ABL Kinase Domain Mutations in Patients with Chronic Myeloid Leukemia in Jordan

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Mutations of the *BCR-ABL* tyrosine kinase domain constitute a major cause of resistance to tyrosine kinase inhibitors in patients with chronic myelogenous leukemia (CML). In this study, we analyzed peripheral blood samples from 185 Jordanian CML patients for ABL mutations, who were on imatinib for a minimum of 6 months regardless of their disease status and over a period of 5 years. Mutations were detected by nested RT-polymerase chain reaction, followed by direct sequencing of the ABL kinase domain. Twelve different point mutations were detected 25 times in 21 patients. The resultant mutations were as follows: four patients have T315I, three of each of the following: L248V, F317L, and G250E, two of each of the following: H396R, M244V, and T277A, and one of each of the following: F311I, M318T, Q252H, F359A, F359I, and Y326H. After patient follow-up, the mutation had disappeared in 12 patients; 3 patients died; 3 patients were not retested; and 3 patients had persistent mutation. The finding of our study is in line with what has been described in the literature. Detecting ABL mutations in chronic phase may lead to positive outcome by modifying treatment.

Introduction

CHRONIC MYELOGENOUS LEUKEMIA (CML) is a clonal myeloproliferative neoplasm; it is characterized by the presence of the Philadelphia chromosome (Ph1) which is the product of the t(9; 22) (q34; q11) translocation (Mauro and Druker, 2001). This translocation results in the *BCR-ABL* gene and the fusion protein that has constitutive tyrosine kinase activity. CML progresses from a relatively benign chronic phase (CP) to an accelerated phase that is characterized by increasing numbers of early hematopoietic cells and additional chromosomal abnormalities (Branford and Hughes, 2006). The disease terminates in blast crisis (BC), which is distinguished by the large number of immature blast cells that populate the bone marrow and peripheral blood (Branford *et al.*, 2006).

Successful development of the *BCR-ABL* tyrosine kinase inhibitor (TKIs) imatinib for chronic myeloid leukemia (CML) marked the beginning of the era in targeted therapy of CML (Maekawa *et al.*, 2007). Over the last decade, imatinib has been the standard of care for CML (Maekawa *et al.*, 2007). Imatinib is able to induce a major cytogenetic response in the majority of CP CML patients (Sawyers *et al.*, 2002). Eighty-five percent of patients treated with imatinib attained a major cytogenetic

response after a median follow-up of 19 months, compared to 22% of patients treated with a nonimatinib combination (Kantarjian *et al.*, 2002). Mutations of the *BCR-ABL* tyrosine kinase domain constitute the major cause of resistance to TKIs in patients with chronic myeloid leukemia occurring in 30% to 90% of patients who develop resistance (Cortes *et al.*, 2007; Ray *et al.*, 2007). Mutations in more than 40 different amino acids have been described, conferring differing levels of resistance to imatinib (Cortes *et al.*, 2007). CP CML patients may develop *BCR-ABL* point mutations after starting the treatment, while others, almost exclusively patients in more advanced phases of the disease, may present mutations already at diagnosis (Luzzatto and Melo, 2002; Roche-Lestienne *et al.*, 2002). Using highly sensitive detection techniques, mutations can be seen even in CP CML patients at diagnosis, but their clinical significance is uncertain. Most resistant patients have acquired point mutations in the ABL kinase domain that render the kinase resistant to imatinib (Melo and Chuah, 2007). Early detection of *BCR-ABL* mutants may identify patients who are likely to become resistant to imatinib therapy (Kantarjian *et al.*, 2008).

All patients with chronic myeloid leukemia on treatment should be monitored by cytogenetics using karyotyping or FISH techniques or with quantitative RT-polymerase chain

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reaction (PCR) (Kantarjian *et al.*, 2002; Reinhold *et al.*, 2003). The resistance testing is useful to help guide CML treatment with alternative therapies (O'Hare *et al.*, 2007). Depending on the exact mutation present, clinical intervention such as increasing dosage of imatinib or using other kinase inhibitors may be effective in controlling the levels of BCR-ABL and the relapse of the disease. Hematopoietic stem cell transplantation may also be an option to be taken into consideration in the presence of specific mutations, such as the T315I mutation, which has been shown to be insensitive to all registered TKIs (Velev *et al.*, 2010).

The aim of this study was to identify ABL mutations in CML patients in a developing country followed for a long period and report on the outcome of the patients developing clones with these mutations.

Materials and Methods

Patients

A total of 185 CML patients who were on imatinib treatment for at least of 6 months regardless of their disease status and who were either followed at the Jordan University Hospital or referred to the Thrombosis and Molecular Hematology Laboratory (TMHL) for real-time quantitative PCR (RQ-PCR) for BCR-ABL transcripts were screened for ABL mutations. The diagnosis of CML was based on cytogenetics, FISH, or molecular method. The study was approved by the investigational review board, and a consent form was obtained from each patient in accordance with the Helsinki declaration. Seventy-six patients were women with a median age of 53.00 ± 12.00 years, and 109 of patients were men with a median age of 58.00 ± 2.00 years as shown in Table 1. The median of age for patients with ABL mutations was 47.3 ± 16 for women ($n=7$) and 43.9 ± 20 for men ($n=14$). All patients were in CP, except two were in AP and one in BC.

The mean duration of disease in the whole population was 93.4 ± 16 months, and the mean duration of disease in patients with ABL mutations ($n=21$) was 37.44 ± 12.00 months. Blood samples were collected between February 2006 and September 2011. The 21 patients with mutations were monitored for disease status for a median of 22.0 ± 12.4 months after initial detection of the mutation. All patients were on imatinib for a minimum of six months. The initial dose of imatinib was 400 mg/day in the patients with CP CML and 800 mg/day in those with accelerated phase. The mean dose of imatinib in all population was 388.53 ± 55.00 mg/day among women ($n=76$) and 390.39 ± 65.00 mg/day among men ($n=109$) as shown in Table 1. The mean dose of imatinib among patients with ABL mutations was 365 ± 65 mg/day for females ($n=7$) and 367 ± 65 mg/day for males ($n=14$).

All 21 patients with mutations were in CP, except 1 who was in BC. Four patients failed to achieve hematologic response, and 17 patients achieved complete hematologic response. Nine patients failed to achieve major molecular response, and 11 patients failed to achieve complete cytogenetic response (CCYR) as shown in Table 4. Monitoring included peripheral blood counts, bone marrow morphology, metaphase karyotyping of bone marrow cells, interphase FISH (bone marrow samples only), and RQ-PCR for quantitation of BCR-ABL transcripts using bone marrow and blood samples.

TABLE 1. DURATION OF CHRONIC MYELOGENOUS LEUKEMIA AND DOSE OF IMATINIB FOR ALL POPULATIONS ($N=185$)

Gender	Mean age (year)	Mean duration of CML (months)	Mean dose of imatinib
F ($n=76$)	53.00 ± 12.00	83.3 ± 22	388.53 ± 55.00
M ($n=109$)	58.00 ± 2.00	98.6 ± 12	390.39 ± 65.00
Total ($n=185$)	56.00 ± 5.00	93.4 ± 16	389.57 ± 58.00

CML, chronic myelogenous leukemia.

RNA extraction and cDNA synthesis

Total RNA was extracted from peripheral blood leukocytes using TRIzol reagent (Life Technologies) according to the manufacturer's instructions. cDNA was synthesized from 1 μ g total RNA in a 20- μ L reaction mixture using random hexamer primers and MMLV reverse transcriptase super-script III enzyme (Invitrogen™).

BCR-ABL quantitation

The quality of the RNA was assessed before sequencing by measurement of the level of BCR-ABL and normal ABL transcripts using RQ-PCR (Kantarjian *et al.*, 2003; Hughes and Branford, 2006). A cut-off level based on the number of detectable ABL gene transcripts in the BCR-ABL cDNA that was quantified by the real-time PCR technique using a Fusion Quant kit (Ipsogen, Inc.) for quantitation of BCR-ABL fusion gene transcripts with normalization to total ABL gene levels according to the manufacturer instructions.

PCR amplification and mutation analysis

The ABL kinase domain of the BCR-ABL fusion gene was amplified using nested RT-PCR, followed by direct sequencing as described previously (Sacha *et al.*, 2003). Briefly, the BCR-ABL allele was amplified using a forward primer that annealed to the BCR exon b2 and a reverse primer that annealed to the exon 7 of the ABL gene. A 675-bp fragment containing the BCR-ABL kinase domain was amplified using a nested PCR, and then the PCR amplification was confirmed by agarose gel electrophoresis and sequenced in both directions to confirm the presence of the mutations using Dye Terminator Chemistry and an ABI 3310 genetic analyzer (Applied Biosystems). The amino acid substitutions were determined using the GenBank accession no. M14752. The sequencing reactions were repeated for confirmation of the positive results.

TABLE 2. DURATION OF CHRONIC MYELOGENOUS LEUKEMIA AND DOSE OF IMATINIB FOR THE (21) PATIENTS WITH MUTATIONS

Gender	Mean age (year)	Mean duration of CML (months)	Mean dose of imatinib
F ($n=7$)	47.3 ± 16	30.25 ± 17.00	365.00 ± 25.00
M ($n=14$)	46.81 ± 17.15	35.4 ± 15.00	367.40 ± 65.00
Total ($n=21$)	44.75 ± 18.2	34.6 ± 16.00	366.26 ± 53.00

TABLE 3. TYPE AND FREQUENCY OF 25 ABL KINASE DOMAIN MUTATIONS

Mutation	No. of patients
M244V	2
L248V	3
G250E	3
Q252H	1
T277A	2
F311I	1
T315I	4
F317L	3
M318T	1
Y326H	1
F359A	1
F359I	1
H396R	2

Results

Mutations were observed in 21 patients of the analyzed population (185). Tables 1 and 2 shows the duration of CML and the dose of imatinib for all 185 study patients and for the patients with mutations, respectively, Table 3 shows the types

and frequencies of ABL kinase domain mutations, and Table 4 shows the disease outcome of patients with ABL mutations after a mean follow-up of 22 ± 12.4 months. The mean duration of disease for 185 patients was 83.3 ± 22.00 for women ($n=76$) and 98.6 ± 12.00 for men ($n=109$) as shown in Table 1. The mean duration of CML for patients with mutations was 30.25 ± 17 for women ($n=7$) and was 35.4 ± 15 months for men ($n=14$) as shown in Table 2. Twelve different point mutations were detected 25 times (13.51%) in 21/185 patients (11.35%); 4 patients have T315I; 3 patients have L248V; 3 patients have G250E; and 3 have F317L; two patients with each of the following mutations: M244V, T277A, and H396R, and one patient with each of the following mutations: M318T, Y326H, Q252H, F311I, F359A, and F359I. Three of the above 21 patients had more than one mutation, one of them had M244V with G250E; the second patient had F317L, M318T, and Y326H, and the third one had T277A and F359A as listed in Table 4.

Discussion

In this study, we intended to test patients who had received at least 6 months of imatinib regardless of the disease status.

TABLE 4. DISEASE OUTCOME OF THE 21 PATIENTS WITH MUTATIONS

Patient No.	Age/sex	Response by 6 months	CML phase	Mutation onset	Treatment strategy after mutation detection	Follow-up analysis
1	72/M	CP	Loss of CCYR	M244V G250E	Nilotinib	G250E
2	32/M	CP	Loss of CCYR	L248V	Imatinib dose increased to 800 mg/day	Negative
3	35/M	CP	Loss of CCYR	L248V	Nilotinib	Negative
4	59/F	CP	Loss of CCYR	F311I	Nilotinib	Negative
5	50/M	BC	Loss of CCYR	T315I	Hydroxyurea	Died
6	31/M	CP	Loss of CCYR	T315I	Hydroxyurea	Not retested ^a
7	29/M	CP	Loss of CCYR	L248V	Imatinib dose increased to 800 mg/day	Negative
8	70/M	CP	Loss of CCYR	F317L M318T Y326H	Imatinib dose increased to 800 mg/day	Negative
9	48/F	CP	Loss of CCYR	H396R	Dasatinib	Negative
10	77/M	CP	Loss of MMR	M244V	Imatinib dose increased to 600 mg/day	M244V
11	72/M	CP	Loss of MMR	G250E	Dasatinib	Died
12	45/M	CP	Loss of MMR	H396R	Imatinib dose increased to 800 mg/day	Negative
13	26/M	CP	Loss of CCYR	F317L	Imatinib dose increased to 800 mg/day	Negative
14	32/M	CP	Loss of MMR	Q252H	Imatinib dose increased to 800 mg/day	Negative
15	42/F	CP	Loss of MMR	T277A	Imatinib dose increased to 800 mg/day	T277A
16	59/F	CP	Loss of MMR	F317L	Nilotinib	Negative
17	50/F	CP	Loss of MMR	T315I	Nilotinib	Not retested ^a
18	31/M	CP	Loss of MMR	T277A F359A	Imatinib dose increased to 800 mg/day	Died
19	23/F	CP	Loss of CCYR	F359I	Nilotinib	Negative
20	36/M	CP	Loss of MMR	G250E	Imatinib dose increased to 800 mg/day	Negative
21	18/M	CP	Loss of CCYR	T315I	Dasatinib	Not retested ^a

^aReason for not retesting: in two patients the follow-up was less than 6 months, and one patient was lost to follow-up.

CP, chronic phase; BC, blast crisis; CHR, complete hematologic response; FHR, failure of hematologic response; MMR, major molecular response; CCYR, complete cytogenetic response.

The samples were initially assessed for the level of *BCR-ABL* and only preceded to mutation analysis if the stored RNA contained a measurable level of *BCR-ABL*, and the control gene level was indicated. Patients tested had either received 6 or more months of imatinib therapy or had developed resistance and ceased therapy after having received imatinib treatment.

The degree of resistance depends on the mutation, with some mutations remaining sensitive to imatinib (Jabbour and Soverini, 2009). Dose escalation of imatinib may overcome resistance in some of these patients. Patients with some mutations may be sensitive to therapy with second-generation TKIs such as nilotinib or dasatinib (Weisberg *et al.*, 2007). The choice of therapy should be guided by multiple factors, including disease phase, patient characteristics, mutational analysis, and the safety profile of the agents (Jabbour *et al.*, 2009).

Current evidence does not support mutation screening on a routine basis unless there is an indication such as loss of response (An *et al.*, 2010). Transient detection of kinase domain mutations has been documented in some patients with stable CCYR, suggesting that the detection of a mutation in the absence of an increase in leukemia burden is uninterpretable (Sherbenou *et al.*, 2007).

Despite the fact that our patients were followed for many years from the diagnosis of CML and for several months after ABL mutation detection, further follow-up is needed to identify the course of the ABL mutation and outcome of these patients. Follow-up for patients with ABL mutations were as follows: the mutations disappeared in 12 patients; 7 patients after the Imatinib dose was increased to 800 mg/day; 2 of them had L248V, 1 patient with each of the following H396R, F317L, G250E, and Q252H, and 1 patient with three mutations F317L, M318T, and Y326H. In four patients after Nilotinib was given, one patient of each of the following mutations: L248V, F311I, F317L and F359I, and in one patient with H396R after Dasatinib was given as shown in Table 4. Three patients were still having the mutation after treatment change: one with T277A mutation, one with G250E mutation and one with M244V mutation. Three patients died, two of them due to disease progression. Three patients were not retested; two of them because of a less-than 6-month follow-up and one patient were lost at follow-up.

In this study, we found three patients with more than one mutation. Patient number 1 and 18 each had two mutations, and patient number 8 had three different point mutations. In patient 18, the mutations conferred a total resistance to imatinib, since it did not respond to increasing the imatinib dose, and subsequently, the patient transformed to acute myeloid leukemia and died. It is possible that these mutations have conferred a gain of function and a second generation of TKIs may overcome this issue. In patient number 1, the mutation seems to be responsive to Nilotinib, since it disappeared after Nilotinib therapy. While in patient Number 8, the mutations have disappeared 2 years after continuous therapy with increased dose of imatinib. We are not aware that such a combination of three mutations has been reported.

The finding of our study concerning *ABL* gene mutation in CML is in line with what has been described in the literature (Soverini *et al.*, 2006; Lewandowski *et al.*, 2009). Mutations were found in 21 of 185 chronic-phase patients (11.35%) treated with imatinib with L248V, G250E, and T315I, being

the more frequently seen. ABL Mutations in our study were widely distributed on the ABL gene as described in a previous study (Markose *et al.*, 2009). We found no cluster of CML in any family.

Detecting ABL mutations in CP may lead to positive outcome by modifying treatment. The screening of ABL mutation is not recommended routinely for patients with CML, but rather it should be limited to a selected group of patients who have a poor or suboptimal response or a loss of CCYR or an increase of BCR-ABL transcript (Sherbenou *et al.*, 2007). Mutation analysis in this situation might influence the course of disease and therapy. Resistance to imatinib may be caused by factors other than ABL mutation. This work did not explore this aspect, since we looked at ABL mutations only. Future work will be directed toward this aspect.

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Author Disclosure Statement

None of the authors have any conflicts of interest regarding this publication.

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