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Pilot study of Bortezomib for Patients with Imatinib-Refractory Chronic Myeloid Leukemia in Chronic or Accelerated Phase

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Abstract

Background—Proteasome inhibitors are anticancer compounds that disrupt the proteolytic activity of the proteasome and lead to tumor cell growth arrest and apoptosis. Bortezomib is a proteasome inhibitor that is currently approved for use in multiple myeloma and mantle cell lymphoma. It induces apoptosis of chronic myeloid leukemia (CML) cells in vitro, but the activity of bortezomib in patients with imatinib-resistant CML is unknown.

Methods—We conducted a pilot trial to evaluate the activity of single agent bortezomib in CML. Seven patients with imatinib-refractory CML were treated with bortezomib at a dose of 1.5 mg/m² on days 1, 4, 8 and 11 every 3 weeks.

Results—The median number of cycles received was 2. No patient had a hematologic or cytogenetic response. Three patients had a temporary decrease in basophil counts associated with therapy with bortezomib. Six patients developed grade 3-4 nonhematological toxicities.

Conclusion—Bortezomib had minimal efficacy and considerable toxicity in patients with imatinib-refractory CML. Further studies should focus on alternative approaches to employ proteasome inhibitors in the treatment of CML, such as in combination with tyrosine kinase inhibitors or as a strategy to eradicate leukemic stem cells.

Keywords

chronic myelogenous leukemia; proteasome inhibitors; bortezomib

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Conflicts of interest

None

Introduction

Therapy with the tyrosine kinase inhibitor (TKI) imatinib (Gleevec, Glivec; Novartis, Basel, Switzerland) is standard of care in patients with newly diagnosed chronic myeloid leukemia (CML).¹ In the last update of the International Randomized Study on Interferon and STI-571 (IRIS) trial, frontline therapy with imatinib in CML in chronic phase (CP) led to a complete cytogenetic response (CCyR) rate of 83% at 8 years, with an overall survival (OS) of 85%.² Despite these excellent results, at least one third of patients have to discontinue therapy with imatinib, mainly because of development of resistance.²

Mechanisms of resistance include development of *BCR-ABL1* mutations, amplification or overexpression of the fusion gene or its protein product, and activation of alternative signaling pathways.³ The main therapeutic options for patients who fail imatinib are the second-generation TKIs dasatinib (Sprycel; Bristol Myers Squibb, New York, NY) and nilotinib (Tasigna; Novartis, Basel, Switzerland). Treatment with these 2nd generation TKIs can induce a major cytogenetic response (MCyR) in approximately 60% of patients in CP who fail imatinib.^{4,5} Although responses with 2nd-generation TKIs are durable in most patients with imatinib-resistant CML in CP, response duration tends to be short in patients with accelerated (AP) and blast phase (BP).⁶⁻⁹ The sequential use of different TKIs can lead to development of clones with multiple *BCR-ABL1* mutations that are resistant to several TKI, including T315I which is resistant to all known available TKIs.^{3,10} It is also clear from in vitro data that none of the available TKIs are able to eradicate the most primitive progenitor leukemic cell, the so called “leukemic stem cell”¹¹⁻¹³. Leukemic stem cells exist in a quiescent state and can persist even in patients who achieved a CCyR with imatinib and other TKI.¹⁴ The persistence of leukemic stem cells is thought to be responsible for relapses in patients who discontinue the use of imatinib even after maintaining a CCyR for many years.¹⁵ Thus, there is room for improvement in the therapy of CML, by developing novel agents that are capable of circumventing resistance to TKIs and possibly eradicating the leukemic stem cell.

The ubiquitin-proteasome system is responsible for degradation of 80% of cytosolic proteins, including proteins associated with cell cycle control and apoptosis.¹⁶⁻²¹ In CML, proteasomal activity is increased compared with normal cells, and CML cells are more susceptible to apoptosis induced by proteasome inhibitors than normal cells.²² *BCR-ABL1* leads to proteasomal degradation of the cyclin dependent kinase inhibitor p27^{Kip1} by activating the phosphatidylinositol 3-kinase (PI3K) pathway.²³⁻²⁵ The c-Abl TK modulator Abi-2 is also degraded by the proteasome.²⁶ Abi-2 levels are undetectable in the bone marrow of patients with CML and Ph+-acute lymphoblastic leukemia (Ph+-ALL) and may contribute to over activity of *BCR-ABL1*.²⁶ The activity of the transcription factor nuclear factor κ B (NFKB1) is also dependent on the proteasome, since ubiquitination of the inhibitor of κ B (IKB) leads to its degradation by the proteasome and permits NFKB1 translocation to the nucleus and induction of apoptosis-resistant genes.²⁰ Constitutive activation and DNA binding of NFKB1 has been demonstrated in CML cells,²⁷ and it seems to contribute to transformation mediated by *BCR-ABL1*.²⁸

There is thus a rationale for exploiting the inhibition of the proteasome as a therapeutic strategy in CML. Proteasome inhibition by tripeptide aldehydes and lactacystin leads to a decrease in the intracellular level of the p210 BCR-ABL1 oncoprotein with subsequent apoptosis in K562 human *BCR-ABL1*-positive cells.²⁹ This is associated with a reduction in BCR-ABL1 phosphorylation and suggests that proteasome inhibition is sufficient to inhibit activity of BCR-ABL1 and subsequently activate apoptosis.²⁹ The boronic acid derivative bortezomib (Velcade; Millennium Pharmaceuticals, Cambridge, MA) is a highly potent and reversible proteasome inhibitor (PI) that is currently approved for therapy of patients with multiple myeloma.³⁰⁻³² Recent data has suggested that bortezomib can inhibit proliferation and induce apoptosis of quiescent CD34+ CML leukemic stem cells.³³ Based on the preclinical activity of bortezomib in CML we present conducted a pilot trial that evaluated the activity of single-agent bortezomib in patients with CML in CP and AP who were resistant to frontline therapy with imatinib.

Patients and Methods

Eligibility

Between September 2001 until June 2003, patients age 18 years or older with Ph-positive CML in CP or AP that had received and failed treatment with imatinib or were intolerant to imatinib were eligible for this trial. Patients should have not received more than 2 previous cytotoxic regimens besides imatinib and/or hydroxyurea. AP was defined as patients having any of the following criteria¹: peripheral blood (PB) or bone marrow (BM) blasts 10% but < 30%²; PB or BM blasts + promyelocytes 20%³; PB or BM basophils 20%⁴; thrombocytopenia < $100 \times 10^9/L$ unrelated to therapy⁵; clonal evolution defined as the presence of additional cytogenetic abnormalities other than the Ph chromosome (Ph chromosome variants or complex Ph chromosome translocations were not considered as an indication of disease acceleration). Other eligibility criteria included¹: life-expectancy of at least 18 weeks²; ECOG performance status 2³; adequate hepatic (bilirubin 1.5 mg/dL) and renal function (creatinine 1.5 mg/dL or creatinine clearance > 60 mL/h)⁴; adequate contraception for women of child-bearing potential and men. Patients should have not received chemotherapy or radiotherapy within 4 weeks before entering the study and should have recovered from adverse events due to agents administered more than 4 weeks earlier. Imatinib was stopped at least 24 hours before starting therapy with bortezomib and the use of hydroxyurea or anagrelide was allowed during the first six weeks of study. This study was approved by the M.D. Anderson Institutional Review Board and conducted in accordance with the principles of the declaration of Helsinki. All patients gave written informed consent before study entry.

Treatment Plan

Patients received bortezomib 1.5 mg/m² as an IV bolus, twice per week for 2 weeks, every 3 weeks (ie, 2 weeks on, 1 week off). Courses were given every 3 weeks provided the granulocyte count had recovered to > $1.5 \times 10^9/L$ and the platelet count to > $50 \times 10^9/L$. If treatment was delayed for less than 1 week, then the next course could be restarted at the full dose. If treatment was delayed for > 1 but < 2 weeks, then treatment could be restarted with a 1 dose level reduction (–1 dose level: 1.3 mg/m²). If treatment was delayed for more than

two weeks, then the patient was to be taken off study. The requirement for a platelet count greater than $50 \times 10^9/L$ was not applicable if the patient started therapy with thrombocytopenia, unless the patient had previously achieved complete hematologic response (CHR). Patients who developed grade 3 or 4 nonhematologic toxicity had the treatment interrupted until the toxicity resolved to grade 1. Therapy was then restarted with a 1 dose-level reduction. Subsequent courses could be restarted early if there was evidence of rapidly progressive disease (ie, doubling of the WBC and to more than $20 \times 10^9/L$). Otherwise, therapy was restarted after 1 week off therapy to complete a 3-week cycle. Patients with no grade 3-4 nonhematologic toxicity and no myelosuppression (ie, neutrophils nadir $> 1.5 \times 10^9/L$ and platelet nadir $> 100 \times 10^9/L$) could have dose escalation by one dose-level (+1 dose level: 1.7 mg/m^2). In the absence of treatment delays due to adverse events, treatment was continued for up to 12 months or until one of the following events¹: disease progression²; lack of response after 6 courses (18 weeks)³; concomitant illness that prevented further treatment administration⁴; severe and unacceptable adverse events⁵; withdraw of consent by the patient⁶; any other change in patient's clinical condition that could preclude him from receiving further therapy as judged by the investigator. Toxicities were evaluated using the National Cancer Institute Common Toxicity Criteria version 2.0.

Evaluation

Baseline studies included medical history with complete physical examination and documentation of all measurable disease (especially spleen and liver size), complete blood count (CBC), comprehensive biochemistry panel (including liver and renal function tests), pregnancy test (for women of childbearing-age) and BM aspiration for morphology and cytogenetics. Cytogenetics included conventional cytogenetic evaluation of 20 metaphases and fluorescent in situ hybridization (FISH) for the *BCR-ABL1* translocation if metaphases not available for cytogenetic analysis. During study a CBC was obtained every 4-7 days during induction and then every 1-4 weeks and a comprehensive biochemistry panel was obtained every two weeks until remission and then every 2-6 weeks. Patients were evaluated with medical history, physical examination and a BM aspiration with cytogenetic study at the time of remission and then every 1-3 months. Responses were classified according to standard criteria.¹

Statistical considerations

The statistical design of Thall and Sung was used.³⁴ The clinical objectives of the trial were to evaluate the safety and efficacy of the experimental treatment while minimizing the number of patients exposed to therapy if it proved ineffective. If the number of responses fell below a specified critical value, then the trial would be terminated early and bortezomib would be rejected as a treatment for further study as single agent in CML. For this purpose, "response" was defined as a CHR. These analyses were performed on the "per protocol patient population" (ie, those patients who received at least one dose of bortezomib). Due to the novelty of imatinib at the time of study design, there was no experience with this population, but it was expected that patients who failed imatinib would have a low probability of response. Based on historical experience among 83 patients with AP treated at MD Anderson over the 5 years before the conduct of this study treated with various

chemotherapy agents the historical previous was set at 9.6%. Patients would be accrued in cohorts of size 5. After the outcomes of each cohort was evaluated, accrual would stop if it was unlikely that, based on the data from the trial thus far, bortezomib provided at least a 15% improvement over the historical rate. Thus, the trial would be terminated if responses were observed in 0/10, 0/15, 1/20, or 2/25. Otherwise, a maximum of 30 patients would be entered. OS was calculated from date treatment started until death. Patients alive at last follow-up were censored. OS was estimated by Kaplan-Meier method³⁵ in Statistica, version 6.0 (Tulsa, OK).

Results

Patient characteristics

A total of 7 patients (2 males and 5 females) were enrolled and their clinical characteristics are outlined in Table 1. Their median age was 69 years (range, 53-74 years). Three patients were in CP and four patients were in AP. Criteria for the diagnosis of AP were more than 20% BM basophils (N = 2) and clonal evolution (N = 3) (one patient had both criteria). Median time from diagnosis to bortezomib was 96 months (range, 42-129). Before bortezomib, patients had previously received a median of two therapies (range, 2-3). All patients had received previous therapy with imatinib and interferon- α , and two patients additionally had received therapy with farnesyl transferase inhibitors (tipifarnib and lonafarnib, one each). Reasons for discontinuing imatinib were resistance in 6 patients and intolerance in one. Best response to imatinib was CHR (N = 5) and minor cytogenetic response (N = 1). The median percentage of Ph+ metaphases at start of therapy was 100% (range, 50-100%). Three patients had additional cytogenetic abnormalities besides the Ph chromosome, including monosomy 7, t(3;21)(q21;q22) and +der(22)t(9;22) in one case each.

Response and Outcomes

Median time on study was 39 days (range, 4-46). The median number of administered cycles was two (range < 1-2), and the median number of administered doses was 7 (range, 2-8). Five patients received concomitant therapy with hydroxyurea to control WBC counts during study. No patient achieved a CHR or partial hematologic response, and no patient achieved a cytogenetic response. Among 4 patients who presented with elevated (> 10%) PB basophils, 3 patients (UPIN 1, 4 and 7) had a greater than 50% decrease in basophil counts (Figure 1). Two of these patients (UPIN 1 and 4) were receiving concomitant hydroxyurea. All patients were eventually taken off study because of toxicity (N = 2; grade 3 fatigue and grade 3 allergic reaction in one patient each), lack of response (N = 1), toxicity and lack of response (N = 2; grade 3 diarrhea and grade 3 diarrhea, nausea and vomiting in one patient each), transformation to myeloid blast phase (N = 1), and one patient was lost to follow-up. As of this report, six of seven patients had died. The median OS from the start of therapy with bortezomib was 8.6 months (range, 4-44 months). Two patients had a prolonged survival after discontinuing bortezomib (28 and 44 months), and both of these patients had received further therapy with a second-generation TKI (nilotinib).

Toxicities and Dose adjustments

Five patients (71%) experienced grade 3-4 hematologic toxicities, including anemia (N = 3), neutropenia (N = 3) and thrombocytopenia (N = 3) (three patients developed more than one toxicity). These events were transient and resolved upon treatment delays or discontinuation. Grade 3-4 nonhematological developed in six patients (86%), including diarrhea (N = 2), fatigue (N = 2), and dizziness, myalgia and allergic reaction (one patient each). Only one patient developed peripheral neuropathy, which was grade 1. Bortezomib was reduced by one dose level in two patients due to grade 3 toxicity (diarrhea and myalgia, respectively). Five patients required a treatment interruption or dose delay because of toxicity (diarrhea [N = 2], neuropathy, fatigue and neutropenia in one patient each). Toxicity was the cause of treatment discontinuation in four patients, including one patient (no. 5) that only received two doses and had to stop treatment because of grade 3 allergic reaction. No deaths were attributed to the study drug.

Discussion

This study evaluated the efficacy of single agent bortezomib in the treatment of patients with imatinib-resistant CML. The population of patients chosen was highly resistant to imatinib, since none had achieved a MCyR. This study was conducted before the broad availability of 2nd-gen TKIs, when no standard therapy was available for this patient population. However, even with modern therapy with 2nd-gen TKI, the lack of a previous MCyR is a risk factor predicting lower probability of response to therapy.³⁶

Unfortunately, we failed to demonstrate that bortezomib had any efficacy against CML in this group of patients, since no patient had a hematologic response. Three patients had a decrease in basophil counts after starting therapy with bortezomib, but two of these patients were receiving concomitant hydroxyurea, which makes it difficult to discern the value of this observation. Nonetheless, it can be concluded that the activity of bortezomib was minimal in this setting, and in face of the results of 2nd-gen TKI (nilotinib, dasatinib) in the same patient population, it is unlikely that bortezomib will face further development as a single agent in the treatment of imatinib-resistant CML.

Patients in our trial developed significant toxicity with bortezomib, and 57% of patients stopped trial because of side effects. Patients in our population were elderly (median age 69 years), and the incidence of grade 3-4 adverse events in a recently reported clinical trial of elderly patients (≥ 65 years) with MM that received bortezomib combined with melphalan and prednisone was 81%, which is comparable to our results.³² It should also be mentioned that the dose used in our study (1.5 mg/m²) was higher than the dose current approved for patients with MM (1.3 mg/m²), which might have also contributed to the toxicity observed. Recent reports suggest that weekly doses of bortezomib in patients with MM are associated with similar response and improved toxicity profile.³⁷ Only one patient in our study developed peripheral neuropathy, but patients in our study received a median of only two cycles of bortezomib. In MM, the incidence of peripheral neuropathy increases with cumulative doses of bortezomib, and apparently reaches a plateau after a total dose of 30 mg/m².^{38,39} Furthermore, severe neuropathy secondary to bortezomib is more common in patients who have baseline neuropathy, and compared with patients with CML, patients with

MM more often receive drugs which can cause neuropathy (vincristine, thalidomide) and the disease itself can also lead to peripheral neuropathy.^{38,39}

As used in this trial, bortezomib failed as a single agent in patients with imatinib-resistant CML. However, recent studies have suggested that bortezomib, may have significant activity in CML. Bortezomib has been shown to induce apoptosis in vitro of CML and Ph+-ALL cell lines harboring wild-type or mutant genotypes of *BCR-ABL1* at similar concentrations.⁴⁰ This suggests that bortezomib acts by a non-ATP competitive mechanism in CML.⁴⁰ Albero et al reported that bortezomib inhibits the proliferation of *BCR-ABL1*-expressing cells.⁴¹ This appears to be mediated through inactivation of NFKB2 and a decrease in the phosphorylation of Rb.⁴¹ This in turn leads to an increase in caspase-dependent apoptosis. Importantly, this effect is independent of the presence or absence of mutations of *BCR-ABL1*. In addition, bortezomib may induce apoptosis of primitive, CD34+/CD38- leukemic stem cells at concentrations similar to those achieved in clinic.³³ This effect is unlikely to be observed in the setting in which we used bortezomib in this clinical trial. One possibility would be to use bortezomib in combination with a TKI, such as imatinib or dasatinib. However, there is in vitro data indicating that bortezomib and imatinib are synergistic when bortezomib is given before the TKI, but antagonistic when given simultaneously, which would pose another obstacle in devising a treatment regimen with these two drugs since TKI are usually administered continuously to patients with CML.⁴⁰ Another approach would be to administer bortezomib to patients with minimal residual disease after being successfully treated with a TKI, aiming to potentially cure CML by eradication of the leukemic stem cell.³³ Even though there is in vitro data supporting such approach, to date no study has evaluated this treatment in vivo.³³ Other PI could also be investigated. Bortezomib reversibly inhibits the $\beta 1$ and $\beta 5$ subunits of the proteasome, while the new PI carfilzomib inhibits irreversibly the $\beta 5$ subunit of the proteasome.⁴² Carfilzomib has clinical activity in patients with relapsed MM after failing therapy with bortezomib,⁴³ and could potentially be exploited in CML after failing several TKI.

Conclusion

Bortezomib as a single agent has minimal efficacy in the treatment of patients with imatinib-resistant CML. Further studies should focus on alternative approaches to employ PI in the treatment of CML, such as in combination with TKI or as a strategy to eradicate residual leukemic stem cells.

REFERENCES

1. Baccarani M, Cortes J, Pane F, et al. Chronic myeloid leukemia: an update of concepts and management recommendations of European LeukemiaNet. *J Clin Oncol.* 2009; 27:6041–51. [PubMed: 19884523]
2. Deininger M, O'Brien SG, Guilhot F, et al. International Randomized Study of Interferon Vs STI571 (IRIS) 8-Year Follow up: Sustained Survival and Low Risk for Progression or Events in Patients with Newly Diagnosed Chronic Myeloid Leukemia in Chronic Phase (CML-CP) Treated with Imatinib. *Blood.* 2009; XXX:114. [abstract] Abstract 1126.
3. Apperley JF. Part I: mechanisms of resistance to imatinib in chronic myeloid leukaemia. *Lancet Oncol.* 2007; 8:1018–29. [PubMed: 17976612]

4. Hochhaus A, Baccarani M, Deininger M, et al. Dasatinib induces durable cytogenetic responses in patients with chronic myelogenous leukemia in chronic phase with resistance or intolerance to imatinib. *Leukemia*. 2008; 22:1200–6. [PubMed: 18401416]
5. Kantarjian HM, Giles F, Gattermann N, et al. Nilotinib (formerly AMN107), a highly selective BCRABL tyrosine kinase inhibitor, is effective in patients with Philadelphia chromosome-positive chronic myelogenous leukemia in chronic phase following imatinib resistance and intolerance. *Blood*. 2007; 110:3540–6. [PubMed: 17715389]
6. Apperley JF, Cortes JE, Kim DW, et al. Dasatinib in the treatment of chronic myeloid leukemia in accelerated phase after imatinib failure: the START a trial. *J Clin Oncol*. 2009; 27:3472–9. [PubMed: 19487385]
7. Cortes J, Kim DW, Raffoux E, et al. Efficacy and safety of dasatinib in imatinib-resistant or -intolerant patients with chronic myeloid leukemia in blast phase. *Leukemia*. 2008; 22:2176–83. [PubMed: 18754032]
8. Giles FJ, Larson RA, Kantarjian HM, et al. Nilotinib in Patients (pts) with Philadelphia Chromosome-Positive (Ph+) Chronic Myelogenous Leukemia in Blast Crisis (CML-BC) Who Are Resistant or Intolerant to Imatinib. *Blood*. 2007; XXX:110. [abstract] Abstract 1025.
9. le Coutre P, Ottmann OG, Giles F, et al. Nilotinib (formerly AMN107), a highly selective BCR-ABL tyrosine kinase inhibitor, is active in patients with imatinib-resistant or -intolerant accelerated-phase chronic myelogenous leukemia. *Blood*. 2008; 111:1834–9. [PubMed: 18048643]
10. Shah NP, Skaggs BJ, Branford S, et al. Sequential ABL kinase inhibitor therapy selects for compound drug-resistant BCR-ABL mutations with altered oncogenic potency. *J Clin Invest*. 2007; 117:2562–9. [PubMed: 17710227]
11. Copland M, Hamilton A, Elrick LJ, et al. Dasatinib (BMS-354825) targets an earlier progenitor population than imatinib in primary CML but does not eliminate the quiescent fraction. *Blood*. 2006; 107:4532–9. [PubMed: 16469872]
12. Graham SM, Jorgensen HG, Allan E, et al. Primitive, quiescent, Philadelphia-positive stem cells from patients with chronic myeloid leukemia are insensitive to STI571 in vitro. *Blood*. 2002; 99:319–25. [PubMed: 11756187]
13. Jorgensen HG, Allan EK, Jordanides NE, Mountford JC, Holyoake TL. Nilotinib exerts equipotent antiproliferative effects to imatinib and does not induce apoptosis in CD34+ CML cells. *Blood*. 2007; 109:4016–9. [PubMed: 17213283]
14. Bhatia R, Holtz M, Niu N, et al. Persistence of malignant hematopoietic progenitors in chronic myelogenous leukemia patients in complete cytogenetic remission following imatinib mesylate treatment. *Blood*. 2003; 101:4701–7. [PubMed: 12576334]
15. Rousselot P, Huguier F, Rea D, et al. Imatinib mesylate discontinuation in patients with chronic myelogenous leukemia in complete molecular remission for more than 2 years. *Blood*. 2007; 109:58–60. [PubMed: 16973963]
16. Cayrol C, Ducommun B. Interaction with cyclin-dependent kinases and PCNA modulates proteasome-dependent degradation of p21. *Oncogene*. 1998; 17:2437–44. [PubMed: 9824154]
17. Chadebecq P, Briccese L, Baldin V, Vidal S, Valette A. Phosphorylation and proteasome-dependent degradation of Bcl-2 in mitotic-arrested cells after microtubule damage. *Biochem Biophys Res Commun*. 1999; 262:823–7. [PubMed: 10471409]
18. Clurman BE, Sheaff RJ, Thress K, Groudine M, Roberts JM. Turnover of cyclin E by the ubiquitin-proteasome pathway is regulated by cdk2 binding and cyclin phosphorylation. *Genes Dev*. 1996; 10:1979–90. [PubMed: 8769642]
19. Pagano M, Tam SW, Theodoras AM, et al. Role of the ubiquitin-proteasome pathway in regulating abundance of the cyclin-dependent kinase inhibitor p27. *Science*. 1995; 269:682–5. [PubMed: 7624798]
20. Palombella VJ, Rando OJ, Goldberg AL, Maniatis T. The ubiquitin-proteasome pathway is required for processing the NF-kappa B1 precursor protein and the activation of NF-kappa B. *Cell*. 1994; 78:773–85. [PubMed: 8087845]
21. Reed SI. The ubiquitin-proteasome pathway in cell cycle control. *Results Probl Cell Differ*. 2006; 42:147–81. [PubMed: 16903211]

22. Magill L, Lynas J, Morris TC, Walker B, Irvine AE. Proteasome proteolytic activity in hematopoietic cells from patients with chronic myeloid leukemia and multiple myeloma. *Haematologica*. 2004; 89:1428–33. [PubMed: 15590391]
23. Andreu EJ, Lledo E, Poch E, et al. BCR-ABL induces the expression of Skp2 through the PI3K pathway to promote p27Kip1 degradation and proliferation of chronic myelogenous leukemia cells. *Cancer Res*. 2005; 65:3264–72. [PubMed: 15833859]
24. Gesbert F, Sellers WR, Signoretti S, Loda M, Griffin JD. BCR/ABL regulates expression of the cyclin-dependent kinase inhibitor p27Kip1 through the phosphatidylinositol 3-Kinase/AKT pathway. *J Biol Chem*. 2000; 275:39223–30. [PubMed: 11010972]
25. Jonuleit T, van der Kuip H, Miething C, et al. Bcr-Abl kinase down-regulates cyclin-dependent kinase inhibitor p27 in human and murine cell lines. *Blood*. 2000; 96:1933–9. [PubMed: 10961897]
26. Dai Z, Quackenbush RC, Courtney KD, et al. Oncogenic Abl and Src tyrosine kinases elicit the ubiquitin-dependent degradation of target proteins through a Ras-independent pathway. *Genes Dev*. 1998; 12:1415–24. [PubMed: 9585502]
27. Hamdane M, David-Cordonnier MH, D'Halluin JC. Activation of p65 NF-kappaB protein by p210BCR-ABL in a myeloid cell line (P210BCR-ABL activates p65 NF-kappaB). *Oncogene*. 1997; 15:2267–75. [PubMed: 9393872]
28. Reuther JY, Reuther GW, Cortez D, Pendergast AM, Baldwin AS Jr. A requirement for NF-kappaB activation in Bcr-Abl-mediated transformation. *Genes Dev*. 1998; 12:968–81. [PubMed: 9531535]
29. Dou QP, McGuire TF, Peng Y, An B. Proteasome inhibition leads to significant reduction of Bcr-Abl expression and subsequent induction of apoptosis in K562 human chronic myelogenous leukemia cells. *J Pharmacol Exp Ther*. 1999; 289:781–90. [PubMed: 10215653]
30. Richardson PG, Barlogie B, Berenson J, et al. A phase 2 study of bortezomib in relapsed, refractory myeloma. *N Engl J Med*. 2003; 348:2609–17. [PubMed: 12826635]
31. Richardson PG, Sonneveld P, Schuster MW, et al. Bortezomib or high-dose dexamethasone for relapsed multiple myeloma. *N Engl J Med*. 2005; 352:2487–98. [PubMed: 15958804]
32. San Miguel JF, Schlag R, Khuageva NK, et al. Bortezomib plus melphalan and prednisone for initial treatment of multiple myeloma. *N Engl J Med*. 2008; 359:906–17. [PubMed: 18753647]
33. Heaney NB, Pellicano F, Zhang B, et al. Bortezomib induces apoptosis in primitive chronic myeloid leukemia cells including LTC-IC and NOD/SCID repopulating cells. *Blood*. 2010; 115:2241–50. [PubMed: 20068223]
34. Thall PF, Sung HG. Some extensions and applications of a Bayesian strategy for monitoring multiple outcomes in clinical trials. *Stat Med*. 1998; 17:1563–80. [PubMed: 9699230]
35. Kaplan E, Meire P. Nonparametric estimation from incomplete observations. *J Am Stat Assoc*. 1958; 53:457–81.
36. Jabbour E, Kantarjian H, O'Brien S, et al. Predictive Factors for Response and Outcome in Patients (pts) Treated with Second Generation Tyrosine Kinase Inhibitors (2-TKI) for Chronic Myeloid Leukemia in Chronic Phase (CML-CP) Post Imatinib Failure. *Blood*. 2009; XXX:114. [abstract] Abstract 509.
37. Bringhen S, Larocca A, Rossi D, et al. Efficacy and safety of once weekly bortezomib in multiple myeloma patients. *Blood*. 2010 XXX.
38. Argyriou AA, Ionomou G, Kalofonos HP. Bortezomib-induced peripheral neuropathy in multiple myeloma: a comprehensive review of the literature. *Blood*. 2008; 112:1593–9. [PubMed: 18574024]
39. Richardson PG, Briemberg H, Jagannath S, et al. Frequency, characteristics, and reversibility of peripheral neuropathy during treatment of advanced multiple myeloma with bortezomib. *J Clin Oncol*. 2006; 24:3113–20. [PubMed: 16754936]
40. Gatto S, Scappini B, Pham L, et al. The proteasome inhibitor PS-341 inhibits growth and induces apoptosis in Bcr/Abl-positive cell lines sensitive and resistant to imatinib mesylate. *Haematologica*. 2003; 88:853–63. [PubMed: 12935973]

41. Albero MP, Vaquer JM, Andreu EJ, et al. Bortezomib decreases Rb phosphorylation and induces caspase-dependent apoptosis in Imatinib-sensitive and -resistant Bcr-Abl1-expressing cells. *Oncogene*. 29:3276–86. XXX. [PubMed: 20305692]
42. Kuhn DJ, Chen Q, Voorhees PM, et al. Potent activity of carfilzomib, a novel, irreversible inhibitor of the ubiquitin-proteasome pathway, against preclinical models of multiple myeloma. *Blood*. 2007; 110:3281–90. [PubMed: 17591945]
43. Jagannath S, Vij R, Stewart AK, et al. Initial Results of PX-171-003, An Open-Label, Single-Arm, Phase II Study of Carfilzomib (CFZ) in Patients with Relapsed and Refractory Multiple Myeloma (MM). *Blood*. 2008; XXX:112. [abstract] Abstract 864.

Figure 1. Basophil decrease associated with therapy with bortezomib in patients no. 1 (A), 4 (B) and 7 (C).

Figure 2.
Overall Survival of patients on trial

Table 1**Patients' Baseline Characteristics**

UPIN	Age, y (Sex)	Phase	Best Response IM	Hb, g/dL	WBC, $\times 10^9/L$	Platelets, $\times 10^9/L$	PB Blasts, %	BM Blasts, %	PB Baso, %	BM Baso, %	Ph+, %
1	63 (F)	AP	CHR	8.7	11.1	636	0	0	28	36	100
2	53 (M)	AP	CHR	9.8	53.2	158	5	4	6	0	100
3	75 (F)	CP	No Response	11.3	3.9	287	0	0	1	8	88
4	74 (F)	CP	CHR	10.6	7.2	589	0	0	19	19	100
5	72 (F)	CP	miCyR	10.4	8.8	2289	10	0	12	9	50
6	69 (M)	AP	CHR	6.8	4.6	224	0	4	6	15	100
7	50 (F)	AP	CHR	8.3	4.8	117	0	7	51	8	100

Baso – Basophils; BM – bone marrow; CHR – complete hematologic response; IM – imatinib; miCyR – minor cytogenetic response PB – peripheral blood; Ph+ – percentage of Ph+ cells in 20 metaphases at start of therapy; UPIN – unique patient identifier; WBC – white blood cell count