Dasatinib: a new step in molecular target therapy

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The chimeric BCR-ABL gene, originated by the Philadelphia chromosome, encodes a fusion protein, BCR-ABL, bearing unregulated tyrosine kinase activity, the pivotal pathogenetic step of chronic myeloid leukemia (CML). Imatinib, an inhibitor of the BCR-ABL tyrosine kinase, significantly improves the outcome of patients with CML. Although the majority of CML patients are responsive to imatinib, a subset of patients loses the response and some progress to accelerated- or blast-phase CML. The understanding of mechanisms of imatinib resistance has led to the development of novel BCR-ABL inhibitors; among these, dasatinib emerged as the most promising, being approximately 300-fold more potent than imatinib; it also inhibits SRC family kinases. Preliminary data, after the introduction of dasatinib in clinical trials, in patients with CML, suggest that this drug is safe and well tolerated; furthermore, the majority of patients with imatinib-resistant disease achieved objective responses, although the durability of responses remains to be defined. Recently, dasatinib emerged as a potent inhibitor of imatinib-resistant protein tyrosin kinase (KIT) activation loop mutants and it is able to induce apoptosis in mast cell and leukemic cell lines expressing these mutations. The preclinical data concerning its activity on several human solid tumor lines widen new opportunities for their use outside CML.

Key words: Bcr-abl kinases, chronic myeloid leukemia, dasatinib, target therapy

Introduction

Recent progress in understanding the molecular basis of cancer enabled the development of molecular targeted therapies, which has already changed, in some cases, the outcome of the neoplastic disease. Dasatinib (BMS-354825) is a novel, oral, multi-targeted inhibitor of BCR-ABL and SRC family kinases rationally designed for the treatment of chronic myeloid leukemia (CML).

A disease model for designing new intelligent drugs

CML is characterized by the presence of a constitutively activated form of the ABL tyrosine kinase that has been linked to malignant transformation [1]. The BCR-ABL chimeric gene arises from a t(9;22)(q34;q11) translocation producing the Philadelphia chromosome [2]. The translocation involves the ABL proto-oncogene, normally on chromosome 9, and a previously unknown gene on chromosome 22, later termed BCR for breakpoint cluster region. The deregulated Ab1 tyrosine kinase activity, consequent to the BCR-ABL transcript, emerged as a pivotal event in the CML pathogenesis, being its constitutive tyrosine kinase activity, capable to transform hematopoietic cells both in vitro and in vivo [3]. In the late 1980’s, among the compounds with inhibitory activity against protein kinases, STI571 (imatinib mesylate) emerged as a promising compound capable to inhibit all the ABL tyrosine kinases, including BCR-ABL [4]. Imatinib also inhibited signaling of the ligand-activated platelet-derived growth factor receptor (PDGFR). Furthermore, it potently inhibited autophosphorylation of the KIT receptor upon binding of its cognate ligand, stem-cell factor, and suppresses KIT autophosphorylation in a cell line established from a patient with a gastrointestinal stromal tumor (GIST) with an activating KIT mutation. In contrast, no activity was seen in a cell line derived from human malignant mastocytosis with a mutation in the activation loop (A-loop) of the kinase (D816V) [5]. Phase I trials with imatinib began in June of 1998 and phase II studies began in late 1999 using imatinib as a single agent for all stages of CML. The drug was approved for first-line treatment of CML in the USA and Europe. Imatinib has revolutionized the treatment of chronic myelogenous leukemia: 5-year data from the pivotal International Randomized Study (IRIS) trial [6] suggest an impending plateau in progression-free survival (PFS); the natural history of CML, particularly for patients in the chronic phase, has been dramatically changed. Nevertheless, although responses in the chronic phase tend to be durable, relapse after an initial response is common in patients with more advanced disease [7].

Resistance to imatinib

There is mounting evidence that CML stem cells are not eliminated by imatinib in vivo [8]; Ph+ CD34+ cells and long-term culture-initiating cells are still detectable in responding patients, achieving the complete cytogenetic
remission. Although imatinib has shown great efficacy in the treatment of CML, resistance and intolerance represent major clinical concerns, particularly in patients with advanced disease; the estimated 2-year incidence of imatinib resistance is about 45% in accelerated phase (AP); after 4 years of imatinib treatment, 75% of CML-AP patients developed resistance. The update of the IRIS indicated that approximately 30% of the 553 imatinib-treated patients with CML-chronic phase (CP) had unsatisfactory therapeutic effect. Patients attaining complete cytogenetic response (CCR) or major cytogenetic response (MCR) by 3 months, resulted to be 94% and 87% free from progression to AP or blast crisis (AP/BC), respectively, versus 55% for the remaining patients; but for patients with advanced disease, rates of resistance and relapsing disease during imatinib are dramatically higher, occurring in 75% or more of AP patients and in about 95% of myeloid BC patients [9]. Therefore, patients with BC-CML still have poor outcome with 3–6 months of overall survival from the onset of BC; indeed, allogeneic stem-cell transplantation can induce durable remission in <10% of these patients, while imatinib yields sustained complete hematologic responses (CHR) in <15%. Primary resistance to imatinib is particularly common in BC; about 95% of patients in blast crisis developed resistance to imatinib at 4-year follow-up and the median survival was 6.9 months [10]. Imatinib resistance may be mediated by BCR-ABL point mutations (that impair the drug binding to BCR-ABL), by gene BCR-ABL amplification or by overexpression of BCR-ABL-independent pathways, e.g. SRC family kinase activation. Alternative mechanisms include overexpression of the P-glycoprotein efflux pump, dysregulation of src family of tyrosine kinases (SFK) activity and activation of other pathways [11, 12]. Therefore, overall (complete plus partial) response rates and quality and durability of responses to imatinib are dependent on the disease status; moreover, an important long-term concern is the high rate of molecular refractoriness. In a recent study only 4% of patients with CCR resulted to be negative for BCR-ABL on at least one occasion [13]. It can be easily supposed that nearly all patients treated with imatinib harbor detectable minimal residual disease and can therefore be considered to have ‘primary molecular resistance’ which is often clinically silent.

The extensive understanding of the mechanism of imatinib resistance has prompted the search for alternate Bcr-Abl inhibitors

To date, mutations at 17 different amino acid positions within the BCR-ABL kinase domain have been associated with clinical resistance to imatinib in CML patients [14]. Two promising new Bcr-Abl inhibitors for treating imatinib-resistant CML are currently being evaluated in clinical trials: the selective Abl inhibitor AMN107 (nilotinib) and the dual Src/Abl inhibitor BMS-354825 (dasatinib). AMN107 and BMS-354825 are 20-fold and 325-fold more potent than imatinib against cells expressing wild-type Bcr-Abl, respectively, and similar improvements are maintained for all imatinib-resistant mutants tested in vitro, with the exception of T315I [15]. Nilotinib was developed by rational drug design on the basis of the structure of an Abl–imatinib complex; like imatinib, nilotinib binds to the inactive Abl kinase conformation with increased affinity. Dasatinib is an inhibitor of SRC family kinases; this property allows dasatinib to overcome resistance conferred by SRC family kinase activation; moreover dasatinib binds Bcr-Abl with less stringent conformational requirements than imatinib and is able to inhibit all Bcr-Abl kinase domain mutants observed in relapsed patients with the exception of T315I. Dasatinib binds both active and inactive conformations of Abl; therefore, compared with imatinib, dasatinib exhibits increased potency but reduced selectivity. The spectrum of dasatinib inhibitory activity also includes ephrin family kinases, PDGFR-β and c-KIT [16].

**How dasatinib overcomes the resistance to imatinib**

Imatinib binds to the ATP-binding site of BCR-ABL only when the A-loop of the kinase is in the inactive or ‘closed’ conformation. One mechanism of acquired resistance to imatinib in CML is the development of mutations of the BCR-ABL A-loop, stabilizing the kinase A-loop in the active conformation, thus preventing imatinib binding. Dasatinib structure imposes less stringent conformational requirements on ABL for kinase inhibition, being capable to interact with the ATP-binding site of BCR-ABL, irrespective of the conformation of the A-loop. In biochemical assays, dasatinib showed potent ATP-competitive inhibitory activity with broad-spectrum antiproliferative activity against hematological and solid tumor cell lines. Shah et al. [14] profiled the activity of dasatinib against a panel of cell lines expressing wild-type or imatinib-resistant BCR-ABL. Dasatinib inhibited the kinase activity of 14 of 15 imatinib-resistant BCR-ABL mutants. The only imatinib-resistant BCR-ABL isoform resistant to dasatinib was the T315I mutant. Dasatinib did not inhibit growth of bone marrow progenitors, isolated from healthy volunteers, but it inhibited by 60%–80% the growth of bone marrow progenitors isolated from CML patients, with either imatinib-sensitive (nonmutant BCR-ABL) or imatinib-resistant disease. Differently from imatinib, dasatinib is not a substrate of multidrug P-glycoprotein efflux pumps; moreover, dasatinib may inhibit other downstream additional kinases [17].

**Dasatinib in CML**

**Phase I trials.** Talpaz et al. [18] tested dasatinib in 84 patients: 72 of them resistant to imatinib; dasatinib (at doses ranging from 15 to 240 mg/day) was administered in four-week treatment cycles; the main toxic effect was myelosuppression, requiring treatment interruption in about 60% of patients, but generally resolved after achieving the cytogenetic response (Table 1). Fifteen patients had pleural effusions not attributable to known causes, considered treatment related, successfully managed with diuretics, thoracentesis or pleurodesis. In seven patients, transitory grade 3–4 abnormalities in liver function tests were observed and asymptomatic hypocalcemia was noted in about 60% of patients. A CHR was achieved in 37 of
40 patients with CP-CML, and major hematologic responses were seen in 31 of 44 patients with AP-CML, BC-CML or Ph+ acute lymphoblastic leukemia (ALL). Responses were maintained in 95% of patients with CP-CML and in 82% of patients with AP-CML, with a median follow-up >12 months and >5 months, respectively.

**phase II clinical trials**

Cortes [19] enrolled 116 patients with imatinib-resistant or -intolerant BC-CML; dasatinib was given at 70 mg b.i.d. with dose escalation to 100 mg b.i.d. for poor initial response or dose reductions to 50 and 40 mg b.i.d. for toxicity. Severe myelosuppression was common, but manageable; non-hematologic toxic effects were usually mild. At 8 months follow-up, dasatinib induced MHR in 34% (myeloid BC) and 31 (lymphoid BC) of these patients, respectively. The majority (86%) were CCRs. Most importantly, these responses were durable: 88% and 46% of myeloid BC and lymphoid BC-CML patients had not progressed at 8 months follow-up. Guiltot et al. [20] conducted a multicenter study in 174 patients with AP-CML resistant or intolerant to imatinib; preliminary assessment of efficacy and safety, performed on the first 107 patients (with follow-up 28 months), showed 81%, 64% and 39% of overall, major and CHR, respectively, with 33% and 24% of MCR and CCR. Myelosuppression was significant with grade 3–4 thrombocytopenia and neutropenia in 79% and 69% of patients, respectively. Non-hematologic toxic effects were generally mild to moderate, with diarrhea (46%), peripheral edema (27%) and pleural effusion (16%). Hochhaus [21] recently published a multicenter study in 186 patients with imatinib-resistant or -intolerant CP-CML receiving dasatinib (70 mg b.i.d.). Escalation to 90 mg dasatinib b.i.d. was permitted for patients who had disease progression. At 8 months follow-up, dasatinib induced notable responses, with 90% and 52% of patients achieving CHR and MCR, respectively. Responses were long lasting with only 2% of events in patients achieving MCR. Dasatinib also induced molecular responses, reducing BCR-ABL/ABL transcript ratios from 66% at baseline to 2.6% at 9 months. Non-hematologic adverse events were generally mild to moderate. In a recent study on 23 patients with CML (19 of them in AP or BC), dasatinib was used after treatment failure with both imatinib and nilotinib; among 13 patients responding to dasatinib, 10 achieved CHR and seven cytogenetic response (two complete), suggesting that dasatinib may be active in some patients after failure with both imatinib and nilotinib [22].

<table>
<thead>
<tr>
<th>Reference</th>
<th>No. of patients treated</th>
<th>Status of disease (CP-AP-BC/CML)</th>
<th>Median follow-up (months)</th>
<th>Hematologic responses (percent)</th>
<th>Major cytogenetic responses (percent)</th>
<th>Duration of response (percent) (at the median follow-up)</th>
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<tr>
<td>M. Talpaz</td>
<td>84</td>
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<td>&gt;12</td>
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<td></td>
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<td>33 BC-CML (10 ALL Ph+)</td>
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<td>34 (myeloid BC)</td>
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<td>46</td>
<td>46 (lymphoid BC) PFS</td>
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<td>90</td>
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<td>J. Cortes</td>
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<td>34 (myeloid BC)</td>
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<td>31 (lymphoid BC)</td>
<td>46</td>
<td>46 (lymphoid BC) PFS</td>
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<tr>
<td>F. Guilhot</td>
<td>174 (107)</td>
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<td>81</td>
<td>64</td>
<td>81 PFS</td>
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<td>H. M. Kantarjian</td>
<td>150 randomized (101 dasatinib 150 mg and 49 imatinib 800 mg)</td>
<td>AP-CML</td>
<td>15</td>
<td>93 (dasatinib)</td>
<td>52 (dasatinib)</td>
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<td>Quintas-Cardama</td>
<td>23</td>
<td>4 CP-CML</td>
<td>4</td>
<td>82 (imatinib), P = 0.034</td>
<td>33 (imatinib), P = 0.023</td>
<td>24 (TF at 6 months)</td>
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<td>10 AP-CML</td>
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<td>9 BC-CML</td>
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ALL, acute lymphoblastic leukemia; AP, accelerated phase; BC, blastic crisis; CCR, complete cytogenetic response; CML, chronic myeloid leukemia; CP, chronic phase; MCR, major cytogenetic response; PFS, progression-free survival.
Ph is well known that patients with T315I do not respond at all to mutants and M351 mutants) and A-loop (H396 mutants)—it is well known that patients with T315I do not respond at all to dasatinib. In the phase I trial of dasatinib in CML and ALL Ph+ resistant to or intolerant of imatinib, the resistance was invariably associated with the presence or the emergence of the T315I BCR-ABL mutation and this mutation was identified in several patients at the time of relapse [18]. Recently in BCR-ABL clones, isolated from patients receiving dasatinib, the F317 residue was found to be frequently involved other than T315, and four different mutations were identified (F317I/V/L/C), implicating F317 as another potential vulnerable site for this drug [24].

dasatinib outside CML

Although imatinib is a potent inhibitor of the kinase activity of wild-type isoforms, including KIT and GIST-associated mutant KIT isoforms, most KIT A-loop mutations are resistant to imatinib as the drug only binds to the inactive conformation of KIT [5]. Some KIT A-loop mutations have been found in association with acute myelogenous leukemia (AML), systemic mastocytosis, in a subset of sinonasal natural killer/T-cell non-Hodgkin’s lymphoma, in seminoma/dysembryonoma and in imatinib-resistant GIST. In the case of mast cell disorders, seminoma and AML, the most frequent KIT mutation involves the A-loop (so-called D816V mutation). This mutation results in a constitutive activation of KIT kinase; therefore, consistent with the structural model of imatinib binding to KIT, the kinase activity of these mutants is always resistant to imatinib [17]. Dasatinib has showed the ability to bind to wild-type KIT at a concentration approximately 20-fold lower than that required for imatinib; preliminary data suggest that dasatinib can effectively inhibit KIT-D816V, and it could be effective in blocking the neoplastic growth in most patients with systemic mastocytosis [25] and in patients with GIST [26]. Kinase domain mutations have also been described in lung cancer, GIST, and the hyperesinophilic syndrome with resistance to kinase inhibitors. Elevated levels of Src kinase expression have been found in a variety of human epithelial cancers, and the levels of expression or activation generally correlate with disease progression. Reduction of c-Src expression by dasatinib can suppress tumor growth in human breast cancer cell lines, in human prostate cancer cells, in colon cancer lines and in lung cancer lines [27]. Outside the neoplastic diseases, preliminary observations suggest dasatinib ability to inhibit the PDGF in different tissue targets, as previously demonstrated with imatinib; PDGF is a potent mitogen for smooth muscle cells and it plays a prominent role in fibrotic processes. In a recent study, dasatinib showed to be 67-fold more potent than imatinib in inhibiting PDGFR; these results suggest dasatinib as potential new therapy for vascular obstructive diseases such as restenosis [28]. Recent data suggesting the role of PDGFR in the pathogenesis of chronic graft versus host disease (GVHD) suggest the possibility to evaluate the activity of imatinib or dasatinib also in this setting [29].

conclusions and perspectives

The addition of dasatinib to the armamentarium for CML therapy, only few years after imatinib introduction, can be truly considered quite an unique event as no other cancer has ever seen so rapid development of a highly effective second-line drug following such highly effective, first-line therapy. In the clinical trials dasatinib showed impressive results also in patients with imatinib-resistant and advanced-phase CML, being its major adverse effect a reversible myelosuppression, which typically resolved in patients attaining cytogenetic remission. Phase II studies for dasatinib supported rapid approval of the compound in the last year for CML and ALL Ph+; the recommended dose is 70 mg b.i.d.; ongoing trials continue to explore dosing options for dasatinib. Preclinical studies have identified several BCR-ABL mutations that confer resistance to dasatinib but not to imatinib, providing a rationale to explore combination therapy as initial treatment of CML [30]. It can be reasoned that successful long-term treatment of CML will require a cocktail of kinase inhibitors, like the anti-HIV-retroviral therapy.

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