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Orijinal Makale **Aytan ve ark.**

Kronik Miyeloid Lösemide Mutasyonların Araştırılması ve Klinikle Olan İlişkilerinin İncelenmesi

Investigation of Mutations in Chronic Myeloid Leukemia and Assessment of the Relation between Mutations and the Clinical Response

Pelin Aytan[1], Çiğdem Gereklioğlu[2], Birol Güvenç[3]

[1]Adana Baskent University Faculty of Medicine, Department of Hematology

[2]Adana Baskent University Faculty of Medicine, Department of Family Medicine

[3]Cukurova University Faculty of Medicine, Department of Hematology

Correspondence Author

Pelin AYTAN

Address: Fatih Mah, 30014 Sok. Yali Apt. 7/14, Mezitli, MERSİN

Phone: 05327803572

E mail: drpelinaytan@gmail.com

Özet

AMAÇ: Kliniğimizde Kronik Myeloid Lösemi (KML) tanısı ile takip ve tedavi edilen hastalarda mutasyon durumlarının incelenmesi ve verilen tedaviye olan yanıtın araştırılması.YÖNTEM: Araştırmamızda kliniğimizde takip edilen tüm KML hastaları (n:100) çalışmaya davet edilmiş, 6 hastaya ulaşılamadığından, 2 hasta da çalışmaya katılmak istemediklerinden çalışma dışı bırakılmıştır. Bu hastaların bilgilerine medikal kayıtlardan ve hasta muayenelerinden ulaşılmıştır. Hastalarda en sık görülen mutasyonlardan beş tanesi - T315I, Y253H, E255K, E255V ve M351T mutasyonları - çalışıldı. İnterferon-alfa, imatinib, nilotinib ve dasatinib uygun durumlarda birinci, ikinci ve üçüncü basamak tedavi olarak verildi. Hastalık fazı, hematolojik ve major moleküler yanıtlar, yanıt alınıncaya dek geçen zaman, yeni ilaç ihtiyacı ve yan etkiler değerlendirildi. Mutasyon varlığı ile hastalarda elde edilen majör moleküler yanıt arasındaki ilişki değerlendirildi.BULGULAR: İncelenen hasta grubunda mutasyon oranı % 3.3 olarak tespit edildi. Çalışmamızda mutasyonu olan 2 hastada T315I mutasyonu mevcut idi ve bu hastalarda nilotinib ve dasatinib tedavileri etkili olmadı. Bu hastalar halen akselere fazda idi. Yine diğer M351T mutasyonu olan hastada da bu ilaçlar ile majör moleküler yanıt elde edilemedi. ABL kinaz domain mutasyonları arasında T315I mutasyon varlığı imatinib, dasatinib ve nilotinibe en yüksek direnci gösterir. SONUÇ: Bu çalışmada da mutasyon mevcudiyetinin uygulanan tedavi ile elde edilen majör moleküler yanıt üzerine negatif etkili olabileceğini tespit edilmiştir.

Anahtar Kelimeler: Kronik Myeloid Lösemi,Tirozin kinaz inhibitörleri, BCR-ABL

Abstract OBJECTIVE: To assess the mutation status of the patients with Chronic Myeloid Leukemia (CML) who have been treated and followed-up in our clinics and to assess the response to the treatments.METHODS: All the patients with the diagnosis of CML (n:100) were invited, 6 patients who could not be reached and 2 patients who did not want to participate were excluded. The data were gathered from files and from patient examinations. The most common 5 mutations - T315I, Y253H, E255K, E255V and M351T mutations - were assessed. Interferon-alpha, imatinib, nilotinib and dasatinib were given as first, second and third line therapies where appropriate. The disease phases, hematologic and major molecular responses, commencement time for response, new drug requirements and adverse effects were evaluated. The association between the presence of mutations and rate of the major molecular response were analyzed.RESULTS: The prevalence of the mutations in the studied population was found to be 3.3%. T315I mutation was detected in 2 patients and no response could be obtained with either nilotinib or dasatinib in these two patients. These patients were still in the accelerated phase. In one patient with M351T mutation major molecular response could not be obtained with these two drugs. Among ABL kinase domain mutations presence of T315I mutation is associated with the highest degree of resistance to imatinib, dasatinib and nilotinib. CONCLUSION: In this study it was found that presence of mutations may affect the rate of major molecular response negatively.
Key Words: Chronic Myeloid Leukemia, tyrosine kinase inhibitors, BCR-ABL

INTRODUCTION

Chronic myeloid leukemia (CML) is a stem cell disorder characterized by abnormal clonal overgrowth of myeloid precursor cells and consists 15% of leukemia in adulthood. Its incidence is 1-2/100 000. The disorder may be seen at every age although most prevalent is at age 67. BCR-ABL fusion gene is formed as the result of the reciprocal translocation between 9 and 22 (1). The product of this gene, p210 peptide, has thyrosine kinase activity. This protein contains NH'terminal domain of BCR and COOH- terminal domain of ABL. The mechanism of conversion of p210 peptide from benign form to malignant form is still not fully understood. However binding of BCR sequence to ABL results in 3 critical functional alteration: 1) ABL protein becomes very active as thyrosine kinase enzyme, 2) DNA protein binding capacity of ABL decreases, 3) ABL's binding to cyto-sceletalactine micro-filaments increases. These effects lead to increased proliferation, influence differentiation and inhibit apoptosis.

CML has three stages as chronic phase (85%), accelerated phase (10%) and blastic phase (5%). Patients in chronic phase of CML progresses to an advanced stage within 3-5 years unless treated (2). Sokal scoring system which uses age, size of the

spleen, platelet count and blast ratio in peripheral blood and Hansfort's prognostic scoring system in which eosinophil and basophil counts in peripheral blood have importance are the two scoring systems which are used for risk assessment (3,4) World Health Organization (WHO) uses hematologic, cytogenetic and molecular responses for assessing response to therapy (5,6). Molecular mutation analysis should certainly be examined in case of suboptimal response or irresponsiveness and before switching to another thyrosine kinase inhibitor or the other therapies.

The aim of the present study is to investigate response to therapy and the association between the presence of mutation and response to therapy in CML patients.

MATERIAL AND METHOD

The study was conducted prospectively with the patients who were being followed up at Hematology Clinic of Cukurova University Medical School with diagnosis of CML between 2004 and 2013. Patients who were regularly coming for follow ups and whose medical records could be reached were included in the study. A total of 100 patients who met inclusion criteria were invited with phone calls and informed consent was obtained from the patients who agreed for participation after being informed about the study. Six patients could not be reached and two patients did not agree for participation so remaining 92 patients were included in the study. Medical data were obtained from patient files and physical examination of the patients. Ethics committee approval was obtained from Non-interventional Clinical Researches Committee of Cukurova University prior to the study.

Hematologic response was evaluated according to European LeukemiaNet criteria (5,6,7) as "complete response", "partial response" and "irresponsiveness". Major molecular response was defined as ≤0.1% BCR-ABL/ABL ratio according to international scale. BCR-ABL level was examined in Hematology Laboratory using Roche LightCycler® t(9;22) Quantification Kit which enables quantitative assessment of BCR-ABL/G6PDH and uses non-nested PCR method to minimize contamination risk before treatment and at every 3 months after treatment in all patients. Patients were re-evaluated with regard to disease stage before every treatment step.

Routine examinations and tests were performed in the patients who came to the clinic between January 2012 and October 2013. Five ml of blood was drawn into EDTA tube for mutation analysis and

stored at -20 C°. DNA isolation was done after all blood had been collected. Roche High Pure PCR Template Preparation Kit (patient number x 100 microliter) elution solution was put into an empty eppendorf and heat block for DNA isolation. (Patient number x 40 microliter) Proteinase K was removed. Afterwards 200 µL of binding buffer + 200 µL of whole blood + 40 µL of proteinase K were added into screwed eppendorfs. The mixture was mixed well and waited at 70 C° for 10 min. 100 µL of isopropanol was put onto the tubes taken from heat block and mixed well. Eppendorf pipet was adjusted to 560 µL and the whole mixture was transferred to the numbered filter tubes (so as the collecting tube was at the bottom and the filter tube was on the top) and centrifuged at 8000 rpm for 1 min, the tube stayed at the bottom was discarded. 500 µL of inhibitor removal buffer was put and centrifuged at 8000 rpm for 1 min, the tube stayed at the bottom was discarded. 500 µL of wash buffer was put and centrifuged at 8000 rpm for 1 min, the tube stayed at the bottom was discarded, centrifuged at 13 000 rpm for 10 sec, the tube stayed at the bottom was discarded. Filter tubes were put into the capped eppendorf tubes on which name and number were written. 100 µL of elution buffer in heat block was put into the tubes, this procedure was performed quickly as heat is important, centrifuged at 8000 rpm for 1 min. Filter tubes were discarded. The DNA obtained from the eppendorf tubes in the bottom was stored at -20 C°.

Five mutations (E255K, E225V, M351T, T315I, Y253H) in c-DNAs were examined in accordance with the instructions of the manufacturer using LightSNIP 121913448-49-57-59 and 61 which were synthesized with TIBMOLBIOL. Study protocol is as follows.

REAL TIME PCR

Preparation of Reagent Mix

Each LightSNIP which was synthesized by TIBMOLBIOL for RS121912448-49-57-59 and 61 and found in lyophilized form was melted in 100 µL PCR grade water in accordance with the recommendations of the manufacturer.

The same mix protocol was used by selecting the proper Reagent Mix for five mutations. The prepared reaction mix was loaded to 96-well plate which is proper for Roche LightCycler 480 II Real Time PCR system with 15 µL mix in each. Afterwards 5 µL of DNA sample was taken and loaded to the wells and a total of 20 µL volume was reached. PCR Grade water was also added as negative control for each mutation. The plate was covered with Sealing Foil. Afterwards the plate was

loaded with the protocol proper for LightCycler 480 II system (8). The same heat protocol was used for all of five mutations.

Analysis stage

Analyses were done using LightCycler 480 II software by selecting TM CALLING analysis type and by examining MC (melting curve).

Statistical Analysis

Statistical analysis was done using SPSS program (SPSS v.15.0, demo, Illinois, USA). Kolmogorov-Smirnov test was used for assessing normality distribution of parametric data. Median (minimum-maximum) values were used for the data not normally distributed. Kruskal-Wallis test was used for comparison of more than two groups and Mann-Whitney U test was used for paired groups. Logarithmic conversion was applied to BCR-ABL values and normal distribution was obtained. Paired samples t test was used for pre- and post-treatment comparisons in paired groups. Chi-square test was used for comparison of non-parametric data. A p level of <0.05 was taken as statistically significant.

RESULTS

While 45 out of 92 patients (49%) were female, 47 (51%) were male. Mean age was 49.3±15.3 years. While the longest duration of follow up was since 1995, median time was 3 years (0-18 years). Diabetes mellitus + hypertension and cardio-vascular diseases were the most common co-morbid conditions with the ratio of 5.4% and 4.3%, respectively. Splenomegaly was detected in 70.7% of the patients at the time of diagnosis.

The patients were seen to have been treated with interferon-alpha (12 patients) as first line treatment when Imatinib was not available in our country and with Imatinib thereafter (80 patients). First line medications were used for median 18.5 months (min 2-max 108 months). Table 1 presents the outcomes of first line medications. As seen in the table, chronic phase was the most common phase at the time of diagnosis (89.2%) and 4.3% of the patients were in blastic phase. Hematologic response was obtained in 90.2% of the patients. Of the patients, 33.7% developed major molecular response with first line medication and molecular response was found to start at median 12 months (min 2 months- max 72 months). Second line treatment was required due to absence of molecular response in 50 out of 92 patients

(54.3%). The most common adverse effect was found as hematologic toxicity with first line treatment (8.7%).

Data of 50 patients who required second line treatment are presented in Table 2. Vast majority of the patients were in blastic phase at the beginning of second line treatment (92%). Second line treatment included Nilotinib, Dasatinib and Imatinib. Nilotinib was the most commonly used second line treatment (48%). Median duration of using second line treatment was 23.5 months (min 3-max 108 months). Hematologic response was obtained in 86% of the patients and major molecular response was obtained in 50% of the patients. Median time to major molecular response was 12 months (min 3-max 72 months). A total of 20 patients (40%) required third line treatment. The most common adverse effect was hematologic toxicity and elevated liver enzymes with second line treatment.

Table 1. Data for first line treatment

		N	%
Disease phase	Chronic phase	82	89.2
	Accelerated phase	6	6.5
	Blastic phase	4	4.3
First line treatment	Imatinib	80	87
	Interferon-α	12	13
Hematologic response to first line treatment			
	Yes	83	90.2
	No	9	9.8
Major molecular response to first line treatment			
	No	61	66.3
	Yes	31	33.7
Commencement time for major molecular response			
	Median (month)	12	
	Range (month)	2-72	
New drug requirement			
	Yes	50	54.3
	No	42	45.7
Indication for new drug requirement			
Major molecular irresponsiveness		43	86
Insufficient major molecular response		5	10
Intolerance		2	4
Adverse effect with first line treatment			
None		82	89.1
Hematologic toxicity		8	8.7
Hematologic toxicity and edema		1	1.1
Elevated liver enzymes		1	1.1

Table 2. Data about second line treatment

		N	%
Disease phase at the time of second line treatment			
	Chronic phase	46	92
	Accelerated phase	4	8
	Blastic phase	0	0
Second line treatment			
	Nilotinib	24	48
	Dasitinib	14	28
	Imatinib	12	24
Hematologic response to second line treatment			
	Yes	43	86
	No	7	14
Major molecular response to second line treatment			
	No	25	50
	Yes	25	50
Commencement time for major molecular response			
	Median (month)	12	
	Range (month)	3-72	
New drug requirement			
	Yes	20	40
	No	30	60
Adverse effect with second line treatment			
	No	42	84
	Hematologic toxicity	3	6
	Others	2	4
	Elevated liver enzymes	3	6

Data about third line treatment are presented in Table 3. Chronic phase was the most common phase (70%). Dasatinib was the most preferred third line treatment (55%). While hematologic response was obtained in 70% of the patients, ratio of major molecular response was 55%. Median duration for using third line treatment was 5 months (min 2- max 52 months). Median time to major molecular response was 12 months (min 2- max 48 months). Hematologic toxicity was the most common adverse effect with third line treatment.

Table 3. Data about third line treatment

		N	%
Disease phase at the time of third line treatment			
	Chronic phase	14	70
	Accelerated phase	5	25
	Blastic phase	1	5
Third line treatment			
	Nilotinib	6	30
	Dasatinib	11	55
	İmatinib	3	15
Hematologic response to third line treatment			
	No	6	30
	Yes	14	70
Major molecular response to third line treatment			
	No	9	45
	Yes	11	55
Commencement time for major molecular response			
	Median (month)	12	
	Range (month)	2-48	
Adverse effect with second line treatment			
	No	15	75
	Hematologic toxicity	3	15
	Pancreatitis	1	5
	Elevated liver enzymes	1	5

Table 4 shows the comparison of first, second and third line drugs with regard to major molecular response. While major molecular response could be obtained in no patients with interferon-alpha, it could be obtained in 38.8% (31/80) of the patients who were using Imatinib as first line treatment and the difference was statistically significant (p=0.007, chi-square) (Table 4). However no difference was detected between second and third line treatments with regard to major molecular response (p=0.116 and p=0.616, respectively) (Table 4).

Table 4. Comparison of first, second and third line drugs with regard to major molecular response

Molecular response		No	Yes	p*
First line drug	Imatinib	49	31	0.007
	Interferon-α	12	0	
Second line drug	Nilotinib	13	11	0.116
	Dasatinib	9	5	
	Imatinib	3	9	
Third line drug	Nilotinib	3	3	0.616
	Dasatinib	4	7	
	Imatinib	2	1	

*chi-square test

The analysis of the time to commencement of major molecular response is presented in Table 5. While major molecular response could be obtained in the patients who used only Imatinib, major molecular response could not be obtained in the ones who used Interferon. Median time to obtain major molecular response was 14 months (min 2-max 72 months) in 31 patients who developed major molecular response with Imatinib. When the time to obtain major molecular response with second line therapies was compared between drugs, the time was found significantly shorter with Dasatinib (Table 5). No difference was found between third line therapies with regard to time to major molecular response.

Table 5. Comparison of the drugs with regard to time to major molecular response

	Nilotinib	Dasatinib	Imatinib	p*
As second line therapy	12 (5-20)	5 (3-20)**	12 (12-72)	0.022
As third line therapy	12 (12-36)	6 (3-48)	36 (36)	0.866

*: Kruskal Wallis test
**: Mann-Whitney U test, significantly different from Imatinib, p=0.03

Mutations were detected in 3 out of 92 patients. Two patients had T315I and another had M351T mutation. Comparison of mutations and major molecular responses to first, second and third line therapies is presented in Table 6. While major molecular response could be obtained in no patients who had mutation

when using second line therapies (0%), this ratio was found as 46.8% in the patients who did not have a mutation (p=0.037). While major molecular response was not obtained in the patients who did not have a mutation when using third line therapies, this ratio was found as 100% in the patients who had a mutation (p=0.063).

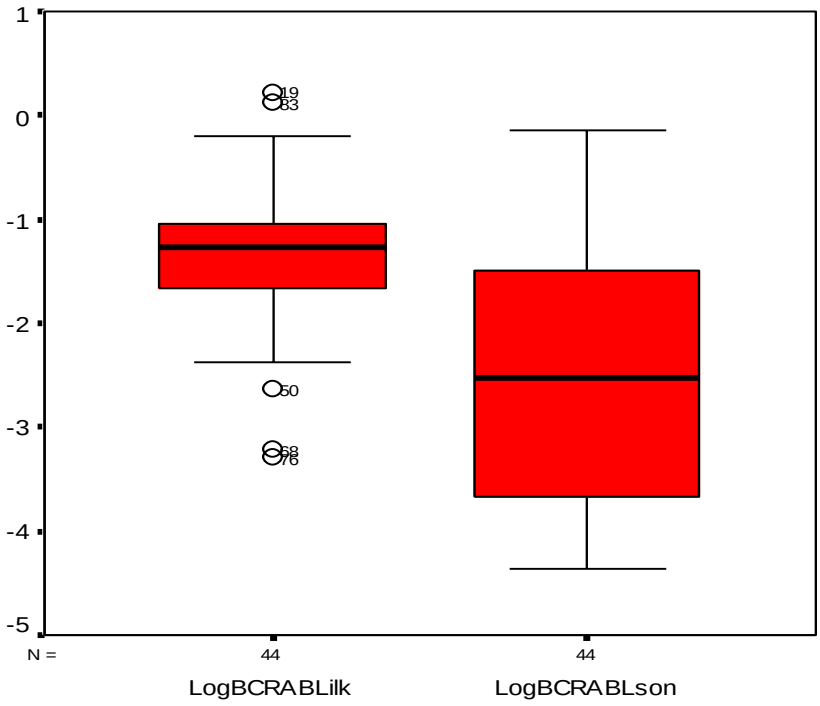
Table 6. Comparison of mutations and major molecular responses to first, second and third line therapies

Mutation		No	Yes	p*
Major molecular response with the first drug	No	58	3	0.113
	Yes	31	0	
Mutation		No	Yes	
Major molecular response with the second drug	No	22	3	0.037
	Yes	25	0	
Mutation		No	Yes	
Major molecular response with the third drug	No	7	2	0.063
	Yes	11	0	

*chi-square test

Logarithmic BCR-ABL values before and after treatment are shown in Figure 1. BCR-ABL values were seen to reach 0 after treatment in 48 patients. While pre-treatment logarithmic BCR-ABL value was log -1.41 ± 0.71, it was found as log -2.51 ± 1.16 after treatment and the difference was statistically significant (paired samples t test, p<0.0001).

Figure 1. Log BCR-ABL values before and after treatment



DISCUSSION

The present study has investigated the presence of the most frequent five BCR-ABL mutations and the association between the presence of mutations and response to therapy in CML patients who were being followed up in our clinic. The ratio of mutations was found as 3.3% and the presence of mutations was found to have a negative effect on major molecular response.

The association between the presence of BCR-ABL mutation and response to therapy has been investigated frequently in recent years. Prevalence of BCR-ABL mutations varied between 36% and 55.7% in patients who were irresponsive to Imatinib therapy in the studies abroad (9). However no studies are available in literature conducted in our country about this issue. We have detected the prevalence of the most common mutations as 3.3% in CML patients who were living in Adana province and around

where our clinic is located. Mutation (one T315I and M351T mutation) was present in 2 out of 12 patients (16.7%) who were using interferon as first line therapy and who did not respond to therapy. There was T351I mutation in one out of 38 patients (2.6%) who were using Imatinib as first line therapy.

Failure with therapy is evaluated as primary resistance and divided as primary hematologic resistance and primary cytogenetic resistance. Secondary resistance is defined as the loss in hematologic and cytogenetic response after achieving response to therapy. While hematologic resistance is detected in 2-4% patients, cytogenetic resistance is detected in 15-25% patients and is more common. Mutations in BCR-ABL are rarely responsible for primary resistance. Recent studies have associated primary response and elevated metabolism gene, prostaglandin-endoperoxidase synthase 1, cyclooxygenase 1and this may be used as a marker for differentiation of the patients who have primary resistance to Imatinib (10). Imatinib resistance may be divided into two categories as BCR-ABL-dependent and BCR-ABL-independent.

Group 1 includes amplification or over-expression, or point mutation of protein product and ABL sequence (11,12). Group 2 includes multi-drug resistance expression and over-expression of Src kinase (13). BCR-ABL-dependent mechanisms are common. They include point mutations detected in 50% of the patients who develop clinical resistance to Imatinib (14,15).

Basal mutation detection does not have a contribution for estimating response to therapy and should not be routinely used in CML patients (16). An association was not shown between basal mutation value and response, progression-free survival and disease-free survival in patients who are treated with Imatinib using high sensitivity DNA sequence technique. Other studies have verified that pre-treatment mutation detection does not indicate insensitivity to Imatinib (9,17,18). Similarly in this study, a significant association was not detected between the presence of mutation and major molecular response in patients who use Imatinib and Interferon as first line therapy.

Redaelli et al. have reported in vitro activity of Nilotinib, Dasatinib and Bosutinibin against 18 BCR-ABL mutations (19). The most common 8 mutations T315I, Y253F/H, E255D/K/R/V, M351T, G250A/I/E, F359C/L/V, H396P/R and M244V mutations are detected in 85% of the patients. Mutations are divided as sensitive, moderately resistant and highly

resistant using semi-maximal inhibitor concentration (IC50). There is a consensus about that these data enable the patients to select the correct TKI therapy for the correct patient. Nilotinib should be selected for the patients who have hypertension, atypical infection and pulmonary disease, F317L, V299L or Q252H mutations; Dasatinib should be selected for the patients who have E255K/V, Y253H and F359C/V mutations. Omacetaxin, AP24534, like clinical trial drugs or hematopoietic stem cell transplantation should be considered in patients who have T315I mutation.

Although Dasatinib and Nilotinib are ineffective against T315I BCR-ABL, this mutation seems to influence the advanced stages of CML. Major molecular response could not be obtained with Nilotinib or Dasatinib in two patients who had T315I mutation. The other therapies (AP24534, Omecetaxin) may yield good outcomes in patients who have T315I mutation. Stem cell transplantation may be performed in patients who are irresponsive to second or third line TKI therapies or who have T315I mutation. Hematologic and cytogenetic response is the same in patients who have or who do not have mutation. Event-free survival was reported as 83% and disease-free survival was reported as 90% in six year follow up20. Resistance may develop in the ratio of 1-17% in some newly diagnosed patients in chronic phase despite significant responses with Imatinib (20,21). The mutations in kinase field of BCR-ABL (domain) are the most common mechanism of Imatinib resistance in CML patients.

More potent TKIs like Dasatinib and Nilotinib have been developed to overcome Imatinib resistance. They have been effective against most Imatinib-resistant KD mutation except T315I (22,23). In our study, two patients had T315I mutation and Nilotinib and Dasatinib were not found effective in these patients. These patients were still in accelerated phase. Major molecular response could not be obtained in another patient with M351T mutation.

Interestingly, in vitro sensitivity of mutations was minimally effective on event-free and disease-free survival in patients with advanced disease. This may be explained with resistance's being multi-factorial in advanced stages. It is often together with the activation of additional and the other potential oncogenic pathways (24,25).

More than 100 BCR-ABL mutations were detected (24,26). While most of them are susceptible to Imatinib (27), some are related with reduced

response and resistance however presence of mutation does not always indicate resistance (24).

Although mutation analysis may have a high cost, cost of a treatment failure may be greater. T315I mutation which hinders Imatinib binding and impairs ATP binding package is the best characterized mutation.

Switching to second line TKIs is another way to overcome Imatinib resistance. M244V, G250E and M351T which are the most frequent BCR-ABL mutations except T315I are highly sensitive both to Nilotinib and Dasatinib in vitro (23). However major molecular response could not be obtained with Nilotinib and Dasatinib in the only patient who had M351T mutation.

Mutation test is recommended for the patients who have ongoing Imatinib-resistant CML and in Imatinib-resistant patients who develop clinical progression under second line TKI therapy as the result of better understanding the association between BCR-ABL mutation development and response to therapy (28-30). However care should be paid when making a clinical decision. Current studies indicate that all mutations do not have clinical and prognostic importance (15). Low amount of mutant colons are present in many patients who are not resistant to therapy (9,18,24). Further purified techniques and better understanding the optimal clinical way are required if mutation tests would be considered as a part of therapy for CML.

The aim of CML treatment is 100% survival and a good quality of life. Although stopping is considered as an attractive result of a successful treatment, few patients can achieve a stable full molecular response. Interrupting therapy should be considered in female patients who have major molecular response and who wish to have a safe pregnancy period (31).

Outcomes of alloHSCT are not influenced by previous Imatinib treatment (32-34). Current data do not show transplant-related toxicity in patients who had previously treated with Nilotinib or Dasatinib (35,36).

Treatment recommendations: Hydroxyurea may be used for a short time or when TKIs are not recommended. Interferon alpha may be a treatment option during pregnancy as Imatinib cannot be used at the beginning of pregnancy and during whole pregnancy31. Interferon alpha may also be used when Imatinib is not proper in low risk patients. Imatinib is used in the dose of 400 mg daily in chronic phase CML patients, Nilotinib and

Dasatinib are selected when tolerance develops against the drug. AlloHSCT is recommended in patients in accelerated phase and blastic phase or when second line TKIs fail in patients with T315I mutation.

A successful treatment with TKIs should not be terminated. The doses below standard dose should not be used except presence of significant side effects.

Complete hematologic response and complete cytogenetic response are low in advanced CML patients (37). Loss of response develops in 6% of the patients in chronic phase and resistance develops against standard dose Imatinib (1). This ratio is higher in advanced stage (24,37-40).

While hematologic resistance develops in 2-4% of the patients, cytogenetic resistance is higher (15-25%) (41). BCR-ABL mutations are rarely responsible for primary resistance (42). Secondary resistance is defined as the loss in hematologic and cytogenetic response after achieving response to therapy. Presence of BCR-ABL mutation does not always lead to clinical resistance. In fact, the magnitude of resistance depends on Imatinib susceptibility of the mutation (26).

Level of basal mutation was shown not to have a benefit on response in newly diagnosed CML patients (16) and should be applied routinely. Additional studies have verified that detection of mutation prior to treatment would not yield information about irresponsiveness to Imatinib (9).

The mutations which develop resistance to Dasatinib include T315A, V299L and F317L/C/V, the mutations which develop resistance to Nilotinib include Y253H, E255K/V, L273M and F359V.

Studies have shown that mutations were not detected in some part of the patients who had progressive disease (9,43). Additional studies have shown that patients with complete cytogenetic response may develop BCR-ABL mutation and may maintain complete cytogenetic response (18,44).

Basal mutation tests are not required as basal BCR-ABL mutations cannot determine the outcome of Imatinib treatment. Alternative approaches are required in case of failure with Imatinib treatment. The dose of Imatinib may be increased but it does not have an effect in patients who developed no hematologic or cytogenetic response or in the patients who had a known Imatinib resistant mutation.

The main goal is to develop a scoring system which is based on many factors which would most accurately estimate response to therapy and which can predict patient outcomes. This scoring system includes one or more of the following: ECOG (Eastern Cooperative Oncology Group) performance score, previous cytogenetic response to Imatinib, basal mutations with low susceptibility to a certain TKI, basophili, Sokal score and recurrent neutropenia (45-47).

The present study has some limitations. First is the small number of patients (n:92), the mutations could be detected in only three patients. The most common 5 mutations have been examined in accordance with the magnitude of the financial support. Second is evaluating hematologic and molecular responses, but not cytogenetic response. These issues should be considered when interpreting the results of the study.

The results of the study indicate that presence of mutations negatively influences response to therapy and the difference is statistically significant in first line therapy and very close to statistical significance in second and third line therapy. This may result from the fact that two out of three mutations was T315I which is the most resistant mutation type. All mutations are not associated with drug resistance, as in the other studies. Response to therapy may be related with mutation type, as in this study. So it may be concluded that detection of basal mutation is not required in CML patients. Detection of mutation may contribute to determine second line TKIs in Imatinib-resistant cases. Unnecessary medications and loss of sources could be overcome when a highly resistant mutation is detected.

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