

Frequency of *BCR-ABL* gene mutations in Polish patients with chronic myeloid leukemia treated with imatinib

A final report of the MAPTEST study

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KEY WORDS

BCR-ABL gene mutations, chronic myeloid leukemia, imatinib

ABSTRACT

INTRODUCTION The presence of *BCR-ABL* oncogene mutations in patients with chronic myeloid leukemia (CML) may be responsible for the failure of tyrosine kinase inhibitor treatment.

OBJECTIVES The aim of the study was to evaluate the frequency of *BCR-ABL* gene mutations in patients with CML (the MAPTEST study) treated with imatinib (IM).

PATIENTS AND METHODS Direct sequencing analysis of *BCR-ABL* gene was performed in 92 patients treated with IM for more than 3 months. The mean time of IM treatment was 18 months. At the time of the analysis, 75 patients were in the first chronic phase (CP), 4 in the second CP, 5 in the acceleration and 8 in the blastic phase. Fifty-seven patients (62%) were treated with IM at a daily dose of 400 mg and 35 patients with higher doses (600 or 800 mg daily). Inclusion criteria were based on the European Leukemia Net definitions for failure and suboptimal response to IM.

RESULTS Twelve mutations were detected in 11 of 92 patients, including 4 mutations (36.7%) diagnosed during CP, 3 (27.3%) in acceleration, and 4 (36.7%) in blast crisis. In 1 patient with lymphoid blast crisis of CML coexisting F359V and Y253F mutations were detected. In the whole group mutations were detected in 2 of 5 patients (40%) with primary resistance (M351T, F359V + Y253F) and in 9 of 87 patients (10.3%) (E255K, T315I-3x, M351T, E355G, F359V-2x) with acquired resistance to IM.

CONCLUSIONS The study confirmed the usefulness of *BCR-ABL* gene mutation screening in patients with CML resistant to IM therapy.

INTRODUCTION There is increasing evidence supporting a role of *BCR-ABL* kinase activity in the induction of genomic instability in chronic myeloid leukemia (CML) cells by multiple mechanisms, including generation of reactive oxygen

species.¹ Clinical and laboratory data suggest that prolonged exposure to *BCR-ABL* kinase activity probably leads to development of clonal disease and acquisition of *BCR-ABL* kinase domain (KD) mutation in the exposed cells.^{2,3}

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Several observations suggested that *BCR-ABL* KD mutation occurs during natural disease course, irrespective of tyrosine kinase inhibitor (TKI) therapy.⁴ However, it cannot be excluded that TKI therapy results in the selection of resistant clones originating from leukemic stem cells. This concept might be supported by the data obtained using a very sensitive allele specific polymerase chain reaction (PCR) method that confirm the presence of the same mutation at relapse and in pre-treatment samples.⁵ Therefore, the *BCR-ABL* KD mutation monitoring in patients with CML initially treated with imatinib (IM) and presenting suboptimal response or resistance to TKI has become of importance to design further therapeutic strategy.⁶ According to the clinical guidelines developed by the National Comprehensive Cancer Network and the European Leukemia Net (ELN)^{7,8} these patients should be screened for the presence of *BCR-ABL* KD mutations; however, there is no consensus regarding the techniques and data reporting.⁹ Until now, several techniques proved to be useful in mutation detection, i.e., direct sequencing,¹⁰ subcloning and sequencing,² denaturing high-performance liquid chromatography,¹¹ pyrosequencing,¹² denaturing gradient gel electrophoresis,¹³ fluorescence PCR and peptide nucleic acid clamping,¹⁴ allele-specific oligonucleotide PCR,⁵ SEQUENOM Mass Array,¹⁵ and the most recent – high-resolution melt curve analysis.¹⁶⁻¹⁸

Apart from the quantitative *BCR-ABL* transcript analysis, which provides essential data on the efficacy of TKI treatment, mutation detection is also important. Mutation-specific drug inhibitory potency (inhibitory concentration for 50% [IC₅₀]) is particularly useful in decision-making process when therapy should be switched to one of the second generation TKIs. Out of all the available methods, direct sequencing is the most common and reliable approach.⁹

The aim of the study was to evaluate the frequency of *BCR-ABL* gene mutations in patients with CML treated with IM (the MAPTEST study). We also focused on the standardization of procedures, especially sample collection, transport and storage, and sequencing method protocol, used in all the participating centers.¹⁹

PATIENTS AND METHODS Blood samples had been collected in 15 Polish hematology departments (Gdańsk, Opole, Warszawa, Wrocław, Białystok, Poznań, Toruń, Olsztyn, Zielona Góra, Katowice, Rzeszów, Kielce, Bydgoszcz, Zamość, Łódź, and Kraków) from September 1, 2007 to March 31, 2008. A mutational study was performed in 3 centers (Gdańsk, Poznań, and Warszawa).

The analysis of *BCR-ABL* gene was performed in 92 patients with CML (mean age 51.9, median 55 years, age range 21–76 years) treated with IM for more than 3 months. The mean time of IM treatment was 18 months. Eighteen patients were previously treated with hydroxyurea (20–50 mg

TABLE 1 Characteristics of the study population (n = 92)

Characteristics	n
disease phase	
chronic	79
first	75
second	4
acceleration	5
blastic transformation	8
lymphoid	2
myeloid	6
imatinib resistance	
primary	5
acquired	87
imatinib daily dose	
400 mg	57
600 or 800 mg	35

per kg body weight), interferon α (3 times a week 3 mln/m² subcutaneously [s.c.]) alone or in combination with arabinoside cytosine (10–20 mg/m² s.c. from day 1 to 10, every month).

At the time of the analysis, 75 patients (81.5%) were in the first chronic phase (CP), 4 (4.3%) in the second CP, 5 (5.4%) in the accelerated and 8 (8.7%) in the blastic phase (6 myeloid and 2 lymphoid). At the time of evaluation, 57 patients (62%) were treated with IM at a daily dose of 400 mg. The remaining 35 patients (38%) were treated with higher IM doses (600 or 800 mg daily). Characteristics of the study group are shown in **TABLE 1**.

A mean time from the diagnosis of CML to mutation screening was 46.5 months (range 3–212 months, median 36 months). Inclusion criteria for the MAPTEST study were based on the ELN criteria for failure and suboptimal response and are presented in **TABLE 2**.⁶

Clinical evaluation of patients was performed monthly with physical examination and laboratory monitoring, including complete peripheral blood count. Classic cytogenetic analysis was conducted every 6 months. The result was considered as conclusive, if the number of evaluable metaphases exceeded 20. Molecular analysis included quantitative measurement of *BCR-ABL* transcript level every 3 to 6 months. Results were expressed as a ratio of *BCR-ABL* to reference gene (mainly *c-ABL*) transcript's copy number on the basis of an intralaboratory standard.

The definition of responses was based on the widely accepted criteria.⁸ Complete hematologic response (CHR) was diagnosed, if a patient was free of the signs and symptoms of the disease, and white blood count and platelets did not exceed an upper laboratory limit. Complete cytogenetic response (CCyR) was defined as the absence of Philadelphia (Ph)-positive metaphases. Major cytogenetic response was defined as a decrease in the number of Ph-positive metaphases

TABLE 2 The inclusion criteria for the MAPTEST study

Duration of treatment (months)	Hematological response	Cytogenetic response	Molecular response	Event
3	lack of CHR or HR	–		–
6	–	lack of MCyR		–
12	–	lack of CCyR	–	–
18	–	–	lack of MMR	
any time	–	–	–	progression to acceleration/ blastic phase loss of hematologic, cytogenetic or molecular response

Abbreviations: CCyR – complete cytogenetic response, CHR – complete hematologic response, HR – hematologic response, MCyR – major cytogenetic response, MMR – major molecular response

to less than 35%. Molecular response was evaluated by determining the amount of *BCR-ABL* transcript in individual patients with real-time PCR. Molecular evaluation was performed every 3 to 6 months as mentioned above. Major molecular response (MMR) was diagnosed, if normalized *BCR-ABL* gene transcript levels decreased by 3 logarithmic units (or below 0.1%).

In the case of loss of some response (i.e., hematological, cytogenetic, or molecular), acquired resistance was diagnosed. Primary resistance (or primary refractoriness) was present when IM did not induce a respective level of response described above.²⁰

To detect *BCR-ABL* KD mutation, 2 directional sequencing studies were performed according to Branford and Hughes protocol² using BigDye Terminator v3.1 Cycle Sequencing Kit and Abi Prism 3130 Genetic Analyzer (Applied Biosystems). Recommendations concerning blood collection, logistics, and sample delivery were published previously by Sacha et al.¹⁹

RESULTS The most common indication for mutation screening in the analyzed group of patients treated with TKI was loss of cytogenetic response (18.5%), lack of MMR after 18 months of IM treatment (15.2%), loss of MMR (13.4%), lack of CCyR after 12 months (10.9%), lack of CCyR after 18 months (9.8%), and lack of CHR or hematologic response (HR) after 3 months (5.4%).

Among 92 patients with CML, 12 mutations were detected in 11 patients, including 4 mutations diagnosed during CP, 3 in acceleration, and 4 in blastic crisis (1 lymphoid and 3 myeloid). In 1 patient with lymphoblastic crisis of CML, co-existing F359V + Y253F mutations were found (TABLE 3).

In the whole group, mutations were detected in 2 subjects with primary resistance (M351T, F359V + Y253F) and in 9 subjects with acquired resistance (E255K, T315I-3x, M351T, E355G, F359V-2x). In a subgroup of patients in the first CP (n = 74) treated with IM and symptoms of primary resistance, a mutation (M351T) was detected only in 1 patient. Similar analysis performed in a subgroup of patients with secondary resistance revealed mutations in 2 patients (M351T and T315I).

DISCUSSION Acquisition of mutations within the KD of *BCR-ABL* has become an intriguing issue regarding TKI resistance in CML. To date, more than 100 distinct point mutations encoding for single amino acid substitutions in the *BCR-ABL* KD have been detected in patients with CML resistant to IM therapy.^{9,10,21-25} Many others have also been generated in vitro by random mutagenesis of *BCR-ABL*.^{21,26}

Mutation sites are clustered in 4 regions of *BCR-ABL* tyrosine kinase, including the phosphate-binding loop (P-loop), IM-binding site,

TABLE 3 The occurrence of mutations in a cohort of patients in relation to the phase of chronic myeloid leukemia

Mutation	Site	Chronic phase	Acceleration phase	Lymphoid blastic crisis	Myeloid blastic crisis	Case number
E255K	P-loop	0	0	0	1 (9.1%)	1
E255V	P-loop	0	1 (9.1%)	0	0	1
T315I	imatinib-binding site	2 (18.2%)	0	0	1 (9.1%)	3
M351T	SH2-contact	2 (18.2%)	0	0	0	2
E355G	substrate-binding region	0	1 (9.1%)	0	0	1
F359V	substrate-binding region	0	1 (9.1%)	1 (9.1%)	0	2
F359V + Y253F	substrate-binding region + P-loop	0	0	1 (9.1%)	0	1
Total		4	3	2	2	11

catalytic domain, and activation loop. Mutations can affect the TKI and *BCR-ABL* tyrosine kinase interactions directly or indirectly, if their presence shifts the thermodynamic equilibrium from inactive toward active conformation of the enzyme. This may reduce TKI efficacy.^{20,27}

The frequency of *BCR-ABL* gene mutations in patients resistant to IM ranges from 40% to 90%, depending on the definition of resistance, methodology of detection, and CML phase.^{10,28-32} In the GIMEMA study, *BCR-ABL* mutations were found in 127 of 297 evaluable patients (43%). Defects were present in 27% of patients with CP (14% treated with IM frontline, 31% treated with IM after interferon [IFN] failure), 52% with accelerated phase, 75% with myeloid blast crisis, and 83% with lymphoid blast crisis/Ph-positive acute lymphoblastic leukemia. *BCR-ABL* mutations were documented in 30% of patients with primary resistance (44% hematologic and 28% cytogenetic) and in 57% of those with acquired resistance, including 23% of patients who lost CHR, 55% of patients who lost HR, and 87% of patients who progressed to accelerated or blast crisis phases. Despite a large spectrum of detected mutations, amino acid substitutions at 7 residues (M244V, G250E, Y253F/H, E255K/V, T315I, M351T, and F359V) accounted for 85% of all resistance-associated aberrations.³³

Jabbour et al. identified 66 mutations in 23 amino acids in 36% of patients resistant to IM therapy (62 of 171). P-loop mutations were the most common (36%). By multivariate analysis, factors associated with acquisition of mutations included older age, prior IFN therapy, and accelerated or blast phase at the time of IM failure. Interestingly, mutation status had no effect on patient survival. However, when survival was evaluated from the initiation of therapy, non-P-loop mutations together with duration of response and transformation at the time of IM failure were all associated with shorter survival.³⁴

In our study group, a relatively small number of mutations of *BCR-ABL* gene were found. However, when patients in acceleration or blastic phases were taken into consideration, the frequency of mutations was similar to that reported by other authors (7/13, 53.8%). Low incidence of *BCR-ABL* gene mutations in our cohort might be related to the characteristics of the study patients, i.e., predominance of patients treated up-front with IM, high percentage of patients in CP (>80%), and a relatively short follow-up. Our results, however, are consistent with the previously reported data that showed that mutations of *BCR-ABL* gene seem to be less common in patients treated with IM since the diagnosis, and the frequency increases with acquisition of TKI resistance.³⁴⁻³⁸

The frequency of TKI resistance in CML patients treated with IM is approximately 15%; however, *BCR-ABL* mutations are present in approximately 8% of patients.^{39,40} Based on the recently published data, mutations with IC_{50} for IM ≤ 150 nM (M244V, L248V, G250E, Q252H, E275K,

D276G, F317L, M351T, E355A, E355G, L387F, F486S) were detected in approximately 23% of CML patients with TKI resistance. Fourteen percent of patients had mutations (F359C/V – 6% E255K/V – 4%, Y253H – 4%) moderately ($IC_{50} > 150$ nM) and 3% (T315I) highly resistant ($IC_{50} > 10\,000$ nM) to IM.^{40,41} These data confirm the results of our study on the frequency and distribution of *BCR-ABL* gene mutations among patients treated up-front with IM.

Finally, it should be highlighted that reported frequency of *BCR-ABL* gene mutations in CML patients depends on the sensitivity of techniques used to detect molecular aberrations. Some laboratory techniques allow to identify 1 mutated in 100,000 wild-type sequences, while the sensitivity of others could discriminate an abnormal one out of 3 normal templates. Using direct sequencing (sensitivity in the order of 20%–30%), the mutant clone can only be identified when it constitutes a considerable proportion of the total template. From the practical point of view, low sensitivity of direct sequencing in mutation detection is very useful. It allows to detect only the “driver” mutation(s) responsible for the disease progression or TKI resistance.

In conclusion, the MAPTEST study was the first global project to determine the frequency of *BCR-ABL* gene mutations in Polish CML patients. A relatively low frequency of detected mutations may be related to the study group characteristics (a large number of newly diagnosed patients). However, this fact does not affect clinical relevance of the obtained data. Identification of mutations with in-vitro determined sensitivity (IC_{50}) to TKI (IM, dasatinib, nilotinib) could help to choose an appropriate second-line inhibitor for further treatment.²⁷ We also hope that the experience gained in the MAPTEST study will result in wider implementation of molecular methods used in clinical evaluation of patients treated with TKI.

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Częstość mutacji genu *BCR-ABL* u polskich chorych na przewlekłą białaczkę szpikową leczonych imatynibem

Raport końcowy badania MAPTEST

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SŁOWA KLUCZOWE

imatynib, mutacje genu *BCR-ABL*, przewlekła białaczka szpikowa

STRESZCZENIE

WPROWADZENIE Obecność mutacji onkogenu *BCR-ABL* u chorych z przewlekłą białaczką szpikową (PBSz) może być odpowiedzialna za niepowodzenie terapii inhibitorami kinazy tyrozynowych.

CELE Ocena częstości występowania mutacji genu *BCR-ABL* u chorych na PBSz (badanie MAPTEST) leczonych imatynibem (IM).

PACJENCI I METODY Ocenę przeprowadzono stosując technikę bezpośredniego sekwencjonowania u 92 pacjentów z rozpoznaniem PBSz leczonych IM > 3 miesiące. Średni okres trwania terapii IM wynosił 18 miesięcy. W czasie oceny 75 pacjentów było w pierwszej fazie przewlekłej, 4 w drugiej fazie przewlekłej, 5 w okresie akceleracji, a 8 pacjentów było w okresie kryzy blastycznej. 57 pacjentów (62%) leczono IM w dawce 400 mg dziennie, a 35 – wyższymi dawkami IM (600 lub 800 mg dziennie). Kryteria włączenia do badania oparto na definicji odpowiedzi suboptymalnej oraz niepowodzenia terapii IM według European Leukemia Net.

WYNIKI W grupie 92 pacjentów z PBSz wykryto 12 mutacji u 11 chorych, w tym u 4 (36,7%) w fazie przewlekłej, 3 (27,3%) w okresie akceleracji i 4 (36,7%) w fazie kryzy blastycznej. W jednym przypadku przełomu limfoblastycznego potwierdzono jednoczesne występowanie mutacji F359V i Y253F. W całej grupie badanych chorych leczonych IM mutacje wykryto u 2 z 5 (40%) pacjentów z opornością pierwotną (M351T, F359V + Y253F) i u 9 z 87 (10,3%) z opornością nabytą (E255K, T315I-3x, M351T, E355G, F359V-2x).

WNIOSKI Badanie potwierdziło przydatność analizy obecności mutacji genu *BCR-ABL* u chorych z PBSz u których terapia IM była nieskuteczna.

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