

# Bortezomib-induced Apoptosis in the Treatment of Non-small Cell Lung Cancer

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## Abstract

Despite advances in the past few decades, current chemotherapy regimens have had only limited efficacy with modest survival benefit and significant toxicity in non-small cell lung cancer (NSCLC) patients. Novel therapies, especially those targeting cell signaling pathways, are now under extensive investigation against many tumor types. Proteasome inhibition, one of the novel cancer therapies, has been shown to have widespread ability to induce apoptosis in vitro in a broad spectrum of tumor cell lines. Bortezomib (Velcade) is the first proteasome inhibitor to enter clinical trials. It has demonstrated encouraging efficacy against multiple myeloma, where it is now an approved therapy. In in vitro studies of lung cancer, bortezomib has been found to induce marked apoptosis by itself or when used together with other novel therapy agents or chemotherapy agents. Despite these promising results, bortezomib has not shown great efficacy against lung cancer in clinical trials. In this review, we describe the role of the proteasome in cell homeostasis and apoptosis, the molecular mechanisms of bortezomib-induced apoptosis in preclinical studies of NSCLC cells, and its efficacy to date in the clinical trials. We consider possible reasons why proteasome inhibition may not be as effective in lung cancer and ways in which the efficacy might be improved in the future. ( J Intern Med Taiwan 2009; 20: 106-119 )

**Key Words :** TRAIL, Proteasome inhibitor, Acquired resistance, Multicellular resistance, Three-dimensional spheroid

## Introduction

Lung cancer, which can be classified into non-small cell lung cancer (NSCLC) and small cell lung cancer (SCLC), is the leading cause of cancer-related death in the world<sup>1</sup>. NSCLC, which accounts for 80 to 85% of all lung cancers, is distinguished from SCLC by its clinical course and its response to chemotherapy and radiotherapy<sup>2</sup>. More than 70% of

NSCLC patients present with unresectable locally advanced or metastatic disease<sup>3</sup>, so chemotherapy with or without radiotherapy remains the mainstay of lung cancer treatment. Despite advances in the past few decades, current chemotherapy regimens have only limited efficacy with modest survival benefit and significant toxicity in NSCLC patients. Novel therapies, especially those targeting cell

signaling pathways, are now under extensive investigation against many tumor types. Proteasome inhibition, one of the novel cancer therapies, has been shown to have widespread ability to disrupt tumor cell homeostasis without inducing toxicity in normal cells and to induce apoptosis in vitro in a broad spectrum of tumor cell lines<sup>4</sup>. Indeed, when either used alone or combined with other approaches, proteasome inhibition has generated intense interest as a therapeutic approach targeting cancer.

Bortezomib (Velcade) is the first proteasome inhibitor to enter clinical trials. It has demonstrated encouraging efficacy against multiple myeloma, where it is now an approved therapy<sup>5</sup>. In in vitro studies of lung cancer, bortezomib also has shown promise; bortezomib has been found to regulate many molecules that are associated with tumor progression and treatment resistance, such as NF  $\kappa$  B<sup>6</sup>, pro-apoptotic and anti-apoptotic Bcl-2 family proteins<sup>7</sup>, and p53<sup>8</sup>. In addition, bortezomib often induces apoptosis by itself or when used together with other novel therapy agents or chemotherapy agents<sup>9</sup>. Because of the promising results in these preclinical studies, bortezomib has now been tested in many clinical trials of NSCLC<sup>10-19</sup>. In this review, we describe the role of the proteasome in cell homeostasis and apoptosis, the molecular mechanisms of bortezomib-induced apoptosis in preclinical studies of NSCLC cells, and its efficacy to date in the clinical trials. We consider possible reasons why proteasome inhibition has not been as effective in lung cancer as in multiple myeloma and strategies by which the efficacy could potentially be improved in the future.

## The role of apoptosis in cancer treatment

### Defects in apoptosis contribute to the development and treatment resistance of cancer

Although cancer was initially thought to

result from uncontrolled proliferation driven by oncogenes, there is increasing evidence that the ability to evade apoptosis is also important for cancer to develop<sup>20</sup>. Whereas inhibition of apoptosis is not sufficient to cause cancer by itself, we have learned from animal models that, when inhibition of apoptosis is combined with activation of growth stimulatory oncogenes, cancers can then develop<sup>21</sup>. As cancer cells acquire the ability to evade apoptosis, they also acquire resistance to the apoptosis that is induced by chemotherapeutic agents<sup>22</sup>. Although chemotherapeutic agents can induce cell cycle arrest, senescence, and autophagy<sup>23</sup>, emerging evidence suggests that many chemotherapeutic agents induce cancer cell death through apoptosis<sup>22</sup>. Furthermore, resistance to chemotherapy has been linked directly to defects in apoptosis<sup>24</sup>. The hope for improved cancer therapy depends on a detailed understanding of apoptotic pathways so that strategies to induce apoptosis or to lower the apoptotic resistance against standard therapies can be developed<sup>25</sup>.

### Molecular machinery of apoptosis

Apoptosis is a programmed cell death that involves a series of biochemical events leading to a variety of morphological changes, including blebbing, cell shrinkage, nuclear fragmentation, chromatin condensation, and chromosomal DNA fragmentation<sup>26</sup>. The mechanisms of apoptosis are highly complicated and involve a cascade of activation of caspases, a family of calcium-dependent cysteine proteases. Caspases can be divided into initiator and effector caspases. Initiator caspases, such as caspase 8 and caspase 9, are activated following several initial apoptotic stimuli and then activate the effector caspases. Activation of the effector caspases, such as caspase 3, caspase 6, and caspase 7, results in the enzymatic cleavage of cellular proteins leading to apoptosis (Figure 1). As one would expect, there is extensive regulation and control over the activation of these caspases.

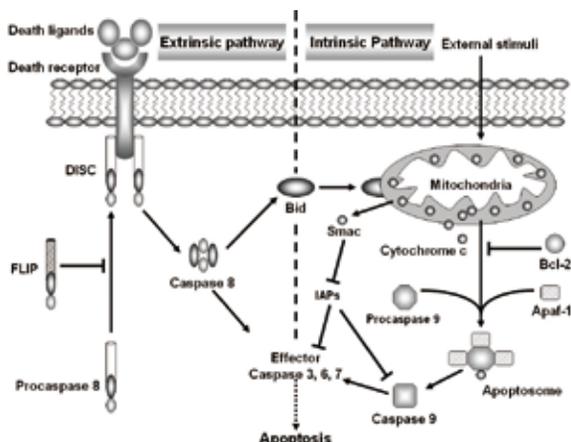


Fig. 1. A simplified schematic representation of the two major apoptotic pathways. Figure modified from Shivapurkar et al<sup>22</sup>.

There are at least 2 major pathways (the extrinsic and intrinsic pathway) that act on the mitochondria to initiate apoptosis and multiple signals that modulate their activity (e.g. p53 and other damage pathways and survival pathways).

### The extrinsic pathway

The extrinsic pathway initiates apoptosis via the interaction of transmembrane receptors and death ligands, such as Fas, TNF- $\alpha$ , and tumor necrosis factor-related apoptosis-inducing ligand (TRAIL)<sup>27</sup>. After the death ligands bind to their corresponding transmembrane receptors, a death-inducing signaling complex (DISC) is formed that then recruits and cleaves the initiator caspase, caspase 8<sup>28</sup>. This death receptor-mediated apoptosis can be inhibited by FLIP, a protein that competes with caspase 8 thereby interfering with the initiation of apoptosis<sup>29</sup>. In addition, FLIP may have effects on the intrinsic pathway (see below) where, in overexpression studies, FLIP can interfere with Bax<sup>30</sup>. Despite this intriguing information, further studies will be needed to confirm whether FLIP acts beyond its well recognized functions in inhibiting death receptor signaling via caspase 8.

### The intrinsic pathway

The intrinsic pathway initiates apoptosis via

the loss of mitochondrial membrane potential and release of multiple pro-apoptotic proteins, including cytochrome c and Smac/DIABLO, from the mitochondria into the cytosol<sup>31</sup>. Following release into the cytosol, cytochrome c forms a multiprotein complex with Apaf-1 and procaspase 9, called an apoptosome. After forming the apoptosome, procaspase 9 is activated and then activates the effector caspases. Smac facilitates apoptosis by inhibiting the IAPs (inhibitor of apoptosis proteins), which can bind and inhibit active caspase 9 and the effector caspases.

The intrinsic and extrinsic pathway are linked together, so that proteins in one pathway can affect those in the other pathway<sup>32</sup>. Ultimately, the intrinsic and extrinsic pathways converge at the mitochondria, a major integrator of apoptotic signals and effector of apoptosis.

### Mitochondrial machinery

Mitochondria regulate apoptosis by integrating diverse death signals via a family of related molecules called Bcl-2 family proteins. The Bcl-2 family proteins can be divided into anti-apoptotic and pro-apoptotic groups. The best known anti-apoptotic Bcl-2 family proteins include Bcl-2, Bcl-xL, Bcl-w, A1 and Mcl-1. The major pro-apoptotic Bcl-2 family proteins are Bax and Bak, which are required to mediate apoptosis<sup>33,34</sup>. There is also a subset of BH3-only molecules that reside normally in the cytoplasm, including Bid, Bad, Bim, Bik, PUMA, and Noxa, that can mediate various pro-apoptotic signals, whether from the loss of adherence, disruption of cytoskeleton or DNA damage, and translocate to the mitochondria where they can then trigger apoptosis by interacting with the anti- and the pro-apoptotic Bcl-2 proteins. One persuasive theory to explain the interaction of these proteins holds that the anti-apoptotic Bcl-2 proteins function by binding and buffering the BH3-only proteins thereby preventing them from binding to and activating the pro-apoptotic proteins, Bax and

Bak<sup>35</sup>. With sufficient activation of the BH3-only molecules or a sufficient reduction in the anti-apoptotic buffering, BH3-only molecules can then bind and activate Bax and Bak, leading to mitochondrial outer membrane permeabilization (MOMP) and the release of proapoptotic proteins as described, such as cytochrome c and Smac/DIABLO<sup>36</sup>.

### The p53 and other stress pathways

The tumor suppressor p53 plays a critical role in maintaining the integrity of cellular DNA. In the presence of extensive DNA damage, p53 functions as a regulatory molecule either to facilitate DNA repair or to initiate apoptotic cell death<sup>37</sup>. The p53 protein, as a damage sensor, may facilitate or induce apoptosis by actions upon both the intrinsic and extrinsic pathway<sup>38</sup>. In the intrinsic pathway, p53 activates pro-apoptotic members of Bcl-2 family proteins as for example by transcriptional activation of Bax<sup>39</sup> and by activating the BH3-only molecules, Puma and Noxa<sup>40</sup>. In the extrinsic pathway, p53 transactivates receptors for death ligands Fas<sup>41</sup> and DR5<sup>42</sup>. Of course, many tumors have inactive p53, thereby enabling them to evade apoptosis via this pathway. In such cells without a functioning p53 pathway, the JNK pathway may mediate similar pro-apoptotic signals; for example, our laboratory has shown that the JNK pathway activates the BH3-only molecule, Bim<sup>43</sup>.

### Survival pathways

In contrast, pro-survival signaling pathways tend to inhibit apoptosis in cells. Such survival pathways may be important anti-apoptotic mechanisms in tumor cells, in which many such pathways are known to be hyperactive. NF  $\kappa$  B is one important survival pathway found to be activated in many cancer cells. NF  $\kappa$  B -regulated genes are involved in the regulation of apoptosis, proliferation, and differentiation<sup>44</sup>. NF  $\kappa$  B is normally inhibited by I  $\kappa$  B, and the major mechanism of NF  $\kappa$  B activation involves the

proteasomal degradation of the inhibitory I  $\kappa$  B following its phosphorylation and ubiquitination<sup>44</sup>. After the degradation of I  $\kappa$  B, NF  $\kappa$  B translocates into the nucleus and promotes the transcription of many target genes. NF  $\kappa$  B activation inhibits apoptosis in most cell systems by inducing expression of anti-apoptotic proteins such as Bcl-2, Bcl-XL, A1, cIAPs, as well as pro-survival cytokines such as IL-6<sup>45</sup>. Another key survival pathway is the PI3K/Akt pathway which is upregulated in many cancers and also plays a role in the inhibition of apoptosis<sup>46</sup>. Activation of the PI3K/Akt pathway inhibits apoptosis in part by the phosphorylation and inactivation of Bad<sup>47</sup> and caspase 9<sup>48</sup> and the stimulation of the NF  $\kappa$  B pathway<sup>49</sup>. In addition, activation of the PI3K/Akt pathway stabilizes MDM2<sup>50</sup>, which blocks p53 activation and thus prevents p53-mediated apoptosis. Other survival signaling pathways such as MAPK are also involved in the regulation of apoptosis<sup>51</sup>. Survival pathways tend to be hyperactive in tumors and thus serve as excellent targets for anti-cancer therapies<sup>52</sup>.

Many proteins in the apoptosis and survival pathways just described are regulated either directly or indirectly by proteasomal degradation. Inhibition of the proteasome appears to upset the balance between the pro- and anti-apoptotic proteins, often in a pro-apoptotic direction<sup>6,8</sup>. Therefore, the proteasome has been investigated in preclinical and clinical studies as a novel target for cancer therapy.

## The role of the proteasome in apoptosis

Proteasomes are large barrel-like protein complexes that are primarily localized in the cytosol but also can be found in the nucleus, endoplasmic reticulum, and plasma membrane<sup>53</sup>. The main functions of the proteasome are to degrade unneeded or damaged proteins and to regulate the turnover of short-lived proteins that control cell-

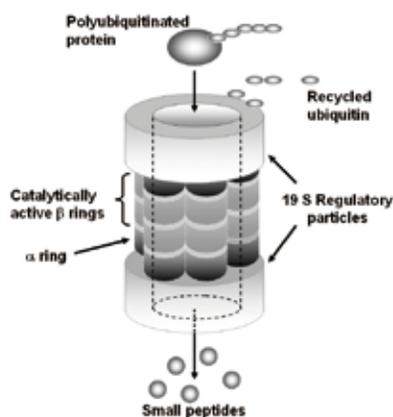


Fig.2. The structure of proteasome and the ubiquitins-proteasome pathway for protein degradation. Proteins are tagged with chain of ubiquitins. The resulting polyubiquitinated proteins are recognized by the 19S regulatory particles, which unfold the protein and remove the ubiquitin from the proteins. The proteins are then degraded to peptides of seven to eight amino acids long.

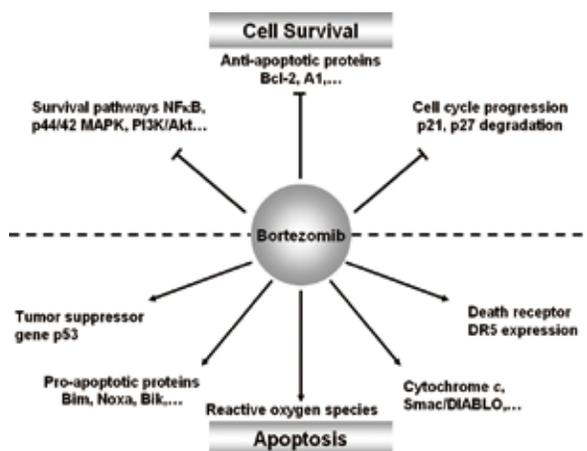


Fig.3. A simplified schema of the molecular mechanism of bortezomib-induced apoptosis.

cycle progression, signal transduction, the inflammatory process and ultimately apoptosis<sup>53</sup>. More than 80% of all cellular proteins are processed by the proteasome<sup>54</sup>.

The proteasome consists of two 19S regulatory particles and a 20S "core" of four stacked rings around a central pore (Figure 2). The two inner  $\beta$  rings contain six active proteolytic sites on the interior surface of the rings, and the two

outer  $\alpha$  rings function as a "gate" through which proteins enter the  $\beta$  rings. Proteins are marked for degradation by being tagged with chains of small proteins called ubiquitins. The resulting polyubiquitinated proteins are recognized by the regulatory particles and enter the central pore of the proteasome where they are degraded to peptides of seven to eight amino acids long. These peptides will be further degraded into amino acids that can later be used to synthesize new proteins. By control of intracellular protein levels, the proteasomal degradation pathway is essential for many cell processes<sup>53</sup>.

## The molecular mechanisms of bortezomib-induced apoptosis

By its myriad effects on the cell, the proteasome may have multiple ways by which it induces apoptosis (Figure 3). It is possible that different mechanisms predominate in different tumors or that multiple mechanisms interact in each tumor to induce apoptosis. Even if we only consider NSCLC, several mechanisms have been identified by which bortezomib and proteasome inhibition induce apoptosis.

### I. Bortezomib inhibits survival pathways.

The NF  $\kappa$  B pathway is thought to be an important target of bortezomib<sup>55</sup>. By blocking the activation of the NF  $\kappa$  B pathway by preventing the proteasomal degradation of I $\kappa$ B, bortezomib may enhance the response to chemotherapy<sup>56</sup>. In one study of lung cancer lines, for example, NF  $\kappa$  B was upregulated by treating the NSCLC cell lines A549 and H157 with the chemotherapeutic agent, gemcitabine, leading to enhanced transcription of all NF  $\kappa$  B -regulated genes and a resistance to gemcitabine<sup>57</sup>. Bortezomib, which inhibited this NF  $\kappa$  B activation, was able to sensitize these cell lines to gemcitabine-induced apoptosis. In this and in many studies, bortezomib is able to enhance the response of

resistant tumor cells to chemotherapy<sup>58</sup>. Despite the consistent evidence that bortezomib inhibits the activation of NF- $\kappa$ B pathway, it remains unclear whether this inhibition is the major mechanism of bortezomib-induced apoptosis.

Indeed, bortezomib may inhibit other survival pathways. For example, there is evidence that bortezomib inhibits the PI3K/Akt pathway and the p44/42 MAPK pathway<sup>59,60</sup>. By these actions, bortezomib may also reduce tumor cell resistance to apoptosis<sup>51</sup>.

## **II. Bortezomib alters the balance of pro-apoptotic and anti-apoptotic proteins.**

There is increasing evidence that an important mechanism of bortezomib-induced apoptosis is the regulation of the balance of pro-apoptotic and anti-apoptotic Bcl-2 family proteins<sup>7</sup>. In general, bortezomib up-regulates BH3-only proteins Bim, Noxa and Bik, and down-regulates anti-apoptotic Bcl-2 family proteins Bcl-2 and A1<sup>61-64</sup>. Whether bortezomib induces up-regulation of pro-apoptotic proteins Bax and BH3-only protein PUMA is still controversial and may depend on the different cell types used in the experiments<sup>61,65</sup>. Although bortezomib has also been shown to up-regulate the anti-apoptotic Bcl-2 family protein Mcl-1 in NSCLC<sup>62,66</sup>, the net effect of bortezomib appears to be to shift the balance in a pro-apoptotic direction. Indeed, as we discuss later, because the upregulation of Mcl-1 may limit the pro-apoptotic actions of bortezomib, current approaches are underway to inactivate Mcl-1 in hopes of enhancing the pro-apoptotic effects of bortezomib.

## **III. Bortezomib enhances stress pathways, p53 and JNK.**

Because the degradation of p53 can be mediated by the ubiquitin-proteasome pathway<sup>67</sup>, bortezomib treatment results in a concentration- and time-dependent accumulation of p53<sup>38</sup>. This accumulation of p53 may promote apoptosis through activation of both the extrinsic and intrinsic

apoptosis pathways<sup>38</sup> or may induce cell cycle arrest. In one preclinical study of NSCLC, bortezomib induced more apoptosis in a cell line with wild-type p53 (H460) than in a cell line with mutant p53 (H322), suggesting that bortezomib induced apoptosis via a p53-dependent pathway<sup>8</sup>. Nonetheless, the same study showed evidence of p53-independent pathways, suggesting multiple mechanisms of apoptosis induction in lung cancer cells. JNK, the other major stress pathway, is directly activated by proteasome inhibition and the JNK pro-apoptotic activation of Bim may enhance the effects of proteasome inhibition on Bim protein accumulation<sup>43,68</sup>.

## **IV. Bortezomib arrests the cell cycle**

In general, bortezomib induces apoptosis in proliferating but not in quiescent cells<sup>4</sup>, suggesting that the cell cycle may play a role in the apoptotic effect of bortezomib. Controlled transitions between cell cycle stages depend on the timely activation of cyclin/cyclin-dependent kinase (CDK) complexes. The CDK inhibitors p21 and p27 are short-lived proteins that induce cell cycle arrest by inhibiting the formation of CDK-cyclin complex. Because p21 and p27 are degraded by the proteasome<sup>69</sup>, bortezomib increases the levels of these two CDK inhibitors, an increase which can result in cell cycle arrest. In NSCLC cell lines, bortezomib treatment was shown to arrest the cell-cycle in the G2-M phase by increasing the levels of the CDK inhibitors and cyclins A and B<sup>8</sup> and this arrest was associated with an increased apoptosis. In another study, introduction of antisense p27 oligonucleotides into an squamous carcinoma cell line blocked the proteasome inhibitor-induced apoptosis<sup>70</sup>, suggesting that accumulation of p27 was the key to the cell cycle arrest and apoptosis.

## **V. Bortezomib has multiple additional effects**

There is some evidence that bortezomib may increase the generation of reactive oxygen species. In experiments using the NSCLC cell line H460,

bortezomib exposure increased the generation of reactive oxygen species and cytochrome c release<sup>71</sup>. In another study, bortezomib combined with a histone deacetylase inhibitor synergistically enhanced the generation of reactive oxygen species in NSCLC and induced marked apoptosis<sup>72</sup>. Of interest, the antioxidant agent Tiron which inhibited the bortezomib-induced reactive oxygen species generation, also blocked cytochrome c release and the bortezomib-induced apoptotic cell death<sup>71,73</sup>. Thus generation of reactive oxygen species may also have a role in the induction of bortezomib-induced apoptosis perhaps by altering mitochondrial membrane potential and the release of cytochrome c from mitochondria<sup>71</sup>.

Multiple other mechanisms may play a role. For example, in NSCLC cell lines, bortezomib was also found to induce increased surface expression of death receptors DR5 and thus might sensitize cells to TRAIL-induced apoptosis despite up-regulation of the expression of the apoptosis inhibitor, survivin<sup>74</sup>.

#### Summary of Bortezomib Actions Leading to Apoptosis

Thus, many preclinical studies have shown promising results in the induction of apoptosis in NSCLC cell lines and encouraged the use of bortezomib in NSCLC clinical trials. However, for unknown reasons, bortezomib has had its greatest benefit in hematologic malignancies, especially in multiple myeloma.

## Bortezomib in cancer clinical trials

### Multiple myeloma shows the best response to bortezomib

Bortezomib has been highly effective in patients with multiple myeloma. In relapsed or refractory multiple myeloma, bortezomib alone caused an overall response rate of 41-43%<sup>75,76</sup>, and the overall responses were increased to 64-90% when bortezomib was combined with other

chemotherapy<sup>77,78</sup>. In newly diagnosed multiple myeloma, bortezomib alone caused a overall response rate of more than 40%<sup>79</sup>. When bortezomib was combined with other chemotherapy in newly diagnosed multiple myeloma as front-line treatment, the overall response rates were more than 80-90%<sup>80-82</sup>. This benefit was associated with only minimal toxicity, mostly thrombocytopenia and electrolyte abnormalities<sup>83</sup>. The effect of bortezomib thus was targeted to the tumor cells with little collateral damage to normal tissues.

### NSCLC: Reality has not yet lived up to its promise

In contrast to the encouraging results of bortezomib in multiple myeloma, the response to bortezomib in patients with NSCLC has not been so promising. A summary of the results of bortezomib in NSCLC clinical trials is listed in Table 1. One clinical trial of bortezomib in previously treated advanced NSCLC showed only modest single-agent activity with an 8% partial response rate, and the complete response rate was 0%<sup>10</sup>. When bortezomib was combined with docetaxel, the partial response was 9%, and again the complete response rate was 0%<sup>10</sup>. Besides docetaxel, bortezomib has also been used in combination with other chemotherapeutic agents and targeted therapy. When combined with gemcitabine/carboplatin, bortezomib induced an overall response rate of 21% with a complete response rate of 2% in patients with chemotherapy-naive stage IV and selected stage IIIB (pleural effusion) NSCLC<sup>12</sup>. When combined with EGFR tyrosine kinase inhibitor erlotinib in a phase II clinical trial, bortezomib did not show sufficient activity and the trial was halted<sup>13</sup>. Toxicities included grade 3 or 4 adverse effects such as fatigue, neutropenia, and dyspnea,<sup>10</sup> with a high incidence of neutropenia when bortezomib was used in combination with docetaxel (53%). In general, the clinical result of bortezomib treatment has been disappointing with only a 4-13% partial response rate when used as single agent, and 8-35%

Table 1: Results of Bortezomib in NSCLC Clinical Trials

Phase	Stage of NSCLC	Response		References
		Bortezomib single agent	Combined therapy	
I	Refractory NSCLC	CR: 0%, PR: 12.5% (n=8)	N/A	Aghajanian <sup>14</sup>
I	Stage IIIB/IV	N/A	Bortezomib + Docetaxel CR: 0%, PR: 7.7% (n=26)	Lara <sup>15</sup>
I	Advanced NSCLC	N/A	Bortezomib + Irinotecan (n=6) CR: 0%, PR: 0%	Ryan <sup>16</sup>
I	Advanced NSCLC	N/A	Bortezomib + Pemetrexed CR: 0%, PR: 12.5% (n=16)	Davis <sup>17</sup>
I	Advanced NSCLC	N/A	Bortezomib + Gemcitabine/Carboplatin CR: 0%, PR: 35% (n=26)	Davies <sup>18</sup>
IB	Stage IIIB/IV	N/A	Bortezomib + Gemcitabine/Cisplatin CR: 0%, PR: 33% (n=27)	Voortman <sup>11</sup>
II	Previously treated stage IIIB/IV	CR: 0%, PR: 8% (n=75)	Bortezomib + Docetaxel CR: 0%, PR: 9% (n=80)	Fanucchi <sup>10</sup>
II	Chemonaive stage IIIB/IV	N/A	Bortezomib + Gemcitabine/Carboplatin CR: 2%, PR: 19% (n=114)	Davies <sup>12</sup>
II	Previously treated stage IIIB/IV	N/A	Bortezomib + Erlotinib CR: 0%, PR: 8.3% (n=24)	Lynch <sup>13</sup>
II	Stage IIIB/IV	CR: 0%, PR: 3.8% (n=26)	N/A	Stevenson <sup>19</sup>

CR: complete response rate, PR: partial response rate

when combined with other chemotherapeutic agents<sup>72-81</sup>.

### Possible explanations for the inefficacy of bortezomib in lung cancer clinical trials

Despite the promising result of bortezomib treatment in patients with multiple myeloma and in preclinical studies of NSCLC cell lines, the response to bortezomib in NSCLC clinical trials has been unexpectedly disappointing. In considering this, we propose various possible explanations for this different treatment response to bortezomib between multiple myeloma and lung cancer. These possible explanations might provide clues for future study to improve the clinical response of NSCLC to bortezomib or to develop other novel therapies.

#### Possible specific mechanisms of bortezomib resistance in NSCLC: Molecular differences bet-

#### ween the two tumors

It is possible that there are differences in the molecular consequences of bortezomib treatment between lung cancer and multiple myeloma. By reviewing the mechanisms just described for the action of bortezomib, we present some interesting differences that have been reported between multiple myeloma and lung cancer studies.

As mentioned, bortezomib is known to alter the balance of pro-apoptotic and anti-apoptotic Bcl-2 family proteins. In general, after bortezomib treatment, there is increased expression of pro-apoptotic BH3-only proteins, such as Bim, PUMA, Noxa, and Bik and decreased expression of anti-apoptotic Bcl-2 family proteins, such as Bcl-2 and A1<sup>7</sup>. However, in lung cancer cell lines H460 and SW 1573 following bortezomib treatment, Mcl-1 was found to be up-regulated<sup>62,66</sup>, while Mcl-1 was shown to be down-regulated and cleaved in multiple

myeloma cells MM.1S, NCI-H292, and U266<sup>84,85</sup> and perhaps also in patients with multiple myeloma<sup>86</sup>. Indeed, the Mcl-1 expression appeared to determine the sensitivity to the bortezomib; for example, in one bortezomib-resistant multiple myeloma cell RPMI-8226, down-regulation of Mcl-1 sensitized the cells to bortezomib-induced apoptosis<sup>85</sup>. This finding suggests that the different effects on Mcl-1 after bortezomib treatment might explain why bortezomib would be more effective in multiple myeloma but not in NSCLC. It would also suggest that interference with Mcl-1 could enhance the responses of lung cancer to bortezomib.

Anti-apoptotic molecules such as FLIP may be elevated by bortezomib differently in the two malignancies. Indeed, in one study, following bortezomib, FLIP was down-regulated in hematopoietic malignancies, such as multiple myeloma<sup>87</sup>. In contrast, FLIP was up-regulated in NSCLC after bortezomib exposure<sup>74</sup>. Indeed, FLIP, best known as an inhibitor of death receptor signaling, may have anti-apoptotic effects at the mitochondria and, if so, FLIP upregulation may inhibit the response to chemotherapy<sup>30</sup>. Again, if this were found to be a general response in lung cancer, interference with FLIP might improve the response of lung cancer to bortezomib.

Bortezomib-induced upregulation of p53 may have different effects because of inherent differences in the mutation and functional inactivation of p53 in the two tumors. A mutation of p53 gene is found in less than 20% of multiple myeloma patients<sup>88</sup> while, on the other hand, mutations of the p53 gene and the resultant loss of tumor suppressor function are found in about 50% of NSCLC<sup>89</sup>. Mutations of the p53 gene can inactivate the protein and remove the key sensor of DNA damage<sup>90</sup> or of other stress signals, such as hypoxia and oncogene hyperexpression, that can induce apoptosis through p53<sup>91</sup>. Indeed, cell lines with mutant p53 are less sensitive to bortezomib-

induced apoptosis than cell lines with wild-type p53<sup>8</sup>. Thus, even if bortezomib successfully upregulates p53, the high incidence of p53 mutation in NSCLC might account for a lower efficacy of bortezomib in NSCLC than in tumors with a lower incidence of p53 mutations.

#### **Possible general mechanisms of bortezomib resistance in NSCLC: Differences between hematopoietic and solid tumors**

Clearly, there are general differences between any hematopoietic and any solid tumor that should be considered. Indeed, many solid tumors demonstrate an apoptotic resistance that appears to be common among solid tumors, and can be modeled *in vitro* using 3D models. The mechanisms of this type of resistance, often called acquired or multicellular resistance<sup>92</sup>, are less well understood than the specific molecular effects discussed above. We will consider a few of them.

Solid tumors may have less drug penetrance and thus a lower effective drug concentration than hematopoietic tumors<sup>93</sup>. In at least one dosing study, however, bortezomib was found to have good drug penetration in many tissues, including bone marrow, kidney, liver and lung<sup>94</sup>. In fact, the maximal bortezomib concentrations in lung and bone marrow after single or repeat dose administration were similar. Although this study did not test the penetrance into the interior of a tumor itself, this study suggests that penetrance would be adequate. These dosing studies however do not address whether the drug that penetrates may function well in the interior of a solid tumor, where acidosis and hypoxia may limit the response. In addition, as mentioned, bortezomib works best against proliferating cells; if so, the less active core of a solid tumor may not respond well to bortezomib, even if the drug successfully reaches the tumor center. However, despite the many possible differences we can envision between the two tumor types, there are currently no data to point to a key

difference that explains the lack of effect of bortezomib in lung cancer.

When compared to non-solid hematopoietic malignancies, solid tumors display a broad resistance that may be a consequence of their 3D shape. Such broad resistance to treatment may be mimicked in the laboratory when monolayers are allowed to grow into small 3D aggregates of cells called spheroids<sup>92,95</sup>. Although the reason why bortezomib induces significant apoptosis in preclinical studies using NSCLC cell lines grown as monolayers but has poor response rate in clinical trials of lung cancer patients remains uncertain, one possibility is that the cells in monolayer culture do not exhibit the apoptotic resistance of the actual tumors. Indeed, the 3D multicellular spheroid model is thought to retain many of the characteristics of the tumor and to display resistance to chemotherapy and radiotherapy more similar to the actual tumor<sup>96</sup>. Of interest, we have recently found that NSCLC cells are sensitized to apoptosis by bortezomib when they are grown as monolayers, but acquire a high level of resistance to bortezomib after they form multicellular spheroids. We are now investigating the features in the 3D spheroids that account for this apoptotic resistance. Our hope is that manipulations in vitro that restore sensitivity to bortezomib may be translated ultimately into the clinic.

## Conclusion

Bortezomib is one of the novel therapeutic agents considered for lung cancer. In preclinical studies of NSCLC, bortezomib induces apoptosis through alteration of many signaling pathways. In addition, when combined with other chemotherapy agents or novel molecular targeted agents, bortezomib shows additive or synergistic activities. These promising results of preclinical studies have promoted the use of bortezomib in NSCLC clinical trials. In contrast to the significant response of

bortezomib in multiple myeloma, the response of bortezomib as a single agent or in combination with other chemotherapy agents or targeted therapies in NSCLC clinical trials has been disappointing. Some clinical trials of bortezomib in NSCLC are still ongoing, and the results of these trials might determine whether bortezomib has a role in the treatment of NSCLC. Further investigations to identify differences between bortezomib-sensitive tumors, such as multiple myeloma, and lung cancer may help to elucidate the essential molecular mechanism of bortezomib-induced apoptosis and the means to enhance the response to bortezomib in lung cancer.

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# Bortezomib引發細胞凋亡在非小細胞肺癌治療之應用

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## 摘 要

雖然癌症化學治療藥物在過去十數年間有顯著進步，非小細胞肺癌的化學治療卻仍沒有令人滿意的療效，而且常伴隨著明顯的藥物副作用。目前有許多標靶治療藥物被研究於治療癌症，其中蛋白解體抑制劑(proteasome inhibitor)被發現可以導致許多腫瘤細胞株發生細胞凋亡(apoptosis)。Bortezomib (Velcade)是第一個進入臨床試驗的蛋白解體抑制劑，由於對多發性骨髓瘤療效顯著，因此已被核准用於治療多發性骨髓瘤。實驗也發現單獨使用Bortezomib或同時合併其他標靶或化學治療藥物可引發肺癌細胞株發生顯著的細胞凋亡，故Bortezomib也進入臨床試驗治療非小細胞肺癌，但這些臨床試驗結果卻不如預期中理想。本文中我們描述蛋白解體在細胞恆定及細胞凋亡機轉中的角色、Bortezomib導致癌細胞株發生細胞凋亡的機轉、以及在非小細胞肺癌臨床試驗之療效，並進一步探討Bortezomib在非小細胞肺癌臨床試驗療效不如預期的可能原因、以及未來如何改善其療效之可能研究方向。