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Proteasome inhibitors: a therapeutic strategy for haematological malignancy

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1. ABSTRACT

The proteasome is a multicatalytic enzyme complex responsible for the regulated degradation of intracellular proteins. In recent years, inhibition of proteasome function has emerged as a novel anti-cancer therapy. Proteasome inhibition is now established as an effective treatment for relapsed and refractory multiple myeloma and offers great promise for the treatment of other haematological malignancies, when used in combination with conventional therapeutic agents. Bortezomib is the first proteasome inhibitor to be used clinically and a second generation of proteasome inhibitors with differential pharmacological properties are currently in early clinical trials. This review summarises the development of proteasome inhibitors as therapeutic agents and describes how novel assays for measuring proteasome activity and inhibition may help to further delineate the mechanisms of action of different proteasome inhibitors. This will allow for the optimized use of proteasome inhibitors in combination therapies and provide the opportunity to design more potent and therapeutically efficacious proteasome inhibitors.

2. THE UBIQUITIN PROTEASOME PATHWAY

In eukaryotic cells, proteins are in a dynamic equilibrium in which degradation is as important as protein synthesis. For a long time protein degradation was believed to serve primarily as a means of eliminating misfolded, damaged or mutant proteins, whose accumulation might be harmful to the cell. Over the past 20 years it has become clear that that degradation of cellular proteins is a highly complex and tightly regulated process that plays a central role in regulating cellular function and maintaining homeostasis. The ubiquitin proteasome pathway (UPP) represents the major pathway for intracellular protein degradation. More than 80% of cellular proteins are degraded through this pathway including those involved in controlling a broad array of cellular processes such as cell cycle, apoptosis, transcription, signal transduction and antigen presentation. Degradation of a protein via the UPP involves two distinct and successive pathways. Proteins destined for proteolysis are initially tagged by the conjugation of multiple monomers of the 76 amino acid protein ubiquitin. This requires the action of three classes of enzymes – E1 (ubiquitin-activating enzyme), E2

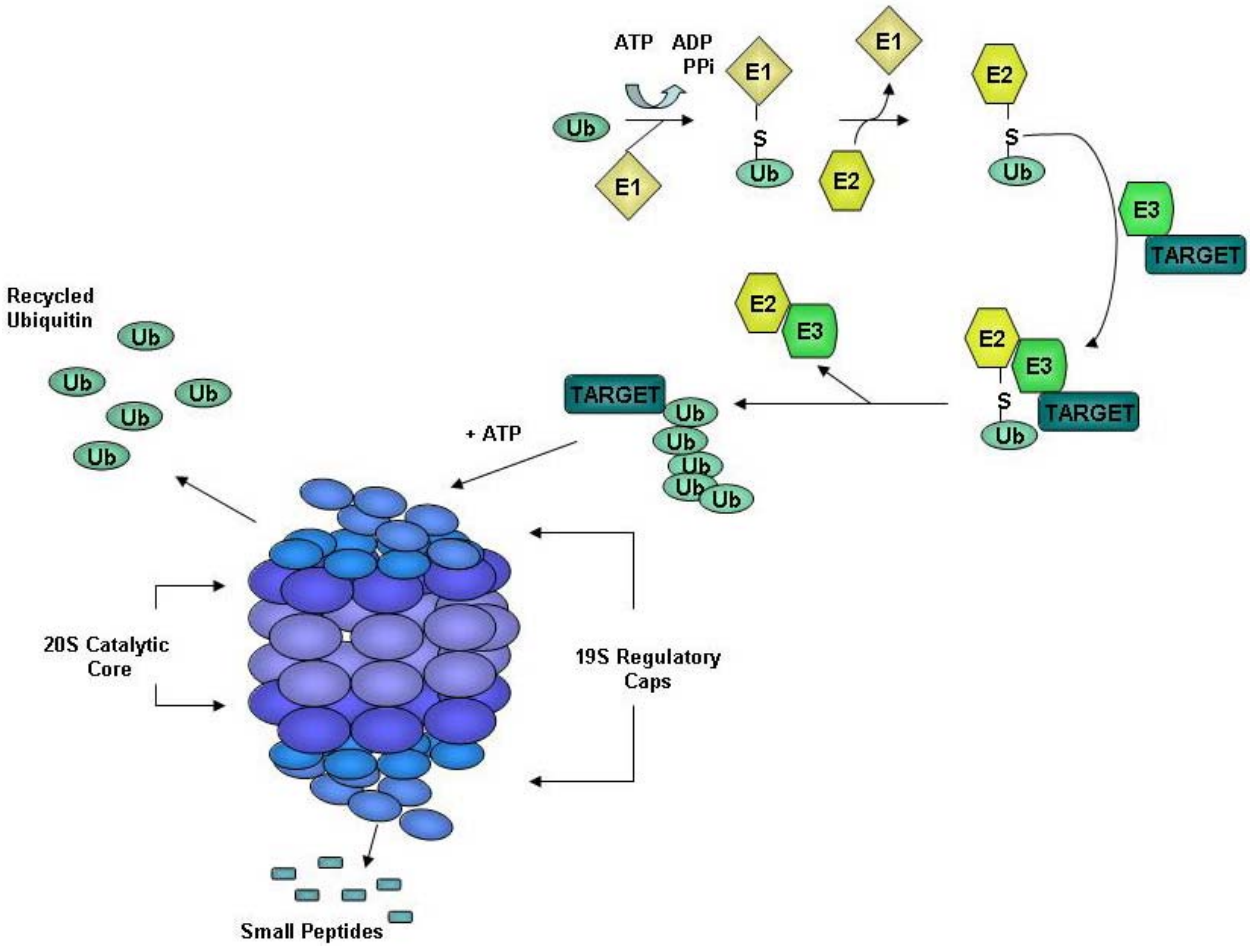

Figure 1. Outline of the ubiquitin proteasome pathway. An E1 ubiquitin-activating enzyme binds ubiquitin (Ub), which is then transferred to an E2 ubiquitin-conjugating enzyme. An E3 ubiquitin ligase subsequently recruits the target protein and mediates the transfer of ubiquitin to the protein. The successive conjugation of ubiquitin moieties generates a polyubiquitin chain that functions as a signal to target the protein to the 26S proteasome for degradation.

(ubiquitin-conjugating enzyme) and E3 (ubiquitin ligase). A single E1 enzyme (UBA 1) activates ubiquitin by forming a thiol ester bond between the E1 active-site located cysteine residue and the C-terminal glycine residue of ubiquitin, in an ATP-dependent step (1). Following activation, ubiquitin is then transferred to one of at least twenty E2 enzymes via the formation of an additional thiol ester bond (2). Finally, E2 shuttles ubiquitin either directly or in cooperation with an E3 enzyme to a lysine residue in the target protein, thereby forming an isopeptide bond between ubiquitin and the protein. There are more than 500 E3 enzymes characterised to date, each of which recognises specific motifs in a small number of corresponding protein substrates (3). Thus, the combination of E2 and E3 enzymes confers an exquisite specificity to the selection of proteins for degradation. The successive conjugation of ubiquitin moieties generates a polyubiquitin chain that acts as a signal to target the protein for degradation by the 26S proteasome. This process is illustrated in Figure 1.

2.1. The 26S Proteasome

The 26S proteasome is a large (1500-2000 kDa) multicatalytic complex that is present in the nucleus and cytoplasm of all eukaryotic cells. It is composed of a central 20S catalytic core and one or two 19S regulatory caps. The 20S core particle is a cylindrical structure made up of 28 subunits arranged into four stacked rings. The two outer rings are composed of seven different alpha-subunits and the two inner rings contain seven different beta-subunits. The catalytic sites are localised to three of the beta-subunits and face inwards to the central cavity (4). Access to the catalytic chamber is through axial channels formed at the centre of the alpha-rings (5). Substrates gain access to the catalytic chamber by binding of the 19S regulatory particle to one or both ends of the 20S proteasome. Ubiquitin-tagged proteins are recognised by the 19S particle, where ubiquitin tags are removed and ATPases with chaperone-like activity then unfold the protein substrates and feed them into the inner catalytic compartment of the 20S proteasome cylinder (6).

The catalytic subunits of the proteasome contain an N-terminal threonine residue, whose hydroxyl group serves as a nucleophile to attack peptide bonds in target proteins (7). Catalytic activities of eukaryotic proteasomes

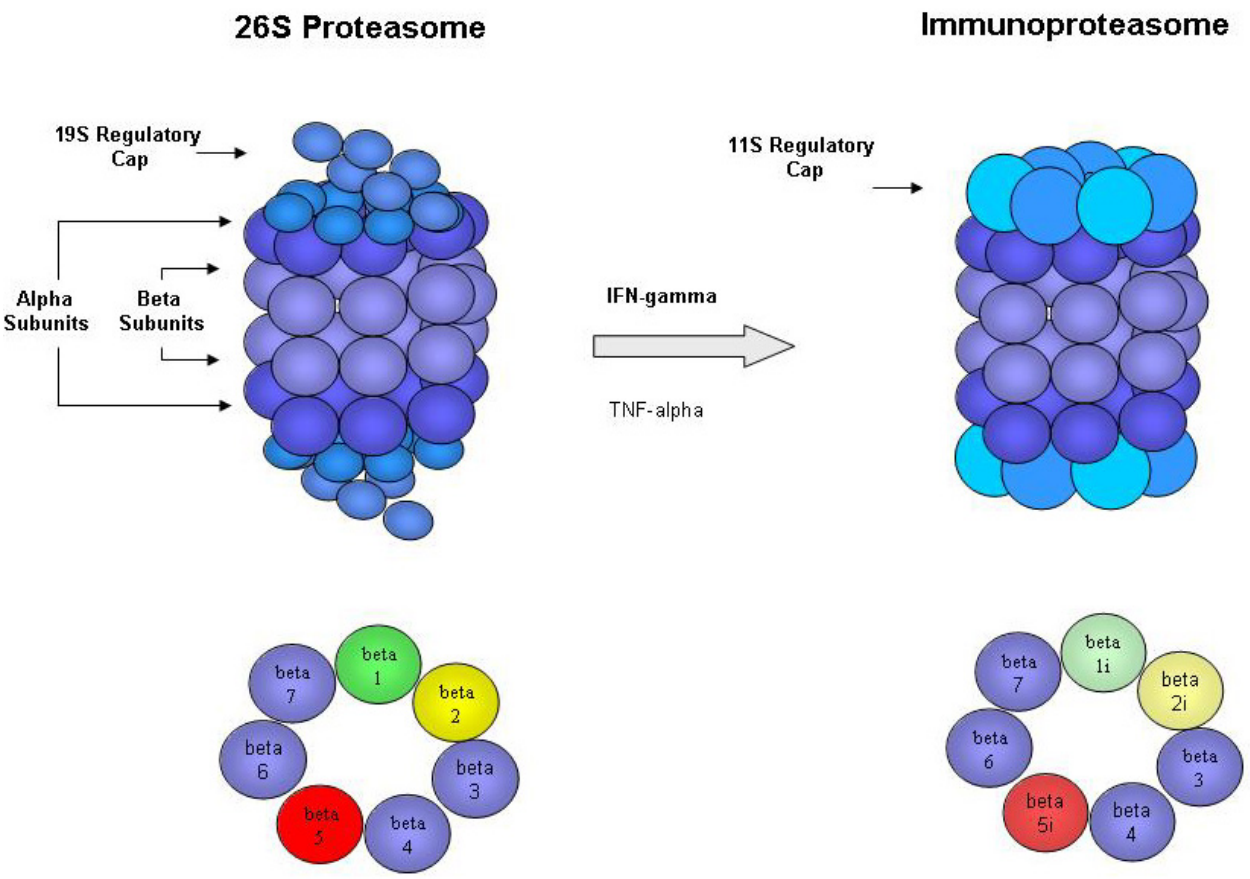

Figure 2. Composition of the proteasome. The 26S proteasome is composed of a 20S catalytic core and two 19S regulatory caps. The 20S core particle is made up of two outer rings containing seven different alpha subunits and two inner rings containing seven different beta subunits. Catalytic subunits are localized to the beta 1, beta 2 and beta 5 subunits. IFN-gamma, and to a lesser extent TNF-alpha, induce expression of an additional set of catalytic beta-subunits, beta 1i, beta 2i and beta 5i, which replace the constitutive catalytic beta subunits. 19S caps are also replaced with 11S regulatory caps to form a new proteolytic particle, the immunoproteasome.

are classified into three major categories, based upon preference to cleave a peptide bond after a particular amino acid residue. These activities are referred to as chymotrypsin-like (CT-L), trypsin-like (T-L) and peptidylglutamyl peptide hydrolysing (PGPH) and are associated with three distinct subunits beta 5, beta 2 and beta 1, respectively (8, 9). The CT-L activity cleaves substrates after hydrophobic residues, the T-L activity cleaves after basic residues and the PGPH (also known as caspase-like) activity cleaves after acidic residues (10). The CT-L activity has traditionally been thought of as the rate-limiting step in protein degradation. However, the individual roles of the three activities in the functioning of the proteasome are still under investigation. There is conflicting evidence on how each activity contributes to the overall biological process. Early mutational studies in yeast suggested a hierarchy of the catalytic activities in degradation, whereby the CT-L activity was deemed to be of most importance (8, 11, 12). These studies demonstrated that inactivation of CT-L sites resulted in significant impairment of cell growth and a large reduction in the degradation of model substrates. In contrast, inactivation of T-L sites caused only mild growth defects and reduced the degradation of two out of four model substrates. No changes were found in strains where the PGPH sites were mutated. Contrary to these studies in yeast, several groups have demonstrated a significant role for T-L and PGPH activity in mammalian cells. Oberdorf *et al* showed that all three active beta subunits can independently contribute to the degradation of cystic fibrosis transmembrane conductance regulator (13). In addition, Cardozo and Michaud established an important role for both T-L and PGPH activities in the degradation of tau proteins (14). More recently, Kisselev *et al* found that the relative importance of the three activities depended on the protein being degraded (15). They also showed that when one active site was inhibited, protein degradation remained processive and the number of peptides generated did not change. These studies suggest that all three catalytic sites make a significant contribution to protein degradation.

2.2. The Immunoproteasome

An alternative proteasome species can be formed in response to cytokine signalling. Interferon-gamma (IFN-gamma) and tumour necrosis factor-alpha (TNF-alpha) induce the expression of an additional set of beta-subunits, beta 1i (LMP2), beta 2i (MECL1) and beta 5i (LMP7), which replace constitutive subunits beta 1, beta 2 and beta 5, to form a new proteolytic particle, the immunoproteasome (Figure 2). This

Table 1. Major Classes of Proteasome Inhibitors

Class	Compound	Binding Mechanism	Catalytic Sites Targeted	Reference
Synthetic Inhibitors				
Peptide Aldehydes	MG-132 Z-LLE-CHO	Reversible Reversible	Predominantly CT-L Predominantly CT-L	18 19
Peptide Alpha-Keto Aldehydes	BzLLLCOCHO	Reversible	CT-L, T-L, PGPH	20, 21
Peptide Vinyl Sulfones	NLVS AdaAhx3L3VS	Irreversible Irreversible	Predominantly CT-L CT-L, T-L, PGPH	22 23
Peptide Boronates	MG-262 Bortezomib	Reversible Reversible	Predominantly CT-L Predominantly CT-L	24
Natural Inhibitors				
Lactacystin and Structurally Related Compounds	Lactacystin Omuralide NPI-0052	Irreversible Irreversible Irreversible	CT-L, T-L, PGPH CT-L, T-L, PGPH CT-L, T-L, PGPH	25 26 27
Epoxyketones	Eponemycin Epoxomicin Carfilzomib	Irreversible Irreversible Irreversible	CT-L, PGPH Predominantly CT-L Predominantly CT-L	28 29 30

proteasome species is so called as the catalytic activities are altered to favour the generation of antigenic peptides (epitopes) for presentation by the major histocompatability (MHC) class 1 mediated immune response (16). IFN-gamma also induces the synthesis of the 11S regulatory cap (16). This complex is composed of three alpha and four beta subunits arranged in a ring-shaped structure that associates with the alpha-rings of the immunoproteasome to open the gated channel. In contrast to the 19S caps, 11S structures do not recognise ubiquitinated proteins or require ATP. The catalytic subunits of the immunoproteasome are particularly abundant in immune-related cells and have been found to be expressed in haemopoietic tumours such as multiple myeloma (MM) (17).

3. THE PROTEASOME AS A DRUG TARGET

Proteasome inhibitors were initially synthesized as *in vitro* probes to investigate the function of the proteasome's proteolytic activities. As the essential role of the UPP in cellular function became evident, proteasome inhibitors began to elicit attention as potential therapeutic agents, in particular as anti-cancer drugs. Interest in proteasome inhibitors, both as a research tool and as therapeutic agents, has increased rapidly in the past decade. Consequently, a lot of effort has been put into the design of new inhibitors with varying specificities and modes of action. The proteasome inhibitors can be categorised into two main groups – synthetic proteasome inhibitors and natural products. The synthetic inhibitors are based on short peptide sequences in which the C-terminus has been replaced with a pharmacophore such as an aldehyde, a vinyl sulphone or a boronate. The C-terminal pharmacophore is responsible for binding reversibly or irreversibly with the catalytic threonine of the proteasome. Natural products have provided both peptide and non-peptide proteasome inhibitors. Among these, the best characterised are lactacystin, epoxomicin, eponomycin and their derivatives. The major classes of proteasome inhibitors and their specificity for catalytic subunits are listed in Table 1. Figure 3 shows the chemical structures and pharmacophores of the proteasome inhibitors.

The concept of proteasome inhibition as a novel treatment for malignant disease led to a multitude of preclinical studies with these agents. Many of the initial studies documenting proteasome inhibitor-mediated apoptosis used cells of haemopoietic origin. Imajoh-Ohmi *et al* were the first to suggest that inhibition of proteasome function had an anti-tumour effect (31). They showed that incubation of the human leukaemic U937 cell line with the natural proteasome inhibitor lactacystin, led to apoptosis. Shortly after, Shinohara *et al* and Drexler used peptide aldehyde inhibitors to induce apoptosis in the MOLT T-cell leukaemia and HL60 promyelocytic leukaemia cell lines, respectively (32, 33). The first *in vivo* demonstration of anti-tumour activity of proteasome inhibitors used a human xenograft model of Burkitt's lymphoma. The peptide aldehyde inhibitor, Z-LLF-CHO, was shown to delay tumour progression and induce apoptosis without any obvious adverse effects (34). Furthermore, proteasome inhibitors were reported to induce preferential apoptosis of malignant cells, with relative sparing of normal controls. These and other studies validated the proteasome as a target for anti-cancer therapy.

3.1. Bortezomib: A Novel Therapeutic Agent

Despite promising results, the utility of many of the available inhibitors was limited to laboratory studies because of a relative lack of potency, specificity or stability. This led to the development of a series of dipeptide boronic acids, which were more potent and selective than many previously available inhibitors (24). These inhibitors were initially screened *in vitro* against the National Cancer Institute's (NCI) panel of 60 cancer cell lines. The proteasome inhibitors displayed a unique pattern of cytotoxicity, with little similarity to other cytotoxic agents in the NCI's database. On the basis of its potency and cytotoxicity, Bortezomib (also known as PS-341 or Velcade) was identified as the best candidate for further testing (35). The activity of this drug was tested both *in vitro* and in murine and human xenograft models of various tumour types. Bortezomib proved to be particularly successful in MM and phase I through to phase III clinical trials confirmed its efficacy in this disease (36-39). Bortezomib was approved by the Food and Drug Administration (FDA) in 2003 and the European Agency for the Evaluation of Medicinal Products (EMEA) in 2004 for the treatment of relapsed and refractory MM and has recently received full approval for the treatment of MM patients who have received at least one prior therapy (40).

Table 2. Published Studies of Bortezomib Combination Therapy for Multiple Myeloma

Combination	Stage of Trial	Treatment Stage	Overall Response	Reference
Bortezomib and Dexamethasone	Phase I	Relapsed / Refractory	80%	48
	Phase II	Previously Untreated	66%	49
	Phase II	Previously Untreated	82.5%	50
Bortezomib and Pegylated Liposomal Doxorubicin	Phase I	Advanced Haematalogical Malignancies	73% in MM	51
	Phase III	Relapsed / Refractory	44%	52
Bortezomib and Melphalan	Phase I/II	Relapsed / Refractory	68%	53
Bortezomib, Thalidomide and Dexamethasone	Phase I/II	Relapsed / Refractory	53%	54
	Phase I/II	Previously Untreated	87%	55
Bortezomib, Doxorubicin and Dexamethasone	Phase I/II	Previously Untreated	95%	56
Bortezomib, Melphalan and Prednisone	Phase I/II	Previously Untreated	89%	57
Bortezomib, Melphalan, Prednisone and Thalidomide	Phase I/II	Relapsed / Refractory	67%	58
Bortezomib, Arsenic Trioxide and Ascorbic Acid	Phase I/II	Relapsed / Refractory	27%	59
Bortezomib, Dexamethasone and Cyclophosphamide	Phase II	Relapsed / Refractory	90%	60

Table 3. Ongoing Clinical Studies of Bortezomib Combination Therapy for Haematological Malignancies

Combination	Stage of Trial	Disease
Bortezomib and Tipifarnib	Phase II	Acute Myeloid Leukaemia
Bortezomib and Tipifarnib	Phase I	Acute Leukaemia and Chronic Myeloid Leukaemia
Bortezomib, Mitoxantrone and Etoposide	Phase I/II	Relapsed / Refractory Acute Lymphoblastic Leukaemia
Bortezomib and Idarubicin	Phase I	Elderly and Relapsed Acute Myeloid Leukaemia
Bortezomib and Doxorubicin Hydrochloride Liposome	Phase I/II	Myeloid or Lymphoid Leukaemia, Multiple Myeloma, Hodgkin's and Non Hodgkin's Lymphoma
Bortezomib and Chemotherapy	Phase I/II	Pediatric Acute Lymphoblastic Leukaemia
Bortezomib, Rituximab, Cyclophosphamide and Prednisone	Phase I/II	Relapsed / Refractory Non Hodgkin's Lymphoma
Bortezomib and Flurarabine +/- Rituximab	Phase I	Relapsed / Refractory Non Hodgkin's Lymphoma and Chronic Lymphocytic Leukaemia
Bortezomib, Idarubicin and Cytosine Arabinoside	Phase I	Acute Myeloid Leukaemia

Information on these trials can be found via http://www.clinicaltrials.gov

Promising results have also been seen with bortezomib in trials of chronic lymphocytic leukaemia (CLL) (41), follicular lymphoma, mantle cell lymphoma (42-44), Waldenstrom's macroglobulinaemia (45, 46) and plasma cell leukaemia (47). Furthermore, inhibition of proteasome function with bortezomib has been demonstrated to both sensitise tumour cells to conventional chemotherapy and to overcome chemotherapy resistance. This has led to a number of clinical trials to investigate the activity of bortezomib in combination with conventional and novel therapies for the treatment of MM (Table 2). Based on the results of these combination studies and encouraging pre-clinical data, more recent studies evaluating the use of bortezomib combination therapies have been initiated in patients with a variety of other haematological malignancies (Table 3).

3.2. Development of Novel Proteasome Inhibitors

Clinical studies with bortezomib have validated the proteasome as a novel and legitimate therapeutic target. However, the use of bortezomib can be limited by toxicity, unresponsive disease or resistance suggesting the need for other proteasome inhibitors with enhanced activity. Recent studies have focused on the development of novel proteasome inhibitors as therapeutic agents. Novel compounds are currently in clinical trials and are described below.

NPI-0052, also known as Salinosporamide A, is a natural proteasome inhibitor derived from the marine actinomycete *Salinospora tropica* (61). NPI-0052 is structurally related to the lactacystin-derived proteasome inhibitor Omuralide, but is distinguished by the presence of a uniquely methylated C3 ring juncture, chlorinated alkyl group at C2 and cyclohexane ring at C2 (Figure 3). It is distinct from bortezomib in its chemical structure, effects on proteasome activities, mechanisms of action and toxicity profile against normal cells. In contrast to bortezomib which reversibly binds to the CT-L and PGPH activities, NPI-0052 binds irreversibly to all three catalytic activities of the proteasome. While bortezomib acts through both the caspase-8 and caspase-9 apoptotic signalling cascades, NPI-0052 induced cell death relies primarily on caspase-8 mediated signalling pathways. Furthermore bortezomib is administered intravenously, whilst NPI-0052 is orally bioactive. *In vitro* studies demonstrated that NPI-0052 induces apoptosis in MM cells resistant to conventional therapies and to bortezomib; animal tumour model studies show that this compound is well tolerated and prolongs survival (27). A phase I trial examining the safety, pharmacokinetics and pharmacodynamics of NPI-0052 in patients with relapsed or refractory MM is currently ongoing.

Epoxomicin, a member of the epoxyketone family of natural peptide proteasome inhibitors, is derived from *Actinomycetes*. This family inhibits proteasome activity through a unique mechanism, by binding to both the hydroxyl and amino groups of the catalytic site threonine residue (62). The simplistic structure of these linear peptides and their high specificity for the proteasome has resulted in the development and synthesis of epoxyketone-related proteasome inhibitors possessing higher potency or novel inhibitory specificities. Carfilzomib (PR-171) is a novel epoxomicin-based proteasome inhibitor, with improved pharmaceutical

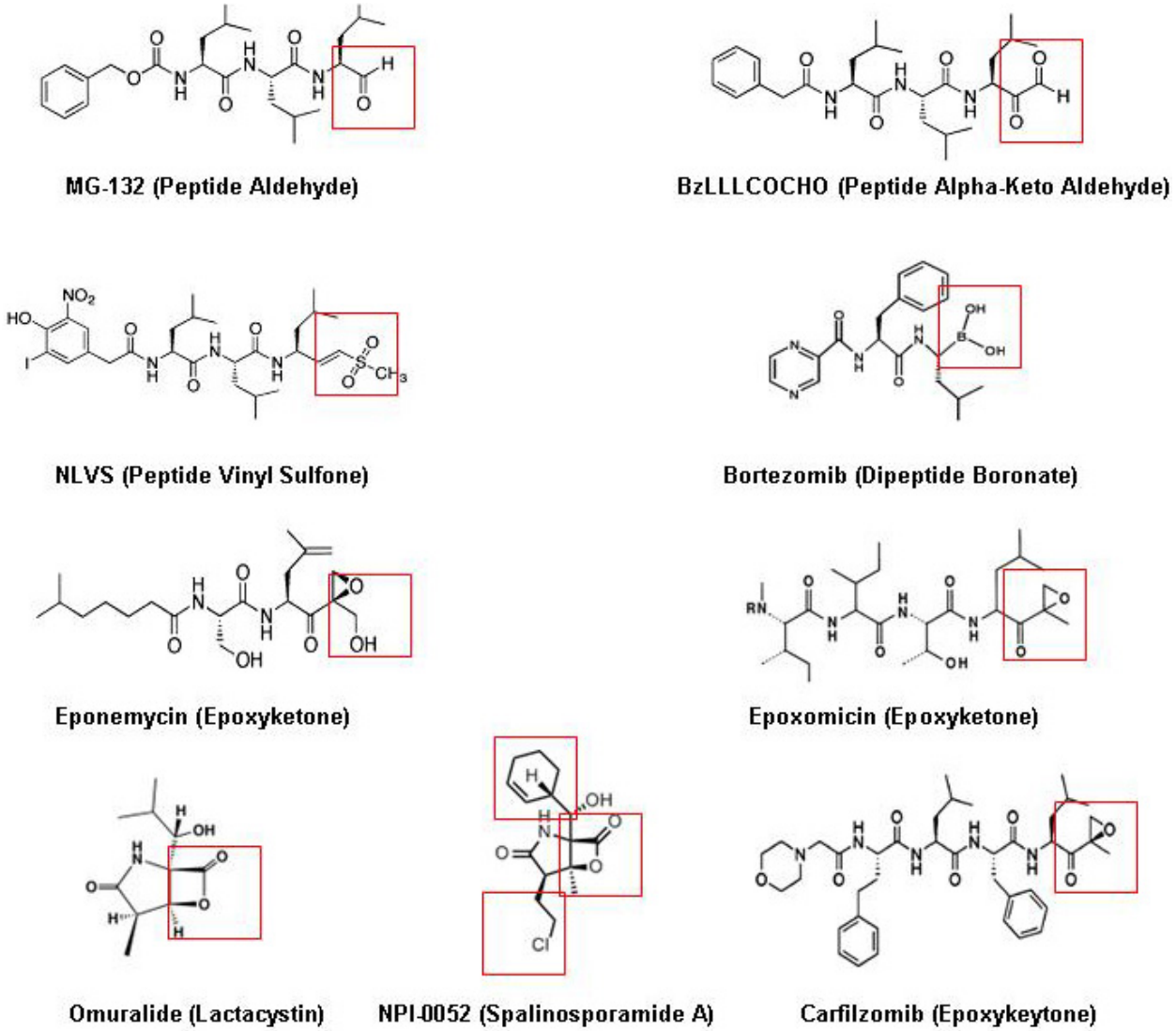

Figure 3. Chemical structures of the major classes of proteasome inhibitors. The pharmacophores of each inhibitor (marked by a red box) are as follows - MG-132 : aldehyde, BzLLCOCHO : alpha-keto aldehyde, NLVS : vinyl sulfone, Bortezomib : boronate, Eponemycin : alpha, beta-epoxyketone, Epoxomicin : alpha, beta-epoxyketone, Omuralide : beta-lactone, NPI-0052 : beta-lactone, chloroethyl group, cyclohexane ring, Carfilzomib : alpha, beta-epoxyketone.

properties. In contrast to bortezomib it is an irreversible inhibitor, specific for the CT-L activity of the proteasome and its immunoproteasome counterpart LMP7. *In vitro*, carfilzomib showed increased efficacy compared to bortezomib and was active against both bortezomib resistant MM cell lines and samples from patients with clinical bortezomib resistance. Activity of carfilzomib in human tumour xenograft models demonstrated tolerability, efficacy and dosing flexibility (30, 63). Two phase I clinical trials of carfilzomib in MM and non-Hodgkin's lymphoma (NHL), comparing two dose-intensive schedules, are currently underway.

Finally, a novel approach that may prove promising is the use of inhibitors that are specific for the catalytic activities of the immunoproteasome. The immunoproteasome is present in many haemopoietic-derived cells, even in the absence of exogenous cytokines. Inhibitors specific for this proteasome species could have the ability to induce apoptosis in some haematological malignancies, with relative sparing of normal tissues. MM in particular is known to express a high level of immunoproteasome subunits, therefore, specific inhibition of immunoproteasome activity may be useful in this disease and toxicities associated with bortezomib, such as peripheral neuropathy and gastrointestinal effects, might be decreased or abolished. Ho *et al* have described the development of probes specific for the LMP2 catalytic subunit of the immunoproteasome (64). Further studies to evaluate these agents as potential drug candidates are underway.

4. METHODS TO PROFILE PROTEASOME ACTIVITY

With proteasome inhibitors already in use in the clinic, a better understanding of proteasome expression and

activity in normal and diseased states is required for the development of improved therapeutic strategy. Altered expression of proteasomes in haemopoietic malignancies was first demonstrated by Kumatori *et al* in 1990 (65). Using immunohistochemistry, immunoblotting and Northern blot analysis, this group demonstrated that expression of proteasomes in a variety of haemopoietic tumours was higher than in normal blood cells. Several groups have since developed enzyme-linked immunoabsorbant assays (ELISAs) to detect circulating 20S proteasome components in serum or plasma samples (66-69). Two of these groups have reported elevated levels of proteasomes in tumour cells and serum in small cohorts of patients with haematological malignancies (67, 69). Recently, a larger study in MM patients reported that increased serum proteasome concentrations correlate with advanced disease and are an independent prognostic factor in MM (70).

The above studies have all measured the quantity of proteasomes present within a sample, however, accurate methods to assess individual catalytic activities and the inhibitory action of proteasome inhibitors on the activities are also required. The individual activities of proteasome subunits can be analysed in two main ways. They are most commonly assessed using fluorogenic peptide substrates specific for CT-L, T-L and PGPH activities. These substrates are composed of three to four amino acid peptides with a fluorogenic reporter group (e.g. AMC) at the C terminus. The proteasome cleaves an amide bond between an amino acid and the reporter group, resulting in the release of fluorescence (71). This provides a fast and convenient means to monitor the individual activities of the proteasome. Using this technique, Magill *et al* observed elevated levels of CT-L activity in primary myeloid leukaemia and MM cells, compared to normal mononuclear cells (72). Our group has also profiled the contributions of the three catalytic activities in MM and lymphoma cell lines using fluorogenic substrates and found that the balance of activities is dependent on cell type (21). Furthermore, this assay is frequently employed to measure the ability of proteasome inhibitors to block different active sites.

One drawback of the fluorogenic substrate assays is that they do not distinguish between constitutive and immunoproteasome activity. Therefore, a more specific assay has been developed to complement this method. Selective inhibitors of the proteasome have been modified as probes to label the active subunits. These activity-based probes offer an advantage over the conventional substrate-based assays, in that they do provide insight into the constitutive / immunoproteasome ratio within cell types and also offer a more direct assessment of the subunit specificity and potency of proteasome inhibitors. A number of peptide vinyl sulfones have been modified to function as active site-directed probes of the proteasome. They were initially synthesized with either a radio-isotope (23) or an azide group (73) attached to label active proteasomes, however, addition of these labels makes the compounds impermeable to cells. Subsequently, a small hapten, dansyl, was attached to the vinyl sulfone inhibitors to retain cell permeability and provide a method to label proteasome subunits in intact cells (74). The use of antibodies against the dansyl moiety allows the detection of labelled subunits by SDS-PAGE and Western blot analysis. More recently, this probe has been further optimized by replacing the dansyl moiety with high-quantum fluorophores (75, 76). This modification allows direct in-gel detection of proteasomal subunits and also enables monitoring of the proteasome in living cells. Kraus *et al* employed one of these activity-based probes to profile the activities of the constitutive and immunoproteasome in a variety of primary human leukaemia cells (77). They demonstrated proteasome activity to be upregulated in some, but not all, samples as compared to primary monocytes and observed significant variability in the patterns of individual activities between different samples. Furthermore, this group demonstrates an upregulation of active subunits in bortezomib resistant cells and hypothesize that the relative contribution of CT-L and T-L activities to the overall activity profile may influence the degree of bortezomib sensitivity in haematological malignancies. These activity-based probes are also being increasingly used to investigate the specificity of proteasome inhibitors for the proteasome's catalytic activity.

5. SUMMARY AND PERSPECTIVE

The past decade has witnessed major progress in our understanding of the role of the UPP in protein regulation in eukaryotic cells. Concurrently, inhibition of proteasome activity has proven to be a viable anti-cancer therapy with particular efficacy being demonstrated in haematological malignancies such as MM and NHL. There are currently a myriad of pre-clinical and clinical studies underway, evaluating the efficacy of bortezomib alone and in combination with other agents. A number of novel proteasome inhibitors are also in early clinical trials. The translation of proteasome inhibitors from laboratory studies to the clinic has been rapid and the mechanisms underlying this therapy are not fully understood. The development of novel activity-based probes has provided new tools to investigate the role of the individual catalytic activities of the proteasome in protein degradation. This will provide the opportunity to rationally design targeted, potent and less toxic second-generation proteasome inhibitors. It is clear that the proteasome is, and will continue to be, an important therapeutic target in haematological malignancies.

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Abbreviations: UPP: ubiquitin proteasome pathway, CT-L: chymotrypsin-like, T-L: trypsin-like, PGPH: peptidylglutamyl peptide hydrolyzing, IFN-gamma: interferon-gamma, TNF-alpha: tumour necrosis factor-

alpha, MHC: major histocompatability, MM: multiple myeloma, NCI: national cancer institute, FDA: food and drug administration, EMEA: European agency for the evaluation of medicinal products, CLL: chronic lymphocytic leukaemia, ELISA: enzyme-linked immunosorbant assay, NHL: non-Hodgkin's lymphoma

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