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Review Article

The Role of Proteasome Inhibition in Nonsmall Cell Lung Cancer

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Lung cancer therapy with current available chemotherapeutic agents is mainly palliative. For these and other reasons there is now a great interest to find targeted therapies that can be effective not only palliating lung cancer or decreasing treatment-related toxicity, but also giving hope to cure these patients. It is already well known that the ubiquitin-proteasome system like other cellular pathways is critical for the proliferation and survival of cancer cells; thus, proteosome inhibition has become a very attractive anticancer therapy. There are several phase I and phase II clinical trials now in non-small cell lung cancer and small cell lung cancer using this potential target. Most of the trials use bortezomib in combination with chemotherapeutic agents. This paper tends to make a state-of-the-art review based on the available literature regarding the use of bortezomib as a single agent or in combination with chemotherapy in patients with lung cancer.

1. Introduction

One of the common strategies for cancer therapy is the targeting of cell homeostasis leading to deregulation of cell processes necessary for survival. In recent years, one of the novel approaches has been the deregulation of protein homeostasis through the obstruction of intracellular protein degradation. This has been done by targeting the ubiquitin-proteasome system (UPS). The UPS plays a central role in the targeted destruction of cellular proteins, including cell cycle regulatory proteins. Because these pathways are critical for the proliferation and survival of all cells, and in particular cancerous cells, proteasome inhibition is a very attractive anticancer therapy [1].

The first element of this pathway being investigated as a target is the proteosome. Because the proteasome degrades about 80% of all intracellular proteins [2], the use of a proteasome inhibitor triggers a mixed repertoire of tumor-suppressing and prosurvival pathways in cancer cells [3]. Its inhibition disturbs the critical intracellular balance between proapoptotic and antiapoptotic signals shifting it towards tumor growth inhibition, apoptosis, and decreased metastasis.

The proteasome inhibitor PS-341 (bortezomib), an already approved agent for the treatment of multiple myeloma, is under evaluation in clinical trials against various malignancies. Here we will review preclinical and clinical data involving this novel anticancer mechanism focusing primarily in the work that has been done in lung cancer. Bortezomib has been tested as single agent and most recently in combination with chemotherapeutic and targeted agents. Multiple targets that directly interact with the proteasome have been described and may represent future focuses of more research and possibly therapeutic development.

2. Action of the Ubiquitin-Proteasome System

The UPS regulates many normal cellular processes including signal transduction, cell cycle control, transcriptional regulation, inflammation, and apoptosis through protein degradation [4]. It requires a series of highly regulated and complex intracellular activities that have not been completely

Table 1: Antitumor and autoprotective mechanisms triggered by proteasome inhibition. Possible antitumor mechanisms of proteasome inhibitors.

(i) Accumulation of p53, p21, and p27
(ii) Differential effects on pro- and antiapoptotic members of Bcl-2 family
(iii) Downregulation of XIAP and survivins
(iv) Inhibition of inducible NF-κB activity
(v) Accumulation of misfolded proteins and endoplasmic reticulum stress
(vi) Induction of oxidative stress
(vii) Activation of bone morphogenetic protein signaling
(viii) Inhibition of protein translation
(ix) Inhibition of telomerase activity
(x) Downregulation of PI3 K/Akt signaling
(xi) Upregulation of death receptor
(xii) Histone acetylation
(xiii) Repression of E2F
(xiv) Inhibition of IL-6-mediated signaling
(xv) Suppression of FoxO and FoxMl proteins
(xvi) Tubulin stabilization
(xvii) Induction of mitotic catastrophe
(xviii) Inhibition of epithelial-mesenchymal transition
(xix) Inhibition of angiogenesis
(xx) Immunosensitization of cancer cells to the cytotoxicity of lymphocytes
(xxi) Increased genomic instability after exposure to ionizing radiation
(xxii) Overcoming multidrug resistance by inhibition of pglycoprotein
Autoregulatory mechanisms against proteasome inhibition
(i) Induction of macroautophagy
(ii) Activation of constitutive NF-κB activity
(iii) Activation of EGFR signaling
(iv) Stat3 phosphorylation
(v) Akt phosphorylation
(vi) Induction of hsp72 and AKR1B10
(vii) Upregulation of glutathione synthesis

Adapted from Wu et al. [3].

elucidated. In general, proteins are targeted for recognition and for subsequent degradation by the proteasome via the attachment of multiple ubiquitin molecules. In order to do this, there are several preparatory steps before proteins are presented to the proteasome. The first step involves the activation of ubiquitin by the formation of a thioester bond with the ubiquitin-activating enzyme (E1) in an ATP-dependent reaction. Then, E1 delivers the activated ubiquitin to the E2 ubiquitin-conjugating enzyme. Finally, E3 ligases transfer ubiquitin from E2 to a lysine residue in the substrate protein [5]. An ubiquitin chain subsequently forms and presents the protein to the 26 S proteasome. It is important to note, however, that these preparatory steps are not used for the degradation of all proteins. Some proteins such as calmodulin and troponin C undergo degradation by the proteasome via ubiquitin-independent pathway [6]. Ultimately, the protein enters the proteasome, ubiquitin is released (if the protein required preubiquination), and the protein is degraded.

The degradation of proteins inside the proteasome is similar to the degradation of proteins by intestinal digestive enzymes. In fact, the proteasome is considered to have chymotrypsin-like, trypsin-like, and peptidyl-glutamyl peptide-hydrolyzing- (PHGH-) like activity. The 26 S proteasome is a large multicatalytic complex that is comprised of a 20 S core catalytic component (the 20 S proteasome) capped at one or both ends by a 19 S regulatory component [1]. The 19 S lid serves as an entry portal for the proteins, which are then subjected to adenosine triphosphate (ATP) hydrolysis within the base. ATPases unfold and linearize large proteins before they undergo catalysis within the core. Allosteric interactions guide the intricate sequencing of proteolytic reactions within the core, which ultimately produces oligopeptides that can be recycled within the cell [6].

3. Bortezomib's Inhibition of the Proteasome

Because peptide boronic acids inhibit serine proteases such as chymotrypsin by mimicking substrate binding at the active site, it was postulated that they might inhibit the proteasome by binding to the chymotrypsin-like site in the 20 S core [1]. Adams synthesized 13 boronic acid proteasome inhibitors and tested them for their ability to inhibit cell growth against the panel of 60 cell lines from the National Cancer Institute. One compound, bortezomib, the boronic acid derivative which was later called bortezomib, was potent and was active against a broad range of cancer cell lines, including nonsmall cell lung, colon, central nervous system, melanoma, ovarian, renal, prostate, and breast cancers, and had a unique cytotoxicity profile, compared with the NCI's historical file of 60,000 compounds [1]. Since the publication of this study in 1999, bortezomib has been tested in numerous *in vitro* and *in vivo* models of several cancers including NSCLC [6].

4. Results of the Inhibition of the Ubiquitin-Proteasome System by Bortezomib

Numerous proteins are degraded by the proteasome, so multiple cellular processes are affected by proteasome inhibition. Therefore, the activity of bortezomib in different cancers may involve a variety of molecular mechanisms (see Table 1) [3]. Nevertheless, one protein that has been clearly implicated in the efficacy of bortezomib is NF-κB.

The proteasome has a direct role in allowing the cell to progress through the cell cycle by degrading cell cycle regulatory proteins and an indirect role by regulating the availability of transcriptional activators [1]. One transcriptional activator believed to have a central role in mediating many of the effects of proteasome inhibition is the transcriptional activator NF-κB [1]. This transcriptional activator is involved

Table 2: *In vitro* studies with bortezomib.

Carcinoma	Cell lines	Effects	References
Multiple myeloma	MM.1S, MM.1R, Dox40, MR20, LR5, RPMI8226, IM-9, U266, ARH-77, Hs Sultan	IkBa degradation, inhibited IL-6-triggered activation of p42/44 MAPK as well as TNF-a induced activation of NF-κB,	Hideshima et al., 2001 [7]
Mantle cell lymphoma	Mino, DB (sp53), Molt-4, L-428	NF-κB activation, bcl-xL and bfl/A1 inhibition, and bcl-2 cleavage	Pham et al., 2003 [8]
NSCLC	H460, H322, H358, H157, A549	Cell cycle arrest at G2-M; Bcl-2 phosphorylation and cleavage; p53 stabilization; induction of p21Cip; increase in cyclins A and B; activation of CDKs; mitochondrial cytochrome c release; activation of caspase pathway; apoptosis; NF-κB Downregulation	Ling et al., 2002 [9], 2003 [10], and 2003 [11]; Denlinger et al., 2004 [12]
Prostate	PC-3 (p53 null)	Cell cycle arrest at G2-M; increase in p21Cip; inhibition of CDK4 activity; PARP cleavage; apoptosis	Adams et al., 1999 [13]
	LNCaP-Pro5	Activation of caspase-3; apoptosis	Williams et al., 2003 [14]
Pancreatic	MIA-PaCa-2	Enhanced cytotoxic effects of gemcitabine; reduced NF-κB activation; reduced Bcl-2 expression without affecting Bax or Bak; PARP cleavage; apoptosis	Bold et al., 2001 [15]
	BxPC3	Cell cycle arrest in G0–G1; increase in p21Cip; caspase-3 activation; apoptosis	Shah et al., 2001 [16]
SCCHN	UM-SCC-9, UM-SCC-11B	Cell cycle arrest in G2-M and S phases; increase in p21Cip; apoptosis; (PARP cleavage shown in murine SCCHN lines); NF-κB Downregulation	Sunwoo et al., 2001 [17]
Ovarian	SKOV 3	Induction of p21Cip; inactivation of Bcl-xL; Downregulation of XIAP; PARP cleavage; activation of caspase pathway; apoptosis	Frankel et al., 2000 [18]
Breast	MCF-7	Cytotoxicity (molecular markers not determined)	Teicher et al., 1999 [19]
Colorectal	LOVO, KM12L4, WiDR	Inhibits chemotherapy-induced NF-κB activation; enhances chemotherapy-induced apoptosis; stabilizes p53, p21Cip; p27Kip	Cusack Jr. et al., 2001 [20]

Adapted from Ludwig et al. [21].

in inflammatory and immune responses, and its signaling pathways are implicated in tumor development [1].

This proto-oncogenic NF-κB pathway requires proteasomal activity. Under normal conditions, NF-κB factors are retained in an inactive state in the cytoplasm by the inhibitors of NF-κBs (IκBs). In order to be freed from this inhibition, IκBs need to be phosphorylated, polyubiquitylated, and degraded by the proteasome. Bortezomib downregulates NF-κB signaling by blocking IκB degradation [5], and this seems to be its prevalent mechanism of action, especially in multiple myeloma and certain solid tumors [21]. Inhibition of NF-κB reduces the expression of proinflammatory response genes and upregulates the cyclin-dependent kinase inhibitors p21Cip1 and p27Kip1, resulting in increased apoptosis in tumor cells [5].

Other important ways in which apoptosis is induced by bortezomib in various models was the induction of phosphorylation and subsequent cleavage of the antiapoptotic factor Bcl-2, the Upregulation of CDK inhibitors, such as p21Cip, stabilization of p53 [21], and interference with the unfolded protein response (UPR) leading to endoplasmatic reticulum stress and thus increased apoptosis [22]. Additionally, bortezomib sensitizes resistant solid tumor cells to TNF-like apoptosis, inducing ligand- (TRAIL-) induced apoptosis, probably by increasing the levels of death receptors DR4 and DR5 [23].

5. *In Vitro* Studies Showing the Effect of Bortezomib in Cancer

Extensive preclinical research has been conducted with bortezomib to elucidate its mechanism of action and to examine its activity. In cell culture, bortezomib induces apoptosis in both hematologic and solid tumor malignancies (see Table 2).

6. Proteasome Inhibitor Targets in Lung Cancer

As in part mentioned above, multiple targets of proteasome inhibition with different cellular effects have been identified,

among those the very important transcription factor directly involved in apoptosis resistance and expression of adhesion molecules is NF-κB. Usually inactive intracellularly due to binding to IkBα, it becomes activated after exposure to cytokines, stress, and receptor signaling, leading to apoptosis resistance, increase in growth factors, angiogenesis, and possible tumor metastasis. NF-κB activation is blocked via proteasome inhibition decreasing downstream signaling thus decreasing cell survival and growth [17]. Overexpression of the antiapoptotic protein Bcl-2 leads to chemoresistance; bortezomib causes downregulation of Bcl-2 via phosphorylation in NSCLC [9, 24], as well as decreased transcription of the Bcl-2 promoter, decreased Bcl-2 level, and induced apoptosis in SCLC [25]. An upregulation of Bax a proapoptotic mediator has proven beneficial leading to an increase benefit from proteasome inhibitor by decreasing Bcl-2/Bax ratio [15].

Cell cycle arrest in G2M phase can be induced by bortezomib in NSCLC which is in part due to accumulation of P53, which is crucial for transcription of genes involved in cell cycle and DNA synthesis [36]. The absence of cyclin-dependent kinase inhibitor p27 acts as poor prognostic factor in NSCLC, bortezomib causes upregulation of p21 and p27 kinase inhibitor leading to arrest of cell cycle inhibiting cyclin A and cyclin E [10, 24, 36].

Bortezomib has been also shown to enhance tumor necrosis factor related apoptosis inducing ligand (TRAIL) induced apoptosis in human cancer cells, bortezomib induced caspase 8 dependent apoptosis, cooperated with trail to induce apoptosis and up-regulated death receptor 5 (DR5) expression in NSCLC cells, which correlated with increased apoptosis by PS-341 and enhancement of TRAIL-induced apoptosis in NSCLC. On the other hand, c-FLIP and surviving levels were elevated after exposure to bortezomib, which in turn protects cells from bortezomib-induced apoptosis [37].

6.1. Phase I Single Agent Proteasome Inhibitors in Lung Cancer. Aghajanian et al. evaluated the safety and pharmacodynamic behavior of bortezomib in patients with histologically confirmed solid tumors who had been heavily pretreated and for which no other therapeutic options were available [38]. Forty-three patients were enrolled after eligibility criteria were met, and informed consent was signed; patients with 14 histologically different tumor types entered the study; among those, 8 patients had documented NSCLC. Prior treatment included a median number of 4 prior chemotherapy regimens, and 12 subjects had received radiation therapy as primary treatment for their malignancy. Forty-three patients received a total of 89 cycles of therapy given twice weekly for 2 consecutive weeks and followed by 1-week recovery period, doses ranged from 0.13 to 1.56 mg/m^2/dose (9 dose levels), with a median number of 2 courses given per patient.

Toxicities were minimal in the first five dose level groups; no hematological dose limiting toxicity was reported, with an increase in the incidence of thrombocytopenia and neutropenia at higher doses. Dose limiting nonhematological toxicities were reported and consisted mainly of diarrhea and painful sensory neuropathy; 2 out of 12 patients treated at the 1.56/m^2 dose developed grade 3 diarrhea and also another 2 out of 12 patients in the same dose group and one in a lower dose group (1.30 mg/m^2) developed grade 3 painful sensory neuropathy which had worsened from prior preexisting symptoms. All these patients had been exposed to taxanes and either carboplatin or cisplatin as prior therapies.

Pharmacodynamic studies revealed a dose-related inhibition of 20 S proteasome activity at higher doses, no significant difference in the mean percentage of inhibition at the 4 different dosing days after 1 hour of drug administration; complete recovery of proteasome activity to baseline was evident prior to drug administration on days 4, 8, and 11 indicating no apparent change to drug sensitivity towards bortezomib-induced proteasome inhibition. Proteasome activity also evaluated at 24 h after day 1 and day 8 dosing which showed recovery but not back to baseline values.

One partial response was seen in a patient with NSCLC who had received prior therapy with six cycles of paclitaxel and carboplatin, two cycles of gemcitabine, three of mitomycin and vinblastine, four weekly docetaxel, and eight weekly methotrexate doses, with disease progression on all of the above regimens; a 50% reduction in the size of bilateral pulmonary nodular infiltrate was seen, with a duration of three months, patient symptoms improved as well, but had to discontinue treatment after three cycles due to painful sensory neuropathy. Stable disease was seen in 3 patients with other tumor types with a mean duration of 4 months.

Dy et al. conducted another phase I and pharmacologic trial of two schedules of bortezomib in patients with advanced cancer [39]; the trial enrolled a total of 44 patients with multiple different tumor types. Of those 2 patients had lung cancer, most of them consisted of colorectal and kidney tumors followed by pancreatic and prostate cancer. 73 courses of therapy with 6 different dose levels (ranging from 0.5 to 1.70 mg/m^2) were administered; 28 patients received study treatment twice weekly for 4 out of 6 weeks, but due to increased toxicity on this schedule, 16 additional patients received study treatment only twice weekly for 2 out of every three weeks. The median number of courses given per patient was 2 in both schedules.

Hematological toxicities related to treatment grade >2 were anemia and thrombocytopenia, most of them occurring in schedule one. Reversible thrombocytopenia was dose limiting for both schedules at 1.60 and 1.70 mg/m^2 dose, no bleeding complications were associated with such nor need for platelet transfusion. Mild leukopenia was observed in one patient in schedule two. Most nonhematological toxicities were reported as mild to moderate consisting of fatigue, diarrhea, nausea, anorexia, sensory neurotoxicity, rash, and vomiting for schedule one; sensory neurotoxicity was dose limiting in one patient in this schedule. Similar side effects were reported in schedule two with the exception of rash and sensory neuropathy; two cases of grade 3 diarrhea were reported in schedule two which improved with dose reductions and the use of loperamide.

Forty-one patients out of the 44 enrolled were assessable for antitumor activity; partial regression (>50%) of a perinephric plasmacytoma was observed in one patient before

cycle 2 of treatment and was sustained for 4 months; five patients had stable disease in at least one evaluation. There was as in the previously described study a dose-dependant increase in the degree of proteasome inhibition after 1 hour of drug administration with a recovery of proteasome activity of 85% at 24 hours except in those receiving 1.50 mg/m^2 on schedule one where a 35% inhibition was still observed at 24 hours. A 549 human NSCLC cells showed a marked increase in p53 levels for 24 hours after exposure to bortezomib.

6.2. Phase II Single Agent Proteasome Inhibitor in Lung Cancer. Stevenson et al. conducted a phase II pharmacodynamic study using single agent bortezomib in patients with advanced stage NSCLC who had received less or equal to one prior regimen [40]. 23 patients were enrolled and received bortezomib at 1.3 to 1.5 mg/m^2 dosing on days 1, 4, 8 and 11 every 21 days; results revealed one patient having partial response, and 9 patients had stable disease, lasting more than 4 cycles in 5 of the patients. Most common grade 3 toxicities included nausea and vomiting, sensory neuropathy, constipation, rash, and thrombocytopenia. Evaluation of p65 and phosphorylated p65 (pp65) by western blot analysis in 12 patients revealed no change in total p65, the ratio of p65/pp65 was also unaffected across the entire group, but significantly decreased in patients with grade 3 toxicity at 30 minutes with nadir at 4 hours and recovery at 24 hours. They were unable to achieved clinical significance with these results.

The role of bortezomib was evaluated in relapsed or refractory extensive stage small cell lung cancer (SCLC) by Lara et al. in the Southwest Oncology Group (SWOG) phase II trial (S0327) [41]; 56 patients with histologically or cytologically confirmed diagnosis or SCLC with evidence of measurable disease, good performance status, and adequate end organ function who had received prior platinum containing regimens and who had not received prior bortezomib were enrolled. Treatment was administered on days 1, 4, 8, and 11 every 21 days at a dose of 1.3 mg/m^2 with dose reductions to 1.0 mg/m^2 if toxicities graded at 3 or 4 based on the National Cancer Institute Common Toxicity Criteria (CTC) version 2.0. Primary end point was response rate (RR); secondary end points included time to progression (TTP) and overall survival (OS). In terms of sensitivity to platinum-based therapy, the patients were well distributed: 28 with platinum sensitive (relapse >90 days after platinum) and 28 with platinum refractory (progression during or < or equal to 90 after platinum). Partial response was observed in one patient and stable disease in two patients in the platinum refractory group; most patients (83%) had disease progression and/or developed symptomatic deterioration; early death was observed in one patient on each group. Three patients were not assessable for response due to other reasons. Median progression-free survival (PFS) and OS for the platinum refractory group were 1.1 and 3.1 months, respectively; in the platinum sensitive group, median PFS was 1.2 months and OS 2.9 months. The 6-month PFS rate was 10% and 0% for the platinum refractory and platinum sensitive group, respectively, and overall 6-month survival was 25% for both strata. Side effects exceeding grade 2 were fatigue and thrombocytopenia; one death possibly related to bortezomib was reported consisting of dyspnea which led to respiratory failure. Pretreatment samples were analyzed via immunhistochemistry; two out of eight patients had abnormally low p27 levels, five had low BAX levels, and six had abnormally high Bcl-2. Bcl-xl was abnormally expressed in a high percentage in all 8 specimens. Patients had at least two of these markers abnormally expressed in their tumors with five patients having 3 proteins abnormally expressed.

These and other studies showed that bortezomib as a single agent has limited activity with single agent responses up to 8% only [42].

7. Bortezomib Combinations in NSCLC

More recently in combination with chemotherapy, bortezomib has shown its most encouraging activity [42]. Recent phase I studies have shown that bortezomib combinations are generally well tolerated and have little addition in toxicity as compared to chemotherapy alone (Table 3). More importantly, there has been a significant increase in survival observed with the use of bortezomib in combination. Work from Davies et al. showed that bortezomib plus gemcitabine/carboplatin resulted in a notable survival benefit (11 months overall survival) in patients with advanced NSCLC [32].

Work remains to be done to determine if more combinations of bortezomib with other chemotherapy regimens or with targeted therapies will yield further survival advantages. Thus far, results with docetaxel, docetaxel + cetuximab, pemetrexed, and erlotinib show modest results at best (Table 3). There are interesting results for example about the combination of erlotinib and bortezomib. Piperdi et al. [43] found that in H358 bronchoalveolar cells, the combination is neither additive nor synergistic in the NSCLC cell lines studied. The choice of schedule may be very important in combining erlotinib with bortezomib, and further *in vivo* studies are required to further evaluate this combination.

Also there is ongoing research looking for predictive markers of bortezomib sensitivity. Voortman et al. [44] showed that the proteasomal as well as apoptotic phenotype determines bortezomib sensitivity in NSCLC cells. There is a preclinical rationale to combine proteasome inhibition with proapoptotic agents as well as agents promoting a more favorable proteasomal phenotype to overcome this resistance.

8. Conclusion

Ubiquitin-proteasome system is critical for the proliferation and survival of cancer cells, and its inhibition by proteasome inhibitors such as bortezomib has become a very attractive anticancer therapy. Bortezomib has proven to be active against a broad range of cancer cell lines including NSCLC, and it has been tested in numerous *in vitro* and *in vivo* NSCLC models. Current phases I and II studies are showing the possibility to have a new targeted therapy for NSCLC

Table 3: Chemotherapy combinations with bortezomib.

Combination	Study	Dose/schedule	Results	Reference
Docetaxel	Bortezomib Plus Docetaxel in NSCLC and Other Solid Tumors: A Phase I California Cancer Consortium Trial	Patients with NSCLC and r solid tumors were enrolled in cohorts of three over six dose levels. Each cycle was 3 weeks and consisted of one docetaxel infusion (day 1) and four bortezomib injections (days 1, 4, 8, and 11)	The MTD was 1.0/75 mg/m^2. The combination was well tolerated. Two patients with NSCLC achieved a PR (6%), and seven (19%) patients achieved SD (6 patients with NSCLC)	Lara Jr. et al. 2006 [26]
	Randomized Phase II Study of Bortezomib Alone and Bortezomib in Combination with Docetaxel in Previously Treated Advanced Nonsmall-Cell Lung Cancer	Patients were assigned to bortezomib 1.5 mg/m^2 (arm A) or bortezomib 1.3 mg/m^2 plus docetaxel 75 mg/m^2 (arm B). A treatment cycle of 21 days comprised four bortezomib doses on days 1, 4, 8, and 11, plus, in arm B, docetaxel on day 1	RORR were 8% in arm A and 9% in arm B. DCR rates were 29% in arm A and 54% in arm B. Median TTP was 1.5 months in arm A and 4.0 months in arm B. One-year survival was 39% and 33%, and OS was 7.4 and 7.8 months in arms A and B, respectively	Fanucchi et al. 2006 [27]
Docetaxel + Cetuximab	Randomized Phase II Trial of Docetaxel Plus Cetuximab or Docetaxel Plus Bortezomib in Patients with Advanced Nonsmall-Cell Lung Cancer and a Performance Status of 2: CALGB 30402	Docetaxel 30 mg/m^2 on days 1, 8, and 15 every 28 days in combination with either cetuximab 400 mg/m^2 loading dose followed by 250 mg/m^2 weekly (D + C) or bortezomib 1.6 mg/m^2 on days 1, 8, and 15 every 28 days (D + B) for up to 4 cycles. Patients with responding or stable disease continued cetuximab or bortezomib until progression	ORR response rates were 13.3% and 10.3% for D + C and D + B, respectively. Median PFS was 3.4 months in the D + C arm and 1.9 months in the D + B arm. 6-month PFS were 27.8% and 13.8% and 5.0 and 3.9 months for median survival, respectively. Grade 3/4 hematologic toxicity was 16% for D + C and 21% for D + B, whereas nonhematologic toxicities were observed in 63% and 44% of patients, respectively. Neither combination met the prespecified PFS end point to justify further research in this setting	Lilenbaum et al. 2009 [28]
Carboplatin + Paclitaxel and XRT	Phase I Trial of Carboplatin/Paclitaxel/Bortezomib and Concurrent Radiotherapy followed by Surgical Resection in Stage III Nonsmall Cell Lung cancer	Bortezomib was administered on days 1, 4, 15, and 18 during the 6-week induction chemoradiotherapy. Cohorts of three patients were entered. All patients were to receive consolidation chemotherapy with carboplatin AUC = 6 and paclitaxel 200 mg/m^2	12 patients in three cohorts were enrolled. The addition of bortezomib was well tolerated, with no unexpected toxicities during the induction phase. However, there were 3 postoperative deaths (two pneumonitis and one from failure of the bronchopulmonary flap). The trial was halted as a consequence of these toxicities	Edelman et al. 2010 [29]

Table 3: Continued.

Combination	Study	Dose/schedule	Results	Reference
Gemcitabine + Cisplatin	A Parallel Dose-Escalation Study of Weekly and Twice-Weekly Bortezomib in Combination with Gemcitabine and Cisplatin in the First-Line Treatment of Patients with Advanced Solid Tumors (Phase I study)	Patients were assigned to increasing doses of bortezomib days 1 and 8 (weekly schedule) or days 1, 4, 8, and 11 (twice-weekly schedule), in addition to gemcitabine 1,000 mg/m^2 days 1 and 8 and cisplatin 70 mg/m^2 day 1, every 21 days. Maximum of six cycles	Weekly bortezomib 1.0 mg/m^2 plus gemcitabine 1,000 mg/m^2 and cisplatin 70 mg/m^2 is the recommended phase II schedule. Of 34 evaluable patients, 13 achieved PR, 17 SD, and 4 PD	Voortman et al. 2007 [30]
Gemcitabine + Carboplatin	The Proteasome Inhibitor Bortezomib in Combination with Gemcitabine and Carboplatin in Advanced Nonsmall Cell Lung Cancer: A California Cancer Consortium Phase I Study	Bortezomib was administered on days 1, 4, 8, and 11, after gemcitabine on days 1 and 8, and carboplatin on day 1 of a 21-day cycle. Three escalating dose levels were evaluated: bortezomib 1.0 mg/m^2/gemcitabine 800 mg/m^2, bortezomib 1.0 mg/m^2/gemcitabine 1000 mg/m^2, and bortezomib 1.3 mg/m^2/gemcitabine 1000 mg/m^2, in combination with carboplatin AUC 5.0	The MTD was defined as bortezomib 1.0 mg/m^2, gemcitabine 1000 mg/m^2, and carboplatin AUC 5.0. The most common grade 3/4 toxicities were thrombocytopenia (rarely associated with bleeding), and neutropenia. Nine of 26 patients (35%) achieved PR, and eight patients had SD	Davies et al. 2008 [31]
	Bortezomib Plus Gemcitabine/Carboplatin As First-Line Treatment of Advanced Nonsmall Cell Lung Cancer A Phase II Southwest Oncology Group Study (S0339)	Stage IIIB/IV NSCLC, performance status 0-1, and no history of brain metastasis received up to six 21-day cycles of gemcitabine 1000 mg/m^2, days 1 and 8, carboplatin area under curve 5.0, day 1, and bortezomib 1.0 mg/m^2, days 1, 4, 8, and 11	114 patients (52% adenocarcinoma, 85% stage IV) OS was 11 months; 1-year and 2-year survival rates were 47% and 19%, respectively. Median PFS was 5 months; 1-year PFS rate was 7%. ORR was 23%, and DCR rate was 68%	Davies et al. 2009 [32]
Pemetrexed	Phase I Study of Two Different Schedules of Bortezomib and Pemetrexed in Advanced Solid Tumors with Emphasis on Nonsmall Cell Lung Cancer	Two separate dose-escalating arms (arm A and arm B) were conducted simultaneously. Patients received pemetrexed on day 1 (D1) (500–600 mg/m^2 IV) every 21 days. In arm A, bortezomib was given twice weekly (0.7–1.3 mg/m^2 on D 1, 4, 8, and 11). In arm B, bortezomib was given weekly (1.0–1.6 mg/m^2 on D 1 and 8)	Of 26 evaluable patients, 2 patients had PR (1 in arm A and 1 in arm B), 13 had SD (7 in arm A and 6 in arm B), and 11 had PD (6 in arm A and 5 in arm B). Of the 16 patients with NSCLC, 2 (12.5%) had PR and 9 had SD, for a DCR of 68.8%. Phase II dose for arm A is pemetrexed 500 mg/m^2 and bortezomib 1.3 mg/m^2 twice weekly. For arm B, the recommended dose is pemetrexed 500 mg/m^2, bortezomib 1.6 mg/m^2 weekly	Davies et al. 2007 [33]

Table 3: Continued.

Combination	Study	Dose/schedule	Results	Reference
	A Randomized Phase II Study of Bortezomib and Pemetrexed, in Combination or Alone, in Patients with Previously Treated Advanced Nonsmall-cell Lung Cancer	Pemetrexed (500 mg/m^2) on day 1 plus bortezomib (1.6 mg/m^2) on days 1 and 8 (Arm A) or pemetrexed (500 mg/m^2) on day 1 (Arm B) or bortezomib (1.6 mg/m^2) on days 1 and 8 (Arm C) of a 21 day cycle	In previously treated NSCLC the addition of bortezomib to pemetrexed was well tolerated but offered no statistically significant response or survival advantage versus pemetrexed alone, while bortezomib alone showed no clinically significant activity	Scagliotti et al. 2010 [34]
Erlotinib	A Randomized Phase II Study of Erlotinib Alone and in Combination with Bortezomib in Previously Treated Advanced NSCLC	Erlotinib 150 mg/d alone (arm A; $n = 25$) or in combination with bortezomib 1.6 mg/m^2, days 1 and 8 (arm B; $n = 25$) in 21-day cycles	ORR were 16% in arm A and 9% in arm B; DCR were 52 and 45%, respectively. The study was halted at the planned interim analysis due to insufficient clinical activity in arm B. Median PFS and OS were 2.7 and 7.3 months in arm A, and 1.3 and 8.5 months in arm B. Six-month survival rates were 56.0% in both arms; 12-month rates were 40 and 30% in arms A and B, respectively. ORR to erlotinib ± bortezomib was significantly higher in patients with EGFR (50 versus 9% for wild type). Insufficient activity was seen with erlotinib plus bortezomib in patients with relapsed/refractory advanced NSCLC to warrant a phase III study of the combination	Lynch et al. 2009 [35]

PR: partial response, CR: complete response, ORR: overall response rate, PFS: progression-free survival, OS: overall survival, DCR: disease control rate (CR + PR + SD), MTD: maximum tolerated dose.

combining this bortezomib with available chemotherapeutic agents. Prospective phase III trials are needed to validate the use of this agent in NSCLC.

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