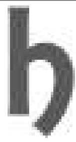

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Ubiquitin-proteasome system is a sensitive target in Ph+ leukemia

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A B S T R A C T

The ubiquitin-proteasome system is an attractive target for anticancer drug development in several cancer including Chronic Myeloid Leukemia Ph+ (CML). The Bcr/Abl tyrosine kinase, the hallmark of CML, by multiple signaling/survival pathways downstream, including the ubiquitin-proteasome system, contributes to leukemic transformation Several key proteins that regulate important cellular processes such as proliferation and apoptosis are regulated by proteasome-dependent proteolysis. For these reasons, proteasome inhibitors represent a relatively new class of antineoplastic agents that act by interfering with the catalytic 20S core of the proteasome, thereby preventing the elimination of diverse cellular proteins targeted for degradation, including BCR-ABL. There are several classes of proteasome inhibitors including peptide aldehydes such as MG-132, the dipeptidyl boronic acid bortezomib, etc, with increased interest in the clinical development. Currently, the clinical utility of proteasome inhibitors in leukemia in general, and in CML in particular, remains relatively unexplored In this review we explore the molecular basis for use of this drugs in CML and the preliminary clinical utility of proteasome inhibitors in CML.

Key words: ubiquitin-proteasome system, NFKB, PS-341, phase 1, biomarkers, CML, ALL Ph+, chronic myeloid leukaemia, Bcr-Abl, imatinib.

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Chronic myelogenous leukemia (CML) is a myeloproliferative disorder characterized by the t (9;22) translocation, resulting in the expression of a fusion oncoprotein, Bcr/Abl, which exhibits constitutively active kinase activity.[1] Constitutive activation of the Bcr/Abl kinase signals to a variety of downstream survival pathways, including the mitogen-activated protein kinase kinase (MEK)/extracellular signal-regulating kinase (ERK) cascade, Akt, signal transducers and activators of transcription (STATs), cell adhesion molecules such as E-selectin, intercellular adhesion molecule-1 (ICAM-1) and nuclear factor B (NF- B), among others.[2-4] The Bcr/Abl tyrosine kinase by multiple signaling/survival pathways downstream of it contributes to leukemic transformation and has been shown to render such cells highly resistant to apoptosis induced by noxious stimuli, including cytotoxic drugs, compared with Bcr/Abl leukemic cells.[4-10] The identification of Bcr/Abl as the pathophysiologic lesion of CML prompted the development of STI571, a tyrosine kinase inhibitor that inhibits the Bcr/Abl, c-Kit, and to a lesser extent, other kinases.[11] STI571 (imatinib mesylate; Gleevec) inhibits the growth of and induces apoptosis in Bcr/Abl-positive leukemia cells *in vitro*[12,13] and has proved to be highly active, when administered orally in patients with CML.[14] However, the emergence of STI571 resistance[15-17] in CML patients initially responsive to this agent18 has led to the search for additional approaches to the treatment of this disease.

The ubiquitin-proteasome pathway and NF-κB signaling in CML

The ubiquitin-proteasome pathway plays an important role in regulating the cell cycle, neoplastic growth, and metastasis.[19] A number of key regulatory proteins are temporally degraded during the cell cycle by the ubiquitin-proteasome pathway; the ordered degradation of these proteins is required for the cell to progress through the

cell cycle to mitosis. One of the targets of ubiquitin-proteasome–mediated degradation is the tumor suppressor p53,[20] which acts as a negative regulator of cell growth. In addition, the ubiquitin-proteasome pathway is required for transcriptional regulation. Nuclear factor-κB (NF-κB) is a key transcription factor, whose activation is regulated by proteasome-mediated degradation of the inhibitor protein IκB.[21] Cell adhesion molecules such as E-selectin, intercellular adhesion molecule-1 (ICAM-1), and vascular cell adhesion molecule-1 (VCAM-1) are regulated by NF-κB[22] and are involved in tumor metastasis and angiogenesis *in vivo*. In addition, NF-κB is required in a number of cell lines[23] to maintain cell viability by activating transcription of a number of antiapoptotic genes. Consistent with this view, inhibiting NF-κB activation by stabilizing the IκB protein makes cells more sensitive to environmental stress and cytotoxic agents, ultimately leading to apoptosis.

Proteasome signaling inhibitor

Proteasome inhibitors represent a relatively new class of antineoplastic agents that act by interfering with the catalytic 20S core of the proteasome, thereby preventing the elimination of diverse cellular proteins targeted for degradation.[24] For reasons that are incompletely understood, proteasome inhibitors effectively induce apoptosis in tumor cells, but are relatively sparing of their normal counterparts.[25] Of the many cellular perturbations induced by proteasome inhibitors, interference with NF-κB signaling has been the subject of intense scrutiny.[26] Interest in the clinical development of proteasome inhibitors has been sparked by recent findings indicating that bortezomib exhibits significant activity in patients with multiple myeloma.[27] There are several classes of proteasome inhibitors including peptide aldehydes such as MG-132,28 as well as the dipeptidyl boronic acid bortezomib (Velcade, formerly known as PS-341; Millenium Pharmaceuticals, Cambridge, MA), which is a more specific inhibitor of the proteasome.[29]

PS-341 (bortezomib, Velcade) is a small, cell permeable molecule that specifically and selectively inhibits the proteasome by binding tightly to the enzyme's chymotrypsin-like active sites. Notably, inhibition of 20S proteasome activity can be reliably and directly detected after treatment with PS-341 *in vitro, in vivo*, and *ex vivo* in cells and tissues. Chemically, PS-341 is a modified dipeptidyl boronic acid derived from leucine and phenylalanine. The average GI_{50} of PS-341 across the 60 cell lines was 3.8 nmol/L. The pattern of growth inhibition and cytotoxicity was unique, suggesting that PS-341 represents a novel class of cytotoxic compound.

Cilloni et Al. reported *in vitro* study clearly demonstrated that the IKK inhibitor PS1145 is able to induce growth arrest and apoptosis in cell lines and in BM cells from CML patients. This effect sound in Imatinib resistant cells treated with the association of Imatinib and PS1145. This is probably due to the fact that PS1145 may acts with different mechanisms of action, not only by the block of the phosphorilation of the 32/36 serine residues of IKBα in the cytoplasm but probably it increases the amount of nuclear IKB that plays an important apoptotic role within the nucleous. It may probably induce an increase of nuclear IKB which is generally phosphorilated by ABL or, such as in these cases, by BCR-ABL. The tyrosine phosphorilated IKB may induce apoptosis by the arrest of nuclear NF-kB function. The addition of Imatinib is probably necessary since it induces the nuclear shuttle of BCR-ABL. This could explain the fact that resistant cells are more sensitive to the combination of the two drugs since the active form of BCR-ABL, as that present in resistant cells, is able to phosphorilate IKBα within the nucleous and to lead the cell toward programmed cell death. The combination of Imatinib and the IKB inhibitor could therefore represent a valid approach to be tested *in vivo* for the treatment of CML patients resistant to Imatinib therapy.

Clinical studies with Bortezomib in CML (Phase I)

Based on striking phase II data[30,31] PS-341 has recently been approved for the therapy of refractory multiple myeloma by the U.S. Food and Drug Administration. A number of phase I trials have been done with varying schedules of PS-341. Phase I studies tested weekly and twice weekly schedules for 2 to 4 weeks[28, 32-34] repeated every 3 to 6 weeks. In general, PS-341 given on these schedules was well tolerated. Nonhematologic toxicities such as fatigue, diarrhea, nausea, vomiting, and sensory neuropathy were likewise observed in all study. Thrombocytopenia was dose limiting in the phase I trials that employed the twice-weekly schedule for 4 of 6 weeks, (as reported by Orlowski *et al.,*[28] and Cortes *et al.,*[32]). These studies, conducted among patients with hematologic malignancies, established a lower MTD compared with study conducted in a mixed population comprised mainly of patients with solid tumors. Altered bone marrow function among patients with hematologic malignancies and reduced tolerability for dose-dependent thrombocytopenia are probable explanations. On the other hand, phase I trials of PS341 using different schedules conducted among patients with solid tumors,[33,34] dose-limiting thrombocytopenia did not occur. Although this may in part be attributable to the subset of patients with hematologic malignancies in this patient population, a schedule-dependent effect on platelets is a tenable hypothesis. Cortes et co-workers[32] undertook a phase I and pharmacologic study among patients with advanced malignancies with PS-341 given as an i.v. bolus twice weekly for 4 weeks of

every 6 weeks (schedule I). As the MTD reached with schedule I in patients with refractory hematologic malignancies was lower[28,32] in comparison with other schedules tested among patients with solid tumors in phase I trials,[33,34] an alternate schedule of PS341 given twice weekly 2 weeks of every 3 weeks (schedule II) was likewise evaluated. In addition, this study aimed to describe the toxicities of PS-341. Twenty-eight patients received PS-341 twice weekly for 4 of 6 weeks (schedule I). while 16 additional patients received PS-341 twice weekly for 2 of every 3 weeks (schedule II). The most common toxicity was thrombocytopenia, which was dose limiting at 1.7 mg/m^2 (schedule I) and 1.6 mg/m^2 (schedule II), respectively. Sensory neuropathy was dose-limiting in a patient in schedule I. Grade 3 toxicities for schedule I were constipation, fatigue, myalgia, and sensory neuropathy. Grade 3 toxicities for schedule II were dehydration resulting from diarrhea, nausea and vomiting, fatigue, hypoglycemia, and hypotension. The maximum tolerated dose was 1.5 mg/m^2 for both schedules. Reversible dose-dependent decreases in 20S proteasome activity in PBMCs were observed, with 36% inhibition at 0.5 mg/m^2, 52% at 0.9 mg/m^2, and 75% at 1.25 mg/m^2. Accumulation of proteasome-targeted polypeptides was detected in tumor samples after treatment with PS-341. A patient with multiple myeloma had a partial response. The Authors concluded that PS-341 given 1.5 mg/m^2 twice weekly for 2 of every 3 weeks is well tolerated and should be further studied in hematological malignances including CML.

Bortezomib in combination with conventional or exploratory chemotherapy

Histone deacetylase inhibitors (HDIs) constitute a diverse group of compounds that promote histone acetylation, chromatin uncoiling, and transcription of a variety of genes involved in multiple cellular processes, including differentiation:[35] their role in the treatment of CML remains to be defined. Previous studies have indicated that exposure of tumor cells to HDIs such as phenylbutyrate leads to inactivation of NF-κB.[36] Such findings raise the possibility that coadministration of HDIs with proteasome inhibitors, which also interrupt this pathway,[23] might be associated with enhanced antitumor activity. Currently, no information is available concerning interactions between clinically relevant HDIs and proteasome inhibitors in leukemia cells in general, and Bcr/Abl$^+$ leukemia cells in particular. To address this issue, C. Yu *et al.,* have examined the effects of treatment of Bcr/Abl$^+$ cells (K562 and LAMA 84), including those resistant to STI571, with HDIs in combination with the proteasome inhibitor bortezomib. They report that these agents interact in a highly synergistic manner to induce mitochondrial injury, caspase activation, and apoptosis in Bcr/Abl$^+$ cells, and that these events are associated with multiple perturbations in signaling and survival pathways, including inhibition of p21^{CIP1} induction, potentiation of Jun kinase (JNK) phosphorylation, and interference with NF-κB DNA binding. Moreover, the HDI/bortezomib regimen potently induces apoptosis in continuously cultured and primary Bcr/Abl$^+$ cells that are resistant to STI571, as well as in Bcr/Abl leukemic cells. Together, these findings suggest that an approach combining clinically relevant HDIs with proteasome inhibitors warrants further investigation as a therapeutic strategy for both Bcr/Abl$^-$ leukemias as well as those that are Bcr/Abl$^+$ and otherwise resistant to standard cytotoxic agents. Y. Dai *et al.,* explored the antiproliferative effect of the combination of bortezomib and flavopiridol in CML cell lines. They found that the two drugs interact synergistically to induce apoptosis in chronic myeloid leukemia cells resistant to imatinib mesylate through both Bcr/Abl-dependent and -independent mechanisms Cyclin-dependent kinase (CDK) inhibitor flavopiridol and the proteasome inhibitor bortezomib were examined in Bcr/Abl$^+$ human leukemia cells. Co-exposure of K562 or LAMA84 cells to subtoxic concentration of flavopiridol (150-200 nM) and bortezomib (5-8 nM) resulted in a synergistic increase in mitochondrial dysfunction and apoptosis. These events were associated with a marked diminution in nuclear factor κB (NF-κB)/DNA binding activity; enhanced phosphorylation of SEK1/MKK4 (stress-activated protein kinase/extracellular signal-related kinase 1/mitogen-activated protein kinase kinase 4), c-Jun N-terminal kinase (JNK), and p38 mitogen-activated protein kinase (MAPK); down-regulation of Bcr/Abl; and a marked reduction in signal transducer and activator of transcription 3 (STAT3) and STAT5 activity. In imatinib mesylate-resistant K562 cells displaying increased Bcr/Abl expression, bortezomib/flavopiridol treatment markedly increased apoptosis in association with down-regulation of Bcr/Abl and BclxL, and diminished phosphorylation of Lyn, Hck, CrkL, and Akt. Parallel studies were performed in imatinib mesylate-resistant LAMA84 cells exhibiting reduced expression of Bcr/Abl but a marked increase in expression/activation of Lyn and Hck. Flavopiridol/bortezomib effectively induced apoptosis in these cells in association with Lyn and Hck inactivation. The capacity of flavopiridol to promote bortezomib-mediated Bcr/Abl down-regulation and apoptosis was mimicked by the positive transcription elongation factor-b (P-TEFb) inhibitor DRB (5,6-dichloro 1-β-D-ribofuranosylbenzinida-sole). Finally, the bortezomib/flavopiridol regimen also potently induced apoptosis in Bcr/Abl$^-$ human leukemia cells. Collectively, these findings suggest that strategy combining bortezomib and other drugs including flavopiridol warrants further examination in chronic myelogenous leukemia and related hematologic malignancies.[38]

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