

Monitoring T315I mutation in chronic myeloid leukemia by amplification refractory mutation system PCR

Monitorizarea mutației T315I în leucemia mieloidă cronică prin ARMS (*amplification refractory mutation system*) PCR

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Abstract

The introduction of imatinib mesylate has revolutionized the treatment of chronic myeloid leukemia. Even though experience with imatinib mesylate is limited, clinical resistance has already been observed. In order to overcome imatinib resistance, more potent tyrosine kinase inhibitors such as nilotinib and dasatinib have been developed, with demonstrable activity against most BCR-ABL domain mutations with the notable exception of T315I mutation. Moreover, with the increased use of newer tyrosine kinase inhibitors it has been suggested that the spectrum of mutations may change, possibly selecting the pan-resistant T315I. That is why mutational analysis should be done on a regular basis or at any time the clinical and hematological aspects change, in order to give the patient the best therapy available.

Key words: imatinib, tyrosine kinase, mutation, T315I

Rezumat

Introducerea imatinib mesylate a revoluționat tratamentul leucemiei mieloide cronice. Deși experiența cu imatinib mesylate este limitată, deja a fost observată rezistența la tratament. Pentru a putea depăși această rezistență, s-au sintetizat inhibitori de tirozin kinază mai potenți, cum sunt nilotinib și dasatinib, cu activitate demonstrată împotriva majorității mutațiilor BCR-ABL, cu excepția mutației T315I. Mai mult, se crede că utilizarea noilor inhibitori de tirozin kinază ar putea determina schimbarea spectrului mutațiilor, posibil cu selectarea mutației pan-rezistente T315I. Din aceste motive, analiza mutațională trebuie efectuată regulat sau în orice moment în care aspectele clinic și hematologic se modifică, pentru a putea oferi pacientului cea mai bună terapie.

Cuvinte cheie: imatinib, tirozin kinaza, mutație, T315I

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Introduction

Chronic myeloid leukemia (CML) is a chronic myeloproliferative disorder characterized by clonal proliferation of hematopoietic stem cells, by the presence of a quasi specific abnormality - the Philadelphia chromosome or its molecular counterpart, the BCR-ABL fusion gene - and by a two phase evolution pattern^{4,12}.

After the initial description of CML more than 150 years ago, little progress has been made in its treatment until the recognition of the tyrosine kinase activity of the BCR-ABL protein which led to the discovery of a new series of molecules targeted against BCR-ABL protein, with tyrosine kinase (TK) inhibitory effect¹.

Tyrosine kinase inhibitors (TKI) and mechanisms of resistance to TKI

One of the TK inhibitors- imatinib mesylate has a high and relatively specific biochemical activity, acceptable toxicity profile and was thus rapidly introduced in clinical practice¹. The introduction of imatinib mesylate for the treatment of CML has improved patient outcome and management of this disease, being at present the standard of care for the initial therapy of patient with CML¹⁰. The IRIS trial, comparing imatinib with the previous therapeutic golden standard alpha-interferon, showed that in imatinib-treated patients the rate of complete hematological remissions was 98% and the rate of complete cytogenetic remission was 87% after a 5 year follow-up¹⁰. Unfortunately, some of the patients treated with imatinib with an initial response, relapsed and patients in advanced phases CML had less favorable results (suboptimal or failure), all of these resulted in an intensive search for resistance mechanisms. Two other TKI, dasatinib and nilotinib, have currently been approved for CML patients failing imatinib therapy, and are able to induce hematological, cytogenetic and molecular remissions in most patients resistant to imatinib. However, it

is still unclear whether the responses to second generation TKIs are durable. There is also a subset of patients who do not respond to these agents^{11,14}.

The mechanisms of resistance indentified so far include: ABL kinase domain mutations interfering with imatinib binding, overexpression and amplification of BCR-ABL, clonal evolution and decreased imatinib bioavailability and cell exposure^{1,5,6}. Among them, point mutation in the BCR-ABL kinase domain appear to be the most common, occurring in 30% to 90% patients who develop resistance. Mutations in more than 40 different amino acids have been described, conferring different levels of resistance^{3,10}.

It is now accepted that the expansion of the clone carrying ABL kinase mutations may be associated with resistance to imatinib and in some cases precedes the progression to advanced phases of CML⁷.

The initial controversy on whether these mutations are induced or selected by TK inhibitory molecules has found its solution in some reports showing that few mutated cells can be observed in imatinib naïve patients¹⁰. Some studies showed that cells that were less sensitive to imatinib *in vitro* harbored BCR-ABL mutations *in vivo*, and more, they would rapidly develop mutations under the pressure of imatinib¹. Overall frequency of mutation appears to increase with sequential use of TK inhibitors³.

The kinase domain mutations should be identified as early as possible because they may indicate the need to change the therapeutic strategy⁷.

There is currently no universally accepted consensus on when patients should be screened for kinase domain mutations, which technique should be used and how the data should be reported⁷.

The only current recommendations available include mutational analysis in all patients presenting in advanced phase and for

those in chronic phase immediately in any case of treatment failure or suboptimal response¹. This would include patients who fail to achieve complete hematologic response at 3 months, minimal cytogenetic response at 6 months, major cytogenetic response at 12 months⁷. There is no evidence whether patients in chronic phase with a high Sokal or Hasford score should be screened more frequently than others.

Mutational analysis is important because not all mutations have the same biochemical and clinical properties. While the biochemical resistance of some mutations can be overcome by dose increase, while some mutations are functionally irrelevant, other P-loop mutations and T315I mutation confer a very high level of resistance¹. Among the P-loop mutations, the most resistant are Y253F, Y253H, E255V, E255K and T315I with biochemical imatinib IC₅₀>5000 nM and some of them with cellular IC₅₀ >10000 nM (IC₅₀ indicates the concentration that inhibits by 50% the biochemical TK activity of *BCR-ABL* and suppresses by 50% the growth of Ph¹ cell lines). In the catalytic domain, F359V is the most resistant mutation to imatinib. Of note, the mean trough plasma level of imatinib in patients treated with 400 mg daily is 1460 nM¹.

The T315I mutation: methods of detection and prognostic significance

Despite the fact that there are more than 40 different ABL mutations described to date, only T315I mutation (substitution of threonine to isoleucine at residue 315 in the heart of imatinib binding pocket) is associated to resistance to all available TK inhibitors⁵, so it is of greatest interest to diagnose, from monitoring and prognostic point of view.

The amplification refractory mutation system PCR (ARMS-PCR) technique is suitable for rapid and routine screening of T315I and with a satisfying cost/ effectiveness value, especially where other more sophisticated techniques would be more difficult to perform.

This technique, which sometimes goes by under alternative names, such as allele-specific PCR (AS-PCR) or sequence-specific PCR, makes use of three primers: a primer wild-type allele specific, a primer mutant-allele specific and a common primer, respectively. The primers wild-type and mutant allele specific usually differ by only one nucleotide, which gives them a high specificity to the corresponding wild-type or mutant allele. In order to obtain a non-competitive PCR reaction, the 2 allele-specific primers are combined with the common primer in separate reaction tubes.

Roche-Lestienne et al¹³ developed an ARMS-PCR for studying the most common ABL gene mutation conferring resistance to the first and second generation TK inhibitors, which is T315I. In order to determine the sensitivity of their assay, the authors made serial dilutions of 100ng DNA from imatinib-resistant patients found positive for T315I mutation and from healthy individuals. A signal corresponding to the mutant-allele was observed even after a 10.000 fold dilution, that is, assuming that 100ng DNA used for each PCR reaction represents the DNA from around 15.000 nucleated cells, the ARMS-PCR technique is able to detect 1.5 T315I mutant cells out 15.000 wild-type cells. Meanwhile, the strong specificity of the method was demonstrated by the constant lack of amplification of mutant-allele specific fragment in healthy individuals who served as controls for the study¹³.

Taken in consideration the high sensitivity of the ARMS-PCR technique, it can be used periodically during the treatment as a screening test for T315I mutation in all the CML patients resistant to TK inhibitors treatment⁸. However, T315I mutation has been found in a subset of CML patients before the initialisation of treatment with TK inhibitors, suggesting that in some cases, this mutation is pre-existing to therapy, which creates a clonal selection of the minor population of mutant cells⁶.

None of the multiple second generation ABL inhibitors available is active against T315I mutation. Recent clinical trials with MK-0457, an Aurora kinase inhibitor with activity against T315I are currently on hold due to concerns about cardio toxicity⁵.

Several others compounds active against T315I are currently in preclinical development: SGX393, AP24534, XL228, DCC-2036. Their use in the management of CML, at which moment of the disease evolution and whether alone or in combination is not clear yet. However, they are clearly needed for all the patients harboring T315I, who currently have limited alternative options⁵.

Conclusion

Under these circumstances, the presence of T315I is an indication for allogeneic bone marrow transplantation, despite the high rates of complications and mortality of the procedure. Periodic analysis of the T315I mutation should be mandatory in all patients receiving TKI treatment since it is a relatively simple and non-expensive procedure. Its early detection would be of great importance in the timely decision to start preparations of allogeneic stem cell transplantation or to include the patient in clinical trials with new molecules.

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