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SHORT COMMUNICATION

Sequential development of mutant clones in an imatinib resistant chronic myeloid leukaemia patient following sequential treatment with multiple tyrosine kinase inhibitors: an emerging problem?

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Abstract With the increasing use of new tyrosine kinase inhibitors it has been suggested that the spectrum of kinase domain mutations may change and possible selection of new resistant clones may occur. We describe a Ph + chronic myeloid leukaemia (CML) patient with primary resistance to imatinib who received without success sequential therapy with multiple TKIs, and developed sequential emergence of kinase domain mutations after these treatments.

Keywords Chronic myeloid leukaemia · Resistance · Tyrosine kinase inhibitors · Mutations

Introduction

The 5-year results of the IRIS study provided an indication of the prevalence of imatinib resistance in Ph + chronic myeloid leukaemia patients treated with this drug. Imatinib treatment was discontinued in 31% of patients, with an estimated 14% of the entire cohort showing resistance [1]. Several mechanisms contribute to imatinib resistance, including BCR–ABL mutations, increased expression of BCR–ABL through gene amplification, decreased intracellular drug concentrations caused by drug efflux proteins (such as OCT1), clonal evolution, and over-expression of Src kinases involved in BCR–ABL-independent activation of alternative pathways, such as Lyn and Hck [2]. However, 40% of resistance is attributed to the emergence of clones expressing mutated forms of BCR–ABL, with amino acid substitutions in the ABL-kinase domain that impair imatinib binding [3]. To overcome imatinib resistance, more potent tyrosine kinase inhibitors (TKIs) such as nilotinib, dasatinib and bosutinib have been developed with activity against most of the BCR/ABL mutations, with the notable exception of the T315I mutation [4]. With the increased use of these newer drugs, it has also been suggested that the spectrum of the kinase mutations may change [5]. We describe here a Ph + CML patient with persisting primary resistance to all the TKIs used in sequence, who dynamically developed sequential mutant clones with disappearance of those previously detected.

Case report

A 55-year-old woman was referred to our Institute in 1996 because of leukocytosis (75×10^9/l), thrombocytosis (716×10^9/l) and splenomegaly. Bone marrow analysis showed an hyperplasia of granuloblastic cell population without blasts, and cytogenetic analysis revealed a standard t(9;22) translocation in all the analysed metaphases. Molecular RT-PCR analysis detected the b3a2 type of transcript thus confirming the diagnosis of chronic myeloid leukaemia in chronic phase, intermediate Sokal risk. After an initial phase of cytoreduction with hydroxyurea, the patient was started on alpha-interferon (INF) at the maximum

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tolerated dose of 9 MUI/day. Several limiting side effects, such as bone and abdominal pain, tiredness and depressive syndrome, were recorded during this treatment. A stable complete haematological response was not reached (platelets >500 $\times$ 10^9/l and spleen still appreciable) and the drug was discontinued in March 1997, with only cytoreductive therapy being continued. With the availability of imatinib in 2000, the patient was started on this drug, at the standard dose of 400 mg/day, in November of the same year. A complete haematological response (CHR) was obtained in 1 month. During imatinib treatment, skin rush and fluid retention were recorded, grade 1 according to WHO. The patient did not reach cytogenetic response and lost CHR in December 2002. From August 2003 to September 2005 the patient was treated without success with combination of high dose imatinib (600 mg/day) and hydroxyurea, but cytogenetic analysis displayed the persistence of Ph chromosome in all the analysed metaphases. At that time a mutational analysis with denaturing-high performance liquid chromatography (DHPLC) showed the presence of M244V mutation of p-loop site of kinase domain. The methodology used for mutation detection was the following: after RNA extraction and reverse transcription, overlapping fragments covering the entire kinase domain were generated by nested PCR and screened by DHPLC. In positive cases, a direct sequencing was performed.

Nilotinib was started at the dose of 400 mg twice a day in October 2005, and CHR was obtained after 1 month of therapy. Impaired glucose tolerance and ocular toxicity were recorded and only a minor cytogenetic response was obtained after 7 months of therapy. After 13 months of therapy a progression of disease to accelerated phase was detected and a second mutational screening analysis performed at that time revealed the absence of M244 V and the appearance of M351T mutation.

In December 2006, dasatinib was started at compassionate use at the dose of 70 mg twice a day. The patient experienced several episodes of haematological toxicity of grade 3 and different non- haematological side effects (nausea, diarrhoea, skin rush and fever) that required a reduction of the dose to 50 mg/day. Patient returned to CP of disease but did not obtain any cytogenetic response. After 14 months of therapy, a third mutational analysis was performed which revealed the disappearance of M351T mutation and the acquisition of a new F317L mutation. After 3 months from detection of the last mutant clone, patient lost CHR and progressed again to accelerated phase. Imatinib was restarted at the dose of 400 mg/day, with the programme of carrying out a sequential therapy with alternating imatinib/nilotinib and imatinib/dasatinib schedule. Unfortunately, after 1 month of therapy the patient experienced electrocardiographic alterations with sub-endocardic ischaemia that led to a definitive suspension of the tyrosine kinase inhibitors. At the present time, the patient is on cytoreductive and supportive care in CP of her disease.

Discussion

Acquisition of kinase domain mutations is the most frequently identified mechanism of resistance to imatinib and was recently reported especially in patients with clinically resistant disease after receiving a second or a third line therapy with TKIs. Cortes et al. [5] described the development of new different mutations in 29 patients resistant to a second line TKI and found that mutations persisting or developing following switch to new TKI were at sites also found in prior in vitro mutagenesis assays. In our case, which was resistant to high dose imatinib, DHPLC revealed a M244V mutation: the prognostic significance of this mutation is still uncertain. According to Jabbour et al. [6] this mutation is part of p-loop region and has no definite significance on overall survival, whereas according to the Adelaide group [7] the mutation occurs outside the p-loop region and is usually relatively innocuous. In the recent study reported by Khorashad et al. [8], this type of mutation was very frequent also in patients who reached complete cytogenetic response and did not have any adverse effect on progression free survival (PFS). In our case, following nilotinib resistance development, DHPLC detected the regression of the KD mutation present before the start of therapy and revealed a new M351T mutation; differently from our observation, Cortes et al. [5] reported that this type of KD mutation was usually lost, and not acquired with a second line TKI. Furthermore, the appearance of M351T mutation in our case was accompanied by disease progression. O'Hare et al. [9] reported the occurrence of mutations in BAF3 cell line in vitro and described that there are few differences in mutants sensitivity to imatinib and nilotinib, particularly for M351T: due to structural differences between these two drugs, this residue comes in close proximity to imatinib, but is less critical for coordinating binding of nilotinib. We observed this mutation, which occur rarely under nilotinib, after 13 months of treatment. Dasatinib therapy allowed complete regression of M351T and of disease accelerated phase, but again drug resistance and appearance of a third F317L mutation were noted. A recent observation showed that the emergence or pre-existence of F317L mutation may be associated with resistance to dasatinib: in fact, amino acid substitutions in the side chain of phenylalanine 317 directly interact with the pyrimidine and thiazole rings of dasatinib and F317L has been shown to induce a 9-to 13.5-fold increase of dasatinib IC_{50} [10]. Also in our patient, the presence of this mutation was associated with resistance to dasatinib. In accordance with our observation are the results of a study by Bradeen et al. who compared the incidence and type of mutations

emerging in *N*-ethyl-*N*-nitrosourea exposed p210 positive cells after imatinib, dasatinib and nilotinib. These authors found that F317L mutation had an incidence of 2% after imatinib treatment, of 10% after dasatinib treatment and of 0% after nilotinib at a wide range of concentrations, implicating that F317 is a potentially vulnerable site for dasatinib, but not for nilotinib [11].

Korashad et al. [8] suggested that KD mutations should be regarded as an event clinically analogous to the development of new cytogenetic abnormalities and that they may be equivalent to clonal evolution. The identification of a KD mutation might be an indication for changing therapy and a mutational screening was suggested by the authors even in case of incomplete cytogenetic responses and in patients in CCR but with modest reductions in transcript levels, especially those with high Sokal risk at diagnosis [8]. As suggested by Shah et al. [12], secondary KD mutations may be more common in patients primarily resistant or relapsed after second line TKI thus reinforcing the idea that targeted kinases have a critical role in propagating the malignant clone. Sequential therapy in patients with primary resistance to imatinib may carry the risk of creating and/or selecting novel kinase alleles with enhanced oncogenic potency. Thus, if acceptably and safe, frontline concomitant combinations of different ABL inhibitors in TKI naïve patients are warranted which should be capable to effectively prevent BCR/ABL KD mutations as a mechanism responsible for disease resistance.

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