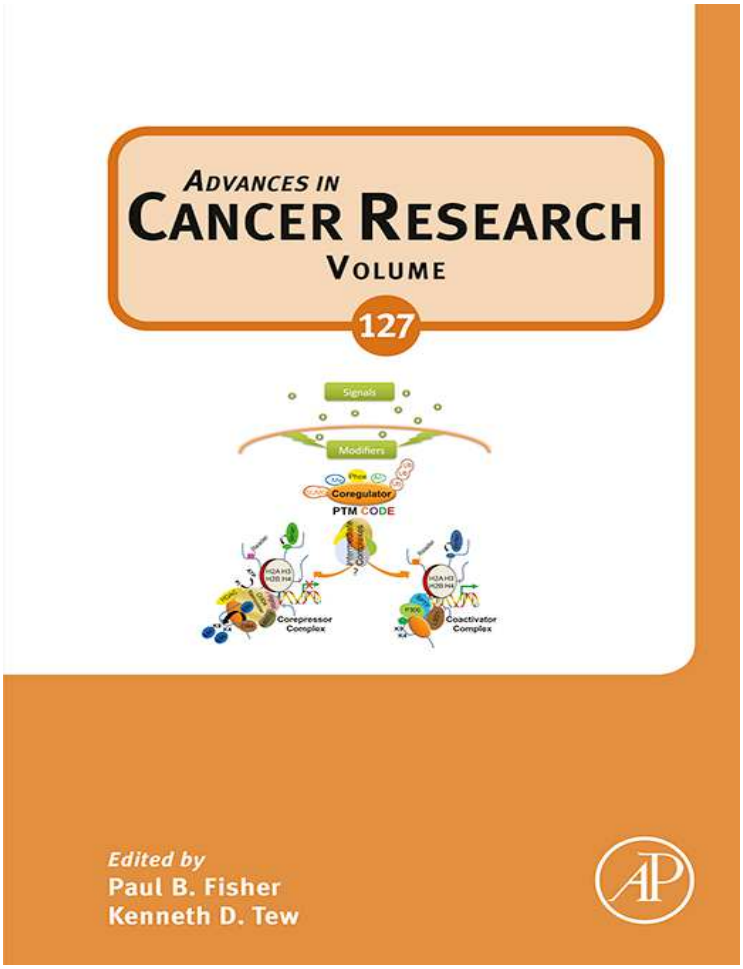

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CHAPTER FIVE

Emerging Therapeutic Strategies for Overcoming Proteasome Inhibitor Resistance

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Contents

Abstract

The debut of the proteasome inhibitor bortezomib (Btz; Velcade®) radically and immediately improved the treatment of multiple myeloma (MM), an incurable malignancy of the plasma cell. Therapeutic resistance is unavoidable, however, and represents a major obstacle to maximizing the clinical potential of the drug. To address this challenge, studies have been conducted to uncover the molecular mechanisms driving Btz resistance and to discover new targeted therapeutic strategies and combinations that restore Btz activity. This review discusses the literature describing molecular adaptations that confer Btz resistance with a primary disease focus on MM. Also discussed are the most recent advances in therapeutic strategies that overcome resistance, approaches that include redox-modulating agents, murine double minute 2 inhibitors, therapeutic monoclonal antibodies, and new epigenetic-targeted drugs like bromodomain and extra terminal domain inhibitors.

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1. INTRODUCTION

The remarkable activity of the proteasome inhibitor (PI) bortezomib (Btz; Velcade®) was first recognized in an initial phase I clinical trial where Orlowski and colleagues observed a complete response in a multiple myeloma (MM) patient with advanced disease (Orlowski et al., 2002). Accelerated regulatory approval was then granted by the FDA in 2003 following two landmark phase II clinical studies in patients with advanced staged MM (Jagannath et al., 2004; Richardson et al., 2003). In these studies, 35% of patients, all of whom had progressive disease following at least three therapies, achieved a measurable response with the average response duration lasting 1 year. Today, Btz is a cornerstone in the treatment of MM for which it is approved as a first-line therapy and is a ubiquitous component of the multidrug cocktails that are used in the clinical management of MM. Prior to the introduction of Btz, MM was a highly aggressive and deadly form of cancer, and minimal advances in treatment had been made since the first trial of melphalan in the early 1960s (Bergsagel, 2014; Bergsagel, Sprague, Austin, & Griffith, 1962). While MM remains incurable today, the development of novel agents such as Btz has substantially improved survival times and quality of life. The development story of Btz serves as a blueprint for navigating the time and resource-intensive path of bench-to-bedside translational research and is a shining example of success in the era of targeted cancer therapy. The details of this story have been discussed in depth elsewhere and will not be the focus of this review (Allen, 2007; Sanchez-Serrano, 2005, 2006). Rather, the emphasis will be on a rapidly expanding literature of molecular strategies that effectively combat therapeutic resistance to Btz. This is an important topic given that, despite the initial effectiveness of Btz, nearly all patients progress to a refractory stage, and therapeutic resistance has emerged as a clear obstacle to maximizing the clinical benefit of the drug. There are multiple distinct molecular strategies capable of enhancing the activity of Btz and restoring sensitivity to resistant cells, and many of these approaches are positioned for immediate clinical evaluation as they involve existing FDA approved drugs or new molecular entities already in development with established toxicity profiles. Over the years, numerous studies have been conducted on the mechanisms of Btz resistance and scores of molecular-targeted approaches have been evaluated in combination with Btz. Likewise, multiple reviews have been published on the topic. To avoid being duplicative, this review will focus

primarily on more recent advances and the treatment options on the horizon for patients with Btz refractory MM. New topics and targets covered include inhibitors of redox regulation, murine double minute 2 (MDM2) inhibitors, and epigenetic modulators like bromodomain and extra terminal domain (BET) inhibitors.

2. MECHANISMS OF BTZ RESISTANCE

The human 26S proteasome is a large (~2.4 MDa) multisubunit protein complex, consisting of a 19S regulatory cap and base and a 20S catalytic core arranged in a cylinder that resembles a stack of rings. The inner two of four stacked rings contain the seven β subunits (β1, β2, β3, etc.), which are the catalytic sites responsible for carrying out the three proteolytic activities of the proteasome. The three enzymatic activities are characterized by their preference for cleaving peptides with specific amino-acid sequence motifs and are named the chymotrypsin-like, trypsin-like, and caspase-like proteolytic activities. For a more in depth review of proteasome structure and function, Adams (2004) and Bhattacharyya, Yu, Mim, and Matouschek (2014) are recommended. The β5 subunit, encoded by the PSMβ5 gene, is the direct molecular binding target of Btz. Binding of Btz to PSMβ5 inhibits the chymotrypsin-like activity of this specific proteasome subunit and is believed to trigger cell death through a host of downstream effects including the inhibition of NFκB signaling via stabilization of IκB, and the activation of multiple stress pathways including the unfolded protein response, endoplasmic reticulum stress, oxidative stress, and the activation of stress signaling kinases like c-Jun N-terminal kinase (JNK; Fig. 1). Cellular models of Btz resistance have shed light on the molecular mechanisms that confer resistance to Btz. Changes in PSMβ5 structure and expression, microenvironmental factors like physical and paracrine interactions with bone stromal cells, and alterations in apoptosis and autophagy signaling are at the core of those changes (Fig. 2). The majority of studies that have investigated these resistance mechanisms have focused on Btz. However, as second- and third-generation PIs activate a common set of downstream pathways and effectors, and in many cases target the same catalytic subunit of the proteasome as Btz, these resistance mechanisms have implications for the use of next-generation PIs as well.

2.1 PSMβ5 Gene Mutations

Gene mutations that alter amino-acid sequences in drug-binding pockets of target proteins are one established mechanism of therapeutic resistance to

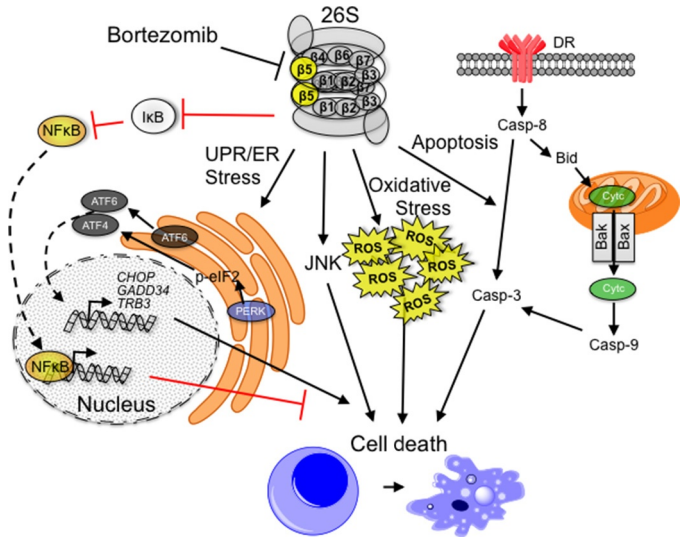

Figure 1 Pleiotropic anti-MM activity of Bortezomib. Inhibition of proteasomal chymotrypsin-like activity by Btz sets in motion a series of events that ultimately lead to the death of MM plasma cells. Btz inhibits NFκB prosurvival signaling by stabilizing the NFκB repressor IκB. Several cellular stress pathways are stimulated, including the unfolded protein response (UPR) and endoplasmic reticulum (ER) stress pathway that culminate in the transcription of proapoptotic genes (i.e., *CHOP*, *GADD34*, *TRB3*, *PUMA*, *NOXA*, and *BIM*). Btz induces the generation of reactive oxygen species (ROS) resulting in an oxidative shift in cellular redox balance and apoptosis. Stress kinases such as c-Jun N-terminal kinase (JNK) are activated along with the extrinsic and intrinsic apoptosis pathways leading to a cascade of caspase activation, the loss of mitochondrial membrane potential, and release of cytochrome c into the cytosol, further potentiating the activation of the terminal caspase, caspase-3.

targeted cancer agents. This was observed in patients with Philadelphia chromosome positive chronic myelogenous leukemia (CML) patients undergoing treatment with c-Abl tyrosine kinase inhibitor imatinib (Gleevec®). Imatinib is highly efficacious in this group of patients due to the expression of a mutant Bcr–Abl fusion protein with constitutive activity. Mutations in the kinase domain of c-Abl near the region of imatinib binding appear in CML patients that have relapsed following chronic imatinib exposure (Branford et al., 2002; Gorre et al., 2001; Roche-Lestienne et al., 2002; Roumiantsev et al., 2002; Shah et al., 2002). It was determined that these point mutations reduce or completely preclude the binding of imatinib to c-Abl. In an analogous situation, mutations in the Btz-binding pocket of PSMβ5 have been identified in MM cell lines following prolonged exposure

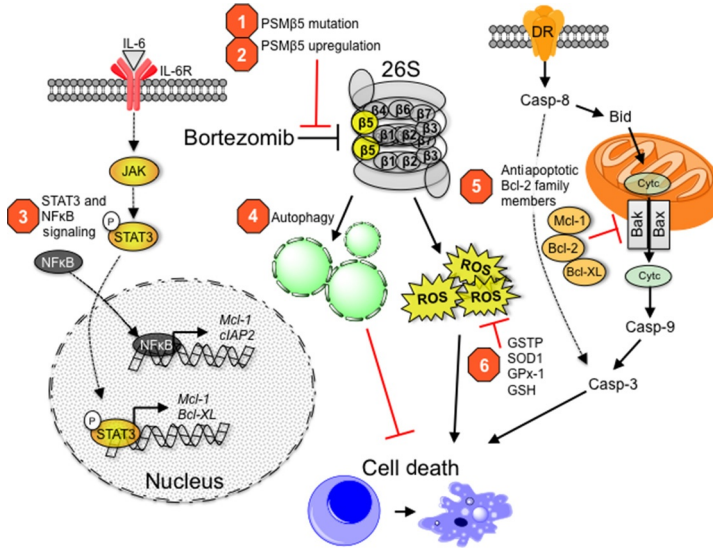

Figure 2 Mechanisms of resistance to Bortezomib. (1) Mutations in the Btz-binding pocket of the PSMβ5 proteasomal subunit appear in cells that have been exposed to Btz for prolonged periods of time. These mutations disrupt Btz binding to PSMβ5 thereby reducing the activity of the drug. (2) Resistant cells upregulate PSMβ5 and other proteasomal subunits as an adaptive response to prolonged Btz treatment. (3) Cellular signaling events such as cytokine (i.e., IL-6) induced or constitutive STAT3 activation and NFκB signaling drive the expression of prosurvival genes, including the antiapoptotic Bcl-2 family member Mcl-1. Mcl-1 is a potent inhibitor of apoptosis known to confer resistance to Btz. (4) Cells may enter into an autophagic state to avoid the cytotoxic effects of Btz. Although autophagy is a catabolic process, it is a protective state under certain conditions and serves as an alternative degradative pathway in the presence of proteasome inhibition. (5) Overexpression of the antiapoptotic Bcl-2 family members, particularly Mcl-1 and Bcl-XL, inhibits the apoptotic pathway and allows cells to escape the cytotoxic effects of Btz. (6) Cellular antioxidants such as GSH neutralize ROS levels to prevent toxicity associated with oxidative stress. The generation of ROS by Btz is critical to the cytotoxic activity of Btz. Redox enzymes such as GSTP, SOD1, and GPx-1 are upregulated in resistant cells, allowing them to neutralize harmful levels of oxidative stress.

to Btz (Ri et al., 2010). Similar findings were reported in non-MM cell models of acquired Btz resistance (Lü, Chen, et al., 2008; Lü et al., 2009; Lü, Yang, et al., 2008; Oerlemans et al., 2008; Verbrugge et al., 2013). These mutations also impart cross-resistance to next-generation PIs (Verbrugge et al., 2012). Multiple PSMβ5 mutations have been identified and display varying degrees of resistance (Lü et al., 2009). Mutations, such as the Ala49Thr modification, occur in regions of PSMβ5 that are critical to Btz

binding (Groll, Berkers, Ploegh, & Ovaa, 2006). The clinical significance of these mutations has been challenged given that they have not been detected in MM patient samples from patients that have relapsed following Btz treatment. Two published reports failed to detect the same PSMβ5 mutations that were identified in MM cell models in patients, and there was no correlation between patient responsiveness to Btz and PSMβ5 single nucleotide polymorphisms (Lichter et al., 2012; Politou et al., 2006; Ri et al., 2010). The study by Lichter and colleagues reported on sequencing from 16 post-Btz treatment samples, of which three were paired pre- and post-Btz. A potentially confounding variable in this study was that 10 of the 16 post-Btz treatment samples were from patients that were nonresponders to Btz to begin with. However, the fact that none of the mutations identified in cell models were identified in 16 samples casts doubt on the relevance of these mutations in the clinical setting. Additional studies on this topic are needed to boost the statistical power of the clinical data set, and if confirmed, to reconcile the discrepancy between the genetics of Btz resistance in cell models versus patients.

2.2 Upregulation of Proteasomal Subunits

In addition to mutation of the PSMβ5 gene, upregulation of PSMβ5 (wild type and/or mutant) and other proteasomal subunits is associated with Btz resistance. Overexpression of PSMβ5 was detected at the mRNA and protein levels in MM cells that were resistant to Btz and epoxomicin (Balsas, Galán-Malo, Marzo, & Naval, 2012), and upregulation of PSMβ5 along with β1 and β2 subunits and the 11S regulator complex were reported in Btz-resistant MM cell lines (Rückrich et al., 2009). Similar results were reported in cell types of non-MM origin (Lü, Chen, et al., 2008; Lü, Yang, et al., 2008; Oerlemans et al., 2008), although Oerlemans and colleagues did not observe any appreciable upregulation of PSMβ5 at the mRNA level, suggesting a posttranscriptional mechanism. RNAi-mediated repression of PSMβ5 partially restored Btz sensitivity in resistant cells, demonstrating a role as a driver of the resistance phenotype (Oerlemans et al., 2008). Data supporting the existence of this mechanism in Btz refractory patients are limited, but one study confirmed upregulation of PSMβ5 following treatment with a Btz-based regimen (Shuqing, Jianmin, Chongmei, Hui, & Wang, 2011). The precise mechanism by which PSMβ5 upregulation contributes to Btz resistance is not entirely clear, and reports from the literature are somewhat contradictory. Lü, Chen, et al. (2008)

and Lü, Yang, et al. (2008) observed an increase in the cellular chymotrypsin-like proteasome activity in Btz-resistant PSMβ5-overexpressing cells. In this case, it is reasonable to hypothesize that more PSMβ5 and an increase in proteasome activity would necessitate more Btz to inhibit the proteasome to the same degree. However, other studies did not report any changes in chymotrypsin-like proteolytic activity in Btz-resistant cells that overexpress PSMβ5 (Oerlemans et al., 2008). In this study, they did not observe upregulation of other proteasome subunits such as PSMβ1, PSMβ2, or PSMα7, demonstrating that the upregulation of PSMβ5 is not coincident with increased proteasomal density and activity but rather a selective increase in this one particular subunit. They further showed that the increased PSMβ5 proteins did not exist as free subunits in the cytosol but remained in the high molecular weight cellular fraction, suggesting that excess PSMβ5 is associated with the proteasome or forms high molecular weight aggregates. One theoretical role of excess PSMβ5 subunits in mediating resistance is that it acts to scavenge available pools of Btz. However, the existing data from the Oerlemans study do not support this hypothesis as resistant cells showed nearly identical inhibition of chymotrypsin-like proteasome activity when treated with Btz compared to sensitive cells. What also remains controversial is *how* PSMβ5 becomes upregulated. The Oerlemans study concluded that a posttranscriptional mechanism was at play due to no detectable changes in mRNA levels. By comparison, Lu et al. reported an increase in PSMβ5 mRNA, which they concluded was driven by gene amplification as determined by metaphase cytogenetics and fluorescence *in situ* hybridization. Balsas et al. showed that Btz resistance was associated with aneuploidy, a finding that somewhat supports the possibility that changes in PSMβ5 copy number or changes in the chromosomal architecture surrounding the PSMβ5 gene could be the cause of PSMβ5 gene upregulation. These studies present a case that PSMβ5 upregulation at least contributes to Btz resistance, although the mechanistic explanation for that role remains unclear.

2.3 Apoptotic Resistance and Autophagy

The anti-MM activity of Btz is attributed to pleiotropic effects with the induction of programmed cell death/apoptosis being the primary mode of cell death. Early studies into the cytotoxic effects of Btz delineated the sequence of events triggered by Btz leading to MM cell death. Principal among those events was the activation of apoptosis via the intrinsic

mitochondrial pathway and/or the extrinsic pathway, which couples death receptors to activation of the apical caspase, caspase-8 (Hideshima et al., 2003; Mitsiades et al., 2002). Treatment of cells with the pan caspase inhibitor ZVAD-FMK partially blocked Btz-induced cell death, demonstrating the contribution of this pathway to the cytotoxic effects of the drug. The importance of apoptotic regulators in modulating Btz sensitivity was further supported in additional studies showing synergy between Btz and apoptotic inducers including tumor necrosis factor-related apoptosis inducing ligand (Mitsiades, Mitsiades, Poulaki, Anderson, & Treon, 2001; Mitsiades, Mitsiades, Poulaki, Chauhan, et al., 2001), and inhibitors of antiapoptotic Bcl-2 family members (Chen et al., 2014; Pei, Dai, & Grant, 2003; Trudel et al., 2007). One of those prosurvival Bcl-2 family members, myeloid cell leukemia-1 (Mcl-1), is a pivotal molecule regulating the sensitivity of MM cells to Btz. Mcl-1 overexpression has been reported as a general trait of MM (Derenne et al., 2002; Wuillème-Toumi et al., 2005), and specifically associated Btz resistance (Balsas et al., 2009; Nencioni et al., 2005). As an important determinant of sensitivity to Btz, Mcl-1 was shown to be cleaved and converted from an antiapoptotic protein of approximately 40 kDa to a proapoptotic 28 kDa form following Btz treatment (Podar et al., 2008). Mcl-1 acts by blocking the activity of the proapoptotic family members, which produce apoptotic signals through the mitochondrial release of cytochrome *c* and subsequent activation of caspase-9 and caspase-3. To target the prosurvival/antiapoptotic Bcl-2 family members (i.e., Bcl-2, Bcl-XL, and Mcl-1), inhibitors have been designed to block their interaction with proapoptotic members (i.e., Bax, Bak, and Bim). BH3 mimetic Bcl-2 inhibitors include ABT-737, ABT-199, and obatoclax (GX015-070). ABT-737 specifically binds to and inhibits Bcl-2 and Bcl-XL, and ABT-199 targets Bcl-2 only. Both drugs, however, fail to inhibit the activity of Mcl-1, a presumed limitation for the treatment of MM and enhancing the activity of Btz. Despite this limitation, the combination of ABT-737 or ABT-199 with Btz has shown promise in preclinical models of lymphoma (Johnson-Farley, Veliz, Bhagavathi, & Bertino, 2015; Paoluzzi et al., 2008; Touzeau et al., 2011). The fact that similar studies targeting MM are lacking may be an indication that the combination is less active against MM. The characteristically high expression of Mcl-1 in MM cells supports this possibility. Obatoclax, on the other hand, offers activity against all the antiapoptotic family members, including Mcl-1. Obatoclax enhances Btz sensitivity and reverses resistance (Nguyen et al., 2007; Pérez-Galán, Roué, Villamor, Campo, & Colomer, 2007; Pérez-Galán

et al., 2008), but these studies too were conducted using non-Hodgkin's lymphoma cell lines. Subsequently, a phase I/II clinical trial of obatoclax and Btz in mantle cell lymphoma (MCL) patients reported disappointing results, calling into question whether the promising preclinical activity of the combination would be translated into clinical benefit. Others have begun to develop specific Mcl-1 inhibitors, including the marinopyrrole drug, maritoclax (Doi et al., 2012), which acts to specifically disrupt the interaction between Mcl-1 and Bim. Future studies evaluating these Mcl-1 targeted drugs in combination with Btz in MM are well justified.

Autophagy is the process by which cells engulf and breakdown organelles and other cytoplasmic components. Although autophagy is a catabolic process, it has been associated with cell survival and resistance to anticancer therapy including Btz. Autophagy as a mechanism of resistance to Btz was first proposed when Btz was found to induce autophagy in MM cells (Hoang, Benavides, Shi, Frost, & Lichtenstein, 2009), and Btz-resistant cells exhibited four times higher levels of autophagy compared to their isogenic sensitive counterparts (Jagannathan, Malek, Vallabhapurapu, Vallabhapurapu, & Driscoll, 2014). Initially, however, it was determined that the use of the autophagy inhibitor 3-methyladenine in combination with Btz was actually antagonistic rather than synergistic (Hoang et al., 2009), suggesting that autophagy contributes to the cytotoxic effects of Btz rather than to resistance. On the other hand, other classes of autophagy inhibitors such as macrolide antibiotics were shown to enhance the activity of Btz (Moriya et al., 2013). Combination of the antimalarial agent and inhibitor of autophagy, hydroxychloroquine, with histone deacetylase (HDAC) inhibitors and the BH3 mimetic, Bcl-2 inhibitor ABT737 together enhanced the activity of Btz dependent on inhibition of protective autophagy (Chen et al., 2014). One clinical trial of hydroxychloroquine combined with Btz in refractory MM patients has been conducted to date (Vogl, Stadtmauer et al., 2014). No conclusions related to efficacy could be made from this single arm study, but responses were observed in 28% of patients. So while autophagy is generally considered a mechanism of resistance to Btz in MM, additional studies are required to determine how this knowledge will be translated into improved therapy for patients. A current limitation may be that hydroxychloroquine and its predecessor, quinacrine, are the only clinically available autophagy inhibitors, and these compounds have autophagy-independent mechanisms of action including the induction of lysosome-mediated apoptosis (Boya et al., 2003; Sui et al., 2013).

3. APPROACHES TO OVERCOMING BTZ RESISTANCE

3.1 Next-Generation Proteasome Inhibitors

The approval and success of Btz has paved the way for the development of second-generation PIs. These new PIs tout improved pharmacology, clinical efficacy, and reduced toxicity as they were designed with increased binding affinity for proteasomal subunits, favorable pharmaceutical properties such as oral bioavailability, and fewer adverse events. New PIs include carfilzomib (Kyprolis®), an epoxomicin derivative and irreversible inhibitor of proteasomal chymotrypsin-like activity; oprozomib (ONX0912), an orally bioavailable derivative of epoxomicin and carfilzomib; ixazomib (MLN9708), an orally bioavailable boronic acid derivative; marizomib (NPI0052; Salinosporamide A), a natural product from the marine bacteria *Salinispora tropica* that binds to PSMβ5 as well as β1 and β2 subunits; and delanzomib (CEP18870), an orally bioavailable and irreversible inhibitor of the proteasomal chymotrypsin-like protease activity. Notably, carfilzomib earned FDA approval for the treatment of refractory MM in 2012 based on phase II data demonstrating a 23.7% response rate, median response duration of 7.8 months, and median overall survival (OS) of 15.6 months (Siegel et al., 2012). The indication for carfilzomib is for patients whose disease has progressed following at least two therapies, including Btz and an immunomodulatory agent (IMiD). The measurable response elicited by carfilzomib in approximately 1/4 of refractory patients suggests that it retains activity in Btz-resistant patients, or at least a portion of them. The major difference in activity between Btz and carfilzomib is that carfilzomib is an irreversible inhibitor of the chymotrypsin-like activity of the 20S proteasome, whereas Btz has reversible binding kinetics. With regard to toxicity, the percentage of patients experiencing peripheral neuropathy, the most common dose-limiting event for Btz, is low with carfilzomib treatment, measured at 13.9% in a cohort of 526 patients from four separate clinical trials, many of whom reported preexisting peripheral neuropathy at baseline (Siegel et al., 2013). Additional studies have shown similar response rates for carfilzomib in multidrug regimens that include lenalidomide and dexamethasone (Stewart et al., 2015). In this trial, roughly 65% of patients were refractory to Btz; however, the specific response rate for the Btz refractory cohort was not separated from the Btz naïve group. Nevertheless, given that the majority of patients enrolled in the study were refractory to Btz, it can be inferred that carfilzomib was effective in at least a

portion of patients with Btz-resistant disease. Similar clinical responses were seen with oprozomib, which exhibited measurable but modest activity in Btz refractory patients. In a phase Ib trial, a 14.3% overall response rate (ORR) was observed in Btz refractory patients ($n=7$) and a 25% ORR was observed in phase II ($n=12$). There are other important clinical considerations (i.e., tolerability) of the new drug, but focusing only on response rate, it is clear that while oprozomib provides benefit to a fraction of Btz refractory MM patients, the majority of them remain unresponsive (Vij et al., 2014). These available data for next-generation PIs suggest that resistance mechanism(s) that impact Btz activity may limit the effectiveness of these newer PIs. At the molecular level, this hypothesis is supported by cell models of PI resistance, where cells that have acquired resistance to Btz show cross-resistance to other PIs including carfilzomib and oprozomib (de Wilt et al., 2012; Franke et al., 2012; Stessman et al., 2013, 2014). Therefore, the identification and understanding of Btz resistance mechanisms and strategies to overcome them are not only critical for maximizing the activity of Btz, but will likely benefit next-generation PIs like carfilzomib and oprozomib.

3.2 Redox Signaling

The regulation of reduction and oxidation reactions (i.e., redox) and maintenance of redox homeostasis are critical to the survival and function of all cells. It is particularly important for MM plasma cells, which are naturally specialized for the mass production and secretion of immunoglobulin (Ig) proteins, a process that generates oxidative stress as a by-product. Ig molecules are large multisubunit proteins held together by intra- and interchain disulfide bonds and noncovalent interactions (Liu & May, 2012), and their folding is oxidative by nature. The proper folding of one Ig molecule requires the formation of approximately 100 disulfide bonds. One plasma cell is capable of synthesizing thousands of Ig molecules per second (Shimizu & Hendershot, 2009), meaning that one Ig-producing MM cell may produce roughly 100,000 disulfide bonds per second (Cenci & Sitia, 2007; Hendershot & Sitia, 2005). Molecular oxygen serves as the electron acceptor for each disulfide bond reaction, yielding the production of reactive oxygen species (ROS). To neutralize the increased ROS load associated with Ig synthesis and folding, plasma cell differentiation is accompanied by an intracellular antioxidant response, with the major adaptation being increased synthesis of glutathione (GSH) (Cullinan & Diehl, 2004; Harding et al., 2003), a tripeptide composed of the amino acids cysteine,

glutamate, and glycine that is the major endogenous antioxidant of all cells. The thiol functional group of cysteine is critical to the antioxidant properties of GSH, as sulfur is a flexible atom capable of donating electrons to reduce free radicals [i.e., hydrogen peroxide (H_2O_2)] or oxidized proteins, lipids, and nucleic acids. GSH increases the viability and growth of MM cells in culture, and other thiol-containing molecules, such as beta mercaptoethanol, are commonly added to the culture media of plasmacytoma cell lines and antibody-producing hybridomas (de St Groth, 1983; Merten, Keller, Cabanie, Litwin, & Flamand, 1989; Schneider, 1989; Shacter, 1987), further emphasizing the importance of maintaining redox balance in these cell types. Because the natural biology of MM cells as secretory cells predisposes them to high levels of oxidative stress (Cenci & Sitia, 2007), redox signaling is an attractive therapeutic target/pathway for MM.

In addition to being a generally promising therapeutic strategy in the treatment of MM, redox-targeted approaches are also effective Btz-sensitizing agents, capable of restoring sensitivity to resistant cells and enhancing the activity of Btz and other PI therapies. PIs deplete cellular pools of GSH and upregulate the expression of redox enzymes such as glutamate–cysteine ligase, heme oxygenase-1, and GST-pi (Nerini-Molteni, Ferrarini, Cozza, Caligaris-Cappio, & Sitia, 2008; Usami et al., 2005), suggesting that changes in redox-modulating enzymes are an adaptive response to PI therapy. Btz-resistant MM cell lines overexpress important redox-regulating enzymes including copper-zinc superoxide dismutase (CuZnSOD or SOD1), glutathione peroxidase-1 (GPx-1), and GSH (Salem, McCormick, Wendlandt, Zhan, & Goel, 2015). SOD1 and GPx-1 are key antioxidant enzymes involved in scavenging excess levels of ROS by catalyzing reactions that neutralize superoxide anion and H_2O_2, respectively. The ectopic expression of SOD1 leads to Btz resistance in MM cells, confirming that upregulation of this one redox modulator is sufficient to protect cells from Btz-induced cell death (Salem et al., 2015). ROS are generated by Btz treatment in a variety of cancer cell types (Fribley, Zeng, & Wang, 2004; Ling, Liebes, Zou, & Perez-Soler, 2003), and antioxidants such as *N*-acetyl cysteine (NAC) protect cells from Btz-induced death (Pérez-Galán et al., 2006; Salem et al., 2015; Yu, Rahmani, Dent, & Grant, 2004). Taken together, these studies form a mechanistic link between Btz sensitivity and oxidative stress. They suggest that cells induce a compensatory and protective redox response to block the cytotoxic effects of PIs, and provide rationale for targeting redox signaling as

an approach to enhancing the activity of PIs and restoring PI sensitivity to refractory cells. Further evidence of the promise of targeting redox pathways for the treatment of MM comes from studies demonstrating the overexpression of the antioxidant and phase II detoxification enzyme, glutathione *S*-transferase-pi (GSTP), in >80% of patients with MM and monoclonal gammopathy of undetermined significance (Petrini et al., 1995; Stella et al., 2013). These studies showed that GSTP expression significantly increased following therapy or correlated with therapeutic response to agents that included Btz, suggesting a role in treatment sensitivity/resistance. The GSTP gene is located on the long arm of chromosome 11 (11q13), which is a frequently translocated chromosomal locus in MM due to aberrant and oncogenic Ig heavy-chain gene (IgH, 14q32) translocations. IgH translocations are one of the most common and earliest oncogenic events in MM, and the fact that their breakpoints localize to the locus of an important redox regulatory enzyme with high frequency further implicates the redox pathway in disease pathogenesis. In MCL, a form of non-Hodgkin's lymphoma characterized cytogenetically by the t(11;14) IgH translocation, GSTP expression is highly expressed in histological samples (Bennaceur-Griscelli et al., 2004; Thieblemont et al., 2008), and the inhibition of GSTP enhances the activity of Btz (Rolland, Raharijaona, Barbarat, Houlgatte, & Thieblemont, 2010). Similar examination of GSTP expression in MM clinical samples from patients with and without t(11;14) translocations is needed, but these parallel studies in MCL suggest that GSTP may be a viable molecular drug target for MM and in combination with Btz. GST family members carry out their protective detoxification process via the direct conjugation of GSH to target electrophiles. More recent advances demonstrate that GSTs have broader biological roles unrelated to detoxification. GSTP, for example, associates with and regulates the activity of mitogen-activated protein kinases including the stress signaling kinase c-Jun N-terminal kinase (JNK; Adler et al., 1999; Wang, Arifoglu, Ronai, & Tew, 2001). GSTP catalyzes the conjugation of GSH to protein cysteines (i.e., the process of S-glutathionylation), a posttranslational modification that alters protein function. S-Glutathionylation influences the activity of a variety of proteins involved in diverse cellular processes from the regulation of energy metabolism and calcium homeostasis to signal transduction and redox, indicating the widespread importance of this process (Tew & Townsend, 2011a, 2011b, 2012). With regard to the proteasome, Demasi, Shringarpure, and Davies (2001) were first to show that proteasomal subunits were S-glutathionylated, an effect that specifically

affected activity of the chymotrypsin-like protease activity in purified preparations of 20S proteasome extracted from mammalian cells. They concluded from their study that PIs like lactacystin alter global S-glutathionylation levels and specifically enhance S-glutathionylation of the proteasome itself. Additional studies conducted using purified 20S proteasomes from *Saccharomyces cerevisiae* showed that S-glutathionylation of the proteasome was sensitive to redox states and predominantly affected the chymotrypsin-like protease activity relative to trypsin-like and caspase-like activities (Demasi et al., 2013). The specific role of GSH and S-glutathionylation was confirmed as this effect was reversed by enzymes such as glutaredoxin 2 and other oxidoreductases that catalyze deglutathionylation, the reverse reaction of GSTs (Silva et al., 2008). S-Glutathionylation appears to enhance 20S proteasome function by promoting an "open gate" conformation of the structure (Silva et al., 2012), thereby enhancing the proteolytic efficiency of the complex. In contradiction to this finding is that oxidative stress was shown to impair the ATP-dependent activity of the 26S proteasome (Reinheckel, Ullrich, Sitte, & Grune, 2000), and S-glutathionylation of RPN1 and Rpn2, subunits of the 19S regulatory particle of the 26S proteasome, inhibits rather than enhances proteolytic activity of the proteasome (Zmijewski, Banerjee, & Abraham, 2009). These seemingly opposing findings may be reconciled by the fact that oxidative stress is known to disengage the 20S core particle from the 19S regulatory unit, effectively increasing the pool of free 20S proteasome, which are capable of degrading proteins in an ATP-independent manner (Grune et al., 2011; Wang, Yen, Kaiser, & Huang, 2010). It has been proposed that this regulation evolved as an adaptive response to increased oxidative stress, enabling cells to increase their capacity to degrade oxidized proteins (Demasi et al., 2014, 2013). It is clear from this collection of studies that changes in redox and levels of S-glutathionylation have a direct impact on the activity of the proteasome; however, it is not clear if and how this affects the activity of Btz, or if this process contributes to the resistance phenotype in MM. Future studies should address these questions.

Several redox-targeted agents are in preclinical development or are actively used in the clinic for the treatment of cancer (Tew & Townsend, 2011a, 2011b). GST-targeted agents include the GSTP1 inhibitor TLK199 (ezatiostat, Telintra), the GSTP-activated prodrug TLK286 (canfosfamide, Telcyta), nitric oxide (NO) generating prodrugs like JS-K, which has demonstrated promising preclinical activity in MM models and

synergized with Btz (Kiziltepe et al., 2007), PABA/NO, mimetics of oxidized GSH (GSSG) like NOV-002, and the metal chelator disulfiram (Antabuse®), which was originally developed in the 1950s to treat alcoholism. Other classes of pro-oxidant chemotherapeutic agents include thiol reactives like arsenic trioxide (As_2O_3; a.k.a. ATO), which has been approved for clinical use in the treatment of acute promyelocytic leukemia (Wang & Chen, 2008). The combination of ATO and Btz was shown to be synergistic in MM and other hematological cancer cell lines (Campbell et al., 2007; Canestraro et al., 2010; Jung, Chen, & McCarty, 2012; Wen et al., 2010; Yan et al., 2007). A phase I/II study combining ATO, Btz, and ascorbic acid in heavily pretreated MM patients showed good tolerability and preliminary signs of efficacy (Berenson et al., 2007), whereas other trials showed no added benefit of combining ATO with Btz (Sharma et al., 2012). Therefore, the benefit of ATO in the treatment of MM and as a enhancer of Btz activity is not strongly supported, although the statistical power of the data sample size has been questioned (He et al., 2014). Alternative strategies for targeting redox to overcome Btz resistance include the inhibition of mucin 1 C-terminal subunit using a novel cell penetrating peptide inhibitor of MUC1-C GO-203 (Yin, Kufe, Avigan, & Kufe, 2014). GO-203 was shown to deplete GSH levels and induce ROS through a mechanism involving downregulation of the p53-inducible regulator of glycolysis and apoptosis (TIGAR).

3.3 MDM2 Inhibitors

MDM2 and its human ortholog, HDM2, are E3 ubiquitin ligases best known for their roles in regulating the stability and activity of p53. Given the critical tumor suppressor function of p53, dubbed the "guardian of the genome" (Lane, 1992), the MDM2–p53 regulatory axis is widely accepted as a promising target for cancer drug development (Chène, 2003). MDM2 directly interacts with p53 and marks it for proteasomal degradation via ligation of ubiquitin tags. Various forms of cellular stress disrupt the MDM2:p53 interaction resulting in the stabilization and derepression of p53. The stabilization of p53 is followed by posttranslational modifications and downstream binding and transactivation of target genes that are involved in a host of cellular processes including cell cycle arrest and apoptosis (El-Deiry, 1998; Meek & Anderson, 2009; Vousden & Prives, 2009). *In lieu* of a physiological stressor that disrupts MDM2 repression of p53 naturally, pharmacological approaches have been devised to interfere with this

interaction with the goal of artificially activating p53 for cancer therapy. A number of small-molecule drugs have been designed with high affinity for the p53-binding pocket of MDM2 and act to displace p53 leading to its stabilization and increased transcriptional activity. In addition to being a general approach to cancer therapy, there have been several reports that MDM2 inhibitors are potent Btz sensitizers in MM. Saha and colleagues observed enhanced activity of Btz in MM cell lines and primary patient plasma cells that were co-treated with Nutlin3a (Saha et al., 2010), the first in a class of potent and specific MDM2 inhibitors (Vassilev et al., 2004). With similar results, work by others (Ooi et al., 2009) found that the combination of Nutlin3a and sublethal concentrations of Btz was effective against Btz-sensitive MM cells as well as a variety of epithelial tumor types. Our group showed that in MM cells with acquired resistance to PIs, including Btz, MDM2 inhibition is effective molecular strategy for restoring Btz sensitivity (Stessman et al., 2014). Nutlin3a was also shown to be a augment the activity of Btz in models of MCL, a form of non-Hodgkin's lymphoma that shares cytogenetic anomalies, such as the t(11;14) IgH translocation, with MM (Jin et al., 2010; Tabe et al., 2009). In addition to Nutlins, second-generation MDM2 inhibitors, such as the small-molecule MI63, enhance the activity of Btz in MM cells (Gu et al., 2014). There are now several classes of MDM2 inhibitors in development, and human trials combining them with Btz in refractory MM patients will determine the clinical utility of this approach.

The molecular mechanism(s) that underlie the synergy between MDM2 inhibition and Btz treatment appear to be multifactorial. Given that MDM2 regulates p53 stability through the ubiquitin–proteasome pathway, it is intuitive that the combination of these agents would converge mechanistically to generate a robust anti-MM effect. In support of this theory, early studies investigating the anti-MM activity of Btz showed that p53 upregulation and phosphorylation at the Ser15 residue were initiated by Btz treatment (Hideshima et al., 2003). The Ser15 modification on p53 disrupts the interaction with MDM2 (Shieh, Ikeda, Taya, & Prives, 1997), mimicking the pharmacological activity of MDM2. These results were further supported by Saha et al. (2010) who reported Btz-induced upregulation of the p53 target genes, p21/WAF1, MDM2, PUMA, and Bax, effects that were synergistically enhanced by the cotreatment with Nutlin3a. The activity of Nutlin3a and Btz is most profound in wild-type p53-expressing cells. This is due to the fact that the effects of MDM2 are mitigated in cells that have

lost wild-type p53 function, as the stabilization of a p53 protein that lacks functionality would fail to evoke downstream transcriptional events that are essential to the activity of p53 pathway activation. This is potentially a general limitation to the class of MDM2 inhibitors due to the high prevalence of somatic p53 mutations in human cancer (Baker et al., 1989; Hollstein, Sidransky, Vogelstein, & Harris, 1991). Many of these mutations carry loss of function, interfering with the ability of p53 to bind consensus DNA-binding sites and induce transcription of target genes. There are, however, reports that the combination of MDM2 inhibitor and Btz is effective in p53-deficient cells, implicating p53-independent mechanisms. Our group showed synergy between Nutlin3a and Btz and especially with carfilzomib in mutant p53-expressing U266 cells (Stessman et al., 2014). Two- to threefold higher concentrations of Nutlin3a were required to bring out the same effect that was observed in wild-type p53-expressing cells, so it is important to note that p53 mutation, while not an excluding factor, was a limiting factor. Similar results were reported in p53 mutant MCL cells through a mechanism involving posttranscriptional upregulation of the proapoptotic effector NOXA (Jin et al., 2010; Tabe et al., 2009). In MM patients, mutations in p53 are significantly less frequent compared to other tumor types. Genomic studies have shown that p53 mutations are rare in MM, being observed in only 3% newly diagnosed patients (Chng et al., 2007; Preudhomme et al., 1992). In addition to point mutations in the p53 gene that lead to inactivating amino-acid substitutions, complete loss of one or both p53 alleles through chromosomal deletions is also observed in cancer. In MM, deletion of the chromosomal arm where p53 is located (17p) is detected in approximately 10% of newly diagnosed patients and is an indicator of very poor prognosis (Boyd et al., 2011; Chen, Tai, et al., 2012; Chen, Qi, Saha, & Chang, 2012; Fonseca et al., 2003; Lodé et al., 2010). Interestingly, it was shown that in the cohort of patients with 17p deletions, the remaining allele of p53 was prone to mutation with 37% of patients presenting with mutations (Lodé et al., 2010). By comparison, no p53 mutations were detected in patients with an intact chromosome 17p, an observation that has been confirmed by others (Chng et al., 2007). So it seems that loss of p53 function by point mutation or loss of chromosome 17p is a relatively rare event in MM, affecting less than approximately 10% of patients. Thus, p53 deficiency is not likely to be a limiting factor in MM patients, making the use of MDM2 inhibitors as combination therapies with Btz and other PIs a promising approach.

3.4 Il-6/STAT3 Signaling Axis

Early studies investigating the role of MM autocrine and paracrine growth factor and cytokine signaling identified interleukin-6 (IL-6) as a potent inducer of MM plasma cell growth and survival in cell culture (Kawano et al., 1988; Klein et al., 1989). Signal transducer and activator of transcription 3 (STAT3) signaling is a critical effector pathway downstream of interleukin-6 receptor (IL-6R) activation (Lütticken et al., 1994; Wegenka, Buschmann, Lütticken, Heinrich, & Horn, 1993; Zhong, Wen, & Darnell, 1994). In MM cells, STAT3 functions in both an IL-6-dependent and -independent manner, and constitutive STAT3 activation has been associated with oncogenesis and the protection from apoptosis (Bharti et al., 2004; Bromberg et al., 1999; Catlett-Falcone et al., 1999; Dalton & Jove, 1999). STAT3 signaling has been implicated in resistance to several MM therapies (Alas & Bonavida, 2003), and studies have correlated increased STAT3 expression and signaling with Btz resistance. Enforced expression of the CKS1B gene, a gene mapping to the short arm of chromosome 1 (1q21), led to induction of the STAT3 phosphorylation and resistance to Btz (Shi et al., 2010). STAT3 was connected to Btz responsiveness in other studies where inhibition of IL-6 signaling using the IL-6-targeted monoclonal antibody CNTO328 (siltuximab) abrogated STAT3 activity and enhanced Btz sensitivity in MM cells (Voorhees et al., 2007). Despite promising preclinical results and the strong molecular rationale for targeting IL-6 and the IL-6 receptor in combination with Btz, clinical trials conducted to date have not demonstrated overwhelming benefit for combining the two agents. A recent phase I study of single agent siltuximab, an IL-6-targeted monoclonal antibody (MAb), in Japan showed good tolerability and activity in refractory MM patients (Suzuki et al., 2015), although a phase II, double-blind, placebo-controlled trial of siltuximab in combination with Btz showed no significant improvement in progression-free survival (PFS) or OS compared to siltuximab plus placebo (Orlowski et al., 2015). Similar results were observed when siltuximab was added to a multidrug regimen that included Btz. In this study, too, no improvements in clinical outcomes in MM patients were seen (San-Miguel, Bladé, et al., 2014). Other possible approaches include the use of anti-IL-6R targeted mAbs as opposed to blocking the function of the soluble cytokine. Tocilizumab, originally named myeloma receptor antibody due to its promise as an MM therapeutic, is one such molecule. However, there are no published results of clinical trials conducted with tocilizumab in MM, alone or in combination with Btz. The IL-6/IL-6R signaling axis is just one pathway

that activates STAT3 in MM cells. An alternative strategy is to disrupt STAT3 signaling through the use of the multikinase inhibitor sorafenib (Nexavar®), a Raf kinase inhibitor that has multiple molecular targets and multifactorial antitumor effects in cells including the inhibition of STAT3 signaling (Ramakrishnan et al., 2010). The inhibition of STAT3 by sorafenib is independent of Raf kinase inhibition, which was determined by Chen et al. (2011) who synthesized sorafenib derivatives that lacked binding affinity for the Raf kinase domain but retained the capacity to inhibit STAT3. Inhibition of STAT3 by sorafenib and derivatives was proposed to occur via activation of the Src homology protein tyrosine phosphatase SHP-1 (Chen, Tai, et al., 2012; Chen, Qi, et al., 2012), which inhibits STAT3 phosphorylation. Numerous preclinical studies have demonstrated a promising anti-MM activity of sorafenib, both as a single agent and in combination with Btz (Kharaziha et al., 2012; Ramakrishnan et al., 2010; Udi et al., 2013). The most consistently observed molecular event triggered by sorafenib in these studies was an inhibition of STAT3 phosphorylation levels and concomitant downregulation of Mcl-1, which is a critical target gene of STAT3 and a known inhibitor of Btz-induced cell death in MM cells (Bhattacharya, Ray, & Johnson, 2005; Carpenter & Lo, 2014; Puthier, Bataille, & Amiot, 1999). Two clinical trials have evaluated sorafenib in MM patients. The first was a phase I study of sorafenib and Btz in patients with advanced malignancies (Kumar et al., 2013). Only 1 of the 14 enrolled patients had MM, and efficacy was not evaluated as an end point in the study, but the regimen was well tolerated. The second trial was a phase II in refractory MM that evaluated sorafenib as a monotherapy (Srkalovic et al., 2014). No responses were detected, results that may discourage additional studies evaluating the activity of sorafenib and Btz. Other strategies to target STAT3 in MM include Janus kinase inhibitors (Li et al., 2010; Monaghan, Khong, Burns, & Spencer, 2011; Ramakrishnan et al., 2010; Scuto et al., 2011), STAT3 peptidomimetics (Turkson et al., 2004), and STAT3-targeted antisense oligonucleotides (Hong et al., 2013).

3.5 Therapeutic Monoclonal Antibodies

The anti-CD38 MAb, daratumumab (JNJ54767414, HuMax® CD38), was recently granted Breakthrough Therapy Designation by the FDA for MM that is refractory to a PI and IMiD. CD38 is a cell surface glycoprotein with cyclic ADP ribose hydrolase activity, but its biological roles are just beginning to be understood. CD38 expressed at high levels in malignant lymphoid tumor cells and especially in MM plasma cells (Lin, Owens, Tricot, &

Wilson, 2004), whereas the majority of normal resting lymphocytes and pluripotent hematopoietic progenitor cells do not express CD38. A predominant path by which daratumumab kills MM cells is through antibody-dependent cell-mediated cytotoxicity (ADCC) and complement-dependent cytotoxicity. Daratumumab also has direct effects on MM cells and was shown to enhance the activity of existing MM therapies including Btz (van der Veer et al., 2011). Ongoing clinical studies combining daratumumab with Btz will determine the utility of this combination in Btz-resistant patients (NCT02136134, NCT02195479, and NCT01998971) although it is clear from the clinical data that daratumumab has significant activity when administered as a single agent in this patient population (Laubach, Tai, Richardson, & Anderson, 2014).

The anti-CS1 MAb elotuzumab is another promising new agent in the treatment of MM, displaying positive data in clinical studies, both alone and in combination with approved drugs like Btz. Cs1 is a cell surface protein belonging to the Ig superfamily. It was first found to be overexpressed in malignant plasma cells compared to normal plasma cells (Hsi et al., 2008), making it a logical target for MM therapy. The anti-MM activity of elotuzumab is attributed to NK cell-dependent ADCC, direct effects on MM cell survival and proliferation, and by blocking the adhesion of MM cells to BMSCs (Collins et al., 2013; Hsi et al., 2008; Tai et al., 2008). A synergistic interaction between Btz and elotuzumab (formerly HuLuc63), as Btz enhanced the ADCC killing of MM cells *in vitro* and enhanced the anti-MM response in preclinical mouse models (van Rhee et al., 2009). In a phase I trial of Elotuzumab and Btz in refractory MM patients, the combination showed a remarkable response rate of 48% and in two of three patients that were refractory to Btz (Jakubowiak et al., 2012). Other promising antibody-based therapeutics for the treatment of MM include antibody–drug conjugates such as the CD138-targeted antibody BT062 (Indatuximab ravtansine), the anti-B cell maturation antigen-targeted antibody GSK2857916 (Tai et al., 2014), and the aforementioned IL-6/IL-6R-targeted siltuximab and tocilizumab.

3.6 Bromodomain and Other Epigenetic Targets

BET family members (BRD2, BRD3, BRD4, and BRDT) are an exciting new class of epigenetic drug targets. These proteins facilitate the initiation and elongation phases of transcription by binding to activated chromatin at acetylated lysine residues. The recognition of activated chromatin by these

so-called epigenetic "readers" promotes the recruitment of the RNA polymerase II complex to sites of active transcription. Bromodomain inhibitors, such as JQ1 (Filippakopoulos et al., 2010), were shown to repress the expression and function of c-Myc, which is one of the most dysregulated oncogenes in MM (Affer et al., 2014; Kuehl & Bergsagel, 2012; Shou et al., 2000). The BRD4 bromodomain was found to occupy regions of regulatory DNA termed super enhancers due to the large size and number of bound transcription factors compared to normal gene enhancers. Super enhancers associate with genes that are critical to MM pathogenesis including the aforementioned c-Myc, IRF4, PRDM1, and XBP-1 (Lovén et al., 2013). As a consequence of this, disruption of BRD4 binding to super enhancers by JQ1 is active against MM cell lines and patient plasma cells (Delmore et al., 2011). Evidence supporting the use of bromodomain inhibitors in combination with Btz comes from a clinical study showing that newly diagnosed MM patients with c-Myc gene abnormalities were more likely to develop resistance to Btz plus dexamethasone therapy and exhibited a significantly shorter PFS (Sekiguchi et al., 2014). These findings suggest that c-Myc activity contributes to Btz resistance, providing rationale for the use of bromodomain inhibitors as a strategy to block c-Myc activity. However, a consensus on the extent and precise role of c-Myc in mediating responsiveness to Btz and PI therapy is debatable. The oncogenic role of c-Myc is well accepted, but depending on the context, c-Myc can act as a proapoptotic signal (Fuhrmann et al., 1999). This was shown in the response to Btz, where c-Myc was shown to regulate NOXA-induced apoptosis following Btz treatment (Nikiforov et al., 2007), and to be a key determinant in Btz-induced apoptosis in MM cells (Chen et al., 2010; Nawrocki et al., 2008). Based on these studies, the combination of a bromodomain inhibitor and Btz would be antagonistic rather than synergistic. A caveat to that conclusion is that while c-Myc expression and activity are highly sensitive to treatment with bromodomain inhibitors, c-Myc is not their sole target. BET proteins are global regulators of gene transcription and BET inhibitors affect the recruitment of basal transcriptional machinery to a large set of genes. In fact, MYC-independent molecular signatures in response to the quinolone BET inhibitor I-BET151 have been reported (Chaidos et al., 2014). Two studies support the use of a bromodomain inhibitor in combination with Btz for the treatment of Btz refractory MM. A recent study demonstrated synergistic interaction between Btz and the bromodomain inhibitor CPI203 (Siegel et al., 2014, ASH abstract 4702), and the combination of JQ1 and Btz was more active than either agent alone in serially

transplanted Btz-resistant cells from the Vk*MYC transgenic mouse model of MM (Chesi et al., 2012). Additional studies should further evaluate the potential of a bromodomain/Btz combination using MM models of resistance in order to establish rationale for the combination in prospective clinical trials in Btz refractory MM patients.

HDAC enzymes are another class of epigenetic modulator with established potential as anticancer therapeutic targets. HDACs negatively regulate the acetylation of lysine residues on histone tails to alter chromatin structure and ultimately gene transcription. Acetylated histones are generally associated with a less coiled chromatin structure and increased rates of transcription; therefore, HDAC inhibitors, which promote histone acetylation, act by affecting global transcription in tumors cells and impacting on a variety of genes and pathways that are important for cell survival, proliferation, apoptosis, differentiation, and metabolism, among others. HDAC inhibitors are potent anti-MM agents and significantly enhance the effects of Btz. For example, the pan HDAC inhibitors vorinostat and panobinostat synergized with Btz in cell culture and animal models of MM (Chesi et al., 2012; Hideshima, Richardson, & Anderson, 2011; Maiso et al., 2006; Pei, Dai, & Grant, 2004; Stessman et al., 2013). An original phase I clinical trial of vorinostat and bortezomib in relapsed patients showed promise including partial responses achieved in three of nine patients that were refractory to Btz (Badros et al., 2009). Similar signs of efficacy were reported by other groups in phase I trials (Weber et al., 2012), although the results of subsequent double-blind, placebo-controlled studies showed that the addition of vorinostat to Btz only modestly improved PFS in a large randomized cohort of patients (Dimopoulos et al., 2013). Furthermore, there was no significant improvement in PFS in the vorinostat versus control group in patients that had received prior PI therapy. Slightly more positive results were reported for the combination of panobinostat and bortezomib in a phase Ib study (San-Miguel et al., 2013) and a large, multicenter, placebo-controlled study of panobinostat, dexamethasone, and Btz versus placebo, dexamethasone and Btz (San-Miguel, Hungria, et al., 2014). Based on an approximated 4-month improvement in PFS, the FDA recently granted accelerated approval of panobinostat. More recent advances include the development of HDAC6 isoform-specific inhibitors, such as ACY-1215. HDAC6 has been shown to regulate the formation and function of aggresomes, which are cellular structures that degrade and clear polyubiquitinated proteins as an alternative pathway to the proteasome. The combination of HDAC6 gene knockdown or treatment with the HDAC6 inhibitors tubacin and ACY-1215 with Btz was found to be synergistic in preclinical models of MM

(Hideshima et al., 2005; Santo et al., 2012). Clinical trials combining ACY-1215 and Btz are now in progress and preliminary results show good tolerability and evidence of responses in Btz refractory patients (Raje et al., 2012 poster 4061; Vogl, Raje, et al., 2014 poster 4764). Figure 3 provides an overview of the various classes of drugs with potential as Btz sensitizing agents.

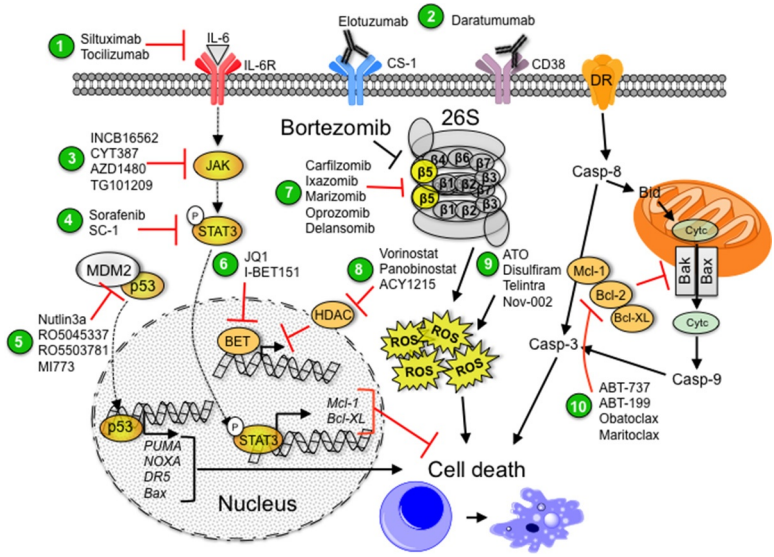

Figure 3 Bortezomib sensitizing therapeutic agents. (1) MAbs targeting IL-6 or IL-6R suppress IL-6 signaling and STAT3 activation, an important signaling network in MM cells. (2) MAbs elotuzumab and daratumumab targeting novel cell surface antigens such as CS-1 and CD38, respectively, have shown promise in patients with Btz refractory MM and have the potential to enhance the activity of Btz. (3) STAT3 is an important prosurvival signal in MM. Strategies to inhibit STAT3 include small-molecule inhibitors of the Janus kinase (JAK), the upstream activator of STAT3, or other means such as the use of (4) sorafenib and its derivatives. (5) MDM2 inhibitors are potent Btz sensitizers that restore Btz sensitivity to resistant cells. (6) BET bromodomain inhibitors are promising new drugs that disrupt the recruitment of transcriptional machinery to super enhancers that regulate the expression of MM oncogenes. (7) Next-generation proteasome inhibitors exhibit clinical activity in a portion of Btz refractory patients. (8) HDAC inhibitors synergize with Btz in preclinical models, although the clinical activity of pan HDAC inhibitors (i.e., vorinostat and panobinostat) has been limited. The HDAC6-selective inhibitor ACY-1215 is currently in clinical trials. (9) Increasing evidence suggests that alterations in redox signaling are key contributors to the Btz resistance phenotype, making redox-modulating agents' prime candidates for trials in Btz refractory patients. (10) Antiapoptotic Bcl-2 family members confer apoptotic resistance and reduce the cytotoxic effects of Btz. BH3 mimetic inhibitors, particularly those that block the activity of Mcl-1, are promising agents for enhancing/restoring the apoptotic effects of Btz treatment.

4. CONCLUDING REMARKS

Btz was a revolutionary advancement in the treatment of MM and remains a cornerstone of MM therapy today. A limitation to Btz is that the depth and duration of response vary between patients, and all patients ultimately stop responding due to the emergence of treatment resistance. Studies have shed light on the molecular mechanisms that drive acquired resistance to Btz, establishing the rationale for new targeted therapeutic approaches to be used in combination with Btz. Next-generation PIs retain activity in a portion of Btz refractory patients. However, in those patients that are nonresponders, the strategies that enhance the activity of Btz may be exploited with similar benefit, as Btz and next-generation PIs have the same molecular target and downstream effector pathways. There has been a surge of new agents for the treatment of MM over the past 10 years, providing more treatment options for MM patients than ever before. Currently, there are 1907 clinical studies registered with clinicaltrials.gov for MM, and 183 of those trials incorporate Btz in refractory patients. The expanding preclinical literature on molecular mechanisms of resistance and new targets in Btz-resistant MM will serve as rationale to guide these and future trial designs. Lastly, this area of research has the potential to deliver biomarkers and predictive signatures of response to Btz and PI therapy that will personalize treatment decisions and guide patient selection for new trials.

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REFERENCES

Adams, J. (2004). The proteasome: A suitable antineoplastic target. *Nature Reviews. Cancer*, *4*, 349–360.

Adler, V., Yin, Z., Fuchs, S. Y., Benezra, M., Rosario, L., Tew, K. D., et al. (1999). Regulation of JNK signaling by GSTp. *The EMBO Journal*, *18*, 1321–1334.

Affer, M., Chesi, M., Chen, W. D., Keats, J. J., Demchenko, Y. N., Tamizhmani, K., et al. (2014). Promiscuous MYC locus rearrangements hijack enhancers but mostly super-enhancers to dysregulate MYC expression in multiple myeloma. *Leukemia*, *28*, 1725–1735.

Alas, S., & Bonavida, B. (2003). Inhibition of constitutive STAT3 activity sensitizes resistant non-Hodgkin's lymphoma and multiple myeloma to chemotherapeutic drug-mediated apoptosis. *Clinical Cancer Research*, *9*, 316–326.

Allen, S. (2007). *The Velcade story. Retrieved from: http://www.boston.com/business/healthcare/articles/(2007)/05/06/the_velcade_story/?page=full*.

Badros, A., Burger, A. M., Philip, S., Niesvizky, R., Kolla, S. S., Goloubeva, O., et al. (2009). Phase I study of vorinostat in combination with bortezomib for relapsed and refractory multiple myeloma. *Clinical Cancer Research*, *15*, 5250–5257.

Baker, S. J., Fearon, E. R., Nigro, J. M., Hamilton, S. R., Preisinger, A. C., Jessup, J. M., et al. (1989). Chromosome 17 deletions and p53 gene mutations in colorectal carcinomas. *Science*, *244*, 217–221.

Balsas, P., Galán-Malo, P., Marzo, I., & Naval, J. (2012). Bortezomib resistance in a myeloma cell line is associated to PSMβ5 overexpression and polyploidy. *Leukemia Research*, *36*, 212–218.

Balsas, P., López-Royuela, N., Galán-Malo, P., Anel, A., Marzo, I., & Naval, J. (2009). Cooperation between Apo2L/TRAIL and bortezomib in multiple myeloma apoptosis. *Biochemical Pharmacology*, *77*, 804–812.

Bennaceur-Griscelli, A., Bosq, J., Koscielny, S., Lefrère, F., Turhan, A., Brousse, N., et al. (2004). High level of glutathione-S-transferase pi expression in mantle cell lymphomas. *Clinical Cancer Research*, *10*, 3029–3034.

Berenson, J. R., Matous, J., Swift, R. A., Mapes, R., Morrison, B., & Yeh, H. S. (2007). A phase I/II study of arsenic trioxide/bortezomib/ascorbic acid combination therapy for the treatment of relapsed or refractory multiple myeloma. *Clinical Cancer Research*, *13*, 1762–1768.

Bergsagel, P. L. (2014). Where we were, where we are, where we are going: Progress in multiple myeloma. *American Society of Clinical Oncology Educational Book/ASCO*, 199–203.

Bergsagel, D. E., Sprague, C. C., Austin, C., & Griffith, K. M. (1962). Evaluation of new chemotherapeutic agents in the treatment of multiple myeloma. IV. L-Phenylalanine mustard (NSC-8806). *Cancer Chemotherapy Reports*, *21*, 87–99.

Bharti, A. C., Shishodia, S., Reuben, J. M., Weber, D., Alexanian, R., Raj-Vadhan, S., et al. (2004). Nuclear factor-kappaB and STAT3 are constitutively active in CD138+ cells derived from multiple myeloma patients, and suppression of these transcription factors leads to apoptosis. *Blood*, *103*, 3175–3184.

Bhattacharya, S., Ray, R. M., & Johnson, L. R. (2005). STAT3-mediated transcription of Bcl-2, Mcl-1 and c-IAP2 prevents apoptosis in polyamine-depleted cells. *The Biochemical Journal*, *392*, 335–344.

Bhattacharyya, S., Yu, H., Mim, C., & Matouschek, A. (2014). Regulated protein turnover: Snapshots of the proteasome in action. *Nature Reviews. Molecular Cell Biology*, *15*, 122–133.

Boya, P., Gonzalez-Polo, R. A., Poncet, D., Andreau, K., Vieira, H. L., Roumier, T., et al. (2003). Mitochondrial membrane permeabilization is a critical step of lysosome-initiated apoptosis induced by hydroxychloroquine. *Oncogene*, *22*, 3927–3936.

Boyd, K. D., Ross, F. M., Tapper, W. J., Chiecchio, L., Dagrada, G., Konn, Z. J., et al. (2011). The clinical impact and molecular biology of del(17p) in multiple myeloma treated with conventional or thalidomide-based therapy. *Genes, Chromosomes & Cancer*, *50*, 765–774.

Branford, S., Rudzki, Z., Walsh, S., Grigg, A., Arthur, C., Taylor, K., et al. (2002). High frequency of point mutations clustered within the adenosine triphosphate-binding region of BCR/ABL in patients with chronic myeloid leukemia or Ph-positive acute lymphoblastic leukemia who develop imatinib (STI571) resistance. *Blood*, *99*, 3472–3475.

Bromberg, J. F., Wrzeszczynska, M. H., Devgan, G., Zhao, Y., Pestell, R. G., Albanese, C., et al. (1999). Stat3 as an oncogene. *Cell*, *98*, 295–303.

Campbell, R. A., Sanchez, E., Steinberg, J. A., Baritaki, S., Gordon, M., Wang, C., et al. (2007). Antimyeloma effects of arsenic trioxide are enhanced by melphalan, bortezomib and ascorbic acid. *British Journal of Haematology*, *138*, 467–478.

Canestraro, M., Galimberti, S., Savli, H., Palumbo, G. A., Tibullo, D., Nagy, B., et al. (2010). Synergistic antiproliferative effect of arsenic trioxide combined with bortezomib in HL60 cell line and primary blasts from patients affected by myeloproliferative disorders. *Cancer Genetics and Cytogenetics*, *199*, 110–120.

Carpenter, R. L., & Lo, H. W. (2014). STAT3 target genes relevant to human cancers. *Cancers*, *6*, 897–925.

Catlett-Falcone, R., Landowski, T. H., Oshiro, M. M., Turkson, J., Levitzki, A., Savino, R., et al. (1999). Constitutive activation of Stat3 signaling confers resistance to apoptosis in human U266 myeloma cells. *Immunity*, *10*, 105–115.

Cenci, S., & Sitia, R. (2007). Managing and exploiting stress in the antibody factory. *FEBS Letters*, *581*, 3652–3657.

Chaidos, A., Caputo, V., Gouvedenou, K., Liu, B., Marigo, I., Chaudhry, M. S., et al. (2014). Potent antimyeloma activity of the novel bromodomain inhibitors I-BET151 and I-BET762. *Blood*, *123*, 697–705.

Chen, S., Blank, J. L., Peters, T., Liu, X. J., Rappoli, D. M., Pickard, M. D., et al. (2010). Genome-wide siRNA screen for modulators of cell death induced by proteasome inhibitor bortezomib. *Cancer Research*, *70*, 4318–4326.

Chen, M. H., Qi, C. X. Y., Saha, M. N., & Chang, H. (2012). p53 nuclear expression correlates with hemizygous TP53 deletion and predicts an adverse outcome for patients with relapsed/refractory multiple myeloma treated with lenalidomide. *American Journal of Clinical Pathology*, *137*, 208–212.

Chen, K. F., Tai, W. T., Hsu, C. Y., Huang, J. W., Liu, C. Y., Chen, P. J., et al. (2012). Blockade of STAT3 activation by sorafenib derivatives through enhancing SHP-1 phosphatase activity. *European Journal of Medicinal Chemistry*, *55*, 220–227.

Chen, K. F., Tai, W. T., Huang, J. W., Hsu, C. Y., Chen, W. L., Cheng, A. L., et al. (2011). Sorafenib derivatives induce apoptosis through inhibition of STAT3 independent of Raf. *European Journal of Medicinal Chemistry*, *46*, 2845–2851.

Chen, S., Zhang, Y., Zhou, L., Leng, Y., Lin, H., Kmieciak, M., et al. (2014). A Bim-targeting strategy overcomes adaptive bortezomib resistance in myeloma through a novel link between autophagy and apoptosis. *Blood*, *124*, 2687–2697.

Chène, P. (2003). Inhibiting the p53-MDM2 interaction: An important target for cancer therapy. *Nature Reviews. Cancer*, *3*, 102–109.

Chesi, M., Matthews, G. M., Garbitt, V. M., Palmer, S. E., Shortt, J., Lefebure, M., et al. (2012). Drug response in a genetically engineered mouse model of multiple myeloma is predictive of clinical efficacy. *Blood*, *120*, 376–385.

Chng, W. J., Price-Troska, T., Gonzalez-Paz, N., Van Wier, S., Jacobus, S., Blood, E., et al. (2007). Clinical significance of TP53 mutation in myeloma. *Leukemia*, *21*, 582–584.

Collins, S. M., Bakan, C. E., Swartzel, G. D., Hofmeister, C. C., Efebera, Y. A., Kwon, H., et al. (2013). Elotuzumab directly enhances NK cell cytotoxicity against myeloma via CS1 ligation: Evidence for augmented NK cell function complementing ADCC. *Cancer Immunology, Immunotherapy*, *62*, 1841–1849.

Cullinan, S. B., & Diehl, J. A. (2004). PERK-dependent activation of Nrf2 contributes to redox homeostasis and cell survival following endoplasmic reticulum stress. *The Journal of Biological Chemistry*, *279*(2010), 8–17.

Dalton, W. S., & Jove, R. (1999). Drug resistance in multiple myeloma: Approaches to circumvention. *Seminars in Oncology*, *26*(5 Suppl. 13), 23–27.

de St Groth, S. F. (1983). Automated production of monoclonal antibodies in a cytostat. *Journal of Immunological Methods*, *57*, 121–136.

de Wilt, L. H., Jansen, G., Assaraf, Y. G., van Meerloo, J., Cloos, J., Schimmer, A. D., et al. (2012). Proteasome-based mechanisms of intrinsic and acquired bortezomib resistance in non-small cell lung cancer. *Biochemical Pharmacology*, *83*, 207–217.

Delmore, J. E., Issa, G. C., Lemieux, M. E., Rahl, P. B., Shi, J., Jacobs, H. M., et al. (2011). BET bromodomain inhibition as a therapeutic strategy to target c-Myc. *Cell*, *146*, 904–917.

Demasi, M., Hand, A., Ohara, E., Oliveira, C. L., Bicev, R. N., Bertoncini, C. A., et al. (2014). 20S proteasome activity is modified via S-glutathionylation based on intracellular redox status of the yeast Saccharomyces cerevisiae: Implications for the degradation of oxidized proteins. *Archives of Biochemistry and Biophysics*, *557*, 65–71.

Demasi, M., Netto, L. E., Silva, G. M., Hand, A., de Oliveira, C. L., Bicev, R. N., et al. (2013). Redox regulation of the proteasome via S-glutathionylation. *Redox Biology*, *2*, 44–51.

Demasi, M., Shringarpure, R., & Davies, K. J. (2001). Glutathiolation of the proteasome is enhanced by proteolytic inhibitors. *Archives of Biochemistry and Biophysics*, *389*, 254–263.

Derenne, S., Monia, B., Dean, N. M., Taylor, J. K., Rapp, M. J., Harousseau, J. L., et al. (2002). Antisense strategy shows that Mcl-1 rather than Bcl-2 or Bcl-x(L) is an essential survival protein of human myeloma cells. *Blood*, *100*, 194–199.

Dimopoulos, M., Siegel, D. S., Lonial, S., Qi, J., Hajek, R., Facon, T., et al. (2013). Vorinostat or placebo in combination with bortezomib in patients with multiple myeloma (VANTAGE 088): A multicentre, randomised, double-blind study. *The Lancet Oncology*, *14*, 1129–1140.

Doi, K., Li, R., Sung, S. S., Wu, H., Liu, Y., Manieri, W., et al. (2012). Discovery of marinopyrrole A (maritoclax) as a selective Mcl-1 antagonist that overcomes ABT-737 resistance by binding to and targeting Mcl-1 for proteasomal degradation. *The Journal of Biological Chemistry*, *287*, 10224–10235.

El-Deiry, W. S. (1998). Regulation of p53 downstream genes. *Seminars in Cancer Biology*, *8*, 345–357.

Filippakopoulos, P., Qi, J., Picaud, S., Shen, Y., Smith, W. B., Fedorov, O., et al. (2010). Selective inhibition of BET bromodomains. *Nature*, *468*, 1067–1073.

Fonseca, R., Blood, E., Rue, M., Harrington, D., Oken, M. M., Kyle, R. A., et al. (2003). Clinical and biologic implications of recurrent genomic aberrations in myeloma. *Blood*, *101*, 4569–4575.

Franke, N. E., Niewerth, D., Assaraf, Y. G., van Meerloo, J., Vojtekova, K., van Zantwijk, C. H., et al. (2012). Impaired bortezomib binding to mutant β5 subunit of the proteasome is the underlying basis for bortezomib resistance in leukemia cells. *Leukemia*, *26*, 757–768.

Fribley, A., Zeng, Q., & Wang, C. Y. (2004). Proteasome inhibitor PS-341 induces apoptosis through induction of endoplasmic reticulum stress-reactive oxygen species in head and neck squamous cell carcinoma cells. *Molecular and Cellular Biology*, *24*, 9695–9704.

Fuhrmann, G., Rosenberger, G., Grusch, M., Klein, N., Hofmann, J., & Krupitza, G. (1999). The MYC dualism in growth and death. *Mutation Research*, *437*, 205–217.

Gorre, M. E., Mohammed, M., Ellwood, K., Hsu, N., Paquette, R., Rao, P. N., et al. (2001). Clinical resistance to STI-571 cancer therapy caused by BCR-ABL gene mutation or amplification. *Science*, *293*, 876–880.

Groll, M., Berkers, C. R., Ploegh, H. L., & Ovaa, H. (2006). Crystal structure of the boronic acid-based proteasome inhibitor bortezomib in complex with the yeast 20S proteasome. *Structure*, *14*, 451–456.

Grune, T., Catalgol, B., Licht, A., Ermak, G., Pickering, A. M., Ngo, J. K., et al. (2011). HSP70 mediates dissociation and reassociation of the 26S proteasome during adaptation to oxidative stress. *Free Radical Biology & Medicine*, *51*, 1355–1364.

Gu, D., Wang, S., Kuiatse, I., Wang, H., He, J., Dai, Y., et al. (2014). Inhibition of the MDM2 E3 ligase induces apoptosis and autophagy in wild-type and mutant p53 models of multiple myeloma, and acts synergistically with ABT-737. *PLoS One*, *9*, e103015.

Harding, H. P., Zhang, Y., Zeng, H., Novoa, I., Lu, P. D., Calfon, M., et al. (2003). An integrated stress response regulates amino acid metabolism and resistance to oxidative stress. *Molecular Cell*, *11*, 619–633.

He, X., Yang, K., Chen, P., Liu, B., Zhang, Y., Wang, F., et al. (2014). Arsenic trioxide-based therapy in relapsed/refractory multiple myeloma patients: A meta-analysis and systematic review. *OncoTargets and Therapy*, *7*, 1593–1599.

Hendershot, L. M., & Sitia, R. (2005). Immunoglobulin assembly and secretion. In T. Honjo, F. W. Alt, & M. Neuberger (Eds.), *Molecular biology of B cells* (pp. 261–273): Amsterdam, The Netherlands: Elsevier Science.

Hideshima, T., Bradner, J. E., Wong, J., Chauhan, D., Richardson, P., Schreiber, S. L., et al. (2005). Small-molecule inhibition of proteasome and aggresome function induces synergistic antitumor activity in multiple myeloma. *Proceedings of the National Academy of Sciences of the United States of America*, *102*, 8567–8572.

Hideshima, T., Mitsiades, C., Akiyama, M., Hayashi, T., Chauhan, D., Richardson, P., et al. (2003). Molecular mechanisms mediating antimyeloma activity of proteasome inhibitor PS-341. *Blood*, *101*, 1530–1534.

Hideshima, T., Richardson, P. G., & Anderson, K. C. (2011). Mechanism of action of proteasome inhibitors and deacetylase inhibitors and the biological basis of synergy in multiple myeloma. *Molecular Cancer Therapeutics*, *10*, 2034–2042.

Hoang, B., Benavides, A., Shi, Y., Frost, P., & Lichtenstein, A. (2009). Effect of autophagy on multiple myeloma cell viability. *Molecular Cancer Therapeutics*, *8*, 1974–1984.

Hollstein, M., Sidransky, D., Vogelstein, B., & Harris, C. C. (1991). p53 mutations in human cancers. *Science*, *253*, 49–53.

Hong, D. S., Younes, A., Fayad, L., Fowler, N. H., Hagemeister, F. B., Mistry, R., et al. (2013). A phase I study of ISIS 481464 (AZD9150), a first-in-human, first-in-class, antisense oligonucleotide inhibitor of STAT3, in patients with advanced cancers. *Journal of Clinical Oncology*, *31*, 8523.

Hsi, E. D., Steinle, R., Balasa, B., Szmania, S., Draksharapu, A., Shum, B. P., et al. (2008). CS1, a potential new therapeutic antibody target for the treatment of multiple myeloma. *Clinical Cancer Research*, *14*, 2775–2784.

Jagannath, S., Barlogie, B., Berenson, J., Siegel, D., Irwin, D., Richardson, P. G., et al. (2004). A phase 2 study of two doses of bortezomib in relapsed or refractory myeloma. *British Journal of Haematology*, *127*, 165–172.

Jagannathan, S., Malek, E., Vallabhapurapu, S., Vallabhapurapu, S., & Driscoll, J. J. (2014). Bortezomib induces AMPK-dependent autophagosome formation uncoupled from apoptosis in drug resistant cells. *Oncotarget*, *5*, 12358–12370.

Jakubowiak, A. J., Benson, D. M., Bensinger, W., Siegel, D. S., Zimmerman, T. M., Mohrbacher, A., et al. (2012). Phase I trial of anti-CS1 monoclonal antibody elotuzumab in combination with bortezomib in the treatment of relapsed/refractory multiple myeloma. *Journal of Clinical Oncology*, *30*, 1960–1965.

Jin, L., Tabe, Y., Kojima, K., Zhou, Y., Pittaluga, S., Konopleva, M., et al. (2010). MDM2 antagonist Nutlin-3 enhances bortezomib-mediated mitochondrial apoptosis in TP53-mutated mantle cell lymphoma. *Cancer Letters*, *299*, 161–170.

Johnson-Farley, N., 1, Veliz, J., Bhagavathi, S., & Bertino, J. R. (2015). ABT-199, a BH3 mimetic that specifically targets Bcl-2, enhances the antitumor activity of chemotherapy, bortezomib and JQ1 in "double hit" lymphoma cells. *Leukemia & Lymphoma*, 1–7.

Jung, H. J., Chen, Z., & McCarty, N. (2012). Synergistic anticancer effects of arsenic trioxide with bortezomib in mantle cell lymphoma. *American Journal of Hematology*, *87*, 1057–1064.

Kawano, M., Hirano, T., Matsuda, T., Taga, T., Horii, Y., Iwato, K., et al. (1988). Autocrine generation and requirement of BSF-2/IL-6 for human multiple myelomas. *Nature*, *332*, 83–85.

Kharaziha, P., De Raeve, H., Fristedt, C., Li, Q., Gruber, A., Johnsson, P., et al. (2012). Sorafenib has potent antitumor activity against multiple myeloma in vitro, ex vivo, and in vivo in the 5T33MM mouse model. *Cancer Research*, *72*, 5348–5362.

Kiziltepe, T., Hideshima, T., Ishitsuka, K., Ocio, E. M., Raje, N., Catley, L., et al. (2007). JS-K, a GST-activated nitric oxide generator, induces DNA double-strand breaks, activates DNA damage response pathways, and induces apoptosis in vitro and in vivo in human multiple myeloma cells. *Blood*, *110*, 709–718.

Klein, B., Zhang, X. G., Jourdan, M., Content, J., Houssiau, F., Aarden, L., et al. (1989). Paracrine rather than autocrine regulation of myeloma-cell growth and differentiation by interleukin-6. *Blood*, *73*, 517–526.

Kuehl, W. M., & Bergsagel, P. L. (2012). MYC addiction: A potential therapeutic target in MM. *Blood*, *120*, 2351–2352.

Kumar, S. K., Jett, J., Marks, R., Richardson, R., Quevedo, F., Moynihan, T., et al. (2013). Phase 1 study of sorafenib in combination with bortezomib in patients with advanced malignancies. *Investigational New Drugs*, *31*, 1201–1206.

Lane, D. (1992). Cancer. p53, guardian of the genome. *Nature*, *358*, 15–16.

Laubach, J. P., Tai, Y. T., Richardson, P. G., & Anderson, K. C. (2014). Daratumumab granted breakthrough drug status. *Expert Opinion on Investigational Drugs*, *23*, 445–452.

Li, J., Favata, M., Kelley, J. A., Caulder, E., Thomas, B., Wen, X., et al. (2010). INCB16562, a JAK1/2 selective inhibitor, is efficacious against multiple myeloma cells and reverses the protective effects of cytokine and stromal cell support. *Neoplasia*, *12*, 28–38.

Lichter, D. I., Danaee, H., Pickard, M. D., Tayber, O., Sintchak, M., Shi, H., et al. (2012). Sequence analysis of β-subunit genes of the 20S proteasome in patients with relapsed multiple myeloma treated with bortezomib or dexamethasone. *Blood*, *120*, 4513–4516.

Lin, P., Owens, R., Tricot, G., & Wilson, C. S. (2004). Flow cytometric immunophenotypic analysis of 306 cases of multiple myeloma. *American Journal of Clinical Pathology*, *121*, 482–488.

Ling, Y. H., Liebes, L., Zou, Y., & Perez-Soler, R. (2003). Reactive oxygen species generation and mitochondrial dysfunction in the apoptotic response to Bortezomib, a novel proteasome inhibitor, in human H460 non-small cell lung cancer cells. *The Journal of Biological Chemistry*, *278*, 33714–33723.

Liu, H., & May, K. (2012). Disulfide bond structures of IgG molecules: Structural variations, chemical modifications and possible impacts to stability and biological function. *MAbs*, *4*, 17–23.

Lodé, L., Eveillard, M., Trichet, V., Soussi, T., Wuillème, S., Richebourg, S., et al. (2010). Mutations in TP53 are exclusively associated with del(17p) in multiple myeloma. *Haematologica*, *95*, 1973–1976.

Lovén, J., Hoke, H. A., Lin, C. Y., Lau, A., Orlando, D. A., Vakoc, C. R., et al. (2013). Selective inhibition of tumor oncogenes by disruption of super-enhancers. *Cell*, *153*, 320–334.

Lü, S., Chen, Z., Yang, J., Chen, L., Gong, S., Zhou, H., et al. (2008). Overexpression of the PSMB5 gene contributes to bortezomib resistance in T-lymphoblastic lymphoma/leukemia cells derived from Jurkat line. *Experimental Hematology*, *36*, 1278–1284.

Lü, S., Yang, J., Chen, Z., Gong, S., Zhou, H., Xu, X., et al. (2009). Different mutants of PSMB5 confer varying bortezomib resistance in T lymphoblastic lymphoma/leukemia cells derived from the Jurkat cell line. *Experimental Hematology*, *37*, 831–837.

Lü, S., Yang, J., Song, X., Gong, S., Zhou, H., Guo, L., et al. (2008). Point mutation of the proteasome beta5 subunit gene is an important mechanism of bortezomib resistance in

bortezomib-selected variants of Jurkat T cell lymphoblastic lymphoma/leukemia line. *The Journal of Pharmacology and Experimental Therapeutics*, *326*, 423–431.

Lütticken, C., Wegenka, U. M., Yuan, J., Buschmann, J., Schindler, C., Ziemiecki, A., et al. (1994). Association of transcription factor APRF and protein kinase Jak1 with the interleukin-6 signal transducer gp130. *Science*, *263*, 89–92.

Maiso, P., Carvajal-Vergara, X., Ocio, E. M., López-Pérez, R., Mateo, G., Gutiérrez, N., et al. (2006). The histone deacetylase inhibitor LBH589 is a potent antimyeloma agent that overcomes drug resistance. *Cancer Research*, *66*, 5781–5789.

Meek, D. W., & Anderson, C. W. (2009). Posttranslational modification of p53: Cooperative integrators of function. *Cold Spring Harbor Perspectives in Biology*, *1*, a000950.

Merten, O. W., Keller, H., Cabanie, L., Litwin, J., & Flamand, B. (1989). Development of a serum-free medium for hybridoma fermentor cultures. In R. E. Spier, J. R. Griffiths, J. Stephenné, & P. J. Crooy (Eds.), *Advances in animal cell biology and technology for bioprocesses* (pp. 263–268). United Kingdom: Butterworths.

Mitsiades, N., Mitsiades, C. S., Poulaki, V., Anderson, K. C., & Treon, S. P. (2001). Concepts in the use of TRAIL/Apo2L: An emerging biotherapy for myeloma and other neoplasias. *Expert Opinion on Investigational Drugs*, *10*, 1521–1530.

Mitsiades, N., Mitsiades, C. S., Poulaki, V., Chauhan, D., Fanourakis, G., Gu, X., et al. (2002). Molecular sequelae of proteasome inhibition in human multiple myeloma cells. *Proceedings of the National Academy of Sciences of the United States of America*, *99*, 14374–14379.

Mitsiades, C. S., Treon, S. P., Mitsiades, N., Shima, Y., Richardson, P., Schlossman, R., et al. (2001). TRAIL/Apo2L ligand selectively induces apoptosis and overcomes drug resistance in multiple myeloma: Therapeutic applications. *Blood*, *98*, 795–804.

Monaghan, K. A., Khong, T., Burns, C. J., & Spencer, A. (2011). The novel JAK inhibitor CYT387 suppresses multiple signalling pathways, prevents proliferation and induces apoptosis in phenotypically diverse myeloma cells. *Leukemia*, *25*, 1891–1899.

Moriya, S., Che, X. F., Komatsu, S., Abe, A., Kawaguchi, T., Gotoh, A., et al. (2013). Macrolide antibiotics block autophagy flux and sensitize to bortezomib via endoplasmic reticulum stress-mediated CHOP induction in myeloma cells. *International Journal of Oncology*, *42*, 1541–1550.

Nawrocki, S. T., Carew, J. S., Maclean, K. H., Courage, J. F., Huang, P., Houghton, J. A., et al. (2008). Myc regulates aggresome formation, the induction of Noxa, and apoptosis in response to the combination of bortezomib and SAHA. *Blood*, *112*, 2917–2926.

Nencioni, A., Hua, F., Dillon, C. P., Yokoo, R., Scheiermann, C., Cardone, M. H., et al. (2005). Evidence for a protective role of Mcl-1 in proteasome inhibitor-induced apoptosis. *Blood*, *105*, 3255–3262.

Nerini-Molteni, S., Ferrarini, M., Cozza, S., Caligaris-Cappio, F., & Sitia, R. (2008). Redox homeostasis modulates the sensitivity of myeloma cells to bortezomib. *British Journal of Haematology*, *141*, 494–503.

Nguyen, M., Marcellus, R. C., Roulston, A., Watson, M., Serfass, L., Murthy Madiraju, S. R., et al. (2007). Small molecule obatoclax (GX15-070) antagonizes MCL-1 and overcomes MCL-1-mediated resistance to apoptosis. *Proceedings of the National Academy of Sciences of the United States of America*, *104*, 19512–19517.

Nikiforov, M. A., Riblett, M., Tang, W. H., Gratchouck, V., Zhuang, D., Fernandez, Y., et al. (2007). Tumor cell-selective regulation of NOXA by c-MYC in response to proteasome inhibition. *Proceedings of the National Academy of Sciences of the United States of America*, *104*, 19488–19493.

Oerlemans, R., Franke, N. E., Assaraf, Y. G., Cloos, J., van Zantwijk, I., Berkers, C. R., et al. (2008). Molecular basis of bortezomib resistance: Proteasome subunit beta5 (PSMB5) gene mutation and overexpression of PSMB5 protein. *Blood*, *112*, 2489–2499.

Ooi, M., Hayden, P. J., Kotoula, V., McMillin, D. W., Charalambous, E., Daskalaki, E., et al. (2009). Interactions of the Hdm2/p53 and proteasome pathways may enhance the antitumor activity of bortezomib. *Clinical Cancer Research*, *15*, 7153–7160.

Orlowski, R. Z., Gercheva, L., Williams, C., Sutherland, H., Robak, T., Masszi, T., et al. (2015). A phase 2, randomized, double-blind, placebo-controlled study of siltuximab (anti-IL-6 mAb) and bortezomib versus bortezomib alone in patients with relapsed or refractory multiple myeloma. *American Journal of Hematology*, *90*, 42–49.

Orlowski, R. Z., Stinchcombe, T. E., Mitchell, B. S., Shea, T. C., Baldwin, A. S., Stahl, S., et al. (2002). Phase I trial of the proteasome inhibitor PS-341 in patients with refractory hematologic malignancies. *Journal of Clinical Oncology*, *20*, 4420–4427.

Paoluzzi, L., Gonen, M., Bhagat, G., Furman, R. R., Gardner, J. R., Scotto, L., et al. (2008). The BH3-only mimetic ABT-737 synergizes the antineoplastic activity of proteasome inhibitors in lymphoid malignancies. *Blood*, *112*, 2906–2916.

Pei, X. Y., Dai, Y., & Grant, S. (2003). The proteasome inhibitor bortezomib promotes mitochondrial injury and apoptosis induced by the small molecule Bcl-2 inhibitor HA14-1 in multiple myeloma cells. *Leukemia*, *17*, 2036–2045.

Pei, X. Y., Dai, Y., & Grant, S. (2004). Synergistic induction of oxidative injury and apoptosis in human multiple myeloma cells by the proteasome inhibitor bortezomib and histone deacetylase inhibitors. *Clinical Cancer Research*, *10*, 3839–3852.

Pérez-Galán, P., Roué, G., López-Guerra, M., Nguyen, M., Villamor, N., Montserrat, E., et al. (2008). BCL-2 phosphorylation modulates sensitivity to the BH3 mimetic GX15-070 (Obatoclax) and reduces its synergistic interaction with bortezomib in chronic lymphocytic leukemia cells. *Leukemia*, *22*, 1712–1720.

Pérez-Galán, P., Roué, G., Villamor, N., Campo, E., & Colomer, D. (2007). The BH3-mimetic GX15-070 synergizes with bortezomib in mantle cell lymphoma by enhancing Noxa-mediated activation of Bak. *Blood*, *109*, 4441–4449.

Pérez-Galán, P., Roué, G., Villamor, N., Montserrat, E., Campo, E., & Colomer, D. (2006). The proteasome inhibitor bortezomib induces apoptosis in mantle-cell lymphoma through generation of ROS and Noxa activation independent of p53 status. *Blood*, *107*, 257–264.

Petrini, M., Di Simone, D., Favati, A., Mattii, L., Valentini, P., & Grassi, B. (1995). GST-pi and P-170 co-expression in multiple myeloma. *British Journal of Haematology*, *90*, 393–397.

Podar, K., Gouill, S. L., Zhang, J., Opferman, J. T., Zorn, E., Tai, Y. T., et al. (2008). A pivotal role for Mcl-1 in Bortezomib-induced apoptosis. *Oncogene*, *27*, 721–731.

Politou, M., Karadimitris, A., Terpos, E., Kotsianidis, I., Apperley, J. F., & Rahemtulla, A. (2006). No evidence of mutations of the PSMB5 (beta-5 subunit of proteasome) in a case of myeloma with clinical resistance to Bortezomib. *Leukemia Research*, *30*, 240–241.

Preudhomme, C., Facon, T., Zandecki, M., Vanrumbeke, M., Laï, J. L., Nataf, E., et al. (1992). Rare occurrence of P53 gene mutations in multiple myeloma. *British Journal of Haematology*, *81*, 440–443.

Puthier, D., Bataille, R., & Amiot, M. (1999). IL-6 up-regulates mcl-1 in human myeloma cells through JAK/STAT rather than ras/MAP kinase pathway. *European Journal of Immunology*, *29*, 3945–3950.

Raje, N., Hari, P. N., Vogl, D. T., Jagannath, S., Orlowski, R. Z., Supko, J. G., et al. (2012). Rocilinostat (ACY-1215), a selective HDAC6 inhibitor, alone and in combination with bortezomib in multiple myeloma: Preliminary results from the first-in-humans phase I/II study. In *ASH annual meeting*, Abstract 4061.

Ramakrishnan, V., Timm, M., Haug, J. L., Kimlinger, T. K., Wellik, L. E., Witzig, T. E., et al. (2010). Sorafenib, a dual Raf kinase/vascular endothelial growth factor receptor inhibitor has significant anti-myeloma activity and synergizes with common anti-myeloma drugs. *Oncogene*, *29*, 1190–1202.

Reinheckel, T., Ullrich, O., Sitte, N., & Grune, T. (2000). Differential impairment of 20S and 26S proteasome activities in human hematopoietic K562 cells during oxidative stress. *Archives of Biochemistry and Biophysics*, *377*, 65–68.

Ri, M., Iida, S., Nakashima, T., Miyazaki, H., Mori, F., Ito, A., et al. (2010). Bortezomib-resistant myeloma cell lines: A role for mutated PSMB5 in preventing the accumulation of unfolded proteins and fatal ER stress. *Leukemia*, *24*, 1506–1512.

Richardson, P. G., Barlogie, B., Berenson, J., Singhal, S., Jagannath, S., Irwin, D., et al. (2003). A phase 2 study of bortezomib in relapsed, refractory myeloma. *The New England Journal of Medicine*, *348*, 2609–2617.

Roche-Lestienne, C., Soenen-Cornu, V., Grardel-Duflos, N., Laï, J. L., Philippe, N., Facon, T., et al. (2002). Several types of mutations of the Abl gene can be found in chronic myeloid leukemia patients resistant to STI571, and they can pre-exist to the onset of treatment. *Blood*, *100*, 1014–1018.

Rolland, D., Raharijaona, M., Barbarat, A., Houlgatte, R., & Thieblemont, C. (2010). Inhibition of GST-pi nuclear transfer increases mantle cell lymphoma sensitivity to cisplatin, cytarabine, gemcitabine, bortezomib and doxorubicin. *Anticancer Research*, *30*, 3951–3957.

Roumiantsev, S., Shah, N. P., Gorre, M. E., Nicoll, J., Brasher, B. B., Sawyers, C. L., et al. (2002). Clinical resistance to the kinase inhibitor STI-571 in chronic myeloid leukemia by mutation of Tyr-253 in the Abl kinase domain P-loop. *Proceedings of the National Academy of Sciences of the United States of America*, *99*, 10700–10705.

Rückrich, T., Kraus, M., Gogel, J., Beck, A., Ovaa, H., Verdoes, M., et al. (2009). Characterization of the ubiquitin-proteasome system in bortezomib-adapted cells. *Leukemia*, *23*, 1098–1105.

Saha, M., Jiang, H., Jayakar, J., Reece, D., Branch, D. R., & Chang, H. (2010). MDM2 antagonist nutlin plus proteasome inhibitor velcade combination displays a synergistic anti-myeloma activity. *Cancer Biology & Therapy*, *9*, 936–944.

Salem, K., McCormick, M. L., Wendlandt, E., Zhan, F., & Goel, A. (2015). Copper-zinc superoxide dismutase-mediated redox regulation of bortezomib resistance in multiple myeloma. *Redox Biology*, *4C*, 23–33.

Sanchez-Serrano, I. (2005). Translational research in the development of bortezomib: A core model. *Discovery Medicine*, *5*, 527–533.

Sánchez-Serrano, I. (2006). Success in translational research: Lessons from the development of bortezomib. *Nature Reviews. Drug Discovery*, *5*, 107–114.

San-Miguel, J., Bladé, J., Shpilberg, O., Grosicki, S., Maloisel, F., Min, C. K., et al. (2014). Phase 2 randomized study of bortezomib-melphalan-prednisone with or without siltuximab (anti-IL-6) in multiple myeloma. *Blood*, *123*, 4136–4142.

San-Miguel, J. F., Hungria, V. T., Yoon, S. S., Beksac, M., Dimopoulos, M. A., Elghandour, A., et al. (2014). Panobinostat plus bortezomib and dexamethasone versus placebo plus bortezomib and dexamethasone in patients with relapsed or relapsed and refractory multiple myeloma: A multicentre, randomised, double-blind phase 3 trial. *The Lancet Oncology*, *15*, 1195–1206.

San-Miguel, J. F., Richardson, P. G., Günther, A., Sezer, O., Siegel, D., Bladé, J., et al. (2013). Phase Ib study of panobinostat and bortezomib in relapsed or relapsed and refractory multiple myeloma. *Journal of Clinical Oncology*, *31*, 3696–3703.

Santo, L., Hideshima, T., Kung, A. L., Tseng, J. C., Tamang, D., Yang, M., et al. (2012). Preclinical activity, pharmacodynamic, and pharmacokinetic properties of a selective HDAC6 inhibitor, ACY-1215, in combination with bortezomib in multiple myeloma. *Blood*, *119*, 2579–2589.

Schneider, Y. J. (1989). Optimization of hybridoma growth and monoclonal antibody secretion in a chemically defined, serum- and protein-free culture medium. *Journal of Immunological Methods*, *116*, 65–77.

Scuto, A., Krejci, P., Popplewell, L., Wu, J., Wang, Y., Kujawski, M., et al. (2011). The novel JAK inhibitor AZD1480 blocks STAT3 and FGFR3 signaling, resulting in suppression of human myeloma cell growth and survival. *Leukemia*, *25*, 538–550.

Sekiguchi, N., Ootsubo, K., Wagatsuma, M., Midorikawa, K., Nagata, A., Noto, S., et al. (2014). The impact of C-Myc gene-related aberrations in newly diagnosed myeloma with bortezomib/dexamethasone therapy. *International Journal of Hematology*, *99*, 288–295.

Shacter, E. (1987). Serum-free medium for growth factor-dependent and -independent plasmacytomas and hybridomas. *Journal of Immunological Methods*, *99*, 259–270.

Shah, N. P., Nicoll, J. M., Nagar, B., Gorre, M. E., Paquette, R. L., Kuriyan, J., et al. (2002). Multiple BCR-ABL kinase domain mutations confer polyclonal resistance to the tyrosine kinase inhibitor imatinib (STI571) in chronic phase and blast crisis chronic myeloid leukemia. *Cancer Cell*, *2*, 117–125.

Sharma, M., Khan, H., Thall, P. F., Orlowski, R. Z., Bassett, R. L., Jr., Shah, N., et al. (2012). A randomized phase 2 trial of a preparative regimen of bortezomib, high-dose melphalan, arsenic trioxide, and ascorbic acid. *Cancer*, *118*, 2507–2515.

Shi, L., Wang, S., Zangari, M., Xu, H., Cao, T. M., Xu, C., et al. (2010). Over-expression of CKS1B activates both MEK/ERK and JAK/STAT3 signaling pathways and promotes myeloma cell drug-resistance. *Oncotarget*, *1*, 22–33.

Shieh, S. Y., 1, Ikeda, M., Taya, Y., & Prives, C. (1997). DNA damage-induced phosphorylation of p53 alleviates inhibition by MDM2. *Cell*, *91*, 325–334.

Shimizu, Y., & Hendershot, L. M. (2009). Oxidative folding: Cellular strategies for dealing with the resultant equimolar production of reactive oxygen species. *Antioxidants & Redox Signaling*, *11*, 2317–2331.

Shou, Y., Martelli, M. L., Gabrea, A., Qi, Y., Brents, L. A., Roschke, A., et al. (2000). Diverse karyotypic abnormalities of the c-myc locus associated with c-myc dysregulation and tumor progression in multiple myeloma. *Proceedings of the National Academy of Sciences of the United States of America*, *97*, 228–233.

Shuqing, L., Jianmin, Y., Chongmei, H., Hui, C., & Wang, J. (2011). Upregulated expression of the PSMB5 gene may contribute to drug resistance in patient with multiple myeloma when treated with bortezomib-based regimen. *Experimental Hematology*, *39*, 1117–1118.

Siegel, M. B., Davare, M. A., Liu, S. Q., Spurgeon, S. E., Loriaux, M. M., Druker, B. J., et al. (2014). 4702 the bromodomain inhibitor CPI203 demonstrates preclinical synergistic activity with bortezomib in drug resistant myeloma. In *ASH annual meeting*, Abstract 4702.

Siegel, D., Martin, T., Nooka, A., Harvey, R. D., Vij, R., Niesvizky, R., et al. (2013). Integrated safety profile of single-agent carfilzomib: Experience from 526 patients enrolled in 4 phase II clinical studies. *Haematologica*, *98*, 1753–1761.

Siegel, D. S., Martin, T., Wang, M., Vij, R., Jakubowiak, A. J., Lonial, S., et al. (2012). A phase 2 study of single-agent carfilzomib (PX-171-003-A1) in patients with relapsed and refractory multiple myeloma. *Blood*, *120*, 2817–2825.

Silva, G. M., Netto, L. E., Discola, K. F., Piassa-Filho, G. M., Pimenta, D. C., Bárcena, J. A., et al. (2008). Role of glutaredoxin 2 and cytosolic thioredoxins in cysteinyl-based redox modification of the 20S proteasome. *The FEBS Journal*, *275*, 2942–2955.

Silva, G. M., Netto, L. E., Simões, V., Santos, L. F., Gozzo, F. C., Demasi, M. A., et al. (2012). Redox control of 20S proteasome gating. *Antioxidants & Redox Signaling*, *16*, 1183–1194.

Srkalovic, G., Hussein, M. A., Hoering, A., Zonder, J. A., Popplewell, L. L., Trivedi, H., et al. (2014). A phase II trial of BAY 43-9006 (sorafenib) (NSC-724772) in patients with relapsing and resistant multiple myeloma: SWOG S0434. *Cancer Medicine*, *3*, 1275–1283.

Stella, F., Weich, N., Panero, J., Fantl, D. B., Schutz, N., Fundia, A. F., et al. (2013). Glutathione S-transferase P1 mRNA expression in plasma cell disorders and its correlation with polymorphic variants and clinical outcome. *Cancer Epidemiology*, *37*, 671–674.

Stessman, H. A., Baughn, L. B., Sarver, A., Xia, T., Deshpande, R., Mansoor, A., et al. (2013). Profiling bortezomib resistance identifies secondary therapies in a mouse myeloma model. *Molecular Cancer Therapeutics*, *12*, 1140–1150.

Stessman, H., Lulla, A., Xia, T., Mitra, A., Harding, T., Mansoor, A., et al. (2014). High-throughput drug screening identifies compounds and molecular strategies for targeting proteasome inhibitor-resistant multiple myeloma. *Leukemia*, *28*, 2263–2267.

Stewart, A. K., Rajkumar, S. V., Dimopoulos, M. A., Masszi, T., Špička, I., Oriol, A., et al. (2015). Carfilzomib, lenalidomide, and dexamethasone for relapsed multiple myeloma. *The New England Journal of Medicine*, *372*, 142–152.

Sui, X., Chen, R., Wang, Z., Huang, Z., Kong, N., Zhang, M., et al. (2013). Autophagy and chemotherapy resistance: A promising therapeutic target for cancer treatment. *Cell Death & Disease*, *4*, e838.

Suzuki, K., Ogura, M., Abe, Y., Suzuki, T., Tobinai, K., Ando, K., et al. (2015). Phase 1 study in Japan of siltuximab, an anti-IL-6 monoclonal antibody, in relapsed/refractory multiple myeloma. *International Journal of Hematology*, *101*(3), 286–294.

Tabe, Y., Sebasigari, D., Jin, L., Rudelius, M., Davies-Hill, T., Miyake, K., et al. (2009). MDM2 antagonist nutlin-3 displays antiproliferative and proapoptotic activity in mantle cell lymphoma. *Clinical Cancer Research*, *15*, 933–942.

Tai, Y. T., Dillon, M., Song, W., Leiba, M., Li, X. F., Burger, P., et al. (2008). Anti-CS1 humanized monoclonal antibody HuLuc63 inhibits myeloma cell adhesion and induces antibody-dependent cellular cytotoxicity in the bone marrow milieu. *Blood*, *112*, 1329–1337.

Tai, Y. T., Mayes, P. A., Acharya, C., Zhong, M. Y., Cea, M., Cagnetta, A., et al. (2014). Novel anti-B-cell maturation antigen antibody-drug conjugate (GSK2857916) selectively induces killing of multiple myeloma. *Blood*, *123*, 3128–3138.

Tew, K. D., & Townsend, D. M. (2011a). Regulatory functions of glutathione S-transferase P1-1 unrelated to detoxification. *Drug Metabolism Reviews*, *43*, 179–193.

Tew, K. D., & Townsend, D. M. (2011b). Redox platforms in cancer drug discovery and development. *Current Opinion in Chemical Biology*, *15*, 156–161.

Tew, K. D., & Townsend, D. M. (2012). Glutathione-s-transferases as determinants of cell survival and death. *Antioxidants & Redox Signaling*, *17*, 1728–1737.

Thieblemont, C., Rolland, D., Baseggio, L., Felman, P., Gazzo, S., Callet-Bauchu, E., et al. (2008). Comprehensive analysis of GST-pi expression in B-cell lymphomas: Correlation with histological subtypes and survival. *Leukemia & Lymphoma*, *49*, 1403–1406.

Touzeau, C., Dousset, C., Bodet, L., Gomez-Bougie, P., Bonnaud, S., Moreau, A., et al. (2011). ABT-737 induces apoptosis in mantle cell lymphoma cells with a Bcl-2high/Mcl-1low profile and synergizes with other antineoplastic agents. *Clinical Cancer Research*, *17*, 5973–5981.

Trudel, S., Li, Z. H., Rauw, J., Tiedemann, R. E., Wen, X. Y., & Stewart, A. K. (2007). Preclinical studies of the pan-Bcl inhibitor obatoclax (GX015-070) in multiple myeloma. *Blood*, *109*, 5430–5438.

Turkson, J., Kim, J. S., Zhang, S., Yuan, J., Huang, M., Glenn, M., et al. (2004). Novel peptidomimetic inhibitors of signal transducer and activator of transcription 3 dimerization and biological activity. *Molecular Cancer Therapeutics*, *3*, 261–269.

Udi, J., Schüler, J., Wider, D., Ihorst, G., Catusse, J., Waldschmidt, J., et al. (2013). Potent in vitro and in vivo activity of sorafenib in multiple myeloma: Induction of cell death, CD138-downregulation and inhibition of migration through actin depolymerization. *British Journal of Haematology*, *161*, 104–116.

Usami, H., Kusano, Y., Kumagai, T., Osada, S., Itoh, K., Ko- bayashi, A., et al. (2005). Selective induction of the tumor marker glutathione S-transferase P1 by proteasome inhibitors. *The Journal of Biological Chemistry*, *280*, 25267–25276.

van der Veer, M. S., de Weers, M., van Kessel, B., Bakker, J. M., Wittebol, S., Parren, P. W., et al. (2011). The therapeutic human CD38 antibody daratumumab improves the anti-myeloma effect of newly emerging multi-drug therapies. *Blood Cancer Journal*, *1*, e41.

van Rhee, F., Szmania, S. M., Dillon, M., van Abbema, A. M., Li, X., Stone, M. K., et al. (2009). Combinatorial efficacy of anti-CS1 monoclonal antibody elotuzumab (HuLuc63) and bortezomib against multiple myeloma. *Molecular Cancer Therapeutics*, *8*, 2616–2624.

Vassilev, L. T., Vu, B. T., Graves, B., Carvajal, D., Podlaski, F., Filipovic, Z., et al. (2004). In vivo activation of the p53 pathway by small-molecule antagonists of MDM2. *Science*, *303*, 844–848.

Verbrugge, S. E., Al, M., Assaraf, Y. G., Niewerth, D., van Meerloo, J., Cloos, J., et al. (2013). Overcoming bortezomib resistance in human B cells by anti-CD20/rituximab-mediated complement-dependent cytotoxicity and epoxyketone-based irreversible proteasome inhibitors. *Experimental Hematology & Oncology*, *2*, 2.

Verbrugge, S. E., Assaraf, Y. G., Dijkmans, B. A., Scheffer, G. L., Al, M., den Uyl, D., et al. (2012). Inactivating PSMB5 mutations and P-glycoprotein (multidrug resistance-associated protein/ATP-binding cassette B1) mediate resistance to proteasome inhibitors: Ex vivo efficacy of (immuno)proteasome inhibitors in mononuclear blood cells from patients with rheumatoid arthritis. *The Journal of Pharmacology and Experimental Therapeutics*, *341*, 174–182.

Vij, R., Savona, M., Siegel, D. S., Kaufman, J. L., Badros, A., Ghobrial, I. M., et al. (2014). Clinical profile of single-agent oprozomib in patients (Pts) with multiple myeloma (MM): Updated results from a multicenter, open-label, dose escalation phase 1b/2 study [abstract]. *Blood*, *124*, 34.

Vogl, D. T., Raje, N., Hari, P., Jones, S. S., Supko, J. G., Leone, G., et al. (2014). Phase 1B results of ricolinostat (ACY-1215) combination therapy with bortezomib and dexamethasone in patients with relapsed or relapsed and refractory multiple myeloma (MM). In *ASH annual meeting*, Abstract 4764.

Vogl, D. T., Stadtmauer, E. A., Tan, K. S., Heitjan, D. F., Davis, L. E., Pontiggia, L., et al. (2014). Combined autophagy and proteasome inhibition: A phase 1 trial of hydroxychloroquine and bortezomib in patients with relapsed/refractory myeloma. *Autophagy*, *10*, 1380–1390.

Voorhees, P. M., Chen, Q., Kuhn, D. J., Small, G. W., Hunsucker, S. A., Strader, J. S., et al. (2007). Inhibition of interleukin-6 signaling with CNTO 328 enhances the activity of bortezomib in preclinical models of multiple myeloma. *Clinical Cancer Research*, *13*, 6469–6478.

Vousden, K. H., & Prives, C. (2009). Blinded by the light: The growing complexity of p53. *Cell*, *137*, 413–431.

Wang, T., Arifoglu, P., Ronai, Z., & Tew, K. D. (2001). Glutathione S-transferase P1-1 (GSTP1-1) inhibits c-Jun N-terminal kinase (JNK1) signaling through interaction with the C terminus. *The Journal of Biological Chemistry*, *276*, 20999–21003.

Wang, Z. Y., & Chen, Z. (2008). Acute promyelocytic leukemia: From highly fatal to highly curable. *Blood*, *111*, 2505–2515.

Wang, X., Yen, J., Kaiser, P., & Huang, L. (2010). Regulation of the 26S proteasome complex during oxidative stress. *Science Signaling*, *3*, ra88.

Weber, D. M., Graef, T., Hussein, M., Sobecks, R. M., Schiller, G. J., Lupinacci, L., et al. (2012). Phase I trial of vorinostat combined with bortezomib for the treatment of relapsing and/or refractory multiple myeloma. *Clinical Lymphoma, Myeloma & Leukemia*, *12*, 319–324.

Wegenka, U. M., Buschmann, J., Lütticken, C., Heinrich, P. C., & Horn, F. (1993). Acute-phase response factor, a nuclear factor binding to acute-phase response elements, is rapidly activated by interleukin-6 at the posttranslational level. *Molecular and Cellular Biology*, *13*, 276–288.

Wen, J., Feng, Y., Huang, W., Chen, H., Liao, B., Rice, L., et al. (2010). Enhanced antimyeloma cytotoxicity by the combination of arsenic trioxide and bortezomib is further potentiated by p38 MAPK inhibition. *Leukemia Research*, *34*, 85–92.

Wuillème-Toumi, S., Robillard, N., Gomez, P., Moreau, P., Le Gouill, S., Avet-Loiseau, H., et al. (2005). Mcl-1 is overexpressed in multiple myeloma and associated with relapse and shorter survival. *Leukemia*, *19*, 1248–1252.

Yan, H., Wang, Y. C., Li, D., Wang, Y., Liu, W., Wu, Y. L., et al. (2007). Arsenic trioxide and proteasome inhibitor bortezomib synergistically induce apoptosis in leukemic cells: The role of protein kinase Cdelta. *Leukemia*, *21*, 1488–1495.

Yin, L., Kufe, T., Avigan, D., & Kufe, D. (2014). Targeting MUC1-C is synergistic with bortezomib in downregulating TIGAR and inducing ROS-mediated myeloma cell death. *Blood*, *123*, 2997–3006.

Yu, C., Rahmani, M., Dent, P., & Grant, S. (2004). The hierarchical relationship between MAPK signaling and ROS generation in human leukemia cells undergoing apoptosis in response to the proteasome inhibitor Bortezomib. *Experimental Cell Research*, *295*, 555–566.

Zhong, Z., Wen, Z., & Darnell, J. E., Jr. (1994). Stat3: A STAT family member activated by tyrosine phosphorylation in response to epidermal growth factor and interleukin-6. *Science*, *264*, 95–98.

Zmijewski, J. W., Banerjee, S., & Abraham, E. (2009). S-glutathionylation of the Rpn2 regulatory subunit inhibits 26 S proteasomal function. *The Journal of Biological Chemistry*, *284*, 22213–22221.