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Proteasome inhibition: potential benefits in the treatment of cancer

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Abstract

Proteasome inhibition is a novel and promising strategy for the treatment of cancer. Bortezomib is a boronic acid dipeptide that potently and reversibly inhibits the 26S proteasome. Following intravenous (IV) injection, bortezomib is cleared with a rapid initial half-life of less than 10 min followed by a longer elimination half-life of 5–15 h. A pharmacodynamic assay has been developed to measure bortezomib activity at the level of the proteasome. Data from phase I trials show that bortezomib induces predictable, dose-related, reversible inhibition of proteasome activity. A phase I trial designed to evaluate the safety and pharmacodynamics of bortezomib administered as a twice-weekly IV bolus for 2 weeks followed by a 1-week rest was conducted in 43 patients with advanced solid tumours. Dose-limiting toxicities (DLTs) at the dose of 1.56 mg/m^2 included diarrhoea and sensory neuropathy. A partial response was seen in one patient with non-small cell lung cancer. In a second phase I trial, bortezomib was administered twice weekly for 4 weeks followed by a 2-week rest to 27 patients with refractory haematological malignancies. DLTs included fatigue, hyponatraemia, and hypokalaemia. Among nine patients with myeloma, there was one complete response and a reduction in paraprotein level and/or marrow plasmacytosis in the remaining eight. Additionally, two partial responses were seen in patients with lymphoma. Thus, in phase I testing, bortezomib demonstrated promising clinical activity. It is biologically active and generally well tolerated. Based on these promising early results, bortezomib is now being actively investigated for the treatment of multiple myeloma, non-Hodgkin's lymphoma, and a variety of solid tumours.

Keywords: Proteasome; Bortezomib; Multiple myeloma; NF-κB; Dipeptides; Boronic acids

1. Introduction

Proteasome inhibition is a novel and promising strategy for the treatment of cancer. The ubiquitin–proteasome pathway is responsible for the degradation of numerous proteins regulating critical cellular functions such as transcription, cell division, cytokine production, and apoptosis [1]. Preclinical studies show that proteasome inhibitors may be more cytotoxic to tumour cells than normal cells [1]. In particular, compared with normal cells, cancer cells appear to be more sensitive to the pro-apoptotic effects of proteasome inhibition [2]. As discussed in detail by Dr. Kenneth Anderson [*Eur J Cancer Suppl*], proteasome activity is required for activation of the transcription factor nuclear factor-κB (NF-κB). Proteasome inhibition prevents the activation of NF-κB by chemotherapy and radiotherapy, resulting in enhanced sensitivity to these tumouricidal agents and increased apoptosis in cancer cells *in vitro* [3].

Bortezomib is a novel boronic acid dipeptide that potently and selectively inhibits the 26S proteasome. Importantly – given the fundamental metabolic role of the ubiquitin–proteasome pathway – bortezomib-induced inhibition of the proteasome is reversible [4]. In the National Cancer Institute *in vitro* screen, bortezomib showed substantial cytotoxicity against a broad range of human tumour cells [4]. The antitumour effects of bortezomib have been confirmed in a wide range of murine xenograft models [2].

Based on this preclinical profile, the early clinical studies of bortezomib were designed to determine whether bortezomib induces predictable and dose-dependent proteasome inhibition in patients, to assess the pharmacodynamic and pharmacokinetic characteristics of bortezomib, and to identify the toxicities associated

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with bortezomib administration. In addition, the phase I trials were designed to determine whether bortezomib demonstrates evidence of antitumour efficacy.

2. Pharmacodynamics

In animal models, bortezomib is rapidly removed from the vascular compartment and distributed widely, quickly approaching the limits of detection. This suggests that traditional plasma pharmacokinetic parameters would not be appropriate for measuring its activity [5–7]. A pharmacodynamic assay was therefore developed to measure and monitor activity at the level of the proteasome [6]. The method has been shown to be sensitive, specific, accurate, and precise [6].

In primates treated with twice-weekly injections of bortezomib for 4 weeks, proteasome activity in white blood cells was significantly decreased at dose levels of 0.54, 0.8, and 1.2 mg/m^2, as compared with controls. Recovery to control activity levels occurred within 72 h. At the maximum tolerated dose of 0.8 mg/m^2, the percentage of proteasome inhibition was 76%. Beyond this level, animals developed hypotension and deaths were observed [5]. In addition, in animal models continuous proteasome inhibition produces unacceptable toxicity (Millennium Pharmaceuticals, Inc. Data on file).

Fig. 1 shows the pooled clinical data from several early clinical studies with bortezomib [7]. The curve is sigmoidal, demonstrating that bortezomib produces a predictable and dose-related inhibition of proteasome activity. In these studies, the half-life of bortezomib-induced proteasome inhibition [(PD)$t_{1/2}$] varied from 8 to 24 h [7]. Thus, an interval of 72 h between dosing should permit nearly full recovery of proteasome function [8].

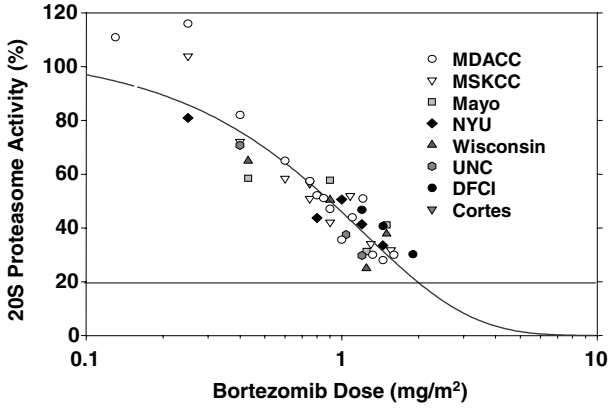

Fig. 1. Summary of pharmacodynamic data from phase I trials of bortezomib. Degree of inhibition of proteasome activity in whole blood lysate vs. bortezomib dose, 1 h following bortezomib administration [7].

3. Pharmacokinetics

Following IV injection, bortezomib is cleared with a very rapid α-half-life of less than 10 min ($t_{1/2\alpha} < 10$ min), followed by a longer elimination half-life of 5–15 h ($t_{1/2\beta}$ 5–15 h). Bortezomib is extensively distributed in tissues with a large volume of distribution ($V_d = 400$–600 L) [9]. In most organs, there is a predictable dose-related increase in exposure, but bortezomib does not cross the blood–brain barrier [4].

The major pathway of bortezomib elimination is cytochrome-mediated hepatic metabolism. However, bortezomib is a poor inhibitor of cytochrome P450 in human microsomes, and there are no specific recommendations for dose adjustment with other P450-metabolised medications. Studies are ongoing to determine the extent to which bortezomib may safely be administered to patients with significant hepatic dysfunction. The pharmacokinetic characteristics of bortezomib in patients with significant renal compromise are also being evaluated.

4. Phase I results in patients with solid tumours

A phase I trial was conducted in patients with histologically documented solid tumours refractory to standard therapy [5]. Eligibility criteria included age $\geqslant 18$ years; Karnofsky performance status $\geqslant 70$; adequate bone marrow reserve; and adequate hepatic, renal, and cardiac function. The goals of the study were to define the safety, DLTs, and pharmacodynamic behaviour of bortezomib administered as a twice-weekly IV bolus for 2 weeks, followed by a 1-week rest. Tumour cases included eight patients with non-small cell lung cancer (NSCLC), six with colon cancer, five with head and neck cancer, and four each with melanoma, ovarian, prostate, and renal cancer. Median patient age was 53 years, median Karnofsky performance status was 80, and patients had received a median of four prior chemotherapy regimens [5].

Patients received a total of 89 cycles of bortezomib at doses ranging from 0.13 to 1.56 mg/m^2 [5]. No dose-limiting haematological toxicity was seen at any dose level [5]. The two DLTs seen at the highest dose level were diarrhoea and sensory neuropathy. Two of the 12 patients treated with 1.56 mg/m^2 developed grade 3 diarrhoea. The diarrhoea lasted for 18–36 h after bortezomib dosing and resolved with loperamide treatment [5]. Diarrhoea on subsequent cycles was successfully prevented with the use of prophylactic loperamide [5]. In addition, two other patients treated at the 1.56 mg/m^2 dose level experienced grade 3 sensory neuropathy. Both patients had previously received known neurotoxic agents (paclitaxel, cisplatin, and carboplatin) and had preexisting neuropathy at the time of study entry (grade

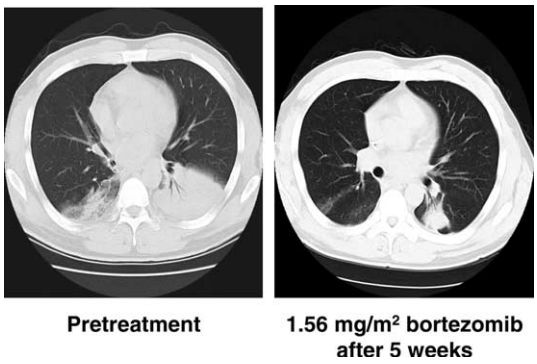

Fig. 2. Computed tomography of the chest in a patient with bronchioloalveolar carcinoma at baseline and following two cycles of treatment with bortezomib [5].

1 in the first patient and grade 2 in the second patient). The neuropathy improved or resolved in both patients with cessation of bortezomib treatment [5].

Pharmacodynamically, a clear dose-related inhibition of proteasome activity was seen at 1 h following IV administration of bortezomib. Assays performed on samples drawn immediately before treatment on days 4, 8, and 11 showed recovery of proteasome activity to baseline. The mean percentages of proteasome inhibition by dose level were: 31% at 0.4 mg/m^2; 42% at 0.6 mg/m^2; 48% at 0.75 mg/m^2; 57% at 0.9 mg/m^2; 46% at 1.08 mg/m^2; 65% at 1.3 mg/m^2; and 68% at 1.56 mg/m^2 [5].

One partial response was documented in a patient with NSCLC (bronchioloalveolar type). The patient had progressed on treatment with paclitaxel and carboplatin; he subsequently progressed on additional treatment with gemcitabine, mitomycin and vinblastine, docetaxel, and methotrexate. The patient's tumour symptoms of cough, bronchorrhea, and intermittent hemoptysis resolved after the first cycle of bortezomib. As shown in Fig. 2, chest computerised tomography after two cycles of therapy showed a 50% reduction in bilateral pulmonary infiltrative masses [5]. The patient stopped bortezomib treatment after three cycles due to sensory neuropathy. The duration of response was 3 months. One patient each with malignant melanoma, nasopharyngeal carcinoma, and renal cell carcinoma had stable disease as their best response. The median duration of stable disease was 4 months [5].

5. Phase I results in patients with haematological malignancies

A second phase I trial was conducted in patients with refractory haematological malignancies [8]. Adult patients with a pathologically confirmed haematological malignancy refractory to standard therapy were eligible for enrollment. Additional eligibility criteria included: (1) Eastern Cooperative Oncology Group (ECOG) performance status 0–2; (2) expected survival $\geqslant 6$ weeks; (3) adequate cardiovascular and hepatic function; (4) serum creatinine $\leqslant 2.5$ mg/dL; and (5) WBC $\geqslant 2000/mm^3$; absolute neutrophil count $\geqslant 1000/mm^3$; haemoglobin $\geqslant 8.0$ g/dL; platelet count $\geqslant 50{,}000/mm^3$. The objectives of the study were to determine the maximum tolerated dose and DLTs of bortezomib administered to patients with refractory haematological malignancies as an IV bolus twice weekly for 4 weeks, followed by a 2-week rest [8].

A total of 27 patients were enrolled and treated at 4 dose levels ranging from 0.40 to 1.38 mg/m^2. Diagnoses included multiple myeloma (11 patients), Waldenström's macroglobulinaemia (1), non-Hodgkin's lymphoma (10), Hodgkin's disease (4), and myelodysplasia (1). No DLTs were observed at the first three dose levels of 0.40, 1.04, and 1.20 mg/m^2. At the 1.38 mg/m^2 dose level, two of the five patients experienced DLTs, consisting of grade 3 hyponatraemia and grade 3 fatigue. Because of these DLTs, the 1.20 mg/m^2 cohort was expanded, but two of the four additional patients experienced DLTs, including grade 3 fatigue and grade 3 hypokalaemia. Enrollment was then continued at the 1.04 mg/m^2 dose level [8].

The most common adverse events overall were thrombocytopaenia (74% of patients), fatigue (59%), and nausea (52%). Thrombocytopaenia was the most common grade 3 event, occurring in 10 patients (37%) during cycle 1. Patients who entered the study with less than normal platelet counts were at greatest risk for thrombocytopaenia. Overall, five (19%) patients developed treatment-emergent peripheral neuropathy, considered bortezomib-related in three cases. All these patients had received prior therapy with potentially neurotoxic agents, including vinca alkaloids and thalidomide [8].

Bortezomib induced a dose-dependent inhibition of proteasome function compared with pretreatment controls. The dose levels of 0.40, 1.04, 1.20, and 1.38 mg/m^2 produced inhibitions of 36%, 60%, 65%, and 74%, respectively. As shown in Fig. 3, significant proteasome inhibition was seen within 1 h of dosing, after which the level of inhibition slowly decayed [8]. The level of inhibition returned toward baseline by 72 h. No cumulative increase in proteasome inhibition was noted with multiple cycles of bortezomib treatment, suggesting that the 72-h interval was adequate to allow for recovery of normal function [8].

Nine of the 12 patients with plasma cell dyscrasias completed at least one full cycle of therapy and were assessable for response. A complete response was documented in a patient with IgG-κ myeloma treated at the 1.04 mg/m^2 dose level. The patient was refractory to prior treatment with vincristine, doxorubicin, and dexamethasone, followed by topotecan and dexamethasone.

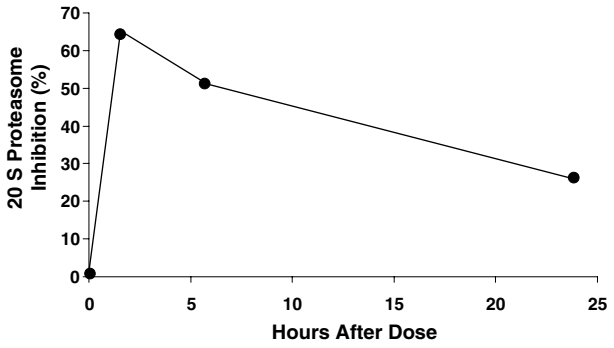

Fig. 3. Bortezomib pharmacodynamics as a function of time. 20S proteasome inhibition 1, 6, and 24 h after bortezomib administration in patients treated at 1.04 mg/m^2 ($n = 12$) (means ± SEM) [8].

Before starting bortezomib, bone marrow biopsy showed 41% plasma cells (Fig. 4(a)) [8]. Pretreatment immunoglobulins included an IgA of 25 mg/dL (normal 40–390), IgM of 15 (normal 25–210), and IgG of 3011 (normal 525–1650). Following cycle 1 of bortezomib, bone marrow plasmacytosis declined to 1% (Fig. 4(b)) [8]. IgA and IgM rose to 69 and 44 mg/dL, respectively, and IgG declined to 1215. Immunofixation showed large monoclonal IgG heavy-chain and κ-light-chain bands (Fig. 4(c)) [8]. After three cycles of treatment, the immunofixation took on a polyclonal pattern indicative of a complete response (Fig. 4(d)) [8]. The patient received four cycles of bortezomib in total. In follow-up, a faint monoclonal band reappeared after 6 months, but the patient remained asymptomatic without further antimyeloma therapy 1 year later. The other eight patients with myeloma experienced either a minor response or stable disease. Partial responses were also observed in one patient with mantle cell lymphoma and one patient with refractory follicular lymphoma [8].

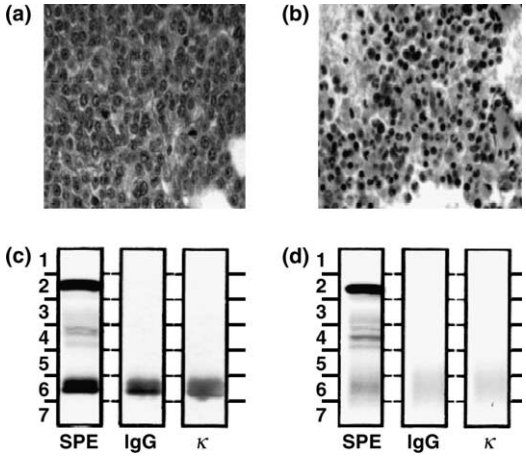

Fig. 4. Response of a patient with IgG myeloma to bortezomib. Patient's bone marrow biopsy before therapy (a) and after cycle 1 of bortezomib (b). Patient's serum protein electrophoresis (SPE) and immunofixation for IgG and κ-light chains before therapy (c) and after cycle 3 of bortezomib (d) [8].

6. Combined analysis of safety and tolerability

Overall, more than 200 patients have been treated with bortezomib in phase I trials. In a pooled analysis of the phase I data, the most commonly reported adverse events were fatigue and gastrointestinal symptoms, including nausea, vomiting, constipation, and diarrhoea (Millennium Pharmaceuticals, Inc. Data on file). These symptoms (more often seen at doses >1.3 mg/m^2) were generally grades 1–2 and led to treatment discontinuation in only 2–3% of cases. Peripheral neuropathy occurred less frequently, but was potentially dose limiting. The incidence of neuropathy, which tended to occur in patients who had received prior treatment with neurotoxic agents, was also dose-related (Millennium Pharmaceuticals, Inc. Data on file).

Based on the phase I results, a dose schedule of bortezomib twice weekly for 2 weeks followed by a 10-day rest was recommended for further clinical development. The dose of 1.3 mg/m^2 given according to this schedule was selected for the large phase II trial of bortezomib as a single agent in patients with relapsed and refractory multiple myeloma (SUMMIT).

7. Summary and conclusions

In early clinical trials, the novel proteasome inhibitor bortezomib shows promising antitumour activity in patients with both solid tumours and haematological malignancies. Bortezomib is biologically active, producing predictable, dose-related, and reversible proteasome inhibition. Treatment with bortezomib is generally well tolerated. The most common adverse effects include gastrointestinal symptoms and fatigue. Peripheral neuropathy occurs less frequently, mainly in patients with prior exposure to neurotoxic agents. Side effects are generally dose-related, mild to moderate in severity, and reversible upon dose reduction and/or cessation of bortezomib therapy.

Based on the promising early clinical trial results, bortezomib is being actively investigated for treatment of multiple myeloma, non-Hodgkin's lymphoma, and a variety of solid tumours. These studies include an evaluation of the use of bortezomib in patients with impaired renal function, and an analysis of hepatic impairment is also planned.

Conflict of interest statement

Prof. Kaye has received honoraria from Millennium Pharmaceuticals, Inc. and Johnson & Johnson.

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