Bulletin of the *Transilvania* University of Braşov
Series VI: Medical Sciences • Vol. 16 (65) No. 2 – 2023
https://doi.org/10.31926/but.ms.2023.65.16.2.7

CHALLENGES OF THE TREATMENT WITH TYROSINE KINASE INHIBITORS IN CHRONIC MYELOID LEUKEMIA – CASE PRESENTATION

A.P. BENEA[1] A. M. ILEA[2] C. M. GAVRIS[3]

Abstract: *We present the case of a 33-year-old male patient diagnosed with chronic phase chronic myeloid leukaemia in June 2008. According to treatment protocols enforced at that time, the patient started oral treatment with imatinib 400 mg/day, reaching a major molecular response (MMR) in 18-month time. The molecular response improved over the time, with an undetectable BCR-ABL1 transcript level after 36 -48 months of treatment. However, the profound molecular response did not last, requiring a switch to the second line of therapy after 75 months of imatinib. Taking nilotinib 400 mg x2/day, the patient achieved a major molecular response in 12 months, and after 18 months of treatment the level of BCR-ABL1 became undetectable. However, two vasooclusive events occurred in 2018 – a transient left parietal ischemic stroke and a left femoral artery occlusion – and in accordance with ESMO 2017 recommendations, nilotinib treatment was interrupted. The treatment free remission decision was made with monthly monitoring of the BCR-ABL1 transcript level, but since the 4th month of monitoring, the deep molecular response disappeared. Administration of dasatinib, the third tyrosine kinase inhibitor, resulted in a deep molecular response which currently lasted until now, during the 58 months of treatment. The presented case represents the evolution of the concept of treatment in this haemato-oncological disease during the last decade.*

Key words: *chronic myeloid leukaemia, imatinib, nilotinib, dasatinib, molecular response, treatment free remission, vascular side effects.*

1. Introduction

Chronic myeloid leukaemia is a clonal disease resulting from the neoplastic transformation of the pluripotent stem cell, the characteristic of the disease being

[1] Hematology Ward of the County Clinical Hospital of Emergency, Brasov, Romania; Collaborator of the Faculty of Medicine, *Transilvania* University of Braşov
[2] Department of Fundamental Disciplines, Faculty of Medicine, *Transilvania* University of Braşov, Romania; County Clinical Hospital of Emergency, Braşov
[3] Department of Internal Medicine, Faculty of Medicine, *Transilvania* University of Braşov, Romania; County Clinical Hospital of Emergency, Brasov
* Corresponding author: alina_roscaro@yahoo.com

the uncontrolled proliferation of elements of the myeloid series (neutrophil granulocytes in particular) and to a lesser extent of the elements of the erythroid series, megakaryocytic and lymphoid elements [1].

Chronic myeloid leukaemia is the first pattern of neoplasia associated with a specific cytogenetic abnormality, mutual unbalanced translocation between chromosomes 9 and 22-t(9;22) [2,3]. The result of this translocation is the appearance of the BCR-ABL1 chimera molecule. The BCR-ABL1 fusion gene is the genetic marker of chronic myeloid leukaemia, used to monitor the effectiveness of the treatment and the minimal residual disease in the blood [4,].

Most cases of chronic myeloid leukaemia (85-90%) are diagnosed in the chronic phase [5].

Imatinib treatment resulted in a rate of survival similar to that of the general population. The overall survival rate of those treated with imatinib is about 10 years in 83.5% of the cases [7].

Despite the significant results achieved with imatinib, it was observed that approximately 15-25% of patients did not achieve molecular optimal response (major molecular response BCR-ABL1 level <0.1%), another percentage of 7-15% developed a secondary resistance [8].

For patients who failed to respond to the treatment with imatinib 400 mg/day, there are other treatment options: nilotinib, dasatinib, bosutinib, asciminib or ponatinib [9]. Ponatinib is indicated only in patients with T315I mutation.

Nilotinib, a structural analogue of imatinib, with an affinity of binding to the ATP, on the BCR-ABL1 gene which is 30-50 times higher, at a dose of 400 mg x2/day induced in patients in the chronic phase of chronic myeloid leukaemia with a therapeutic failure to imatinib, a haematological response rate of 100% and an overall cytogenetic response rate of 63%, of which 38% represents a complete cytogenetic response [9,10].

Dasatinib, an inhibitor of BCR-ABL1 kinase, but also of the Src family of kinases, is 350 times more powerful than imatinib. In the DASISION trial, as a first-line therapy, dasatinib 100 mg/day, compared with imatinib 400 mg/day, at 5 years of follow-up, induced a disease-free progression rate of 84%. The rate of progression to blast flare and accelerated phase was of 4.6% with dasatinib versus 7.8% with imatinib [11].

Currently, according to ELN recommendations, all three tyrosine kinase inhibitors – imatinib, nilotinib, dasatinib – can be used as the first-line therapy in chronic myeloid leukaemia. The choice of tyrosine kinase inhibitor should be based on several aspects: patient age, risk class, treatment objectives (achieving sustained deep molecular response and possibility of interrupting treatment), patient comorbidities and side effects [12,13].

Of the three, imatinib is the most safety, with moderately mild side effects: peripheral oedema, weight gain, fatigue, myalgia. Dasatinib should be avoided in patients with lung disease and the risk of pleurisy and pulmonary hypertension. Nilotinib is responsible for hyperglycemia, dyslipidemia, QT prolongation and severe vascular events: peripheral arterial occlusive disease, progressive atherosclerosis, ischemic strokes and ischemic heart disease [14].

Prospective analysis by Kim et al. of 159 patients treated with imatinib or nilotinib monitored for peripheral arterial occlusion

disease showed that 35.7% of patients treated with first line nilotinib and 26% of those treated with second line nilotinib developed peripheral arterial occlusive disease compared with only 6.3% of those treated with imatinib [15]. However, the rate of Vaso occlusive events in the ENEST-nd trial at 3 years of follow-up is only 1.3% [16,17].

The major goal of treatment in chronic myeloid leukaemia today is to interrupt the treatment - treatment free remission (TRF-) in patients who achieved a deeply sustained molecular response. The first study whose TRF target was the STIM-stop imatinib trial [18]. In the ENEST freedom study, in patients treated with nilotinib as the first line for at least 3 years and who achieved sustained deep molecular response (MR4 – BCR-ABL transcript level <0.01% and MR4.5 – BCR-ABL1 transcript level <0.0032%), it was found that one year after interrupting the treatment, 51.6% of patients were in major or deep molecular response, respectively 48.9% after 2 years [19]. Treatment free remission gives patients the chance to have a better quality of life, with reduced side effects associated to long-term treatment.

2. Clinical Case

We present the case of B.F. patient, male, diagnosed in June 2008 at the age of 33 with chronic myeloid leukemia-chronic phase, in the hematology department of Brasov County Clinical Hospital of Emergency, Romania. The patient presented himself in the emergency department of the hospital on June 25, 2008, for an altered general condition, asthenia, headache, generalized bone pain, quasi-permanent pain with pressure character in the upper abdominal floor, gastric fullness, night sweats, weight loss about 8 kg in the last 2 months. The patient is a non-smoker, with no significant pathological family or personal history. At the clinical examination, hepatomegaly is detected at 5 cm below the costal margin, splenomegaly at 12 cm below the costal margin.

Automatic blood count reveals leukocytosis (L - 404,3x10^9/L) with a left deviation of the leukocyte formula, with eosinophilia and basophilia, moderate microcytic anemic syndrome (Hb - 8.3 g/dL; Hct - 24.6%; VEM - 59 fl), platelets within normal limits (Plt - 274x10^9/L).

The capillary blood smear has the typical appearance of leukocytosis and neutrophilia with left deviation of the leukocyte formula up to myeloblast level (13%) and 2 pickups at myelocyte-metamyelocyte (16%), respectively non-segmented-segmented (47%), eosinophilia (2%) and basophilia (6%) levels.

Biochemical: LDH - 801 IU, liver, and kidney functions within normal limits; ferritin - 397 ng/mL (normal).

Abdominal ultrasound examination confirms a 28 cm splenomegaly and a hepatomegaly with homogeneous structure.

The bone marrow aspirate reveals marked hypercellularity, myeloid hyperplasia, myeloid/erythroid ratio = 100/2, with 2% myeloblasts, 15% promyelocytes, 4% basophils and 3% eosinophils.

Cytogenetic examination, performed by chromosomal banding technique and analyzing 20 metaphases, reveals 100% the presence of the Philadelphia chromosome. Qualitative and quantitative molecular analysis reveals the major transcript type b2a2 and the fusion gene BCR-ABL1 100%. Based on the clinical and laboratory data, the diagnosis of BCR-ABL1

positive chronic myeloid leukemia was established in the chronic phase, in the Sokal low risk class (value 0.61) and an EUTOS high risk (value -102) [20, 21, 22].

Table 1

Clinical -paraclinical characteristics of the patient at the beginning of the disease

Age, y	**33**
Sex	Male
Blood film (cells)	
Hemoglobin (g/dL)	8.3
Leucocytes (x10^9/L)	404.3
Blast cells (%)	13
Basophils (%)	6
Platelets (x10^9/L)	274
Cytogenetics (100%)	100

According to the national and international treatment protocols for chronic myeloid leukemia – chronic phase, in 2008 cytoreductive treatment with hydroxicarbamide 2 grams/day for 2 months was initiated. Subsequently, the first-line tyrosine kinase inhibitor imatinib (glivec) was administered 400 mg/day orally.

One month after taking imatinib the patient achieved complete hematological response. The persistence of a mild microcytic anemic syndrome and the presence of red blood cells in the target with basophilic punctures on the peripheral blood smear raised the suspicion of a hemoglobinopathy. Hemoglobin electrophoresis confirms a minor betathalassemia (HbA2 - 5.5%) under normal ferritin values.

After 3 months of treatment, the patient achieved complete hematological response, and the BCR-ABL1 transcript level decreased to only 33.7%. Further monitoring of the patient was performed by molecular biology (RT-PCR, Ritus Biotec) every 6 months. BCR-ABL1 transcript levels at 6, 12, 18, 24 months were 1.91%, 1.128%, 0.1%, o.1%. Basically, the patient achieved a major molecular response (MMR) after 18 months, which according to treatment monitoring criteria at that time was an optimal response (23). Continuing treatment with imatinib 400 mg/day induced a deep molecular response over time after 36 months and 48 months, with undetectable BCR-ABL1 transcript levels. After 60 months of imatinib treatment the patient was in MMR, but the BCR-ABL1 transcript was detectable at a value of 0.056%. The monitoring of BCR-ABL1 transcript levels was slowly increasing, so that after 72 months, a level of 0.151% was detected, and after 75 months 1.128%, which defined the loss of major molecular response according to ELN criteria. Patient non-compliance with treatment was excluded and imatinib plasma levels were within normal range (0.610 µg/mL). It was decided to switch to the second line of treatment with a tyrosine kinase inhibitor.

Fig. 1. *Evolution of the molecular response under imatinib (the first line of treatment)*

Mutational analysis performed prior to the choice of the second-line tyrosine kinase inhibitor revealed the presence of V290L mutation and absence of T315I mutation. In accordance with this result of mutational analysis and patient profile, young, without cardiovascular comorbidities and with good glycemic

profile, nilotinib 400 mgx2/day was chosen.

Table 2

The characteristics of the patient when starting the second tyrozin kinase inhibitor

Age, y	**39**
FBC	Complete hematologic response
RT-PCR (BCR-ABL1%)	1.128
Plasma level of imatinib (µg/mL)	0.610
Mutational exam	V299L positive T315I negative
Smoker	No
Cardiovascular risk factors	No
Metabolic profiles	Normal

The treatment with nilotinib was effective, with a major molecular response achieved after 12 months, and after 18 months, at serial tests, the patient was in deep molecular response with an undetectable transcript level.

Monthly monitoring of the pulse, blood pressure, ECG and every 3 months of lipid and carbohydrate profile revealed no changes. Nilotinib was well tolerated without requiring dose adjustments.

In July 2018, at the age of 43, after 40 months of treatment with nilotinib 400 mg x 2/day, a left facial paresis suddenly appeared and a motor deficit at the upper and lower right limb. At his presentation at the neurology emergency room, the patient has a sinusal rhythm, with a frequency of 72 BM, and a blood pressure of 160/80 mmHg.

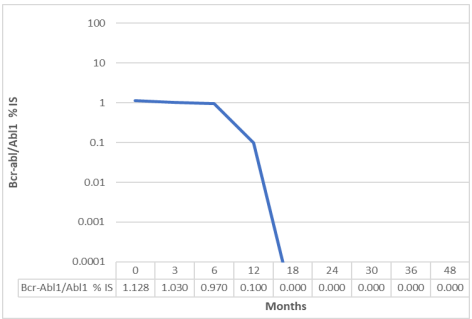

Fig. 2. *Evolution of the molecular response under nilotinib (the second line)*

Biochemical were in normal range. Brain MRI performed in emergency – left parietal ischemic accident without changes on the angio sequence. Carotid Eco Doppler did not reveal significant stenoses in the carotid arteries. Cardiac ultrasound: normal parameters with LVEF 60%.

The cerebral ischemic episode was resolved completely within 12 hours, and it was considered a transient event. Nilotinib treatment was interrupted for one-month; permanent platelet antiaggregant, diuretic and lipid-lowering treatment was administered.

Because of the recovery from the transient ischemic cerebral event was complete and because the implementation of interruption of nilotinib in Romania in patients who had been in sustained deep molecular response for 24 months had not yet been implemented, it was decided to continue the treatment with nilotinib 400 mg x2/day.

In November 2018, after 4 months after the ischemic event, and 3 months after reintroducing nilotinib in the treatment scheme, the patient came to the ambulatory for the monthly prescription complaining of pain in the left lower limb while walking which had started approximately 2 weeks before, with

progressive increasing pain. Clinically, the left popliteal pulse and left radial pulse were absent.

The imaging / Angio CT examination performed in emergency with the suspicion of acute ischemia of the left pelvic limb detects a left femoral artery occlusion by thrombosis, having a length of 33 cm; On the right, millimetric parietal thromboses with cumulative tiered stenosis up to 45% of the middle third were detected.

The patient was urgently sent to the vascular surgery department where left proximal femur-popliteal bypass was performed with no. 7 Dacron prosthesis, with a favorable postoperative evolution. Nilotinib treatment was permanently stopped, and he was recommended to take double platelet antiaggregating medication and to continue the lipid-lowering treatment.

The permanent nilotinib treatment free remission was based on the ESMO 2017 recommendations [25]. The patient being in sustained molecular response, but especially presenting 2 vasoocclusive events, met the criteria for permanent interruption of nilotinib treatment, but with monthly monitoring of BCR-ABL1 transcript levels.

After the first 3 months of nilotinib interruption, BCR-ABL1 transcript levels were undetectable. Starting with the 4th month, the BCR-ABL1 transcript was detectable by RT-PCR, initially at a value of 0.0047% (which did not require retaking any treatment), but after a slowly progressive growth after 7 months of nilotinib remission, the patient lost the major molecular response, with a transcript level of 0.13%.

According to ESMO 2017 criteria, MMR loss requires reintroduction of kinase inhibitor therapy. Due to severe vaso-occlusive side effects with nilotinib, the patient being negative for the T315I mutation, treatment with dasatinib 100 mg/day was initiated. After 3 months of treatment with dasatinib the patient regained MMR, having a BCR-ABL1 transcript of 0.06%.

The patient is currently in a deeply sustained molecular response (constant undetectable transcript level for the 24 months) with a good quality of life. It should be noted that the only periods in which the patient was hospitalized were those at the beginning of the disease and those imposed by the 2 vascular events.

3. Discussions

Chronic myeloid leukaemia is a disease that occurs in all age groups, the average age at diagnosis being 64 years, young age representing a negative prognostic factor [26,27].

The presented case is that of a young adult, diagnosed with chronic myeloid leukaemia in the chronic phase, Sokal low risk, EUTOS high risk according to European LeukemiaNet (ELN) criteria [18]. According to studies, placing the patient in the high EUTOS score class implies achieving a major deep molecular response later than in the low-risk patients. The average time is 21 months in the high-risk patients, compared to just 14.8 months in the low-risk patients [29].

Initiation of the treatment with imatinib 400 mg/day in 2008 was in accordance with the-ELN criteria specific for those times. The monitoring of the therapeutic response was performed by molecular biology (RT-PCR) at 3 months initially, then at 6 months, related to the way of reimbursing the costs of investigations in

Romania, although ELN guidelines provide for a continuous monitoring at every 3 months. According to ELN 2009 criteria, the patient achieved optimal response in 18 months, which supported to continue treatment at the same dose of imatinib [23].

However, looking retrospectively, based on ELN 2013 criteria, the patient was in 2009, 12 months after treatment, in the warning category [12]. With a BCR-ABL1 transcript level of 1.128%, the patient did not achieve a major molecular response. Moreover, the BCR-ABL1 transcript level at 3 months of treatment of 33.7%, over 10% that would have been optimal, and at 6 months over 1%, warned an early risk of a delayed achievement of the major molecular response. The consequences of the delayed achievement of optimal molecular response under tyrosine kinase inhibitor, especially in the first 12 months of treatment, are associated with genetic instability, risk of acquisition of new mutations in the kinase domain and decreased rates of disease-free progression [30, 31].

Continuing treatment with imatinib 400 mg/day according to 2009 criteria, achieved a deep molecular response (MMR, RM 4.5) after 24 months, the delayed response mentioned in the specialized literature was not confirmed.

The choice of the second-line therapy was influenced by the fact that the patient is young, without cardiovascular or metabolic comorbidities, but especially because of the mutational analysis. Response to nilotinib 400 mgx2/day as the second-line therapy was optimal, with a recovery of a major molecular response after 12 months and deep molecular response MR4.5 after 18 months [12].

The optimal molecular response with nilotinib was associated, despite the patient profile without cardiovascular comorbidities and rigorous monitoring, with vasoocclusive events: ischemic stroke and left femoral artery occlusive disease. At the time of vasooscuslusive events, apart from the patient being overweight, no cardiovascular risk factors were identified. We mention that being known from the specialized literature, nilotinib is associated with vasoocclusive events and progression of atherosclerotic lesions, patients being asymptomatic in the period prior to vasoocclusive events, in addition to brain MRI performed at the time of stroke, additional cardiological investigations and carotid Eco Doppler were performed, all of them being within the normal limits.

The pathogenesis behind the arterial occlusive events in patients treated with nilotinib is represented by the inflammatory status: increased level of interleukin 6 and 10, ox LDL, C reactive protein and tumor necrosis factor (TNF alfa). In this case the CRP level was 3 fold increased during the first arterial occlusive event and 4 fold increased during the second episode [34]. In 2018, after 42 months of continuous treatment with nilotinib, the patient being in deep molecular response (undetectable BCR-ABL1 transcript level) lasted for 24 months, in accordance with ESMO guidelines and the nilotinib product leaflet approved by EMA, it was decided to stop all treatment with treatment free remission (tyrosine kinase) inhibitor and monthly monitoring BCR-ABL1 transcript levels [25; Novartis Pharmaceuticals Corporation 2017a.b]. The recurrence of the BCR-ABL1 transcript during the 4th month of monitoring and loss of the major

molecular response in the 7th month was estimated in a certain way, considering that the patient had been treated with 2 tyrosine kinase inhibitors [19, 32].

During the treatment free remission period the patient was monitored very closely, and the fact that the treatment was reinforced as soon as the major molecular response was lost, gave the patient the chance to achieve the major molecular response for the third time in the disease evolution, after 3 months of treatment with dasatinib. Reaching deep MR within the first 6 months of dasatinib treatment was estimable as dasatinib is a more powerful tyrosine kinase inhibitor than nilotinib. We consider that the particularities of this case are: the deep molecular responses (major and MR4,5) achieved with all three tyrosine kinase inhibitors that currently represent the first-line therapy, the two vasoocclusive events in a young patient without cardiovascular comorbidities that once again raised questions about the safety profile of nilotinib and the good quality of life, the patient being socially active all these years, excepting the periods of hospitalization at the beginning of the disease and subsequently on the occasion of vasoocclusive complications.

The patient is currently in deep molecular response under dasatinib 100 mg/day administered for 58 months (4 years and 10 months), with an overall survival rate of 15 years.

4. Conclusions

This case of chronic phase-chronic myeloid leukaemia at the beginning, in a young patient, with evolution of 15 years, illustrates the evolution in the last decade of the concept of treatment in this haemato-oncological disease. In nowadays the choice of 3 tyrosine kinase inhibitors (imatinib, nilotinib, dasatinib) as the first line CML treatment gives patients the chance to rapidly achieve deep molecular response and treatment free remissions [18].

References

1. Faderl S, Talpaz M, Estrov Z, Kantarjian HM. Chronic myelogenous leukemia: biology and therapy. *Am Intern Med*.1999; 131:207-219.
2. Rowley JD. Letter: a new consistent chromosomal abnormality in chronic myelogenous leukemia identified by quinacrine fluorescence and Giemsa staining. *Nature*. 1973; 243(5405):290-293.
3. Pane F, Frigerl F, Sindona M, et al. Neutrophilic chronic myeloid leukemia: a distinct disease with a specific molecular marker (BCR/ABL with C3/A2 junction). *Blood*. 1996; 88: 2410-4.
4. Hughes TP, Kaeda J, Branford S, et al: International randomized study of interferon versus STI571 (IRIS) study group. Frequency of major molecular responses to imatinib or interferon alfa plus cytarabine in newly diagnosed chronic myeloid leukemia. *N Engl J Med*. 2003:249(15): 1423-32.
5. Cortes J. Natural history and staging of chronic myelogenous leukemia. *Hematol Oncol Clin N Am*. 2004; 18:568-584.
6. Kantarjian H. O' Brian S. Jabour E, et al. Improved survival in chronic myeloid leukemia since the introduction of imatinib therapy: a single institution historical experience. *Blood.* 2012; 119(9):1981-7.

7. Hochaus A, Larson RA, Guilhot F, et al. Long term outcomes of imatinib treatment for chronic myeloid leukemia. *N Engl J Med.* 2017; 376:917-27.
8. Bixby D, Talpaz M. Mechanism of resistance to tyrosin kinase inhibitors in chronic myeloid leukemia and recent therapeutic strategy to overcome resistance. *ASH Educational Book*. 2009:461-76.
9. Kantarjian H, Giles F, Wunderle L, et al. Nilotinib in imatinib resistant CML and Philadelphia chromosome positive ALL. *N Engl J Med*.2006; 354:2542-51.
10. Hochaus A, Saglio G, Hughes TP, et al. Long-term benefits and risks of frontline nilotinib versus imatinib for chronic myeloid leukemia in chronic phase: 5 years update of the randomized ENEST-nd trial. *Leukemia* 2016; 30:1044-51.
11. Cortes JE, Saglio G, Kantarjian HM, et al. Final 5 year study results of DASISION: the dasatinib versus imatinib study in treatment of naïve chronic myeloid leukemia patients trial. *J Clin Oncol*. 2016; 34: 2333-40.
12. Baccarani M, Deininger MW, Rosti G, et al. European LeukemiaNet recommendations for the management of chronic myeloid leukemia. *Blood.* 2013; 122:872-84.
13. Saussele S, Krauss MP, Hehlman R, et al. Impact of comorbidities on overall survival in patients with chronic myeloid leukemia results on the randomized CML-Study IV. *Blood*. 2015; 126:42-9.
14. Quintas-Cardama A, Kantarjian H, Cortes J. Nilotinib associated vascular events. *Clin Lymphoma Myeloma Leuk.* 2012; 12(5):337-40.
15. Kim TD, Rea D, Schwartz M, Grille P, Nicolini FE, Rosti G, et al. Peripheral artery occlusive disease in chronic phase chronic myeloid leukemia patients treated with nilotinib or imatinib*. Leukemia*. 2013; 27(6):1316-21.
16. Larson RA, Hochhaus A, Hughes TP, Clark RE, Etienne G, Kim DW, et al. Nilotinib vs imatinib in patients with newly diagnosed Philadelphia chromosome-positive chronic myeloid leukemia in chronic phase: ENEST-nd 3 year follow-up. *Leukemia*. 2012; 26(10):2197-203.
17. Gardembas M, Neumi S, Corby A, Omarjee L, et al. Impact of nilotinib treatment on subclinical cardiovascular biomarkers. *J Clin Oncol.* 2015; 33(suppl):abstr 7045.
18. Mahon FX, Rea D, Guilhot J, et al. Discontinuation of imatinib in patients with chronic myeloid leukemia who have maintained complete molecular remission for at least 2 years: the prospective multicenter Stop Imatinib (STIM) trial. *Lancet Oncol.* 2010; 11:1029-35.
19. Hocchaus A, Masszi T, Giles FJ, et al. Treatment-free remission following frontline nilotinib in patients with chronic myeloid leukemia in chronic phase: results from the ENEST freedom study. *Leukemia.* 2017; 31:1525-31.
20. Deininger M.W.N. Wintrobe's Clinical Hematology, 14th Edition, 2018; 1737-1753.
21. Sokal JE, Cox EB, Baccarani M, et al. Prognostic discrimination in "good risk"chronic granulacytic leukemia. *Blood*.1984; 63:789-99.
22. Hasford J, Baccarani M, Hoffman V, et al. Predicting cytogenetic response

and subsequent progression free survival in 2060 patients with CML on imatinib treatment: the EUTOS score. *Blood*. 2011; 118:686-92.

23. Baccarani M, Cortes J, Pane F, et al. Chronic myeloid leukemia: an update concepts and management recommendations of European LeukemiaNet. *J Clin Oncol*. 2009; 27(35):6041-51.
24. Soverini S, Hocchaus A, Nicolini F.E., 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.
25. Hocchaus A, Saussele S, Rosti G, et al. Chronic Myeloid Leukemia:ESMO Clinical Practice Guidelines for diagnosis, treatment and follow-up. *Annals of Oncology*. 2017, 28(4 iv41-iv51).
26. Kalmanti L, Saussele S, Lauseker M, et al. Young patients with chronic myeloid leukemia do well in spite of poor prognostic indicators: results from the randomized CML study IV. *Ann Haematol*. 2014;93: 71-80.
27. Pemmaraju N, Kantarjian H, Shan J, et al. Analysis of outcomes in adolescents and young adults with chronic myelogenous leukemia treated with upfront tyrosin kinase inhibitor therapy. *Hematol J*. 2012; 97(7):1029-35.
28. Hehlmann R, Hochhaus A, Baccarani M; European LeukemiaNet. Chronic myeloid leukemia. *Lancet.* 2007; 370:342-50.
29. Saussele S, Hoffman V, Lauseker M, et al. Prediction of molecular response of chronic phase CML patients by the EUTOS score: Results of the randomized CML study IV. 2011; *Blood*. 118(21):3762-37.
30. Harrington P, Kizilors A, de Lavallade H. The role of early molecular response in the management of chronic phase CML. *Curr Hematol Malig Rep*. 2017; 12(2):79-84.
31. Ribeiro BF, Vergilio BR, Miranda EC, et al. BCR-ABL1 Transcript levels at 3 and 6 months are better for identifying chronic myeloid leukemia patients with poor outcome in response to second line TKI inhibitors after imatinib failure: A report from single institution. *Acta Haematol*. 2015: 134(4): 248-54.
32. Ross MD, Masszi T, Gomez Casares MT, et al. Durable treatment-free remission in patients with chronic myeloid leukemia in chronic phase following frontline nilotinib: 96-week updates of the ENEST freedom study. *Journal of Cancer Research and Clinical Oncology*. 2018; 144:945-54.
33. Hochhaus A, Gambacorti-Passerini C, Abloud C, et al. Bosutinib for preateted patients with chronic phase chronic myeloid leukemia; primary results of the phase 4 BUOND study. *Leukemia*. 2020; 34(8):2125-37.
34. Sicuranza A, Ferrigno I, Abruzzesse E, et al. Pro-Inflammatory and Pro-Oxidative Changes During Nilotinib Treatment in CML Patients: Results of a Prospective Multicenter Front-Line TKIs Study (KIARO Study). *Front Oncol* 2022; 12:835563.