# BCR-ABL Transcript Level and Neutrophil Alkaline Phosphatase Activity in CML Patients Treated with Imatinib

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**ABSTRACT**

The efficacy of imatinib mesylate has been demonstrated in patients with chronic phase of chronic myeloid leukemia (CML) as well as in advanced phase disease. The aim of this study was to evaluate the molecular response to 12 months of imatinib treatment as assessed by logarithmic reduction in BCR-ABL transcription levels, in Turkish chronic phase CML patients. Seventy-seven chronic phase CML patients were included in this multicenter, retrospective study. All patients received 12 months of oral 400 mg/day imatinib treatment. Hematological, major molecular and complete molecular response rates were evaluated after 12 months of treatment. In addition, neutrophil alkaline phosphatase (NAP) activities before and after treatment of 15 patients were also analyzed.

At 12 months of treatment, hematological, major molecular and complete molecular response rates were 87%, 58.5% and 32.5%, respectively. Major molecular response rates did not significantly differ among Sokal risk groups. In addition, a significant increase in NAP levels were seen after treatment compared to baseline ($p = 0.001$). Although there was a negative correlation between NAP activity and BCR-ABL transcript level, this was not statistically significant ($r= -0.340$, $p=0.06$).

Twelve months of imatinib treatment resulted in a molecular response in substantial proportion of CML patients without any difference among risk groups. In addition, NAP score may serve as a tool for the clinical follow-up of the response to imatinib treatment in CML patients.

**Keywords:** Chronic myeloid leukemia, Imatinib mesylate, Neutrophil alkaline phosphatase

*This study was performed in Akdeniz University, Suleyman Demirel University and Ankara Etlik Ihtisas Hospitals.*

## ÖZET

**İmatinib ile Tedavi Edilen Hastalarda BCR-ABL Transkript Seviyesi ve Nötrofil Alkalen Fosfataz Aktivitesi**

Kronik ve ileri evre kronik miyeloid lösemili (KML) hastalarda imatinib mesilatın etkinliği gösterilmiştir. Bu çalışmada kronik fazda olan Türk KML hastaların 12 aylık imatinib mesilat tedavisine moleküler cevabın (BCR-ABL transkript seviyelerindeki logaritmik azalma) analiz edilmesi amaçlanmıştır.

Bu çok merkezli çalışma 77 kronik faz KML hastalarından oluşmaktadır. Tüm hastalar 12 ay boyunca oral 400 mg imatinib tedavisi aldı. Oniki aylık tedavi sonrası hematolojik, majör ve tam moleküler yanıtlar incelendi. Ayrıca, tedavi öncesi ve sonrası 15 hastanın nötrofil alkalen fosfataz (NAP) aktiviteleri de incelendi.

Oniki aylık tedavi sonrası hematolojik, majör ve tam moleküler yanıt oranları sırasıyla %87, %58.5 ve %32.5 idi. Sokal risk grupları arasında majör moleküler yanıt oranları açısından anlamlı fark yok idi. Ayrıca, tedavi sonrası NAP seviyesinde anlamlı artış görüldü (p=0.001). NAP aktivitesi ve BCR-ABL seviyesi arasında negatif korelâsyon olmasına rağmen bu ilişki istatistiksel olarak anlamlı değildi (r= -0.340, p=0.06).

KML hastaların değişik risk grupları arasında 12 aylık imatinib tedavi sonrası majör moleküler yanıt açısından fark yok idi. Ayrıca, NAP skoru imatinib tedavisine klinik yanıtı değerlendirmede bir araç olarak kullanılabilir.



**Anahtar Kelimeler:** Kronik miyeloid lösemi, İmatinib mesilat, Nötrofil alkalen fosfataz


## INTRODUCTION

Chronic myeloid leukemia (CML) is the first malignancy associated with a specific chromosome abnormality. The Philadelphia (Ph) chromosome, the result of a t(9;22) reciprocal translocation, leads the juxtaposition of DNA sequences from the BCR and ABL genes.[1,2] BCR-ABL gene encodes different proteins, usually p210 in CML, with dysregulated tyrosine kinase activity causing leukemogenesis.[3,4]

Until the last decade, the prospect for patients diagnosed with CML had been relatively unfavorable. Imatinib mesylate (Gleevec, Novartis, Basel, Switzerland) is the first therapy to target tyrosine kinase activity. The introduction of imatinib has led not only to more favorable outcomes, but has driven the development of advances in monitoring response to therapy at molecular level. Indeed, the exquisite sensitivity of molecular monitoring using quantitative reverse-transcriptase polymerase chain reaction (RQ-PCR) as well as real-time PCR (RT-PCR) has led the ability to further refine our understanding of how long CML responds to imatinib and other BCR-ABL targeted therapies. The level of molecular response (MR) at 12 months was confirmed to be predictive of long-term clinical outcomes.[5,6]

Neutrophil alkaline phosphates (NAP) is an enzyme that catalyzes the hydrolysis of various monophosphate esters and mainly stored on the luminal side of the secretary vesicles in cytoplasm.[7] Although the biological role of this enzyme in neutrophils has remained unclear, value of the NAP activity examination for the differential diagnosis of certain hematological disorders has been well documented.[8] Abnormally low NAP activities are preferentially seen in CML and paroxysmal nocturnal hemoglobinuria.[9]

In this multicenter retrospective study, we evaluated molecular response to 12 months of imatinib treatment among Turkish chronic phase CML patients. NAP activities of 15 patients before and after treatment were also analyzed.

## PATIENTS and METHODS

In this multicenter-retrospective study, we analyzed 77 patients who were diagnosed as chronic phase CML according WHO criteria and treated with imatinib between 2003 and 2006.[10] None of the patients had a previous treatment other than hydroxyurea before imatinib. Of these 77 patients, 33 (42.9%) were woman and 44 (57.1%) were man. They had a median age of 53 ± 15 years (range 17-78).

Before the start of treatment, patients were evaluated with history, physical exam, complete blood count (WBC) with differential, blood chemistry and RT-PCR. All patients also had a pretreatment bone marrow evaluation for morphology and cytogenesis. In addition, baseline NAP activities were studied 15 patients. All of the patients were in early

chronic phase and had received 400 mg/day imatinib orally during 12 moths. In addition to the evaluation of the hematological response at 3 months, all patients were evaluated for their hematological and molecular responses at 12 months of the treatment. In addition, NAP activities of 15 patients were evaluated at 12 months.

After the RNA isolation and reverse transcriptase reaction, RT-PCR was performed by using LightCycler and LightCycler t(9;22) Quantification Kit (Roche Molecular Systems, Branchburg, NJ, USA) according to manufacturers instructions. All of the centers which included in the study used the same protocol and kit. G6PDH is the housekeeping gene of this kit. Primers and probes were included in the kit and they designed to detect b3a2, b2a2 and e1a2 fusion transcripts, covering 95% of the described t(9;22) translocations. Major MR is defined if there was more than 3-log decrease in BCR-ABL gene levels according to beginning levels. Undetectable transcription level after two consecutive assays is defined as complete MR.

NAP activity was evaluated by the method defined by Kaplow reference (Sigma-Aldrich Chemie GmbH, Germany).[8]

### Statistical Analysis

Statistical analyses were carried out using the Statistical Package for Social Sciences software (SPSS for Windows, Release 13.0). Chi-squared test, Fisher's exact test, Wilcoxon signed ranks test and Spearman bivariate correlation coefficient were used for the analyses of the data. A p value below 0.05 was considered as statistically significant.

## RESULTS

At the time of the diagnosis, the mean hemoglobin level and platelet number and white blood cell count of the patients were 11.4 ±2.2 g/dl, 471.221 ± 296.272 /mm$^3$, 131.801 ± 100.969 / mm$^3$, respectively. Except four patients, the prognostic score of Sokal et al. were determined.[11] Of 73 patients, 9 (12.3%) patients were in low risk group, and 36 (49.3%) patients in intermediate risk group and 28 (38.4%) patients in high risk group.

At the 3 months treatment all patients were in hematologic remission. At the 12 months of treatment, 67 (87%) patients were in hematologic remission. Hematologic response was lost in 10 (13%) patients. The rate of the hematologic remissions was 88.8 %, 90.9 % and 78.3 % in low-, intermediate-, and high-risk patients, respectively ($p>0.05$). At the end of 12 months, ≥ 3 log reduction in BCR-ABL transcript level (major MR) was seen 45 (58.5%) patients, 3 log < reduction in 22 (28.5%) patients and unchanged in 10 (13%) patients. Complete MR was seen in 25 (32.5%) patients. According to Sokal risk classification, the distribution of MR to the treatment of 73 patients was presented in Table 1. There was no statistically difference in the rate of major MR between low-, intermediate-, and high-risk patients ($p >0.05$).

All of NAP activities of 15 patients were under.normal level at the beginning of the treatment. Except one patient, the NAP activities of the 14 patients were in the normal range at the 12 months treatments. All of those patients were in hematologic remission rates at the 3 and 12 months of treatment. All of those patients had reduction in BCR-ABL transcript levels. Major MR was seen in 7 patients

**Table 1.** The distribution of molecular response to the treatment of patients according to Sokal risk classification (9)

| | Reduction in BCR-ABL transcription level | | | |
|---|---|---|---|---|
| | ≥ 3 log (major molecular response) | 3 log < | Unchanged | Total |
| Low risk group | 4 (44.4%) | 4 (44.4%) | 1 (11.1%) | 9 (100 %) |
| Intermediate risk group | 23 (63.9%) | 10 (27.8%) | 3 (8.3%) | 36 (100 %) |
| High risk group | 15 (53.6 %) | 8 (28.6%) | 5 (17.8%) | 28 (100 %) |

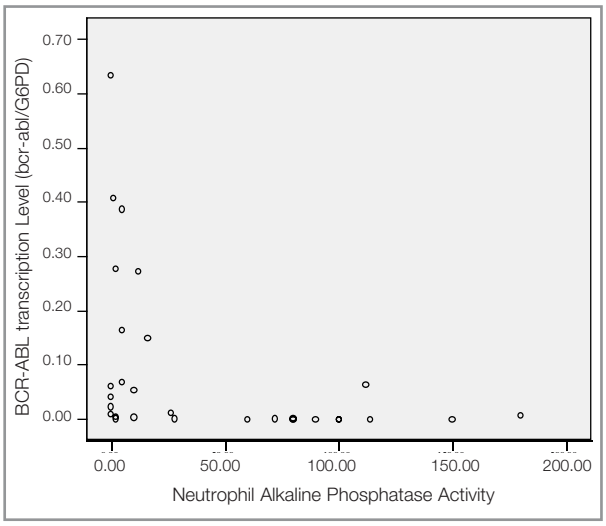


**Figure 1.** The distributions of the neutrophil alkaline phosphates activities and BCR-ABL transcription

and 6 of those patients were in complete MR. The patient, who had low level NAP activity at 12 months treatment, was in hematologic remission and reduction in BCR-ABL level was under 3 log in this patient. There were statistically difference in NAP activities and BCR-ABL transcript levels between pretreatment and 12 months of the treatment of these patients [2.0 (0-16) vs. 80.0 (10-180) and 0.0680 (0.0033-0.6325) vs. 0.0001 (0.0-0.0627) : median (min-max) respectively; p = 0.001 and p = 0.008]. Because there was no linear relation between NAP activities and BCR-ABL transcript levels logarithmic transformations on variables were made (Figure 1). Although there was a negative correlation between NAP activity and BCR-ABL transcript level, this was not statistically significant (r= -0.340, p=0.06).

## DISCUSSION

The superiority of imatinib mesylate over interferon and other traditional treatment has been shown in variety of prospective randomized international studies.[12-14] Major molecular remission after 12 months of imatinib therapy lead continuous remission and better prognosis.[6,13-19] And it was shown to be the better predictor of survival free of progression to accelerated and blast phase CML than the level of the cytogenetic response. Patients with a complete cytogenetic response accompanied by a 3 ≥ log reduction in BCR-ABL transcripts had a progression survival rate of 100% compared to a rate of 95% of patients with < 3 log reduction (p< 0.007). Patients with a complete cytogenetic response have a progression free survival of 97% compared to 95% in patients with only a partial response. One plausible explanation is that the molecular analysis is more precise in discriminating between response subgroups than cytogenetic analysis.[6,13] Although imatinib has been used in the treatment of the CML patients since 2001, the number of the studies including MR to imatinib treatment from different countries still is low. In those studies, complete hematologic response rates and major MR rates at 12 months imatinib treatment were over 90 % and 40-62%, respectively.[12,13,16,20] On the other hand, complete MR ranged from 4% to 34%.[6,16,20-24] We suppose that according to different racial groups of the patients response rates can be variable due to gene polymorphisms in different countries.

In this multicenter-retrospective study, the molecular response at 12 months of imatinib treatment was evaluated for the first time in Turkish CML patients. Sixty-seven (87%) patients were in hematologic remission at 12th months of treatment. Major MR to treatment was seen in 45 (58.4%) patients. Of these 45 patients, 25 (32.5%) patients were in complete MR. The percentage of the patients having hematologic response, major and complete MR at 12th months treatment are comparable to the others studies.[6,12,13,16,20-24]

The Sokal RR has also been reported to predict the response to treatment and overall survival.[11] In the IRIS study, rates of major MR at 12-months among cases with complete cytogenetic responses were 66%, 45% and 38% in low-, intermediate-, and high-risk patients, respectively (p= 0.007).[13] In our study, rates of major MR at 12-months were 44%, 63%, and 53.6% in low-, intermediate-, and high-risk patients, respectively. We did not find any statistically significant difference between risk subgroups in terms of major molecular response rates (p> 0.05). This contrasting finding may be due to the low number of cases in the low-risk group of the present study. On the other hand, similar to that previous study, the rate of the patients not respon-

ding to the treatment was also higher in our high-risk group (Table 1).[13]

Decreased NAP synthesis is a classical feature of CML and increase during an infectious or inflammatory complication or clinical remission with chemotherapy.[25] Low NAP activity of neutrophil of the patients with CML has been thought to be as a consequence of NAP messenger RNA deficiency.[26] In the present study, NAP activity of 15 patients was under normal level before the treatment. After the treatment, all of those patients were in hematologic remission. Major MR was seen in 7 patients and 6 of those patients were in complete MR. In all except one patient, NAP activities returned to the normal levels. A significant change was observed after treatment in NAP activity compared to baseline ($p< 0.01$). The same significantly change was also present in BCR-ABL transcript levels of those patients between pretreatment and 12 months of the treatment ($p= 0.008$). The relation between NAP activities and BCR-ABL transcript levels was not linear. Because of that, logarithmic transformations on variables were made (Figure 1). Although statistically was not significant, but very near to signifance level, there was a negative correlation between NAP activity and BCR-ABL transcript level ($r= -0.340$, $p= 0.06$).

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