≤0.001% IS) reduction in BCR-ABL transcript level.
- Treatment failure with primary resistance to imatinib (n=2) (Table I).

For BCR-ABL1/ABL1 ratio decay kinetics, we found a rapid decay profile in patients who achieved deep molecular responses (DMRs) very rapidly (Figure 2 & Figure 3).

These two patients had MMR-like molecular responses within 6 and 9 months. They are male, with an age of 74 years for the patient in whom MMR was noted at six months and 55 years for the other patient. Both subsequently had deep molecular responses maintained throughout treatment with imatinib (Figure 3).

2. Results of qualitative assessment of molecular response:

a. Response under 1st line therapy:

Time to MMR was variable between 6 months to 18 months with a mean of 9 months.

- At three months of treatment: 10 patients were evaluated. 60% of patients were in optimal response with a 1 log (10) reduction (≤10% IS). One patient was put on 2 nd line therapy following the occurrence of drug-induced toxidermia. One patient was in alert status with a transcript level >10% IS, and 30% of patients were in failure.
- At six months of treatment: 9 patients were evaluated, and 66.7% were in optimal response with a reduction of 2 log (10) (≤1% IS), including one patient who was in alert status at the third month. Among these six patients, two were in MMR. 33.3% were still in treatment failure (<1 log (10)). At 12 months of treatment: 9 patients were evaluated; 33.3% had achieved an optimal response, 2 had a PMR, and one was still in MMR. Three patients went to alert status, and three patients did not achieve a minimum 2 log (10) reduction level and are considered treatment failures. These patients were switched to 2nd line treatment.
- At 18 months of treatment: 6 patients were evaluated, and 83.3% had an optimal response. 30% of patients had an MMR, one had a PMR, and one had an alert.
- At 24 months of treatment: 4 patients were evaluated, three patients had an optimal response with an MMR, and one patient was on alert.

b. Response under 2nd line treatment:

- Response at three months: 4 patients were evaluated. 75% had an optimal response, and 25% had a warning.
- Response at six months: 4 patients were evaluated. 25% had an optimal response with MMR, 50% had a warning, and 25% were in therapeutic failure. We noted one patient who stopped treatment during the fourth and fifth month due to supply difficulties.
- Response at 12 months: 4 patients were evaluated. 25% had an optimal response with therapeutic failure in 3 patients (75%). One patient was put on 3rd line treatment.
- Response at 18 months: 3 patients were at 18 months of treatment. 33.3% were in optimal response, and 66.6% were still in failure.
- Response at 24 months: 2 patients were at 24 months of treatment. Both patients were still in failure. The decision was made to switch to a 3rd line treatment.

c. Response to 3rd line therapy:

- Response at three months of treatment: 2 patients were evaluated. The optimal response in 1 patient (50%) and a warning in the other patient (50%).
- Response at six months of treatment: 1 patient (50%) had a warning, and the other patient (50%) had a treatment failure.
- Response at 12 months of treatment: Both patients were in failure
- Response at 18 months of treatment: The two patients were still in failure

3. Determinants of significant molecular response:

For gender, among the seven patients who achieved MMR, we noted a male predominance with a sex ratio (M/F) of 2.5. The median age of patients who obtained MMR was 55 years, with extremes ranging from 46 to 74 years.

For the ELTS prognostic score, we noted a higher proportion of obtaining an MMR in low-risk patients (100%) than in intermediate- or high-risk patients (50%). However, this result is inconclusive: Odds ratio = 1.25 (95% CI: [0.07-22.68]). For early molecular responses at 3 and 6 months, patients in optimal response at three months had MMRs of 66.6% and 83.3%, respectively, at 12 and 18 months. Patients in optimal response at six months had MMR at 12 and 18 months for 57.1 and 85.7%, respectively (Figure 4, Figure 5, Figure 6 & Figure 7). However, no patient who had failed at six months had achieved MMR.

4. Survival:

During follow-up (72 Months), there were no deaths. Therefore, the estimated probability of death-free survival at 72 months is 100%. The events considered are loss of molecular response, death, and accelerated or blast phase transformation. The probability of event-free survival (EFS) is 70%.

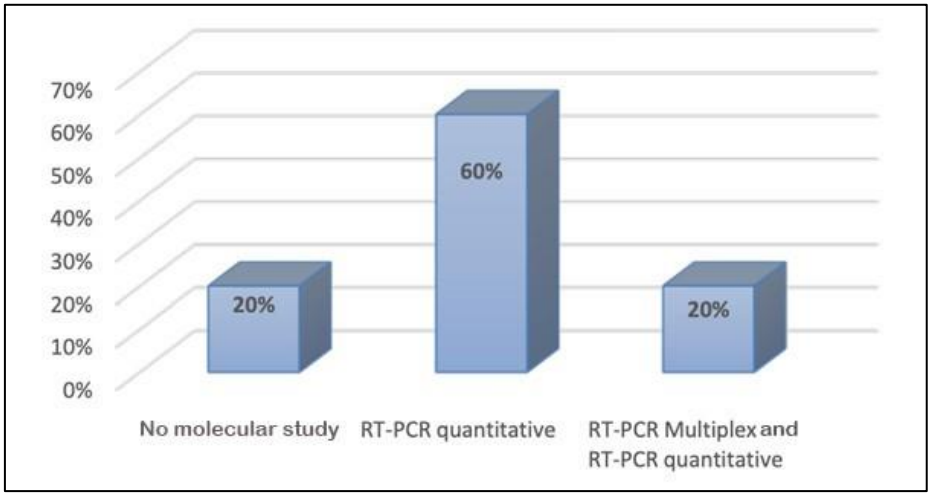

Figure 1: Characteristics of the molecular study in the patients studied

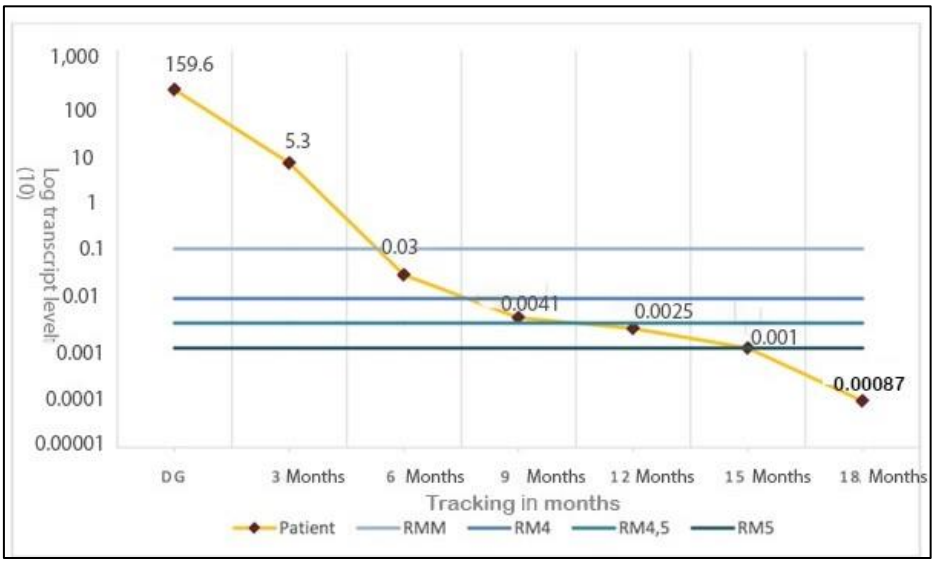

Figure 2: Profile showing a rapid decrease of the BCR-ABL1 transcript level in one patient in our series, reaching after six months of treatment an MMR

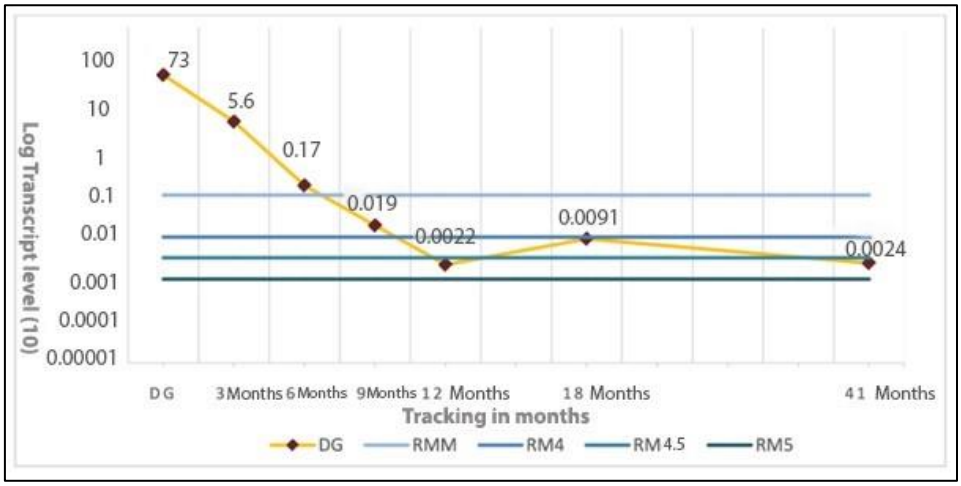

Figure 3: Profile showing a rapid decrease in BCR-ABL1 transcript level in one patient in our series, reaching MMR after nine months of treatment

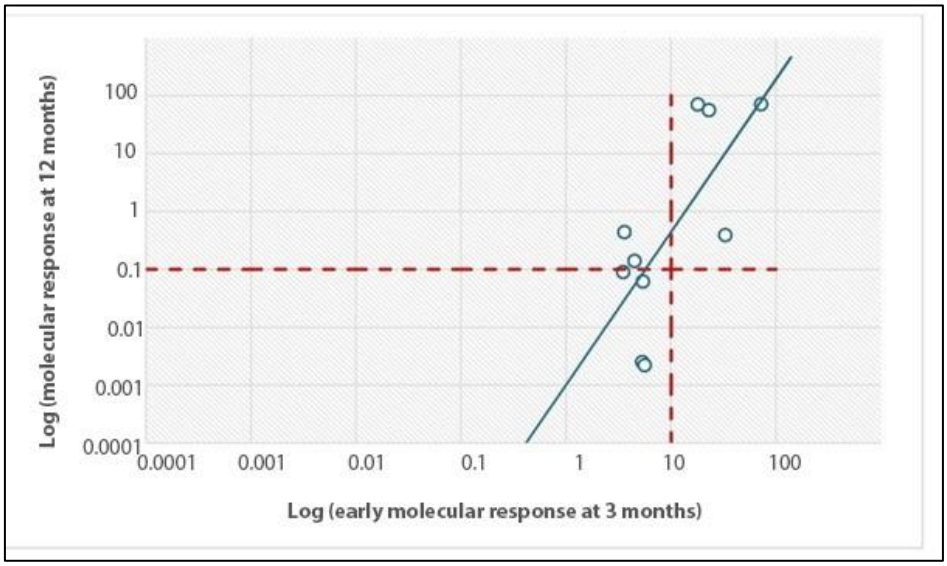

Figure 4: MMR at 12 months based on the early response at three months

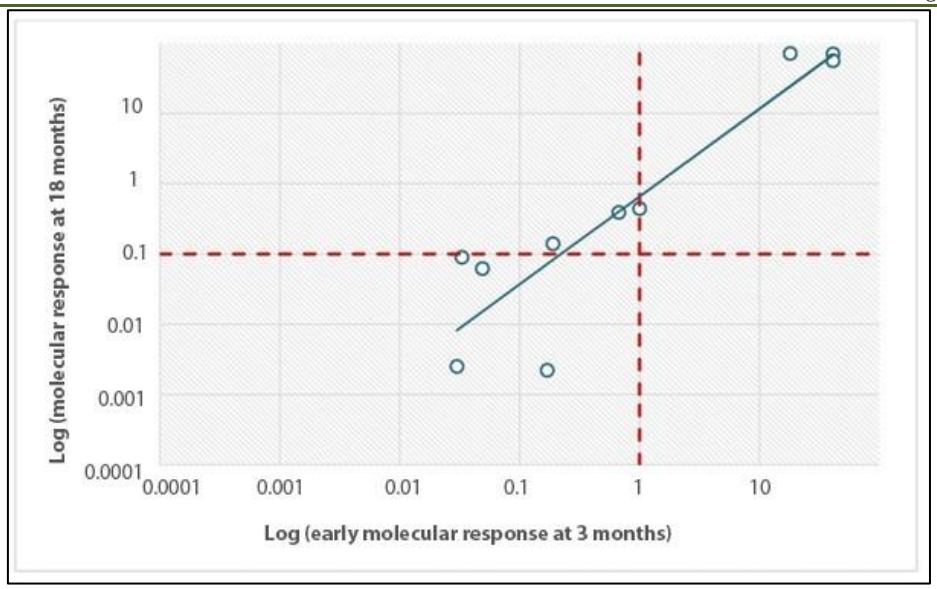

Figure 5: MMR at 18 months based on the early response at three months

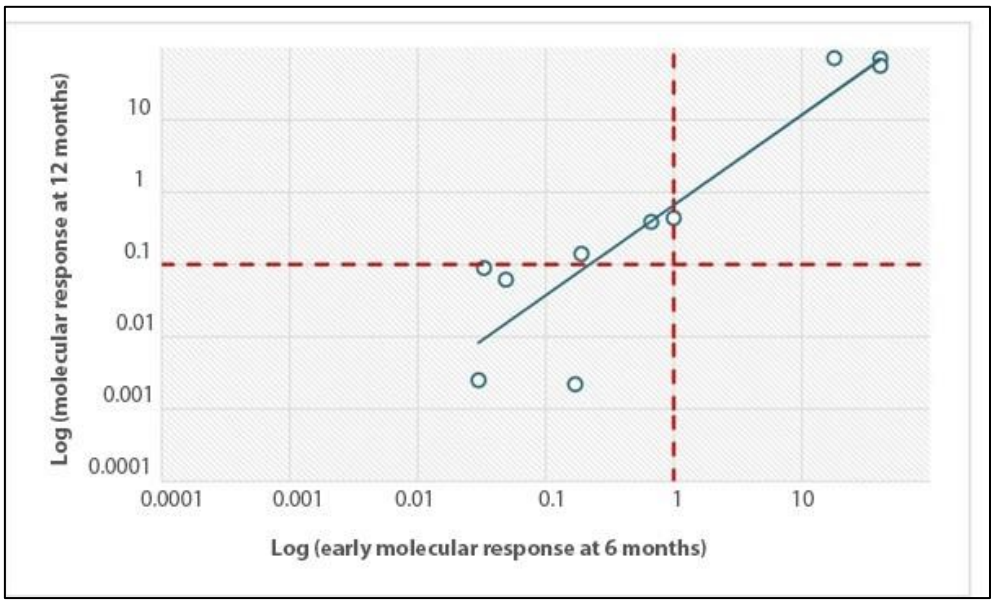

Figure 6: MMR at 12 months based on the early response at six months

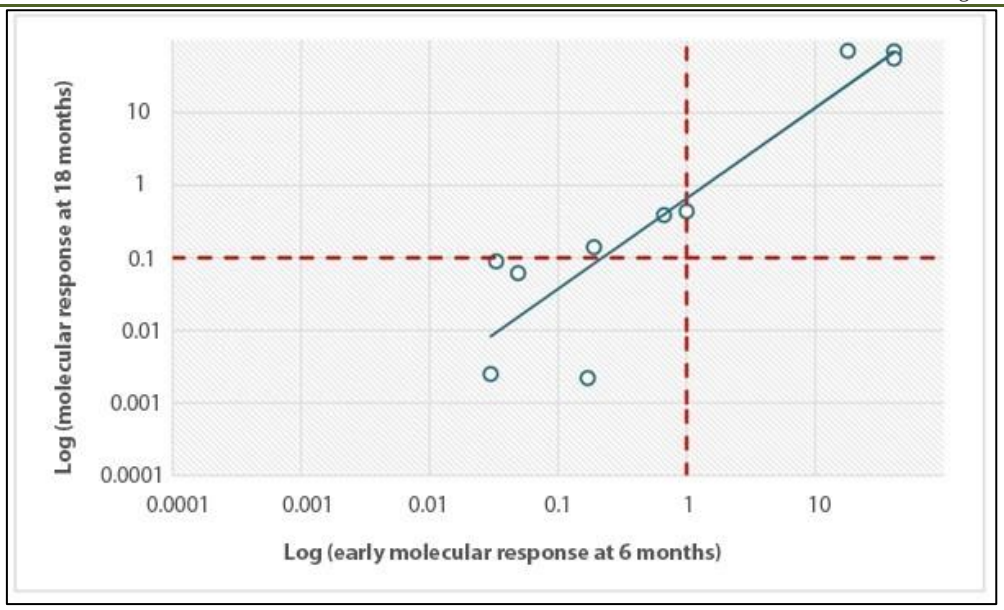

Figure 7: MMR at 18 months based on the early response at six months

Table 1: Characteristics of molecular responses to treatment in our series

		Molecular responses				
		RMPr	**RMM**	**RMP**		
				RM4	**RM4.5**	**RM5**
Percentage		70%	70%	30%	20%	20%
Leadtime	3 months	n=6	-	-	-	-
	6 months	n=1	n=3	-	-	-
	9 months	-	n=1	n=1	-	-
	12 months	-	-	-	n=2	-
	15 months	-	n=1	n=1	-	n=1
	18 months	-	n=2	-	-	-
	24 months	-	-	n=1	-	-
	45 months	-	-	-	-	n=1
Average time		3 months	9 months	15 months		

Table 2: Comparison of blood count data in several series

Series	**White blood cells**				**Platelets**	
	Average value (G/L)	**Extremes (G/L)**	**100,000-4000,000 /mm3**	**>400,000 /mm3**	**Average value (G/L)**	**Extremes (G/L)**
Chikkodi *et al*., [19]	138.3	[4.1-697]	-	-	326	[85-1,819]
El Mouhdi	239	[27-413]	75%	15%	298	[116-1,071]
Lang *et al*., [22]	95	[9.2-598]	-	-	395	[89-4,800]
Agharbi	-	[20-900]	13%	52%	-	[154-800]
Our study	166.9	[57.9-270]	90%	0%	482.2	[168-1,607]

Table 3: Comparison of karyotype results according to the different series

Studies	**t(9 ;22)**	**t(9 ;22) negative**	**ACA**
Hoffmann *et al*., [10]	86.9%	3.7%	9.4%
Smith *et al.*,	86.4%	8.6%	5.0%
Mukibi *et al*.,	95%	5%	0%
Djouadi *et al*.,	95.3%	0.9%	3.8%
El Mouhdi	73.6%	26.4%	2.56%
Our study	100%	0%	0%

DISCUSSION

CML constitute about 15% of newly diagnosed leukemias in adults [2]. It is related to an acquired genetic abnormality in a hematopoietic stem cell: a balanced reciprocal translocation between the long arms of chromosomes 9 and 22: t (9;22) (q34; q11). Chromosome 22, shortened by material exchange, is called chromosome Ph1 and is found in about 95% of CML patients [7, 8]. CML is an insidious disease and is most often discovered incidentally during a routine CBC. It may also be revealed by MPS, found in more than 50% of chronic-phase CML patients, or by other general signs that are not specific to the disease [2].

I-Characteristics of the population:

Our study showed a male/female gender ratio of 2.3. In the literature, several studies have shown a clear male predominance in the distribution of CML with a gender ratio (M/F) close to 2 [2]. On the other hand, another study [9] did not show any difference in the distribution of CML according to sex in the sample studied.

Age is essential in conditioning the prognosis and, consequently, therapeutic decisions [4]. CML can develop at any age, although its peak incidence occurs during adulthood [3]. Our study showed a mean age of diagnosis of 59.2 years. This result is consistent with data from the EUTOS [10] and SIMPLICITY [11] registries of European and American populations in which the mean age at diagnosis is 57 years. However, there is a discrepancy with the results established in populations from developing and underdeveloped countries where CML occurs mainly in young subjects below the age of 50 years [12].

The use of TKIs and various molecular biology tools has revolutionized the management of CML. However, several factors, including access to care, cost of drugs, means of follow-up, and medical coverage, impact the overall survival of patients [13]. In our study, all of our patients had medical insurance allowing them to obtain TKIs and regular follow-ups with the GeneXpert technique.

In the literature, CML is often discovered incidentally by routine CBC. Symptomatology consisting of left hypochondrial heaviness, fever, weight loss, and night sweats has also been reported and is mainly seen in patients with more advanced diseases [14-16]. In our study, the anemic syndrome was the most frequent reason for consultation, found in 60% of cases. Its presence is partly related to the delay in diagnosis. The non-routine practice of blood count in our context is the probable cause of this difference. On clinical examination, MPS was present in 50% of our patients. This is consistent with the results of the international randomized interferon study (IRIS) [17]. On the other hand, none of our patients presented hepatomegaly or adenopathy, and this is contrary to the results of other series [18-20].

II-Biological diagnosis:

The blood count is essential because it allows for evoking the diagnosis. In our series, hyperleukocytosis is frank in all our patients. This is in agreement with the literature series. Moreover, thrombocytosis is very frequent in our series. However, no thrombotic events were reported in our patients, which is consistent with the literature [2].

On the other hand, anemia was noted in 80% of the cases, thus agreeing with the literature results [20, 21]. Finally, the reading of the blood smear revealed the presence of myeloma. This result agrees with the literature data where the importance of myeloma increases with the increase of leukocytosis [22].

In our series, the myelogram was performed in all patients, showing a marrow rich in granular and megakaryocytic cells in all cases, which is consistent with the literature [2]. This myelogram also allowed the classification of patients according to the evolutionary phases of the disease.

Conventional cytogenetics (bone marrow karyotype) is essential. It allows the detection and quantification of the percentage of Ph+ cells carrying the t (9;22) translocation (q34; q11) in 90% of CML patients at the time of diagnosis [23] (Table III).

In our study, 80% of the patients underwent quantitative PCR at the time of diagnosis using the GeneXpert automated system. In the literature, the use of quantitative methods at diagnosis remains debated. The authors of the NCCN guidelines recommend performing QR-PCR at the outset and using qualitative PCR only if there is a discrepancy between cytogenetic testing results and quantitative PCR [24]. This is useful since the quantitative study of BCR-ABL transcript levels not only allows the detection of the most frequent transcripts (M-BCR) but also establishes a baseline value for the study of decline kinetics that influences therapeutic decisions during follow-up. Furthermore, some studies have reported the association of high transcript levels at diagnosis with a lower probability of response [25].

However, routine assessment by QR-PCR is not considered necessary at this stage, according to ELN recommendations [4]. Indeed, quantitative PCR, particularly the GeneXpert technique has several limitations; in addition to the high cost of the cartridges, it only allows the detection of b2a2 and b3a2 transcripts. This test is, therefore, not suitable for screening patients at diagnosis [26]. Consequently, it is currently recommended to perform a qualitative PCR at diagnosis, particularly a multiplex PCR capable of

amplifying and identifying several transcripts simultaneously in a single PCR reaction [27, 28].

In our series, only 20% of patients had access to multiplex RT-PCR. It showed the co-expression of both types of transcripts e13a2 and e14a2 (b2a2 and b3a2) in all samples analyzed. This co-expression is explained by the process of alternative splicing [29]. One study showed that the b3a2 transcript was the most frequent, its overall proportion was 54.5%, and the simultaneous expression of the e13a2 and e14a2 transcripts constituted only 7.6% of the variants detected, with a predominant distribution in Australia (18.7%) and less frequent in Asia (2.4%) [30]. Variability in the incidences of different transcripts across regions is also observed in several studies; different genetic profiles, environmental factors, and the detection sensitivity of the different methods used may be the cause [31].

Currently, TKIs are the recommended first-line treatment. However, using a short course of hydroxyurea chemotherapy is possible while awaiting diagnostic confirmation [4]. Following these recommendations, 80% of patients in our series received pre-phase chemotherapy. The first-line drugs effective in newly diagnosed chronic-phase CML are imatinib, dasatinib, nilotinib, and bosutinib. The choice depends on risk score, toxicity profile, age, tolerance, and comorbidities [3].

III. Molecular monitoring:

Currently, molecular biology is the reference means for monitoring therapeutic responses. Indeed, due to their insufficient sensitivity for the study of responses, the indication of cytogenetic methods remains restricted to patients with atypical translocations, rare or atypical BCR-ABL transcripts, therapeutic resistance or failure, and during the progression of the disease towards an accelerated or blastic phase [4].

1. Means:

a. Monitoring of molecular responses: GeneXpert:

In our study, the monitoring of therapeutic responses was based on the GeneXpert technique. Its sensitivity and speed of execution allowed clinical decisions to be made promptly. Indeed, this method allows reproducible detection of low levels of transcripts with a proven limit of detection (LOD) and quantification (LOQ) of 4.5 log (<0.0032%). The possibility of inter-laboratory standardization with no need for a calibration line is also among its advantages [32, 33].

b. Detection of transcript mutations:

Despite the many advantages of the GeneXpert test, it is still unable to ensure a complete follow-up, especially in patients who are in failure and who require, in addition to the quantification of the BCR-ABL transcript, more detailed explorations, including the search for mutations in the transcript, which requires molecular biology techniques.

In our series, among the three unsuccessful patients, two patients could benefit from the search for the T315I mutation by direct double-strand sequencing using the Sanger method. This technique was long considered the reference method for mutational analysis of the kinase domain of BCR-ABL [34].

2. Assessment of molecular responses:

a. Quantitative assessment:

Ten patients underwent molecular follow-up according to ELN recommendations [4]. This follow-up allowed us to stratify these patients according to the definitions of response to treatment and to detect those resistant to the initial treatment.

The heterogeneity of disease expression means that the kinetics of BCR-ABL transcript decay vary from patient to patient, depending on their response to treatment. This was one of the findings of the IRIS trial, in which about 45% of the original cohort discontinued treatment because of inefficacy or intolerance [17]. Response to treatment is therefore not uniform, suggesting the existence of other intrinsic factors incriminated in this variability.

b. Qualitative assessment:

The ELN recommendations [4] have established three levels of response that should be considered when making treatment decisions. The optimal response is associated with an excellent long-term outcome and indicates the continuation of current therapy. Failure indicates the need to change treatment to avoid disease progression. Warning indicates the need for close monitoring to allow for appropriate changes in the event of failure [35, 36].

3. Determinants of major molecular responses:

Despite the significant improvement in survival and management of patients on TKIs, early prognostic evaluation is necessary to consider a possible therapeutic change and ensure a favorable disease course.

In our work, we studied the profile of patients who obtained MMR based on the following factors: gender, age, ELTS prognostic score, and BCR-ABL1 transcript expression levels at 3 and 6 months. Other factors have also been studied in the literature, including transcript type and BCR- ABL1 transcript downregulation dynamics.

a. Gender:

One of the findings of our study was that men have a higher MMR rate than women (gender ratio M/F = 2.5). However, this finding is disputed in the literature where studies report a significantly higher rate of MMR

in women compared to men [37-39]. This difference in MMR distribution by gender was explained by better adherence to treatment by women than men [40].

b. Age:

The role of age in the achievement of MMR remains controversial in the literature, mainly due to the interplay of several factors including the difference in pharmacokinetics, adherence to treatment, and the variability of tolerance of TKIs by age. In this sense, Marin et al. [40] demonstrated in a series of 87 patients that elderly subjects had a higher rate of adherence to treatment than young subjects.

On the other hand, a Czech study including 103 patients showed higher hematological toxicity in elderly subjects in the 61 to 86 age group with a lower frequency of MMR at 18 months compared to subjects in the 19 to 60 age group (63% versus 79%) [24].

Finally, another study by Lin *et al*., [39] reported a higher frequency of MMR in patients aged 61 to 71 with fewer relapses (7.1%).

c. Early molecular responses:

Determination of BCR-ABL transcript levels by GeneXpert assay after initiation of TKI therapy has a long-term impact. Indeed, the detection of rMPR (BCR-ABL levels ≤ 10% at three or/and ≤ 1% at six months of treatment) is a good predictor of treatment response with better overall survival, progression-free survival, as well as a higher incidence of major molecular response [18]. This explains the incorporation of BCR-ABL levels at 3, 6, and 12 months in the ELN and NCCN recommendations for monitoring treatment response [3, 4].

Our result agrees with that published in the IRIS study [17] where 100% of patients who had rMR at three months achieved MMR at 24 months without developing treatment resistance after 30 months. The same finding was reported by Branford et al. in a study including 55 patients treated with imatinib; patients who had 3-month rMMPR had a significantly better MMR rate at 24 months compared with those who had a ≤2 log10 reduction at three months (100 vs. 54%, p <0.001) [18].

4. Determinants of failure and survival:

Our results are consistent with data in the literature. Men generally have a higher probability of failure than women [38-40]. Several studies have also reported a statistically significant correlation between prognostic scores and the occurrence of failure [35, 26], with a better predictive value of the ELTS score compared to the Sokal score [31, 40].

In contrast, in our study, no failure was observed in patients who had rMDR at 3 or 6 months, and this following the results of different studies reporting the value of molecular response at three months and six months in characterizing patients at high risk of failure or relapse and those with a better probability of PFS [32, 37].

It is important to note that the interpretation of failures must necessarily take into consideration not only the quality of molecular responses but also the patient's profile, clinical course, and socio-economic background. These factors define the goal of treatment and allow for a more realistic and individualized approach to patient management, and are especially relevant in ambiguous situations where a global vision is necessary to avoid disease progression and ensure patient survival [3].

CONCLUSION

CML is a model in onco-hematology. It represents the first malignant disease whose chromosomal abnormality has been described. Its leukemogenesis has been exceptionally well elucidated, allowing significant therapeutic advances. It is the first disease in which targeted molecular therapy has been successfully used.

The knowledge of the molecular basis of this disease has also allowed the development of more sensitive and robust techniques to ensure the management of patients from diagnosis to follow-up, in particular multiplex RT-PCR, RQ-PCR and its automated version, the GeneXpert test, as well as the various tools for the detection of tyrosine kinase domain mutations, namely direct sequencing and high throughput sequencing.

Based on the results of our study, we conclude that the GeneXpert technique, by its sensitivity, standardization, and automation, provides a powerful tool to ensure the diagnosis of patients carrying major transcripts and to evaluate the molecular response, thus allowing faster identification of patients in failure or at high risk of failure and a better understanding of the prognosis in order to establish an optimal therapeutic strategy.

However, more than the GeneXpert test is required to study the complete molecular profile of the patient and ensure a targeted and individualized therapeutic approach. Hence the need to introduce the multiplex RT-PCR technique at diagnosis, allowing the detection of all known BCR-ABL1 molecular rearrangements to propose an adequate molecular follow-up. The implementation of high throughput sequencing is also desirable to characterize the disease's mutational profile and subsequently guide the treatment choice.

Declaration of interest: The authors declare having no conflict of interest

REFERENCES

1. Arber, D. A., Orazi, A., Hasserjian, R., Thiele, J., Borowitz, M. J., Le Beau, M. M., ... & Vardiman, J. W. (2016). The 2016 revision to the World Health Organization classification of myeloid neoplasms and acute leukemia. *Blood, The Journal of the American Society of Hematology*, *127*(20), 2391-2405.
2. Leguay Tet Mahon, F. X. (2005). Leucémie myéloïde chronique. *EMC - Hématologie*, *2*(3), 187-205. https://www.em-consulte.com/article/35751/leucemie-myeloide-chronique
3. Deininger, M. W., Shah, N. P., Altman, J. K., Berman, E., Bhatia, R., Bhatnagar, B., ... & Sundar, H. (2020). Chronic myeloid leukemia, version 2.2021, NCCN clinical practice guidelines in oncology. *Journal of the National Comprehensive Cancer Network*, *18*(10), 1385-1415.
4. Hochhaus, A., Baccarani, M., Silver, R. T., Schiffer, C., Apperley, J. F., Cervantes, F., ... & Hehlmann, R. (2020). European LeukemiaNet 2020 recommendations for treating chronic myeloid leukemia. *Leukemia*, *34*(4), 966-984.
5. Hughes, T., & Branford, S. (2006). Molecular monitoring of BCR–ABL as a guide to clinical management in chronic myeloid leukaemia. *Blood reviews*, *20*(1), 29-41.
6. Cross, N. C. (2009). Standardisation of molecular monitoring for chronic myeloid leukaemia. *Best practice & research Clinical haematology*, *22*(3), 355-365. https://blood-cancer.com/cml-leukemia?utm_source=google&utm_medium=cpc&utm_campagn=Blood-2023 CML&utm_agid=148258544066&utm_adgroup=CML&utm_term=cml&device=c&gclid=Cj0KCQjwy9kBhCHARIsAHpBjHi8yadnf3_Bv8ZfiM55JZQKLKcpNz0GosixpHI_9-ZUHsGhK8bxkLwaAhp8EALw_wcB.
7. Yu, S., Cui, M., He, X., Jing, R., & Wang, H. (2017). A review of the challenge in measuring and standardizing BCR-ABL1. *Clinical Chemistry and Laboratory Medicine (CCLM)*, *55*(10), 1465-1473.
8. Stukan, I., Gryzik, M., Hoser, G., Want, A., Grabowska-Pyrzewicz, W., Zdioruk, M., ... & Wojda, U. (2022). Novel Dicarboximide BK124. 1 Breaks Multidrug Resistance and Shows Anticancer Efficacy in Chronic Myeloid Leukemia Preclinical Models and Patients' CD34+/CD38− Leukemia Stem Cells. *Cancers*, *14*(15), 3641.
9. Chong, S. L., Asnawi, A. W. A., Leong, T. S., Tan, J. T., Law, K. B., Hon, S. L., ... & Tan, S. M. (2021). Impact of timely BCR-ABL1 monitoring before allogeneic stem cell transplantation among patients with BCR-ABL1-positive B-acute lymphoblastic leukemia. *Blood research*, *56*(3), 175-183.
10. Hoffmann, V. S., Baccarani, M., Hasford, J., Lindörfer, D., Burgstaller, S., Sertic, D., ... & Hehlmann, R. (2015). The EUTOS population-based registry: incidence and clinical characteristics of 2904 CML patients in 20 European Countries. *Leukemia*, *29*(6), 1336-1343.
11. Hehlmann, R., Cortes, J. E., Zyczynski, T., Gambacorti-Passerini, C., Goldberg, S. L., Mauro, M. J., ... & Paquette, R. L. (2019). Tyrosine kinase inhibitor interruptions, discontinuations and switching in patients with chronic-phase chronic myeloid leukemia in routine clinical practice: SIMPLICITY. *American journal of hematology*, *94*(1), 46-54.
12. Malhotra, H., Radich, J., & Garcia-Gonzalez, P. (2019). Meeting the needs of CML patients in resource-poor countries. *Hematology 2014, the American Society of Hematology Education Program Book*, *2019*(1), 433-442.
13. Jabbour, E. J., Siegartel, L. R., Lin, J., Lingohr-Smith, M., Menges, B., & Makenbaeva, D. (2018). Economic value of regular monitoring of response to treatment among US patients with chronic myeloid leukemia based on an economic model. *Journal of Medical Economics*, *21*(10), 1036-1040.
14. Rohrbacher, M., & Hasford, J. (2009). Epidemiology of chronic myeloid leukaemia (CML). *Best practice & research Clinical haematology*, *22*(3), 295-302.
15. Cortes, J. (2020). How to manage CML patients with comorbidities. *Blood*, *136*(2), 2507-2512 (PMID: 33236757).
16. Thompson, P. A., Kantarjian, H. M., & Cortes, J. E. (2015, October). Diagnosis and treatment of chronic myeloid leukemia in 2015. In *Mayo Clinic Proceedings* (Vol. 90, No. 10, pp. 1440-1454). Elsevier.
17. O'Brien, S. G., Guilhot, F., Larson, R. A., Gathmann, I., Baccarani, M., Cervantes, F., ... & Druker, B. J. (2003). Imatinib compared with interferon and low-dose cytarabine for newly diagnosed chronic-phase chronic myeloid leukemia. *New England Journal of Medicine*, *348*(11), 994-1004.
18. Nasser, A., Hussein, A., Chamba, C., Yonazi, M., Mushi, R., Schuh, A., & Luzzatto, L. (2021). Molecular response to imatinib in patients with chronic myeloid leukemia in Tanzania. *Blood advances*, *5*(5), 1403-1411.
19. Chikkodi, S. V., Malhotra, P., Naseem, S., Khadwal, A., Prakash, G., Sahu, K. K., ... & Varma, S. (2015). Factors affecting early molecular response in chronic myeloid leukemia. *Clinical Lymphoma Myeloma and Leukemia*, *15*, S114-S119.
20. Pagnano, K. B. B., Miranda, E. C., Delamain, M. T., Duarte, G. O., de Paula, E. V., Lorand-Metze, I., & de Souza, C. A. (2017). Influence of BCR-ABL transcript type on outcome in patients with chronic-phase chronic myeloid leukemia treated with imatinib. *Clinical Lymphoma Myeloma and Leukemia*, *17*(11), 728-733.
21. Marcé, S., Xicoy, B., García, O., Cabezón, M., Estrada, N., Vélez, P., ... & Grupo Español de Leucemia Mieloide Crónica (GELMC). (2021). Impact of BCR-ABL1 transcript type on response, treatment-free remission rate and survival in chronic myeloid leukemia patients treated with imatinib. *Journal of clinical medicine*, *10*(14), 3146.

22. Lang, A. S., Mounier, M., Roques, M., Chretien, M. L., & Boulin, M. (2015). A retrospective study of the prescribing and outcomes of tyrosine kinase inhibitors in chronic myeloid leukaemia over a period of more than 10 years. *Journal of Clinical Pharmacy and Therapeutics*, *40*(4), 391-397.
23. Wang, Y. L., Bagg, A., Pear, W., Nowell, P. C., & Hess, J. L. (2001). Chronic myelogenous leukemia: laboratory diagnosis and monitoring. *Genes, Chromosomes and Cancer*, *32*(2), 97-111.
24. Maute, C., Gauthier, A., Day, G. J., & Cayuela, J. M. (2018). Xpert® BCR-ABL Ultra¥ Demonstrated Good Inter-Laboratory Reproducibility in Three Consecutive GBMHM External Quality Assessments. *Blood*, *132*(Supplement 1), 3018-3018. https://doi.org/10.1182/blood-2018-99-117447.
25. Zhang, J., Wang, Y., Wang, J., Hu, J., Chen, S., Jin, J., ... & Hou, M. (2018). Early BCR-ABL1 decline in imatinib-treated patients with chronic myeloid leukemia: results from a multicenter study of the Chinese CML alliance. *Blood Cancer Journal*, *8*(7), 61.
26. Jobbagy, Z., van Atta, R., Murphy, K. M., Eshleman, J. R., & Gocke, C. D. (2007). Evaluation of the Cepheid GeneXpert BCR-ABL assay. *The Journal of Molecular Diagnostics*, *9*(2), 220-227.
27. Hehlmann, R. (2020). Chronic myeloid leukemia in 2020. *Hemasphere*, *4*(5).
28. Hehlmann, R. (2020). The new ELN recommendations for treating CML. *Journal of Clinical Medicine*, *9*(11), 3671.
29. Han, J. J. (2023). Treatment-free remission after discontinuation of imatinib, dasatinib, and nilotinib in patients with chronic myeloid leukemia. *Blood research*, *58*(S1), S58-S65.
30. Baccarani, M., Castagnetti, F., Gugliotta, G., Rosti, G., Soverini, S., Albeer, A., ... & International BCR-ABL Study Group. (2019). The proportion of different BCR-ABL1 transcript types in chronic myeloid leukemia. An international overview. *Leukemia*, *33*(5), 1173-1183.
31. Zhang, X. S., Gale, R. P., Huang, X. J., & Jiang, Q. (2022). Is the Sokal or EUTOS long-term survival (ELTS) score a better predictor of responses and outcomes in persons with chronic myeloid leukemia receiving tyrosine-kinase inhibitors?. *Leukemia*, *36*(2), 482-491. https://doi.org/10.1038/s41375-021-01387-y
32. Francis, J., Dubashi, B., Sundaram, R., Pradhan, S. C., & Chandrasekaran, A. (2015). Influence of Sokal, Hasford, EUTOS scores and pharmacogenetic factors on the complete cytogenetic response at 1 year in chronic myeloid leukemia patients treated with imatinib. *Medical Oncology*, *32*, 1-6. https://doi.org/10.1007/s12032-015-0665-0
33. Soverini, S., Rosti, G., Baccarani, M., & Martinelli, G. (2014). Molecular monitoring. *Current hematologic malignancy reports*, *9*(1), 1-8.
34. Soverini, S., Hochhaus, A., Nicolini, F. E., Gruber, F., Lange, T., Saglio, G., ... & Martinelli, G. (2011). 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, The Journal of the American Society of Hematology*, *118*(5), 1208-1215.
35. Kantarjian, H. M., Giles, F., Gattermann, N., Bhalla, K., Alimena, G., Palandri, F., ... & Le Coutre, P. (2007). Nilotinib (formerly AMN107), a highly selective BCR-ABL tyrosine kinase inhibitor, is effective in patients with Philadelphia chromosome–positive chronic myelogenous leukemia in chronic phase following imatinib resistance and intolerance. *Blood, The Journal of the American Society of Hematology*, *110*(10), 3540-3546.
36. Yuan, T., Lai, Y. Y., Qin, Y. Z., Shi, H. X., Huang, X. J., Hou, Y., & Jiang, Q. (2020). Analysis of the efficacy and influencing factors of nilotinib or dasatinib as second-or third-line treatment in patients with chronic myeloid leukemia in the chronic phase and accelerated phase. *Zhonghua xue ye xue za zhi= Zhonghua Xueyexue Zazhi*, *41*(2), 93-99.
37. Mahon, F. X., & Etienne, G. (2014). Deep molecular response in chronic myeloid leukemia: the new goal of therapy?. *Clinical Cancer Research*, *20*(2), 310-322.
38. Branford, S., Yeung, D. T., Ross, D. M., Prime, J. A., Field, C. R., Altamura, H. K., ... & Hughes, T. P. (2013). Early molecular response and female sex strongly predict stable undetectable BCR-ABL1, the criteria for imatinib discontinuation in patients with CML. *Blood, The Journal of the American Society of Hematology*, *121*(19), 3818-3824.
39. Lin, H. X., Sjaarda, J., Dyck, J., Stringer, R., Hillis, C., Harvey, M., ... & Sadikovic, B. (2016). Gender and BCR-ABL transcript type are correlated with molecular response to imatinib treatment in patients with chronic myeloid leukemia. *European journal of haematology*, *96*(4), 360-366.
40. Marin, D., Bazeos, A., Mahon, F. X., Eliasson, L., Milojkovic, D., Bua, M., ... & Khorashad, J. S. (2010). Adherence is the critical factor for achieving molecular responses in patients with chronic myeloid leukemia who achieve complete cytogenetic responses on imatinib. *Journal of clinical oncology*, *28*(14), 2381-2388.