Genetics & Applications

Institute for Genetic Engineering and Biotechnology University of Sarajevo

Research Article Open access

How we monitor the therapy effectiveness in patients with chronic myeloid leukemia - A Single center experience

Semir Mešanović[1]*, Azra Jahić[2], Samira Hasić[2], Vlastimir Simendić[2], Haris Šahović[2]

[1]*University Clinical Center Tuzla, Policlinic for Laboratory Diagnostics, Pathology Department, Tuzla, Bosnia and Herzegovina*
[2]*University Clinical Center Tuzla, Clinic for hematology and stem cell transplantation, Tuzla, Bosnia and Herzegovina*

DOI: 10.31383/ga.vol7iss1ga02

***Correspondence**
E-mail:
mesanovic_semir@yahoo.com

Received
January, 2023
Accepted
April, 2023
Published
April, 2023

Keywords

Chronic myeloid leukemia, Molecular monitoring, Polymerase chain reaction, Tyrosine kinase inhibitor

Abstract

Chronic myeloid leukemia (CML) is a hematological disorder characterized by increased proliferation of the granulocytic cell lineage. We diagnose CML by presence of the breakpoint cluster region-abelson (*BCR-ABL1*) oncogene using the quantitative real-time polymerase chain reaction (QRT-PCR). This method provides an accurate and unambiguous follow-up of the treatment response to tyrosine kinase inhibitors (TKIs). This study aimed to determine the molecular response to the first and second generation of TKIs in first and second line of treatment using the QRT-PCR method. We conducted a retrospective study on 48 CML patients treated with the first and second generation of TKIs in first and second-line treatment. Treatment responses have been followed-up every 3 months using the QRT-PCR method. Patients were divided into three groups according to molecular responses to the first line of TKIs. Results obtained in this study showed that the first group of patients did not achieve major molecular response (MMR) in the first 18 months of TKI treatment. The second and third group of patients achieved MMR and deep molecular response (DMR) in the first 18 months of TKI treatment. These results indicate that patients with MMR and DMR in the first 18 months of TKIs treatment had a favourable clinical course of the disease. Inadequate molecular responses to the first line of TKIs can be improved with in increase of the dose of TKIs or by switching to other TKIs. Continuous and timely molecular monitoring of TKI's response in CML patients provides a careful observation of the disease's course and a proper treatment approach.

Introduction

Chronic myeloid leukemia (CML) is a clonal myeloproliferative disease of hematopoietic stem cell lineage characterized by presence of the Philadelphia chromosome (Ph). Redistribution of chromosomal segments in the Ph translocation t(9;22)(q34;q11) results in the breakpoint cluster region-abelson (*BCR-ABL1*) oncogene (Nowell et al., 1960; Rowley, 1973). The product of this fusion gene is an oncoprotein (p210) whose tyrosine kinase activity and inhibition of apoptosis stimulates the overproduction of white blood cells (Čolović et al., 1999). Looking back through history, there have been several revolutionary approaches to CML treatment. The most important discovery in the treatment of CML was tyrosine kinase inhibitors (TKIs).

At the beginning of the 21st century, the strategy of developmental therapy of CML has been based on special molecules that can target and block the gene. The 2-phenylaminopyrimidine, named STI571, and later imatinib mesylate (Glivec; Novartis), proved to be the most potent inhibitor of tyrosine kinase activity of the pathological *BCR-ABL1* oncoprotein (Melo et al., 2007). Clinical studies have shown very low toxicity and high effectiveness of imatinib at a dose of 400 mg once daily. Therefore, as a first generation of TKIs, imatinib represented the gold standard of the first line of treatment for newly diagnosed CML (Druker et al., 2001). In addition to imatinib, nilotinib, a second generation of TKIs, has become available as well for CML treatment. Nilotinib (Tasigna, AMN 107, Novartis) is an aminopyrimidine, a competitive selective *BCR-ABL1* tyrosine kinase inhibitor. This drug has shown very potent inhibition of tyrosine kinase activity in cellular autophosphorylation. An important role of nilotinib has been an inhibitory action on imatinib-resistant mutated cells as well (Melo et al., 2007). In our country, nilotinib has been used as the first line of treatment for CML in younger patients, and as a second line of treatment for imatinib-resistant patients. Due to its potency, TKI treatment leads to complete cytogenetic remission within 12 months. After achieving complete cytogenetic remission, there is a need for more sensitive methods in CML monitoring. The quantitative real-time polymerase chain reaction method (QRT-PCR) provides the most adequate information about the amount of pathological *BCR-ABL1* transcript. The number of *BCR-ABL1* transcripts correlates with the number of constitutively expressed gene transcripts (*ABL1*) used on that occasion. The result of quantification has been expressed by the logarithm of the decrease of *BCR-ABL1/ABL1* ratio. A reduction of 3.0 logarithms of the *BCR-ABL1/ABL1* ratio or more within 12 months has been named a major molecular response (MMR). A decrease in this ratio of 4.0 log, 4.5 log and 5.0 log, or undetectable *BCR-ABL1* with a minimum number of *ABL1* transcript (10000-31999, 32000-99999, ≥100 000 as follows) represents a deep molecular response (DMR). The results of studies have shown a positive correlation between DMR and a high probability of long-term survival without disease progression (Cross et al., 2015).

Material and methods

This was a retrospective study conducted on 48 CML patients of which 30 were female and 18 were male diagnosed and treated at the University Clinical Center Tuzla (UCC Tuzla) for a period of five years from September 2017 to September 2022. The study has been approved by the Ethics Committee of UCC Tuzla (02-09/2-71/22). The median age of patients in the sample was 52 years (range 21-79 years).

A total of 48 CML patients have started treatment with hydroxyurea in order to reduce the white blood cell count (WBC). Most of the patients, 62.5% (30/48) have started first-line treatment with the first generation of TKIs, imatinib, at a dose of 400 mg once daily, while 37.5% of patients (18/48) have started first-line treatment

with second generation of TKIs, nilotinib, at a daily dose of 600 mg b.i.d.

According to inclusion criteria, the study included patients diagnosed with CML and treated with TKIs for at least 18 months, with regular molecular monitoring every 3 months using QRT-PCR method. Based on the level of molecular response in the first 18 months, patients were divided into three groups. The first group consisted of 4 patients with no molecular response. Group II included 8 patients who achieved more than 3 log reduction, while 36 patients made up group III which achieved DMR.

Ribonucleic acid (RNA) was isolated from peripheral blood cells, using TRIzol® reagent (Thermo Fisher Scientific Inc., Carlsbad, CA ,USA) following the single-step RNA isolation protocol (Müller et al., 2008). Synthesis of complementary deoxyribonucleic acid (cDNA) was performed using High Capacity DNA reverse transcription kit (Thermo Fisher Scientific Inc., Carlsbad, CA, USA). The QRT-PCR method was performed using a Light Cycler 2.0 PCR machine (Roche). Amplification of *ABL1* as a control gene and fusion *BCR-ABL1* gene was performed using a commercial Fusion quant *BCR-ABL* Mbcr kit (Qiagen Ipsogen, France).

The results of the quantification analysis and the BCR-*ABL1/ABL1* ratio were multiplied by the conversion factor (CF) to be expressed according to the International Scale (An International Scale-IS) (Cross et al., 2015). Patients were monitored every 3 months for a period of 60 months at the Department of molecular pathology of UCC Tuzla.

Results and Discussion

In the first group of patients, 8.3% (4/48) did not manage to achieve a 3.0 log reduction in the first 18 months of treatment (Table 1). After an increment of imatinib dose from 400 mg to 600 or 800 mg/day, 50% of the patients (2/4) in this group managed to achieve MMR in the next 12 months of follow-up, while 50% of the patients (2/4) who failed to respond to an increase in the dose of imatinib were switched to nilotinib 300 mg orally twice daily. Six months later, one of these patients (½) managed to achieve 1.8 log *BCR-ABL1* reduction, while the other patient (½) achieved 2.3 log *BCR-ABL1* reduction. None of these patients relapsed during the follow-up and both have managed to maintain the achieved values of *BCR-ABL1* reduction.

Table 1. Follow up results in the 1st group (NO MMR in 18 months follow-up (n=4)

Therapy strategy	log BCR-ABL1 reduction	No. of patients with MMR (in 60 months)
Increased dose IM (n=2)	3.0	2
Therapy switch (IM/NIL) (n=2)	1.8 2.3	0

In the second group of patients, 16.66% (8/48) achieved MMR in the first 18 months of treatment (Table 2). In total 87.5% (7/8) of these patients achieved and maintained DMR during five years of follow-up, i.e. 4.0 log and higher. None of these patients relapsed during follow-up. Due to the impossibility of achieving MMR in the first line of treatment to imatinib at a dose of 400 mg, one patient (1/8) (12.5%) in this group was switched to nilotinib. Twenty-four months later, this patient managed to achieve and maintain DMR during five years of follow-up with a 4.0 log reduction (MR 4.0).

In the third group of patients, 75% (36/48) managed to achieve DMR (> 4 log; MR4.0) in the first 18 months of treatment. Fifty per cent (18/36) of these patients were treated with imatinib and 50% (18/36) were treated with nilotinib in first-line treatment (Table 3). All patients achieved MMR in the period from 6 to 12 months after TKI therapy has been started. During the follow-up period, 16.66% of patients in this group (6/36) achieved an undetectable *BCR-ABL1* fusion, which refers to a molecular response with a 5.0 log reduction (MR 5.0). None of these patients relapsed during follow-up.

Table 2. Follow up results in the 2nd group (MMR in 18 months follow-up n=8)

Therapy strategy	log BCR-ABL1 reduction	No. of patients withMR (in 60 months)
Therapy continuation (n=7)	>4.0 log	7
Therapy switch (IM-NIL) (n=1)	4.0 log	1

Table 3. Follow up results in the 3rd group (DMR in 18 months follow-up n=36)

Therapy strategy	log BCR-ABL1 reduction	No. of patients with MR (in 60 months)
Therapy continuation(n=32)	>5.0 log (undetectable BCR-ABL1)	6
	>4log	26
Molecular relapse of the disease. Therapy switch (n=4)		
IM-NIL (n= 2)	>4.0 log	2
NIL-IM (n=2)	MMR	2

At some time points, the remaining 83.33 % of patients (30/36) in this group had a detectable disease at the molecular level. In total 13.33% (4/30) of them have lost MMR at the median of 22 months of follow-up (19-24 months). Two patients from this group (2/4; 50%), initially treated with imatinib, were switched to nilotinib 600 mg daily as per protocol. Six months later, they all managed to achieve and maintain DMR during the follow-up of the study. Other two patients with molecular relapse, treated with nilotinib in first-line, were switched to imatinib 400 mg once daily. Both patients regained a MMR after three months of receiving imatinib and continued in MMR at the last follow-up without a disease progression.

The remaining 86.66% of patients (26/30) in the third group of patients with DMR had no molecular relapse at any point in time and they all had a stable measurable DMR (MR4.0-MR5.0) during the follow-up.

The discoveries of the molecular pathogenesis of CML and TKIs were epoch-making discoveries that changed the diagnostic and therapeutic approach to the disease. Until recently, the treatment response in CML patients was measured by the number of Ph-positive cells using the cytogenetic banding technique, according to the recommendations of the ELN (Baccarani et al., 2013).

However, QRT-PCR soon has become the only method for quantitative measurement of remission, and the results of the analysis were expressed in accordance with the IS (Cross et al., 2015). Periodic measurement of the amount of *BCR-ABL1* fusion gene expressed as the ratio of *BCR-ABL1/ABL1*, every 3 months has been used to assess the treatment response in each patient.

Analysis of the clinical course of the disease, complete blood cell count, results of bone marrow cytogenetic analysis and QRT-PCR findings are ELN criteria for a proper assessment of the treatment response of patients with CML (Hochhaus et al., 2020).

This study emphasizes the importance of timely and continuous molecular monitoring of treatment response in patients diagnosed with CML.

Summarizing the results of our study, the first group of patients did not have an adequate response to the applied imatinib and did not meet the European Leukemia Net (ELN) criteria for MMR (Cross et al., 2015). According to the ELN guidelines, patients who do not have the expected therapeutic response to TKI need a different therapeutic approach, i.e. a higher dose of TKI or treatment options with other TKIs (Hochhaus et al., 2020). Our results have shown that an increased dose of imatinib induced a molecular response in two patients, while the remaining two patients had no improvement in the response to an increased dose of imatinib. Therefore, in these patients, imatinib was replaced by nilotinib, but without an improvement in the response. However, none of these two patients had a progression of the disease during the follow-up of the study.

The introduction of the second and third generation (Scemblix, Novartis) of TKIs depends on the failure of previously applied TKI. Although their use provides significant rates of favourable responses, the presence of point mutations in the *ABL1* part of the *BCR-ABL1* oncogene can have an inhibitory effect on this group of TKIs, especially in cases of a point mutation T315I (Hughes et al., 2006).

The second and third group of patients managed to achieve molecular responses defined as MMR and DMR. During the five-year follow-up, some of these patients achieved unmeasurable BCR-*ABL1/ABL1* values, while some had a disease recurrence and did not meet the criteria for a favourable long-term prognosis (Hochhaus et al., 2008). Despite this, it is necessary to monitor the course of the disease continuously, even including patients with undetectable *BCR-ABL1*.

The results of the third group of patients have shown that even in patients with DMR, the therapeutic response may be lost and they may

experience disease relapse due to the acquired resistance to TKI. However, the loss of DMR, not so rarely, occurs in patients, who from time to time, do not take regularly TKIs. Also, some patients use medications that can have an inhibitory effect on the mechanism of action of TKIs. This is the reason why this group of patients must be monitored regularly to predict the unfavourable course and the possible relapse of the disease.

A comparison of all three groups of patients according to molecular responses has shown the most frequent fluctuations of responses in the first and second group when imatinib has been used in first-line treatment. A stable molecular response has been achieved in the third group where as many as 83.33% (32/36) of patients achieved DMR. Sixteen of them (16/32) received nilotinib as the first treatment option for CML. Results of numerous studies have shown greater efficiency of nilotinib in comparison with imatinib in first-line treatment of CML (Hochhaus et al., 2017; Saussele et al., 2018).

The results of our study are in accordance with recommendations of continuous every three months monitoring of TKIs effect, especially in patients with an unstable molecular response, and as well in cases of switching to a new TKI (Gu´erin et al., 2014). Monthly molecular monitoring is strongly recommended in specific scenarios, such as in the discontinuation of TKIs due to pregnancy and the first six months of follow-up of treatment-free remission (TFR) (Hochhaus et al., 2017).

Conclusion

Periodic and continuous three months molecular monitoring of patients includes an assessment of the clinical course of the disease and also the measurement of the present fusion *BCR-ABL1* gene. Quantitative *BCR-ABL1* PCR test of peripheral blood gives us a measure of the pathological fusion gene relative to the internal control gene and represents the gold standard in CML monitoring. Thus, the degree of leukemic cell burden is translated into critically important prognostic information. Strict adherence to treatment recommendations and accurate measurement of the *BCR-ABL1* fusion transcript is inversely proportional to the likelihood of the disease progression. Patients remain clinically stable regardless of whether their *BCR-ABL1* transcripts continue to be positive or negative during the regular follow-up examinations. Careful monitoring of the molecular responses helps in choosing an adequate TKI and optimizes an overall treatment response in CML patients.

Conflict of interest

The authors declare that they don't have any conflict of interest.

References

Baccarani M, Deininger MW, Rosti G, Hochhaus A, Soverini S, Apperley JF, Cervantes F, Clark RE, Cortes JE, Guilhot F, Hjorth-Hansen H, Hughes TP, Kantarjian HM, Kim DW, Larson RA, Lipton JH, Mahon FX, Martinelli G, Mayer J, Müller MC, Niederwieser D, Pane F, Radich JP, Rousselot P, Saglio G, Saußele S, Schiffer C, Silver R, Simonsson B, Steegmann JL, Goldman JM, Hehlmann R (2013) European LeukemiaNet recommendations for the management of chronic myeloid leukemia. Blood 8;122(6):872-84.

Čolović M, Janković G (1999) Maligne bolesti krvi. Zavod za udžbenike i nastavna sredstva, Beograd.

Cross NC, White HE, Colomer D, Ehrencrona H, Foroni L, Gottardi E, Lange T, Lion T, Machova Polakova K, Dulucq S, Martinelli G, Oppliger Leibundgut E, Pallisgaard N, Barbany G, Sacha T, Talmaci R, Izzo B, Saglio G, Pane F, Müller MC, Hochhaus A (2015) Laboratory recommendations for scoring deep molecular responses following treatment for chronic myeloid leukemia. Leukemia 29(5): 999-1003.

Druker BJ, Talpaz M, Resta DJ, Peng B, Buchdunger E, Ford JM, Lydon NB, Kantarjian H, Capdeville R, Ohno-

Jones S, Sawyers CL (2001) Efficacy and safety of a specific inhibitor of the BCR-ABL tyrosine kinase in chronic myeloid leukemia. N Engl J Med 5;344(14):1031-7.

Gu´erin A, Chen L, Dea K, Wu EQ, Goldberg SL (2014) Association between regular molecular monitoring and tyrosine kinase inhibitor therapy adherence in chronic myelogenous leukemia in the chronic phase. Curr Med Res Opin 30(7):1345-1352.

Hochhaus A, Druker B, Sawyers C, Guilhot F, Schiffer CA, Cortes J, Niederwieser DW, Gambacorti-Passerini C, Stone RM, Goldman J, Fischer T, O'Brien SG, Reiffers JJ, Mone M, Krahnke T, Talpaz M, Kantarjian HM (2008) Favorable long-term follow-up results over 6 years for response, survival, and safety with imatinib mesylate therapy in chronic-phase chronic myeloid leukemia after failure of interferon-alpha treatment. Blood 1;111(3):1039-43.

Hochhaus A, Masszi T, Giles FJ, Radich JP, Ross DM, Gómez Casares MT, Hellmann A, Stentoft J, Conneally E, García-Gutiérrez V, Gattermann N, Wiktor-Jedrzejczak W, le Coutre PD, Martino B, Saussele S, Menssen HD, Deng W, Krunic N, Bedoucha V, Saglio G (2017) Treatment-free remission following frontline nilotinib in patients with chronic myeloid leukemia in chronic phase: results from the ENESTfreedom study. Leukemia 31(7):1525-1531.

Hochhaus A, Baccarani M, Silver RT, Schiffer C, Apperley JF, Cervantes F, Clark RE, Cortes JE, Deininger MW, Guilhot F, Hjorth-Hansen H, Hughes TP, Janssen JJWM, Kantarjian HM, Kim DW, Larson RA, Lipton JH, Mahon FX, Mayer J, Nicolini F, Niederwieser D, Pane F, Radich JP, Rea D, Richter J, Rosti G, Rousselot P, Saglio G, Saußele S, Soverini S, Steegmann JL, Turkina A, Zaritskey A, Hehlmann R (2020) European LeukemiaNet 2020 recommendations for treating chronic myeloid leukemia. Leukemia 34(4):966-984.

Hughes T, Deininger M, Hochhaus A, Branford S, Radich J, Kaeda J (2006) 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 108(1):28-37.

Melo JV, Goldman JM (2007) Myeloproliferative disorders. Springer 182-2015.

Müller M, Schrenk T, Hochhaus A (2008) Trizol RNA-Isolation. III. Medizinische Universitätsklinik, Forschungslabor, Fakultät fur klinische Medizin Mannheim der Universität Heidelberg. Unpublished internal company document.

Nowell PC, Hungerford DA (1960) A minute chromosome in human granulocytic leukemia. Science 132:1497.

Rowley JD (1973) A new consistent chromosome abnormality in chronic myelogenous leukaemia identified by quinacrine fluorescence and Giemsa banding. Nature 243:290–293.

Saussele S, Richter J, Guilhot J, Gruber FX, Hjorth-Hansen H, Almeida A, Janssen JJWM, Mayer J, Koskenvesa P, Panayiotidis P, Olsson-Strömberg U, Martinez-Lopez J, Rousselot P, Vestergaard H, Ehrencrona H, Kairisto V, Machová Poláková K, Müller MC, Mustjoki S, Berger MG, Fabarius A, Hofmann WK, Hochhaus A, Pfirrmann M, Mahon FX (2018) EURO-SKI investigators. 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 19(6):747-7