Artigo / Article

Frequency of the *BCR/ABL* rearrangements and associated alterations detected by FISH during monitoring of patients taking imatinib mesylate in isolation

Freqüência do rearranjo BCR/ABL e alterações associadas detectadas por FISH no monitoramento de pacientes em uso exclusivo do mesilato de imatinibe

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*The introduction of imatinib mesylate as treatment of chronic myelogenous leukemia has saved many patients, but the success of therapy is hampered by resistance and possible non-destruction of the malignant clone. This article describes the cytogenetic responses and abnormal cytogenetic patterns involving the ABL and BCR genes detected by FISH in patients who use exclusively imatinib. The results showed that other alterations involving the BCR and ABL genes do not seem to be related to resistance to the drug as they occur in low frequencies and can not be associated to the cytogenetic response or to the time of treatment. Moreover, the response to imatinib seems to be individual and unpredictable, independent of the time of treatment and of its initiation after diagnosis. Rev. bras. hematol. hemoter. 2006;**28**(2):115-119.*

Key words: *Chronic myelogenous leukemia; imatinib; FISH.*

Introduction

Chronic myelogenous leukemia (CML) is a malignant myeloproliferative disorder originating from a pluripotent hematopoietic stem cell that acquires a Philadelphia (Ph) chromosome encoding the *BCR/ABL* oncogeneic fusion protein, acting tyrosine kinase, essential for leukemic transformation.[1]

The natural course of the disease is usually characterized by three sequential phases, chronic, accelerated and blast crisis, during which progressive resistance to therapy can be acquired.[2]

Recently the treatment of CML has been revolutionized by the introduction of imatinib mesylate. This is the treatment of choice for the chronic phase of the disease and is used in CML as the first therapeutic option after diagnosis in cases where other treatments failed or in cases where it is not possible to perform bone marrow transplantation.[3] Imatinib stabilizes an inactive conformation of the *BCR-ABL* protein and related kinase and suppresses the growth of *BCR-ABL* expressing CML progenitor cells by the blockade of the ATP-binding site of the kinase domain of the protein.[4,5,6]

The success of imatinib is hampered by acquired resistance, which occurs over months to years as a result of the selection of a subclone bearing mutations in the kinase domain.[7]

This outcome confirms the molecular basis for the success of imatinib, but also points to issues that will likely emerge with longer term use.[8] In some patients clonal cytogenetic aberrations can appear including an escape strategy of leukemic clones from the action of the drug.[9]

Frequent periodic monitoring of a patient's response to therapy has become an essential component of disease evaluation, allowing possible alterations to drug dose schedules, addition of other agents, or the change to other

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therapies, including allogeneic bone marrow transplantation.[10]

This article describes the frequencies of *BCR-ABL* (cytogenetic responses) and of abnormal cytogenetic patterns involving *ABL* and *BCR* genes detected by Fluorescence *in situ* Hybridization (FISH) in interphasic nuclei of leukemic cells in bone marrow of Brazilian patients with CML exclusively taking imatinib mesylate.

Materials and Methods

Cells aspirated from bone marrow of 28 patients with CML were collected at different intervals of from 5 to 34 months after the beginning of treatment with imatinib, according to doctor's requests. All the patients had the *BCR/ABL* rearrangement detected at diagnosis and were being treated continuously and exclusively with imatinib mesylate.

The cells were left for 24 hours in a non-stimulated culture in RPMI1640 medium with 20% fetal cow serum. FISH assays were performed with the DNA dual-color, dual fusion probes for *BCR-ABL* (LSI/BCR labeled in SpectrumGreen and LSI/ABL labeled in SpectrumOrange, Vysis). The protocols for hybridization and detection were made according to Estécio *et al* (2002)[11] and the manufacturer's instructions. At least 800 cells were analyzed per patient per test and the data were described according to the International System for Human Cytogenetic Nomenclature (ISCN 1995).[12] As controls, cells from lymphocyte cultures of healthy individuals were utilized and none presented with the two established signals. Cases with *BCR/ABL* rearrangement frequencies of over 0.5% were considered positive.[13] The evaluation of the cytogenetic response followed the criteria described by TBAKHI (2002):14 complete, defined as non-detectable Ph+/*BCR-ABL*+ cells; partial, in which there are 1-34% of Ph+/*BCR-ABL*+ cells; minor, in which there are 35-94% of Ph+/*BCR-ABL*+ cells and no response in which more than 95% of Ph+/*BCR-ABL*+ cells are present in bone marrow. The cases with <35% of Ph+/*BCR-ABL*+ cells were also considered as major response, defined as the sum of complete and partial responses.

Results

Table 1 shows the relative frequency of the *BCR/ABL* rearrangement and of other alterations observed in each case, in different periods after the beginning of therapy using imatinib. With the exception of case 25 in blast crisis, the others were in the chronic phase of the disease when they started imatinib treatment.

The time of treatment with imatinib and the period from diagnosis until the start of treatment was very heterogeneous in the studied series. This variation made the observation irregularities in the cytogenetic response to

Table 1
Relative frequency of the *BCR/ABL* rearrangement and of other alterations observed in each case

Case	DF	TDT	TTI	*BCR-ABL* %	Other alterations %
1	CP	39	10	51.0	7.0
2	CP	15	8	0	0
			9	0	0
			20	0	2;0
3	CP	16	7	80.0	0
			17	31.0	0
			24	52.0	0
			34	9.0	0
4	CP	16	12	1.0	1.5
5	CP	28	12	0	0
			27	0	0
6	CP	31	10	0	0
			17	0	0
7	CP	66	14	66.0	2.0
8	CP	72	7	89.0	0
9	CP	43	12	5.0	0
10	CP	58	15	1.0	10.5
11	CP	20	11	31.0	0
12	CP	6	7	4.0	0
13	CP	4	12	1.0	0
			16	1.0	0
14	CP	3	6	0	0
			14	0	0
15	CP	21	15	56.0	0
16	CP	3	5	4.0	0
			15	60.0	0
17	CP	41	26	0	0
			31	0	0
18	CP	3	8	26.0	0
19	CP	5	16	3.0	0
			27	27.0	0
20	CP	2	6	0	0
			11	0	0
21	CP	2	5	3.0	0.5
22	CP	2	5	0	0
23	CP	2	6	0	0
24	CP	2	6	6.0	0
25	BC	35	15	73.5	0
26	CP	40	8	23.0	0
27	CP	48	13	8.0	0
28	CP	14	12	1.0	0

Phase of disease at the beginning of treatment (DF); time (months) from diagnosis of CML until the start of treatment (TDT); time (months) of treatment with imatinib (TTI), CP= Chronic phase; BC= Blast crisis

the drug possible reflecting individual and unexpected responses.

For ten patients, cytogenetic investigations were made more than once and in eight of them the frequencies obtained after variable periods of treatment were zero, low or progressively reduced, suggesting a good therapeutic response. Two patients presented with progressively higher rearrangement frequencies, suggesting worsening of the disease.

In eight (28.6%) patients no *BCR*/*ABL* rearrangement was identified, suggesting complete cytogenetic response. In case 2, even though the patient achieved complete cytogenetic response at eight and nine months after treatment with imatinib, at 20 months the patient presented with 2% of cells with another previously non-detected alteration.

In 15 (53.6%) cases the frequency of the rearrangement was low (1% to 31%) indicating partial cytogenetic response, observed in a period varying from 5 to 34 months of treatment. Seven (25%) patients presented high frequencies of the rearrangement (35% to 94%), that is a minor response, obtained from 7 to 24 months of treatment. Cases 3 and 16 presented with variable cytogenetic responses and were included in the two last categories. However, a major cytogenetic response was observed in 82.2% of the patients.

Six cases (21.4%) showed the *BCR*/*ABL* re-arrangement associated with other alterations, such as additional simple fusions and extra copies of *ABL* or *BCR* in non-polyploid cells not present at diagnosis, suggesting clonal evolution of the disease with involvement of the same genes and chromosomes. Case 2 (3.6%) presented with an extra copy of the *BCR* gene without the presence of the typical rearrangement of the disease. Table 2 shows the types and frequencies of the other alterations found.

Table 2
Description and frequency of other alterations involving the *BC*R and/or *ABL* genes

Case	Alteration	%
1	FFFRG/FFFFRG	7.0
2	GGG	2.0
4	FRG	1.5
7	FRG	2.0
10	FRG FRRG	6.5 4.0
21	GGG	0.5

F = fusion, R = red (*ABL* gene), G = green *(BCR* gene)

Discussion

Imatinib mesylate has given efficient results in all phases of LMC, although the responses are more significant and lasting in patients in the chronic phase.[15] Compared to other treatment methods, cytogenic responses with imatinib occur much more rapidly, in many cases within three and six months of treatment.[16] In the cases studied here, patients in the chronic phase had varying responses to the drug which were not associated to the time of treatment. The patient in the blast crisis phase did not improve with imatinib.

A shorter interval between CML diagnosis and the initiation of imatinib is predictive of a better molecular response to therapy.[17] Eight cases of this study took imatinib shortly after diagnosis (from 2 to 3 months) and four of these (14, 20, 22 and 23) presented complete cytogenetic responses in at least the first six months of the treatment. However, another three (18, 21 and 24) achieved partial responses and the other patient (16) had partial and later minor response at different times of treatment.

Really, it is not clear if the resulting cytogenetic remissions with the use of the drug are lasting or if the mesylate imatinib can eliminate the malignant primitive progenitors from which the disease arises.[18]

The origin of CML begins in a hematopoietic stem cell, a target population that is largely quiescent, which either does not express *BCR-ABL* or is unaffected by the BCR-ABL expression. Quiescent Ph+ stem cells are insensitive to mesylate imatinib treatment in vitro. This suggests that the Ph+ cells may not be eliminated by mesylate imatinib treatment in CML patients. Long-term mesylate imatinib mono-therapy may be required to continually suppress leukemic development but this may lead to the generation and selection of *BCR-ABL* resistant escape mutants.[9]

Results for the chronic phase of CML obtained during efficacy and safety studies are outstanding, but few data are available about long-term effects.[19] Some authors described cytogenetic relapse after the interruption of the treatment with imatinib. Mauro *et al*.[20] reported two cases of CML in which imatinib therapy, which sustained complete cytogenetic response, was ceased after 14 months and the disease relapsed. Usuki *et al*.[21] described a case in which complete cytogenetic response was maintained for 24 months with imatinib, followed by cytogenetic relapse after cessation of imatinib therapy. In the cases studied here, the patients who achieved complete cytogenetic response, sustained for periods varying from 5 to 31 months, were all maintained in therapy exactly because of the possibility of relapse after the interruption of the treatment.

Most patients in the chronic phase maintain lasting responses however, many in blast crisis fail to respond and the evolution of the disease is quick as was observed in this study. When CML progresses to "blast" crisis, cytogenetic changes in addition to the Ph chromosome are often evident as can explain the less efficacious effects of imatinib.[8]

Patients who do not present cytogenetic responses after six months of therapy are generally defined as having

primary resistance to imatinib and are likely to present mutations in *ABL*.[22] Kinase domain mutations are the most commonly identified mechanism associated with relapse. Many of these mutations decrease the sensitivity of *ABL* kinase to imatinib, thus accounting for resistance to imatinib.[5,15] This was not evaluated in this study, nor was the presence of other cytogenetic alterations without the involvement of the 9 and 22 chromosomes.

In several cases the appearance of other alterations of clonal chromosomal abnormalities after therapy using imatinib have also been reported, but their incidence, etiology and prognosis remain unclear.[23] Our patients who presented with other alterations involving the *BCR* and *ABL* genes do not seem to have developed them as a result of resistance to imatinib as, apart from the alterations occurring in low frequencies, they were not be correlated to the cytogenetic response or to the time of treatment using the drug.

Deletions of der(9) have been recognized in 9% to 15% of patients with CML over recent years. These deletions are usually large, involving several megabases and are thought to occur at the time of the Ph chromosome translocation rather than during disease progression.[24] Alterations, as observed in case H14 (one fusion, two red signals and one green signal) have been described as resulting from the microinsertion of *ABL* in the *BCR*, of complex rearrangements including deletion of adjacent flanking sequences and of complex rearrangements involving other chromosomes.[25] However, a single fusion can be related to deletions from derivative chromosome 9.[26] The presence or absence of alterations do not change the response to imatinib. The alterations observed in the other cases that presented fusions, a green signal and a red signal could be related to the loss of one of the chromosomes derived from the translocation.

In spite of the Brazilian series being small, the frequencies of the cytogenetic response to imatinib mesylate were equal as previously described by other authors.[18,27] This response to imatinib, which was good in the majority of the cases, seems to be individual and unpredictable, independently of the phase, the initiation and time of treatment using the drug.

Resumo

*A introdução do mesilato de imatinibe como tratamento da leucemia mielóide crônica tem salvado muitos pacientes, mas o sucesso da terapia tem sido prejudicado pela resistência e possível não destruição do clone maligno. Este artigo descreve a resposta citogenética e padrões citogenéticos anormais envolvendo os genes ABL e BCR detectados por FISH em pacientes em uso exclusivo de imatinibe. Os resultados mostraram que outras alterações envolvendo os genes BCR e ABL não parecem estar relacionadas à resistência à droga, elas ocorrem em baixas freqüências e podem não estar associadas à resposta citogenética ou ao tempo de tratamento. Contudo, a resposta ao imatinibe parece ser individual e imprevisível, independente do tempo e do início do tratamento após o diagnóstico. Rev. bras. hematol. hemoter. 2006;**28**(2):115-119.*

Palavras-chave: *Leucemia mielóide crônica; imatinibe; FISH.*

References

1. Latagliata R, Breccia M, Carmosino I, Sarlo C, Montefusco E, Mancini M, *et al*. Elderly patients with Ph+ chronic myelogenous leukemia (CML): results of imatinib mesylate treatment. Leuk Res 2005;29(3):287-291.
2. Petzer AL, Gunsilius E. Hematopoietic stem cells in chronic myeloid leukemia. Arch Med Res 2003;34(6):496-506.
3. Calabretta B, Perrotti D. The biology of CML blast crisis. Blood 2004; 103(11):4010-22.
4. Borthakur G, Cortes JE. Imatinib mesylate in the treatment of chronic myelogenous leukemia. Int J Hematol 2004; 79(5):411-19.
5. Deininger M, Buchdunger E, Druker BJ. The development of imatinib as a therapeutic agent for chronic myeloid leukemia. Blood 2005; 105(7):2640-53.
6. Dewar AL, Cambareri AC, Zannettino AC, Miller BL, Doherty KV, Hughes TP, *et al*. Macrophage colony stimulating factor receptor, c-fms, is a novel target of imatinib. Blood. 2005;105(8):3.127-32.
7. Burgess MR, Skaggs BJ, Shah NP, Lee FY, Sawyers C. Comparative analysis of two clinically active *BCR-ABL* kinase inhibitors reveals the role of conformation-specific binding in resistance PNAS . Proc Natl Acad Sci USA 2005;102(9):3.395-400.
8. Sausville EA. Imatinib for chronic myelogenous leukaemia: a 9 or 24 carat gold standard? Lancet 2003;361(9367):1.400-1.
9. Wong S, Witte ON. The *BCR-ABL* story: Bench to bedside and back. Annu Rev Immunol 2004;22:247-306.
10. Kantarjian HM, Talpaz M, Cortes J, O'Brien S, Faderl S, Thomas D. Quantitative polymerase chain reaction monitoring of BCR-ABL during therapy with imatinib mesylate (STI571; Gleevec) in chronic-phase chronic myelogenous leukemia. Clin Cancer Res 2003;9:160-166.
11. Estécio MRH, Fett-Conte AC, Varella-Garcia M, Fridman CC, Silva AE. Molecular and cytogenetic analyses on Brazilian youths with pervasive developmental disorders. J Autism Dev Disord 2002;32:35-42.
12. ISCN: An international system for human cytogenetic nomenclature. Mitelman, F. ed. Karger, Basel, 114p., 1995.
13. Aoun P, Wiggins M, Pickering D, Foran J, Rasheed H, Pavletic Sz, *et al*. Interphase fluorescence in situ hybridization studies for the detection of 9q34 deletions in chronic myelogenous leukemia: a practical approach to clinical diagnosis. Cancer Genet Cytogenet 2004;154(2):138-43.
14. Tbakhi A. Qualitative and quantitative detection of residual disease in chronic myelogenous leukaemia. Histopathology 2002;41(2):83-87.
15. Corbin AS, La Rosee P, Stoffregen EP, Druker BJ, Deininger MW. Several *BCR-ABL* kinase domain mutants associated with imatinib mesylate resistance remain sensitive to imatinib. Blood 2003;101(11):4611-14.
16. Deininger MWN. Cytogenetic studies in patients on imatinib. Sem Hematol 2003;40(2):50-55.
17. Roche-Lestienne C, Darré S, Lai JL, Bacón T. Factors predicting molecular and cytogenetic response in chronic myeloid leukemia patients treated with imatinib. Haematologica 2005;90: 131-3.
18. Usui N. Imatinib therapy for patients with chronic myelogenous leukemia. Gan To Kagaku Ryoho 2005;32(3):397-403.
19. Gozzetti A, Tozzuoli D, Crupi R, Fanelli A, Gentili S, Bocchia M, *et al*. A novel t(6;7)(p24;q21) in a chronic myelocytic leukemia in complete cytogenetic remission after therapy with imatinib mesylate. Cancer Genet Cytogenet 2004;148:152-4.

20. Mauro MJ, Druker BJ, Maziarz RT. Divergent clinical outcome in two CML patients who discontinued imatinib therapy after achieving a molecular remission. Leuk Res 2004;28:71-3.

21. Usuki K, Tijima K, Iki S, Urabe A. CML cytogenetic relapse after cessation of imatinib therapy. Leuk Res 2005;29(2):237-8.

22. Agirre X, Fontalba A, Andreu EJ, Odero MD, Larrayoz MJ, Montiel C. Lack of *BCR/ABL* point mutations in chronic myeloid leukemia patients in chronic phase before imatinib treatment is not predictive of response. Haematologica 2003;88(12):1425-6.

23. Terre C, Eclache V, Rousselot P, Imbert M, Charrin C, Gervais C, *et al*. Report of 34 patients with clonal chromosomal abnormalities in Philadelphia-negative cells during imatinib treatment of Philadelphia-positive chronic myeloid leukemia. Leukemia 2004;18(8):1340-46.

24. Quintas-Cardama A, Kantarjian H, Talpaz M, O'brien S, Garcia-Manero G, Verstovsek S, *et al*. Imatinib mesylate therapy may overcome the poor prognostic significance of deletions of derivative chromosome 9 in patients with chronic myelogenous leukemia. Blood 2005;105(6): 2281-6.

25. Haigh S, Cuthbert G. Fluorescence in situ hybridization characterization of different cryptic *BCR-ABL* rearrangements in chronic myeloid leukemia. Cancer Genet Cytogenet 2004;155(2):132-7.

26. Robinson HM, Martineau M, Harris RL, Barber KE, Jalali GR, Moorman AV, *et al*. Derivative chromosome 9 deletions are a significant feature of childhood Philadelphia chromosome positive acute lymphoblast leukaemia. Leukemia 2005;19(4):564-71.

27. Turkina AG, Khprpshko ND, Druzhkova GA, Zingerman BV, Zakharova ES, Chelysheva EIU, *et al*. Therapeutic efficacy of imatinib mesylate (Glivec) in chronic phase of myeloid leukemia. Ter Arkh 2003; 75(8):62-7.

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