



The combination therapy of imatinib and dasatinib achieves long-term molecular response in two imatinib-resistant and dasatinibintolerant patients with advanced chronic myeloid leukemia

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Abstract

For patients with chronic myeloid leukemia (CML) failing imatinib therapy, second-generation tyrosine kinase inhibitors (TKIs) are recommended. Here, we describe two patients with advanced CML who failed imatinib therapy and did not tolerate the recommended dose of dasatinib, but then achieved a major molecular response with the combination of imatinib and dasatinib with no significant extramedullary toxicity. Our observations suggest that combination of TKIs may provide an additive/synergistic antileukemic effect.

Keywords: chronic myeloid leukemia, tyrosine kinase inhibitors, resistance, combined therapy

Introduction

Imatinib is the frontline therapy for chronic-phase chronic myeloid leukemia (CML) because it demonstrates high complete cytogenetic response (CCyR) and major molecular response (MMR) rates. Only a minority of patients achieve undetectable levels of *BCR-ABL* transcripts^[1]. However, a further 20%-25% of those who do achieve complete hematologic response (CHR) or CCyR, or both, may eventually stop responding and acquire resistance during exposure to imatinib^[2]. In advanced-phase CML (i.e., accelerated phase and blast crisis), 38% of the patients treated with imatinib achieved major cytogenetic response (MCR)^[3]. However, responses tend to be transient in most of the responders. Dasatinib and nilotinib are potent tyrosine kinase inhibitors (TKIs) with activity against many

imatinib-resistant CML clones. For patients with CML who become or are inherently resistant to imatinib therapy, dose escalation and the second-generation TKIs need to be considered based on the *BCR-ABL* mutation profile and the patient's disease history^[4], with the notable exception of the T315I mutation. With the increasing use of TKIs, it has been suggested that the spectrum of kinase domain mutations may change and possible selection of new resistant clones may occur. More recently, experimental and computational works suggest that any of the pair-wise cross-resistance of imatinib, dasatinib, and nilotinib could overcome and prevent resistance^[5-6]. In this case study, we report two patients showing resistance to imatinib and intolerance to recommended-dose dasatinib, who were treated on combined standard-dose imatinib and reduced-dose dasatinib with no significant extramedullary toxicity.

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Our observations confirm a very recent report showing that combination of TKIs may provide an additive/synergistic antileukemic effect.

Case report

A 40-year-old man was diagnosed with CML in August 2001. He commenced imatinib at an initial dose of 400 mg/day while the disease was progressing, with a platelet count of $1000 \times 10^9/L$, in April 2004. After 18 months of treatment with imatinib, he achieved a partial cytogenetic response (30%Ph+ metaphase) and commenced imatinib at 600 mg/day. The dynamics of *BCR-ABL* fusion clones by fluorescence *in situ* hybridization (FISH) on a peripheral blood specimen using dual probes for the *BCR* and *ABL* genes showed 0.6% in June 2006 and 36% in October 2006. Cytogenetic analysis showed 100% Ph+ metaphases in January 2007. He then commenced dasatinib at 70 mg b.i.d. for 10 days, but experienced dyspnea and a feeling of impending death. Ultrasonography showed moderate pleural and pericardial effusion. Dasatinib therapy was discontinued and the dyspnea improved significantly soon after. Although he had an HLA-matched sibling, he declined allogeneic stem cell transplantation. At that point, direct sequencing of PCR-RFLP-amplified *BCR-ABL1* products did not detect any mutation. He commenced imatinib at 800 mg/day in February 2007. Five months later, the patient lost hematologic response, showing persistent high platelet values ($>900 \times 10^9/L$), administration of dasatinib of 70 mg q.o.d and imatinib of 600 mg/day was initiated in September 2007. This resulted in a CHR and CCyR after 1 month and 8 months, respectively, and the regimen was very well tolerated with no side effects. The *BCR-ABL1/ABL1* ratio in peripheral blood decreased to less than 0.01% by RT-PCR (four log sensitivity) in July 2008 (10 months on combination imatinib with dasatinib). He remained in persistent MMR at the 40 month regular follow-up. In January 2011, the *BCR-ABL1/ABL1* ratio in peripheral blood dramatically increased to 31.1% when the *BCR-ABL* T315I mutation proved positive by DNA sequencing. The patient then received 2 cycles of treatment consisting of low-dose aclarubicin, cytarabine and G-CSF, but had a poor outcome.

The second patient was a 71-year-old man diagnosed with CML in September 2002. Following 3 years of hydroxyurea and alpha interferon therapy, he commenced imatinib at 400 mg/day and achieved a partial cytogenetic response (20%Ph+ metaphases) after 10 months. The disease progressed to the accelerated phase, which was detected by observing bone marrow morphology and cytogenetic analysis of 39% Ph+

metaphases in October 2006. Imatinib was sequentially escalated to a dose of 600 mg/day. After 3, 6, and 9 months of imatinib therapy, *BCR-ABL* fusion clones by FISH were 43%, 43.3%, and 4%, respectively. With no mutations detected, he commenced imatinib of 800 mg/day until September 2007, at which time he had failed to achieve CHR. White blood cell (WBC) count was $15.2 \times 10^9/L$ with 89% myeloid blasts in the differential. Meanwhile, bone marrow morphology and immunophenotyping showed malignant blasts positive for CD13, CD117, CD34, and CD33, without expressing other lymphoid markers. Cytogenetic analysis showed 47, XY, +8, t(9;22)(q34;q11), i(17q) in 20 metaphases. He commenced dasatinib at 70 mg b.i.d., but 3 days later exhibited dyspnea and cardiopalmus. Further examination by a computed tomography scan showed a great quantity of pleural effusion with an albumin level of 39.4 g/L. Constrained symptoms improved significantly with diuretics, thoracentesis, and temporary interruption of dasatinib. He subsequently commenced dasatinib of 70 mg once daily in October 2007. However, after 4 days of dasatinib therapy dyspnea recurred. At this point, myeloblasts decreased to 1% in the peripheral blood smear. Subsequently, the patient was started with a combination of imatinib of 600 mg/day and dasatinib of 70 mg q.o.d., with favorable toleration. A CCyR and MMR were achieved at 3 and 12 months after combined therapy, respectively. On his last assessment in August 2013 (66 months on combination of imatinib with dasatinib), *BCR-ABL* transcripts were undetectable in every 3 month exam.

Discussion

Resistance to imatinib is likely to be a multifactorial process. The predominant cause of acquired resistance to imatinib is reactivation of *BCR-ABL* kinase activity via kinase domain mutations, amplification of the *BCR-ABL* genomic locus, and is independent of *BCR-ABL*^[4]. Secondary clonal abnormalities have been observed to develop in approximately 5% of patients achieving CCyR. It has been suggested that alternative genetic aberrations synergize with *BCR-ABL* in the evolution of imatinib resistance^[7]. Although the mechanism is not clear, the disease progression may be associated with clonal evolution, which was also verified in our second patient.

The optimal treatment for patients failing imatinib treatment is imatinib dose escalation, a second-generation TKI, allogeneic stem cell transplantation, or other antileukemia agents, such as homoharringtonine^[4]. A sequential use of imatinib, followed by either dasatinib or nilotinib on an empirical basis has provided therapeutic

options for patients who presented with imatinib resistance^[8]. Dasatinib is a synthetic small-molecule inhibitor of Src-family kinases, which inhibits the binding of Abl kinase to BCR-ABL, irrespective of ABL conformation. *In vitro*, dasatinib presents 325-fold greater activity against native BCR-ABL, when compared with imatinib, and has shown efficacy against all imatinib-resistant *BCR-ABL* mutations with the exception of T315I. It is also more potent than imatinib in inhibiting nonmutated *BCR-ABL* kinase activity. Different kinase domain mutation profiles have been recovered with imatinib, dasatinib, and nilotinib in mutagenesis assays. Breccia *et al.*^[9] described a case of persisting primary resistance to three TKIs used in sequence, and dynamically developed sequential mutant clones with disappearance of those previously detected. The patient developed M244V mutation after imatinib therapy, but acquisition of a new M351T mutation and disappearance of M244V mutation after nilotinib therapy and acquisition of a new F317L mutation and disappearance of M351T after dasatinib therapy. Recently, Cortes *et al.*^[8] reported that 29 of 112 patients (26%) who received either dasatinib or nilotinib after imatinib failure developed new kinase domain mutation. Mutations that confer resistance to dasatinib, but not to imatinib, have been identified *in vitro*. For example, V299L mutation, also involving a contact point, was reported in patients who failed dasatinib, but rarely in patients with imatinib therapy. Therefore, the rational approach for the use of several TKIs is to tailor these agents to tackle specific *BCR-ABL1* mutant clones according to the individual mutations detected at each time-point during the course of therapy.

One attractive strategy for minimizing the onset of acquired drug resistance is to use a combined Abl inhibitor approach^[18]. Computational work has suggested that a combination of different drugs against CML could similarly overcome the resistance problem, even during advanced stages^[19]. Further studies using computational method have shown that combining two of imatinib, dasatinib, and nilotinib can provide an advantage over using one drug alone^[5]. However, combining three TKI drugs may not lead to any further advantage when compared with the combination of two drugs^[8]. Recently, Hiwase *et al.*^[6] found that the combination of low-dose dasatinib and nilotinib induced significantly higher cell death than that induced by each individual drug alone *in vitro*. Thus, the most potential benefit of the new therapeutical approach that is it may target a wider range of resistant clones than a single agent, and thereby prohibit or delay the onset of acquired drug resistance. Interestingly and surprisingly, imatinib works at all advanced stages of the disease

because blast crisis is usually attributed to additional genetic changes conferring BCR-ABL-independent pathways of proliferation and maturation arrest^[2]. For a combined Abl inhibitor approach to work, it is crucial that imatinib does not interfere with the ability of the Src/Abl inhibitor to access its binding site within the *BCR-ABL* kinase domain, even when coadministered with imatinib at concentrations above typical clinical levels. Furthermore, in some cases, additive antiproliferative effects are observed.

Thus, owing to the enhanced inhibitory activity, combined Abl inhibitor therapy could lead to eradication of a higher proportion of residual leukemic cells and, potentially, decreasing BCR-ABL-dependent molecular disease persistence in CML^[9]; this improves the molecular response rates in newly diagnosed patients and those with imatinib-resistant CML. Thus, if acceptable, frontline concomitant combinations of different Abl inhibitors in TKIs naïve patients are warranted, taking into consideration that the use of TKIs combinations will lead to increased toxicity in normal tissues in the clinical setting. In view of efficacy and safety, the administration of standard dose of imatinib at 600 mg once daily combined with reduced dose of dasatinib at 70 mg q.o.d. to our two patients resulted in the rapid achievement of CCyR, which is still ongoing and accompanied by MMR. To the best of our knowledge, this is the first report on the use of imatinib-dasatinib combinations for patients with imatinib-resistant CML in advanced phase.

The recommended daily dose of dasatinib for patients with chronic-phase CML is from 70 mg b.i.d. to 100 mg once daily. Dasatinib of 100 mg once daily retains the efficacy of 70 mg twice daily with less toxicity; however, survival was significantly better with dasatinib of 100 mg daily. A recent *in vitro* study by Hiwase *et al.*^[6] showed that the combination of low-dose dasatinib and nilotinib may provide an additive/synergistic antileukemic effect, which may be of particular relevance to TKI therapy and expressing ABCB1. Dasatinib is a substrate of ABCB1^[6]. Based on our *in vivo* and other experimental findings^[5-6], we speculate that a combination of imatinib and dasatinib may be synergistic, and imatinib-mediated inhibition of ABCB1 may enhance the intracellular uptake and retention (IUR) of dasatinib. This would be analogous to the described^[6] synergy between nilotinib and imatinib or dasatinib and nilotinib, which we speculated to be owing to an imatinib-mediated increase in nilotinib IUR, a nilotinib-mediated increase in dasatinib IUR^[6].

Both our patients were rescued from switching to standard-dose imatinib and reduced-dose dasatinib combination therapy without new combination-related

side effect and obtained longer-term benefit. As it has been recently pointed out in an excellent review^[4], dasatinib was efficacious and the results did not differ significantly across dasatinib doses. Intermittent target inhibition with TKIs may preserve the efficacy and reduce adverse events^[4,9]. Administering imatinib and dasatinib simultaneously produced a marked reduction in cells expressing *BCR-ABL* or some mutant except normal cell and primary CML cells expressing mutant T315I. Moreover, there does not appear to be cross-intolerance between dasatinib and imatinib^[3]. Consistent with the study of O'Hare *et al*^[10], our results showed that the combined Abl inhibitors do not exhibit increased toxicity when compared with single agents.

Based on our data, it can be postulated that imatinib affects malignant cells, while dasatinib has a critical role in inhibiting or eradicating the several subclones (mutant ABCB1-overexpressing cells, and/or resistant-imatinib malignant clone). In addition, malignant clones associated with resistance to dasatinib may be vulnerable to imatinib, thus decreasing the overall chances of resistance. Additive/synergistic antiproliferative effects from imatinib-dasatinib combination could lead to eradication of a higher proportion of residual leukemic cells. Thus, the data presented here suggest that a combined imatinib-dasatinib approach can be employed for further clinical trials in the subset of patients with TKI resistance and stem cell refractoriness, in addition to opening a field for research on the use of any of the pairwise cross-resistant combinations of these powerful agents.

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