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Review

The proteasome: A worthwhile target for the treatment of solid tumours?

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ABSTRACT

Proteasomes have a fundamental function since they degrade numerous different proteins, including those involved in the regulation of the cell cycle. Proteasome inhibition is a novel approach to the treatment of solid tumours. PS-341 (bortezomib) is a small, cell-permeable molecule that selectively inhibits the proteasome binding it in a reversible manner. The proteasome has been established as an important target in haematologic malignancies and has been approved for the treatment of multiple myeloma. Bortezomib induces apoptosis of malignant cells through the inhibition of NF-κB and stabilisation of proapoptotic proteins. In preclinical studies, bortezomib also promoted chemo and radiosensitisation of malignant cells *in vitro* and inhibited tumour growth in murine xenografts models. The single-agent and combination studies of bortezomib in solid tumours are detailed.

1. Introduction

The balance between protein synthesis and degradation is essential for normal cellular functioning. The proteasome is a multicatalytic enzyme complex that degrades several intracellular proteins by a targeted and controlled mechanism.[1,2] The ubiquitin-proteasome pathway degrades intracellular proteins which mediate various cellular functions such as transcription, stress response, cell cycle regulation, oncogenesis, ribosome biogenesis, cellular differentiation, and DNA repair.[3] The capacity of proteasome for degradation of tumour-suppressing and proapoptotic protein targets known to be dysregulated in many human malignancies provides the rationale for its selection as a target for cancer therapy. Moreover, preclinical studies have shown that proteasome inhibition decreases proliferation, induces apoptosis, enhances the activity of chemotherapy and radiation, and reverses chemoresistance in a variety of haematologic and solid malignancy models *in vitro* and *in vivo*.

PS-341 (bortezomib) is the first proteasome inhibitor investigated in clinical trials. It is approved in the United States and Europe for treating multiple myeloma patients who have received at least one prior therapy. Two phase II trials have shown that treatment with bortezomib, alone or in combination with dexamethasone, produced durable responses with meaningful survival benefits in patients with recurrent and/

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or refractory multiple myeloma.[4,5] In the APEX phase III study, comparing bortezomib and dexamethasone in patients with multiple myeloma who had had a relapse after one to three previous therapies, the proteasome inhibitor yielded a rate of 6% complete and 32% partial responses versus 1% and 17%, respectively, for dexamethasone. The median time to progression was significantly increased from 106 days with dexamethasone to 189 days with bortezomib and the 1-year overall survival was also higher in the bortezomib arm (80% versus 66%).[6]

Bortezomib has also shown activity in preclinical studies of a variety of solid tumours, such as breast, gastric, colon, non-small lung cancer (NSCLC), pancreas, and this has prompted several phase I/II clinical studies. Moreover, additional understanding of the mechanisms of action of proteasome inhibitors has led to their incorporation into combination regimens with both standard chemotherapeutics and novel agents. Taken together, these studies demonstrate the power of rational drug design and development to provide novel effective therapies for patients with haematological and solid malignancies.

In this paper we mainly focus on the way the proteasome works, and on the anticancer effects of bortezomib, with particular emphasis on preclinical and clinical studies in solid tumours.

2. Mechanism of action of proteasome

The ubiquitin-mediated proteasome pathway regulates a group of intracellular proteins that govern cell cycle, tumour growth, and survival (Fig. 1). This pathway is the principal mechanism of degradation for short-lived cellular regulatory proteins, including p53, cyclins and the cyclin-dependent kinase (CDK) inhibitors p21 and p27, the oestrogen receptor, and the inhibitor (IκB) of nuclear transcription factor kappa B (NF-κB).[7–11] 26S proteasome consists of a multisubunit, cylindrical complex including a 20S core catalytic component and 19S regulatory particles that contain polyubiquitin-binding sites and isopeptidase activity for the cleavage and release of ubiquitin from the protein substrate.[12] The proteasome requires adenosine triphosphate (ATP) hydrolysis and regulates multicatalytic protease that selectively degrades polyubiquinated proteins. These proteins are degraded by a multistep process that involves specific protein ligases. The first step includes protein mark with a chain of small polypeptides named ubiquitin; ubiquitin-activating enzyme (E1) activates ubiquitin molecule to the protein and, consequently, a long polypeptide chain of ubiquitin moieties is formed; finally, the multi-enzyme proteolytic complex 26S proteasome degrades protein into small fragments in an ATP-dependent manner.[3,13] In particular, the proteasome degrades a wide range of protein substrates involved in cell cycle regulation, apoptosis and other cellular functions. Controlled transitions between cell cycle stage depend on the timely activation of cyclins and CDK complexes. CDKs are serine/threonine kinases that are activated upon association with regulatory cyclin subunits at specific phases during cell-cycle progression. Expression of specific cyclins is regulated differentially by proteasome degradation during each phase of the cell cycle. In addition, the activity of CDKs is regulated further by a variety of inhibitor factors, such as $p21^{Cip1}$ $p27^{Kip1}$, that are able to prevent the formation of a variety of CDK-cyclin complexes and to arrest cell-cycle progression; both $p21^{Cip1}$ and $p27^{Kip1}$ are also proteasomal substrates.[14] The tumour suppressor protein p53 is another important substrate for proteasomal degradation. Activated p53 arrests cells in the G1-phase and promotes apoptosis to allow elimination of damaged cells through induction of the proapoptotic protein Bax, which, in turn, is also a proteasomal substrate. Taken together, these findings suggest that proteasome inhibition results in the stabilisation of p53, $p21^{Cip1}$, $p27^{Kip1}$ and Bax, dysregulation of cell-cycle progression and, finally, apoptosis.[15]

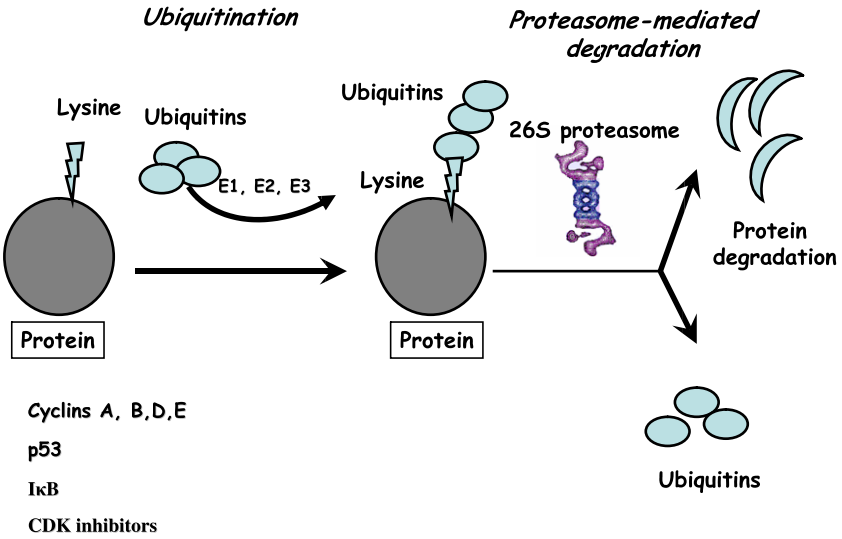

Fig. 1 – The ubiquitin-proteasome pathway is shown. On the left, the ubiquitination mechanism is explained: polyubiquitinated tails are added to specific lysine moieties on the protein. On the right, the proteasome-mediated degradation is shown: ubiquitinated proteins are degraded by the 26S proteasome. I-κB: nuclear factor-kappa B inhibitor; CDK: cyclin-dependant kinase; E1: ubiquitin-activating enzyme; E2: ubiquitin-conjugating enzyme; E3: ubiquitin ligase.

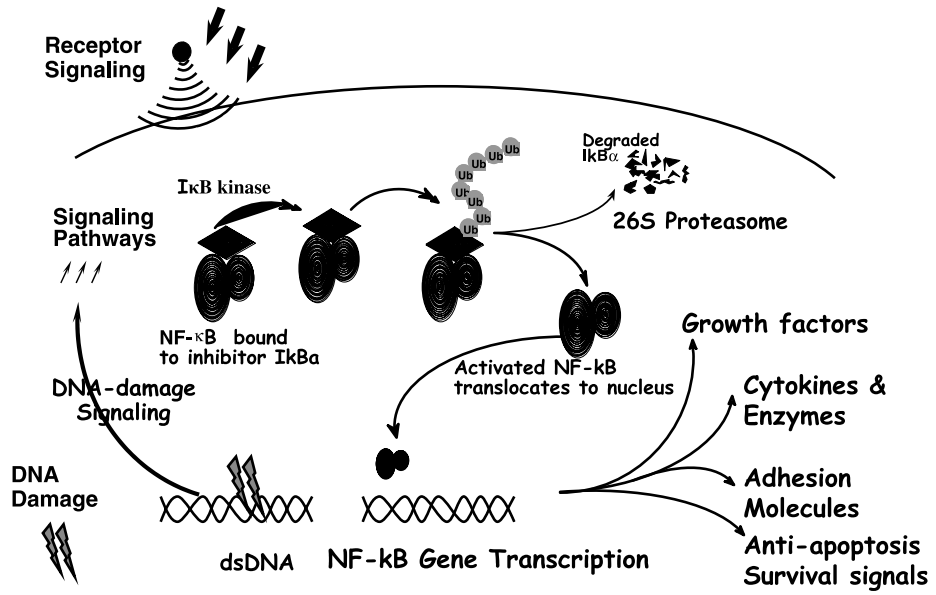

Fig. 2 – *NF-κB* activation pathway is shown. Several factors induce degradation of *IκB* by the proteasome. Once released from IκB inhibition, NF-κB translocates to the nucleus to activate genes that protect the cell from apoptosis, promote cell growth, stimulate angiogenesis. NF-κB: Nuclear factor-κB; I-κB: nuclear factor-kappa B inhibitor; dsDNA: DNA double-strand.

The proteasome is also important in modulating the activity of NF-κB (Fig. 2). This nuclear factor regulates various immune and inflammatory responses, but it may also play a main role in tumourigenesis by stimulating cell proliferation, blocking apoptosis, inducing angiogenesis. So the dysregulation of this pathway is probably an important component of uncontrolled cell growth in some malignancies. In fact, in quiescent cells, its regulatory protein inhibitor, IκB, binds to NF-κB in the cytoplasm and prevents its translocation into the nucleus. The NF-κB pathway is activated by a variety of cellular stress signals, chemo and radiotherapy, which lead to phosphorylation of a serine residue on IκB, that targets it for ubiquitination and proteasomal degradation. This process allows activated NF-κB subunit to translocate into the nucleus, where it induces expression of a variety of genes encoding cell adhesion molecules and antiapoptotic factors.[16,17] NF-κB has also been implicated in controlling gene expression of endothelial cell surface adhesion molecules, such as intercellular adhesion molecule 1, vascular cell adhesion molecule, and E-selectin,[18] which are involved in tumour metastasis and angiogenesis.[19]

3. Preclinical studies of proteasome inhibitor

3.1. *Single-agent and combination studies with bortezomib*

Based on the unique potential for cellular regulation via the ubiquitin-proteasome pathway, proteasome inhibitors have been developed and shown to be potentially cytotoxic against a variety of cancer cell lines *in vitro* and in *in vivo* models. Bortezomib is a novel dipeptide boronate, cell-permeable molecule that selectively inhibits the proteasome by binding it in a reversible manner. Moreover, bortezomib induces expression and increases stability of p53 and up-regulated p53-induced gene expression implicated in the induction of apoptosis.[20,21] The G_2/M phase arrest by bortezomib was shown to occur via drug-induced stabilisation of p53 protein and induction of p21 and MDM2 proteins, as well as the accumulation and stimulation of G_2/M phase-related regulators such as cyclins A and B.[22] The activity of bortezomib in solid tumours *in vivo* has been evaluated in a variety of xenograft models. Intravenous single-agent bortezomib at a dose of 1.0 mg/kg given weekly or twice weekly reduced tumour growth in nude mice bearing palpable prostate or pancreatic tumours by 50–80%.[23,24]

Bortezomib also increases the sensitivity of tumour cells to chemotherapy and radiation and reverses chemoresistance. In fact, experimental evidence strongly implicates the activity of NF-κB in promoting chemoresistance, tumour metastasis and angiogenesis. The sensitivity of chemoresistant myeloma cells to chemotherapeutic agents was markedly increased (100,000–1,000,000-fold) when combined with a noncytotoxic dose of bortezomib without affecting normal haematopoietic cells; so, these results suggest that inhibition of NF-κB with bortezomib may overcome chemoresistance.[24,25] In colon carcinoma cells, bortezomib inhibited the radiation-induced increase in NF-κB and enhanced radiosensitivity.[26]

The ability of proteasome inhibitors to target NF-κB was one rationale for the use of these agents alone, but also provided a basis for combination regimens. Many chemotherapeutics induce NF-κB and thereby activate an antiapoptotic program that, if inhibited, can enhance the antitumour activity of the chemotherapeutic.[27] Inhibition of the proteasome was shown initially to increase the efficacy of CPT-11 (irinotecan) through blockade of NF-κB in a model of colon cancer.[28] In another study, gemcitabine caused a 59% reduction of pancreatic cancer volume compared with control,[29] while the combination of gemcitabine and bortezomib increased growth inhibition to 75%.

Proteasome inhibition with bortezomib in combination with other agents was able to enhance chemosensitivity, overcome chemoresistance, and in some cases induce synergistic anti-myeloma effects *in vitro*.[25,30] Modulation of proteasome function may also enhance the therapeutic effects of some chemotherapeutics through other pathways, including inhibition of maturation of P-glycoprotein,[31] and suppression of the cell's DNA damage repair pathways.[32]

3.2. *Cross talk between proteasome and tyrosine kinase pathway*

The dysregulation of a variety of pathways, such as NF-κB, epidermal growth factor receptor (EGFR) and Ras/PI3K/Akt, is very common in solid tumours. It is known that bortezomib also interferes with the p44/42 mitogen-activated protein kinase (MAPK), a downstream effector of EGFR pathway that communicates proliferative signals, and induces accumulation of cyclin-dependent kinase inhibitors $p21^{Cip1}$ and $p27^{Kip1}$.[33] On the basis of this assumption, a preclinical study was carried out to evaluate the effect of proteasome inhibitor on EGFR survival signalling in pancreatic cancer cells. It was observed that bortezomib up-regulated the phosphorylation of EGFR and other downstream effectors increasing EGFR-dependence. When bortezomib was combined with an EGFR tyrosine kinase inhibitor, (gefitinib or erlotinib), apoptosis was significantly enhanced.[34] In addition to these findings, the antiproliferative activity of bortezomib alone or in combination with either gefitinib or cetuximab, a monoclonal anti-EGFR antibody, was evaluated in human lung, colon, pancreatic and oesophageal cancer cell lines. A significant synergistic antiproliferative effect was observed with the combined treatment of bortezomib and each EGFR-inhibitor, causing an efficient suppression in phosphorylated (p) EGFR, pMAPK, pAkt levels with a parallel significant increase in $p27^{Kip1}$ protein.[35] Moreover, the growth inhibitory effects of the combination of bortezomib plus tipifarnib, a farnesyl transferase inhibitor, were examined in head and neck squamous cell carcinoma lines. The combined treatment resulted in both significantly increased apoptosis and G2-M arrest.[36]

These preliminary results suggest that there is the rational basis to translate into a clinical setting the combination of a proteasome inhibitor with an EGFR inhibitor as a multi-targeted treatment for human cancer.

4. Clinical studies

The clinical feasibility of using bortezomib for treating solid malignancies has been explored in a number of phase I and II studies, the main of which are summarised below.

4.1. *Phase I single agent studies*

A number of phase I trials have been carried out with different schedules of bortezomib. A phase I clinical study evaluated the dose-limiting toxicity (DLT) and maximum-tolerated dose (MTD) of bortezomib as single-agent administered as an intravenous bolus once-weekly for 4 out of 5 weeks (doses ranging from 0.13 to 2.0 mg/m^2), in 53 patients, 48 of whom had advanced androgen-independent prostate cancer. The DLT was seen in two of five patients treated at the 2.0 mg/m^2/dose level, and it included grade 3 diarrhoea in both patients and grade 3 syncope and hypotension in one patient; so, the recommended phase II dose of bortezomib was 1.6 mg/m^2. The inhibition of proteasome activity was partially reversed by the time of the next dose administration with this weekly schedule. Two patients with prostate cancer had prostate-specific antigen response, whereas two patients had partial response in lymph nodes. The biologic activity, such as inhibition of NF-κB related markers, was seen at tolerated doses of bortezomib. The maximum level of 20S inhibition was 70 to 75%, which suggests that the inhibition of proteasome is saturable.[37] Another phase I study tested two different schedules (schedule 1: twice weekly for 4 out of 6 weeks; schedule 2: twice weekly for 2 out of 3 weeks) of bortezomib in 44 patients with advanced cancers. The most common toxicity was thrombocytopenia, which was dose limiting at 1.7 mg/m^2 (schedule 1) and 1.6 mg/m^2 (schedule 2), respectively, whereas the MTD was 1.5 mg/m^2 for both schedules. Moreover, other side effects were fatigue, myalgia, and sensory neuropathy for schedule 1, and dehydration, hypotension, hypoglicemia for schedule 2. A patient with multiple myeloma had a partial response.[38] This schedule should be further examined in phase II trials and may prove useful to be used within combination chemotherapy trials.

Bortezomib was given at a starting dose of 1.0 mg/m^2 on days 1, 4, 8, 11, every 3 weeks in a phase I/II study in 18 patients with unresectable hepatocellular carcinoma. No grade 4 DLTs in cycle 1 occurred, while grade 2/3 toxicities included thrombocytopenia, fatigue and neuropathy. Based on observed toxicities in all cycles, 1.3 mg/m^2 was considered MTD. In 7/15 evaluable patients, a stable disease was observed.[39] The phase I single-agent studies with bortezomib are summarised in Table 1.

4.2. *Phase I combination studies*

Following preclinical studies which highlighted the synergy between bortezomib and taxanes,[40,41] a phase I trial of twice-weekly bortezomib (day 2, 5, 9, 12) and weekly docetaxel was carried out; the recommended doses were 0.8 mg/m^2 and 25 mg/m^2, respectively, every 21 days. The DLTs were thrombocytopenia and febrile neutropenia. Other common side effects were anaemia and fatigue. The clinical activity was modest in this pretreated patient population, since only four patients had stable disease as best observed response.[42] In another phase I study, the combination of paclitaxel and bortezomib was evaluated. Twenty-five patients with advanced solid tumours were treated with escalating doses of weekly bortezomib and paclitaxel. The main toxicities were grade 3 fatigue and neurotoxicity. One patient with advanced pancreatic cancer achieved a partial response, while another patient had stable disease.[43]

Another phase I clinical trial evaluated the safety and biologic effects of bortezomib and irinotecan coadministered in 51 patients. The MTD for the combination regimen was bortezomib 1.3 mg/m^2 twice a week and irinotecan 125 mg/m^2 days 1, 8, followed by a 1-week rest. Overall, the most common grade 3 or 4 nonhaematologic adverse events were fatigue, diarrhoea, nausea, and vomiting, whereas neutrope-

Table 1 – Phase I single agent studies of bortezomib

Patients population	Prostate cancer[37]	Advanced solid tumours[38]	Hepatocarcinoma[39]
N. patient	53	28 + 16	18
Schedule	B: starting dose 0.13 mg/m2 i.v. weekly q 4 every 5 weeks MTD: 1.6 mg/m^2	*Schedule 1:* B twice weekly for 4 out of 6 weeks MTD: 1.7 mg/m^2 *Schedule 2:* Twice weekly for 2 out of 3 weeks MTD: 1.6 mg/m^2	B: starting dose 1.0 mg/m^2 days 1, 4, 8, 11 q 3 weeks MTD: 1.3 mg/m^2
Response	2 PR + 2 PSA responses	1 PR in multiple myeloma	7 SD
Toxicities	Diarrhoea, hypotension	Neuropathy, fatigue	Thrombocytopenia, neuropathy, fatigue

B bortezomib; MTD maximum tolerated dose; SD stable disease; PR partial response.

nia was the most common haematologic event.[44] These results warrant further investigation, especially in cancers that are known to be responsive to irinotecan therapy. Preclinical studies have shown that proteasome inhibitors may overcome tumour resistance to gemcitabine by inducing reduction of NF-κB activity, down-regulation of Bcl-2, and stabilisation of $p21^{Cip1}$ and $p27^{Kip1}$.[29,45] These findings prompted a phase I trial to determine the MTD of escalating doses of gemcitabine (1000 mg/m^2 given once a week for 2 weeks) with bortezomib (1.0 mg/m^2 given twice a week) every 21 days, in 31 patients with advanced solid tumours. This combination was well tolerated with a toxicity profile similar to the other phase I combination studies and exhibited preliminary evidence of antitumour activity as reflected by a partial response in a patient with advanced NSCLC. Notably, this patient had previously had recurrence after combined modality therapy that included gemcitabine.[46] Studies on the intracellular kinetics of gemcitabine phosphorylation, accumulation, and disposition in tumours exposed to both gemcitabine and bortezomib could yield important information about potential synergy between these agents, and mechanisms of action of bortezomib preventing gemcitabine resistance.[47] Another phase I study was carried out to evaluate the combination between 5-fluorouracil (5FU) 500 mg/m^2 and leucovorin (LV) 20 mg/m^2 with starting dose of bortezomib 0.5 mg/m^2 twice weekly for 4 weeks, with 2 weeks rest. Nineteen patients were evaluable for response: one partial response (oesophageal), eight stable disease (seven colorectal, one anal) and ten progressive disease were achieved.[48] In another phase I study, bortezomib was evaluated in combination with gemcitabine and carboplatin in 16 patients with advanced NSCLC, ten of whom were chemo-naïve. The recommended phase II doses for this regimen are: bortezomib 1.0 mg/m^2, gemcitabine 1000 mg/m^2 and carboplatin area under the curve (AUC) = 5. In ten evaluable patients, four partial responses and five stable diseases were achieved.[49]

Finally, 15 patients with advanced ovarian cancer who had received upfront chemotherapy and up to two prior chemotherapy regimens for recurrent disease were treated with a fixed dose of carboplatin (AUC = 5) in combination with escalating dose of bortezomib administered twice weekly for 2 weeks every 21 days. The overall response rate to this combination was 47%, with two complete responses and five partial responses, including one complete response in a patient with platinum-resistant disease.[50] A Gynaecologic Oncology Group phase II trial of single-agent bortezomib in recurrent ovarian cancer is currently ongoing.

Phase I combination studies with bortezomib are summarised in Tables 2 and 3.

4.3. *Phase II single agent studies*

A large number of phase II studies of single-agent bortezomib have been carried out or are currently underway.

A phase II trial of bortezomib was carried out in 27 patients with metastatic malignant melanoma. It was closed at the

Table 2 – Phase I combination studies of bortezomib

Patients population	Advanced solid tumours[42]	Advanced solid tumours[43]	Advanced solid tumours[44]	Advanced solid tumours[46]
N. patient	14	25	51	31
Schedule	B starting dose 0.8 mg/m2 day 2, 5, 9, 12 Docetaxel starting dose 25 mg/m2 day 1, 8 q 3 weeks	B starting dose 0.6 mg/m2 day 2, 5, 9, 12 Paclitaxel starting dose 80 mg/m2 day 1, 8 q 3 weeks	B starting dose 1.3 mg/m2 day twice a week + Irinotecan 125 mg/m^2 days 1,8 q 3 weeks	B starting dose 1.0 mg/m^2 twice a week Gem 1000 mg/m^2 given once a week for 2 weeks q 3 weeks
Response	4 SD	1 PR	10 SD	1 PR, 7 SD
Toxicities	Haematologic	Neurotoxicity, fatigue	Diarrhoea, nausea vomiting	Abdominals pain, Haematologic

B bortezomib; CR complete response; SD stable disease; PR partial response; Gem gemicitabine.

Table 3 – Phase I combination studies of bortezomib

Patients population	Advanced solid tumours[48]	Advanced NSCLC[49]	Advanced ovarian tumours[50]
N. patient	21	16	15
Schedule	B starting dose 0.5 mg/m^2 twice weekly 5-FU 500 mg/m2 LV 20 mg/m2 for 4 weeks out of 6	B starting dose 1.0 mg/m^2 days 1,4, 8, 11 GEM starting dose 800 mg/m^2 days 1, 8 CBCDA AUC 5 day 1	B starting dose 0.75 mg/m^2 days 1, 4, 8, 11 + CBCDA AUC 5 day 1
Response	1 PR, 8 SD	4 PR, 5 SD	2 CR, 5 PR
Toxicities	Abdominals pain, diarrhoea	Myelosoppression	Diarrhoea

B bortezomib; CR complete response; SD stable disease; PR partial response; CBCDA carboplatin; GEM gemicitabine; 5-FU fluorouracil; LV leucovorin.

planned interim analysis due to early evidence of lack of clinical activity. In fact, there were no major clinical responses and only six patients (22%) achieved a stable disease. The median time to disease progression was 1.5 months, with a median overall survival of 14.5 months. Based on these data, further testing of single agent bortezomib in patients with metastatic melanoma is not warranted, but, based on preclinical models of potential synergy with chemotherapy, exploration of combination regimens in this disease may be worthwhile.[51] Similar results were observed in a phase II study in which 25 patients with a variety of recurrent or metastatic sarcomas were included. Due to the lack of clinical activity, the study was closed after the first stage of accrual. Median survival was 10.1 months; thirteen patients had disease progression after a median of 1.4 months, while one confirmed partial response was achieved in a patient with a metastatic retroperitoneal leiomyosarcoma.[52] Another phase II study was carried out in 16 patients with metastatic neuroendocrine tumours, on the basis of preclinical activities of bortezomib in PC-12 neuroendocrine (pheochromocytoma) tumour cell line.[53] Although achieving the surrogate biological end point of 20S proteasome inhibition in peripheral blood mononuclear cells obtained from 15 patients, this study failed to show any objective tumour response. Stable disease was observed in 11 of 16 patients (69%) at the median evaluation time of 12 weeks (range, 3 to 24 weeks).[54] Bortezomib also showed lack of clinical activity in colorectal cancer, since only three patients out of 19 had a stable disease as best observed response.[55]

A phase II study was conducted in metastatic renal carcinoma, based on the putative anti-angiogenic role of bortezomib. In particular, four (11%) of 37 patients in this study achieved a partial response, whereas 14 patients (38%) had stable disease and 19 patients had disease progression. Although the response rate was low, the duration of these responses, which ranged between 8 and 20 months, suggest that bortezomib has an antitumour effect in individual patients with metastatic renal carcinoma.[56] A multi-institution phase II study was conducted in 23 patients with metastatic renal carcinoma. This study was closed after a planned interim analysis revealed only one objective response.[57] Efforts to identify molecular features predicting response may be warranted, in addition to exploration of combination therapy with interferon alfa or new active agents targeting the vascular endothelial growth factor (VEGF) pathway.

Two phase II studies that were conducted in metastatic breast cancer did not report any evidence of clinical activity.[58,59] In one of these studies, pharmacologic and biologic activities were also evaluated in eight of the 12 patients. Although bortezomib was able to inhibit proteasome activity and reduce the circulating levels of IL-6, these biologic effects did not translate into a therapeutic benefit leading to the conclusion that single-agent bortezomib does not have clinically significant activity in metastatic breast cancer. These results can be partially explained by the observation that patients enrolled in this study had particularly aggressive metastatic disease with extremely poor prognosis and low probability of response to additional therapy.[59]

Single-agent bortezomib does not have activity in patients with metastatic solid tumours. Bortezomib is safe at the schedule using in all cited studies (1.5 mg/m^2 twice weekly for 2 weeks every 3 weeks), the most significant clinical adverse event being a peripheral sensory neuropathy. The detailed evaluation of bortezomib-associated neurotoxicity, including autonomic neuropathy, and the value of prophylactic strategies, deserve future investigation. Furthermore, with growing preclinical data demonstrating synergism between bortezomib and cytotoxic chemotherapy, future studies should evaluate bortezomib in combination with chemotherapy in solid tumours but caution should be taken in combining bortezomib with agents that may have overlapping gastrointestinal or neurologic toxicity. An appropriate stratification using novel available technologies should help us in the selection of patients who are likely to respond to bortezomib administered either as single agent or in combination. Phase II studies of single-agent bortezomib are summarised in Tables 4 and 5.

4.4. *Phase II combination studies*

Bortezomib may have significant anti-tumour activity when used in combination with other active conventional agents. The combination of irinotecan and bortezomib in patients with advanced colorectal cancer has been evaluated but no response data are available yet.[60]

A randomised phase II study was conducted in 87 patients with metastatic pancreatic cancer, who were randomised to receive bortezomib alone (1.5 mg/m^2 twice weekly for 2 weeks

Table 4 – Phase II single agent studies of bortezomib

Patients population	Metastatic melanoma[51]	Recurrent or metastatic soft tissue sarcoma[52]	Metastatic neuroendocrine tumours[54]
N. patient	27	25	16
Schedule	B:1.5 mg/m^2 on Days 1, 4, 8, 11 q 3 weeks	B:1.5 mg/m^2 on days 1, 4, 8, 11 q 3 weeks	B:1.5 mg/m^2 on days 1, 4, 8, 11 q 3 weeks
Response	6 SD	1/21 PR	11 SD
Toxicities	Neuropathy, fatigue, thrombocytopenia	Neuropathy, myalgia, fatigue	Neuropathy, diarrhoea, vomiting

B bortezomib; PR partial response; SD stable disease.

Table 5 – Phase II single agent studies of bortezomib

Patients population	Metastatic Colorectal[55]	Advanced renal tumours[56]	Advanced renal tumours[57]	Metastatic breast cancer[58]	Metastatic breast cancer[59]
N. patient	19	37	23	12	12
Schedule	B:1.5 mg/m^2 on days 1, 4, 8, 11 q 3 weeks	B:1.5mg/m^2 on days 1, 4, 8, 11 q 3 weeks	B:1.5 mg/m^2 on days 1, 4, 8, 11 q 3 weeks	B:1.5 mg/m^2 on days 1, 4, 8, 11 q 3 weeks	B:1.5 mg/m^2 on days 1, 4, 8, 11 q 3 weeks
Response	3/19 SD	4 PR; 14 SD	1/21 PR	NO OR	1 SD
Toxicities	Neuropathy, myalgia	Neuropathy	Neuropathy	Fatigue	Thrombocytopenia, fatigue

B bortezomib; CR complete response; SD stable disease; PR partial response OR objective response; NSCLC non-small cell lung carcinoma.

every 3 weeks) or the combination of bortezomib (1.0 mg/m^2 twice weekly for 2 weeks every 3 weeks) plus gemcitabine (1000 mg/m^2 days 1,8 every 3 weeks). The response rate was 0% in the arm with bortezomib alone (42 evaluable patients), with median survival of 2.5 months (95% CI 2.0–3.3) and median time to progression of 1.2 months (95% CI 1.1–1.3). However, four patients achieved a partial response (RR = 10%), in the combination arm (39 evaluable patients). In addition to these findings, the dose of bortezomib was lowered from 1.5 to 1.3 mg/m^2 in the arm with bortezomib alone following the observation of an higher than expected rate of grade 3/4 events, such as haematologic toxicities. Bortezomib used alone or in combination with gemcitabine provided no benefit to patients with metastatic pancreatic cancer. It is possible that the sequence of administration may influence response, but further preclinical work is needed to determine the antineoplastic activity of bortezomib in pancreatic cancer and how this may be translated into future clinical trials.[61]

Fanucchi et al. investigated the safety and efficacy of bortezomib monotherapy (arm A) compared with the combination of bortezomib and docetaxel (arm B) as second-line therapy in 155 patients with locally advanced and metastatic NSCLC. Overall response rate was 8% (90% CI; 3.5% to 15.2%) in arm A and 9% (90% CI; 4.2% to 15.8%) in arm B. Time to response was 36 to 83 days in arm A, with five of six patients responding within 40 days, and 38 to 99 days in arm B, with two of seven patients responding within 41 days.[62] This study was not powered to demonstrate differences between treatment arms; however, bortezomib plus docetaxel seemed to demonstrate modest benefit compared with bortezomib monotherapy. Nevertheless, the combination of bortezomib plus docetaxel was not as active as it might have been expected based on preclinical results. These results were also comparable with the 8.9% response rate reported by Shepherd et al.[63] with erlotinib in a phase III study in second- and third-line advanced NSCLC. Median overall survival of 7.4 months

Table 6 – Phase II combination studies of bortezomib

Patients population	Colorectal carcinoma[60]	Metastatic pancreatic carcinoma[61]	Pretreated NSCLC[62]
N. patient	68	87	75 + 80
Schedule	*Arm 1* B: 1.5 mg/m^2 on days 1, 4, 8, 11 q 3 weeks *Arm 2* B: 1.3 mg/m^2 on days 1, 4, 8, 11 Cpt-11 125mg/m^{2}q 3 weeks	*Arm 1* B: 1.5 mg/m^2 on days 1, 4, 8, 11 q 3 weeks *Arm 2* B: 1.0 mg/m^2 on days 1, 4, 8, 11 Gemcitabine 1000mg/m^{2}q 3 weeks	*Arm 1* B:1.5 mg/m^2 on days 1, 4, 8, 11 q 3 weeks *Arm 2* B:1.3 mg/m^2 on days 1, 4, 8, 11 Docetaxel 75 mg/m^2 q 3 weeks
Response	NA	RR: 0% (arm 1) RR: 10 % (arm 2)	6 PR, 16 SD (arm 1) 7PR, 36 SD (arm 2)
Toxicities	Haematologic, neuropathy, fatigue	Abdominal pain, fatigue, neuropathy	Neutropenia, neuropathy, fatigue

B bortezomib; SD stable disease; PR partial response; RR response rate; NA not available.

and 1-year survival probability of 38.7% with bortezomib monotherapy are also comparable to results from these studies. Additional studies will be needed to determine the most effective way to combine this drug with taxanes as well as other cytotoxic agents in NSCLC. Phase II combination studies of bortezomib are summarised in Table 6.

5. Conclusion

The 26S proteasome acts as a housekeeper to eliminate damaged or misfolded proteins. In addition, many regulatory proteins governing the cell cycle, transcription factor activation, apoptosis, and cell trafficking, are the substrates for proteasome mediated degradation. Five years after entering clinical trials, bortezomib has demonstrated efficacy for the treatment of patients with recurrent and refractory multiple myeloma. The clinical results in multiple myeloma provide proof of concept for proteasome inhibition as an anticancer therapy, and the role of bortezomib in other types of cancer and in different settings is undergoing active investigation. More mature data are awaited eagerly.

Conflict of interest statement

None declared.

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