The Preclinical Discovery and Development of Bortezomib for the Treatment of Mantle Cell Lymphoma

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Abstract

Introduction—Mantle cell lymphoma (MCL) is an incurable, often aggressive B-cell malignancy. Bortezomib (BTZ), the 20S proteasome inhibitor was originally developed and approved for treatment of relapsed refractory multiple myeloma, and subsequently approved for treatment of MCL. BTZ's single-agent activity induces clinical responses in approximately one-third of relapsed MCL patients. BTZ-containing combination therapies have further improved the quality and duration of clinical responses compared to standard chemotherapies in previously untreated MCL patients.

Areas Covered—This review summarizes the discovery, mechanisms of -action and resistance, preclinical-clinical-developments, and FDA approval of BTZ for treatments of MCL.

Expert opinion—Preclinical MCL models demonstrated the apoptotic effect of BTZ through multiple mechanisms, as well as synergistic anti-MCL activity between BTZ and other chemotherapeutics. Single-agent and combinational clinical trials have validated the therapeutic potential of targeting the ubiquitin proteasome system (UPS) in MCL. However, inherent and acquired drug resistance remains a significant clinical problem and multiple potential mechanisms have been identified. Next-generation proteasome inhibitors with different pharmacodynamic properties from BTZ may partially address the issue of inherent resistance, with increased response rates noted in some diseases. In addition, upstream UPS components, *e.g.*, E3 ligases or deubiquitinating enzymes, may also be targetable in MCL.

Keywords

ubiquitin–proteasome-system; bortezomib; cancer; drug-development; drug resistance; molecular-targeting; proteasome-inhibitors; mantle cell lymphoma; pre-clinical; clinical trials; targeted therapy

1. Introduction

Recognized by the World Health Organization as a distinct clinical entity in 1994, mantle cell lymphoma (MCL) possesses distinctive: clinical, biological, and molecular characteristics. MCL is considered a generally incurable B-cell malignancy [1]. MCL comprises 6% of all non-Hodgkin's lymphomas, with an incidence of approximately 5,000 cases per year in the United States [1]. The median age of diagnosis of MCL is the mid-60s, with a 3:1 male predominance. Extra-nodal involvement is frequent, and the general prognosis of MCL patients is poor. Although the disease frequently responds to initial treatments, therapy-resistant relapses eventually develop in almost all cases. As such, the median overall survival (OS) remains below three years [2].

The 2003 FDA approval of bortezomib (BTZ; Figure 1) for treatment of Multiple Myeloma (MM) validated the therapeutic potential of targeting the 20S proteasome and the ubiquitin proteasome system (UPS) in cancer and especially in hematologic malignancies [3] (Table 1). BTZ inhibits the 20S core proteasome, resulting in cancer cell death *via* multiple mechanisms; including, induction of reactive oxygen species (ROS), suppression of the unfolded protein response (UPR), accumulation of ubiquitinated proteins, inhibition of the cellular NFκB survival pathway *via* accumulation of IκBα, and stabilization of tumor suppressor proteins such as p21, p27, Bax and p53 [4–5].

Based on the findings and successes of preclinical model studies, BTZ was examined as a potential chemotherapeutic in the treatment of MCL. BTZ induced responses in approximately one-third of patients with previously-treated MCL in a single-agent Phase 2 trial [6]. A subsequent Phase 3 trial comparing a BTZ-containing combination to standard chemotherapeutic regimens demonstrated superior results with regard to quality and duration of patient responses in the BTZ-containing arm [7]. Consequently, BTZ was approved by the FDA for the treatment of relapsed Mantle Cell Lymphoma (MCL) on December 8th, 2006 for patients who have received at least one prior therapy, and on October 9th, 2014 as a frontline therapy for use in previously untreated MCL patients (Table 1) [8].

The intentions of this review are to provide a biological background on the role of the proteasome in cancer, and the molecular rationale of proteasome inhibition in MCL. In addition, the review will provide a historical and scientific accounting of the discovery, preclinical and early clinical development of BTZ as treatment of MCL. Finally, we will examine the potential limitations of BTZ therapy and discuss potential means of addressing these issues.

2. The Role of the Proteasome and the UPS in Cancer

The UPS is a complex, dynamic, and critical system in both normal and cancer cells, and disruption of the UPS, such as by a proteasome inhibitor, is associated with extensive modulation of signaling pathways, cellular activities and apoptosis/autophagy [9–12]. As a posttranslational modification system, the UPS modulates the fate of diverse proteins through the dynamic addition, branching and removal of ubiquitin (Ub) moieties. Ub is a highly conserved and small (76 amino acids, 8.5 kDa) regulatory protein moiety which,

through a series of molecular processes, is linked to lysine residues of targeted proteins. The fate of the target proteins is dependent on the dynamic pattern of ubiquitin branching [13–15].

2.1 The Structure and characteristics of the 26S Proteasome

The 26S proteasome is the central molecular machinery of the UPS, consisting of a 20S catalytic core which enzymatically degrades targeted, ubiquitinated proteins, as well as two 19S regulatory caps which regulate the removal and recycling of the linked Ub chains and linearizing and inserting the targeted proteins into the 20S proteasome for catalytic degradation (Figure 2A). The 20S core is composed of two identical inner β-rings and two identical outer α-rings. Each β-ring contains 3 proteolytic sites on β1, β2 and β5 subunits, responsible for the post-glutamyl peptide hydrolase-like (PGPH) (or caspase-like), trypsin-like, or chymotrypsin-like (CT) activities, respectively (Figure 2A) [16–17].

2.2 Regulators of the UPS: Ubiquitin-Conjugating Enzymes E1, E2 and E3

Regulation of protein ubiquitination involves a multi-step process that transfers Ub-moieties to target proteins by the conjugation and ligation of different lengths and branching patterns of Ub. Ub conjugation occurs *via* an enzymatic cascade, involving three distinct enzymes: (i) Ub-activating (E1), (ii) Ub-conjugating (E2), and (iii) Ub-ligating enzymes (E3 Ligases) (Figure 2B). Protein ubiquitination is initiated by the ATP-dependent formation of a thioester linkage between the C-terminus of the Ub moiety and a cysteine residue of the E1-activating enzyme [13–14]. The Ub moiety is then transferred to an E2-Ubiquitin-conjugating enzyme through the formation of an E1-Ub-E2 complex *via* the formation of a *thio*-ester intermediate complex [15]. Finally, the Ub is transferred from the E2-conjugating enzyme to a target specific E3-ligase bound to a specific protein-substrate directly or *via* a third high-energy *thio*-ester intermediate, which results in the formation of an isopeptide bond between the C-terminus of the Ub and a specific lysine residue on the substrate protein, or expanding a preexisting Ub-chain of variable lengths and branching patterns [15–18].

A complex hierarchal pyramid of E1, E2, and E3 ubiquitin-conjugating enzymes allows for fine-tuned modulation of protein degradation *via* variable ubiquitination and branching patterns, intricately coordinated by differential expression and localization of the components of the UPS [18].

2.3 UPS-Mediated Regulation of Target Specificity and Cellular Fate

The extent and complexity of Ub-branching patterns significantly affects the behavior of the target protein and provides an extensive and diverse set of potential targets for future UPS-targeted drugs distinct from 20S-Core proteasome targeted by BTZ and other PIs [19–20]. The UPS regulates cellular activities and functions through protein binding specificity, and also differential expression and cellular localization of the individual UPS components (such as E1, E2, E3 and Deubiquitinases/DUBs) [15, 18]. For example, the UPS regulates cell-cycle progression and apoptosis through the turnover of key proteins, such as the cyclins, p21 and p53 [21–22]. UPS also plays an essential role in regulating one of the most important cell survival pathways, the NFκB pathway [23–24]. Cancer cells have been shown to utilize the UPS to maintain aberrant cell growth and resistance to apoptosis through

enhanced degradation of the NFκB inhibitor, IκB. Inhibition of the proteasome by BTZ has been shown to induce cell-cycle arrest and apoptotic cell death selectively in human cancer cells such as multiple myeloma [25–26]. Proteasome inhibition causes these effects through modulation of a variety of cellular pathways in cancer cells, specifically, accumulation of p27, Bax, p53 and IκBα, inhibition of NFκB, and induction of the unfolded protein response (UPR) and DNA damage responses (DDR) (Figure 3) [12, 16, 18–22 24–235]. These UPS-mediated effects have been observed in pre-clinical studies across a wide spectrum of tumors, including MM and MCL.

3. Discovery of Bortezomib

BTZ (Figure 1) is a reversible inhibitor of the 20S proteasome. The discovery and development of BTZ, then known as PS-341, a dipeptide boronic acid, was accomplished by Dr. Julian Adams and his team, who sought to inhibit the proteasome's enzymatic functions in order to diminish the aberrant proteasome activity associated with cancer and inflammation [27–28]. Numerous peptidyl aldehydes have demonstrated the ability to inhibit the chymotrypsin (CT)-like activity of the proteasome complex [5]. Utilizing X-ray crystallography, it was discovered that the aldehyde inhibitors formed hemi-acetal adducts with the active site threonine nucleophile of β-subunits (Figure 2A) [28]. In order to further enhance the interaction with the β-subunits, Boron was incorporated into the compound which significantly enhanced the potency of the prototype proteasome inhibitors (PIs). BTZ is one such boron-containing PI.

In vitro, myeloma cells are differentially sensitive to BTZ-induced proteasome inhibition and apoptosis relative to normal cells. One reason for this is that non-malignant cells do not divide as rapidly as cancer cells therefore they are less dependent on the proteasome for protein turnover [4–5 19,]. As mentioned, a specific action of BTZ is the inhibition of NFκB pathway through the stabilization of its inhibitor protein IκB. Myeloma cells specifically depend on NFκB-mediated transcription of cytokine growth factor interleukin-6 (IL6), angiogenesis through vascular endothelial growth factor (VEGF), and the cell adhesion molecule VCAM-1 for adherence of the plasma cells to the stromal tissue in bone marrow [25–26, 30]. Dr. Adams and his collaborators found that even at low nanomolar concentrations, BTZ was highly effective in the abrogation of transcription of NFκβ-dependent genes. BTZ also increases $p27^{Kip1}$ levels, and consequently reduces Cyclin-D1, thus inhibiting cell-cycle progression [31]. Proteasome inhibition by BTZ has also been shown to upregulate pro-apoptotic genes and downregulate anti-apoptotic genes [32–33]. For example, BTZ selectively increases levels of a pro-apoptotic protein NOXA in malignant cells [34–35]. Furthermore, it has been demonstrated that BTZ induces ER stress, ultimately leading to cytochrome C release and subsequent caspase-mediated apoptosis [33, 36]. The key molecular mechanisms of action of BTZ-dependent cancer cell death have been summarized in Figure 3.

4. Molecular Characteristics of MCL

Immuno-phenotyping of MCL typically reveals a $CD5^+$, $CD20^+$, $CD10^-$, $FMC7^+$, $CD23^-$, $CD43^+$, and cyclin $D1^+$ profile [37–38]. Additionally, MCL is characterized by the specific

chromosomal translocation t(11;14)(q13;q32) [39], which results in the overexpression of CCND1. MCL cells have increased expression of the transcription factor SOX11, which has been shown to promote tumor growth of MCL cells *in vivo* and is known to regulate a broad set of transcriptional programs that includes B-cell differentiation, cell proliferation, apoptosis, and angiogenesis [40–41]. MCL cells also exhibit inherent genetic instability with a tendency to accumulate alterations in cell cycle regulatory genes, DNA damage response pathway, and cell survival mechanisms which may contribute to the inevitable development of resistance to therapy seen in patients with MCL [42].

Recent genome-wide studies using next-generation sequencing (NGS) have expanded the understanding of genes and pathways involved in the development of MCL [43]. It has been shown that the most common secondary alteration in MCL is the mutation of the DNA damage sensor ATM, particularly in cells over-expressing SOX11 [40, 42]. Mutations in several chromatin modifiers such as WHSK1 (10%), MLL2 (14%), and MEF2B (3%) have also been detected almost exclusively in MCL cells expressing SOX11. Other aberrations identified include activating mutations in NOTCH1/2 in 10% of tumors associated with an aggressive evolution and somatic mutations in regulatory genes of NFκB pathway in 10% to 15% of MCL [44–45].

5. Preclinical studies of Bortezomib in Mantle Cell Lymphoma

5.1 Molecular mechanisms of Bortezomib activity and resistance in MCL

The cytotoxicity of bortezomib in MCL cells results from the effects of proteasome inhibition on several intracellular mechanisms [46–51]. An analysis of primary MCL cells by Chiarle *et al* showed that the protein levels of the p27 CDK inhibitor is decreased due to degradation by the proteasome [46]. The protein p27 is a negative regulator of the cell cycle, and its inhibition or down-regulation is associated with tumor progression. Although this research group used a different proteasome inhibitor (hemin), it nevertheless linked proteasome inhibition to the diminished tumorigenesis in MCL. BTZ was also shown to cause cell cycle arrest and apoptosis by inhibition of the NFκB pathway in MCL cell lines (Mino and DB-sp53) and primary tumor samples [31]. NFκB, an important signaling pathway regulating cell cycle progression and cell survival, is constitutively expressed in these cell lines and tissues. Inhibition of the proteasome by BTZ down-regulates the expression of NFκB, which can lead to G_1 cell cycle arrest and apoptosis. The NFκB survival pathway may be sustained by protein kinase CK2, which phosphorylates and activates NFκB. Inhibition of CK2 enhances BTZ's cytotoxic effect in MCL cell lines (Granta 519, Jeko-1, and Rec-1) [50–51]. Thus, high levels of CK2 may be involved in BTZ resistance. However, a study comparing BTZ-sensitive and BTZ-resistant MCL cell lines found no correlation between NF-kB activity and resistance status [35]. Furthermore, NF-kB activity varies widely among the sensitive and resistant cell lines [35]. As an example, NF-kB activity is ~2.5-fold higher in Granta cells than it is in UPN1 cells, but Granta is as sensitive to BTZ as UPN1 (35). Therefore, whether NF-kB pathway is involved in BTZ resistance remains unclear.

As further discussed in Section 6, a main problem observed through the use of BTZ is inherent and acquired resistance to the drug. Preclinical studies have suggested several

potential mechanisms involved in development of bortezomib resistance, including: plasmacytic differentiation *via* up-regulation of IRF4 and CD38 and expression of CD138 (52); MCL-initiating or stem-like cell phenotype (lacking of prototypic B-cell marker CD19 (53); a defect in regulation or function of the pro-apoptotic BH3-only protein NOXA (35).

Consistently, targeting these involved pathways would overcome BTZ resistance. Indeed, harnessing NOXA demethylation was shown to be able to overcome BTZ resistance in MCL (54). Furthermore, resistance to BTZ could be overcome by the Hsp90 inhibitor IPI-504 (55), the dual PI3K and mTOR inhibitor NVP-BEZ235 (56), Sorafenib (57), calcium blockers (58), and inhibition of Lyn (59),

In addition, treatment of MCL cell lines (JVM-2, GRANTA-519, JEKO, and REC-1) with BTZ leads to depolarization of the mitochondria membrane, ROS production, and induction of the pro-apoptotic NOXA protein, resulting in apoptosis [35]. NOXA, in particular, appears to be important, as *si*RNA-induced downregulation of NOXA inhibits BTZ-induced apoptosis [35]. PRDM1 (*PR domain zinc finger protein 1)* is a transcription factor that represses the expression of proteins needed for B-cell identity and proliferation and helps to drive B-cells through their final differentiation stage to become antibody-secreting cells. PRDM1 is also a mediator of NOXA-induced apoptosis. Interestingly, PRDM1 was shown to be needed for BTZ-induced apoptosis in MCL cell lines (Mino and Jeko-1) and primary tumor samples [49]. This finding further strengthens the role of NOXA in BTZ-mediated anticancer activity in MM and MCL.

5.2 Rationale for combining BTZ with other chemotherapeutic agents in MCL

Given the fact that the majority of MCL patients do not have a clinical response to single-agent BTZ, and that it had been shown to be easily combined with other antineoplastic agents in the treatment of multiple myeloma, there was interest in developing BTZ-containing combination therapy in MCL.

Work involving several MCL cell lines (Granta-519, HBL-2, and Jeko-1) showed synergistic anti-MCL activity resulting from sequential use of the traditional chemotherapy drug cytarabine and BTZ. This approach was used with some success in two MCL patients treated by the same investigators [51]. A synergistic anti-tumor effect of the HDAC inhibitor vorinostat (SAHA) and BTZ, possibly due to vorinostat-mediated interference of NFκB transcription and signaling, as well as generation of ROS and also caspase activation, in human MCL cell lines (Jeko-1 and Granta-519) was also reported [60]. Treatment of MCL cell lines (Mino, Jeko, Granta, and DBsp53) and primary patient samples with the combination of the JAK/STAT pathway inhibitor Degrasyn and BTZ synergistically inhibits growth and induces apoptosis. In MCL xenograft studies using SCID mice, the drug combination treatment prevents tumor development and prolongs animal survival [61]. Arsenic Trioxide (ATO), which has been shown to upregulate pro-apoptotic proteins and induce apoptosis in MCL through downregulation of the NFκB pathway, also displays synergy with BTZ [62–63]. This has been demonstrated in multiple MCL cell lines (Jeko-1, SP-53, Mino, and REC-1) and primary patient samples.

More recently, a synergistic inhibitory effect of idelalisib (CAL-101) and BTZ on the growth of human MCL cell lines (Z138, HBL-2, and Jeko-1) was reported [64]. Treatment of MCL cell lines with both CAL-101 and BTZ resulted in inhibition of the Akt and NFκB signaling pathways and concurrent increased apoptosis [65]. Idelalisib is a PI3K inhibitor that is FDA-approved for chronic lymphocytic leukemia and follicular lymphoma [65].

Perhaps most importantly, the combination of BTZ and the anti-CD20 monoclonal antibody rituximab was shown to have increased response in MCL cell lines (SP-53, Mino, and Jeko) and patient samples [64]. Combination of BTZ and Rituximab leads to enhanced inhibition of NFκB and Akt signaling pathways and induction of apoptosis. These findings related to rituximab and BTZ in MCL are of particular significance given the central role the antibody plays in clinical management of B-cell lymphomas.

In summary, there is ample pre-clinical evidence that BTZ has synergistic anti-MCL activity with other drugs, including ones with central importance in the clinical management of MCL such as rituximab.

6. Clinical Development of BTZ in MCL treatment

Phase I studies found that the maximally tolerated dose (MTD) of BTZ in previously-treated NHL patients was similar to that for MM: 1.04 to 1.5 mg/m^2 [6, 66–67]. The phase II PINNACLE trial in 2006, in which patients with relapsed/refractory MCL were treated with BTZ monotherapy, showed an overall response rate of 32%. This trial formed the basis for the initial FDA approval of BTZ for the treatment of MCL with at least one prior therapy (Table 1) (6, 68). Subsequent Phase II trials which combined BTZ and rituximab with either purine analogues (69–71) or alkylating agents (72–73) generally demonstrated overall response rates of over 50%. Impressively, the combination of BTZ with a standard alkylator-containing regimen (Rituxan + HyperCVAD) in newly-diagnosed MCL is associated with a response rate of 90–100% (74–75). BTZ-containing front-line combinations in MCL are generally tolerated, with myelosuppression, gastrointestinal side effects, and therapy-emergent peripheral sensory neuropathy seen (74–80). In 2015, the FDA approved a BTZ-based drug combination as the front-line treatment for MCL after a large phase III study demonstrated that compared to R-CHOP (rituximab, cyclophosphamide, doxorubicin, vincristine, and prednisone), the VcR-CAP regimen (BTZ, rituximab, cyclophosphamide, doxorubicin, and prednisone) is associated with more durable disease control and better clinical responses [7–8] (Table 1).

There have been multiple clinical trials utilizing BTZ in combination with other chemotherapeutics in the treatment of MCL, since the initial recognition of BTZ's efficacy in MCL (Table 2). Of particular interest is a trial combining BTZ and rituximab with bendamustine, an alkylating agent, in patients with relapsed/refractory indolent NHL and MCL [73]. This combination was associated with an impressive 83% response rate, and expected gastrointestinal and neuropathic side effects. In contrast, the combination of BTZ, rituximab and lenalidomide was somewhat disappointing. This is a regimen widely used in the initial and subsequent treatment of multiple myeloma, and incorporates another FDA-approved therapy for MCL. Though the combination was tolerable, the overall efficacy

(response rate 45%) was not sufficiently increased to justify further exploration of the combination [81]. Similarly, other recent Phase 1/2 trials combining BTZ with clinically available drugs such as everolimus [82] and temsirolimus [83] did not demonstrate clinical activity commensurate to reported pre-clinical synergy.

There are two main problems encountered from the use of BTZ: (i) toxicities related to proteasomal inhibition and off-target actions, and (ii) inherent and acquired resistance to the drug. Common toxicities associated with BTZ include gastrointestinal side effects, myelosuppression, and neurotoxicity (usually peripheral sensory neuropathy, but occasionally motor or autonomic). A meta-analysis of over 30 myeloma and NHL trials utilizing intravenous BTZ demonstrated a 34% incidence of treatment-associated neuropathy (8% severe) [84]. Weekly, rather than twice-weekly, dosing has not been shown to reduce the frequency or severity of neuropathy in NHL patients [85], though this is held to be true in myeloma. Subcutaneous BTZ administration in myeloma has been shown to reduce neurotoxicity without affecting the anti-cancer effects of the drug [86], and has led to the general use of subcutaneous administration in NHL patients, as well. Several second-generation proteasome inhibitors have been developed, each with unique chemical structure, biochemical properties, binding affinity, binding reversibility, potency and/or selectivity. Carfilzomib (CFZ) has been approved by the FDA for treating MM in 2013. It has been shown that CFZ binds the 20S proteasome with more specificity than BTZ, with little or no off-target activity outside of the proteasome (87). Carfilzomib is somewhat less neurotoxic than BTZ, a finding postulated to be related to the differing on-and off-target binding of the drugs. The free α–amino group required for adduct formation with CFZ does not interact with serine and cysteine proteases that can be inhibited by BTZ (88). CFZ selectively targets the β5 over β1 and β2 subunits in the constitutive 20S proteasome.

Most patients treated with BTZ- and rituximab-containing induction regimens respond to initial therapy but relapses are invariable. Once chemotherapy for relapsed disease is required, response rates are dramatically lower, including general response rates of less than 50% with most BTZ-containing salvage regimens.

BTZ resistance remains to be a great challenge in the field. Although several potential mechanisms have been identified in preclinical studies (**Section 5.1**), they should be confirmed in clinical settings. Other pathways should also be examined as potential strategies to overcome BTZ resistance. It may be possible to target other components of the UPS pathway such as E3 ubiquitin ligase [89] and 19S proteasome-associated DUBs [90–91] to induce anti-tumor responses in BTZ-sensitive and resistant conditions.

7. Conclusions

Traditional therapies for MCL have limited efficacy, with almost all patients eventually relapsing. Recent work is rapidly elucidating the molecular basis of tumorigenesis and drug resistance, with an increasing number of targetable cellular proteins and pathways identified – including ones relevant to the use of BTZ and other proteasome inhibitors. Research involving MCL cell lines, primary tumor samples, and animal models has demonstrated that BTZ inhibits the pro-survival NFκB pathway *via* CK2, induces the pro-apoptotic protein

NOXA *via* PRDM1, and causes mitochondrial membrane depolarization, associated with ROS production and apoptosis induction. As with other therapies, primary and acquired resistance to BTZ is a limitation to its use *versus* MCL. Preclinical work supports the use of BTZ-containing combinations to partially overcome the problem of resistance. Numerous clinical trials in MCL have confirmed that combining BTZ with other anti-lymphoma therapies is both feasible and effective. Future work should focus, at least on: better understanding the mechanisms of action of BTZ responsible for its efficacy and resistance; developing more potent, more specific and less toxic USP inhibitors that have activity in BTZ-resistant systems; deeper insight on the clonal heterogeneity and predictive/prognostic biomarkers of MCL; further identification of specific, critical, essential cellular pathways for this disease. All these could lead to development of novel strategies for selectively targeting MCL and improving its treatment outcomes.

8. Expert Opinion

The key findings described above relate to the biological effects of BTZ in pre-clinical models and the promising results from subsequent clinical trials. The mechanism of BTZ activity is likely due to its effect on multiple intracellular processes. It is not clear which UPS substrate(s) is most important as a mediator of therapeutic response. Further, there may be multiple resistance mechanisms relevant to BTZ therapy in MCL, and these have not been fully elucidated.

Optimization of MCL therapy not only entails identifying the best antineoplastic agents, but also the most effective schedule and duration of therapy. Maintenance therapy with other drugs, namely rituximab, has been shown to prolong disease control in some types of NHL, raising the question as to whether a "continuous therapy" strategy might be helpful in MCL management. Long-term maintenance therapy with BTZ has been shown to be feasible and beneficial in another hematologic malignancy, multiple myeloma. This, as well as the recent development of a more convenient, well-tolerated oral proteasome inhibitor (Ixazomib ®, also called Ninlaro®) may make PI maintenance therapy more feasible in the future. Ultimately, even if combinations containing BTZ or other PIs fail to cure MCL, it is reasonable to hope they may make prolonged disease control possible for more patients.

Continued research into resistance pathways is going to be essential in achieving maximal benefit from BTZ and proteasome inhibitors in general in MCL. It may be possible to circumvent resistance to existing PIs by targeting other components of the UPS. For example, inhibition of some specific E3-ligases would stabilize expression of tumor suppressors IκBα, p27, *etc.*, without generally inhibiting all proteasome activity globally, which is critical to normal cellular activities. Alternatively, new classes of drugs with completely different targets may potentiate the effects of BTZ. As one example, selinexor is a drug which selectively inhibits CRM-1 (also called exportin-1 or XPO-1) thereby blocking shuttling of tumor suppressor proteins out of the cell nucleus [91]. This oral agent has been shown to have preclinical activity in a variety of tumors, including solid tumors, NHL, and multiple myeloma. Preclinical work suggests treatment of PI-resistant myeloma cells with selinexor may partially restore PI-sensitivity [92]. This is being explored in clinical trials in relapsed/refractory myeloma presently [93]. Identifying key pathways and selectively

targeting them in MCL cells (or the surrounding microenvironment, perhaps) should lead to an improved therapeutic index for these drugs. Clonal evolution and MCL cell heterogeneity may continue to pose an obstacle to completely overcoming drug resistance, as it has in other tumors. As we are now in an era in which molecular and genetic profiling of cancer cells is less time- and cost-prohibitive than ever before, it is likely that advances in cancer biology gleaned from other tumor types may be brought to bear against MCL. Targeted therapies approved for other cancers (or even non-malignant disease) may be able to be used *versus* MCL if patient-specific (even clone-specific) susceptibility factors could be identified. These factors are likely to change over the course of a single case of MCL as different therapies are used sequentially.

Acknowledgments

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Article highlights

- The discovery and development of Bortezomib (BTZ), the first FDA approved 20S proteasome inhibitor drug for treating relapsed refractory multiple myeloma, has validated the therapeutic potential of targeting the proteasome and the ubiquitin-proteasome system (UPS) to selectively treat human cancer.

- Mantle cell lymphoma (MCL) is an aggressive B-cell malignancy. Traditional therapies for MCL have met with limited efficacy, with almost all patients eventually experiencing relapse. Therefore, it is important to understand the molecular characteristics of MCL clonal heterogeneity, discover predictive/prognostic biomarkers, and develop new targeted therapies for treating this disease.

- Preclinical MCL models demonstrated potent apoptotic effects of BTZ alone, as well as enhanced synergistic anti-MCL activity between BTZ and other chemotherapeutics.

- BTZ alone induces clinical responses in approximately one-third of relapsed MCL patients, and BTZ-based combination therapies have further improved the quality and duration of clinical responses and enhanced survival rates, compared to standard front-line treatments in previously untreated MCL patients.

- The cytotoxicity of BTZ in MCL cells results from its proteasome inhibitory activity,., through several potential mechanisms, including: accumulation of tumor suppressor proteins (p27, p21, p53 and NOXA), inhibition of the pro-survival NFκB pathway, mitochondrial membrane depolarization, ROS production, cell cycle arrest and apoptosis induction.

- Inherent and acquired BTZ resistance remains the major challenge in MCL therapies. Preclinical studies of BTZ resistance have identified multiple possible mechanisms, such as plasmacytic differentiation, stem-like cell phenotypes, and defects in regulation or functions of NOXA. However, which of these is responsible for clinical BTZ resistance requires further investigation.

- Next-generation proteasome inhibitors with different pharmacodynamic properties from BTZ may partially address the issue of resistance.

- Targeting upstream components of the UPS pathway, such as E3 ubiquitin ligases and deubiquitinating enzymes may be a promising strategy for overcoming proteasome inhibitor resistance and improving the specificity and efficacy of BTZ-based combination therapies for treating patients with MCL.
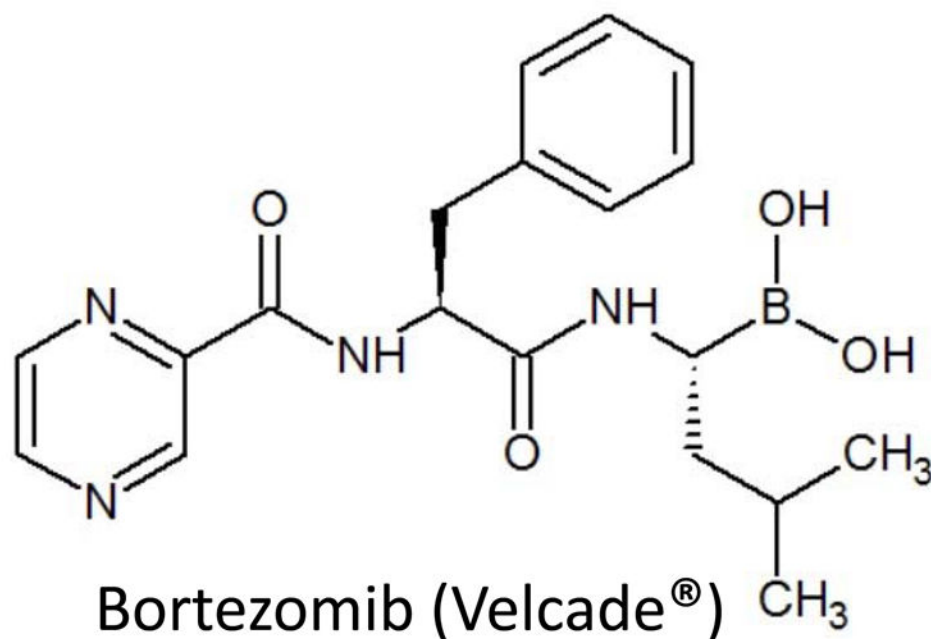
Figure 1. Chemical Structure of BTZ

Bortezomib (BTZ, Trade name Velcade®) is a dipeptide boronic acid which forms non-covalent bonds with the N-terminal hydroxyl groups of threonine residues of the 20S proteasome *via* the Boron atom' activity as an electron receiver, thus forming stable tetrahedral intermediates which inhibit the catalytic activity of specific β-subunits (β5 mainly, and β1) resulting in inhibition of chymotrypsin-like and PGPH-like activities.
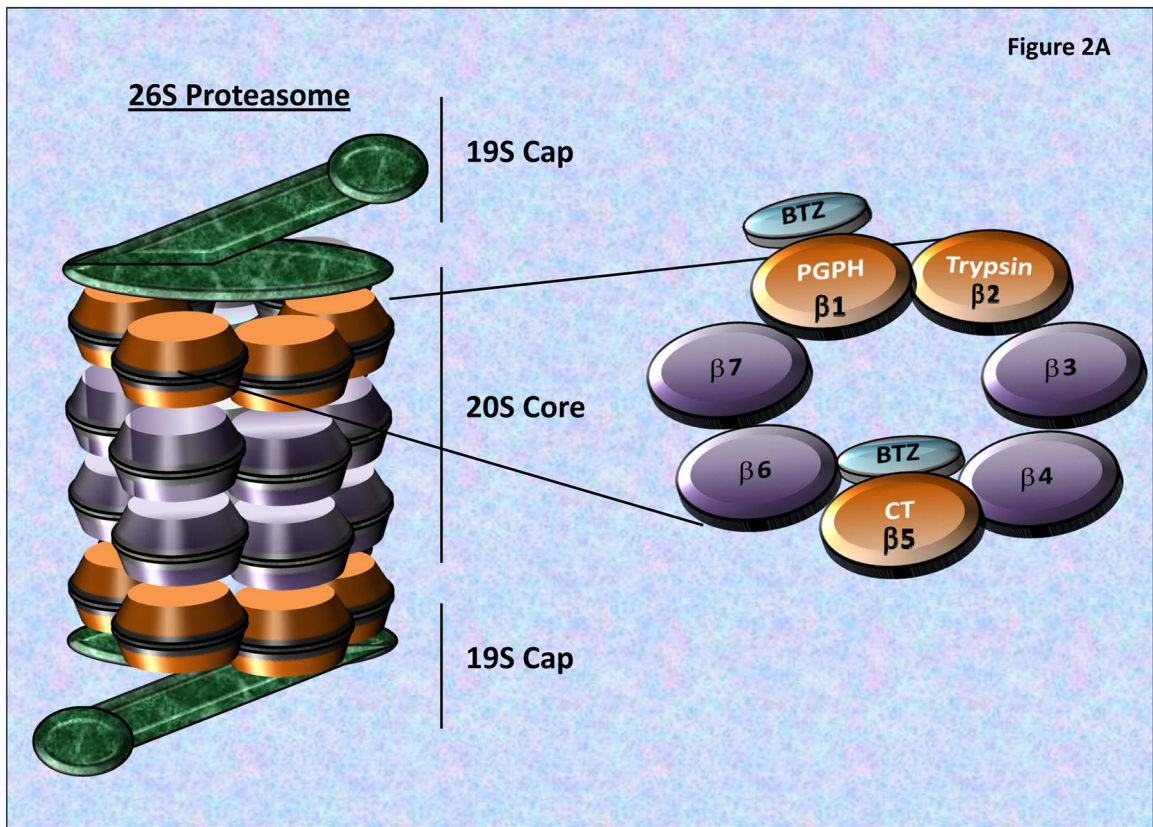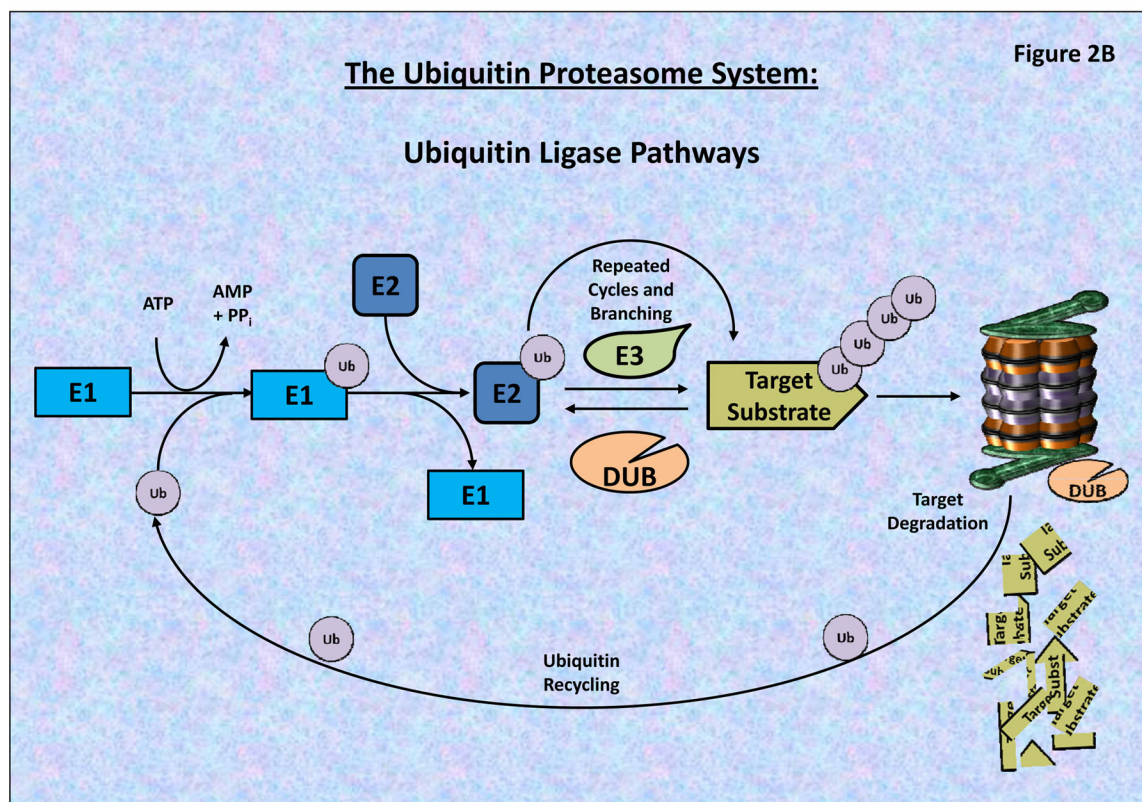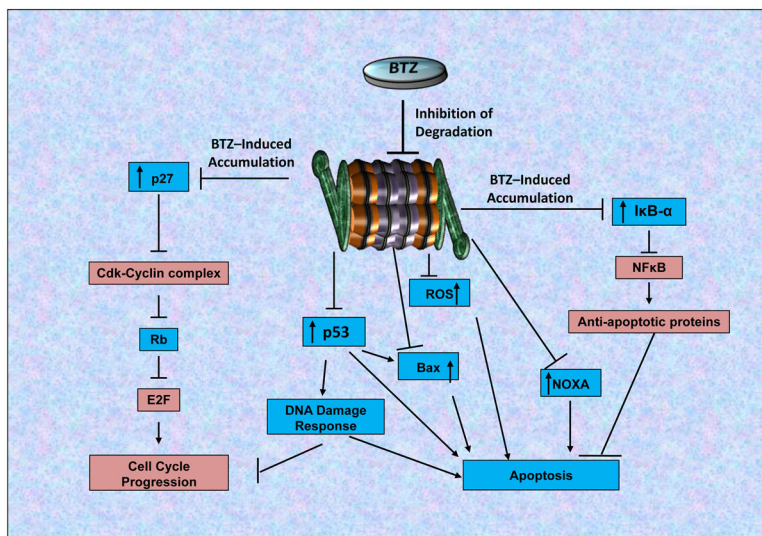
Figure 2A

Figure 2. The 26S Proteasome Structure and Proteolytic Activities
A) The 26S proteasome consists of a 20S catalytic core and two 19S regulatory caps, which removes/recycles linked Ub, linearize and direct targeted proteins into the 20S Core. The 20S core is composed of 2-inner β-rings and 2-outer α-rings. As seen the β-rings contain 3 proteolytic sites (β1, β2 and β5 subunits), possessing post-glutamyl peptide hydrolase-like (PGPH) (or caspase-like), trypsin-like, or chymotrypsin-like (CT) activities, respectively. BTZ primarily binds to the β5 and β1 subunits blocking proteasome activities. B) The Ubiquitin Proteasome System (UPS) is composed of a series of ubiquitinating enzymes which work through the specific and targeted addition and branching of Ubiquitin to proteins which are differentially targeted by different E3-Ligases (which possess target specificity). Through the utilization of ATP, Ub is conjugated to the E1 Ligase, and then transferred to an E2 Ligases which subsequently transfers the Ub Moiety to a target protein or an existing Ub-chain. The complexity of the Ub branching pattern is determined by the interaction of numerous UPS components including the Deubiquitinating enzymes (DUBs) which serve to both recycle as well as modulate the branching patterns of ubiquitinated proteins. Most poly-ubiquitinated proteins then pass through the 19S cap which linearizes the peptide and passes it into the 20S Core proteasome for catalytic degradation.

Figure 3. Mechanisms of Action of BTZ and Cellular Effects and Pathways
The 26S proteasome is responsible for the degradation of numerous critical cellular proteins, thus regulating multiple critical cellular pathways. Depicted, are a few key pathways associated with the proteasome activity and regulated by BTZ-mediated proteasome inhibition. Inhibition of the proteasome results in the stabilization and accumulation of multiple tumor suppressors (Blue) such as: p27, p53, Rb, IκBα, Bax and NOXA as well as ROS. Accumulation of p27 reverses the inhibition on RB, resulting in blockage of cell cycle progression through inhibition of E2F, a critical regulator of cell cycle progression (Oncogene/Oncogenic: Red). Accumulation and stabilization of Bax induces Cytochrome-C dependent apoptosis, and accumulation of IκBα inhibits NFκB, a crucial regulator of cell survival and anti-apoptotic pathways. Induction of NOXA and ROS by BTZ also contribute to its apoptosis-inducing and anti-MCL activities. Therefore, proteasome inhibition by BTZ obstructs three key cancer pathways: (i) Cell cycle progression, (ii) Cell survival pathways, and (iii) Cellular proliferation, the combination of which results in significant inhibition of cancer cell growth.

Table 1

Major events in the clinical development of Bortezomib

Year	Events In Bortezomib Development	Source
1995	Synthesis by a team led by Julian Adams.	Adams, J. et al. Boronic ester and acid compounds. US Patent 5,780,554 (1995).
2000	Phase-I clinical trial shows BTZ is tolerable at 1.04 mg/m². It also shows activity in multiple myeloma.	Orlowski, R. et al. Phase I trial of the proteasome inhibitor PS-341 in patients with refractory hematologic malignancies. J. Clin. Oncol. 20, 4420–4427 (2002).
2003	Phase II clinical trial shows BTZ being active in refractory multiple myeloma. This prompted accelerated FDA approval for BTZ as treatment of refractory multiple myeloma.	Richardson, P. G. et al. A phase 2 study of bortezomib in relapsed, refractory myeloma. N. Engl. J. Med. 348, 2609–2617 (2003).
2005	Phase III trial confirms BTZ activity in multiple myeloma. The FDA approved BTZ for the treatment of multiple myeloma with one prior therapy.	Kane RC et al. United States Food and Drug Administration approval summary: bortezomib for the treatment of progressive multiple myeloma after one prior therapy. Clin Cancer Res. 2006 May 15;12(10):2955–60.
2006	Phase II trial shows BTZ activity in mantle cell lymphoma. The FDA approved BTZ for the treatment of mantle cell lymphoma with at least one prior therapy.	Kane RC et al. Bortezomib for the treatment of mantle cell lymphoma. Clinical cancer research, 13(18 pt 1): 5291–4, 2007.
2008	Phase III trial reports superior activity when combining BTZ with melphalan and prednisone as initial treatment for multiple myeloma. The FDA approved BTZ to be used in the initial treatment of multiple myeloma.	Miguel, J.F.S. et al. Bortezomib plus Melphalan and Prednisone for initial treatment of multiple myeloma. N. Engl. J. Med. 359, 906–917 (2008).
2014	Phase III trial reports at least 59% increased efficacy when BTZ replaces vincristine in a drug combination that includes rituximab, cyclophosphamide, doxorubicin, and prednisone for the initial treatment of mantle cell lymphoma. The FDA approved BTZ for the initial treatment of mantle cell lymphoma.	Robak T. et al. Bortezomib-based therapy for newly diagnosed mantle-cell lymphoma. N. Engl. J. Med. 372:944–953 (2015).

Table 2

Ongoing clinical trials of Bortezomib in Mantle Cell Lymphoma

Name of Study	Status	Condition	Intervention
Bortezomib (Velcade) Plus Rituximab-HyperCVAD in Patients With Mantle Cell Lymphoma	Active, Not recruiting	Mantle Cell Lymphoma	Bortezomib, Rituximab, Cyclophosphamide, Vincristine, Methotrexate, Cytarabine, Doxorubicin
EPOCH-R Chemotherapy Plus Bortezomib to Treat Mantle Cell Lymphoma	Active, Not recruiting	Mantle Cell Lymphoma	EPOCH, Rituximab, Bortezomib
Rituximab, Lenalidomide, and Bortezomib in Mantle Cell Lymphoma	Active, Not recruiting	Mantle Cell Lymphoma	Rituximab, Bortezomib, Lenalidomide
Combination of Ibrutinib and Bortezomib to Treat Patients With Mantle Cell Lymphoma	Recruiting	Mantle Cell Lymphoma	Ibrutinib, Bortezomib
Bortezomib (VELCADE), Cladribine and Rituximab (VCR) in Mantle Cell Lymphoma (PSHCI 10–011)	Recruiting	Mantle Cell Lymphoma	Cladribine, Rituximab, Bortezomib
Trial of Bortezomib, Cytarabine, and Dexamethasone in Mantle Cell Lymphoma	Not Yet Recruiting	Mantle Cell Lymphoma	Bortezomib, Cytarabine, Dexamethasone, Pegfilgrastim
Bortezomib and Lenalidomide in Treating Patients With Relapsed or Refractory Mantle Cell Lymphoma	Active, Not recruiting	Mantle Cell Lymphoma	Bortezomib, Lenalidomide
Bortezomib, Cladribine, and Rituximab in Treating Patients With Advanced Mantle Cell Lymphoma or Indolent Lymphoma	Active, Not recruiting	Lymphoma, mantle cell lymphoma, indolent lymphoma, SLL	Cladribine, Rituximab, Bortezomib
Alisertib, Bortezomib, and Rituximab in Treating Patients With Relapsed or Refractory Mantle Cell Lymphoma or B-cell Low Grade Non-Hodgkin Lymphoma	Recruiting	Recurrent and refractory B-cell non-Hodgkin Lymphoma, recurrent and refractory mantle cell lymphoma	Alisertib, Rituximab, Bortezomib
Bortezomib and Vorinostat in Treating Patients With Recurrent Mantle Cell Lymphoma or Recurrent and/or Refractory Diffuse Large B-Cell Lymphoma	Active, Not recruiting	Recurrent adult diffuse large cell lymphoma, recurrent mantle cell lymphoma	Vorinostat, Bortezomib
Efficacy and Safety of R-HAD Alone or in Combination With Bortezomib in Patients With Relapsed or Refractory MCL	Recruiting	Mantle cell lymphoma	Rituximab, high dose Ara-C, Dexamethasone, Bortezomib

*
Data obtained from clinicaltrials.gov