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Improving upon the promise of targeted therapy of human malignancy: chronic myeloid leukemia as a paradigm

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Abstract The small molecule BCR-ABL-selective kinase inhibitor imatinib is the single most effective medical therapy for the treatment of chronic myeloid leukemia (CML). Although imatinib is highly effective initially and generally well tolerated, for patients who undergo relapse or disease progression few effective therapeutic options exist. Studies have demonstrated that the principal mechanisms of imatinib resistance are BCR-ABL kinase domain point mutation and over-expression. Two novel, potent BCR-ABL inhibitors that harbor promising preclinical activity are undergoing clinical trial evaluation. These agents, dasatinib (BMS-354825) and nilotinib (AMN107), effectively inhibit the activity of nearly all imatinib-resistant BCR-ABL kinase domain mutant forms tested in vitro. Notably, however, neither of these compounds is effective against the imatinib-resistant BCR-ABL/T315I mutation. Early clinical evidence suggests that the T315I mutation might drive the majority of cases who acquire resistance to these second-generation agents. Preclinical efforts to identify an inhibitor of the BCR-ABL/T315I mutation have resulted in demonstration that the Aurora kinase inhibitor VX-680 can bind and inhibit the kinase domain of BCR-ABL/T315I.

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Introduction

While the overwhelming majority of patients with chronic myeloid leukemia (CML) in chronic phase exhibit durable hematologic and cytogenetic responses to imatinib, many patients lose their best response or progress to accelerated or blast phase CML despite continued therapy with imatinib. Studies have demonstrated that patients with chronic phase CML who do not achieve a major cytogenetic response on imatinib are more likely to progress to blast phase [13]. Furthermore, the durability of response in patients who initiate imatinib therapy in accelerated or blast phase CML is significantly lower than that in chronic phase patients.

Definition of imatinib resistance

Primary hematologic resistance [i.e., failure to obtain a complete hematologic response (CHR) despite therapeutic doses of imatinib] occurs in <5% of CML cases through poorly understood mechanisms. Primary cytogenetic resistance is more commonly encountered (in approximately 30% of chronic phase cases) and represents a failure to achieve either major cytogenetic response (<35% Ph-positive marrow metaphases) after 6 months of therapy or complete cytogenetic response after 12 months of therapy. Secondary or "acquired" hematologic and cytogenetic resistance refers to loss of a previously established response. With 42 months of

follow-up, 16% of patients with "early" chronic phase CML (i.e., disease duration <6 months when imatinib was initiated) developed secondary resistance or disease progression [9]. With 48 months of follow-up, the incidence of secondary resistance or progressive disease in chronic phase cases previously treated with interferon-α was 26%; this rate was substantially higher in accelerated (73%) and blast phases (95%) of CML, strongly suggesting that the phase of disease clearly correlates with long-term outcome on imatinib.

Molecular mechanisms of imatinib resistance

Although our understanding of the molecular mechanisms responsible for the relatively rare cases of primary hematologic resistance to imatinib remains poor, the mechanisms of secondary resistance are largely understood and have led to the development of second-generation BCR-ABL inhibitors.

BCR-ABL-dependent resistance

Landmark molecular studies of leukemic cells isolated from blast phase patients, whose responses to imatinib are almost universally transient, revealed that BCR-ABL activity is reactivated at the time of relapse in most cases [8]. Despite the numerous secondary genetic alterations that are undoubtedly present in blast phase disease, disease relapse on imatinib therapy is nearly always conferred by BCR-ABL. Therefore, attempts once again to inhibit BCR-ABL activity in these patients holds considerable therapeutic promise.

Selection for cells that harbor imatinib-resistant BCR-ABL kinase domain mutations clearly represents the most common mechanism of acquired resistance to imatinib, occurring in 50–90% of cases. To date, >40 different mutations have been associated with clinical resistance to imatinib [1, 2, 8, 10, 11, 19, 22, 26]. Co-crystal analysis reveals that imatinib binds to the ABL kinase domain in the inactive, or closed, conformation and induces a variety of conformational changes to the protein upon binding [21]. Although some resistance-associated mutations, most notably BCR-ABL/T315I, occur at amino acid positions implicated in directly contacting imatinib, most imatinib-resistant mutations are believed to prevent the kinase domain from effectively adopting the specific inactive conformation to which imatinib binds [22]. Studies have shown that some mutations confer only a moderate degree of resistance, and as a result dose escalation is predicted to recapture responses in some cases [5, 22, 26]. Moreover, better BCR-ABL inhibitors might prove efficacious in many cases of mutation-driven resistance.

It is estimated that 10% of resistant disease is associated with overproduction of BCR-ABL typically through genomic amplification or the acquisition of additional Ph chromosomes [3, 7, 8, 10, 15]. The intracellular concentrations of imatinib achievable in patients are likely insufficient to inhibit increased levels of BCR-ABL protein in these cells and again, increasing the dose of imatinib or treatment with more potent BCR-ABL inhibitors may be of clinical utility.

BCR-ABL-independent resistance

Although the clear majority of acquired imatinib resistance cases are associated with reactivation of BCR-ABL activity through the mechanisms delineated above, some cases appear to occur through mechanisms independent of BCR-ABL: "primary resistance" [10, 28]. As stated above, 30–50% of blast phase patients do not achieve objective responses to imatinib compared with <5% of chronic phase patients. It is possible that some of this discrepancy may merely represent a higher likelihood of harboring a pretreatment imatinib-resistant BCR-ABL kinase domain mutation in the blast phase as a result of larger tumor burden. Indeed, a substantial fraction of BCR-ABL kinase domain sequences were found to harbor imatinib-resistant mutations prior to imatinib therapy in two of four patients who failed initially to respond to imatinib [22], but a comprehensive analysis of primary resistant cases to confirm this analysis has not been done. It is plausible that cell survival mechanisms capable of operating independently of BCR-ABL are responsible for many cases of primary imatinib resistance, although our understanding of specific mechanisms remains limited. In some cases, reliance on alternative pathways may be responsible for acquired resistance as well, as suggested by studies of primary cells in one case associated with a NUP98/DDX10 fusion gene in addition to the Ph chromosome [28]. Some cell lines established from bone marrow samples obtained from imatinib-resistant patients have implicated SRC gene activation as a mechanism of resistance, but supporting clinical data are lacking [6].

Novel BCR-ABL kinase inhibitors for imatinib-resistant CML

Two investigational small molecule ABL kinase inhibitors, dasatinib (BMS-354825) and nilotinib (AMN107),

have shown considerable efficacy in phase I clinical trials conducted in imatinib-resistant CML patients and are undergoing further evaluation clinically. Response data observed in phase I of both compounds were presented at the 2005 annual meeting of the American Society of Clinical Oncology [20, 25]. Long-term safety and efficacy of these new inhibitors are not known.

Dasatinib is structurally unrelated to imatinib. In co-crystal analyses, dasatinib has been shown to bind to the ABL kinase domain in the active (open) conformation [28]. Preclinical studies have shown that the compound is approximately 300-fold more potent than imatinib and has potent inhibitory activity against nearly all imatinib-resistant mutants tested [16, 24].

The first patients treated with dasatinib had chronic phase CML with hematologic resistance or intolerance to imatinib. The majority of patients were found to have imatinib-resistant BCR-ABL kinase domain mutations at the time of enrollment. The observed CHR rate in patients with resistance ($n = 31$) or intolerance ($n = 8$) to imatinib was 84 and 100%, respectively. The reported major and overall cytogenetic response rates were 35 and 52%, respectively, in imatinib-resistant patients, and 50 and 63%, respectively, in imatinib-intolerant patients. No dose-limiting toxicity was identified, and multinational phase II studies employing a dose of 70 mg bid have completed accrual. Subsequently, the phase I study was amended to allow enrollment of imatinib-resistant and imatinib-intolerant patients with accelerated and blast phase CML as well as Ph-positive acute lymphocytic leukemia (ALL). The CHR rate in ten accelerated phase patients was 50%; overall and complete cytogenetic response rates were 40 and 30%, respectively. Of 34 patients with blast phase/Ph-positive ALL, a CHR rate of 28% with overall and complete cytogenetic responses 56 and 19%, respectively, was observed. Grade 3–4 cytopenia was very common, and pleural effusion constituted the most common grade 3–4 nonhematologic toxicity. Molecular analysis of the BCR-ABL kinase domain of all patients was performed prior to initiation of dasatinib. Imatinib-resistant cases associated with the T315I mutation, which was highly resistant to dasatinib in preclinical studies [16, 24] were not associated with objective responses on dasatinib. In four of five patients with blast phase CML/Ph-positive ALL who developed acquired resistance to dasatinib, the T315I mutation was newly detected at time of relapse [23].

Nilotinib, an aminopyrimidine, is structurally derived from imatinib and was designed to bind more efficiently to the ABL kinase domain. Like imatinib, nilotinib binds the inactive conformation of the ABL kinase domain, but with approximately 25-fold increased potency relative to imatinib. As a result this compound harbors activity against most imatinib-resistant mutations tested, but not the T315I mutation [27].

The nilotinib phase I trial initially enrolled patients with imatinib-resistant accelerated and blast phases of CML and Ph-positive ALL [12, 17]. Nilotinib had significant phase I response activity with a CHR rate of 51% in 50 accelerated phase patients and overall and complete cytogenetic response rates of 38 and 14%, respectively. Of 24 myeloid blast phase cases, 17% achieved CHR with overall and complete cytogenetic responses occurring in 25 and 8% of patients, respectively. Only one of nine patients with lymphoid blast phase CML achieved CHR, while overall and complete cytogenetic responses occurred in 22%. Among ten patients with Ph-positive ALL, 10% achieved CHR and no cytogenetic response was observed. Grade 3–4 hematologic toxicity was commonly seen, and the most common grade 3–4 nonhematologic toxicities were rash and hyperbilirubinemia. The phase I study was subsequently amended to include imatinib-resistant chronic phase CML patients. Analysis of 15 patients revealed achievement of CHR in 80%, with overall and complete cytogenetic response rates of 40 and 13%, respectively [12]. The majority of patients with pretreatment kinase domain mutations responded, but no response was observed in the lone patient on the trial with the T315I mutation. At the present time, little is known about the spectrum of mutations capable of conferring clinical resistance to nilotinib.

Other kinase inhibitors and the BCR-ABL/T315I mutation

Other BCR-ABL-selective inhibitors are currently undergoing early clinical evaluation. For example, two SRC/ABL inhibitors, SKI-606 and NS-187, are in clinical development. Preclinical studies of both agents, however, revealed a lack of activity against the BCR-ABL/T315I mutation. Notably, a similar mutation at the corresponding threonine residue has been identified at the time of relapse in other kinases targeted by imatinib, such as KIT in resistant gastrointestinal stromal tumor cases and PDGFR in resistant cases of hypereosinophilic syndrome. Moreover, cases of acquired resistance to the EGFR inhibitors gefitinib and erlotinib in non-small cell lung cancer patients have been attributed to substitution at the corresponding threonine residue in EGFR [14, 18]. Therefore it appears that CML represents a paradigm for the targeted therapy of human malignancies.

Recent work suggests that the Aurora kinase inhibitor VX-680 is capable of binding to and inhibiting kinase activity of the BCR-ABL/T315I mutation in the low micromolar range [4, 29]. Interestingly, the highly conserved threonine residue that resides at position 315 in BCR-ABL is not present in Aurora kinases; rather, leucine exists at the corresponding position. The bulky non-polar nature of the substituted isoleucine for threonine residue in BCR-ABL/T315I is therefore reflected in Aurora kinases, and selectively inhibiting BCR-ABL/T315I without simultaneously inhibiting Aurora kinases may prove difficult. Clinical evaluation of VX-680 in CML patients harboring BCR-ABL/T315I is ongoing.

Future directions

By their ability to override the majority of imatinib-resistant BCR-ABL kinase domain point mutations in preclinical studies, dasatinib and nilotinib represent important advances in CML targeted therapy [16, 24, 27]. Preliminary evaluation of these agents suggests that the majority of patients with imatinib-resistant chronic phase disease will achieve objective responses. However, the durability of responses with these agents remains undefined. Clearly, the BCR-ABL/T315I mutation is predicted to be highly resistant to these agents and may drive the majority of acquired resistance cases to these compounds. Strategies to control effectively resistance mediated by the T315I mutation represent the next major frontier in the targeted treatment of CML. Compounds such as VX-680, coupled with existing ABL kinase inhibitors, may help improve survival in accelerated and blast phase cases. The safety of combination kinase inhibitor therapy will soon be addressed in clinical trials.

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