The Expanding Role of Proteasome-Based Therapy in the Treatment of Hematologic Malignancies

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ABSTRACT

The Ubiquitin (Ub)+Proteasome pathway is the major cellular pathway for the selective degradation of nuclear and cytosolic proteins. The proteasome is the catalytic core of the Ub+Proteasome pathway and has become an intriguing new target in drug development and cancer therapy for the treatment of hematologic malignancies. Successful pharmacologic inhibition of the proteasome with the boron-containing small molecule bortezomib led to US Food and Drug Administration (FDA) approval for the treatment of multiple myeloma (MM). That clinical success has propelled tremendous interest and application of proteasome inhibition to an increasing number of hematologic malignancies. Inhibition of the proteasome results in the accumulation of multi-ubiquitinated proteins that are normally degraded through the tightly regulated Ub+Proteasome pathway. Such an accumulation leads to the stabilization of numerous cellular proteins that control the cell cycle, growth, proliferation and apoptosis. It is thought that the accumulation of multi-Ub~protein conjugates leads to apoptosis although there are numerous mechanisms proposed to explain how proteasome inhibition leads to cell death. However, not all patients respond to bortezomib-based therapy and moreover, those patients that do respond inevitably develop drug resistance. In addition, the mechanism of action for bortezomib remains incompletely characterized and, thus, newer proteasome inhibitors are needed and are in clinical development. The use of the proteasome inhibitor bortezomib has been expanded successfully from MM to other hematologic malignancies that include various lymphomas, Waldenström’s macroglobulinemia, Amyloidosis and Acute Myeloid Leukemia (AML). The proteasome is a validated therapeutic target and proteasome inhibitors modulate protein stability to effect tumor cell growth control and promote programmed cell death. Targeting the Ub+Proteasome pathway offers great promise in the treatment of hematologic and eventually solid tumor malignancies.
INTRODUCTION

In eukaryotic cells, the vast majority of cytosolic and nuclear proteins are degraded through the Ub+Proteasome pathway [1]. Proteins are targeted for degradation through the covalent ligation to Ub; a highly conserved 76 amino acid polypeptide that is covalently linked in an ATP-dependent manner to target substrates [2,3]. An ATP-dependent high molecular mass complex known as the 26S proteasome then recognizes and degrades the multi-ubiquitinated protein conjugates (Fig. 1). The Ub+Proteasome pathway controls essential cellular processes such as cell-cycle progression, signal transduction, transcriptional regulation and programmed cell death [4-7]. Importantly, the Ub+Proteasome has been linked to cancer biology since it regulates the degradation of cyclins, oncoproteins and tumor suppressors (Fig. 2).

Intracellular protein degradation occurs in two major cellular systems that control the process of protein removal: the lysosomal and the non-lysosomal Ub+Proteasome systems. The discovery of the membrane-bound organelle, the lysosome in the 1950’s was important in establishing the lysosomal pathway for protein degradation. The lysosome was first thought to be the major site of protein degradation due to the action of hydrolases such as the cathepsins. However, further studies showed that most cellular proteins are degraded in a non-lysosomal process, which led to discovery of the Ub+Proteasome system. While lysosomal protein degradation is an exciting area of interest, further discussion is beyond the scope of this review.

Figure 1: The Ubiquitin+Proteasome Pathway for Protein Degradation
In eukaryotes, the 26S proteasome is a ~2.5-MDa structure that consists of over 30 different subunits and catalyzes the ATP-dependent degradation of nuclear and cytosolic proteins [8-12]. The 26S proteasome is a highly complex and mechanistically sophisticated proteolytic machine that recognizes, unfolds, translocates and cleaves multi-ubiquitinated proteins into peptides in a sequential “dis-assembly line” manner. It is reasonable that these multiple activities are enzymatically and physically linked to not only improve efficiency of proteolysis but also to prevent the escape of partially deubiquitinated or partially cleaved protein substrates (Fig. 3). This structure is found in both the cytoplasm and nucleus of all eukaryotic cells [9, 10, 16 and references therein]. The 26S proteasome consists of a barrel-shaped proteolytic core complex, known as the 20S proteasome that harbors the proteolytic activities and is capped at one or both ends by 19S regulatory complexes that fulfill multiple functions [11-17].

The 20S proteasome is a multicatalytic protease that exhibits various peptidase activities to function as the catalytic core the 26S proteasome [21-30]. All peptidase activities for proteolytic cleavage of the protein substrate reside within the 20S structure. In mammalian tissues, the 20S proteasome is comprised of up to 14 different proteins, with each subunit represented twice. The ancestor of the eukaryotic 20S proteasome is the eubacterial and archaeabacterial proteasome. The architectural characteristic of 20S proteasome is its composition of four seven-numbered rings, with two outer rings containing α subunits and two central rings composed of β subunits. It is apparent that the α subunits serve a structural function and the β subunits are responsible for the catalytic activity. These are classified as either α subunits or β subunits based on their similarities to the two subunits found in the 20S proteasome in the archaeabacterium Thermoplasma acidophilum [21-24]. In the archaeabacterial form the α and β subunits form four seven-membered rings that stack on top of each other to form a barrel-shaped structure [21-28]. Interestingly, during this process the number of active site β subunits has been reduced to three yielding six active site β subunits in the eukaryotic proteasome. In parallel, the
rather broad amino acid specificity of the archaeal β subunits has been narrowed down to three more distinct specificities, which are characterized as chymotrypsin-like, trypsin-like and peptidyl-glutamyl-peptide hydrolyzing activity, the latter cleaving after acidic amino acids [21-24]. Numerous proteasome-interacting subunits have been described in various eukaryotic organisms [29].

Figure 3: Structural Models of the Fully Assembled 26S Proteasome

The 19S regulatory complexes function in the recognition, unfolding and translocation of multi-ubiquitinated proteins [30-36]. It is thought that these regulatory complexes facilitate entry of the unfolded Ub~conjugates into the 20S proteasome to promote degradation and also to prevent erroneous or unselective proteolysis [29-36]. Assembly of the 26S proteasome complex and the attachment of the 19S particles is chaperone-mediated and an elaborate multi-step process (Figure 3). The 19S regulatory particle of yeast, which is the most thoroughly studied version is composed of at least 17 different subunits. The 19S complex itself can be split into two different subcomplexes that are known as the base and the lid. Both subcomplexes are linked to each other through subunit Rpn10 (known as S5a in mammals) (Rpn, Regulatory Particle Non-ATPase) (10 and references therein). The base is composed of a ring of six different ATPase subunits of the AAA-type (Rpt1 to Rpt6; Rpt, Regulatory Particle Triple “A” protein), which dock onto the α rings on both ends of the 20S core and two additional non-ATPase subunits, Rpn1 and Rpn2. The lid is composed of eight different subunits, Rpn3 and Rpn5 to Rpn11. Rpt5/S6a, is able to bind to multi-Ub chains, implying that this subunit forms part of the mechanism by which ubiquitylated substrates are recognized by the 26S proteasome [37]. Upon binding to the α ring of the 20S complex, the ATPase ring appears to form a narrow pore to allow the substrate to then enter the catalytic core. The ATPases may function as an anti-chaperone to unfold the protein to allow entry into the catalytic complex [38-40]. The other four subunits of the base subcomplex are all non-ATPases. One of these non-ATPases is Rpn10/Pus1/S5a that contains a Ub-interacting
motif which binds to Ub chains [38,39]. Also, the non-ATPase subunit Uch2/UCH37 subunit is homologous to Ub hydrolases [34,35]. The other two non-ATPase subunits, Rpn1/Mts4/S2 and Rpn2/S1 may play a structural role and link the ATPase ring of the base sub-complex and the lid sub-complex [36-38]. The lid sub-complex consists of eight non-ATPase subunits. Rpn11/Pad1/S13 has been shown to have a novel metalloprotease domain and may play a role in Ub recycling by cleaving the Ub chain from the protein substrate [39]. The function of the other subunits is under investigation but importantly, the lid complex shows a remarkable conservation in its overall structure/subunit composition with two other protein complexes; the COP9/Signalosome and the elF3 complex [41,42].

Selectivity in the Ub-pathway is mediated by E3 ubiquitin ligases that select proteins for degradation. E3 ligases function in concert with E2 Ub-conjugating (UBC) enzymes to elicit Ub attachment to a lysine on the target protein through isopeptide bond formation. The E3 Ub ligase targets specific protein substrates for degradation by the proteasome. In general, the E3 Ub ligase is involved in polyubiquitination. Upon covalent linkage of a first Ub moiety to the substrate, a second is attached to the first, a third is attached to the second, and so forth to mark the substrate for proteasomal degradation. However, there are some ubiquitination events that are limited to mono-ubiquitination, in which only a single Ub is added by the Ub ligase to a substrate molecule. Mono-ubiquitinated proteins are generally not targeted to the proteasome for degradation, but may instead be altered in their subcellular localization, function or binding partners. Further complicating matters, different lysines on Ub can be targeted by an E3 to make conventional (K-48) or alternative, e.g., K-11, K23, chains.

Table I. Selected Proteasome Inhibitors as Therapeutic Agents in Oncology

<table>
<thead>
<tr>
<th>Inhibitor</th>
<th>Compound Class</th>
<th>Mechanism</th>
<th>Status</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lactacystin</td>
<td>β-lactone prodrug</td>
<td>binds the β5 subunit</td>
<td>pre-clinical</td>
</tr>
<tr>
<td>Aclacinomycin</td>
<td>aklavinone</td>
<td>inhibits the ChTL activity</td>
<td>pre-clinical</td>
</tr>
<tr>
<td>Eponemycin</td>
<td>α'β'-epoxyketone</td>
<td>binds covalently the β5, β5i and β1i catalytic subunits of the 20S proteasome and selectively inhibits the 3 major proteasome proteolytic activities at different rates</td>
<td>pre-clinical</td>
</tr>
<tr>
<td>Eponemycin</td>
<td>α'β'-epoxyketone</td>
<td>binds covalently to the β5i, β5, β2i and β2 catalytic subunits and inhibits the ChTL activity</td>
<td>pre-clinical</td>
</tr>
<tr>
<td>PR-39</td>
<td>cathelicidin</td>
<td>highly basic arginine/proline-rich peptide that binds α-7 subunit</td>
<td>pre-clinical</td>
</tr>
<tr>
<td>Bortezomib</td>
<td>boronyl dipeptide</td>
<td>inhibits the ChTL activity</td>
<td>FDA-approved for MM (1st line, Rel, Ref)</td>
</tr>
<tr>
<td>Carfilzomib</td>
<td>epoxyketone</td>
<td>inhibits the ChTL activity</td>
<td>Phase II Rel/Ref MM</td>
</tr>
</tbody>
</table>

| FDA-approved for Mantle Cell Lymphoma |
| Early Phase- Refractory DLBCL |
| Phase II-Waldenstrom’s Macroglobulinemia |
| Phase II- Amyloidosis |
| Phase II Rel Solid Tumors |
TARGETING THE UB-PROTEASOME PATHWAY

Structurally, bortezomib is a modified dipeptidyl boronic acid derived from leucine and phenylalanine and is the first anti-neoplastic proteasome inhibitor. Bortezomib inhibits specifically the chymotrypsin-like activity, but this inhibition is sufficient to block all proteasomal catalytic activity [43, 44]. Proteasome inhibition induces apoptosis of cancer cells that are apparently more susceptible while simultaneously exerting a nontoxic effect on most normal cells. Disruption of the usually tightly regulated Ub+Proteasome system leads to the accumulation of high molecular weight Ub~protein conjugates that trigger apoptosis [45, 46]. Targeted protein degradation is a key mechanism for regulating numerous essential cellular signaling pathways that regulate proliferation, survival, and death. Furthermore, many proteasome substrates are found to be deregulated in tumor cells, including p53, cyclins, p21, p27, BRCA, IκB and NFκB. This makes modulation of proteasome activity an attractive strategy for cancer therapy and proteasome inhibitors have demonstrated antitumor activity alone or in combination with other therapies [47]. Numerous proteasome inhibitors, both naturally-occurring and synthesized are currently in pre-clinical development or early phase clinical trials (Table I).

MECHANISM OF PROTEASOME INHIBITOR INDUCED CELL DEATH

Bortezomib has shown cytotoxic activity against a variety of MM cell lines and in plasma cells from MM patients. A multitude of mechanisms have been proposed for the observed effect of bortezomib in MM cells. These putative mechanisms include promotion of apoptosis, inhibition of activation of nuclear factor-κB (NF-κB) in either or both the myeloma cells or the tumor microenvironment, reduction of adherence of myeloma cells to the bone marrow stroma, blocked production and reduced intracellular signaling of interleukin-6 (IL-6), blocked production and efficacy of angiogenic factors, defective regulation of effectors of apoptosis such as B-cell lymphoma-2 (Bcl-2), overexpression, accumulation and functional alteration in tumor suppressor protein p53 levels and apoptotic capacity, accumulation of unfolded or multi-ubiquitinated immunoglobulin chains, the global accumulation of unfolded or misfolded proteins, the global accumulation of Ub~protein conjugates, the loss of free Ub and, finally, the induction of endoplasmic reticulum (ER) stress. It is noteworthy that bortezomib is effective in other non-MM cell lines that include lymphomas, leukemias and amyloidosis which do not synthesize abundant amounts of immunoglobulins. Furthermore, bortezomib is generally ineffective against most solid tumors and solid tumor cell lines although the NF-κB pathway is dysregulated in these same solid tumors. One attractive hypothesis is that proteasome inhibition prevents the clearance of misfolded proteins by the ER–associated degradation (ERAD) pathway, resulting in enhanced ER stress that leads to cell death.

The transcription factor NF-κB is a tightly regulated positive mediator of T- and B-cell development, proliferation, and survival and the controlled activity of NF-κB is required for the coordination of physiologic immune responses. NF-κB controls the expression of many genes that regulate apoptosis, cell proliferation and differentiation. Deregulation of NF-κB activation is a hallmark of several lymphoid malignancies and is directly linked to advanced disease. Constitutive NF-κB activation can promote continuous lymphocyte proliferation and survival and has recently been recognized as a critical factor in pathogenesis. Various molecular events lead to deregulation of NF-κB signaling in MM, Hodgkin’s disease and various T- and B-cell non-Hodgkin
lymphomas. The pivotal role of the NF-κB pathway in the inhibition of apoptosis, tumor promotion and progression, and the observation that NF-κB is constitutively activated in a large number of hematologic malignancies suggests that NF-κB inhibitors would be useful in cancer therapy. Proteasome inhibitors such as bortezomib may be useful to block activation of the NF-κB pathway. However, it should be noted that NF-κB functions in activation of the innate and adaptive immune responses and that inhibitors of this pathway may also enhance the chemotherapy-induced apoptosis of normal hematopoietic progenitors.

### Table II. Selected Bortezomib Regimens in Hematologic Malignancies

<table>
<thead>
<tr>
<th>Hematologic Malignancy</th>
<th>Year</th>
<th>Patient Population</th>
<th>Patients</th>
<th>Regimen</th>
<th>Response</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Multiple Myeloma</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>- SUMMIT</td>
<td>2003</td>
<td>Relapsed/</td>
<td>202</td>
<td>Bortezomib 1.3 mg/m² on D1, 4, 8, &amp; 11</td>
<td>35 % ORR</td>
</tr>
<tr>
<td></td>
<td></td>
<td>refractory</td>
<td></td>
<td>- 3 week cycle</td>
<td>- 27% CR/PR</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>- Up to 8 cycles (24 weeks)</td>
<td>- 7 % MR</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>- PO Dex (20 mg day of/after) for suboptimal response</td>
<td></td>
</tr>
<tr>
<td>- CREST</td>
<td>2004</td>
<td>Relapsed/</td>
<td>54</td>
<td>Bortezomib 1-1.3 mg/m² on D1, 4, 8, &amp; 11</td>
<td>37% CR/PR</td>
</tr>
<tr>
<td></td>
<td></td>
<td>refractory</td>
<td></td>
<td>- 3 week cycle</td>
<td>- 1 mg Bortezomib</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>- Up to 8 cycles (24 weeks)</td>
<td>50 % CR/PR</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>- PO Dex (20 mg day of/after) for suboptimal response</td>
<td>- 1.3mg Bortezomib</td>
</tr>
<tr>
<td>- VISTA</td>
<td>2008</td>
<td>Initial Treatment</td>
<td>682</td>
<td>MP +/- Bortezomib 1.3mg/m²</td>
<td>&gt;PR - 71% v 35% CR</td>
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<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>- 9 6-week cycles</td>
<td>- 30% v 4%</td>
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<td></td>
<td></td>
<td></td>
<td></td>
<td>- MP given on D1-4</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>- Bor - D1, 4, 8, 11, 22, 25, 29, &amp; 32 – C1-4</td>
<td></td>
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<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>- Bor - D1, 8, 22, &amp; 29 – C5-9</td>
<td></td>
</tr>
<tr>
<td><strong>Mantle Cell Lymphoma</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>- PINNACLE</td>
<td>2006</td>
<td>Relapsed/</td>
<td>155</td>
<td>Bortezomib 1.3 mg/m² on D1, 4, 8, &amp; 11</td>
<td>33-40% ORR</td>
</tr>
<tr>
<td></td>
<td></td>
<td>refractory</td>
<td></td>
<td>- 3 week cycle</td>
<td>- 8 % CR+CRu</td>
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<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>- Up to 1 yr</td>
<td></td>
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<tr>
<td>- Update</td>
<td>2009</td>
<td>Relapsed/</td>
<td>155</td>
<td>Bortezomib 1.3 mg/m² on D1, 4, 8, &amp; 11</td>
<td>32% ORR</td>
</tr>
<tr>
<td></td>
<td></td>
<td>refractory</td>
<td>(55 pts-</td>
<td>- 3 week cycle</td>
<td>- 8 % CR+CRu</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>still f/u)</td>
<td>- Up to 17 cycles/4 cycles beyond CR</td>
<td></td>
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<tr>
<td><strong>DLBCL</strong></td>
<td></td>
<td></td>
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<td></td>
<td></td>
</tr>
<tr>
<td>- DA-EPOCH+Bortezomib 0.7-1.7 mg/m² on</td>
<td>2009</td>
<td>Relapsed/</td>
<td>44</td>
<td>DA-EPOCH +Bortezomib</td>
<td>42% ORR</td>
</tr>
<tr>
<td>D1 &amp; 4</td>
<td></td>
<td>refractory</td>
<td></td>
<td>- 3 weeks cycles</td>
<td>- 23 % CR</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>- Up to 6 cycles</td>
<td>83% v 13% ORR</td>
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<td></td>
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<td></td>
<td></td>
<td></td>
<td>- ABC v. GCB</td>
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<tr>
<td><strong>PTCL</strong></td>
<td></td>
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<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>- Bortezomib 1.3 mg/m² on D1, 4, 8, &amp; 11</td>
<td>2007</td>
<td>Relapsed/</td>
<td>12</td>
<td>Bortezomib 1.3 mg/m² on D1, 4, 8, &amp; 11</td>
<td>67% ORR</td>
</tr>
<tr>
<td></td>
<td></td>
<td>refractory</td>
<td></td>
<td>- 3 week cycle</td>
<td>- 17 % CR</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>- Up to 6 cycles</td>
<td>50% PR</td>
</tr>
<tr>
<td><strong>Waldenstrom’s Macroglobulinemia</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>- Chen et al [84]</td>
<td>2007</td>
<td>Untreated/</td>
<td>27</td>
<td>Bortezomib 1.3 mg/m² on</td>
<td>26% ORR</td>
</tr>
<tr>
<td></td>
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PROTEASOME INHIBITORS IN MULTIPLE MYELOMA

Multiple myeloma (MM) is a B-cell disorder that produces malignant plasma cells and represents the second most common hematological malignancy [48-50]. The incidence and prevalence of MM are similar in the US and Europe with a total of ~40,000 cases diagnosed annually in both parts of the world. The course of MM is characterized by an asymptomatic or subclinical phase before diagnosis, a chronic phase lasting several years and an aggressive terminal phase. MM leads to progressive morbidity and eventual mortality by lowering resistance to infection and causing significant skeletal destruction with bone pain, pathological fractures, and hypercalcemia), anemia, renal failure, neurological complications and hyperviscosity.

The outcome of MM patients treated with conventional chemotherapy with or without high-dose therapy/autologous SCT has not been satisfactory, with median survivals ranging from 2 to 3 years for older patients and from 5 to 6 years for younger patients. Front-line approval of bortezomib was based upon the phase III VISTA (VELCADE as Initial Standard Therapy in MM: Assessment with Melphalan and Prednisone) trial [51-55] (Table II). VISTA was a randomized, international, open-label trial compared VELCADE in combination with the current standard of care versus the standard of care alone in 682 previously untreated patients who were unsuitable for stem cell transplantation. VISTA demonstrated that patients receiving the standard of care plus VELCADE achieved a greater overall survival (OS) rate at 24 months (83%) compared to those who only received standard of care alone (70%). Furthermore, the CR rate in the VELCADE combination arm was 30% vs. 4% in the standard of care arm alone.

Bortezomib was initially approved as a third-line treatment of relapsed and refractory MM by the FDA under the accelerated approval program based upon results of the SUMMIT trial [53]. The SUMMIT trial studied 202 patients who had received at least two prior therapies and showed disease progression. Clinical remissions by SWOG criteria were observed in 17.6% of patients (95CI: 12%, 24%) and response lasted a median time of 1 year. In addition, the CREST trial in 54 subjects with relapsed MM showed similar
positive and promising responses [54-57].

In recent years, the availability of the novel effective immunomodulatory drugs (IMID’s), e.g., thalidomide and lenalidomide, in combination with bortezomib has resulted in a significant improvement in the long-term outcome. Moreover, novel drugs targeting new molecular pathways associated with Myelomagenesis and newly identified targets such as new proteasome inhibitors, the aggresome and histone deacetylases (HDACs) that act through many different mechanisms of action have been developed and are currently being investigated in clinical trials to further improve the outcome of patients with MM [58-60]. The treatment of MM patients with relapsed or refractory disease is even more challenging. Regimens containing IMID’s, particularly bortezomib in combination with IMID’s can be effective “rescue” regimens for patients refractory to primary treatment and were first approved for patients with relapsed or refractory MM. However, the duration of response is limited and all patients will eventually develop progressive disease. Among the next generation of novel drugs, the most promising are the IMID pomalidomide and the proteasome inhibitors carfilzomib (an epoxyketone), NPI-0052 (a bicyclic β-lactone γ-lactam) and a second-generation boron-based proteasome inhibitor MLN-9708. Furthermore, the HDAC inhibitors, particularly vorinostat (SAHA) and panobinostat (LBH 589) are gaining prominence in combination with IMID’s for patients refractory to primary treatment and were first approved for patients with relapsed or refractory MM. Hence this combination is being evaluated in the PANORAMA program (PANobinostat ORAil in Multiple myeloma) Phase III (PANORAMA 1) and Phase II (PANORAMA 2) studies. PANORAMA 2 is a U.S.-based, multicenter, single-arm study to evaluate the efficacy of PAN + bortezomib+ DEX in pts with relapsed or bortezomib-refractory MM. In a phase Ib MM study, combination of oral PAN with bortezomib displayed a predictable and manageable safety profile with limited neurotoxicity and clinical efficacy seen with CR + PR + MR in 68% of pts, including 8 of 13 (62%) bortezomib-refractory pts. The combination of oral PAN and bortezomib has a predictable and manageable safety profile with promising activity in pts with relapsed, refractory MM, including MM refractory to prior bortezomib-based therapy.

BORTEZOMIB USE IN MANTLE CELL LYMPHOMA

Mantle cell lymphoma (MCL) is an aggressive lymphoma that is refractory to most current chemotherapy regimens has a poor outcome, especially when first-line treatment has failed [61, 62]. Several clinical studies have demonstrated that bortezomib has clinical effects on MCL and it appears that new therapeutic strategies such as radioactively labeled antibodies or molecular targeting agents, e.g. bortezomib, are warranted to further improve the clinical outcome of MCL. First line treatment of mantle cell lymphoma includes rituximab-based combination chemotherapy, e.g., hyperCVAD, CHOP, EPOCH [63, 64]. The PINNACLE trial, evaluated the effect of bortezomib in MCL patients that had failed at least one prior treatment. Results demonstrated a substantial response rate of 31% and disease progression was delayed by more than 6 months in >50% of MCL patients [65-68]. The PINNACLE study demonstrated a median time to progression (TTP) of 6.7 months, median time to next therapy (TTNT) of 7.4 months and median overall survival (OS) of 23.5 months. In responding patients, median TTP was 12.4 months, median duration of response (DOR) was 9.2 months, median TTNT was 14.3 months, and median OS was 35.4 months. The one-year survival rate was 69% overall and 91% in responding patients. Important, activity was seen in patients with refractory disease and toxicity was generally

Panobinostat (PAN; LBH589) is a potent pan-deacetylase inhibitor (pan-DACi) proposed to also disrupt aggresome and HSP90 function and thus to promote cytotoxic misfolded protein aggregate formation and eventually cell death. Combination with bortezomib has shown synergistic cytotoxicity in preclinical studies.
manageable. Overall, the study indicated that bortezomib was associated with lengthy responses and notable survival in patients with relapsed or refractory MCL, with considerable TTP and TTNT in responding patients, to suggest a substantial clinical benefit and validated the use of bortezomib-based therapy in the treatment of relapsed and/or refractory MCL. Furthermore, the PINNACLE study was performed in a multicenter international setting and supports the accelerated addition of bortezomib as a treatment for relapsed MCL. Bortezomib was granted approval for treatment of relapsed or refractory MCL in 2006 represented an important step in second-line treatments.

BORTEZOMIB IN DIFFUSE LARGE B CELL LYMPHOMAS

Diffuse large-B-cell lymphoma (DLBCL) is an aggressive form of non-Hodgkin lymphoma (NHL) that accounts for 30% to 40% of the total incidence of NHL [69, 70]. Patients with DLBCL have been divided into 3 groups according to their gene profiling patterns: germinal-center B-cell–like DLBCL (GCB-DLBCL), activated B-cell–like DLBCL (ABC-DLBCL), and mediastinal or unclassified type 1 [71-73]. These subcategories are characterized by distinct differences in survival, responsiveness to various chemotherapies and dependence on signaling pathways, particularly the nuclear factor-kB (NF-κB) pathway. Aside from those patients eligible for allogeneic or autologous SCT, combination chemotherapy offers a potentially curative option for a subset of DLBCL patients. However, responses to cytotoxic chemotherapy, e.g., rituximab with cytoxan, hydroxyrubicin, oncovin, and prednisone, vary considerably depending on multiple factors, including disease stage and genetic profile, among others. In particular, patients with the ABC-DLBCL subtype, which is NF-κB-dependent, appear to have a significantly worse prognosis than the other subtypes. Collectively, these considerations have prompted the search for more effective treatment strategies in DLBCL.

In recent years considerable progress has been made in the treatment of patients with B-cell NHL [74-76]. Although responses can be achieved with combination chemotherapy regimens, a substantial proportion of patients are still not cured. In recent years, the knowledge of the cellular and molecular biology of distinct types of B-cell NHL have led to the development of a new class of drugs that specifically targets unique disease-specific pathways. ABC DLBCL has a worse survival after upfront chemotherapy and is characterized by constitutive activation of the antiapoptotic nuclear factor–kappa B (NF-κB) pathway [78, 79]. Results suggest that bortezomib enhanced the activity of chemotherapy in ABC but not the GCB forms of DLBCL and provided a rational therapeutic approach based on the genetically distinct DLBCL subtypes [78].

A recent study demonstrated that the second-generation irreversible proteasome inhibitor carfilzomib was dramatically potentiated in combination with histone deacetylase inhibitors (HDACi’s) in DLBCL cells. Efficacy was seen in both the ABC- and GCB subtypes of DLBCL [80]. Although the proteasome inhibitor bortezomib has shown very limited single-agent activity in DLBCL, results of a recent study suggest that addition of bortezomib to standard chemotherapy improves outcomes in ABC-DLBCL. In addition, it has been proposed that synergistic interactions between HDAC and proteasome inhibitors in malignant hematopoietic cells reflect inhibition of HDACI-mediated NF-κB activation a phenomenon that may stem from RelA acetylation. However, it is noteworthy that HDACIs blocked NF-κB activation and promoted carfilzomib lethality in both ABC- and GC-type DLBCL cells, arguing against the possibility that this interaction is specific only for DLBCL cells addicted to the NF-κB pathway. In addition, there is evidence that proteasome inhibitor lethality may involve factors other than NF-κB inhibition; and in a recent study that used myeloma cells, bortezomib was unexpectedly shown to actually activate NF-κB. The initial rationale for the use of bortezomib in MM was the potential inhibition of NF-κB activity by blocking proteasomal degradation of IkBα. Bortezomib inhibits inducible NF-κB activity; however, its impact on constitutive NF-κB activity in MM cells is a point of discussion [81, 82]. Recent studies demonstrated that bortezomib-induced NF-κB activation was mediated via the canonical pathway. Moreover, other class of proteasome inhibitors also induced IkBα down-regulation associated with NF-κB activation. Bortezomib triggered phosphorylation of IKBα and
its upstream receptor interacting protein2 (RIP2), whereas the IKKβ inhibitor MLN120B blocked bortezomib-induced IκB downregulation and NF-κB activation, indicating a crucial role for RIP2/IKKβ signaling plays crucial role in bortezomib-induced NF-κB activation. Moreover, IKK inhibitors enhanced bortezomib-induced cytotoxicity. Our studies therefore suggest that bortezomib-induced cytotoxicity cannot be fully attributed to inhibition of canonical NF-κB activity in MM cells. Potentially, the high levels of immunoglobulin production and ER-Golgi protein transport would sensitize MM cells to proteotoxic stress, providing an attractive explanation for bortezomib’s clinical activity and a potential means of identifying bortezomib-based combination approaches that will display even greater antitumor effects [82]. Consequently, the possibility that HDAC inhibitors potentiate the effect of carfilzomib through mechanisms unrelated to NF-κB cannot be excluded.

PERIPHERAL T-CELL LYMPHOMAS (PTCL)

Peripheral T-cell lymphomas (PTCL’s) are a heterogeneous group of generally aggressive cancers that comprise ~15% of all NHL’s. There are multiple forms of aggressive PTCL’ and the current treatment approaches are similar to those used for B-cell lymphomas. These regimens have been used for PTCL patients with either localized or advanced stage disease and with autologous hematopoietic cell transplantation utilized in selected patients as well. Phase II clinical trials of bortezomib have demonstrated promising results in the treatment of patients with B-cell lymphomas [83]. NCCN guidelines now recommend bortezomib as second-line therapy for patients with relapsed or refractory PTCL in those patients that are not candidates for high-dose chemotherapy or autologous stem cell transplant. A multi-center phase II trial of bortezomib combined with a standard chemotherapy regimen in the treatment of previously untreated individuals with PTCL is ongoing.

WALDENSTRÖM’S MACROGLOBULINAEMIA

Waldenström’s macroglobulinemia is a rare type of slow-growing, NHL that results in the overproduction of a monoclonal immunoglobulin (IgM) antibody by abnormal plasma cells. The malignant plasma cells multiply uncontrollably to produce high levels of IgM in the blood and eventually cause hyperviscosity. Treatment of symptoms that develop in WM patients may include plasmapheresis, chemotherapy and newly emerging biological therapies such as bortezomib. Bortezomib demonstrates potent activity against WM primary cells and cell lines [84, 85]. Interestingly, bortezomib has shown activity as a single agent as salvage therapy in WM patients. Moreover, ORR’s have reached 60-80% and major response rates have been observed in up to 50-60% of cases. A recent clinical study found that bortezomib was in fact an active agent in relapsed and refractory WM [86]. In this study, 27 patients with WM received up to eight cycles of bortezomib and all but one of the patients had relapsed or refractory disease. Following therapy, median serum IgM levels declined from 4.6 to 2.1 g/dL (p<0.0001) and the ORR was 85%. Common grade III/IV toxicities were sensory neuropathies (22.2%), leukopenia (18.5%), neutropenia (14.8%), dizziness (11.1%), and thrombocytopenia (7.4%).

In a phase II clinical study [86] evaluated the effectiveness and toxicity of single-agent bortezomib in WM. Symptomatic patients, untreated or previously treated, received bortezomib 1.3 mg/m2 intravenously days 1, 4, 8, and 11 on a 21-day cycle until two cycles past complete response (CR), stable disease (SD) attained, progression (PD), or unacceptable toxicity. A median of six cycles (range of 2 to 39) of bortezomib was administered. Twenty-one patients had a decrease in IgM of at least 25%, with 12 patients (44%) reaching at least 50% IgM reduction. Using both IgM and bi-dimensional criteria, responses included 7 PRs (26%), 19 SDs (70%), and 1 PD (4%). Total response rate was 26% and reductions in IgM levels were prompt with nodal responses lagging. The slower response in nodal disease may require prolonged or dose-adjusted schedule to allow longer treatment and avoid unwanted toxicities.

The recent WMCTG clinical trial 05-180 examined the activity of bortezomib, dexamethasone and rituximab in 23 symptomatic, untreated WM patients [87]. Patients received four cycles of induction with all three agents followed by four more cycles with each given
three months apart. The results demonstrated the bortezomib/ dexamethasone/ rituximab combination produced a durable response with high rates of response and CR. A Phase II trial of weekly bortezomib in combination with rituximab in relapsed/ refractory WM in 37 patients that had received one or more therapies demonstrated that this combination had significant activity and minimal neurological toxicity. It is clear that future studies are warranted and that bortezomib-based therapy holds promise as a leading therapeutic in the treatment of WM.

**PRIMARY SYSTEMIC AMYLOIDOSIS**

AL amyloidosis is the most common form of systemic amyloidosis in the U.S and is a rare disease that occurs in approximately eight of every 1,000,000 persons. It affects males and females equally and usually develops after the age of 40. At least 15 types of amyloidosis have been identified and each type is associated with deposits of a different protein. It is noteworthy that AL amyloidosis occurs in 5-15% of patients diagnosed with MM. Despite severe difficulties in the diagnosis and treatment of AL amyloidosis, patients with this disease can achieve long-term survival if properly managed. The optimal course of management requires early, accurate diagnosis, correct identification of the amyloid type, institution of prompt, appropriate and effective therapy and close follow-up with careful supportive care. Systemic involvement that affects the heart, kidneys and liver renders patients with worse outcomes and generally confers reduced response to various forms of chemotherapy. Bortezomib as treatment for systemic light chain amyloidosis has been reported in a phase II clinical study [90], a small case series and case reports [89, 91]. NCCN's Drug and Biologics Compendium lists systemic light chain amyloidosis as an indication for bortezomib. However, the NCCN guidelines indicate that this and other treatments for systemic light chain amyloidosis should be provided in the context of a clinical trial. Kastritis and colleagues [91] assessed the activity and feasibility of the combination of bortezomib and dexamethasone (BD) in patients with AL amyloidosis. A total of 18 patients were treated with BD including 7 that had relapsed or progressed after prior therapies. Eleven (61%) patients had 2 or more organs involved and the kidneys and heart were affected in 14 and 15 patients, respectively. Among the evaluable patients, 94% had a hematological response and 44% had a hematological CR, including all 5 patients who had not responded to prior high dose dexamethasone-based treatment and 1 patient under dialysis. Five patients (28%) had a response in at least 1 affected organ. Hematological responses were rapid (median of 0.9 months) and median time to organ response was 4 months. The authors concluded that the combination of BD is feasible in patients with AL amyloidosis. Patients achieve a rapid hematological response and toxicity can be managed with close follow-up and dose adjustment. This treatment may be a valid option for patients with severe heart or kidney impairment.

Wechalekar et al [92] reported preliminary observations on the effectiveness of bortezomib in 20 patients with primary (AL) amyloidosis whose clonal disease was active despite prior treatment with a median of 3 lines of prior chemotherapy that included a thalidomide combination in all cases. Patients received a median of 3 (range of 1 to 6) cycles of bortezomib and 9 (45%) patients received concurrent dexamethasone. Three (15%) patients achieved complete hematological responses, and a further 13 (65%) achieved partial responses. Fifteen (75%) patients experienced some degree of toxicity, which in 8 (40%) cases resulted in discontinuation of bortezomib. Hence, bortezomib shows promise in the treatment of systemic AL amyloidosis.

It is mechanistically relevant that tumor cells that secrete amyloidogenic monoclonal proteins are similar to malignant plasma cells and demonstrate sensitivity to bortezomib. Since the cytotoxic effect of bortezomib on myeloma cells cannot be completely explained by the inhibition of the NF-κB pathway other mechanisms are definitely plausible. It is noteworthy that both amyloidogenic and plasma cells exhibit enhanced protein synthesis activity and protein secretory function. An attractive hypothesis is the accumulation Ub-conjugated proteins results in enhanced ER stress that then triggers apoptosis. Since normal cells may have less synthetic activity they may be less susceptible to the effects of proteasome inhibition. Hence, protein synthesis,
folding and ER transport may be linked to induction of ER stress by bortezomib-induced accumulation of ubiquitinated proteins. Cells with enhanced secretory activity may be further sensitized to proteasome inhibition. Mature plasma cells are terminally differentiated elements of the B lymphocytic lineage with a highly developed ER specialized in immunoglobulin secretion. ER quality control systems prevent mutated, misfolded or unfolded proteins from proceeding to the Golgi and the accumulation of misfolded proteins in the ER lumen initiates the unfolded protein response (UPR). Upon initiation of the UPR, cells then initiate steps to handle the accumulation of unfolded proteins. Protein translation is attenuated, transcription of genes that fulfill protein folding functions such ER resident chaperones and folding enzymes is altered and the ER-specific protein degradation (ERAD) system is activated. Entry of proteins into the ER and the stability of mRNA encoding secretory proteins is selectively inhibited as well. When these rescue mechanisms are not adequate to eliminate misfolded proteins from the ER, apoptotic pathways are then activated.

PROTEASOME INHIBITORS IN ACUTE MYELOID LEUKEMIA

Acute myeloid leukemia (AML) is an immunophenotypically heterogeneous group of diseases, with CD34+ cases being associated with drug resistance and poor outcome [93, 94]. Nearly 80% of patients with AML achieve a complete remission with induction chemotherapy. However, a high proportion of patients relapse, and eventually die of their disease. This is important, particularly due to the heterogeneity of AML, including a wide array of genetic lesions and immunophenotypic profiles. The CD34 antigen identifies early progenitor cells and, accordingly, AML can be divided into immature and mature forms (CD34+ and CD34−, respectively), the former subset associated with drug resistance and poorer outcome, as compared to the more mature CD34− cases. Moreover, at relapse, blast cells usually display a more immature phenotype, as a reflection of drug resistance and it has been suggested that the presence of an immature phenotype with age and cytogenetics represent important prognostic factors in AML.

A recent study demonstrated that bortezomib has marked in vitro activity in both AML cell lines and fresh blast cells obtained from AML patients [93]. Moreover, the antileukemic effect of bortezomib was seen in CD34+ and CD34− AML cells to suggest that proteasome inhibitors may in fact overcome the drug resistance associated with the immature CD34+ phenotype. Importantly, bortezomib was significantly more active than doxorubicin in the immature CD34+ cells, while there were no differences in its action on CD34− cells. Based upon these studies, a detailed analysis of the in vitro activity and mechanism of action of bortezomib on AML cells was performed [94]. Bortezomib was shown to be anti-proliferative and induced apoptosis in chronic phase (CP) CD34+ CML cells at clinically achievable concentrations. Moreover, bortezomib targeted primitive CML cells, with effects on CD34+38−, long-term culture-initiating (LTC-IC) and nonobese diabetic/severe combined immunodeficient (NOD/SCID) repopulating cells. Bortezomib was not selective for CML cells and induced apoptosis in normal CD34+38− cells and the effects against CML cells were seen when bortezomib was used alone or in combination with dasatinib. Bortezomib caused proteasome but not BCR-ABL inhibition and was effective in inhibiting proteasome activity and inducing apoptosis in cell lines expressing BCR-ABL mutations, including T315I. By targeting both TKI-insensitive stem and progenitor cells and TKI-resistant BCR-ABL mutations, bortezomib may offer a potential therapeutic option in certain leukemias.

CONCLUDING REMARKS

The Ub+Proteasome pathway has been validated as a therapeutic target in oncology and revolutionized treatment of certain hematologic malignancies. The small molecule bortezomib was the first proteasome inhibitor to enter clinical use and received FDA approval for the treatment of patients with multiple myeloma, therefore validating inhibition of the proteasome as an anticancer target. Preclinical data also has demonstrated the synergistic effect of bortezomib with other chemotherapeutic agents and the ability to overcome drug resistance of targeted therapy in MM has since led to the development and investigation of more than 30 new
compounds in this disease and in the related plasma cell dyscrasias WM and Amyloidosis. The last decade has marked a new era in the treatment of diseases characterized by B cell disorders and monoclonal gammopathies and a paradigm shift has evolved to employ novel therapeutic agents that target the malignant clone and the surrounding bone marrow microenvironment. The finding that tumor cells are more sensitive to alteration of the Ub+Proteasome pathway and that inhibitors of the proteasome are useful in certain types of cancer therapy suggests that further investigation should provide new therapeutic strategies to attack proliferative disorders. Additionally, the combination of bortezomib with chemotherapeutic agents has yielded high response rates comparable or superior to those achieved in the SCT setting and more applicable to the elderly or frail patient populations. Together, these therapies should lead to higher response rates, more durable responses, less toxicity and prolonged survival for patients, making hematologic malignancies and certain plasma cell dyscrasias increasingly chronic and treatable diseases.

Inhibition of the proteasome peptidase activity prevents the degradation of all proteins normally degraded through the Ub+Proteasome pathway. The demonstrated efficacy of proteasome inhibition on cancer cells was at first surprising, because the proteasome is responsible for the degradation of most intracellular proteins and the initial expectation was that it would be equally toxic to normal cells. Recent evidence that pharmacological inhibition of the Ub+Proteasome pathway can be efficacious in the treatment of human cancers has set the stage for attempts to selectively inhibit the activities of disease-specific components within the pathway. Targeting the proteasome, while proven clinically efficacious, does not allow for this level of specificity. An increase in target specificity could increase the effectiveness of a therapeutic intervention while simultaneously diminishing unwanted side-effects and toxicities. Targeting of other components within the Ub+Proteasome pathway, e.g., E3 ligases, may provide the highest level of specificity and the preclinical successes of the MDM2 inhibitors are encouraging, although no E3 inhibitor has as of yet entered human clinical trials. These important findings highlight the need for further research into the role of Ub+Proteasome system and protein homeostasis in cancer and other diseases. Since the greatest amount of specificity is present in the E3 Ub conjugation step, it is anticipated that drugs that target individual E3 ligases, possibly by blocking substrate binding, are likely to provide the greatest level of selectivity. However, E3’s are unconventional enzymes and the development of specific inhibitors represents a significant challenge. Specifically, it is likely that E3’s bind target substrates stoichiometrically and therefore do not function catalytically to select substrates for ubiquitination. Moreover, somewhat problematic is that each of these E3 ligases targets a finite number of substrates that are used in many cellular processes. Inhibition of E3 Ub ligase function globally is therefore likely to interrupt many diverse pathways while inhibition of a single E3 ligase could have a minimal effect. Pharmacologic targeting of E3 ligases could, in theory, provide an enhanced therapeutic index greater than targeting the proteasome because specific E3s ubiquitinate a small number of proteins, whereas the proteasome degrades all ubiquitinated proteins. A more detailed understanding of this system as well as the impact of targeting specific steps within this pathway has the potential to result in more disease-specific therapies with fewer off-target effects.

The Ub+Proteasome pathway is a highly complex, tightly regulated system that targets most cellular proteins for degradation. Recently, novel parallel pathways have been described that attach Ub-like molecules, e.g., Small Ubiquitin-like MOdifier (SUMO) and NEDD8 to target proteins [95, 96]. Identifying the underlying components and steps in these pathways is essential for the development of novel, mechanism-based drugs. The Ub+Proteasome, Sumoylation and Neddylation pathways are ideal candidates as cytotoxic drugs that block key oncogenic steps in cancer and result in a proapoptotic cancer cell milieu. The therapeutic success of proteasome inhibitors in the treatment of hematological malignancies validates the proteasome as viable therapeutic target. Future efforts will investigate the therapeutic value of bortezomib in combination with other targeted and chemotherapeutic agents, the efficacy of
second-generation proteasome inhibitors and the identification of other components within the Ub+Proteasome pathway that may serve as novel targets. Finally, targeting components in the Sumoylation and Neddylation pathways will also be pursued to yield more selective therapeutics to develop mechanism-based drugs that modulate the Ub+Proteasome pathway.

**LIST OF ABBREVIATIONS**

MM – multiple myeloma  
Ub – ubiquitin  
WM – Waldenström’s macroglobulinemia  
AML – acute myeloid leukemia  
SCT – stem cell transplantation  
CR – complete remission  
PR – partial remission  
SD – stable disease  
PD – progressive disease  

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