# NILOTINIB EFFICACY IN 21 IMATINIB-RESISTANT OR-INTOLERANT T (9;22) POSITIVE CHRONIC MYELOID LEUKEMIA PATIENTS WITH AND WITHOUT ADDITIONAL CHROMOSOMAL CHANGES

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## ABSTRACT

• ***Objective:*** *Clonal cytogenetic aberrations other than Philadelphia chromosome can develop during the course of chronic myeloid leukemia naturally or under the pressure of treatment strategies like interferon, imatinib and dasatinib. Some of them are associated with resistance to treatment and progression to advanced phases of chronic myeloid leukemia.Nilotinib is a second generation thyrosine kinase inhibitor, but its efficacy in chronic myeloid leukemia patients with additional chromosomal changes has not been delineated yet. In this study we evaluated the efficacy of nilotinib in imatinib-resistant or -intolerant t(9;22) positive chronic myeloid leukemia patients with and without additional chromosomal changes*

• ***Material and Method:*** *21 patients (13 females, 8 males) with a median age of 53 were given nilotinib 800 mg bid orally during a median follow up period of 17 months (range 12-25 months). Five patients had additional chromosomal changes.*

• ***Results:*** *In Ph positive patients we had a major cytogenetic response rate of 67% and a complete cytogenetic response rate of 25%. The patients with additional chromosomal changes achieved hematologic, cytogenetic and molecular responses.*

• ***Conclusion:*** *We found out that nilotinib was efficacious in chronic phase CML patients resistant or intolerant to imatinib and it was also successful in CML patients with specific additional chromosomal changes.*

• ***Key Words:*** *chronic myeloid leukemia, nilotinib, tyrosine kinase inhibitors, cytogenetics, clonal aberrations* ***Nobel Med 2010; 6(2): 57-62***

## ÖZET

### İMATİNİBE DİRENÇLİ VEYA ENTOLERANS GÖSTEREN, KROMOZOMAL DEĞİŞİKLİKLERİ OLAN VE OLMAYAN T(9;22) POZİTİF KRONİK MYELOİD LÖSEMİLİ 21 HASTADA NİLOTİNİB'İN ETKİNLİĞİ

• **Amaç:** Kronik miyeloid löseminin seyri sırasında doğal olarak veya interferon, imatinib ve dasatinib gibi tedavide kullanılan ilaçlara bağlı olarak Philadelphia kromozomu dışında klonal aberasyonlar gelişebilir. Bu aberasyonların bir kısmı tedaviye direnç oluşumuyla ve hastalıkta ileri safhalar olan akselere veya blastik fazlara geçiş ile ilişkilidir.

2. kuşak tirozin kinaz inhibitörlerinden olan nilotitinibin ek kromomozom anomalisi olan hastalardaki etkinliği tam ortaya konmuş değildir. Biz bu çalışmada imatinibe dirençli olan veya intolerans gösteren, ek kromozom anomalisi olan veya olmayan kronik miyeloid lösemili hastalarda nilotinibin etkinliğini araştırdık.

• **Materyal ve Metod:** Ortanca yaşları 53 olan toplam 21 hastaya (13 kadın, 8 erkek) 2x400 mg dozunda nilotinib oral yolla uygulandı. Ortanca ilaç uygulama süresi 17 aydı (12-25 ay). Bu hastaların beşinde ek kromozom anomalisi mevcuttu.

• **Bulgular:** Ph pozitif hastalarda majör sitogenetik yanıt oranı %67 ve tam sitogenetik yanıt oranı % 25 bulundu. Ek kromozom anomalisi olan tüm hastalarda hematolojik, sitogenetik ve moleküler yanıtlar gözlendi.

• **Sonuç:** Sonuç olarak imatinibe dirençli olan ve intolerans gösteren hastalarda ve ek kromozom anomalisi olan hastalarda nilotinibin etkili bir tedavi seçeneği olduğunu gözlemledik.



• **Anahtar Kelimeler:** : Kronik miyeloid lösemi, nilotinib, tirozin kinaz inhibitörleri, sitogenetik, klonal aberasyonlar **Nobel Med 2010; 6(2): 57-62**


## INTRODUCTION

Chronic myeloid leukemia (CML) is a myeloproliferative neoplasm and is genetically characterized by the presence of the reciprocal translocation t(9;22)(q34;q11) resulting in a bcr-abl fusion gene on the derivative chromosome 22, which is called Philadelphia (Ph) chromosome. The resulting gene product is BCR/ABL and the deregulated tyrosine kinase activity of this oncoprotein is responsible for leukemogenesis.[1-3] Masked or variant Ph translocations can occur in 5-8% of CML cases. As a result of cryptic rearrangement, masked Ph chromosomes can be found in cases with a normal karyotype or in patients with complex changes.[4]

The use of targeted therapies has revolutionized the outcome of CML at the beginning of the new millennium. Imatinib an inhibitor of BCR/ABL tyrosine kinase achieved major or complete cytogenetic responses and major or complete molecular responses in patients with CML and improved survival.[5] However, several mechanisms can lead to clinical resistance to this drug. Among them point mutations of the ABL kinase domain of bcr-abl fusion gene is the leading cause of resistance.[6] Clonal cytogenetic aberrations have been described in patients with advanced CML and they reflect the natural evolution of the disease. They seem also to be involved in imatinib resistance in CML. In CML patients with cytogenetic response to interferon-α or imatinib, clonal chromosomal aberrations can arise.[7,8] These additional cytogenetic abnormalities occur in both Ph+ and Ph- cells.[9,10]

To overcome the resistance to imatinib second generation tyrosine kinase inhibitors such as nilotinib and dasatinib are developed and are still in the market. In one study dasatinib was found to be efficacious in patients with clonal cytogenetic aberrations that concur with BCR/ABL independent imatinib resistance.[11]

Nilotinib is a derivative of imatinib and has improved target specifity. It was found to be highly active and safe in patients with chronic or accelerated phase CML following resistance or intolerance to imatinib.[12,13] At our center, we used nilotinib in 21 imatinib-resistant or -intolerant CML patients in chronic phase, accelerated phase or with blast crisis. The patients participated in the ENACT (expanding nilotinib access in clinical trials) study.[14]

## MATERIAL and METHOD

The study was approved by the local ethical committee of Istanbul University, Faculty of Medicine and by the ethical committee of the Ministry of Health of Turkey. Twenty one patients (8 male, 13 female) with t (9;22) positive CML after imatinib failure were included in this study. Nineteen patients were Ph positive and two remaining patients had masked Ph translocations with complex karyotype (Table 2). Median age was 53 years (range 28-71) and the median CML duration from diagnosis to nilotinib treatment was 82 months (range 16-127). The patients's characteristics are shown in Table 1. Two patients were in blastic crisis, one patient→

in accelerated phase and 18 patients were in chronic phase. The patients were pretreated with imatinib (n=21), hydroxyurea (n=20), interferon-α and cytosine arabinoside (n=12). They did not receive dasatinib or any other second line tyrosine kinase inhibitor prior to nilotinib administration. Nilotinib was provided by the pharmaceutical company Novartis Pharmaceuticals (Florham Park, NJ) for the ENACT trial.[14] Nilotinib was administered to all patients at a dose of 400 mg twice daily. In case of adverse events, dose modifications were made according to the guidelines of the ENACT trial. Cytogenetic analyses of bone marrow were made by conventional cytogenetics with G-banding technique and interpreted according to the International System for Human Cytogenetic Nomenclature.[15] Hematologic and cytogenetic response criteria have been described

**Table 1:** Patient characteristics

| | CP | AP | BC |
|---|---|---|---|
| Total patient number (n=21) | 18 | 1 | 2 |
| Median age, years (range) | 53 (28-71) | | |
| Male/Female (n) | 8/13 | | |
| Median CML duration from diagnosis to nilotinib treatment, months (range) | 84 (17-137) | | |
| İmatinib resistant /intolerant (n) | 18/3 | | |
| Previously used antineoplastic drugs (other than imatinib) | | | |
| Hydroxyurea | n=20 | | |
| Interferon-α | n=13 | | |
| Cytosine arabinoside | n=12 | | |

CP: Chronic phase, AP: Accelerated phase, BC: Blast crisis

**Table 2:** Cytogenetics and cytogenetic responses to nilotinib treatment

| Patient number | Initials | Disease phase before nilotinib | Duration of CML/nilotinib usage (months) | Karyotype | Response to nilotinib |
|---|---|---|---|---|---|
| 1 | SO | BC | 137/3 | 46, XX, t(9;22)(q34;q11) | No response |
| 2 | EO | CP | 17/29 | 46, XY, t(9;22)(q34;q11) | Complete CyR |
| 3 | BB | CP | 91/25 | 46, XX, t(9;22)(q34;q11) | Complete HR, Ph %100 |
| 4 | SY | BC | 21/2 | 46, XY, t(9;22)(q34;q11) | No response |
| 5 | GY | CP | 83/18 | 46, XX, t(9;22)(q34;q11) | Partial CyR, Ph 20% |
| 6 | CM | CP | 55/16 | 46, XX, t(9;22)(q34;q11) | Minimal CyR , Ph 90% |
| 7 | AA | AP | 101/5 | 46, XY, t(9;22)(q34;q11) | No response |
| 8 | SG | CP | 136/20 | 46,XX, t(1;4)(p36.3;q21) | Complete HR |
| 9 | IA | CP | 120/2 | 46, XX, t(9;22)(q34;q11) | Discontinued because of QT prolongation |
| 10 | FG | CP | 125/17 | 46, XY, t(9;22)(q34;q11.2)(23)/46, idem, t(5;12;15)(q33;q14;p13)(2)/45, idem, +8, i(17q(1),c(28) | Complete HR, Ph 100% |
| 11 | AIM | CP | 31/2 | 46, XX, t(9;22)(q34;q11) | Patient discontinued treatment |
| 12 | MY | CP | 80/17 | 46, XX, t(9;22)(q34;q11) | Complete HR, Ph 100% |
| 13 | IG | CP | 109/17 | 47, XY ,t(9;22)(q34;q11), del22(q11.2) | Complete CyR, complete molecular response, bcr-abl (-) |
| 14 | ZK | CP | 38/15 | 46, XY, t(9;22)(q34;q11) | Complete CyR, complete molecular response bcr-abl (-) |
| 15 | MD | CP | 91/14 | 46, XX, t(17;22) (p13;q11), del19(p13.2) | Complete HR response |
| 16 | AAŞ | CP | 28/14 | 46, XY, t(9,22)(q34;q11) 47, XY,+8 | Partial CyR, Ph 30 % |
| 17 | LV | CP | 72/13 | 46, XX, t(9;22)(q34;q11) | Partial CyR, Ph 10% |
| 18 | MT | CP | 97/13 | 46, XY, t(9;22)(q34;q11) | Minor CyR, Ph 60% |
| 19 | HB | CP | 105/2 | 46, XX, t(9;22)(q34;q11) | Minor CyR, Ph 60%, Discontinued because of pancytopenia due to hemophagocytosis |
| 20 | IÇ | CP | 84/2 | 46, XX, t(9;22)(q34;q11) | Patient discontinued treatment |
| 21 | HT | CP | 123/12 | 46, XY, t(9;22)(q34;q11) | Partial CyR, Ph 10% |

CyR: cytogenetic response, HR: hematologic remission

**Table 3 :** Outcome of the patients with nilotinib treatment

| | CP n=18 | AP n=1 | BC n=2 |
|---|---|---|---|
| **Continuing treatment** | 14 | 0 | 0 |
| **Discontinued treatment** | 4 | 1 | 2 |
| Progression | 0 | 1 | 2 |
| Adverse events | 2 | 0 | 0 |
| Abnormal Lab | 0 | 0 | 0 |
| Death | 0 | 0 | 0 |
| Others | 2 | 0 | 0 |

CP: Chronic phase, AP: Accelerated phase, BC: Blast crisis

**Table 4 :** Adverse events with nilotinib treatment

| Adverse event (n) | n=21 | |
|---|---|---|
| | All grades | Grade 3/4 |
| Skin rash | 1 | 0 |
| Hyperbilirubinemia | 1 | 0 |
| Headache | 3 | 0 |
| Nausea | 3 | 0 |
| Pruritis | 1 | 0 |
| ALT-AST elevation | 1 | 0 |
| Lipase elevation | 6 | 0 |
| Hyperglycemia | 1 | 0 |
| Erectile dysfunction | 1 | 0 |
| QT prolongation | 1 | 1 |
| Pancytopenia | 1 | 1 |
| Thrombocytopenia | 2 | 2 |
| Neutropenia | 1 | 1 |

previously. Cytogenetic responses are as follows: complete, Ph positivity of 0%; partial, Ph positivity of 1% to 35%; minor, Ph positivity of 36% to 65%; and minimal, Ph positivity of 66% to 95%. A major cytogenetic response includes complete and partial cytogenetic responses. Cytogenetic responses were based on the percentage of Ph- metaphases among 20 or more metaphase cells in each bone marrow sample.[12] Bcr-abl was amplified with quantitative RT-PCR using t(9;22) quantification kit (Roche Diagnostics, Mannheim, Germany).

## RESULTS

Fourteen patients (67%) are continuing to receive nilotinib without a dose modification. The median duration of their nilotinib usage is 17 months (range 12-25). Among patients that were Ph positive, we achieved a major cytogenetic response rate of 67% (8/12) and a complete cytogenetic response rate of 25%. Two patients with masked Ph translocations and complex karyotypes (patient #8 and patient #15) could not be evaluated for cytogenetic remission. However they are clinically well and have a complete hematologic remission. They are still bcr-abl positive with low quantitative RT-PCR levels. As we do not have their bcr-abl transcript results according to the international scale, data are not shown here.[16] Three patients (two in blastic crisis and one in accelerated phase) did not responded to nilotinib. Two patients withdrew the informed consent and quit the trial. In two patients, we discontinued nilotinib because of adverse events. One adverse event was prolonged pancytopenia due to hemophagocytosis in patient #19. Second adverse event was QT prolongation without arrhythmia in patient #9. Nevertheless nilotinib was discontinued according to the protocol. Outcome of the patients with nilotinib treatment are shown in Table 3. Nilotinib was generally well tolerated and the adverse events, which were easily manageable in the majority of the cases (Table 4).

## DISCUSSION

Nilotinib is a highly selective inhibitor of the BCR/ABL protein and has been shown to be efficacious in imatinib-resistant or -intolerant CML patients in chronic phase and accelerated phase.[12,13] Our study group consisted of CML patients, who had a considerable long duration of disease prior to nilotinib treatment and they were pretreated with imatinib, as well as with hydroxyurea, interferon-α and/or cytosine arabinoside. Among the patients that continued to take the drug (n=14) major cytogenetic response rates and complete cytogenetic response rates were 67% and 25%, respectively. Two patients with blast crisis (patient #1 and #4) and one patient in accelerated phase (patient #7) did not show any response to nilotinib. They did not respond later to dasatinib as well. Thus, these response rates are reflecting the efficacy of nilotinib in our chronic phase CML patients and they are compatible with the previously reported studies.[12,13]

CML patients can acquire additional cytogenetic changes in the natural evolution of the disease to advanced phases. Interferon-α and imatinib can also lead to the development of clonal chromosomal aberrations.[7,8] We observed five patients with additional chromosomal aberrations, which are shown in Table 2 (Patient #8, #10, #13, #15 and #16). The majority of these patients had a markedly long duration of CML prior to nilotinib usage. All of the patients were previously treated with interferon-α and imatinib. We achieved in three out of these 5 patients complete hematologic remission (Patient #8,#10,and#15) with nilotinib treatment. Two of these→

patients were bcr-abl positive, but had masked Ph translocation with additional chromosomal aberrations [Patient #8: 46, XX, t (1;4) (p36.3;q21) and patient #15: 46, XX, t(17;22) (p13;q11), del19 (p13.2)]. These patients were in complete hematologic remission and in better clinical condition in comparison to prior to their previous treatments. However we could not assess the cytogenetic response rate with conventional cytogenetics in these two patients, because they had masked Ph translocations. They had relatively low bcr-abl transcript levels by quantitative RT-PCR.

The patient #10 is in hematologic remission after nilotinib treatment, had a relatively long duration of CML under exposure to hyroxyurea, interferon-α cytosine arabinoside and imatinib. Following a blast crisis, this female patient returned to chronic phase with imatinib, but later on, the disease progressed. She had a complex karyotype and additional translocations, [46, XY, t(9,22)(q34;q11.2)(23)/46, idem, t(5;12;15)(q33;q14;p13) (2)/45, idem, +8, i(17q(1),c(28)]. Ph was in 100% of metaphases positive.

The patient #13 with deletion of the chromosome 22 [46, XY ,t(9;22)(q34;q11), del(22q11.2)] is in complete molecular remission with negative bcr-abl after nilotinib treatment. The patient #16 with trisomy 8 [46, XY, t(9,22)(q34;q11) 47, XY,+8] had a partial cytogenetic response (Ph 30%) after nilotinib treatment.

Taking together, all these five patients with additional chromosomal aberrations responded to treatment and in the last two patients, we could also show a significant cytogenetic and even molecular responses. Trisomy of chromosome 8 was detected in 2 of the 5 cases. This aberration is the most common additional change in CML patients and has no clear prognostic significance in CML with imatinib treatment.[17]

Patient #10 with the most complex karyotype had trisomy 8 in addition to the other abnormalities responded to nilotinib with complete hematologic remission after imatinib failure. The second patient with trisomy 8 (patient #16) achieved a partial cytogenetic response. Patient #10 had also a complex additional translocation t(5;12;15)(q33;q14;p13) in a minor Ph- clone besides other chromosomal aberrations (Table 2). In this case, we were not able to say, whether the Ph translocation was a primary or secondary event. Sometimes the Ph+ clone can be suppressed by tyrosine kinase inhibition and the additional chromosomal aberration can drive myeloproliferation after arising secondarily.[17]

Clonal evolution is a well described feature of imatinib [8,17] and dasatinib treatment.[11,18] The knowledge about clonal evolution in CML with nilotinib is little and insufficient. The treatment duration of our patients was relatively short. A follow up examination of their cytogenetic findings is required to make sufficient conclusions in this field.

## CONCLUSION

In conclusion, nilotinib is efficacious in chronic phase CML patients resistant or intolerant to imatinib and it is also successful in CML patients with specific additional chromosomal changes, which develop during the natural evolution of the disease or under the pressure of previous treatment agents.

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