Significance of the T3151, T317L, E255K AND Y253H BCR-ABL gene mutations in Philadelphia positive Chronic Myeloid Leukemia patients

Philadelhia pozitif Kronik Miyeloid Lösemi Hastalarında T315I, T317L, E255K ve Y253H BCR-ABL gen mutasyonlarının önemi

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Özet

Bu çalışmada; imatinib tedavisi alan Philadelphia pozitif (Ph+) Kronik Myelositer Lösemi (KML) hastalarında ABL geninde en sık gözlenen (imatinib bağlanma bölgesinde bulunan) T315I, F317L ve Y253H ve genin P-Ioop fosfat bağlanma bölgesinde bulunan E255K mutasyonları araştırılmış ve bu mutasyonlar ile klinik parametreler arasında anlamlı bir ilişkinin olup olmadığı araştırılmıştır. 2000-2008 tarihleri arasında 2 merkezde izlenen 54 kronik faz KML hastasında BCR-ABL genindeki T315I, F317L ve Y253H ve P-Ioop fosfat bağlanma bölgesinde bulunan E255K mutasyonları Allel Spesifik Oligonükleotit-Polimeraz Zincir Reaksiyonu (ASO-PCR) ile analiz edilmiştir. Hastaların 12'si erkek, 42'si kadın olup medyan yaş 44.5 (19–78)'dur. Tanıda Sokol risk sınıflamasına göre; hastaların 12'si (%22.2) düşük riskli, 26'sı (% 48.1) orta riskli ve 16'sı (%29.6) yüksek riskli gruptadır. Medyan imatinib kullanım süresi 1.8 (0.3–7) yıldır. İmatinib tedavi yanıtı değerlendirildiğinde; hastaların 24'ü (%44.4) optimal yanıtlı, 10'u (%18.5) suboptimal yanıtlı 20'si (%37) ise yanıtsız olarak değerlendirilmiştir. Medyan total ve progresyonsuz yaşam sürelerine henüz ulaşılmamış olup, 7 yılda beklenen total sağkalım %96, progresyonsuz sağkalım %80 bulunmuştur. Taranan mutasyonlar hastaların 18'inde (%33.3) belirlenmiş olup sırası ile T315I 11 (20.3) ve F317L 9 (%16.6) hastada saptanırken,Y253H ve E255K mutasyonları bulunmamıştır. Mutasyon belirlenen hastaların 2 (%3.7)'sinde T315I ve F317L mutasyonu birlikteliği saptanmıştır. Yanıtsız hastaların %60'ın da mutasyon saptanmıştır (p=0.004). Mutasyon saptanan hastalarda 7 yıllık PFS %62 mutasyon saptanmayanlarda ise % 90 olarak bulunmuştur (p=0.041). T315I mutasyonu saptanan hastalar AKİT'e yönlendirilirken F317L mutasyonu olanlar da nilotinib veya dasatinib tedavisine geçilmiştir. Optimal tedavi şeklinin belirlenmesi açısından; KML hastalarında direnç erken dönemde tanımlanmalı, mutasyon tipi belirlenerek mutasyona göre rasyonel tedavi şekli planlanmalıdır.

Anahtar kelimeler: Kronik Myeloid Lösemi, BCR-ABL geni, mutasyon, imatinib, prognoz.

Abstract

In this study it has been aimed to scan the most frequently observed types of mutations in imanitib treated Philadelphia positive (Ph+) Chronic Myelositer Leukemia (CML) patients, namely the T315I, F317L, Y253H, and the P-Ioop phosphate binding region E255K mutations, in order to demonstrate any significant correlation between the presence of these mutations and the estimations on the relevant clinical parameters. Fifty four chronic phase (CP) CML patients attending two separate centers between 2000 and 2008 were included in this study, after scanning by the ASO-PCR method, to be positive for T315I, F317L, Y253H and E255K mutations in the BCR-ABL gene. Twenty of the patients were men and 42 were women with a median age of 44.5 (19-78). According to the Sokol classification for diagnosis, 12 cases (22.2%) were in the low risk, 26 (48.1%) were in the middle risk and 16 (29.6%) were in the high risk group. Median time of imatinib usage was 1.8 (0.3-7) years. Response to imatinib treatment was optimal in 24 (44.4%) of the patients and suboptimal in 10 (18.5%) of the patients, while 20 (37%) showed resistance. Expected values in 7 years were found to be 96% for median overall survival (OS) and 80% for progression-free survival (PFS). Scanned mutations were observed in 18 (33.3%) patients and T315I and F317L together were detected in 2 patients. Mutations were detected in 60% (p=0.004) of the imatinib resistant patients and the 7-year PFS in this group was 62%, while it was 90% (p=0.041) in patients without detected mutations. While patients with T3151 mutation were recommended allogeneic stem cell transplantation, patients with F317L mutation were started on nilotinib and dasatinib treatment. To determine the optimal treatment type; resistance should be identified in early stages in CML patients and the rational treatment should be planned according to the determined mutation type.

Key words: Chronic Myelogenous or Myeloid Leukemia, BCR-ABL fusion gene, mutation, imatinib, prognosis

INTRODUCTION

Chronic Myeloid leukemia (CML) is a clonal disorder resulting from the malignant transformation of a single pluripotent stem cell (1, 2). CML constitutes 20% of all types of leukemias with an annual incidence of 1 in 100,000 and is mostly seen in the 5th and 6th decades of life with slightly higher frequency in the male

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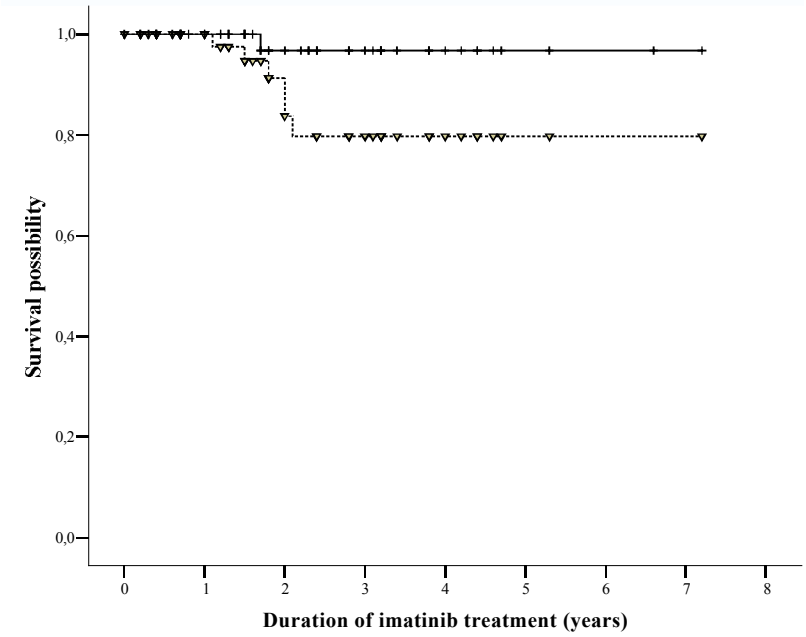
Table 1: Chronic Phase Clinical Parameters of Patients Daignosed with Chronic Myeloid Leukemia

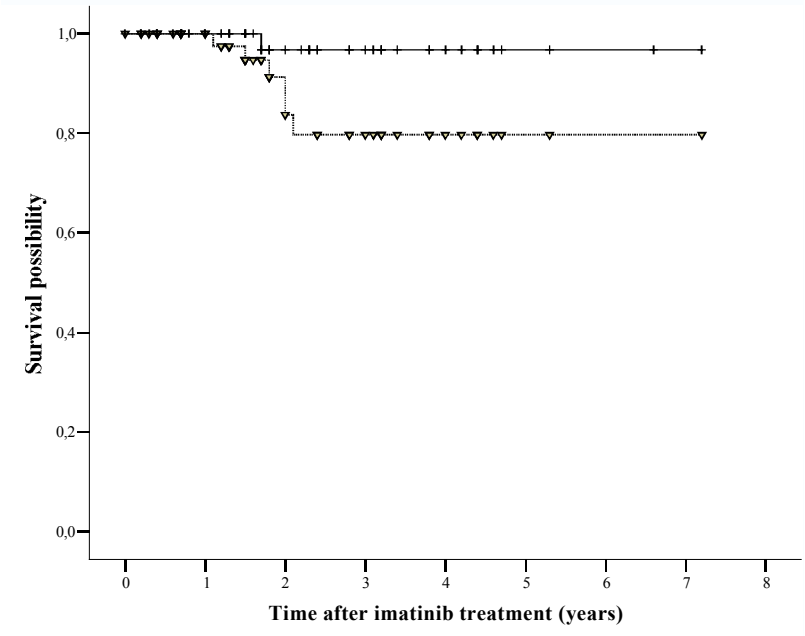
		N	
Number of patients		54	
Age (median)			44.5 (19-78)
Gender- male/female		12 /42	
Splenomegaly	cm*	35 (% 64.8)	4 (0-18)
Haemoglobin	gr/dL*		10.7 (6.7-15.2)
	<11 gr/dL	28 (%51.8)	
Leukocytes	µL*		73210 (4290-335000)
	>10000/µL	53 (%98.1)	
	>100000/µL	13 (%24.1)	
Thrombocytes	10^3/µL*		451 (58-1400)
	>450x10^3/µL	28 (%51.9)	
	<100x10^3/µL	2 (%3.7)	
Sokol risk rating	0 (Low risk)	12 (%22.2)	
	1 (Medium risk)	26 (%48.1)	
	2 (High risk)	16 (%29.6)	
Peripheral eosinophils	>3% n=18	3 (%16.6)	
Peripheral promyelocytes	%* n=24		5 (1-30)
Peripheral blast cells	%* n=24		2.5 (0-5)
Bone marrow blast cells	%* n=54		2.5 (0-5)
Bone marrow reticulin fibre content	n=20		
	1	12 (%60)	
	2	6 (%30)	
	3	2 (%10)	
Therapy before imatinib	Hydroxyurea	26 (%48.1)	
	Hydroxyure-a+interferon	8 (%14.8)	
	None	20 (%37.1)	
Response to imatinib therapy	(optimal)	24 (%44.5)	
(haematologic, cytogenetic and	(suboptimal)	10 (%18.5)	
molecular response together)	No response	20 (%37)	
BCR-ABL point mutations	T315I	11 (%20.3)	
	F317L	9 (%16.6)	
	E255K	0	
	Y253H	0	
* =median			

subpopulation (3, 4). Imatinib is a 2-phenyl-amino-pyrimidine derivative that inhibits the binding of the Abelson (Abl)-specific tyrosine kinase, also known as an inhibitor of signal transduction, that has been the subject of different phase I and phase II studies. Imatinib, with its reliable effects having been demonstrated in interferon (INF)-resistant chronic phase (CP) - CML as well as in accelerated or blast phase cases, has been selected as a first-line treatment agent. It also inhibits the thrombocyte (platelet)-derived growth factor receptor and the stem cell factor (SCF) (1, 3, 5). The most important problem associated with the use of imatinib is primary resistance or acquired resistance to this agent. Diagnosis of primary resistance is based on the absence of complete haematological response (CHR) in 3 months and of major cytogenetic response (MCR) in 6 months or of complete cytogenetic response (CCR) in 12 months (3). Acquired resistance is the loss of the favourable haematological, cytogenetic or molecular response to the treatment (6). Binding of imatinib to the breakpoint cluster region (BCR)-ABL kinase domain is impeded by the formation of mutations in this region which are seen in 30-90% of the patients with acquired imatinib resistance (5, 7). New tyrosine kinase inhibitors have been developed against the occurrence of ABL point mutations in imatinib-resistant CML cases. The rates of incidence of these point mutations in the imatinib resistant CML patients have been ranked as: T315I -15%, E255K- 15%, Y253H- 5% and F317L- 3%. In CML cases positive for T315I mutations the new generation of tyrosine inhibitors are not effective and consquently these patients are being recommended allogeneic stem cell transplant as alternative treatment. In the cases of Y253H and E255K mutations, dasatinib has been observed to be more effective than nilotinib, while in F317L/V mutations dasatinib has been repored to be more effective than nilotinib (8-10).

In this study it has been aimed to scan the most fre-

Figure 1: Relationship of survival with Imatinib use over 7 years





quently observed types of mutations in imanitib treated CML patients, namely the T315I, F317L, Y253H, and the P-Ioop phosphate binding region E255K mutations, in order to demonstrate any significant correlation between the presence of these mutations and the estimations on the relevant clinical parameters.

PATIENTS AND METHODS

Preparation of the patients and the samples

Patients (n=54) diagnosed with Ph (+) CML and healthy controls (n=50) attending Gaziantep University Medical Faculty and Erciyes University Medical Faculty Adult Haematology Polyclinics have been included in this study which has been approved by the Gaziantep University Ethics Committee (07-2007/40). All participants have submitted signed consent forms. Blood samples (2 ml) have been taken in tubes containing EDTA, and DNA separation has been achieved by the use of Invitek Invisorb Spin Blood Midi kit (LN: CA050036). The DNA materials have been stored at -20oC.

Allele-Specific Oligonucleotide–Polymerase Chain Reaction (ASO-PCR)

After proliferating the 4 different sites on the ABL kinase gene for the Y253H, E255K, T315I and F317L mutations by means of the ASO-PCR method, cases positive and negative for mutations were identified by the subsequent separation of the products electrophoretically on 2% agarose gel (11). Genotypes of the samples demonstrating mutation were verified by the SSCP analysis (12).

Statistical Analysis

The SPSS 11.5 package program has been used for the analysis of the findings of the study; Log Rank Test has been used for the comparison of the Overall Survival (OS) and Progression-Free Survival (PFR) with the clinical parameters; Chi-Square test has been used to compare the clinical parameters with the findings on T315I, F317L mutations. $P<0.05$ has been accepted as statistically significant. The data obtained, starting with the t (9;22) positivity, have been compared with the clinical parameters to check the presence or absence of significant correlations.

Table 1: Chronic Phase Clinical Parameters of Patients Daignosed with Chronic Myeloid Leukemia

RESULTS

The estimated clinical parameters of the 54 patients diagnosed with Ph (+) CP-CML at Gaziantep University and Erciyes University, Medical Faculty, Adult Haematology Departments are presented in Table 1. The median follow-up period was 1.8 (0.3-7.2) years for those patients receiving imatinib treatment. The overall survival (OS) for the 7-year imatinib therapy was 97% and the 7-year long progression-free survival

Table 2: Clinical Characteristics of Chronic Myeloid Leukemia Patients Receiving Imatinib

		%
Disease before imatinib Chronic Phase	54	100
7- Year Overall Survival	97%	
Follow-up on patients given imatinib (years)	1.8 (0.3-7.2)	
7- Year overall Survival post imanitib	97%	
7- Year progression-free Survival post imanitib	80%	
Total Mortality (brain oedema 1.7 year after Imanitib)	1	
Progression	6	
Giving Optimal and Suboptimal response	35	
Surviving without response	19	

(PFS) was 80% (Figure 1). During imatinib treatment progression was evident in 6 patients, optimal and suboptimal responses were observed in 35 patients, while 19 gave no response and one case of mortality was recorded (Table 2).

The effect of the clinical parameters of Chronic Phase CML (CP-CML) on survival has been shown in Table 3. In the group given hydroxy urea before imanitib, the 7-year PFS rate was 75%, whereas the rate was 83% in the group given hydroxy urea together with interferon. While the 7-year PFS was 50% in the group with the T315I point mutation, this rate was 86% in the group without this mutation (p=0.0470) (Figure 2).

Statistically significant relationships between T315I BTR-ABL point mutation and the clinical parameters including Sokol risk rating, gender, bone marrow reticulin fibre content, haemoglobin, leukocyte and thrombocyte counts could not be demonstrated. However, a statistically significant relationship between T315I BCR-ABL point mutation and the peripheral eosinophil count % has been observed (p=0.011). Thus, in patients with peripheral eosinophil count above 3% the risk of occurrence of T315I point mutation was increased 28 fold (Table 4). Also, a statistically significant correlation was evident between the response to imatinib treatment and the T315I BCR-ABL point mutation status in the patient group (p=0.002) (Table 4). A statistically significant relation between F317L BCR-ABL point mutation and the Sokol risk rating, gender, peripheral eosinophil %, bone marrow reticulin fibre content, response to imatinib treatment, haemoglobin, leukocyte, and thrombocyte counts could not be detected (data not shown). Despite the absence of statistically significant relationships between the cited clinical parameters and the presence or the absence of mutations, such a statistically significant relationship was found to exist between the response to imanitib and the mutation status (p=0.004) (Table 5).

DISCUSSION

Imatinib is essentially an inhibitor of the ABL-ATP binding site. Therefore, mutations at the BCR-ABL kinase domain impede the binding of imanitib. Among the patients with acquired imanitib resistance 50-60% are positive for BCR-ABL kinase region mutations (7, 13). In this study the Sokol risk rating showed 22.2% (n=12) of the patients to be at low risk, 48.1% (n=26) to be at medium risk and 29.6% (16) of the patients to be at high risk in diagnosis, and a statistically significant relationship between this risk classification and the determined frequency of mutations was not demonstrated (p=0.975). Evaluation of the response to imatinib treatment showed that 44.4% (24) gave optimal response, 18.5% (10) gave suboptimal response, while 37% (20) were without a response. In the study by Hess et al. on 59 Ph(+) CP-CML patients 51% (28) were also without any cytogenetic, molecular or haematological response.14 Similarly, Lehaye et al. have concluded that 29% of their 300 Ph(+) CP-CML patients were without response to treatment. At the end of the 4.5-year treatment with imatinib 45% of these patients were found to be positive for mutations (15). In other studies cited in the literature the incidence of mutations in groups of similar patients have been shown to vary within the 13.4 -/ 48% range (4,16). In our study, over a median 1.8-year observation period BCR-ABL mutations were determined in 33.3% (18) of our patients which is consistent with the reported results in the literature.

Incidence of the T315I point mutation in our group of CP-CML patients was 20.3% and this mutation was demonstrated in 45% (9) of those patients without

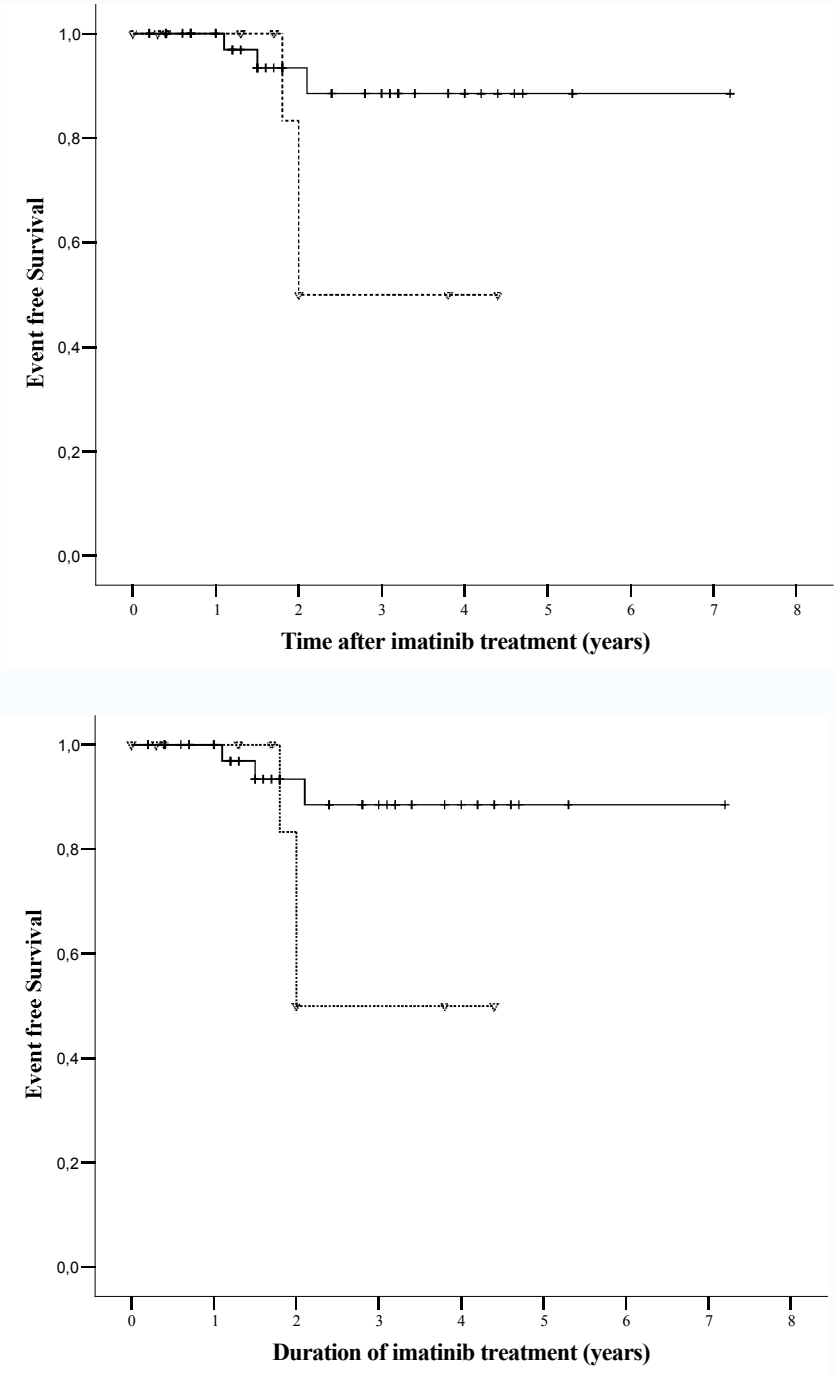
Table 3: The Effect of Clinical Parameters of CP-CML on Survival (Log-rank test)

	N	7-y OS (%)	Log Rank p	7-y PFS (%)	Log Rank P
No of Patients	54	97		80	
Sokol					
0 (Low risk)	12	100	0.1245	80	0.6070
1 (Medium risk)	26	100		85	
2 (High risk)	16	83		66	
Gender					
Female	42	96	0.3384	77	0.5106
Male	12	100		88	
Peripheral eosinophils					
≤%3	15	100		72	0.7518
>%3	3	100		100	
Bonemarrow reticulin fibre content					
1	12	100		67	0.8055
>1	8	100		67	
Response to imatinib treatment					
Optimal	24	100	0.5004	100	0.0095
Suboptimal	10	100		100	
Without response	20	92		54	
Therapy before imanitib					
Hydroxyurea	26	94	0.6969	75	0.7946
Hydroxyurea+interferon	8	100		83	
None	20	100		86	
BCR-ABL point mutation T315I					
Yes	11	100	0.5892	50 (2 y)*	0.0470
No	43	96		86	
BCR-ABL point mutation F317L					
Yes	9	100	0.6610	66	0.2088
No	45	96		83	
BCR-ABL point mutations T315I ± F317L					
Yes	18	100	0.4902	62	0.0610
No	36	95		90	
Sokol: patient age, spleen size, blast percentage in peripheral blood and the thrombocyte count *( ) median year, Y: year OS: Overall Survival; PFS: Progressio-free Survival					

response to the imatinib treatment. Observations on the incidence of the T315I point mutation have been reported by others to be in the range of 16-26% (10, 11, 17), which is consistent with our results. The new tyrosine kinase inhibitors have not been effective in the treatment of T315I-positive cases and the patients have generally been recommended to undergo an allogeneic stem cell transplant (8,18), which has also been our choice of alternative treatment. In this study, incidence of the F317L point mutation has been found to be 16.6% which is higher than the 4-8% range reported in the literature (8, 17-19). In 3.7% (2) of our patients joint presence of the T315I and F317L mutations has been observed which had not been reported previously in the literature.

The E255K and Y253H point mutations have not been demonstrated in our group of CP-CML patients. Branford et al. have shown E255K type of mutation in 22.2% (4) and the Y253H type of mutation in 5% (1) of their 18 patients (17, 19) Similarly, Ouyang et al. have demonstrated the presence of the E255K mutation in 4% (1) and the Y253H mutation in 4% (1)

Figure 2: The efect of T315I mutation on Progression-free Survival



of their 23 CML patients.20 In the general literature the incidence of these mutations have been reported to vary in the range of 4-22%. Thus, it is significant that they have not been demonstable in our group of patients.

The presence of mutations in 60% of the patients without response to imatinib therapy was highly significant (p=0.004). Lehaye et al. have also determined presence of mutations in 50% of 35 CP-CML patients who demonstrated acquired resistance to therapy (15, 16). Similarly, Ernst et al. determined mutations in 50% of 911 CML patients with no response or suboptimal response to imatinib treatment (21); while Soverini et al. (22) also found mutations in 43% of 297 patients not responding to imatinib, with an incidence of 75% mutations in those patients in the blast crisis phase of the disease. These findings are in consistence with our results reported here. In this study, as the median periods for OS and PFS of our patients had not yet been covered, the expected survival rates were determined to be 97% and 80% , respectively. In the study of Lehaye et al. with 300 CP-CML patients, the expected OS was 88%, whereas the 2-year survival in the blast phase of the disease was reported to be 17% (15).

In the IRIS study, which is the definitive phase III trial of imatinib mesylate as frontline therapy for CML, the 5-year OS for patients in the accelerated and the blast phases was 83% and the PFS was 93%. The overall 5-year survival in CML patients was 89% (23). Similarly, Rosti et al. reported a 5-year OS rate of 91% and a PFS rate of 95% in 158 CML patients during the accelerated and blast phase of the disease (24).

The survival expectancies reported in our study are in accordance with the results reported in the literature. Hence, in our patients with the T315I point mutation the 2-year PFS was 50%, which rose to 86% in patients without the mutation (p=0.047). Our results are in accordance with other reports in the literature. Nicolini et al. have reported shorter survival periods for the T315I-positive cases among the 89 imatinib-resistant CML patients they studied (25, 26). Thus, there is a significant difference in the life expactancy of the patients with P-loop and non-P-loop mutations, survival being shorter with the former type of mutation, and the T3151 point mutation representing a negative prognostic factor for the patient (10, 24).

In reference to the Sokol risk rating criteria, the 7-year OS rate was 100% in 12 low risk patients and in 26 medium risk patients, but decreased to 83% (p=0,124) in the 16 high risk patient group. Baccarani et al. reported 54-month survival rates of 94%, 88%, and 81% (p<0.001) in patients classified according to Sokol criteria as low, medium and high risk cases, respectively (26, 27). In the similiarly classified group of 124 CP-CML patients of Lehaye et al., the 2-year overall survival rates were 100%, 98%, and 91%, respective-

Table 4: Comparison of the Clinical Parameters and the T315I BCR-ABL Point Mutation

	n	T315I Negative	T315I positive	Risk	95% Interval	Chi-Square Test p
No of patients	54					
Sokol						
0 (Low risk)	12	10	2			0.885
1 (Medium risk)	26	20	6			
2 (High risk)	16	13	3			
Gender						
Female	42	35	7	0.400	0.94-1.703	0.206
Male	12	8	4			
Peripheral eosinophil						
≤%3	15	14	1	28.000	1.208-648.809	0.011
>%3	3	1	2			
Bone marrow reticulin fibre content						
1	12	11	1	6.600	0.543-80.235	0.110
>1	8	5	3			
Response to imatinib treatment						
Optimal	24	23	1			0.002
Suboptimal	10	9	1			
Without response	20	11	9			
Haemoglobin						
<11 gr/dL	28	24	4	0.452	0.115-1.777	0.249
>11 gr/dL	26	19	7			
Leukocyte						
$<100x10^3/\mu L$	41	31	10	0.258	0.030-2.242	0.193
$>100x10^3/\mu L$	13	12	1			
Thrombocyte						
$>450x10^3/\mu L$	25	21	4	1.833	0.468-7.188	0.381
$<450x10^3/\mu L$	29	22	7			

ly, in the low, medium and high risk cases (15). Thus, our results here have reproduced those given in the literature.

During the 1.8-year median follow-up of our imatinib treated patients 33.3% (18) showed acquired mutations. It has been reported by others that mutations have appeared in 18% of patients who have had less than 4 years of imatinib therapy, with the incidence of mutations rising to 41% among patients who have used imatinib in excess of 4 years (11, 28). Thus, the incidence of mutations increases with the duration of the imatinib therapy. Studies have shown that as the disease phase advances incidence of resistance to therapy also increases. Hence, 37% of our patients have been concluded to be without response to the therapy. Among the 139 CP-CML patients investigated by Lehaye et al. resistance development has been observed to increase from 45% (80 patients) in the accelerated phase to 92% (76 patients) in the blast phase (15). It can be seen that our results are in consistance with those reported by others.

Of the 20 patients who did not respond to imatinib 1 (5%) was lost due to imatinib-related cerebral oedema. In another study with 300 CML patients 4 of 41 (10%) were lost during imatinib therapy (14). In the same study, the mortality rate was 28% and 32% among the non-responding patients in the accelerated phase and the blast phase of the disease, respectively (15, 16). Hence, mortality increases with the advanced phases of the disease. In our study, the 1 patient lost was not responding to treatment, but was not positive for any of the 4 types of mutations investigated. The reason for not responding to imatinib may be mutation(s) at other domains or BCR-ABL gene amplification, alpha-1 acid protein or multidrug resistance gene (MDR-1) causing secondary resistance to therapy (10, 29).

In 19 of the patients we have treated cytogenetic in-

Table 5: Comparison of the Mutations and the Clinical Parameters

	n	Mutation (negative)	Mutasyon (positive)	Risk	95% Interval	Chi-Square test p
No of patients	54					
Sokol						
0 (Low risk)	12	8	4			0.975
1 (Medium risk)	26	17	9			
2 (High risk)	16	11	5			
Gender						
Female	42	28	14	0.000	0.256-3.960	1.000
Male	12	8	4			
Peripheral eosinophils						
≤%3	15	11	4	0.302	0.385-78.573	0.180
>%3	3	1	2			
Bone Marrow reticulin fibre content						
1	12	9	3	0.351	0.720-34.726	0.094
>1	8	3	5			
Response to imanitib treatment						
Optimal	24	21	3			0.004
Suboptimal	10	7	3			
Without response	20	8	12			
Haemoglobin						
<11 gr/dL	28	21	7	0.180	0.143-1.445	0.178
>11 gr/dL	26	15	11			
Leukocyte						
$<100x10^3/\mu L$	41	26	15	0.112	0.123-2.191	0.368
$>100x10^3/\mu L$	13	10	3			
Thrombocyte						
$>450x10^3/\mu L$	28	17	11	0.130	0.555-5.556	0.336
$<450x10^3/\mu L$	26	19	7			

vestigations were carried out due to gene variation and trisomy-8 was determined in 1 (5%). This result agrees with reports in the lierature. Trisomy-8 has been reported to be present in 6 (9%) of the 66 CML patients who did not respond to imanitib treatment (10, 27).

Conclusion

In this study the T315I type of point mutation showed the highest incidence of the BCR-ABL mutations investigated, and at a rate in agreement with those reported in the literature. New tyrosine kinase inhibitors are ineffective in CML cases with T315I type of mutation. Treatment recommended for cases with this type of mutation has been allogeneic stem cell transplantation. In the cases with F317L type of mutation nilotinib treatment has been started as it has been reported to be more effective than dasanitib. PFS was low in the cases with the T3151 mutation (p=0.047). The median imatinib treatment of the patients was 1.8 years and the tendency for increased drug resistance with the prolongation of imatinib treatment cannot be overlooked. In this study BCR-ABL mutations were demonstrated in 60% of the patients who did not respond to imanitib teatment.

PFS rate was found to be low in the non-responding patients (p=0.004). In CML Sokol risk classification has been regarded as a prognostic indicator, and the incidence of mutations is found to be raised with the progress of the disease phase. In our study a significant relationship between the Sokol risk rating and the incidence of mutations investigated was not demonstrable, whereas presence of BCR-ABL point mutations were found to be more significant prognostic determinants in CML. In order to determine the optimal treatment in CML it is necessary to diagnose resistance to treatment at an early phase of the disease and the genotype of the mutation present must be established for therapeutic planning.

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