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The 26S Proteasome Complex: An Attractive Target for Cancer Therapy

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Abstract

The 26S proteasome complex engages in an ATP-dependent proteolytic degradation of a variety of oncoproteins, transcription factors, cell cycle specific cyclins, cyclin-dependent kinase inhibitors, ornithine decarboxylase, and other key regulatory cellular proteins. Thus, the proteasome regulates either directly or indirectly many important cellular processes. Altered regulation of these cellular events is linked to the development of cancer. Therefore, the proteasome has become an attractive target for the treatment of numerous cancers. Several proteasome inhibitors that target the proteolytic active sites of the 26S proteasome complex have been developed and tested for anti-tumor activities. These proteasome inhibitors have displayed impressive anti-tumor functions by inducing apoptosis in different tumor types. Further, the proteasome inhibitors have been shown to induce cell cycle arrest, and inhibit angiogenesis, cell-cell adhesion, cell migration, immune and inflammatory responses, and DNA repair response. A number of proteasome inhibitors are now in clinical trials to treat multiple myeloma and solid tumors. Many other proteasome inhibitors with different efficiencies are being developed and tested for anti-tumor activities. Several proteasome inhibitors currently in clinical trials have shown significantly improved anti-tumor activities when combined with other drugs such as histone deacetylase (HDAC) inhibitors, Akt (protein kinase B) inhibitors, DNA damaging agents, Hsp90 (heat shock protein 90) inhibitors, and lenalidomide. The proteasome inhibitor bortezomib is now in the clinic to treat multiple myeloma and mantle cell lymphoma. Here, we discuss the 26S proteasome complex in carcinogenesis and different proteasome inhibitors with their potential therapeutic applications in treatment of numerous cancers.

Keywords

26S proteasome complex; proteasome inhibitors; cancers

Introduction

The 26S proteasome complex is a non-lysosomal proteolytic machine in eukaryotes (1, 2). It consists of a 20S core particle (CP) and a 19S regulatory particle (RP). The 20S CP confers the proteolytic activities of the proteasome, whereas the 19S RP shows an ATP-dependence and specificity for ubiquitin protein conjugates. The 20S CP resembles a cylinder composed

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of four rings (two α and two β rings) (1, 3). These rings are flush with each other, giving the 20S CP a seven-fold symmetry. Each α ring is composed of seven different α components ($\alpha 1$ – $\alpha 7$). Similarly, seven different β components ($\beta 1$ – $\beta 7$) form a β ring. Three of the seven β -components in the β ring are catalytically active, and are named by their substrate specificities: chymotrypsin-like ($\beta 5$), trypsin-like ($\beta 2$), and post-acidic or caspase-like ($\beta 1$). The chymotrypsin-like activity cleaves proteins after hydrophobic residues, while the trypsin and caspase-like activities cleave after basic and acidic residues, respectively (4, 5). The substrate protein is translocated into the catalytic chamber of the 20S CP with the help of the 19S RP. The substrate protein is targeted to the 26S proteasome via its polyubiquitylation (Figure 1). The ubiquitin chains are added to the protein substrate by three enzymes: ubiquitin-activating E1, ubiquitin-conjugating E2, and ubiquitin-ligase E3 (Figure 1). E1 ubiquitin activating enzyme binds with an ubiquitin molecule, passes the ubiquitin to E2 ubiquitin conjugating enzyme, and E3 ubiquitin ligase enzyme enables the linking of C-terminal glycine residues of ubiquitin to lysine (K) residue on the substrate protein (Figure 1). Polyubiquitylation occurs through the linkage on one of the seven K residues of ubiquitin. The 19S RP recognizes the K48-linked polyubiquitylated-substrate protein, unfolds it, and finally feeds it into the catalytic chamber of the 20S CP for proteolysis in an ATP-dependent manner (6, 7, 8). Further, the 19S RP cleaves off the ubiquitin from the substrate protein, and recycles it for future use. While K48-mediated polyubiquitylated-form of the substrate protein is recognized and degraded by the proteasome, K63-linked polyubiquitylation is not targeted for degradation by the 26S proteasome complex, but plays a crucial role in cellular signaling.

The proteasome complex is found in both the cytoplasm and nucleus of eukaryotic cell where they regulate the ebb and flow of proteins involved in progression through the cell cycle, inflammatory mechanisms, antigen presentation, signal transduction, apoptosis, and other key regulatory cellular processes. Through these processes, the 26S proteasome complex plays a crucial role in maintaining normal cellular functions. The proteasome exists in two isoforms: the constitutive proteasome or the 26S proteasome, and the immunoproteasome (9). While the constitutive proteasome is found in most cells, the immunoproteasome is tissue-specific and abundant in immune-related cells. The immunoproteasome is formed in response to cytokine signaling. The immunoproteasome differs from its more common counterpart in that it contains a variation of the normal β -components. The $\beta 1$, $\beta 2$ and $\beta 5$ components of the constitutive proteasome are replaced by $\beta 1i$ (LMP2), $\beta 2i$ (MECL1 or LMP10) and $\beta 5i$ (LMP7) in the immunoproteasome. The immunoproteasome also has an 11S regulatory structure or PA28 instead of the 19S RP of the 26S proteasome. Stimulation from the γ -interferon (IFN- γ) can instigate the switch of constitutive β -components to the immuno β -components. Similarly, tumor necrosis factor- α (TNF- α) has also been shown to induce the expression of immuno β -components and 11S regulatory cap to form the immunoproteasome. Such modifications help the immunoproteasome to generate antigenic peptides in a major histocompatibility complex (MHC) class I-mediated immune response (10).

The 26S proteasome complex in different cellular events and carcinogenesis

The 26S proteasome regulates many cellular functions, the most prominent of which include the advancement through mitosis, growth, chemotaxis, antigen presentation, angiogenesis, apoptosis, and the expression of several genes which in turn regulate other processes. These mechanisms influenced by the 26S proteasome are some of the processes altered or deregulated in cancers. The most prominent substrates and related molecules of the 26S proteasome involved in cellular processes and carcinogenesis are discussed below.

Nuclear Factor-KappaB (NF- κ B)

NF- κ B was originally discovered as a regulator for the expression of the kappa light-chain gene in murine B-lymphocytes (11). Later on, NF- κ B has been found in nearly all animal cell types. NF- κ B is a transcription factor, and is involved in the activation of the genes encoding for cytokines, chemokines, growth factors, cell-adhesion molecules, and surface receptors (12–15) (Figure 2). Through transcriptional regulation of a number of genes, NF- κ B controls various immune and inflammatory responses. Further, it suppresses apoptosis, and induces angiogenesis, cell proliferation and migration (Figure 2), and thereby plays a crucial role in tumorigenesis (16–18). NF- κ B is a heterodimer of p50 and p65. The 26S proteasome is involved in generating p50 from the precursor protein p105. p50 then binds to p65 and becomes the active dimer or NF- κ B. In the cytoplasm, I κ B binds to NF- κ B and inhibits the translocation of NF- κ B to the nucleus for gene activation (Figure 2). External stimuli (e.g. ionizing and ultraviolet irradiation, pathogens, stress, free radicals, and cytokines) induce phosphorylation of I κ B (Figure 2). This phosphorylation triggers polyubiquitylation of I κ B for degradation by the 26S proteasome complex (Figure 2). The proteasomal degradation of I κ B promotes the translocation of NF- κ B to the nucleus to switch on the transcription of its target genes (Figure 2). Thus, the 26S proteasome complex plays a pivotal role in regulating the function of NF- κ B and associated key intra- and inter-cellular events. Therefore, misregulation of NF- κ B function would lead to various types of cancers. Incidentally, several cancers like breast cancer, myeloma, prostate cancer, and leukemias show constitutive activity of NF- κ B (14, 19–24). This confers chemoresistance and increased aggression in phenotypes through the continued expression of factors associated with anti-apoptosis, angiogenesis, cell growth/proliferation, and metastasis (14, 24–26). In addition to its involvement in cancer, NF- κ B is also linked to inflammatory and autoimmune diseases, septic shock, viral infection, improper immune development, processes of synaptic plasticity and memory as well as neurodegenerative and heart diseases (27–29).

Apoptosis

Cancer is characterized by an uncontrolled growth and spread of abnormal cells. Thus, the induction of apoptosis would promote the killing of abnormal cancer cells. However, cancer cells often have a dysregulation of apoptotic signaling pathways, leading to the suppression of apoptosis. Such an aberrant regulation of apoptosis provides a survival advantage to the cancer cells and therefore resistance to chemotherapy. Intriguingly, the key factors involved in controlling the apoptosis are regulated by the 26S proteasome complex (9, 30). For example, the levels of the pro-apoptotic factors such as p53, Bax, and NOXA are increased following inhibition of the proteolytic function of the 26S proteasome. Further, the inhibition of the proteasome activity has been shown to downregulate the anti-apoptotic factors such as Bcl-2 and IAP (inhibitor of apoptosis) proteins. Therefore, the inhibition of the proteolytic function in cancer cells would promote the apoptosis by upregulating the functions of the pro-apoptotic factors and suppressing the anti-apoptotic factors, thereby killing the cancer cells. Indeed, inhibition of the proteolytic function of the 26S proteasome has been shown to enhance the apoptosis in a number of cancer cells (9) (Figure 3).

As mentioned above, p53 is a pro-apoptotic factor that plays a critical role in apoptosis. Normally an unstable protein, its enhanced levels result in the initiation of the apoptosis cascade. Down-regulation of 26S proteasomal degradation causes the pro-apoptotic accumulation of p53, and hence induction of apoptosis (Figure 3). Activation of an E3 ubiquitin ligase, MDM2, ubiquitylates p53 and subsequently leads to its proteasomal degradation (31). This downregulation of p53 activity leads to tumor progression and drug resistance. Further, in high-risk HPV (human papilloma virus)-related cancer cases, the E6 oncoprotein and E6-AP (E6 associated protein, an E3 ubiquitin ligase) bind to p53, and ubiquitylate it for subsequent 26S proteasomal degradation (32, 33). Thus, the 26S

proteasome complex plays a key role in regulating the level of p53, and through it apoptosis and cancer progression. Therefore, the inhibition of the proteolytic function of the 26S proteasome in cancer cells would augment the level of p53, and would eventually impair the cancer progression by inducing apoptosis (Figure 3). Unsurprisingly, the inhibition of the proteasome activity has been shown to induce p53-dependent apoptosis in renal cancer cell lines (34), colon cancer (35), melanoma, and multiple myeloma (36). Proteasome inhibition has also been demonstrated to activate the downstream target genes of p53 such as Bax, p21, PUMA (p53 upregulated modulator of apoptosis) and Fas ligand (37).

Cell cycle

Regulation of cell cycle progression is a pivotal step in controlling carcinogenesis. In general, rapid cell cycle progression leads to cell proliferation and uncontrolled growth, ultimately developing transformed cancerous cells. Cyclins and cyclin dependent kinases (CDKs) tightly control the progression of cell cycle. However, the functions of several cyclins, CDKs, and their interplay are regulated by the proteolytic activity of the 26S proteasome complex, conferring proteasomal regulation of cell cycle progression in a number of ways. For example, CDK inhibition by tumor suppressor p27 downregulates cyclins D and E, and subsequently negatively controls the cell cycle progression through G1/S phase (38) (Figure 3). The 26S proteasome complex is involved in the degradation of p27, and thus promotes cell cycle progression (Figure 3). The E3 ubiquitin ligase, Skp-2 (S-phase kinase protein 2) targets p27 for ubiquitylation for its 26S proteasomal degradation. Due to this, low levels of p27 would lead to rapid cell cycle progression and hence oncogenesis. In fact, low levels of p27 are observed in various malignancies such as lymphoma, breast, lung, colon, prostate, ovarian, and brain cancers (39). Further, high levels of Skp-2 contributing to enhanced proteasomal degradation of p27 have been demonstrated in several cancers including non-small cell lung carcinoma (40). Similarly, the 26S proteasome is also involved in the regulation of CDK-inhibiting protein p21 levels, hence controlling cell cycle progression (41–43) (Figure 3).

Like cyclins D and E, cyclins A and B are also regulated by the 26S proteasome. The anaphase-promoting complex/cyclosome (APC/C) serves as an E3 ubiquitin ligase, and ubiquitylates both cyclins A and B, marking them for degradation by the 26S proteasome complex (44–46). The 26S proteasomal degradation of cyclins A and B guarantees that the cell completes mitosis and can enter the next cell cycle. In fact, cyclin B is rapidly degraded by the proteasome as the cell exits mitosis (47–49). Clearly, an alteration of the proteasomal degradation of cyclins A and B, or their enhanced level would be correlated with a number of cancers. Indeed, cyclin B has been found to be overexpressed in numerous cancer cell lines (50).

Further, the 26S proteasome complex has been shown to regulate cell cycle progression via an oncogenic transcription factor, Forkhead Box M1 (FoxM1). FoxM1 induces the expression of the genes that are involved in cell cycle progression (Figure 3). It is expressed at a low level in normal cells. However, its overexpression can lead to a rapid cell cycle progression. In fact, FoxM1 has been shown to be overexpressed in numerous cancers such as non-small cell lung carcinoma (51) breast cancer (52), colorectal cancer (53), glioblastomas (54), pancreatic carcinomas (55) and squamous cell carcinomas (56). Additionally, the inhibition of the proteolytic activity of the 26S proteasome has been demonstrated to suppress the expression of FoxM1 and its transcriptional activity in cancer cell lines (57, 58) (Figure 3). Thus, the proteasome plays an important role in regulation of oncogenesis via controlling the expression and activity of FoxM1.

Endoplasmic reticulum (ER) stress

Following the translation of mRNA at the ER, proteins are folded into their functional forms. When proteins are not folded properly, they are directed to 26S proteasomal degradation. If misfolded or unfolded proteins are not degraded by the proteasome, they form aggregates and lead to the ER stress. The ER stress triggers unfolded protein response (UPR) to reduce the accumulation of unfolded proteins and restore the ER function. When protein aggregation or ER stress persists, especially in cancerous cells with high rates of protein synthesis, the UPR signaling switches from the pro-survival to pro-apoptotic. Consequently, the 26S proteasome complex also plays an important role in regulating the ER stress and cell survival. Therefore, inhibition of the proteasomal function in cancer cells would promote apoptosis and have an anti-tumor function (Figure 3). In fact, the inhibition of the proteolytic activity of the 26S proteasome has been shown to induce pro-apoptotic ER stress in multiple myeloma (59), pancreatic (60), head and neck cancer (61), and non-small cell lung carcinoma (62).

Androgen receptor (AR)

AR is a ligand-dependent transcription factor, and belongs to the family of nuclear receptors. It plays an important role during differentiation and growth of the prostate and accompanying urogenital structures (24, 63). In presence of its ligand androgen, it binds to the promoters of a set of genes and regulates their expression (24, 63). With the ability to upregulate or downregulate under certain conditions, AR influences the expression of many genes. Some include: keratinocyte growth factor, probasin, prostate specific antigen (PSA), p21, Kallikrein, ornithine decarboxylase, and the AR gene itself. When the strict regulation of AR slips, it causes tumorigenesis, especially prostate cancer. Androgen plays a crucial role in the development of prostate cancer by activating AR. Prostate cancer is the second leading cause of cancer death among American males. Due to this, great importance is placed on androgen and its part in the development of prostate cancer. When AR is inactivated in AR-dependent prostate cancer, those cells can no longer replicate DNA or enter S phase, causing cell death (24) (Figure 3). Interaction between calmodulin (CaM) and AR can cause CaM-dependent protein kinases to phosphorylate AR, thus manipulating its molecular stability and nuclear localization via the 26S proteasome (24). Proteasome inhibitor MG-132 arrests AR interaction with co-regulators ARA70 or TIF2, as well as preventing nuclear translocation of AR, repressing AR transactivation (24, 63). Further, the inhibition of the proteolytic activity of the 26S proteasome by bortezomib reduces the basal level of AR, and subsequently induces the apoptosis of androgen-dependent human prostate cancer LNCaP cells (24). However, some prostate cancers grow in the absence of androgen, named androgen-independent AR-positive prostate cancer. Interestingly, decreasing active AR levels in these cells still inhibits growth (24). Together, these studies have implicated the 26S proteasome complex in regulating AR expression and function, and hence carcinogenesis. Thus, proteasome inhibition has anti-tumor effects via modulating the activity of AR. Likewise, proteasome inhibition has also been shown to regulate GR (glucocorticoid receptor) and ER (estrogen receptor) target genes in MCF-7 breast cancer cells (64). Further, Alarid and colleagues (65) have recently provided a new link of proteasomal function in estrogen signaling in breast cancers, by demonstrating the repression of ER gene expression in response to proteasome inhibition.

Chemokines

Chemokines are a family of chemotactic cytokines, and cause cells to move along a chemical gradient. This mechanism is very important in terms of angiogenesis, cell migration, and metastasis, all playing enormous roles in cancer (66–69). The role of chemokines in metastasis is the outcome of modified presentation of G-protein coupled receptors. When the production of the chemokine receptor CXCR4 is upregulated in cancer

cells (e.g., metastatic breast cancer, ovarian cancer and malignant melanoma cells), the cell migration is increased towards tissues that constitutively express the cognate chemokines ligand CXCL12, like liver, bone marrow, lymph nodes, and lung. To the contrary, the chemokine CXCL14/BRAK exhibits tumor suppressing activity but its expression is often modified in several cancers including prostate as well as squamous cell carcinomas of the head, neck, and cervix (69–71). The ubiquitin-proteasome system is responsible for degrading chemokine CXCL14 in cancer and other immortalized cells, but the proteasome does not degrade CXCL14 in normal epithelial cells (69). Treatment with proteasome inhibitor lactacystin resulted in the expression of CXCL14 in cancer cell lines that had previously shown impaired CXCL14 expression. Also, when LNCaP prostate cancer cells were treated with proteasome inhibitor MG-132, polyubiquitylated CXCL14 was found. These results demonstrate that cancer cells prevent their own CXCL14 expression as well as degradation by the proteasome. When overexpressed, CXCL14 inhibits angiogenesis and hence tumor growth (69, 72) (Figure 3). Further, normal and lower levels of CXCL14 would increase the chances of dendritic cells penetrating the tumor, leading to detection of the tumor by the immune system (69, 71–73). Thus, CXCL14 has anti-tumor activities via impairing angiogenesis and enhancing the detection of tumor by the immune system. The absence of CXCL14 in head and neck and prostate cancers favors cancer progression. Therefore, the inhibition of the proteolytic function of the proteasome in the cancer cells would increase the level of CXCL14, and hence would produce anti-tumor effects (Figure 3).

Cell-surface receptors

Cell-surface receptors, growth factors and their signaling pathways play important roles in carcinogenesis. Cell-surface receptors have been demonstrated to be ubiquitylated and degraded by the 26S proteasome complex. Proteasomal degradation of the cell-surface receptors is very relevant in cancer chemotherapy. For example, tyrosine kinase receptors are degraded by the 26S proteasome, and such degradation provides anti-tumor activity to herbimycin A which inhibits multiple tyrosine protein kinases (74). Similarly, protein kinase C (PKC) inhibitor downregulates PKC via 26S proteasomal degradation (75, 76). Other cell-surface receptors such as T-cell antigen receptor (TCR) and platelet-derived growth factor (PDGF) are also degraded by the 26S proteasome. These receptors are ubiquitylated in response to ligand binding for proteasomal degradation (77, 78). Thus, the 26S proteasome complex plays an important role in regulating the stabilities of the cell-surface receptors, and hence their signaling pathways.

AP-1

The proto-oncogene products c-JUN and c-FOS interact to form the transcription factor, AP-1 (activator protein 1). These two proto-oncoproteins form AP-1 as a heterodimer of c-JUN and c-FOS or a homodimer of c-JUN, and are known to be degraded by the 26S proteasome complex (79–84). The delta region, a 27 amino acid long segment of c-JUN, enables ubiquitylation and proteasomal degradation of the protein. This segment is missing in v-JUN, the transforming retroviral counterpart of c-JUN. As a result, v-JUN is upregulated, and such an increased stability is very likely to contribute to its oncogenicity (79). Therefore, an impaired proteasomal degradation of c-JUN can lead to oncogenesis. Moreover, the proteasomal degradation of c-JUN is essential to maintain normal function of AP-1 factors. The AP-1 factors play important roles in handling oxidative stresses (85, 86). AP-1 upregulation has been correlated with drug resistance in several cancer cell lines (87–89).

DNA repair

The DNA damage and regulation of its repair mechanisms are strongly correlated with carcinogenesis. The proteasome complex plays a crucial role in DNA repair (90). The inhibition of the proteolytic function would impair DNA repair (Figure 3), and trigger apoptosis. Therefore, DNA damaging agents have been used to kill cancer cells. Further, the combination of DNA damaging agents with proteasome inhibitor would have synergistic effects in killing cancer cells. Indeed, proteasome inhibitors have shown more effective anti-tumor activities when combined with the DNA damaging agents such as radiation and camptothecin (CPT) (9, 90).

MHC-restricted class I antigens

As mentioned above, MHC-restricted class I antigens are vitally processed by the immunoproteasome and presented on the cell surface for recognition by cytotoxic T lymphocytes. Proteins LMP2, LMP7, and LMP10 are interchanged with the three components of the 20S catalytic core to form the immunoproteasome in the instance of IFN- γ induction (91–95). Substitution of LMP2, LMP7, and LMP10 yields more types of peptides expressed on the cell surface (96, 97). Thus, the low levels of LMP2, LMP7, and LMP10 can lead to a decrease in MHC class I-restricted peptide presentation, and cause an escape from immune surveillance, leading to cancer. Indeed, very low levels of LMP2, LMP7, and lower antigen presentation are found in 3 small-cell lung carcinoma lines (98). Further, the mouse T-cell lymphoma cell line SP-3 has been shown to display an underexpression of LMP2 and an impairment of antigen presentation (99). Oncogenic viruses have been found to down-regulate LMP2 and LMP7 upon viral transformation of the cell (100). However, the expression of LMP2 and LMP7 has been shown to be increased with an enhancement of antigen presentation in these cancer cell lines by expressing IFN- γ following transfection (99, 101). By expressing fewer peptides, cancerous cells may avoid detection by the immune system. Thus, the immunoproteasome plays an important role in antigen presentation, and its malfunction would lead to the escape from immune surveillance (Figure 3), and hence cancer. In fact, a low level of immunoproteasome activity is present in certain cancer cells (98–100). A down-regulation of LMP2 and LMP7 has also been observed in hepatocellular carcinoma (102). Likewise, a decreased level of 26S proteasomal activity has also been observed in lung cancer stem-like cells *in vitro* (103). Further, an altered level of the proteasome component MB1 ($\beta 5$) is found in ovarian cancer (104).

As discussed above, the 26S proteasome is involved in nearly every kind of pathway cancer uses for survival and growth. By understanding these pathways and their relationship with the proteasome, it becomes clear that the manipulation of the 26S proteasome in turn would determine the fate of cancer cells. Undoubtedly, the proteasomal inhibition has been proven to be an attractive anti-cancer tool as discussed below.

Proteasome inhibition to treat cancer

When proteasome inhibitors prevent the proteasome from activating NF- κ B, factors of angiogenesis, survival, and growth are down-regulated while apoptosis is up-regulated in multiple tumor cell lines (16, 105–115) (Figure 3). This effect is also noticed in chemotherapy-resistant cells, additionally due to disruption of proteasomal regulation of caspases and Bcl-2. Further, proteasome inhibition enhances the levels of p21 and p27 (116, 117) (Figure 3). Such enhancement inhibits CDKs and consequently arrests cell cycle, halting the growth of cancer cells (Figure 3). The inhibition of the proteolytic function of the 26S proteasome has also been shown to impair the development of new blood vessels from endothelial cells or angiogenesis (Figure 3) that is a vital factor for tumor growth and metastasis (115, 118). Disruption of angiogenesis by proteasome inhibition also occurs by

decreasing microvessel density and the expression of vascular endothelial growth factor (VEGF) (115, 118, 119) (Figure 3). Thus, the proteasome inhibition impairs angiogenesis as well as disturbs cellular homeostasis, hence leading to an anti-tumor activity. Overall, these studies demonstrated that the inhibition of the proteolytic function of the 26S proteasome induces apoptosis and cell cycle arrest, and represses angiogenesis as well as metastasis (Figure 3). In fact, apoptosis and other anti-tumor effects from proteasome inhibition have been observed in various cancer cell lines and xenograft models including lymphoma, leukemia, melanoma, pancreatic, prostate, head and neck, breast, and lung cancers (36, 119–126). Further, the cancer cells are more sensitive to the cytotoxic effects of the proteasome inhibition as compared to the normal cells (127, 128). Also, cessation of all proteasomal function is not required to achieve anti-tumor effects (14, 129). Together, these studies have implicated the proteasome inhibition as an attractive way of treating cancer cells (Figure 3). Therefore, a large number of studies are focused on a variety of proteasome inhibitors for effectively treating cancer.

There is a wide variety of natural and synthetic proteasome inhibitors. These inhibitors are clustered into five groups: peptide aldehydes, peptide vinyl sulphones, peptide boronates, peptide epoxyketones, and β -lactones (lactacystin and its derivatives). Small-molecule proteasome inhibitors mimic the peptide substrates of the active sites in the 20S catalytic core subunit of the 26S proteasome complex. Lactacystin is a microbial metabolite isolated from *Streptomyces*, and is the first compound found to have an inhibiting effect on the proteasome. Lactacystin effectively and irreversibly inhibits the $\beta 5$ -component of the proteasome by selectively modifying N-terminal threonine residues, and also reversibly binds to the $\beta 1$ - and $\beta 2$ -components. MG-132 (Z-Leu-Leu-Leu-aldehyde) and PSI (Z-Ile-Glu-(OtBu)-Ala-Leu-aldehyde) are two of the first proteasome inhibitors synthesized. These are peptide aldehydes that reversibly bind to the $\beta 2$ - and $\beta 5$ components by forming covalent hemiacetal adducts. At high concentrations, they also inhibit calpains and cathepsins proteases. However, these compounds exhibit low specificity and high metabolic instability, limiting to use as research reagents. The peptide vinyl sulfone proteasome inhibitor has a vinyl sulfone group which is less reactive than the aldehyde group of the peptide aldehyde proteasome inhibitor. The vinyl sulfone group irreversibly binds to the active site. One most potent peptide vinyl sulfone proteasome inhibitor is AdaAhx3-LLL-vs. This inhibitor binds to the active sites of both the constitutive and immunoproteasome with almost equal efficiencies (130, 131). Peptide boronates, epoxomicin (peptide epoxyketone), and lactacystin have shown higher specificity to the proteasome, and therefore show the most promise for drug development. Two of the peptide boronates, MG-262 and bortezomib, form more stable tetrahedral intermediates with N-terminal threonine residues of the 20S CP, lending them a greater efficacy.

In addition to the synthetic and natural proteasome inhibitors, a variety of proteasome inhibiting compounds can also be found in foods. Some of these inhibitors are: apigenin, epigallocatechin-gallate (EGCG), and ajoene. Apigenin is a polyphenolic flavone found in a broad range of fruits and vegetables (132, 133). It has demonstrated chemopreventive properties in several cancer models such as lung, skin, cervical, prostate, and leukemia by scavenging the free radicals, anti-inflammation, and proteasome inhibition (134–141). It comes as no surprise that frequent ingestion of apigenin and other polyphenolic compounds correlates with a lowered cancer risk and even a suppression of tumor growth (132, 133). Apigenin achieves its proteasomal inhibiting effect by interrupting the chymotrypsin-like activity of the $\beta 5$ -component of the proteasome (133). In a study using MDA-MB-231 breast cancer cells, Chen et al. (133) found that at the highest concentration tested, apigenin reduced proliferation and viability of cancer cells by 50% after 24 hours. Western blot analysis confirmed that apigenin caused 40% proteasome inhibition, a buildup of ubiquitinated-Bax and IkB α , as well as increased caspase-3, caspase-7, and cleaved PARP

(poly ADP-ribose polymerase) levels, indicating apoptosis (133, 136, 141). In addition to a significant proteasome inhibition, apigenin also seems to induce the expression of death receptor 5 and an apoptosis-inducing TNF-associated ligand in leukemia, prostate, and colon cancer cells without having a toxic effect on normal cells (133, 142). This cancer-targeted toxicity is echoed in animal models *in vitro* and *in vivo* (133). Like apigenin, EGCG present in green, but not black, tea has been demonstrated to inhibit the proteolytic function of the 26S proteasome. It is a polyphenolic compound, and has an anti-tumor activity (143, 144). Further, EGCG has been shown to attenuate the release of pro-inflammatory cytokines, thereby terminating inflammation (143, 144). Likewise, ajoene is an organo sulfur compound present in garlic (130). It has been shown to inhibit the trypsin-like activity of the 20S CP of the proteasome complex (145). It induces apoptosis as well as cell cycle arrest of tumor cells by inhibiting G2/M phase. Consequently, ajoene has cytotoxic effects in tumor cells (146).

Like the proteasome inhibitors found in foods, naturally occurring gallium has also shown anti-neoplastic activity in clinical trials in bladder cancer, lymphomas, and a variety of other malignancies (147–152). Gallium III complex demonstrates anti-tumor activity via the inhibition of the proteasomal activity of the 26S proteasome (152). Further, gallium disturbs iron homeostasis by competing with Fe^{3+} for uptake into cells, the mediator for which is the transferrin receptor system that is overexpressed in cancerous cells (152, 153–155). Chen et al (152) have demonstrated that a certain gallium complex tested inhibited 81% of proteasomal activity in C4–2B prostate cancer cells. This complex also induces apoptosis, as evidenced by PARP cleavage, TUNEL positivity, nuclei condensation, and activation of caspase-3/caspase-7 (152).

Bortezomib: a proteasome inhibitor in the clinic to treat cancer

As mentioned above, the proteasome complex plays crucial roles in many important biological events, and its malfunction is strongly correlated to carcinogenesis. Thus, the proteasome inhibitors have shown a broad spectrum of anti-proliferative and pro-apoptotic activities against haematological and solid tumors. However, many of these proteasome inhibitors have low potency, specificity or stability (156–161). Therefore, new proteasome inhibitors with greater potency and selectivity were developed. Thirteen boron-containing proteasome inhibitors were synthesized, and subsequently screened for anti-cancer activity using a panel of 60 human tumor cell lines of National Cancer Institute, USA (162). One compound showed extremely high potency against a wide range of cancer cell lines. This compound is known as bortezomib, velcade, or PS-341 (originally synthesized as MG-341 at a company called Myogenics, and marketed as velcade by Millennium Pharmaceuticals, Inc., Cambridge, MA, USA) (Table 1). Bortezomib is a water-soluble dipeptide boronic acid which contains pyrazinoic acid, phenylalanine and Leucine with boric acid instead of a carboxylic acid.

Bortezomib is a stable proteasome inhibitor that binds covalently and reversibly with the $\beta 5$ component of the 20S catalytic core subunit of the proteasome forming tetrahedral intermediates on the N-terminal threonine residues (156). Further, it does not have any known activity against other cellular proteases (163). Due to these qualities, bortezomib entered into clinical phase I trials (19, 20, 164–166). In phase I clinical trials, bortezomib demonstrated an effective proteasome inhibition with fair tolerance levels, and thus moved into phase II clinical trials. Bortezomib had success in this clinical phase with refractory multiple myeloma patients (167), spurring its rapid approval by the Food and Drug Administration (FDA) and European Medicines Agency (EMA). Interestingly, in the phase III clinical trials in refractory multiple myeloma, the survival rate of the patients treated with bortezomib exceeded that of the patients treated with dexamethasone (168). Also, this

proteasome inhibitor is consistently able to surmount the factors that normally cause treatment resistance including γ -radiation and the chemotherapeutic agent CPT-11 by preventing the activation of NF- κ B (18, 169). Though accumulation of p53 can initiate apoptosis, bortezomib kills tumor cells independently of p53 levels, even drug resistant multiple myeloma cell lines with mutant p53 (16, 170).

Bortezomib specifically shows a high efficacy in multiple myeloma, non-small lung cancer, mantle cell lymphomas, and pancreatic cancer (125, 164, 167, 171, 172). Though not all tumor cell types react similarly to bortezomib, its substantial activity in a variety of cancer cell lines and tumor types in clinical trials propelled it into FDA approval. The FDA first approved bortezomib in 2003 for the third-line treatment of multiple myeloma (173). Later on, it was approved for the first-line treatment in 2008. It has also been approved in treating mantle cell lymphoma (a fast-growing cancer that begins in the cells of the immune system) in 2006 (174). As the first FDA approved proteasome inhibitor, bortezomib exhibits an around 1000-fold improvement over its aldehyde predecessors and more specificity to the β 5 component of the 20S catalytic core of the proteasome. Although bortezomib has shown a significant anti-tumor activity, it is also used to overcome chemoresistance (175–177). Due to this, bortezomib has been successfully combined with several other agents such as doxorubicin, thalidomide, melphalan, and dexamethasone. Thus, there is a great hope in developing better combinatorial therapy without increasing toxicity in treating numerous cancer patients. Currently, there are a large number of clinical trials going on for combinatorial therapy involving bortezomib against haematological malignancies and solid tumors.

Combinatorial therapies have shown great potential for cancer treatment. The combination of bortezomib with other drugs such as Hsp90 inhibitor, HDAC inhibitor, Akt inhibitor, and lenalidomide have more clinical benefits as compared to bortezomib alone (115, 178). Bortezomib with DNA damaging agent works well with relapsed and/or refractory cancer patients (110, 115, 179, 180). Hsp90 inhibitor has been shown to overcome bortezomib resistance in mantle cell lymphoma (108, 178). Lenalidomide has been combined with steroids, proteasome inhibitors, mTOR (target of rapamycin) inhibitors, humanized monoclonal antibodies, and Akt inhibitors. Lenalidomide with bortezomib or Akt inhibitor has shown very impressive responses in the cancer patients (115). Overall, the combinatorial therapies have shown a very promising results, and the most successful combination is likely to be approved soon to treat cancer patients.

Although bortezomib has been approved to treat multiple myeloma and mantle cell lymphoma patients, it also sensitizes pancreatic cancer cells to ER stress-mediated apoptosis (30, 181–183). Further, an induction of ER stress is a novel strategy to enhance bortezomib-induced apoptosis in pancreatic cancer cells. The combination of bortezomib with HDAC inhibitor, SAHA (Suberoylanilide hydroxamic acid), entered clinical trials in 2007. Additionally, the combination of bortezomib with HDAC6 inhibitor (more specific) may have better clinical benefits in treating pancreatic cancer or other solid malignancies (30, 182).

Though bortezomib kills cancer cells, the cellular mechanisms for clinical efficacy of bortezomib are not clearly known. However, several mechanisms-of-action of bortezomib have been implicated in killing cancer cells, which include disruption of cell adhesion- and cytokine-dependent survival pathways (e.g., NF- κ B signaling pathway), inhibition of angiogenesis, activation of a misfolded protein stress response (or ER stress), upregulation of pro-apoptotic or downregulation of anti-apoptotic genes. DNA microarray analysis revealed upregulation of genes involved in hypoxia, ER stress/UPR, oxidative stress, apoptosis, and amino acid starvation following proteasomal inhibition (184–194). Thus,

bortezomib seems to kill cancer cells by hypoxic response deregulation in tumor cells, mTOR inhibition, and ER stress-induced apoptosis. Further, bortezomib has been shown to upregulate AP-1 activity and activating transcription factor (ATF) families (192, 193, 195–199). ATF4 contributes to apoptosis, thus implicating ATFs in bortezomib-induced apoptosis. Like other cancer therapies, some factors contribute resistance to bortezomib treatment (115). An increased expression of HSPs reduces the efficacy of bortezomib. For example, HSP27 directly correlates with bortezomib resistance (200), and HSP90 inhibition overcomes bortezomib resistance in mantle cell lymphoma (115, 178). Bortezomib has also been shown to promote IFN- α and TRAIL (TNF-related apoptosis-inducing ligand)-induced apoptosis in human bladder cancer cells (201). Therefore, bortezomib manifests its anti-tumor activity via multiple mechanisms.

Bortezomib is metabolized primarily by cytochrome P450 3A4 (202, 203). Though intensely effective in treating many types of cancer, bortezomib is not without its side effects. Bortezomib has a dose limiting toxicity and pain associated with intravenous administration. Patients treated with bortezomib have experienced peripheral neuropathy, pyrexia, adverse gastrointestinal events, myelosuppression, orthostatic hypotension, asthenia, thrombocytopenia, cardiac and pulmonary disorders, and pain (128, 167, 168, 204, 205). Bortezomib is also associated with a high rate of shingles (206). Further, it has not shown promising results in treating solid tumors (9). These facts have demanded the need to develop a new generation of proteasome inhibitors. In this direction, several proteasome inhibitors have been developed, and are currently under clinical trials as presented below.

Proteasome inhibitors in clinical trials to treat cancer

There are several promising proteasome inhibitors that are currently in clinical trials. These are: Carfilzomib (PR-171), ONX0912 (PR-047), Marizomib (NPI-0052), CEP-18770, and MLN9708 (Table 1). Several immunoproteasome inhibitors (Table 2) have also been developed, which have shown impressive results in the pre-clinical studies. These inhibitors are described below.

Carfilzomib

Carfilzomib (also known as PR-171) is an epoxomicin-based proteasome inhibitor with improved pharmaceutical properties. Proteolix Inc. (California, USA) has developed carfilzomib as a second generation proteasome inhibitor to treat multiple myeloma patients (129). Carfilzomib irreversibly binds to the catalytic site of the proteasome, and inhibits the chymotrypsin-like activity. Unlike bortezomib, carfilzomib has shown minimal cross-reactivity with the other catalytic sites of the 20S CP. Further, carfilzomib shows minimal reactivity with other protease classes. Thus, carfilzomib has a better selectivity than bortezomib for chymotrypsin-like activity of the 26S proteasome in *in vitro* and *in vivo* studies (129, 207, 208). Carfilzomib has also shown better tolerability and dosing flexibility in xenograft models (129, 208). Pre-clinical studies indicate that carfilzomib is active against models of solid tumors, lymphomas, and myeloma (129, 208–210). Carfilzomib inhibits cell proliferation, and induces apoptosis which is associated with activation of JNK (c-Jun N-terminal protein kinase), depolarization of mitochondrial membrane, release of cytochrome C, and activation of both intrinsic and extrinsic caspase pathways in patient-derived multiple myeloma cells as well as neoplastic cells from patients with other hematologic malignancies (129, 209, 210). The phase I clinical trials of carfilzomib demonstrated that multiple myeloma patients who have relapsed or progressed following a number of therapies (including bortezomib and stem cell transplant) can also achieve durable anti-tumor responses with carfilzomib. Carfilzomib is well tolerated in patients at doses that suppress chymotrypsin-like proteasome activity by >80% in whole blood. The phase II clinical trials of carfilzomib provided promising results in patients with relapsed or

refractory multiple myeloma. Currently, clinical phase III trials are ongoing for carfilzomib in multiple myeloma (9, 211, 212). Further, carfilzomib is now under clinical phase I trials for acute myeloid leukemia (AML), acute lymphoblastic leukaemia (ALL), and chronic lymphocytic leukaemia (CLL) (9, 129). It is also in phase 1b/II trials in solid tumors (118, 213). Like bortezomib, carfilzomib may work better in combination with other therapies. In fact, it has been shown to function better in leukemia and lymphoma in combination with HDAC inhibitors *in vitro* (214, 215). Carfilzomib has also been shown to interact synergistically with HDAC inhibitors in mantle cell lymphoma cells (211). Furthermore, carfilzomib acts synergistically with dexamethasone, and has shown an increased level of anti-multiple myeloma activity as compared to bortezomib (129).

ONX0912

Both bortezomib and carfilzomib are administered intravenously. However, an oral proteasome inhibitor could be easily administered in the multi-drug treatment regimens. Proteolix, Inc. has developed an oral analogue, ONX0912 (also known as PR-047) that has N-cap with significant pre-clinical anti-tumor activities (216). This agent shows an improved therapeutic window over carfilzomib in experimental animal models. It has been demonstrated to reduce tumor progression and prolong survival in animal models of multiple myeloma, non-Hodgkin's lymphoma and colorectal cancer (216–218). Further, it has been shown to enhance the anti-tumor activity in combination with HDAC inhibitor, lenolidomide and bortezomib (216–218). This proteasome inhibitor is currently under clinical trials. The clinical phase I trials of this compound are also ongoing in advanced solid tumors (9).

NPI-0052

NPI-0052 (also known as salinosporamide A or marizomib) is an irreversible second generation proteasome inhibitor, and orally bioactive (219). It has been developed by Nereus Pharmaceuticals, Inc. (San Diego, CA, USA) (220). It is a non-peptide, β -lactone compound that is related to lactacystin. It has been derived from the marine bacterium *Salinospora tropica* (221), and possesses anti-tumor activity through caspase-8 activation (222, 223). It stimulates apoptosis predominantly via caspase-8-mediated pathway (222, 223). Thus, NPI-0052 induces apoptosis via mechanisms that are unique from those evoked by bortezomib (108, 222, 223). NPI-0052 also differs from bortezomib or carfilzomib in terms of its inhibitory effects on the three major enzymatic activities of the 20S CP. It binds irreversibly to all catalytic sites for proteolysis of the 26S proteasome (223). At the maximum tolerated dose without apparent toxicity, NPI-0052 shows as high as 90% proteasome inhibition as compared to 70% inhibition by bortezomib (169, 219). The proteasome inhibition by NPI-0052 increases progressively over 24 hours, and remained essentially unchanged for 72 hours. On the other hand, the proteasome inhibition by bortezomib reaches the maximum level of inhibition at 1.5 hours, and then significantly decreases over the next 24 hours (219). Therefore, NPI-0052 appears to be a more effective compound in treating cancer patients. The cellular response to NPI-0052 occurs much earlier than bortezomib. Further, it has shown effectiveness in multiple myeloma cell lines that are resistant to bortezomib (223). It has also been demonstrated to be significantly effective in pre-clinical studies in Waldenstrom's macroglobulinemia, acute leukaemia, CLL, prostate, pancreatic and colon cancers (219, 222, 224–228). However, NPI-0052 may be less specific since its analog lactacystin binds to several proteasome subunits as well as inhibits other cellular proteases. Although NPI-0052 blocks a wider range of proteasome activities, it appears to be less toxic to normal cells (223, 229). In mice implanted with human myeloma tumor cells, NPI-0052 was well tolerated and showed prolonged survival as well as significantly reduced the rate of cancer recurrences. Further, the cancer cells were killed more effectively by the combination of NPI-0052 with bortezomib and HDAC inhibitors,

MS-275 and valproic acid (VPA) without additional toxicity to normal cells (222). The clinical phase I trials of NPI-0052 are ongoing in advanced solid tumors, refractory lymphoma and non-small cell lung carcinoma (9).

CEP-18770

It is a boronic acid-based proteasome inhibitor. Like bortezomib, it is a reversible proteasome inhibitor, and primarily inhibits the chymotrypsin-like activity of the proteasome (118, 230). It is a water soluble and orally bioactive proteasome inhibitor (118, 230). CEP-18770 abrogates the production of VEGF in multiple myeloma cells (118). Such a decreased level of VEGF production inhibits cell migration and vasculogenesis from the endothelial progenitors (118). Further, the role of CEP-18770 in angiogenesis is corroborated by its direct inhibitory effect on endothelial cell proliferation, survival, and capillary tubular morphogenesis (118). CEP-18770 has also been shown to promote apoptosis in human multiple myeloma cell lines (118, 230). It is a potent inhibitor of constitutive and TNF- α -triggered NF- κ B activation (118). CEP-18770 has been demonstrated to have a significantly reduced toxicity toward human bone marrow progenitors, bone marrow stromal cells, and normal human intestinal cells as compared to bortezomib (118). Although CEP-18770 has a significant anti-tumor activity, it is more effective in combination with bortezomib and melphalan in animal tumor models (231). The clinical phase I trials of CEP-18770 have been completed for solid tumors and non-Hodgkin's lymphoma (9). Currently, it is under phase I/II clinical trials for multiple myeloma (9).

MLN9708

It is a small molecule boron-containing peptide inhibitor (Millennium Pharmaceuticals, Inc.). In contrast to bortezomib, MLN9708 is orally bioavailable (232). Like bortezomib, it inhibits the chymotrypsin-like activity of the proteasome. However, the proteasome dissociation half-life of MLN9708 is shorter than bortezomib. Thus, it has improved tissue distribution, pharmacokinetics, pharmacodynamics, and anti-tumor activity in xenograft models (233). It is biologically inactive. However, it is hydrolyzed quickly in plasma to MLN2238 that is biologically active. It has shown strong anti-cancer activity against numerous cancer cell lines (232, 233). It has also been demonstrated to be effective in human prostate xenograft, colon cancer and lymphoma models (233). Very recently, Chauhan et al (232) have demonstrated that MLN9708 has synergistic anti-multiple myeloma activity when combined with bortezomib, HDAC inhibitor, lenalidomide or dexamethasone. This proteasome inhibitor is currently in phase I clinical trials in patients with lymphoma and non-haematological malignancies (9). Further, clinical phase I/II trials of MLN9708 for multiple myeloma are ongoing (9).

Immunoproteasome inhibitors

The immunoproteasome is present in immune cells at a lower level. Thus, the inhibition of the immunoproteasome will provide specificity over constitutive proteasome. Such specificity will attenuate the toxicities associated with constitutive proteasome inhibition. Several immunoproteasome inhibitors such as PR-957, PR-924 and IPSI-001 have been developed. Pre-clinical studies of these inhibitors have shown impressive anti-tumor and anti-inflammatory responses. PR-957 (also known as ONX0914) has recently been developed by Proteolix, Inc (234, 235). Like carfilzomib, it is a peptide epoxyketone proteasome inhibitor. It inhibits chymotrypsin-like activity of the immunoproteasome. PR-957 inhibits the functions of IL-1 (interleukin-1), IL-6 and TNF. Further, it blocks the production of IL-23 by activated monocytes and interferon- γ and IL-2 by T cells. Therefore, PR-957 has immunosuppressive effects (234, 235). Hence, PR-957 may be effective against autoimmune diseases in conjunction with cancer treatment. PR-957 induces an anti-

inflammatory response at a low dose as compared to the non-selective inhibitors such as bortezomib and carfilzomib (234, 236, 237). Like PR-957, PR-924 is a peptide epoxyketone proteasome inhibitor, and inhibits chymotrypsin-like activity of the immunoproteasome (238). It impairs the growth of multiple myeloma cell lines and primary tumor cells. It has also been shown to inhibit the tumor growth in animal models without significant toxicities. Unlike PR-957 and PR-924, IPSI-001 is a peptide aldehyde type of inhibitor (239). It inhibits preferentially the β 1i component of the immunoproteasome. It has been shown to inhibit the haematological malignancies in *in vitro* models. It also potently inhibits proliferation in myeloma patient samples (239). Further, IPSI-001 overcomes conventional and novel drug resistance (239). Together, these immunoproteasome inhibitors have great potential to be in the clinic in future with more selectivity and less toxicity.

Concluding remarks

Here, we have discussed the 26S proteasome complex in different key cellular events and carcinogenesis. It is clear from a large number of studies that the 26S proteasome complex regulates a multitude of cellular processes like cell cycle progression, inflammation, antigen presentation, apoptosis, DNA repair, transcription, and indirectly: cell growth, chemotaxis, angiogenesis, and cell adhesion. Many of these mechanisms are altered to the benefit of cancer cells. For this reason, the 26S proteasome complex has become an attractive target for cancer therapy. In fact, the proteasome inhibition has led to an increased apoptosis and other anti-tumor effects such as cell cycle arrest, and inhibition of angiogenesis and metastasis in various cancer cell lines and xenograft models. The proteasome inhibitor bortezomib is in the clinic to treat multiple myeloma and mantle cell lymphoma patients. Several proteasome inhibitors are now in clinical trials to treat multiple myeloma and solid tumors. Additional proteasome inhibitors with different efficacies are being developed and tested for anti-tumor activities. Several proteasome inhibitors have shown significantly improved anti-tumor activities when combined with other drugs such as HDAC inhibitor, Akt inhibitor, DNA damaging agent, Hsp90 inhibitor, and lenalidomide. In summary, proteasome inhibitors alone or in combination with other therapies have shown very promising results to treat cancer patients in the clinic more effectively.

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References

1. Bhaumik SR, Malik S. Diverse regulatory mechanisms of eukaryotic transcriptional activation by the proteasome complex. *Crit Rev Biochem Mol Biol*. 2008; 43:419–33. [PubMed: 19058045]
2. Bhaumik SR. Distinct regulatory mechanisms of eukaryotic transcriptional activation by SAGA and TFIID. *Biochim Biophys Acta*. 2011; 1809:97–108. [PubMed: 20800707]
3. Groll M, Ditzel L, Lowe J, Stock D, Bochtler M, Wolf DH, Huber R. Structure of the 20S proteasome from yeast at 2.4 Å resolution. *Nature*. 1997; 386:463–471. [PubMed: 9087403]
4. Heinemeyer W, Fischer M, Krimmer T, Stachon U, Wolf DH. The active sites of the eukaryotic 20S proteasome and their involvement in subunit precursor processing. *J Biol Chem*. 1997; 272:25200–25209. [PubMed: 9312134]
5. Groll M, Heinemeyer W, Jager S, Ulrich T, Bochtler M, Wolf DH, Huber R. The catalytic sites of 20S proteasomes and their role in subunit maturation: A mutational and crystallographic study. *Proc Nat Acad Sci*. 1999; 96:10975–10983.

6. Groll M, Bajorek M, Kohler A, Moroder L, Rubin DM, Huber R, Glickman MH, Finley D. A gated channel into the proteasome core particle. *Nat Struct Biol.* 2000; 7:1062–1067. [PubMed: 11062564]
7. Groll M, Kim KB, Kairies N, Huber R, Crews CM. Crystal structure of epoxomicin: 20S proteasome reveals a basis for selectivity of alpha, beta—epoxyketone proteasome inhibitors. *J Am Chem Soc.* 2000; 122:1237–1238.
8. Navon A, Goldberg AL. Proteins are unfolded on the surface of the ATPase ring before transport into the proteasome. *Mol Cell.* 2001; 8:1339–1349. [PubMed: 11779508]
9. Crawford LJ, Walker B, Irvine AE. Proteasome inhibitors in cancer therapy. *J Cell Commun Signal.* 2011; 5:101–110. [PubMed: 21484190]
10. Rock KL, Goldberg AL. Degradation of cell proteins and the generation of MHC class I-presented peptides. *Annu Rev Immunol.* 1999; 17:739–799. [PubMed: 10358773]
11. Sen R, Baltimore D. Inducibility of kappa immunoglobulin enhancer-binding protein NFkB by a post-translational mechanism. *Cell.* 1986; 47:921–928. [PubMed: 3096580]
12. Pajonk F, McBride WH. The proteasome in cancer biology and treatment. *Radiat Res.* 2001; 156:447–459. [PubMed: 11604057]
13. Pahl HL. Activators and target genes of Rel/NF-kappaB transcription factors. *Oncogene.* 1999; 18:6853–6866. [PubMed: 10602461]
14. Montagut C, Rovira A, Albanell J. The proteasome: a novel target for anticancer therapy. *Clin Transl Oncol.* 2006; 8:313–7. [PubMed: 16760005]
15. Luqman BS, Pezzuto JM. NFkappaB: a promising target for natural products in cancer chemoprevention. *Phytother Res.* 2010; 24:949–63. [PubMed: 20577970]
16. Hideshima T, Richardson P, Chauhan D, et al. The proteasome inhibitor PS 541 inhibits growth, induces apoptosis, and overcomes drug resistance in human multiple myeloma cells. *Cancer Res.* 2001; 61:5071–6.
17. Sunwoo JB, Chen Z, Dong G, et al. Novel proteasome inhibitor PS-341 inhibits activation of nuclear-factor kappa B, cell survival, tumor growth, and angiogenesis in squamous cell carcinoma. *Clin Cancer Res.* 2001; 7:1419–28. [PubMed: 11350913]
18. Cusack JC Jr, Liu R, Houston M, et al. Enhanced chemosensitivity to CPT-11 with proteasome inhibitor PS-341: implications for systemic nuclear-factor-kappaB inhibition. *Cancer Res.* 2001; 61:3535–40. [PubMed: 11325813]
19. Orlowski RZ, Stinchcombe TE, Mitchell BS, et al. Phase I trial of the proteasome inhibitor PS 341 in patients with refractory hematologic malignancies. *J Clin Oncol.* 2002; 20:4420–7. [PubMed: 12431963]
20. Cortes J, Thomas D, Koller C, et al. Phase I study of bortezomib in refractory or relapsed acute leukemias. *Clin Cancer Res.* 2004; 10:3371–6. [PubMed: 15161691]
21. Ditsworth D, Zong WX. NF-kappaB: Key mediator of inflammation-associated cancer. *Cancer Biol Ther.* 2004; 3:1214–1216. [PubMed: 15611628]
22. McCarty MF. Targeting multiple signaling pathways as a strategy for managing prostate cancer: multifocal signal modulation therapy. *Integr Cancer Ther.* 2004; 3:349–380. [PubMed: 15523106]
23. Sarkar FH, Li Y. Cell signaling pathways altered by natural chemopreventive agents. *Mutat Res.* 2004; 555:53–64. [PubMed: 15476851]
24. Reddy GP, Barrack ER, Dou QP, Menon M, Pelley R, Sarkar FH, Sheng S. Regulatory processes affecting androgen receptor expression, stability, and function: potential targets to treat hormone-refractory prostate cancer. *J Cell Biochem.* 2006; 98:1408–23. [PubMed: 16619263]
25. Aggarwal BB. Nuclear factor-kappaB: The enemy within. *Cancer Cell.* 2004; 6:203–208. [PubMed: 15380510]
26. Monks NR, Biswas DK, Pardee AB. Blocking antiapoptosis as a strategy for cancer chemotherapy: NFkappaB as a target. *J Cell Biochem.* 2004; 92:646–650. [PubMed: 15211562]
27. Gilmore TD, Koedood M, Piffat KA, White DW. Rel/NFkB/IkB proteins and cancer. *Oncogene.* 1996; 13:1367–1378. [PubMed: 8875974]
28. Gilmore TD. NFkB: from basic research to human disease. *Oncogene.* 2006; 25:6679–6899.

29. Perkins ND. Integrating cell-signaling pathways with NF κ B and IKK function. *Nature Rev Mol Cell Biol.* 2007; 8:40–62.
30. McConkey DJ, Zhu K. Mechanisms of proteasome inhibitor action and resistance in cancer. *Drug Resist Update.* 2008; 11:164–179.
31. Momand J, Jung D, Wilczynski S, Niland J. The MDM2 gene amplification database. *Nucleic Acids Res.* 1998; 26:3452–3459.
32. Scheffner M, Wemess BA, Huibregtse JM, Levine AJ, Howley PM. The E6 oncoprotein encoded by human papillomavirus types 16 and 18 promotes the degradation of p53. *Cell.* 1990; 63:1129–1136. [PubMed: 2175676]
33. Scheffner M, Huibregtse JM, Vierstra RD, Howley PM. The HPV- 16 E6 and E6-AP complex functions as a ubiquitin-protein ligase in the ubiquitination of p53. *Cell.* 1993; 75:495–505. [PubMed: 8221889]
34. Vaziri SA, Grabowski DR, Hill J, Rybicki LR, Burk R, Bukowski RM, Ganapathi MK, Gamapathi R. Inhibition of proteasome activity by bortezomib in renal cancer cells is p53 and VHL independent. *Anticancer Res.* 2009; 29:2961–2969. [PubMed: 19661301]
35. Ding WX, Ni HM, Chen X, Yu J, Zhang L, Yin XM. A coordinated action of Bax, PUMA and p53 promotes MG-132—induced mitochondria activation and apoptosis in colon cancer cells. *Mol Cancer Ther.* 2007; 6:1062–1069. [PubMed: 17363499]
36. Qin JZ, Ziffra J, Stennett L, Bodner B, Bonish BK, Chaturvedi V, Bennett F, Pollock PM, Trent JM, Hendrix MJ, Rizzo P, Miele L, Nickoloff BJ. Proteasome inhibitors trigger NOXA-mediated apoptosis in melanoma and myeloma cells. *Cancer Res.* 2005; 65:6282–6293. [PubMed: 16024630]
37. Williams SA, McConkey DJ. The proteasome inhibitor bortezomib stabilizes a novel active form of p53 in human LNCaP-Pro5 cancer cells. *Cancer Res.* 2003; 63:7338–7344. [PubMed: 14612532]
38. Sherr CJ, Roberts JM. CDK inhibitors: positive and negative regulators of G1-phase progression. *Genes Dev.* 1999; 13:1501–1512. [PubMed: 10385618]
39. Chu IM, Hengst L, Joyce M. The CDK inhibitor p27 in human cancer: prognostic potential and relevance to anticancer therapy. *Nat Rev Cancer.* 2008; 8:253–267. [PubMed: 18354415]
40. Inui N, Kitagawa K, Miwa S, Hattori T, Chida K, Nakamura H, Kitagawa M. High expression of Cks1 in human nonsmall cell lung carcinoma. *Biochem Biophys Res Commun.* 2003; 303:978–984. [PubMed: 12670508]
41. Lu Z, Hunter T. Ubiquitylation and proteasomal degradation of the p21(Cip1), p27(Kip1) and p57(Kip2) CDK inhibitors. *Cell Cycle.* 2010; 9:2342–52. [PubMed: 20519948]
42. Xu H, Zhang Z, Li M, Zhang R. MDM2 promotes proteasomal degradation of p21Waf1 via a conformation change. *J Biol Chem.* 2010; 285:18407–14. [PubMed: 20308078]
43. Wang B, Liu K, Lin HY, Bellam N, Ling S, Lin WC. 14–3–3Tau regulates ubiquitin-independent proteasomal degradation of p21, a novel mechanism of p21 downregulation in breast cancer. *Mol Cell Biol.* 2010; 30:1508–27. [PubMed: 20086099]
44. Fung TK, Poon RY. A roller coaster ride with the mitotic cyclins. *Semin Cell Dev Biol.* 2005; 16:335–42. [PubMed: 15840442]
45. Mitra J, Enders GH, Azizkhan-Clifford J, Lengel KL. Dual regulation of the anaphase promoting complex in human cells by cyclin A-Cdk2 and cyclin A-Cdk1 complexes. *Cell Cycle.* 2006; 5:661–6. [PubMed: 16582612]
46. van Leuken R, Clijsters L, Wolthuis R. To cell cycle, swing the APC/C. *Biochim Biophys Acta.* 2008; 1786:49–59. [PubMed: 18544349]
47. Glotzer M, Murray AW, Kirschner MW. Cyclin is degraded by the ubiquitin pathway. *Nature.* 1991; 349:132–138. [PubMed: 1846030]
48. Amon A, Irniger S, Nasmyth K. Closing the cell cycle circle in yeast: G2 cyclin proteolysis initiated at mitosis persists until the activation of G1 cyclins in the next cycle. *Cell.* 1994; 77:1037–1050. [PubMed: 8020094]
49. Brandeis M. The proteolysis of mitotic cyclins in mammalian cells persists from the end of mitosis until the onset of S phase. *EMBO J.* 1996; 15:5280–5289. [PubMed: 8895573]

50. Keyomarsi K, Pardee AB. Redundant cyclin overexpression and gene amplification in breast cancer cells. *Proc Natl Acad Sci USA*. 1993; 90:1112–1116. [PubMed: 8430082]
51. Gialmanidis IP, Bravou V, Amanetopoulou SG, Varakis J, Kourea H, Papadaki H. Overexpression of hedgehog pathway molecules and FOXM1 in non-small cell lung carcinomas. *Lung Cancer*. 2009; 66:64–74. [PubMed: 19200615]
52. Bektas N, Haaf A, Veeck J, Wild PJ, Luscher-Firzlaff J, Hartmann A, Knuchel R, Dahl E. Tight correlation between expression of the Forkhead transcription factor FoxM1 and Her2 in human breast cancer. *BMC Cancer*. 2008; 8:42. [PubMed: 18254960]
53. Yoshida Y, Wang IC, Yoder HM, Davidson NO, Costa RH. The forkhead box M1 transcription factor contributes to the development and growth of mouse colorectal cancer. *Gastroenterology*. 2007; 132:1420–1431. [PubMed: 17408638]
54. Liu M, Dai B, Kang SH, Ban K, Huang FJ, Lang FF, Aldape KD, Xie TX, Pelloski CE, Xie K, Sawaya R, Huang S. FoxM1B is overexpressed in human glioblastomas and critically regulates the tumorigenicity of glioma cells. *Cancer Res*. 2006; 66:3593–3602. [PubMed: 16585184]
55. Wang Z, Banerjee S, Kong D, Li Y, Sarker FH. Downregulation of Forkhead Box M1 transcription factor leads to the inhibition of invasion and angiogenesis of pancreatic cancer cells. *Cancer Res*. 2007; 67:8293–8300. [PubMed: 17804744]
56. Gemenetzi E, Bose A, Riaz AM, Chaplin T, Young BD, Ali M, Sugden D, Thurlow JK, Cheong SC, Teo SH, Wan H, Waseem A, Parkinson EK, Fortune F, The MT. FoxM1 upregulation is an early event in human squamous cell carcinoma and it is enhanced by nicotine during malignant transformation. *PLoS One*. 2009; 4:e4849. [PubMed: 19287496]
57. Bhat UG, Halasi M, Gartel AL. FoxM1 is a general target for proteasome inhibitors. *PLoS One*. 2009; 4:e6593. [PubMed: 19672316]
58. Gartel AL. A new target for proteasome inhibitors: FoxM1. *Expert Opin Investig Drugs*. 2010; 19:235–242.
59. Obeng EA, Carlson LM, Gutman DM, Harrington WJ, Lee KP, Boise LH. Proteasome inhibitors induce a terminal unfolded protein response in multiple myeloma cells. *Blood*. 2006; 107:4907–4916. [PubMed: 16507771]
60. Nawrocki ST, Carwe JS, Pino MS, Highshaw RA, Dunner K, Huang P, Abbruzzese JL, McConkey DJ. Bortezomib sensitizes pancreatic cells to endoplasmic reticulum stress-mediated apoptosis. *Cancer Res*. 2005; 65:11658–11666. [PubMed: 16357177]
61. Fribley A, Wang CY. Proteasome inhibitor induces apoptosis through induction of endoplasmic reticulum stress. *Cancer Biol Ther*. 2006; 5:745–748. [PubMed: 16861900]
62. Morgillo F, D'Aiuto E, Troiani T, Artinelli ME, Cascone T, De Palma R, Orditura M, De Vita F, Ciardiello F. Antitumor activity of bortezomib in human cancer cells with acquired resistance to anti-epidermal growth factor receptor tyrosine kinase inhibitors. *Lung Cancer*. 2011; 71:283–90. [PubMed: 20619923]
63. Jaworski T. Degradation and beyond: control of androgen receptor activity by the proteasome system. *Cell Mol Biol Lett*. 2006; 11:109–31. [PubMed: 16847754]
64. Kinyamu HK, Collins JB, Grissom SF, Hebbar PB, Archer TK. Genome wide transcriptional profiling in breast cancer cells reveals distinct changes in hormone receptor target genes and chromatin modifying enzymes after proteasome inhibition. *Mol Carcinog*. 2008; 47:845–85. [PubMed: 18381591]
65. Powers GL, Ellison-Zelski SJ, Casa AJ, Lee AV, Alarid ET. Proteasome inhibition represses ERalpha gene expression in ER+ cells: a new link between proteasome activity and estrogen signaling in breast cancer. *Oncogene*. 2010; 29:1509–18. [PubMed: 19946334]
66. Rossi D, Zlotnik A. The biology of chemokines and their receptors. *Annu Rev Immunol*. 2000; 18:217–42. [PubMed: 10837058]
67. Belperio JA, Keane MP, Arenberg DA, Addison CL, Ehlert JE, Burdick MD, Strieter RM. CXC chemokines in angiogenesis. *J Leukoc Biol*. 2000; 68:1–8. [PubMed: 10914483]
68. Muller A, Homey B, Soto H, Ge N, Catron D, Buchanan ME, McClanahan T, Murphy E, Yuan W, Wagner SN, Barrera JL, Mohar A, Verastegui E, Zlotnik A. Involvement of chemokine receptors in breast cancer metastasis. *Nature*. 2001; 410:50–6. [PubMed: 11242036]

69. Peterson FC, Thorpe JA, Harder AG, Volkman BF, Schwarze SR. Structural determinants involved in the regulation of CXCL14/BRAK expression by the 26 S proteasome. *J Mol Biol.* 2006; 363:813–22. [PubMed: 16987528]
70. Schwarze SR, Luo J, Isaacs WB, Jarrard DF. Modulation of CXCL14 (BRAK) expression in prostate cancer. *Prostate.* 2005; 64:67–74. [PubMed: 15651028]
71. Shurin GV, Ferris RL, Tourkova IL, Perez L, Lokshin A, Balkir L, Collins B, Chatta GS, Shurin MR. Loss of new chemokine CXCL14 in tumor tissue is associated with low infiltration by dendritic cells (DC), while restoration of human CXCL14 expression in tumor cells causes attraction of DC both in vitro and in vivo. *J Immunol.* 2005; 174:5490–8. [PubMed: 15843547]
72. Shellenberger TD, Wang M, Gujrati M, Jayakumar A, Strieter RM, Burdick MD, Ioannides CG, Efferson CL, El-Naggar AK, Roberts D, Clayman GL, Frederick MJ. BRAK/CXCL14 is a potent inhibitor of angiogenesis and a chemotactic factor for immature dendritic cells. *Cancer Res.* 2004; 64:8262–70. [PubMed: 15548693]
73. Fushimi T, O'Connor TP, Crystal RG. Adenoviral gene transfer of stromal cell-derived factor-1 to murine tumors induces the accumulation of dendritic cells and suppresses tumor growth. *Cancer Res.* 2006; 66:3513–22. [PubMed: 16585175]
74. Sepp Lorenzino L, Ma Z, Lebowitz DE, Vinitsky A, Rosen N. Herbimycin A induces the 20S proteasome- and ubiquitin-dependent degradation of receptor tyrosine kinases. *J Biol Chem.* 1995; 270:16580–16587. [PubMed: 7622464]
75. Philip PA, Harris AL. Potential for protein kinase C inhibitors in cancer therapy. *Cancer Treat Res.* 1995; 78:3–27. [PubMed: 8595146]
76. Lee HW, Smith L, Pettit GR, Vinitsky A, Smith JB. Ubiquitination of protein kinase C- α and degradation by the proteasome. *J Biol Chem.* 1996; 271:20973–20976. [PubMed: 8702857]
77. Hou D, Cenciarelli C, Jensen JP, Nguyen HB, Weissman AM. Activation-dependent ubiquitination of a T cell antigen receptor subunit on multiple intracellular lysines. *J Biol Chem.* 1994; 269:14244–14247. [PubMed: 8188707]
78. Mori S, Kanaki H, Tanaka K, Morisaki N, Saito Y. Ligand-activated platelet-derived growth factor beta-receptor is degraded through proteasome-dependent proteolytic pathway. *Biochem Biophys Res Commun.* 1995; 217:224–229. [PubMed: 8526915]
79. Treier M, Staszewski LM, Bohmann D. Ubiquitin-dependent c-Jun degradation in vivo is mediated by the delta domain. *Cell.* 1994; 78:787–798. [PubMed: 8087846]
80. Tsurumi C, Ishida N, Tamura T, Kakizuka A, Nishida E, Okumura E, Kishimoto T, Inagaki M, Okazaki K, Sagata N, Ishihara A, Tanaka K. Degradation of c-Fos by the 26S proteasome is accelerated by c-Jun and multiple protein kinases. *Mol Cell Biol.* 1995; 15:5682–5687. [PubMed: 7565719]
81. Jariel-Encontre I, Pariat M, Martin F, Carillo S, Salvat C, Piechaczyk M. Ubiquitinylation is not an absolute requirement for degradation of c-Jun protein by the 26S proteasome. *J Biol Chem.* 1995; 270:11623–11627. [PubMed: 7744802]
82. Hermida-Matsumoto ML, Chock PB, Curran T, Yang DC. Ubiquitinylation of transcription factors c-Jun and c-Fos using reconstituted ubiquitinating enzymes. *J Biol Chem.* 1996; 271:4930–4936. [PubMed: 8617766]
83. Musti AM, Treier M, Bohmann D. Reduced ubiquitin-dependent degradation of c-Jun after phosphorylation by MAP kinases. *Science.* 1997; 275:400–402. [PubMed: 8994040]
84. Spataro V, Norbury C, Harris AL. The ubiquitin-proteasome pathway in cancer. *Br J Cancer.* 1998; 77:448–55. [PubMed: 9472642]
85. Schreiber M, Baumann B, Cotton M, Angel P, Wagner EF. Fos is an essential component of the mammalian UV response. *EMBO J.* 1995; 14:5338–5349. [PubMed: 7489723]
86. Pinkus R, Weiner LM, Daniel V. Role of oxidants and antioxidants in the induction of AP-1, NF- κ B, and glutathione S-transferase gene expression. *J Biol Chem.* 1996; 271:13422–13429. [PubMed: 8662787]
87. Moffat GJ, McLaren AW, Wolf CR. Involvement of Jun and Fos proteins in regulating transcriptional activation of the human pi class glutathione S-transferase gene in multidrug-resistant MCF7 breast cancer cells. *J Biol Chem.* 1994; 269:16397–16402. [PubMed: 8206948]

88. Ritke MK, Bergoltz VV, Allan WP, Yalowich JC. Increased c-jun/AP-1 levels in etoposide-resistant human leukemia K562 cells. *Biochem Pharmacol.* 1994; 48:525–533. [PubMed: 8068039]
89. Yao KS, Godwin AK, Johnson SW, Ozols RF, O'Dwyer PJ, Hamilton TC. Evidence for altered regulation of gamma-glutamylcysteine synthetase gene expression among cisplatin-sensitive and cisplatin-resistant human ovarian cancer cell lines. *Cancer Res.* 1995; 55:4367–4374. [PubMed: 7671249]
90. Motegi A, Murakawa Y, Takeda S. The vital link between the ubiquitin-proteasome pathway and DNA repair: impact on cancer therapy. *Cancer Lett.* 2009; 283:1–9. [PubMed: 19201084]
91. Belich MP, Glynn RJ, Senger G, Sheer D, Trowsdale J. Proteasome components with reciprocal expression to that of the MHC-encoded LMP proteins. *Curr Biol.* 1994; 4:769–776. [PubMed: 7820546]
92. Fruh K, Gossen M, Wang K, Bujard H, Peterson PA, Yang Y. Displacement of housekeeping proteasome subunits by MHC-encoded LMPs: a newly discovered mechanism for modulating the multicatalytic proteinase complex. *EMBO J.* 1994; 13:3236–3244. [PubMed: 8045254]
93. Groettrup M, Kraft R, Kostka S, Standera S, Stohwasser R, Kloetzel PM. A third interferon-gamma-induced subunit exchange in the 20S proteasome. *Eur J Immunol.* 1996; 26:863–869. [PubMed: 8625980]
94. Hisamatsu H, Shimbara N, Saito Y, Kristensen P, Hendil KB, Fujiwara T, Takahashi E, Tanahashi N, Tamura T, Ichihara A, Tanaka K. Newly identified pair of proteasomal subunits regulated reciprocally by interferon gamma. *J Exp Med.* 1996; 183:1807–1816. [PubMed: 8666937]
95. Nandi D, Jiang H, Monaco JJ. Identification of MECL-1 (LMP-10) as the third IFN-gamma-inducible proteasome subunit. *J Immunol.* 1996; 156:2361–2364. [PubMed: 8786291]
96. Gaczynska M, Goldberg AL, Tanaka K, Hendil KB, Rock KL. Proteasome subunits X and Y alter peptidase activities in opposite ways to the interferon-gamma-induced subunits LMP2 and LMP7. *J Biol Chem.* 1996; 271:17275–17280. [PubMed: 8663318]
97. Kuckelkom U, Frentzel S, Kraft R, Kostka S, Groettrup M, Kloetzel PM. Incorporation of major histocompatibility complex-encoded subunits LMP2 and LMP7 changes the quality of the 20S proteasome polypeptide processing products independent of interferon-gamma. *Eur J Immunol.* 1995; 25:2605–2611. [PubMed: 7589133]
98. Restifo NP, Esquivel F, Kawakami Y, Yewdell JW, Mule JJ, Rosenberg SA, Bennink JR. Identification of human cancers deficient in antigen processing. *J Exp Med.* 1993; 177:265–272. [PubMed: 8426105]
99. Sibille C, Gould KG, Willard Gallo K, Thomson S, Rivett AJ, Powis S, Butcher GW, De Baetselier P. LMP2+ proteasomes are required for the presentation of specific antigens to cytotoxic T lymphocytes. *Curr Biol.* 1995; 5:923–930. [PubMed: 7583150]
100. Rotem Yehudar R, Groettrup M, Soza A, Kloetzel PM, Ehrlich R. LMP-associated proteolytic activities and TAP-dependent peptide transport for class I MHC molecules are suppressed in cell lines transformed by the highly oncogenic adenovirus 12. *J Exp Med.* 1996; 183:499–514. [PubMed: 8627162]
101. Seliger B, Hohne A, Knuth A, Bernhard H, Meyer T, Tampe R, Momburg F, Huber C. Analysis of the major histocompatibility complex class I antigen presentation machinery in normal and malignant renal cells: evidence for deficiencies associated with transformation and progression. *Cancer Res.* 1996; 56:1756–1760. [PubMed: 8620489]
102. Matsui M, Machida S, Itani-Yohda T, Akatsuka T. Downregulation of the proteasome subunits, transporter, and antigen presentation in hepatocellular carcinoma, and their restoration by interferon-gamma. *J Gastroenterol Hepatol.* 2002; 17:897–907. [PubMed: 12164966]
103. Pan J, Zhang Q, Wang Y, You M. 26S proteasome activity is down-regulated in lung cancer stem-like cells propagated in vitro. *PLoS One.* 2010; 5:e13298. [PubMed: 20949018]
104. Leffers N, Gooden MJ, Mokhova AA, Kast WM, Boezen HM, Ten Hoor KA, Hollema H, Daemen T, van der Zee AG, Nijman HW. Down-regulation of proteasomal subunit MB1 is an independent predictor of improved survival in ovarian cancer. *Gynecol Oncol.* 2009; 113:256–63. [PubMed: 19243813]

105. Karin M, Ben-Neriah Y. Phosphorylation meets ubiquitination: the control of NF-[kappa]B activity. *Annu Rev Immunol.* 2000; 18:621–663. [PubMed: 10837071]
106. Karin M, Cao Y, Greten FR, Li ZW. NF-kappaB in cancer: from innocent bystander to major culprit. *Nat Rev Cancer.* 2002; 2:301–310. [PubMed: 12001991]
107. Tergaonkar V, Pando M, Vafa O, Wahl G, Verma I. p53 stabilization is decreased upon NFkappaB activation: a role for NFkappaB in acquisition of resistance to chemotherapy. *Cancer Cell.* 2002; 1:493–503. [PubMed: 12124178]
108. Mitsiades N, Mitsiades CS, Poulaki V, Chauhan D, Fanourakis G, Gu X, et al. Molecular sequelae of proteasome inhibition in human multiple myeloma cells. *Proc Natl Acad Sci U S A.* 2002; 99:14374–9. [PubMed: 12391322]
109. LeBlanc R, Catley LP, Hideshima T, Lentzsch S, Mitsiades CS, Mitsiades N, et al. Proteasome inhibitor PS-341 inhibits human myeloma cell growth in vivo and prolongs survival in a murine model. *Cancer Res.* 2002; 62:4996–5000. [PubMed: 12208752]
110. Hideshima T, Mitsiades C, Akiyama M, Hayashi T, Chauhan D, Richardson P, et al. Molecular mechanisms mediating antimyeloma activity of proteasome inhibitor PS-341. *Blood.* 2003; 101:1530–4. [PubMed: 12393500]
111. Nakanishi C, Toi M. Nuclear factor-kappaB inhibitors as sensitizers to anticancer drugs. *Nat Rev Cancer.* 2005; 5:297–309. [PubMed: 15803156]
112. Piva R, Belardo G, Santoro MG. NF-kappaB: a stress-regulated switch for cell survival. *Antioxid Redox Signal.* 2006; 8:478–486. [PubMed: 16677091]
113. Chauhan D, Hideshima T, Anderson KC. Targeting proteasomes as therapy in multiple myeloma. *Adv Exp Med Biol.* 2008; 615:251–60. [PubMed: 18437898]
114. Mukherjee S, Raje N, Schoonmaker JA, Liu JC, Hideshima T, Wein MN, et al. Pharmacologic targeting of a stem/progenitor population in vivo is associated with enhanced bone regeneration in mice. *J Clin Invest.* 2008; 118:491–504. [PubMed: 18219387]
115. Anderson KC. Oncogenomics to target myeloma in the bone marrow microenvironment. *Clin Cancer Res.* 2011; 17:1225–33. [PubMed: 21411438]
116. Pagano M, Tam SW, Theodoras AM, et al. Role of the ubiquitin-proteasome pathway in regulating abundance of the cyclin-dependent kinase inhibitor p27. *Science.* 1995; 269:682–685. [PubMed: 7624798]
117. Bloom J, Amador V, Bartolini F, DeMartino G, Pagano M. Proteasome-mediated degradation of p21 via N-terminal ubiquitinylation. *Cell.* 2003; 115:71–82. [PubMed: 14532004]
118. Piva R, Ruggeri B, Williams M, Costa G, Tamagno I, Ferrero D, Giai V, Coscia M, Peola S, Massaia M, Pezzoni G, Allievi C, Pescalli N, Cassin M, di Giovine S, Nicoli P, de Feudis P, Strepponi I, Roato I, Ferracini R, Bussolati B, Camussi G, Jones-Bolin S, Hunter K, Zhao H, Neri A, Palumbo A, Berkers C, Ovaa H, Bernareggi A, Inghirami G. CEP-18770: A novel, orally active proteasome inhibitor with a tumor-selective pharmacological profile competitive with bortezomib. *Blood.* 2008; 111:2765–2775. [PubMed: 18057228]
119. Nawrocki ST, Bruns CJ, Harbison MT, et al. Effects of the proteasome inhibitor PS-341 on apoptosis and angiogenesis in orthotopic human pancreatic tumor xenografts. *Mol Cancer Ther.* 2002; 1:1243–53. [PubMed: 12516957]
120. Adams J. The proteasome as a novel target for the treatment of breast cancer. *Breast Dis.* 2002; 15:61–70. [PubMed: 15687646]
121. Williams S, Pettaway C, Song R, Papandreou C, Logothetis C, McConkey DJ. Differential effects of the proteasome inhibitor bortezomib on apoptosis and angiogenesis in human prostate tumor xenografts. *Mol Cancer Ther.* 2003; 2:835–843. [PubMed: 14555702]
122. Fribley A, Zeng Q, Wang CY. Proteasome inhibitor PS-341 induces apoptosis through induction of endoplasmic reticulum stress-reactive oxygen species in head and neck squamous cell carcinoma cells. *Mol Cell Biol.* 2004; 24:9695–9704. [PubMed: 15509775]
123. Yu C, Rahmani M, Dent P, Grant S. The hierarchical relationship between MAPK signaling and ROS generation in human leukemia cells undergoing apoptosis in response to the proteasome inhibitor Bortezomib. *Exp Cell Res.* 2004; 295:555–566. [PubMed: 15093752]

124. Chen D, Frezza M, Schmitt S, Kanwar J, Dou PQ. Bortezomib as the first proteasome inhibitor anticancer drug: current status and future perspectives. *Curr Cancer Drug Targets*. 2011; 11:239–53. [PubMed: 21247388]
125. Scagliotti G. Proteasome inhibitors in lung cancer. *Crit Rev Oncol Hematol*. 2006; 58:177–189. [PubMed: 16427303]
126. Perez-Galan P, Roue G, Villamor N, Montserrat E, Campo E, Colomer D. The proteasome inhibitor bortezomib induces apoptosis in mantle-cell lymphoma through generation of ROS and Noxa activation independent of p53 status. *Blood*. 2006; 107:257–264. [PubMed: 16166592]
127. Nalepa G, Rolfe M, Harper JW. Drug discovery in the ubiquitin-proteasome system. *Nat Rev Drug Discov*. 2006; 5:596–613. [PubMed: 16816840]
128. Ludwig H, Khayat D, Giaccone G, Facon T. Proteasome inhibition and its clinical prospects in the treatment of hematologic and solid malignancies. *Cancer*. 2005; 104:1794–1807. [PubMed: 16178003]
129. Kuhn DJ, Chen Q, Voorhees PM, Strader JS, Shenk KD, Sun CM, Demo SD, Bennett MK, van Leeuwen FW, Chanan-Khan AA, Orlowski RZ. Potent activity of carfilzomib, a novel, irreversible inhibitor of the ubiquitin proteasome pathway against preclinical models of multiple myeloma. *Blood*. 2007; 110:3281–3280. [PubMed: 17591945]
130. de Bettignies G, Coux O. Proteasome inhibitors: Dozens of molecules and still counting. *Biochimie*. 2010; 92:1530–45. [PubMed: 20615448]
131. Kessler BM, Tortorella D, Altun M, et al. Extended peptide-based inhibitors efficiently target the proteasome and reveal overlapping specificities of the catalytic beta-subunits, *Chem. Biol*. 2001; 8:913–929.
132. Aggarwal BB, Shishodia S. Molecular targets of dietary agents for prevention and therapy of cancer. *Biochem Pharmacol*. 2006; 71:1397–1421. [PubMed: 16563357]
133. Chen D, Landis-Piowar KR, Chen MS, Dou QP. Inhibition of proteasome activity by the dietary flavonoid apigenin is associated with growth inhibition in cultured breast cancer cells and xenografts. *Breast Cancer Res*. 2007; 9:R80. [PubMed: 18300387]
134. Birt DF, Mitchell D, Gold B, Pour P, Pinch HC. Inhibition of ultraviolet light induced skin carcinogenesis in SKH-1 mice by apigenin, a plant flavonoid. *Anticancer Res*. 1997; 17:85–91. [PubMed: 9066634]
135. Gupta S, Afaq F, Mukhtar H. Involvement of nuclear factor-kappa B, Bax and Bcl-2 in induction of cell cycle arrest and apoptosis by apigenin in human prostate carcinoma cells. *Oncogene*. 2002; 21:3727–3738. [PubMed: 12032841]
136. Chen D, Daniel KG, Chen MS, Kuhn DJ, Landis-Piowar KR, Dou QP. Dietary flavonoids as proteasome inhibitors and apoptosis inducers in human leukemia cells. *Biochem Pharmacol*. 2005; 69:1421–1432. [PubMed: 15857606]
137. Zheng PW, Chiang LC, Lin CC. Apigenin induced apoptosis through p53-dependent pathway in human cervical carcinoma cells. *Life Sci*. 2005; 76:1367–1379. [PubMed: 15670616]
138. Liu LZ, Fang J, Zhou Q, Hu X, Shi X, Jiang BH. Apigenin inhibits expression of vascular endothelial growth factor and angiogenesis in human lung cancer cells: implication of chemoprevention of lung cancer. *Mol Pharmacol*. 2005; 68:635–643. [PubMed: 15947208]
139. Choi JS, Choi YJ, Park SH, Kang JS, Kang YH. Flavones mitigate tumor necrosis factor-alpha-induced adhesion molecule upregulation in cultured human endothelial cells: role of nuclear factor-kappa B. *J Nutr*. 2004; 134:1013–1019. [PubMed: 15113938]
140. Sim GS, Lee BC, Cho HS, Lee JW, Kim JH, Lee DH, Pyo HB, Moon DC, Oh KW, Yun YP, et al. Structure activity relationship of antioxidative property of flavonoids and inhibitory effect on matrix metalloproteinase activity in UVA-irradiated human dermal fibroblast. *Arch Pharm Res*. 2007; 30:290–298. [PubMed: 17424933]
141. Chen D, Chen MS, Cui QC, Yang H, Dou QP. Structure proteasome-inhibitory activity relationships of dietary flavonoids in human cancer cells. *Front Biosci*. 2007; 12:1935–1945. [PubMed: 17127432]
142. Horinaka M, Yoshida T, Shiraishi T, Nakata S, Wakada M, Sakai T. The dietary flavonoid apigenin sensitizes malignant tumor cells to tumor necrosis factor-related apoptosis-inducing ligand. *Mol Cancer Ther*. 2006; 5:945–951. [PubMed: 16648565]

143. Nam S, Smith DM, Dou QP. Ester bond-containing tea polyphenols potently inhibit proteasome activity *in vitro* and *in vivo*. *J Biol Chem*. 2001; 276:13322–13330. [PubMed: 11278274]
144. Pajonk F, Riedisser A, Henke M, McBride WH, Fiebich B. The effects of tea extracts on proinflammatory signaling. *BMC Med*. 2006; 4:28. [PubMed: 17140430]
145. Xu B, Monsarrat B, Gairin JE, Girbal-Neuhausser E. Effect of ajoene, a natural antitumor small molecule, on human 20S proteasome activity *in vitro* and in human leukemic HL60 cells. *Fundam Clin Pharmacol*. 2004; 18:171–180. [PubMed: 15066131]
146. Hassan HT. Ajoene (natural garlic compound): a new anti-leukaemia agent for AML therapy. *Leuk Res*. 2004; 28:667–671. [PubMed: 15158086]
147. Bernstein LR. Mechanisms of therapeutic activity for gallium. *Pharmacol Rev*. 1998; 50:665–82. [PubMed: 9860806]
148. Einhorn L. Gallium nitrate in the treatment of bladder cancer. *Semin Oncol*. 2003; 30:34–41. [PubMed: 12776258]
149. Jakupiec MA, Keppler BK. Gallium in cancer treatment. *Curr Top Med Chem*. 2004; 4:1575–83. [PubMed: 15579097]
150. Straus DJ. Gallium nitrate in the treatment of lymphoma. *Semin Oncol*. 2003; 30:25–33. [PubMed: 12776257]
151. Chitambar CR. Apoptotic mechanisms of gallium nitrate: basic and clinical investigations. *Oncology (Huntingt)*. 2004; 18:39–44. [PubMed: 15651176]
152. Chen D, Frezza M, Shakya R, Cui QC, Milacic V, Verani CN, Dou QP. Inhibition of the proteasome activity by gallium(III) complexes contributes to their anti prostate tumor effects. *Cancer Res*. 2007; 67:9258–65. [PubMed: 17909033]
153. Chitambar CR, Seligman PA. Effects of different transferrin forms on transferrin receptor expression, iron uptake, and cellular proliferation of human leukemic HL60 cells. Mechanisms responsible for the specific cytotoxicity of transferrin-gallium. *J Clin Invest*. 1986; 78:1538–46. [PubMed: 3465751]
154. Esserman L, Takahashi S, Rojas V, Warnke R, Levy R. An epitope of the transferrin receptor is exposed on the cell surface of high-grade but not low-grade human lymphomas. *Blood*. 1989; 74:2718–29. [PubMed: 2479430]
155. Chitambar CR. Gallium compounds as antineoplastic agents. *Curr Opin Oncol*. 2004; 16:547–52. [PubMed: 15627016]
156. Adams J, Behnke M, Chen S, et al. Potent and selective inhibitors of the proteasome: dipeptidyl boronic acids. *Bioorg Med Chem Lett*. 1998; 8:333–8. [PubMed: 9871680]
157. Bogyo M, Gaczynska M, Ploegh HL. Proteasome inhibitors and antigen presentation. *Biopolymers*. 1997; 43:269–80. 19. [PubMed: 9316392]
158. Groll M, Koguchi Y, Huber R, Kohno J. Crystal structure of the 20S proteasome:TMC-95A complex: a non-covalent proteasome inhibitor. *J Mol Biol*. 2001; 311:543–8. [PubMed: 11493007]
159. Meng L, Kwok BH, Sin N, et al. Eponemycin exerts its antitumor effect through the inhibition of proteasome function. *Cancer Res*. 1999; 59:2798–801. [PubMed: 10383134]
160. Fenteany G, Standaert RF, Lane WS, et al. Inhibition of proteasome activities and subunit-specific amino-terminal threonine modification by 3' lactacystin. *Science*. 1995; 268:726–31. [PubMed: 7732382]
161. Kozlowski L, Stoklosa T, Omura S, et al. Lactacystin inhibits cathepsin A activity in melanoma cell lines. *Tumour Biol*. 2001; 22:211–5. [PubMed: 11399945]
162. Adams J, Palombella VJ, Sausville EA, Johnson J, Destree A, Lazarus DD, Maas J, Pien CS, Prakash S, Elliott PJ. Proteasome inhibitors: a novel class of potent and effective antitumor agents. *Cancer Res*. 1999; 59:2615–2622. [PubMed: 10363983]
163. Kisselev AF, Goldberg AL. Proteasome inhibitors: from research tools to drug candidates. *Chem Biol*. 2001; 8:759–58. [PubMed: 11514225]
164. Aghajanian C, Soignet S, Dizon DS, et al. A phase I trial of the novel proteasome inhibitor PS341 in advanced solid tumor malignancies. *Clin Cancer Res*. 2002; 8:2505–11. [PubMed: 12171876]

165. Papandreou CN, Daliani DD, Nix D, et al. Phase I trial of the proteasome inhibitor bortezomib in patients with advanced solid tumors with observations in androgen-independent prostate cancer. *J Clin Oncol*. 2004; 22:2108–21. [PubMed: 15169797]
166. Portnow J, Frankel P, Koehler S, Twardowski P, Shibata S, Martel C, Morgan R, Cristea M, Chow W, Lim D, Chung V, Reckamp K, Leong L, Synold TW. A phase I study of bortezomib and temozolomide in patients with advanced solid tumors. *Cancer Chemother Pharmacol*. 2011 Aug 18. [Epub ahead of print].
167. Richardson PG, Barlogie B, Berenson J, et al. A phase 2 study of bortezomib in relapsed, refractory myeloma. *N Engl J Med*. 2003; 348:2609–17. [PubMed: 12826635]
168. Richardson PG, Sonneveld P, Schuster MW, et al. Bortezomib or high-dose dexamethasone for relapsed multiple myeloma. *N Engl J Med*. 2005; 352:2487–98. [PubMed: 15958804]
169. Russo SM, Tepper JE, Baldwin AS Jr, et al. Enhancement of radiosensitivity by proteasome inhibition: implications for a role of NF- κ B. *Int J Radiat Oncol Biol Phys*. 2001; 50:183–93.
170. An WG, Hwang SG, Trepel JB, Blagosklonny MV. Protease inhibitor-induced apoptosis: accumulation of wt p53, p21WAF1/CIP1, and induction of apoptosis are independent markers of proteasome inhibition. *Leukemia*. 2000; 14:1276–1283. [PubMed: 10914553]
171. Kumar A, Hozo I, Wheatley K, Djulbegovic B. Thalidomide versus bortezomib based regimens as first-line therapy for patients with multiple myeloma: a systematic review. *Am J Hematol*. 2011; 86:18–24. [PubMed: 21120867]
172. Papandreou CN, Logothetis CJ. Bortezomib as a potential treatment for prostate cancer. *Cancer Res*. 2004; 64:5036–43. [PubMed: 15289299]
173. Kane RC, Farell AT, Sridhara R, Pazdur R. United States Food and Drug Administration approval summary: bortezomib for the treatment of progressive multiple myeloma after one prior therapy. *Clin Cancer Res*. 2006; 12:2955–2960. [PubMed: 16707588]
174. Kane RC, Dagher R, Farell A, Ko CW, Sridhara R, Justice R, Pazdur R. Bortezomib for the treatment of mantle cell lymphoma. *Clin Cancer Res*. 2007; 13:5291–5294. [PubMed: 17875757]
175. Ma MH, Yang HH, Parker K, et al. The proteasome inhibitor PS-341 markedly enhances sensitivity of multiple myeloma tumor cells to chemotherapeutic agents. *Clin Cancer Res*. 2003; 9:1136–1144. [PubMed: 12631619]
176. Orlowski RZ, Nagler A, Sonneveld P, et al. The combination of pegylated liposomal doxorubicin and bortezomib significantly improves time to progression of patients with relapsed/refractory multiple myeloma compared with bortezomib alone: results from a randomized phase 3 study. *J Clin Oncol*. 2007; 25:3892–3901. [PubMed: 17679727]
177. Mitsiades N, Mitsiades CS, Richardson PG, et al. The proteasome inhibitor PS-341 potentiates sensitivity of multiple myeloma cells to conventional chemotherapeutic agents: therapeutic applications. *Blood*. 2003; 101:2377–2380. [PubMed: 12424198]
178. Roué G, Pérez-Galán P, Mozos A, López-Guerra M, Xargay-Torrent S, Rosich L, Saborit-Villarroya I, Normant E, Campo E, Colomer D. The Hsp90 inhibitor IPI-504 overcomes bortezomib resistance in mantle cell lymphoma in vitro and in vivo by down-regulation of the prosurvival ER chaperone BiP/Grp78. *Blood*. 2011; 117:1270–9. [PubMed: 21106982]
179. Pérez-Galán P, Dreyling M, Wiestner A. Mantle cell lymphoma: biology, pathogenesis, and the molecular basis of treatment in the genomic era. *Blood*. 2011; 117:26–38. [PubMed: 20940415]
180. Fribley AM, Evenchik B, Zeng Q, Park BK, Guan JY, Zhang H, Hale TJ, Soengas MS, Kaufman RJ, Wang CY. Proteasome inhibitor PS-341 induces apoptosis in cisplatin-resistant squamous cell carcinoma cells by induction of Noxa. *J Biol Chem*. 2006; 281:31440–7. [PubMed: 16928686]
181. McConkey DJ, Dinney CP. The molecular, the bad, and the ugly: preventing bladder cancer via mTOR inhibition. *Cancer Prev Res (Phila)*. 2009; 2:1001–2. [PubMed: 19952365]
182. Zhu K, Dunner K Jr, McConkey DJ. Proteasome inhibitors activate autophagy as a cytoprotective response in human prostate cancer cells. *Oncogene*. 2010; 29:451–62. [PubMed: 19881538]
183. Janku F, McConkey DJ, Hong DS, Kurzrock R. Autophagy as a target for anticancer therapy. *Nat Rev Clin Oncol*. 2011; 8:528–39. [PubMed: 21587219]
184. Yew EH, Cheung NS, Choy MS, Qi RZ, Lee AY, Peng ZF, Melendez AJ, Manikandan J, Koay ES, Chiu LL, Ng WL, Whiteman M, Kandiah J, Halliwell B. Proteasome inhibition by

lactacystin in primary neuronal cells induces both potentially neuroprotective and pro-apoptotic transcriptional responses: a microarray analysis. *J Neurochem.* 2005; 94:943–956. [PubMed: 15992382]

185. Mulligan G, Mitsiades C, Bryant B, Zhan F, Chng WJ, Roels S, Koenig E, Fergus A, Huang Y, Richardson P, Trepicchio WL, Broyl A, Sonneveld P, Shaughnessy JD Jr, Bergsagel PL, Schenkein D, Esseltine DL, Boral A, Anderson KC. Gene expression profiling and correlation with outcome in clinical trials of the proteasome inhibitor bortezomib. *Blood.* 2007; 109:3177–3188. [PubMed: 17185464]
186. Poulaki V, Mitsiades CS, Kotoula V, Negri J, McMillin D, Miller JW, Mitsiades N. The proteasome inhibitor bortezomib induces apoptosis in human retinoblastoma cell lines *in vitro*. *Invest Ophthalmol Vis Sci.* 2007; 48:4706–4719. [PubMed: 17898295]
187. Chauhan, D.; Hideshima, T.; Anderson, KC. Targeting proteasome as a therapy in multiple myeloma. In: Khosravi-Far, R.; Whilte, E., editors. *Programmed Cell Death in Cancer Progression and Therapy*. Vol. 12. Springer; 2008. p. 251-260.
188. Tang ZY, Wu YL, Gao SL, Shen HW. Effects of the proteasome inhibitor bortezomib on gene expression profiles of pancreatic cancer cells. *J Surg Res.* 2008; 145:111–123. [PubMed: 17714734]
189. Zhao X, Qiu W, Kung J, Zhao X, Peng X, Yegappan M, Yen-Lieberman B, His ED. Bortezomib induces caspase-dependent apoptosis in Hodgkin lymphoma cell lines and is associated with reduced c-FLIP expression: a gene expression profiling study with implications for potential combination therapies. *Leuk Res.* 2008; 32:275–285. [PubMed: 17659339]
190. Brüning A, Burger P, Vogel M, Rahmeh M, Friese K, Lenhard M, Burges A. Bortezomib treatment of ovarian cancer cells mediates endoplasmic reticulum stress, cell cycle arrest, and apoptosis. *Invest New Drugs.* 2009; 27:543–551.
191. Wang Q, Mora-Jensen H, Weniger MA, Perez-Galan P, Wolford C, Hai T, Ron D, Chen W, Trenkle W, Wiestner A, Ye Y. ERAD inhibitors integrate ER stress with an epigenetic mechanism to activate BH3-only protein NOXA in cancer cells. *Proc Natl Acad Sci U S A.* 2009; 106:2200–5. [PubMed: 19164757]
192. Milani M, Rzymiski T, Mellor HR, Pike L, Bottini A, Generali D, Harris AL. The role of ATF4 stabilization and autophagy in resistance of breast cancer cells treated with Bortezomib. *Cancer Res.* 2009; 69:4415–23. [PubMed: 19417138]
193. Armstrong JL, Flockhart R, Veal GJ, Lovat PE, Redfern CP. Regulation of endoplasmic reticulum stress-induced cell death by ATF4 in neuroectodermal tumor cells. *J Biol Chem.* 2010; 285:6091–100. [PubMed: 20022965]
194. Brüning A, Vogel M, Mylonas I, Friese K, Burges A. Bortezomib Targets the Caspase-Like Proteasome Activity in Cervical Cancer Cells, Triggering Apoptosis That Can be Enhanced by Nelfinavir. *Curr Cancer Drug Targets.* 2011 Jul 15. [Epub ahead of print].
195. Yang Y, Ikezoe T, Saito T, Kobayashi M, Koeffler HP, Taguchi H. Proteasome inhibitor PS-341 induces growth arrest and apoptosis of non-small cell lung cancer cells via the JNK/c-Jun/AP-1 signaling. *Cancer Sci.* 2004; 95:176–80. [PubMed: 14965369]
196. Lauricella M, Emanuele S, D'Anneo A, Calvaruso G, Vassallo B, Carlisi D, Portanova P, Vento R, Tesoriere G. JNK and AP-1 mediate apoptosis induced by bortezomib in HepG2 cells via FasL/caspase-8 and mitochondria-dependent pathways. *Apoptosis.* 2006; 11:607–25. [PubMed: 16528474]
197. Chen Z, Ricker JL, Malhotra PS, Nottingham L, Bagain L, Lee TL, Yeh NT, Van Waes C. Differential bortezomib sensitivity in head and neck cancer lines corresponds to proteasome, nuclear factor-kappaB and activator protein-1 related mechanisms. *Mol Cancer Ther.* 2008; 7:1949–60. [PubMed: 18645005]
198. Rzymiski T, Milani M, Singleton DC, Harris AL. Role of ATF4 in regulation of autophagy and resistance to drugs and hypoxia. *Cell Cycle.* 2009; 8:3838–47. [PubMed: 19887912]
199. Wottawa M, Köditz J, Katschinski DM. Normoxic destabilization of ATF-4 depends on proteasomal degradation. *Acta Physiol (Oxf).* 2010; 198:457–63. [PubMed: 19922526]

200. Chauhan D, Li G, Shringarpure R, Podar K, Ohtake Y, Hideshima T, Anderson KC. Blockade of Hsp27 overcomes Bortezomib/proteasome inhibitor PS-341 resistance in lymphoma cells. *Cancer Research*. 2003; 63:6174–6177. [PubMed: 14559800]
201. Papageorgiou A, Kamat A, Benedict WF, Dinney C, McConkey DJ. Combination therapy with IFN- α plus bortezomib induces apoptosis and inhibits angiogenesis in human bladder cancer cells. *Mol Cancer Ther*. 2006; 5:3032–41. [PubMed: 17172406]
202. Uttamsingh V, Lu C, Miwa G, Gan L. Relative contributions of the five major human cytochromes P450, 1A2, 2C9, 2C19, 2D6 and 3A4, to the hepatic metabolism of the proteasome inhibitor bortezomib. *Drug Metab Dispos*. 2005; 33:1723–1728.
203. Venkatakrishnan K, Rader M, Ramanathan RK, Ramalingam S, Chen E, Riordan W, Trepicchio W, Cooper M, Karol M, von Moltke L, Neuwirth R, Egorin M, Chatta G. Effect of the CYP3A inhibitor ketoconazole on the pharmacokinetics and pharmacodynamics of bortezomib in patients with advanced solid tumors: a prospective, multicenter, open-label, randomized, twoway crossover drug-drug interaction study. *Clin Ther*. 2009; 31:2444–2458. [PubMed: 20110052]
204. Cavaletti G, Jakubowiak AJ. Peripheral neuropathy during bortezomib treatment of multiple myeloma: a review of recent studies. *Leuk Lymphoma*. 2010; 51:1178–87. [PubMed: 20497001]
205. Dispenzieri A. Bortezomib for myeloma much ado about something. *N Engl J Med*. 2005; 352:2546–2548. [PubMed: 15958811]
206. Oakervee HE, Popat R, Curry N, et al. PAD combination therapy (PS-341/bortezomib, doxorubicin and dexamethasone) for previously untreated patients with multiple myeloma. *Br J Haematol*. 2005; 129:755–62. [PubMed: 15953001]
207. Sacco A, Aujay M, Morgan B, Azab AK, Maiso P, Liu Y, Zhang Y, Azab F, Ngo HT, Issa GC, Quang P, Roccaro AM, Ghobrial IM. Carfilzomib-dependent selective inhibition of the chymotrypsin-like activity of the proteasome leads to antitumor activity in Waldenstrom's Macroglobulinemia. *Clin Cancer Res*. 2011; 17:1753–64. [PubMed: 21355079]
208. Demo SD, Kirk CJ, Aujay MA, Buchholz TJ, Dajee M, Ho MN, Jiang J, Laidig GJ, Lewis ER, Parlati F, Shenk KD, Smyth MS, Sun CM, Vallona MK, Woo TM, Molineaux CJ, Bennett MK. Antitumor activity of PR-171, a novel irreversible inhibitor of the proteasome. *Cancer Res*. 2007; 67:6383–6391. [PubMed: 17616698]
209. Jain S, Diefenbach C, Zain J, O'Connor OA. Emerging role of carfilzomib in treatment of relapsed and refractory lymphoid neoplasms and multiple myeloma. *Core Evid*. 2011; 6:43–57. [PubMed: 21654882]
210. Khan ML, Stewart AK. Carfilzomib: a novel second-generation proteasome inhibitor. *Future Oncol*. 2011; 7:607–12. [PubMed: 21568676]
211. Dasmahapatra G, Lembersky D, Son MP, Attkisson E, Dent P, Fisher RI, Friedberg JW, Grant S. Carfilzomib Interacts Synergistically With Histone Deacetylase Inhibitors In Mantle Cell Lymphoma Cells In Vitro And In Vivo. *Mol Cancer Ther*. 2011; 10:1686–97. [PubMed: 21750224]
212. Yang J, Wang Z, Fang Y, Jiang J, Zhao F, Wong H, Bennett MK, Molineaux CJ, Kirk CJ. Pharmacokinetics, Pharmacodynamics, Metabolism, Distribution and Excretion of Carfilzomib in Rats. *Drug Metab Dispos*. 2011 Jul 13.
213. Demo E, Frush D, Gottfried M, et al. Glycogen storage disease type III-hepatocellular carcinoma a long-term complication? *J Hepatol*. 2007; 46:492–498. [PubMed: 17196294]
214. Dasmahapatra G, Lembersky D, Kramer L, Fisher RI, Friedberg J, Dent P, Grant S. The pan-HDAC inhibitor vorinostat potentiates the activity of the proteasome inhibitor carfilzomib in human DLBCL cells in vitro and in vivo. *Blood*. 2010; 115:4478–4487. [PubMed: 20233973]
215. Fuchs O, Provaznikova D, Marinov I, Kuzelova K, Spika I. Antiproliferative and proapoptotic effects of proteasome inhibitors and their combination with histone deacetylase inhibitors on leukemia cells. *Cardiovasc Hematol Disord Drug Targets*. 2009; 9:62–77. [PubMed: 19275578]
216. Roccaro AM, Sacco A, Aujay M, Ngo HT, Azab AK, Quang P, Maiso P, Runnels J, Anderson KC, Demo S, Ghobrial IM. Selective inhibition of chymotrypsin-like activity of the immunoproteasome and constitutive proteasome in Waldenstrom macroglobulinemia. *Blood*. 2010; 115:4051–4060. [PubMed: 20110419]

217. Zhou HJ, Aujay MA, Bennett MK, Dajee M, Demo SD, Fang Y, Ho MN, Jiang J, Kirk CJ, Laidig GJ, Lewis ER, Lu Y, Muchamuel T, Parlati F, Ring E, Shenk KD, Shields J, Shwonek PJ, Stanton T, Sun CM, Sylvain C, Woo TM, Yang J. Design and synthesis of an orally bioavailable and selective peptide epoxyketone proteasome inhibitor (PR-047). *J Med Chem.* 2009; 52:3028–3038. [PubMed: 19348473]
218. Chauhan D, Singh AV, Aujay M, Kirk CJ, Bandi M, Ciccarelli B, Raje N, Richardson P, Anderson KC. A novel orally active proteasome inhibitor ONX 0912 triggers in vitro and in vivo cytotoxicity in multiple myeloma. *Blood.* 2010; 116:4906–4915. [PubMed: 20805366]
219. Cusack JC Jr, Liu R, Xia L, Chao TH, Pien C, Niu W, Palombella VJ, Neuteboom ST, Palladino MA. NPI-0052 enhances tumoricidal response to conventional cancer therapy in a colon cancer model. *Clin Cancer Res.* 2006; 12:6758–64. [PubMed: 17121896]
220. Feling RH, Buchanan GO, Mincer TJ, Kauffman CA, Jensen PR, Fenical W. Salinosporamide A: a highly cytotoxic proteasome inhibitor from a novel microbial source, a marine bacterium of the new genus salinospora. *Angew Chem Int Ed Engl.* 2003; 42:355–357. [PubMed: 12548698]
221. Macherla VR, Mitchell SS, Manam RR, Reed KA, Chao TH, Nicholson B, Deyanat-Yazdi G, Mai B, Jensen PR, Fenical WF, Neuteboom ST, Lam KS, Palladino MA, Potts BC. Structure-activity relationship studies of salinosporamide A (NPI-0052), a novel marine-derived proteasome inhibitor. *J Med Chem.* 2005; 48:3684–3687. [PubMed: 15916417]
222. Miller CP, Ban K, Dujka ME, McConkey DJ, Munsell M, Palladino M, Chandra J. NPI-0052, a novel proteasome inhibitor, induces caspase-8 and ROS dependent apoptosis alone and in combination with HDAC inhibitors in leukemia cells. *Blood.* 2007; 110:267–77. [PubMed: 17356134]
223. Chauhan D, Catley L, Li G, et al. A novel orally active proteasome inhibitor induces apoptosis in multiple myeloma cells with mechanisms distinct from Bortezomib. *Cancer Cell.* 2005; 8:407–19. [PubMed: 16286248]
224. Roccaro AM, Leleu X, Sacco A, Jia X, Melham M, Moreau AS, Ngo HT, Runnels J, Azab A, Azab F, Burwick N, Farag M, Treon SP, Palladino MA, Hideshima T, Chauhan D, Anderson KC, Ghobrial IM. Dual targeting of the proteasome regulates survival and homing in Waldenstrommacroglobulinemia. *Blood.* 2008; 111:4752–4763. [PubMed: 18316628]
225. Miller CP, Rudra S, Keating MJ, Wierda WG, Palladino M, Chandra J. Caspase-8 dependent histone acetylation by a novel proteasome inhibitor, NPI-0052: a mechanism for synergy in leukemia cells. *Blood.* 2009; 113:4289–4299. [PubMed: 19182209]
226. Ruiz S, Krupnik Y, Keating M, Chandra J, Pallidino M, McConkey D. The proteasome inhibitor NPI-0052 is a more effective inducer of apoptosis than bortezomib in lymphocytes from patients with chronic lymphocytic leukemia. *Mol Cancer Ther.* 2006; 5:1836–1843. [PubMed: 16891470]
227. Baritaki S, Chapman A, Yeung K, Spandidos DA, Palladino M, Bonavida B. Inhibition of epithelial to mesenchymal transition in metastatic prostate cancer cells by the novel proteasome inhibitor NPI-0052: pivotal roles of Snail repression and RKIP induction. *Oncogene.* 2009; 28:3573–3585. [PubMed: 19633685]
228. Sloss CM, Wang F, Liu R, Xia L, Houston M, Ljungman D, Palladino MA, Cusack CJ. Proteasome inhibition activates epidermal growth factor receptor (EGFR) and EGFR-independent mitogenic kinase signaling pathways in pancreatic cancer cells. *Clin Cancer Res.* 2008; 14:5116–5123. [PubMed: 18698029]
229. Singh AV, Palladino MA, Lloyd GK, Potts BC, Chauhan D, Anderson KC. Pharmacodynamic and efficacy studies of the novel proteasome inhibitor NPI-0052 (marizomib) in a human plasmacytoma xenograft murine model. *Br J Haematol.* 2010; 149:550–559. [PubMed: 20331453]
230. Dorsey BD, Iqbal M, Chatterjee S, Menta E, Bernardini R, Bernareggi A, Cassara PG, D'Arasmo G, Ferretti E, De Munari S, Oliva A, Pezzoni G, Allievi C, Strepponi I, Ruggeri B, Ator MA, Williams M, Mallamo JP. Discovery of a potent, selective and orally active proteasome inhibitor for the treatment of cancer. *J Med Cham.* 2008; 51:1068–1072.
231. Sanchez E, Li M, Steinberg JA, Wang C, Shen J, Bonavida B, Li ZW, Chen H, Berenson JR. The proteasome inhibitor CEP-18770 enhances the anti-myeloma activity of bortezomib and melphelan. *Br J Haematol.* 2010; 148:569–581. [PubMed: 19958357]

232. Chauhan D, Tian Z, Zhou B, Kuhn DJ, Orlowski RZ, Raje NS, Richardson PG, Anderson KC. In vitro and In vivo Selective Antitumor Activity of a Novel Orally Bioavailable Proteasome Inhibitor MLN9708 Against Multiple Myeloma Cells. *Clin Cancer Res.* 2011 Jun 30.
233. Kupperman E, Lee EC, Cao Y, Bannerman B, Fitzgerald M, Berger A, Yu J, Yang Y, Hales P, Bruzzese F, Liu J, Blank J, Garcia K, Tsu C, Dick L, Fleming P, Yu L, Manfredi M, Rolfe M, Bolen J. Evaluation of the proteasome inhibitor MLN9708 in preclinical models of human cancer. *Cancer Res.* 2010; 70:1970–1980. [PubMed: 20160034]
234. Muchamuel T, Basler M, Aujay MA, Suzuki E, Kalim KW, Lauer C, Sylvain C, Ring ER, Shields J, Jiang J, Shwonek P, Parlati F, Demo SD, Bennett MK, Kirk CJ, Groettrup M. A selective inhibitor of the immunoproteasome subunit LMP7 blocks cytokine production and attenuates progression of experimental arthritis. *Nat Med.* 2009; 15:781–7. [PubMed: 19525961]
235. Basler M, Dajee M, Moll C, Groettrup M, Kirk CJ. Prevention of experimental colitis by a selective inhibitor of the immunoproteasome. *J Immunol.* 2010; 185:634–41. [PubMed: 20525886]
236. Palombella VJ, Conner EM, Fuseler JW, Destree A, Davis JM, Laroux FS, Wolf RE, Huang J, Brand S, Elliott PJ, Lazarus D, McCormack T, Parent L, Stein R, Adams J, Grisham MB. Role of the proteasome and NF- κ B in streptococcal cell wall-induced polyarthritis. *Proc Natl Acad Sci USA.* 1998; 95:15671–15676. [PubMed: 9861028]
237. Qureshi N, Perera PY, Shen J, Zhang G, Lensch A, Splitter G, Morrison DC, Vogel SN. The proteasome as a lipopolysaccharide-binding protein in macrophages: differential effects of proteasome inhibition on lipopolysaccharide-induced signaling events. *J Immunol.* 2003; 171:1515–1525. [PubMed: 12874245]
238. Singh AV, Bandi M, Aujay MA, Kirk CJ, Hark DE, Raje N, Chauhan D, Anderson KC. PR-924, a selective inhibitor of the immunoproteasome subunit LMP-7, blocks multiple myeloma cell growth both in vitro and in vivo. *Br J Haematol.* 2010; 152:155–163. [PubMed: 21114484]
239. Kuhn DJ, Hunsucker SA, Chen Q, Voorhees PM, Orlowski M, Orlowski RZ. Targeted inhibition of the immunoproteasome is a potent strategy against models of multiple myeloma that overcomes resistance to conventional drugs and nonspecific proteasome inhibitors. *Blood.* 2009; 113:4667–4776. [PubMed: 19050304]

Figure 1.

The schematic diagram showing ubiquitylation of substrate protein and its subsequent degradation by the 26S proteasome complex. A ubiquitin activating enzyme (E1) first forms a thio-ester bond with ubiquitin (a highly conserved protein with 76 amino acids) in an ATP-dependent manner. Ubiquitin then binds to a ubiquitin conjugating enzyme (E2). Subsequently, the carboxy-terminus of ubiquitin forms an isopeptide bond with a K residue on the substrate protein in the presence of a ubiquitin ligase enzyme (E3). Multiple ubiquitylation cycles result in polyubiquitylation of the substrate protein. The 26S proteasome complex recognizes, unfolds and degrades polyubiquitylated-substrate protein into small peptides. Ub, ubiquitin.

Figure 2. The schematic diagram showing the regulation NF-κB functions by the 26S proteasome complex.

Figure 3.
The schematic diagram showing the effects of the proteasome inhibition on different pathways, contributing to cancer prevention.

Table 1

26S Proteasome inhibitors with their targets and clinical status.

26S Proteasome inhibitor	Target in 26S proteasome	Clinical status
Bortezomib	Chymotrypsin-like activity	<ul style="list-style-type: none"> • Approved for MM and MCL.
Carfilzomib	Chymotrypsin-like activity	<ul style="list-style-type: none"> • Phase III in MM. • Phase I in AML, ALL, CLL. • Phase Ib/II in solid tumors.
ONX0912	Chymotrypsin-like activity	<ul style="list-style-type: none"> • Phase I in solid tumors
NPI-0052	Chymotrypsin-like, Trypsin-like, Caspase-like activities	<ul style="list-style-type: none"> • Phase I in MM, solid tumors, refractory lymphoma, and non-small cell lung carcinoma.
CEP-18770	Chymotrypsin-like activity	<ul style="list-style-type: none"> • Phase I/II in MM.
MLN9708	Chymotrypsin-like activity	<ul style="list-style-type: none"> • Phase I/II in MM. • Phase I in lymphoma and non-haematological malignancies.

MM, multiple myeloma; MCL, mantle cell lymphoma; AML, acute myeloid leukaemia; ALL, acute lymphoblastic leukaemia; and CLL, chronic lymphocytic leukaemia.

Table 2

Immunoproteasome inhibitors with their targets and pre-clinical results.

Immunoproteasome inhibitor	Target in immunoproteasome	Pre-clinical results
PR-957	Chymotrypsin-like activity	<ul style="list-style-type: none">• Inhibits inflammatory response.
PR-924	Chymotrypsin-like activity	<ul style="list-style-type: none">• Inhibits tumor growth in animal models without significant toxicities.• Inhibits growth of primary cell lines and primary tumor cells.• Anti-tumor activity against MM.
IPSI-001	Caspase-like activity	<ul style="list-style-type: none">• Inhibits hematological malignancies in <i>in vitro</i> models.• Inhibits proliferation in myeloma patient samples.• Overcomes other drug resistance.