THE PROTEASOME: A SUITABLE ANTINEOPLASTIC TARGET

Julian Adams

The proteasome is an abundant multi-enzyme complex that provides the main pathway for degradation of intracellular proteins in eukaryotic cells. As such, it controls the levels of proteins that are important for cell-cycle progression and apoptosis in normal and malignant cells; for example, cyclins, caspases, BCL2 and nuclear factor of κB. A proteasome inhibitor — bortezomib — has been developed that has shown efficacy as an anticancer agent in the clinic. How can targeting such a universal, broadly active cellular component provide the selectivity and specificity that are required for cancer therapeutics?

APOPTOSIS
Programmed cell death is triggered through two main pathways. One involves cell-surface death receptors (such as CD95), which activate caspase-8. The other is initiated by mitochondrial perturbations, leading to release of pro-apoptotic molecules from the intermitochondrial membrane space, which stimulates the caspase-9 proteolytic pathway.

PROTEASE
An enzyme that breaks down a protein by causing cleavage of a peptide chain at a specific position (that is, when the carbonyl group is part of a specific amino acid). Trypsin is a mammalian intestinal protease that specifically hydrolyses at lysine or arginine.

Infinity Pharmaceuticals, Inc., 780 Memorial Drive, Cambridge, Massachusetts 02139, USA.
e-mail: Julian.Adams@ipi.com
doi:10.1038/nrc1361

Tumorigenesis is characterized by aberrant responses to cellular signals that normally regulate cell growth, differentiation, function and programmed cell death (APOPTOSIS)[1]. These cellular processes are, in turn, regulated by the transcription, translation and degradation of regulatory proteins, so their disruption can contribute to tumour formation. The role of protein-degradation pathways has, however, frequently been overlooked. Protein degradation is not a simple process and many regulatory pathways are involved in the control of what, when, where and how proteins are degraded. For example, PROTEASES must be physically segregated or somehow kept in check to prevent indiscriminate polypeptide degradation, and misfolded proteins that should be destroyed must be distinguished from polypeptides that are still in the process of folding.

Cells use incredibly diverse mechanisms to regulate intracellular protein stability and degradation. Although there might be additional mechanisms for intracellular cytosolic protein degradation that have yet to be characterized, two main routes have been identified: lysosomes and proteasomes (FIG. 1). Simplistically, lysosomes can be described as the cellular organelles that are responsible for degrading extracellular and transmembrane proteins, whereas proteasomes degrade intracellular proteins[2]. Lysosomes are cytoplasmic membrane-bound vesicles that enclose proteases and other hydrolytic enzymes. The compartmentalization of these proteolytic enzymes prevents them from degrading cellular proteins in an uncontrolled fashion. Until proteasomes were discovered[3,4] and the scope of their activity was studied, lysosomes were considered to be the principal apparatus through which proteins were degraded within the cytoplasm. It is now understood that proteasomes are key to intracellular protein degradation and that orderly degradation is crucial to the maintenance of normal cell functions.

Structure and localization of the 26S proteasome

The functionally active 26S proteasome is a very large 2.4 MDa ATP-dependent proteolytic complex that is found in the cytoplasm and nucleus of eukaryotic cells. It consists of a 20S core catalytic cylindrical complex capped at both ends by 19S regulatory subunits (FIG. 2)[5–7]. The proteasome identifies proteins that have been targeted for degradation by their UBIQUITIN 'tag', although ubiquitylation is not a prerequisite for degradation for every protein.

The 20S proteasome. The 20S proteasome is a complex of 28 protein subunits that are organized into 4 stacked rings to create a cylindrical structure (for reviews, see REFS 8,9). The top and bottom rings of the 20S proteasome are formed by seven polypeptides, termed the α-subunits because of their sequence similarity. The two inner rings consist of seven β-subunits that form a central chamber — these contain the enzymatically active sites of the proteasome complex. Three (β1, β2 and β5) of the seven β-subunits

Summary

- The proteasome is an abundant, catalytic complex that is found in both the nucleus and cytoplasm of eukaryotic cells.
- The function of the proteasome is to degrade or process intracellular proteins, some of which represent mediators of cell-cycle progression and apoptosis, such as the cyclins, caspases, BCL2 and nuclear factor of κB (NF-κB).
- Malignant cells are more susceptible to certain proteasome inhibitors, which might be explained, in part, by the reversal or bypass of some of the effects of the mutations in cell-cycle and apoptotic checkpoints that have led to tumorigenesis.
- Other explanations for this differential susceptibility include higher dependency of highly proliferative malignant cells on the proteasome system to remove aberrant proteins and the dependence of some tumours on the proteasome-dependent NF-κB activation pathway to maintain drug or radiation resistance.
- In addition to direct apoptotic effects, there is a strong biological basis for using proteasome inhibition to enhance sensitivity to standard chemotherapy and radiation therapy, and to overcome drug resistance.
- The proteasome inhibitor bortezomib has established clinical efficacy and an approved clinical indication for the treatment of relapsed and refractory multiple myeloma — proof of the principle that the proteasome is a suitable antineoplastic target.

perform the proteasome enzymatic activities, which have been characterized as CHYMOTRYPTIC-like (a preference for tyrosine or phenylalanine at the P1 (peptide carbonyl) position), tryptic-like (a preference for arginine or lysine) and post-glutamyl peptidyl hydrolytic-like (or post-acidic, because of a preference for cleavage after aspartate, glutamate or other acidic residues)[10–12]. The catalytic NUCLEOPHILES of these β-subunits are amino-terminal threonine residues, which make the β-subunits unusual compared with other proteinases — catalytic nucleophiles have generally been classified within the serine, cysteine, aspartic or metallo classes based on their mechanism of action. The function of the other four β-subunits is unclear. A complex network of allosteric interactions regulates the sequence of enzymatic activities within the proteasome, which ultimately yields oligopeptides[13,14].

To function *in vivo*, the 20S proteasome requires the association of regulatory units that partially determine the specificity in proteasome function. One such regulatory particle is the 19S regulatory complex[11].

The 19S regulatory complex. The 19S regulatory complex — also known as the 19S cap, 19S regulatory particle or PA700 — is a 700-kDa complex of 20 polypeptide subunits that binds to both ends of the 20S proteasome to form the 26S proteasome. *In vitro*, the 19S regulatory particle seems to consist of two subcomplexes: the 'base', which contains six ATPases and several other polypeptides, and the 'lid'[15]. The base alone is able to degrade peptides and non-ubiquitylated proteins, but the lid is required to degrade ubiquitylated proteins, so providing a greater level of specificity for proteolytic degradation. The intact 26S proteasome catalyses the ubiquitin pathway of ATP-dependent protein degradation[15]. ATP hydrolysis is required both for the formation of the 26S complex and also for the unfolding and linearizing of large proteins to facilitate their entry into the catalytic inner core of the proteasome[11,16]. Not surprisingly, there are some exceptions to the general rule that the 26S complex functions strictly in the energy-dependent proteolysis of ubiquitylated proteins; for example, the RB family of tumour suppressors undergo proteasome-dependent degradation through a ubiquitin-independent pathway, following binding with the human cytomegalovirus pp71 protein[17]. Other notable examples are calmodulin and troponin C[18].

The 11S regulator. Mammalian cells contain another regulatory complex that associates with the 20S proteasome: the 11S regulator or PA28 (REFS 11,19). The 11S regulator consists of a 28-kDa α-subunit and a 28-kDa β-subunit[20,21]. A third sequence-homologous subunit, γ, has different enzymatic properties and does not seem to bind to the other two subunits to form the 11S regulator[22–24]. Binding of 11S to the ends of the 20S proteasome is not dependent on ATP hydrolysis or other cofactors. Also, unlike the 19S cap, 11S does not catalyse the degradation of large proteins. Cell culture and mouse transgenic experiments have demonstrated that interferon-γ induces the expression of 11Sα and 11Sβ (for a review, see REF. 24), and the evidence indicates a role for the 11S complex in promoting the production of antigenic peptides or, perhaps, in facilitating the delivery of peptides to the lumen of the endoplasmic reticulum[24–31]. Indeed, proteasomal cleavage produces the carboxyl termini of immunodominant antigenic peptides, but formation of the N termini of these peptides and efficient presentation on the cell surface requires the activity of aminopeptidases that are located in the endoplasmic reticulum[13].

Proteasome localization. Proteasomes are found in both the nucleus and the cytoplasm of eukaryotic cells — the distribution varies according to cell or tissue types. The significance of differential distribution is now being characterized[6]. Proteasomes seem to undergo cell-cycle-specific redistribution between the nucleus and cytoplasm[32–36]. Experiments in living cells with fluorescently tagged proteasome subunits have shown that active proteasome complexes can enter nuclei through the nuclear pores or during reassembly of the nuclear envelope after mitosis[36]. The signal pathways that regulate intracellular localization of proteasomes and their subunits *in vivo* are still being characterized. In the cytoplasm, proteasomes localize near centrosomes, on the outer surface of the endoplasmic reticulum and in cytoskeletal networks[37,38].

Ubiquitylation. Proteins become tagged with ubiquitin polypeptides during ubiquitylation, which targets them for degradation by the proteasome. However, it is now known that ubiquitylation can also serve as a signal for kinase activation, trafficking and other non-proteolytic activities[39]. Ubiquitin is covalently conjugated to cellular proteins at a lysine residue, and this process requires the actions of three enzymes that act in sequence (for reviews, see REFS 10,40–42).

UBIQUITIN
A 76-amino-acid polypeptide that is highly conserved in eukaryotic cells.

CHYMOTRYPSIN
A mammalian intestinal protease that is specific for phenylalanine, tryptophan and tyrosine.

NUCLEOPHILE
The portion of a molecule that provides an electron pair in a reaction, ultimately causing a bond to break and the formation of a new bond.

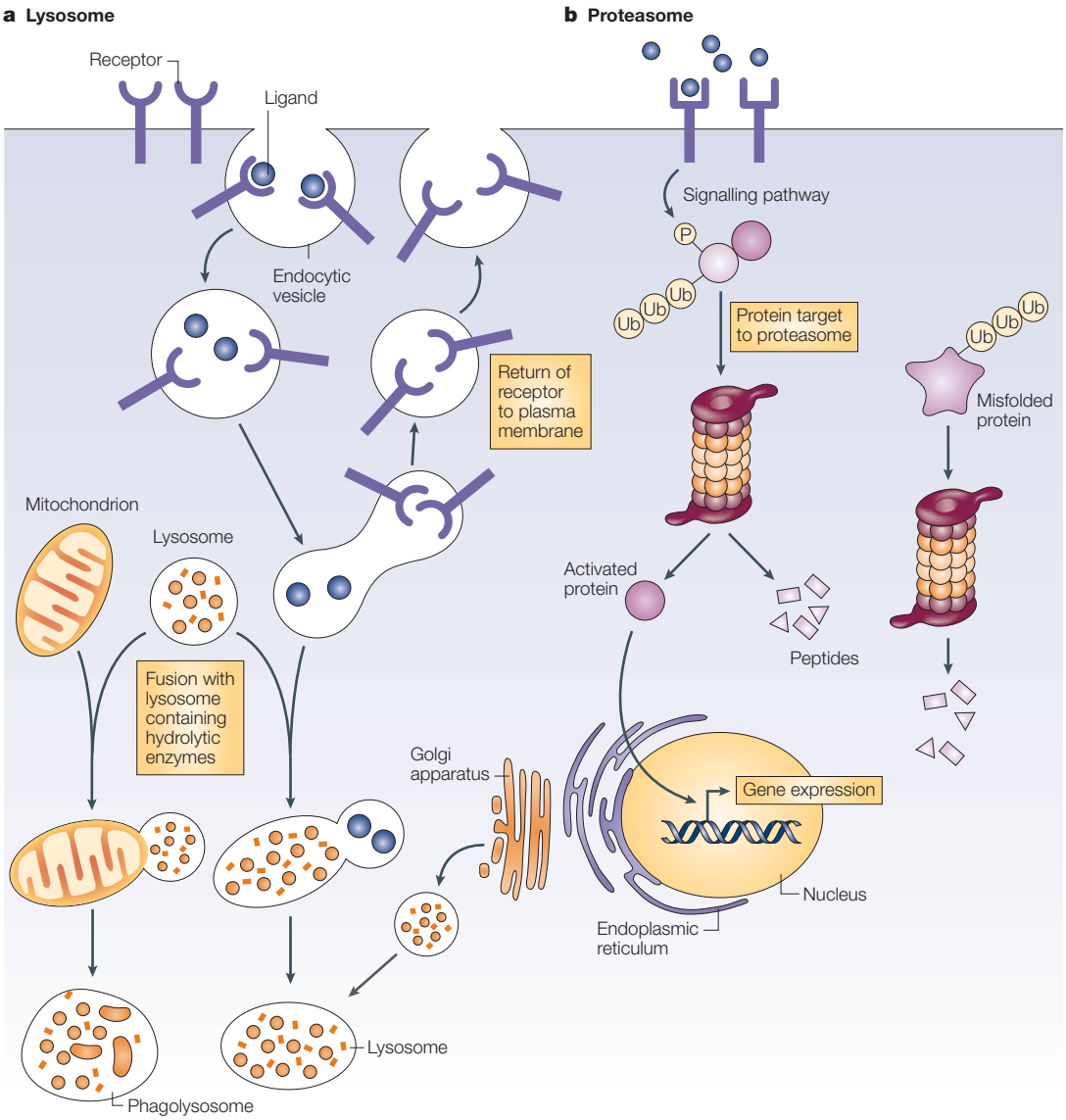

Figure 1 | **Eukaryotic protein degradation.** The two main routes of intracellular protein degradation are the lysosomes and the proteasomes. **a** | Lysosomes are cytoplasmic, membrane-bound vesicles that enclose proteases and hydrolytic enzymes. They degrade extracellular and transmembrane proteins that are taken up by endocytosis — the process by which the cellular plasma membrane invaginates and breaks off internally to transport materials into the cell — and participate in autophagocytosis by fusing with phagosomes. **b** | Proteasomes are primarily involved in degrading intracellular proteins. These might be targeted by phosphorylation following activation of signalling pathways, or recognized because they are misfolded. The proteins are targeted for degradation by their ubiquitin tag.

First, in the initiation step, the (ubiquitin-activating) E1 enzyme binds ubiquitin in the presence of ATP; organisms generally have only one *E1* gene. Second, ubiquitin is transferred from E1 to one of more than 25 E2s (the ubiquitin-conjugating enzymes or ubiquitin-carrier proteins). Hundreds of ubiquitin ligases, or E3s, exist in mammalian cells and each binds to specific protein substrates that have been targeted for proteo-lysis because of a misfolding or conformational change. In the third step, E3, together with E2, catalyses the transfer of ubiquitin from E2 to the substrate. In addition to catalysing the attachment of the first ubiquitin molecule to a protein, these three enzymes also facilitate the attachment of subsequent ubiquitin polypeptides to a lysine residue of the previously conjugated molecule. This process leads to the formation of the polyubiquitin chain that is recognized by the 26S proteasome.

CYCLIN
A short-lived regulatory protein that activates cyclin-dependent kinases to induce cell-cycle progression.

Proteasome function

Many proteasome substrates are known mediators of pathways that are dysregulated with neoplasia (FIG. 3). A large and growing body of evidence indicates that the proteasome affects cell-cycle progression in part by regulating the CYCLINS; in addition, the proteasome can cause increases or decreases in apoptotic activity through effects on caspases, BCL2 activity and nuclear

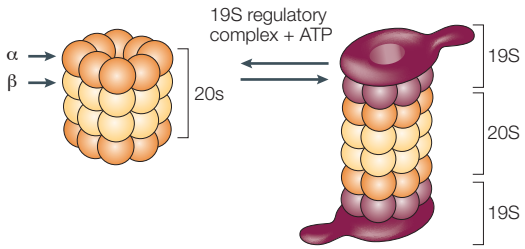

Figure 2 | **Proteasome structure.** A three-dimensional representation of the proteasome multi-enzyme complex. This is composed of the 20S complex, which comprises α- and β-subunits, and two 19S regulatory complexes. Together with ATP, these form the 26S proteasome.

factor of κB (NF-κB). Although abnormalities of these regulatory pathways are observed in malignant cells, specific defects in proteasome structure or function have not been associated with cancers.

Cyclins in cell-cycle progression. Cyclin-dependent kinase (CDK) complexes regulate the progression of eukaryotic cells through the phases of the cell cycle. CDK activity is regulated by short-lived regulatory proteins named cyclins because they are rapidly degraded after exit from the cell cycle. In mammals, different cyclins act at different stages of the cell cycle. Cyclin D and cyclin E act during the G1 (growth) phase, cyclin E and cyclin A during the S (DNA synthesis) phase, and cyclin A and cyclin B during mitosis[43]. In addition to binding with an appropriate cyclin, activation of mammalian CDKs also requires dephosphorylation by a specific member of the CDC25 phosphatase family. The interplay among CDKs, cyclins and CDC25s is highly regulated and includes feedback-loop mechanisms and the activity of CDK inhibitors such as WAF1 (also known as CIP1 and p21) and KIP1 (also known as p27)[44].

The rapid turnover of cyclins involves the ubiquitin–proteasome pathway[45–47]. Furthermore, KIP1 and WAF1 are also degraded by the proteasome. *In vitro* and *in vivo* experiments show that KIP1 in quiescent cells has a lower level of ubiquitylation associated with its longer half-life, compared with KIP1 in proliferating cells[48–50]. The proteasome has also been implicated in regulating the stability of CDC25A[51,52], CDC25B[53] and CDC25C[54] during cell-cycle progression (FIG. 3a). The orderly and temporal degradation of these regulatory molecules is required for continued cell growth. Therefore, inhibition of proteasome-mediated degradation of these molecules might arrest or retard cell growth, and if these effects were specific to or preferentially targeted neoplastic cells, the differential effect could potentially be clinically relevant.

p53 and MDM2. The p53 tumour suppressor is a short-lived protein that is present in low quantities in normal eukaryotic cells. Cellular stresses, such as chemical- or radiation-induced DNA damage, hypoxia and oncogene activation, lead to stabilization and accumulation of p53, which serves as the trigger for diverse cellular responses. Depending on conditions, these responses might involve cell-cycle arrest, DNA repair, differentiation, senescence and apoptosis (for a review, see REF. 55). An estimated 50% of human tumours have mutations in p53, and many other tumours are associated with alterations in the positive (ARF) or negative (MDM2) regulators of p53 (REFS 56–58). Wild-type p53 promotes the expression of MDM2, which, in turn, ubiquitylates p53 and targets it for rapid degradation by the proteasome, so creating an efficient regulatory feedback loop (FIG. 3b)[59–61]. In fact, MDM2 is a ubiquitin ligase E3 for p53 (REFS 62,63). Using proteasome inhibitors to attack cancers that overexpress MDM2 (and therefore inactivate p53 without the need for mutations in p53)[57] might be a good approach to salvaging the p53 regulatory cascade and inducing apoptosis of these tumour cells.

NF-κB pathway. The NF-κB family represents a group of transcription factors that are bound to the inhibitor protein IκB. The association with the inhibitor confines them to inactivity in the cytoplasm (for a review, see REF. 64). Stimulation of cells by chemotherapy, radiation, viruses, cytokines, antigens or oxidants initiates a cascade of signals that leads to IκB phosphorylation, ubiquitylation and subsequent degradation by the 26S proteasome[65,66]. Liberated NF-κB translocates to the nucleus, where it binds to promoter regions of target genes and activates transcription of growth factors (such as the interleukins); cell-adhesion molecules (such as intracellular cell-adhesion molecule and vascular cell-adhesion molecule); angiogenesis factors; enzymes that are induced by stress (such as cyclooxygenase-2, nitric oxide synthase and 5-lipoxygenase); and anti-apoptotic factors (such as inhibitor of apoptosis (IAP) and BCL2) that serve to promote cell growth and differentiation and to protect a cell from apoptosis (FIG. 3c)[67].

The growth-promoting and anti-apoptotic effects of NF-κB make it a rational target for antineoplastic agents. An approach to preventing NF-κB activation is to inhibit proteasome activity, which stabilizes IκB and increases the susceptibility of malignant cells to chemotherapeutic agents or other cellular stresses, such as ionizing radiation.

Caspases. Apoptosis requires a cascade of complex biochemical events that are performed with the participation of a family of cysteine proteases called caspases. Specifically, caspase-8 and caspase-3 are thought to be essential to the apoptosis cascade[68]. More than 70 proteins are caspase substrates during apoptotic cell death. Precursors of caspases are constitutively expressed, but can be rapidly activated by a specific inducer. In malignant cells, caspase activation might be desirable to promote apoptosis. As activation of NF-κB can inactivate caspase-8 (for example, in response to tumour-necrosis factor (TNF) stimulation)[69], inhibition of proteasome activity would prevent the activation of NF-κB, potentiate caspase activity and induce apoptosis.

MDM2
An E3 ubiquitin ligase that targets p53 for degradation through the 26S proteasome. It is also a transcriptional target of p53, functioning in a negative-feedback loop.

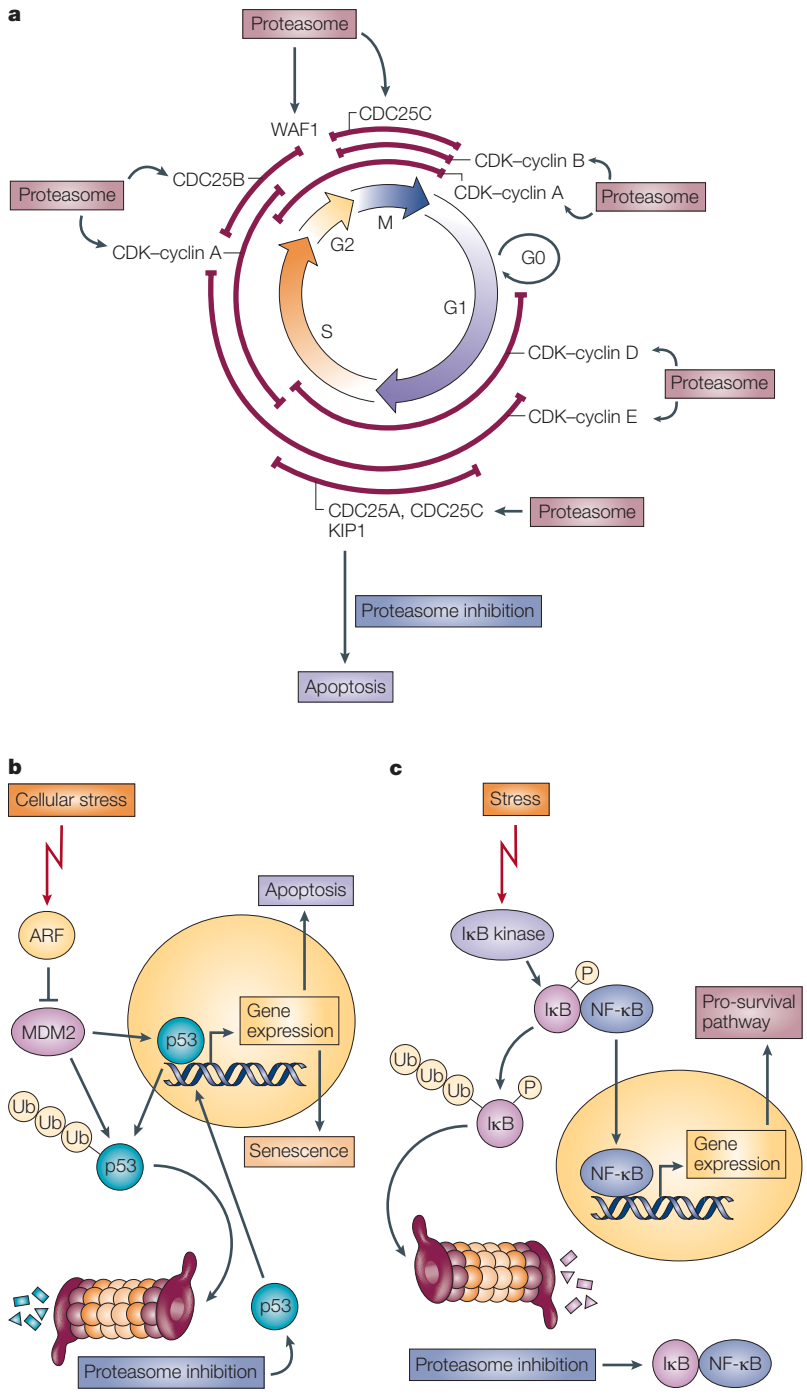

Figure 3 | **Cellular pathways associated with the proteasome. a** | The CDC25 family and the cyclins are intimately involved in cell-cycle regulation and are catabolysed by proteasomes in a tightly controlled sequence. With proteasome inhibition, the regulation of CDC25A, CDC25C, KIP1 and the cyclins is destabilized, which is thought to make the cell more susceptible to apoptosis. **b** | The tumour suppressor p53 accumulates with cellular stress, such as DNA damage, oncogene activation and hypoxia. MDM2 inhibits the activity of p53, in part by exporting it to the cytoplasm where it can be degraded by proteasomes, but also by acting as a ubiquitin ligase. p53 becomes activated following proteasome inhibition, which stimulates p53-mediated tumour-suppressor activity; namely, apoptosis and senescence. **c** | In response to stress, such as neoplasia and chemotherapy, the IκB inhibitor becomes phosphorylated and deactivated by the proteasome. Thereafter, nuclear factor of κB (NF-κB) is expressed and promotes several pro-survival pathways. Proteasome inhibition prevents the activation of NF-κB and increases the susceptibility of the cell to cytotoxic effects of chemotherapy. CDK, cyclin-dependent kinase.

Targeting the proteasome for cancer therapy

The fact that the proteasome is crucial for the execution of many cellular functions indicates that it would be difficult to use it as a target for chemotherapy and yet maintain a tolerable therapeutic index. However, empirical findings indicate that many types of actively proliferating malignant cells are more sensitive to proteasome blockade than non-cancerous cells. For example, compared with quiescent human leukaemic HL60 cells, proliferating HL60 cells are significantly more susceptible to apoptosis that is induced by the proteasome inhibitors *N*-carbobenzoxy-L-leucyl-L-leucyl-norvalinal (LLnV) and *N*-carbobenzoxy-L-isoleucyl-L-γ-*t*-butyl-L-glutamyl-L-alanyl-L-leucinal (PSI)[70]. Similarly, the proteasome inhibitor lactacystin induces apoptosis of radiation-sensitive and radiation-resistant human chronic lymphocytic leukaemia (CLL) cells at doses that do not affect normal lymphocytes[71]. At least a 340-fold higher concentration of PSI was necessary to induce apoptosis in contact-inhibited quiescent cells, compared with actively proliferating primary endothelial cells[72]. Lactacystin and LLnV treatment also induced apoptosis of oral squamous-cell carcinoma cells, but not of normal oral epithelial cells or gingival fibroblasts[73]. Interestingly, treatment with these proteasome inhibitors was associated with an accumulation of KIP1 and reduced expression of the survival protein BCL2, which presumably led to inhibition of cell-cycle progression and eventual apoptosis. More recent experiments demonstrated that lactacystin blocks cell-cycle progression, inhibits degradation of KIP1 and causes apoptosis of activated B-cell CLL (B-CLL) cells with little effect on resting B-CLL cells[74].

Human multiple-myeloma cells are more susceptible than normal peripheral-blood mononuclear cells to growth inhibition and apoptosis that is induced by treatment with the proteasome inhibitor bortezomib (formerly known as PS-341)[75]. Treatment with another proteasome inhibitor — MG-132 — induces apoptosis of acute myelogenous leukaemia (AML) stem cells, but has little effect on normal $CD34^+$ stem cells *in vitro*, despite the fact that both cell lines are usually quiescent with a predominant G0 cell-cycle status[76]. Results of cell culture and *in vivo* xenotransplantation experiments by the same group showed that the combination of MG-132 with the anthracycline idarubicin induces apoptosis of leukaemia stem cells, but not of normal haematopoietic stem cells, by activating p53. Dissection of the contributions made by the proteasome inhibitor and the anthracycline might provide a strong rationale for using this kind of strategy to treat AML, particularly because p53 is mutated in only 9% of specimens that are derived from patients with AML[76,77].

The downstream mechanism for the increased susceptibility of malignant versus normal cells to proteasome inhibitors has not been conclusively determined. There might, in fact, be different mechanisms, which function alone or in concert in different cell types[78]. The biological basis for these mechanisms is related to the underlying characteristics of malignant cells that are

Table 1 | **Proteasome inhibitors**

Agents	Origin	Phase	Comments	References
Aclacinomycin A	Natural	Preclinical	Laboratory use	87
Benzamide (CVT-634)	Synthetic	Preclinical	Laboratory use	88,89
Bortezomib (PS-341)	Synthetic	Clinical, for lymphoma	Approved by the FDA in 2003 for the treatment of relapsed and refractory multiple myeloma	97
Calpain inhibitors I and II	Synthetic	Preclinical	Laboratory use	90,91
Eponemycin	Natural	Preclinical	Laboratory use	92
Epoxomycin	Natural	Preclinical	Laboratory use	93
Lactacystin	Natural metabolite of *Streptomyces*	Preclinical	Specific, but weak, inhibitor that blocks proteasome activity by targeting the catalytic β-subunit; also inhibits cathepsin A; no effect on serine or cysteine proteases	86
MG132	Synthetic	Preclinical	Targets calpains and cathepsins	95–97
NLVS	Synthetic	Preclinical	Vinyl sulphonate tripeptides that also inhibit cathepsins	94
MLN-519 (a lactacystin derivative)	Synthetic	Clinical, Phase I	Safety and tolerability recently evaluated in a placebo-controlled Phase I study in 39 healthy volunteers	98
Ritonavir	Synthetic	FDA-approved for HIV	HIV1 protease inhibitor that also selectively inhibits the proteasome	99

The primary inhibitory target for these compounds is the chymotryptic-like activity of the proteasome[85]. Also reviewed in Refs 8,86,95. FDA, Food and Drug Administration; HIV, human immunodeficiency virus; NLVS, 4-hydroxy-5-iodo-3-nitrophenylacetyl-Leu-Leu-leucinal-vinyl sulphone.

fundamental to cancer development. For example, one aspect of this differential susceptibility is that many types of malignant cells rapidly proliferate and have one or more aberrant cell-cycle checkpoints. Therefore, these cells might accumulate defective proteins at a much higher rate than normal cells, which increases their dependency on the proteasome as a disposal mechanism. Consistent with this hypothesis, Masdehors and colleagues[79] reported that B-CLL cells had a threefold higher level of chymotryptic-like proteasome activity and higher levels of nuclear ubiquitin-conjugated proteins compared with normal lymphocytes. Inhibition of proteasome function would lead to a progressive accumulation of these proteins and the resulting cellular dysfunction could trigger apoptosis. However, this explanation is incomplete because some quiescent malignant cells are more susceptible to proteasome inhibitors than are their proliferating normal counterparts[76].

The susceptibility of malignant cells to proteasome inhibition might be largely explained by the fact that the inhibition of proteasome activity causes a reversal or bypass of some mutational effects in cell-cycle and apoptotic checkpoints that led to tumorigenesis. Given the role of many proteasome substrates (such as cyclins, CDKs, WAF1 and KIP1) in cell-cycle and apoptotic regulatory pathways, proteasome inhibition might have the functional effect of subverting the uncontrolled proliferation and suppression of apoptosis of the malignant cells.

Another factor that influences this differential susceptibility is tied to the NF-κB activation pathway. Abnormal regulation of this pathway has been associated with some malignancies, including acute lymphoblastic leukaemia[80] and multiple myeloma[81,82]. Constitutive activation of the NF-κB pathway is a mechanism for drug resistance that is used by numerous types of solid tumours and haematological malignancies (for a review, see REF. 64). For example, inhibition of NF-κB activation by the proteasome inhibitor *N*-acetyl-L-leucinyl-L-leucinyl-L-norleucinal or by transient transfection of mutant IκBα significantly increased sensitivity to the induction of apoptosis by various death-inducing ligands (DILs; such as TNF-α and doxorubicin) in sensitive and DIL-resistant lymphoid cell lines[83]. Recent experiments indicate that NF-κB has a consensus-binding site for the human MULTIDRUG-RESISTANCE GENE 1 (*MDR1*) and that, *in vitro*, NF-κB transactivates expression of *MDR1*, further supporting a role for NF-κB in drug resistance[84].

Proteasome inhibitors — anticancer applications

Proteasome inhibitors might be natural or synthetic (TABLE 1), and five important classes of proteasome inhibitors exist: peptide aldehydes, peptide vinyl sulphones, peptide boronates, peptide epoxiketones and β-lactones. However, at present, only two compounds are in clinical development because of their antineoplastic (bortezomib) and anti-inflammatory (PS-519, also known as MLN519) properties[85–99]. The natural inhibitor lactacystin and synthetic peptide aldehydes were the first agents to be identified with the ability to inhibit proteasomes (although peptide aldehydes also inhibit other proteases). The rationale behind the development of bortezomib was that substituting the aldehyde with boronic acid would create compounds that could form covalent and reversible complexes with the proteasome with improved potency and selectivity

MULTIDRUG-RESISTANCE GENE 1
A gene that responds to environmental stress by allowing the cell to develop resistance to a large number of cancer chemotherapeutic agents.

compared with their corresponding aldehydes[97]. So, a series of dipeptidyl boronic-acid proteasome inhibitors was developed that was initially screened *in vitro* against the standard National Cancer Institute (NCI) panel of 60 human tumour cell lines[100]. Bortezomib was 1 of the 13 boronic-acid proteasome inhibitors that we selected for further study because it demonstrated promising cytotoxic activity in these *in vitro* assays.

In vitro and *in vivo* studies of proteasome inhibitors in various cancer models have highlighted several properties of this class of agents that make them suitable for anticancer therapy[73–75,86,96,101–115]. The specificity and exact mechanism of action of each proteasome inhibitor are still being elucidated, but much is known about the role of each target in carcinogenesis, angiogenesis and metastasis (for a review, see REF. 86). The ultimate result of inhibiting proteasome activity is apoptosis, increased sensitivity to standard chemotherapy and radiation therapy, and decreased resistance to chemotherapies and irradiation (TABLE 2)[73–75,86,96,101–115]. Much still remains to be learned about the exact sequence of events and how these events are specific to a certain inhibitor and tumour type.

Bortezomib

Preclinical characterization. *In vitro* experiments showed that bortezomib treatment has a cytotoxic effect in various tumour types (such as breast, colorectal, lymphoma, multiple myeloma, ovarian, pancreatic, prostate and squamous cell carcinomas), including some that do not express p53. This indicates that bortezomib can induce apoptosis by a p53-independent mechanism[75,86,100,112,113]. In these experiments, bortezomib treatment also led to an increase in WAF1 levels and an accumulation of cells in the G2–M phase of the cycle before undergoing apoptosis. Experiments with non-small-cell lung cancer (NSCLC) H460 cells showed that bortezomib treatment first leads to BCL2 phosphorylation and cleavage, which is accompanied by G2–M phase arrest, followed by apoptosis[103]. Bortezomib blocks TNF-α-induced NF-κB activation in a dose- and time-dependent fashion in multiple-myeloma cells through degradation of IκBα[116]. Further clues to the apoptotic pathway that is induced by bortezomib have been uncovered by more recent oligonucleotide microarray-based transcriptional profiling of the human multiple-myeloma cell line MM.1S[117]. Bortezomib treatment led to downregulation of transcripts that are associated with important growth and survival pathways (such as insulin-like growth factor 1, its receptor and proteins that regulate its signalling pathways) and upregulation of transcripts involved in both of the main pro-apoptotic pathways (one involving mitochondrial cytochrome *c* release and caspase-9 activation, the other requiring activation of JUN kinase and FAS–caspase-8). Further investigation of how bortezomib mediates its effects against multiple myeloma has confirmed that, at least *in vitro*, bortezomib induces caspase expression[118]. Furthermore, these experiments also showed that bortezomib inhibits DNA repair and induces p53 by phosphorylation and degradation of MDM2, through activation of caspase-3 by caspase-8.

In vivo studies have shown significant antitumour activities. In a human multiple-myeloma xenograft mouse model, bortezomib treatment induced apoptosis and decreased angiogenesis *in vivo*, which led to significant tumour size reduction and increased survival, compared with control mice[119]. Toxicity was minimal, despite the fact that dose-dependent proteasome inhibition was greater in normal tissues than in the tumours. This result further attests to the greater susceptibility of malignant cells to proteasome inhibition. Bortezomib treatment also inhibited angiogenesis and tumour growth of mouse and human squamous-cell carcinomas that were implanted in nude mice[120]. These

Table 2 | **Antineoplastic activities of proteasome inhibitors in human cells**

Inhibitor	Activity	Result	Cell types	References
LLnV, LLnL PSI, MG115	Upregulation of p53 activity in some, but not all, cells	Growth arrest and apoptosis	Fibroblasts	101,102
CEP1612, LLnV	Accumulation of CDK inhibitors KIP1 and WAF1	Growth arrest and apoptosis	Transformed fibroblasts; oral squamous-cell carcinoma	73,96
LLnL, LLL, LLM, lactacystin	Decreased expression and phosphorylation of the apoptotic suppressor BCR–ABL	Growth arrest and apoptosis	Chronic myelogenous leukaemia	104
MG132, LLnL, lactacystin	Activation of SAPK	Growth arrest and apoptosis	IL-2-dependent cell line CTLL-20; IL-3-dependent haematopoietic progenitor cell line Ba/F_3; oligodendroglial cell primary cultures	105–107
Bortezomib	In part, downregulation of NF-κB; reduced expression/ increased phosphorylation and cleavage of BCL2	Growth arrest and apoptosis; restored sensitivity to standard chemotherapy and radiation therapy	Breast, brain, colorectal, lung, ovarian, pancreatic, prostate, squamous-cell and tongue cancer; leukaemia; multiple myeloma; lymphoma	75,103,108–113
Lactacystin, MG132 bortezomib	Activation of caspases	Apoptosis	Chronic lymphocytic leukaemia; chronic myelogenous leukaemia	74,114,115

CDK, cyclin-dependent kinase; IL, interleukin; LLL, *N*-carbobenzoxy-L-leucyl-L-leucyl-L-leucinal; LLM, *N*-acetyl-L-leucyl-L-leucyl-L-methioninal; LLnL, *N*-acetyl-L-leucyl-L-leucyl-norleucinal; LLnV, *N*-carbobenzoxy-L-leucyl-L-leucyl-L-norvalinal; NF-κB, nuclear factor of κB; SAPK, stress-activated protein kinase.

Box 1 | **Multiple myeloma as a target for proteasome inhibitors**

The rationale for using proteasome inhibitors to treat multiple myeloma is based on several types of data (for a review, see REF. 78). First, proteasome inhibitors downregulate the cytokine-induced expression of E-selectin, vascular cell-adhesion molecule 1 (VCAM1) and intracellular cell-adhesion molecule 1 (REF. 128).

VCAM1 is essential for binding multiple-myeloma cells to bone-marrow stromal cells, which results in protection of multiple-myeloma cells from apoptosis[129,130]. Second, proteasome inhibition of NF-κB activation might reduce NF-κB-dependent expression of interleukin-6 (IL-6), which is a growth and survival factor for multiple-myeloma cell lines. IL-6 is also thought to be involved in creating a dexamethasone-resistant phenotype in multiple-myeloma cells[78,131]. Third, the bone marrow of patients with aggressive multiple myeloma shows increased neovascularization[132]. Experiments with the proteasome inhibitor lactacystin indicate that the proteasome might have a role in angiogenesis[133]. More recently, the anti-angiogenic properties demonstrated *in vivo* by bortezomib[117,120] and MG262 (REF. 134; a commercially available proteasome inhibitor used in laboratory research) indicate that one mechanism by which proteasome inhibitors attack multiple myeloma is, in part, to affect its dependence on bone-marrow neovascularization. Finally, studies have shown that bortezomib acts additively or synergistically with other standard chemotherapies, such as dexamethasone, melphalan and pegylated liposomal doxorubicin[75,126,138].

effects were associated with inhibition of NF-κB activation, and expression of the NF-κB-dependent pro-angiogenic growth-regulated oncogene-α and vascular endothelial growth factor. Similar tumour-growth inhibitory and pro-survival results have been reported from studies of bortezomib treatment in human prostate carcinoma[100] and lung[121] mouse xenograft models.

Chemosensitizer and radiosensitizer. The goal of standard chemotherapy and radiotherapy is to induce tumour cells to undergo apoptosis, causing minimal, if any, damage to normal cells. Many tumours become resistant to the apoptotic stimuli of the treatment by producing an inhibitor of apoptosis (a growth promoter) or by expressing the *MDR1* gene . Expression of the anti-apoptotic, pro-growth factor NF-κB is activated by radiotherapy or chemotherapy in many malignant tissues. Specific inhibition of NF-κB activation by gene therapy with an adenovirus containing a modified *IκBα* gene sensitizes cells to chemotherapy, demonstrating that NF-κB is a target for circumventing drug resistance[122]. Given that proteasome inhibition has been shown to block NF-κB activation, this strategy has been tested in preclinical models by various investigators to circumvent tumour drug resistance and enhance chemosensitivity. Hideshima and colleagues[75] showed that bortezomib inhibits growth and induces apoptosis in multiple-myeloma cells isolated from patients with disease resistant to conventional chemotherapy with dexamethasone. Others have shown that low-dose bortezomib sensitizes chemotherapy-resistant multiple-myeloma cells and cells obtained from a patient with relapsed disease to chemotherapy with doxorubicin and melphalan[123]. Unlike the effect of either agent alone, the combination of bortezomib and irinotecan was very effective in inducing apoptosis, inhibiting cell proliferation and blocking NF-κB activation of pancreatic tumour xenografts[108]. Similarly, the addition of bortezomib significantly improved pancreatic xenograft tumour growth inhibition induced by chemotherapy with gemcitabine[124]. Cusack and colleagues[110] demonstrated that proteasome inhibition with bortezomib prevents irinotecan-induced NF-κB activation, increasing chemosensitivity and apoptosis in colorectal cancer cells in tissue culture and in a xenograft model.

Encouraging results have also been obtained in experiments testing the radiosensitizing potential of proteasome inhibitors. Russo and co-workers[109] showed that radiation-induced NF-κB activation was inhibited by pretreatment with bortezomib, which led to decreased cell growth, increased apoptosis, increased radiosensitivity *in vitro* and a greatly improved reduction in colorectal tumour burden in xenograft models. An NCI-sponsored Phase I trial is now assessing the feasibility of bortezomib and radiation dual-modality therapy in patients with recurrence of metastatic, squamous-cell head and neck carcinoma.

Clinical activity. Bortezomib is the first proteasome inhibitor to enter clinical development and has recently been approved for the treatment of multiple myeloma (see below). A tolerable and efficacious dose and schedule of bortezomib in myeloma was defined in Phase I and II studies (TABLE 3)[125–127]. In an initial dose-escalation Phase I trial involving 27 patients with refractory haematological malignancies, Orlowski and colleagues[126] confirmed the preclinical finding that bortezomib induced 20S proteasome inhibition in a dose- and time-dependent manner. Of nine evaluable patients with myeloma, one had a complete response and the remaining eight showed improvement in paraprotein levels and/or marrow plasmacytosis. In another Phase I trial in heavily pretreated patients with advanced solid tumours, one patient with NSCLC experienced a partial response.

The rationale for using bortezomib in the treatment of multiple myeloma relies on data from several avenues of research (BOX 1)[75,78,117,120,128–134]. Based on these preclinical observations and the result of Phase I trials, a large multicentre, Phase II trial was performed in 202 patients with relapsed, refractory multiple myeloma[127]. There was a 35% response rate (complete, partial and minimal responses) as defined by the European Group for Blood and Marrow Transplant[135], including 4% with a complete response (negative by immunofixation) and 6% with a near-complete response (positive by immunofixation testing). As of March 21, 2003, the median overall survival was 17.8 months and the median duration of response was 14.3 months[136]. The results of this trial formed the basis, in part, for approval of bortezomib in 2003 by the United States Food and Drug Administration as a treatment for patients with multiple myeloma who have received two previous lines of therapy and have relapsed on their last therapy (TABLE 3).

Table 3 | **Clinical trials with bortezomib**

Phase	Tumour	Number of patients	Efficacy results	Important adverse events	References
Phase I	Advanced solid tumours	43	One response in non-small-cell lung carcinoma	DLTs were diarrhoea and sensory neurotoxicity	125
Phase I	Refractory haematological malignancies	27	Activity seen against refractory multiple myeloma and non-Hodgkin's lymphoma	DLTs were thrombocytopaenia, hyponatraemia, hypokalaemia, fatigue and malaise	126
Phase II	Relapsed and refractory multiple myeloma	202	Response rate (complete, partial or minimal response) of 35%	Thrombocytopaenia, fatigue, peripheral neuropathy, neutropaenia*	127

*Grade 3 or 4 adverse events occurring in >10% of patients. Phase I trials define tolerable dose and schedule. Phase II trials define efficacy in multiple myeloma with single-agent bortezomib. DLT, dose-limiting toxicity.

A second Phase II study examined the efficacy and safety of 1.0 and 1.3 mg/m^2 bortezomib in 54 patients with multiple myeloma who failed front-line therapy. Preliminary reports indicate a potential dose effect for efficacy and safety[136].

A Phase III trial comparing 1.3 mg/m^2 bortezomib (twice weekly for 2 weeks followed by a 1 week rest) with high-dose oral dexamethasone for patients with relapsed or refractory multiple myeloma has recently been halted based on an interim analysis and the recommendation of an independent data-monitoring committee to allow patients in the high-dose dexamethasone group to choose to receive bortezomib. The results of this trial and of ongoing front-line studies in patients with multiple myeloma will help clarify how to use this agent in earlier-stage disease.

Preliminary reports testing the potential chemosensitizing effects of bortezomib in combination with other chemotherapeutic agents are appearing and the results are promising. In a study to identify an optimal dosing regimen and to begin to study antitumour activity, combination bortezomib and pegylated liposomal doxorubicin (Doxil) was administered to 42 patients with haematological malignancies as reported by Orlowski *et al.*[137]. Of the 22 patients with multiple myeloma who were evaluable for a response, 16 (73%) had a complete or partial response to treatment. Complete or near-complete responses were observed in 5 of 13 patients who previously received an anthracycline-containing regimen, but either had stable disease or had frankly progressed on previous treatment. In another combination dose-finding study, the combination of bortezomib with melphalan provided encouraging activity in patients with relapsed or refractory multiple myeloma[138]. Starting with low doses of each agent, several partial or minor responses were observed, including some responses in patients who had previously progressed on melphalan alone. Dose escalation of bortezomib is ongoing in this study.

Safety. Bortezomib has been safely administered as cyclical therapy (a usual cycle entails twice-weekly treatment for 2 weeks every 3 weeks) for up to 13 cycles in patients with myeloma[136]. Proteasome activity is maximally inhibited 1 hour after dosing, so the rest periods between dosing allows for recovery of proteasome function[126]. The most commonly reported adverse events with bortezomib occurring in 30% of patients enrolled in clinical trials were asthenic conditions (that is, weakness), nausea, diarrhoea, decreased appetite, constipation, thrombocytopaenia, peripheral neuropathy, pyrexia, vomiting and anaemia (see Velcade full prescribing information in online links box). The most frequent moderate to serious adverse events observed with the use of bortezomib in humans have been thrombocytopaenia, fatigue, peripheral neuropathy and neutropaenia. Peripheral neuropathy has been largely manageable with either dose reduction or, in severe instances, discontinuation of treatment[127]. This toxicity has been reversible for most patients following cessation or completion of treatment[139]. Thrombocytopaenia occurs most frequently in patients with low baseline counts and is generally transient, with recovery within the 10 day rest period between cycles[127]. The deaths of two patients were deemed possibly related to treatment with the study drug[127].

Future studies. Trials are in progress to investigate the use of single-agent bortezomib or bortezomib in numerous combinations in various malignancies in addition to multiple myeloma, including head and neck, pancreatic, ovarian, prostate, breast, small-cell and non-small-cell lung, colorectal, renal-cell, bladder, skin (malignant melanoma), stomach, central nervous system, neuroendocrine, lymphoma and other haematological cancers. Emerging data from these clinical investigations indicate that bortezomib might have therapeutic value for certain other malignancies, including non-Hodgkin's lymphoma, and have prompted additional research focus on lymphoma, as well as on colorectal, lung, breast and prostate cancers.

Implications and future directions

The empirical finding that many malignant cells are more susceptible to proteasome inhibition than their normal counterparts is key to achieving a suitable therapeutic ratio. This differential susceptibility can be explained by several mechanisms that might function alone or together in different cell types. One mechanism is the rapid proliferation rate of many tumours, which might make these cells more dependent on proteasomes to remove misfolded or damaged proteins, the accumulation of which might induce apoptosis.

Another, more elegant, mechanism is that inhibition of proteasome activity might reverse or bypass some of the effects of cell-cycle or apoptosis checkpoint mutations that have caused the development or maintenance of the cancerous phenotype. A third mechanism for this differential susceptibility is linked to the dependence of some tumours on the proteasome-dependent pathway by which NF-κB is activated to maintain drug or radiation resistance. Much remains to be learned about how these mechanisms might interact with one another and with other yet-to-be-defined pathways for tumour sensitivity to proteasome inhibitors.

Results of preclinical and clinical studies of proteasome inhibitors indicate that these agents act through various mechanisms to arrest the growth and spread of malignant cells. This antitumour effect is associated with increased expression of several cell-cycle-regulatory proteins (such as KIP1 and WAF1), caspase activation and the blockade of the NF-κB pro-cell-growth pathway, leading to apoptosis and inhibition of angiogenesis. Proteasome inhibitors have also been associated with sensitizing effects when given in association with standard chemotherapy or radiotherapy.

Another area of future research is the development of agents that target regulatory events occurring upstream from the activated proteasome. These potential targets might control phosphorylation or ubiquitylation of proteasome substrates and their regulators. As they have a role in the production of antigenic peptides, proteasome inhibitors are also being studied as potential anti-inflammatory (for example, in arthritis, asthma, multiple sclerosis and psoriasis) and antiviral agents.

1. Hanahan, D. & Weinberg, R. A. The hallmarks of cancer. *Cell* **100**, 57–70 (2000).
2. Ciechanover, A. The ubiquitin–proteasome proteolytic pathway. *Cell* **79**, 13–21 (1994).
3. Hough, R., Pratt, G. & Rechsteiner, M. Purification of two high molecular weight proteases from rabbit reticulocyte lysate. *J. Biol. Chem.* **262**, 8303–8313 (1987).
4. Waxman, L., Fagan, J. M. & Goldberg, A. L. Demonstration of two distinct high molecular weight proteases in rabbit reticulocytes, one of which degrades ubiquitin conjugates. *J. Biol. Chem.* **262**, 2451–2457 (1987).
5. Bortezomib (Velcade) for multiple myeloma. *Med. Lett. Drugs Ther.* **45**, 57–58 (2003).
6. Brooks, P. *et al.* Subcellular localization of proteasomes and their regulatory complexes in mammalian cells. *Biochem. J.* **346**, 155–161 (2000).
This paper demonstrates the complexity of proteasomal populations and localizations in mammalian cells.
7. Peters, J. M., Cejka, Z., Harris, J. R., Kleinschmidt, J. A. & Baumeister, W. Structural features of the 26 S proteasome complex. *J. Mol. Biol.* **234**, 932–937 (1993).
8. Elliott, P. J. & Ross, J. S. The proteasome: a new target for novel drug therapies. *Am. J. Clin. Pathol.* **116**, 637–646 (2001).
9. Baumeister, W., Walz, J., Zuhl, F. & Seemuller, E. The proteasome: paradigm of a self-compartmentalizing protease. *Cell* **92**, 367–380 (1998).
10. Ciechanover, A. The ubiquitin–proteasome pathway: on protein death and cell life. *EMBO J.* **17**, 7151–7160 (1998).
11. DeMartino, G. N. & Slaughter, C. A. The proteasome, a novel protease regulated by multiple mechanisms. *J. Biol. Chem.* **274**, 22123–22126 (1999).
12. Kisselev, A. F., Akopian, T. N., Castillo, V. & Goldberg, A. L. Proteasome active sites allosterically regulate each other, suggesting a cyclical bite-chew mechanism for protein breakdown. *Mol. Cell* **4**, 395–402 (1999).
13. Goldberg, A. L., Cascio, P., Saric, T. & Rock, K. L. The importance of the proteasome and subsequent proteolytic steps in the generation of antigenic peptides. *Mol. Immunol.* **39**, 147–164 (2002).
14. Kisselev, A. F. *et al.* The caspase-like sites of proteasomes, their substrate specificity, new inhibitors and substrates, and allosteric interactions with the trypsin-like sites. *J. Biol. Chem.* **278**, 35869–35877 (2003).
15. Glickman, M. H. *et al.* A subcomplex of the proteasome regulatory particle required for ubiquitin-conjugate degradation and related to the COP9-signalosome and eIF3. *Cell* **94**, 615–623 (1998).
16. Benaroudj, N., Zwickl, P., Seemuller, E., Baumeister, W. & Goldberg, A. L. ATP hydrolysis by the proteasome regulatory complex PAN serves multiple functions in protein degradation. *Mol. Cell* **11**, 69–78 (2003).
17. Kalejta, R. F. & Shenk, T. Proteasome-dependent, ubiquitin-independent degradation of the Rb family of tumor suppressors by the human cytomegalovirus pp71 protein. *Proc. Natl Acad. Sci. USA* **100**, 3263–3268 (2003).
18. Benaroudj, N., Tarcsa, E., Cascio, P. & Goldberg, A. L. The unfolding of substrates and ubiquitin-independent protein degradation by proteasomes. *Biochimie* **83**, 311–318 (2001).
19. Ahn, K. *et al. In vivo* characterization of the proteasome regulator PA28. *J. Biol. Chem.* **271**, 18237–18242 (1996).
20. Ma, C. P., Slaughter, C. A. & DeMartino, G. N. Identification, purification, and characterization of a protein activator (PA28) of the 20 S proteasome (macropain). *J. Biol. Chem.* **267**, 10515–10523 (1992).
21. Mott, J. D. *et al.* PA28, an activator of the 20 S proteasome, is composed of two nonidentical but homologous subunits. *J. Biol. Chem.* **269**, 31466–31471 (1994).
22. Nikaido, T. *et al.* Cloning and nucleotide sequence of cDNA for Ki antigen, a highly conserved nuclear protein detected with sera from patients with systemic lupus erythematosus. *Clin. Exp. Immunol.* **79**, 209–214 (1990).
23. Realini, C. *et al.* Characterization of recombinant REGα, REGβ, and REGγ proteasome activators. *J. Biol. Chem.* **272**, 25483–25492 (1997).
24. Rechsteiner, M., Realini, C. & Ustrell, V. The proteasome activator 11 S REG (PA28) and class I antigen presentation. *Biochem. J.* **345**, 1–15 (2000).
25. Groettrup, M. *et al.* A role for the proteasome regulator PA28α in antigen presentation. *Nature* **381**, 166–168 (1996).
26. Murata, S. *et al.* Immunoproteasome assembly and antigen presentation in mice lacking both PA28α and PA28β. *EMBO J.* **20**, 5898–5907 (2001).
27. Gaczynska, M., Rock, K. L. & Goldberg, A. L. γ-interferon and expression of MHC genes regulate peptide hydrolysis by proteasomes. *Nature* **365**, 264–267 (1993).
28. Bose, S., Brooks, P., Mason, G. G. & Rivett, A. J. γ-interferon decreases the level of 26 S proteasomes and changes the pattern of phosphorylation. *Biochem. J.* **353**, 291–297 (2001).
29. Hill, C. P., Masters, E. I. & Whitby, F. G. The 11S regulators of 20S proteasome activity. *Curr. Top. Microbiol. Immunol.* **268**, 73–89 (2002).
30. Whitby, F. G. *et al.* Structural basis for the activation of 20S proteasomes by 11S regulators. *Nature* **408**, 115–120 (2000).
31. Realini, C., Rogers, S. W. & Rechsteiner, M. KEKE motifs. Proposed roles in protein-protein association and presentation of peptides by MHC class I receptors. *FEBS Lett.* **348**, 109–113 (1994).
32. Palmer, A. *et al.* Subpopulations of proteasomes in rat liver nuclei, microsomes and cytosol. *Biochem. J.* **316**, 401–407 (1996).
33. Amsterdam, A., Pitzer, F. & Baumeister, W. Changes in intracellular localization of proteasomes in immortalized ovarian granulosa cells during mitosis associated with a role in cell cycle control. *Proc. Natl Acad. Sci. USA* **90**, 99–103 (1993).
34. Palmer, A., Mason, G. G., Paramio, J. M., Knecht, E. & Rivett, A. J. Changes in proteasome localization during the cell cycle. *Eur. J. Cell Biol.* **64**, 163–175 (1994).
35. Lafarga, M., Fernandez, R., Mayo, I., Berciano, M. T. & Castano, J. G. Proteasome dynamics during cell cycle in rat Schwann cells. *Glia* **38**, 313–328 (2002).
36. Reits, E. A., Benham, A. M., Plougastel, B., Neefjes, J. & Trowsdale, J. Dynamics of proteasome distribution in living cells. *EMBO J.* **16**, 6087–6094 (1997).
This paper demonstrates the free and rapid diffusion of the vast majority (> 90%) of the proteasome within, but not between, the cytoplasmic and nuclear compartments of the cell, and how the cytoplasmically generated proteasome enters the nucleus.
37. Wojcik, C. & DeMartino, G. N. Intracellular localization of proteasomes. *Int. J. Biochem. Cell Biol.* **35**, 579–589 (2003).
38. Gordon, C. The intracellular localization of the proteasome. *Curr. Top. Microbiol. Immunol.* **268**, 175–184 (2002).
39. Pickart, C. M. Ubiquitin enters the new millennium. *Mol. Cell* **8**, 499–504 (2001).
40. Hicke, L. A new ticket for entry into budding vesicles-ubiquitin. *Cell* **106**, 527–530 (2001).
41. Goldberg, A. L. Protein degradation and protection against misfolded or damaged proteins. *Nature* **426**, 895–899 (2003).
42. Yang, Y. & Yu, X. Regulation of apoptosis: the ubiquitous way. *FASEB J.* **17**, 790–799 (2003).
43. King, R. W., Deshaies, R. J., Peters, J. M. & Kirschner, M. W. How proteolysis drives the cell cycle. *Science* **274**, 1652–1659 (1996).
44. Murray, A. W. Recycling the cell cycle: cyclins revisited. *Cell* **116**, 221–234 (2004).
45. Glotzer, M., Murray, A. W. & Kirschner, M. W. Cyclin is degraded by the ubiquitin pathway. *Nature* **349**, 132–138 (1991).
46. Diehl, J. A., Zindy, F. & Sherr, C. J. Inhibition of cyclin D1 phosphorylation on threonine-286 prevents its rapid degradation via the ubiquitin-proteasome pathway. *Genes Dev.* **11**, 957–972 (1997).
47. Clurman, B. E., Sheaff, R. J., Thress, K., Groudine, M. & Roberts, J. M. Turnover of cyclin E by the ubiquitin–proteasome pathway is regulated by cdk2 binding and cyclin phosphorylation. *Genes Dev.* **10**, 1979–1990 (1996).
48. Pagano, M. *et al.* Role of the ubiquitin–proteasome pathway in regulating abundance of the cyclin-dependent kinase inhibitor p27. *Science* **269**, 682–685 (1995).
49. Shirane, M. *et al.* Down-regulation of $p27^{Kip1}$ by two mechanisms, ubiquitin-mediated degradation and proteolytic processing. *J. Biol. Chem.* **274**, 13886–13893 (1999).
50. Tam, S. W., Theodoras, A. M. & Pagano, M. Kip1 degradation via the ubiquitin–proteasome pathway. *Leukemia* **11**(Suppl 3), 363–366 (1997).
51. Mailand, N. *et al.* Rapid destruction of human Cdc25A in response to DNA damage. *Science* **288**, 1425–1429 (2000).
52. Bernardi, R., Liebermann, D. A. & Hoffman, B. Cdc25A stability is controlled by the ubiquitin-proteasome pathway during cell cycle progression and terminal differentiation. *Oncogene* **19**, 2447–2454 (2000).
53. Baldin, V., Cans, C., Knibiehler, M. & Ducommun, B. Phosphorylation of human CDC25B phosphatase by CDK1–cyclin A triggers its proteasome-dependent degradation. *J. Biol. Chem.* **272**, 32731–32734 (1997).
54. Chen, F. *et al.* Arsenite-induced Cdc25C degradation is through the KEN-box and ubiquitin-proteasome pathway. *Proc. Natl Acad. Sci. USA* **99**, 1990–1995 (2002).

55. Chène, P. Inhibiting the p53–MDM2 interaction: an important target for cancer therapy. *Nature Rev. Cancer* **3**, 102–109 (2003).
56. Soussi, T. The p53 tumor suppressor gene: from molecular biology to clinical investigation. *Ann. NY Acad. Sci.* **910**, 121–137 (2000).
57. Momand, J., Wu, H. H. & Dasgupta, G. MDM2—master regulator of the p53 tumor suppressor protein. *Gene* **242**, 15–29 (2000).
58. Sherr, C. J. & Weber, J. D. The ARF/p53 pathway. *Curr. Opin. Genet. Dev.* **10**, 94–99 (2000).
59. Maki, C. G., Huibregtse, J. M. & Howley, P. M. *In vivo* ubiquitination and proteasome-mediated degradation of p53(1). *Cancer Res.* **56**, 2649–2654 (1996).
60. Kubbutat, M. H., Jones, S. N. & Vousden, K. H. Regulation of p53 stability by Mdm2. *Nature* **387**, 299–303 (1997).
61. Haupt, Y., Maya, R., Kazaz, A. & Oren, M. Mdm2 promotes the rapid degradation of p53. *Nature* **387**, 296–299 (1997).
62. Honda, R., Tanaka, H. & Yasuda, H. Oncoprotein MDM2 is a ubiquitin ligase E3 for tumor suppressor p53. *FEBS Lett.* **420**, 25–27 (1997).
63. Fang, S., Jensen, J. P., Ludwig, R. L., Vousden, K. H. & Weissman, A. M. Mdm2 is a RING finger-dependent ubiquitin protein ligase for itself and p53. *J. Biol. Chem.* **275**, 8945–8951 (2000).
64. Karin, M., Cao, Y., Greten, F. R. & Li, Z. W. NF-κB in cancer: from innocent bystander to major culprit. *Nature Rev. Cancer* **2**, 301–310 (2002).
65. Palombella, V. J., Rando, O. J., Goldberg, A. L. & Maniatis, T. The ubiquitin-proteasome pathway is required for processing the NF-κB1 precursor protein and the activation of NF-κB. *Cell* **78**, 773–785 (1994).
66. Li, C. C., Dai, R. M. & Longo, D. L. Inactivation of NF-κB inhibitor IκBα: ubiquitin-dependent proteolysis and its degradation product. *Biochem. Biophys. Res. Commun.* **215**, 292–301 (1995).
67. Adams, J. Proteasome inhibition: a novel approach to cancer therapy. *Trends Mol. Med.* **8**, S49–S54 (2002).
68. Wang, C. Y., Mayo, M. W., Korneluk, R. G., Goeddel, D. V. & Baldwin, A. S. Jr. NF-κB antiapoptosis: induction of TRAF1 and TRAF2 and c-IAP1 and c-IAP2 to suppress caspase-8 activation. *Science* **281**, 1680–1683 (1998).
An early study demonstrating that proteasome inhibition induces apoptosis in proliferating (but not quiescent and differentiated) leukaemic cells in a manner that is associated with the activation of CPP32 and increased KIP1 levels.
69. Varfolomeev, E. E. *et al.* Targeted disruption of the mouse caspase 8 gene ablates cell death induction by the TNF receptors, Fas/Apo1, and DR3 and is lethal prenatally. *Immunity.* **9**, 267–276 (1998).
70. Drexler, H. C. Activation of the cell death program by inhibition of proteasome function. *Proc. Natl Acad. Sci. USA* **94**, 855–860 (1997).
71. Masdehors, P. *et al.* Increased sensitivity of CLL-derived lymphocytes to apoptotic death activation by the proteasome-specific inhibitor lactacystin. *Br. J. Haematol.* **105**, 752–757 (1999).
72. Drexler, H. C., Risau, W. & Konerding, M. A. Inhibition of proteasome function induces programmed cell death in proliferating endothelial cells. *FASEB J.* **14**, 65–77 (2000).
73. Kudo, Y. *et al.* $p27^{Kip1}$ accumulation by inhibition of proteasome function induces apoptosis in oral squamous cell carcinoma cells. *Clin. Cancer Res.* **6**, 916–923 (2000).
74. Bogner, C. *et al.* Cycling B-CLL cells are highly susceptible to inhibition of the proteasome: involvement of p27, early D-type cyclins, Bax, and caspase-dependent and-independent pathways. *Exp. Hematol.* **31**, 218–225 (2003).
75. Hideshima, T. *et al.* The proteasome inhibitor PS-341 inhibits growth, induces apoptosis, and overcomes drug resistance in human multiple myeloma cells. *Cancer Res.* **61**, 3071–3076 (2001).
76. Guzman, M. L. *et al.* Preferential induction of apoptosis for primary human leukemic stem cells. *Proc. Natl Acad. Sci. USA* **99**, 16220–16225 (2002).
This study shows the ability of proteasome inhibition to combine well with anthracycline therapy to target leukaemic cells *in vitro* and *in vivo*, through NF-κB- and p53-related mechanisms.
77. Stirewalt, D. L. *et al.* FLT3, RAS, and TP53 mutations in elderly patients with acute myeloid leukemia. *Blood* **97**, 3589–3595 (2001).
78. Schenkein, D. Proteasome inhibitors in the treatment of B-cell malignancies. *Clin. Lymphoma* **3**, 49–55 (2002).
79. Masdehors, P. *et al.* Deregulation of the ubiquitin system and p53 proteolysis modify the apoptotic response in B-CLL lymphocytes. *Blood* **96**, 269–274 (2000).
80. Kordes, U., Krappmann, D., Heissmeyer, V., Ludwig, W. D. & Scheidereit, C. Transcription factor NF-κB is constitutively activated in acute lymphoblastic leukemia cells. *Leukemia* **14**, 399–402 (2000).
81. Feinman, R. *et al.* Role of NF-κB in the rescue of multiple myeloma cells from glucocorticoid-induced apoptosis by bcl-2. *Blood* **93**, 3044–3052 (1999).
82. Ni, H. *et al.* Analysis of expression of nuclear factor κB (NF-κB) in multiple myeloma: downregulation of NF-κB induces apoptosis. *Br. J. Haematol.* **115**, 279–286 (2001).
83. Jeremias, I. *et al.* Inhibition of nuclear factor κB activation attenuates apoptosis resistance in lymphoid cells. *Blood* **91**, 4624–4631 (1998).
This paper demonstrates that inhibition of NF-κB activity, either through proteasome inhibition or by mutant IκBα, increases the sensitivity of cells to apoptosis, including cells resistant to apoptosis, following treatment with various agents.
84. Bentires-Alj, M. *et al.* NF-κB transcription factor induces drug resistance through MDR1 expression in cancer cells. *Oncogene* **22**, 90–97 (2003).
85. Adams, J., Palombella, V. J. & Elliott, P. J. Proteasome inhibition: a new strategy in cancer treatment. *Invest. New Drugs* **18**, 109–121 (2000).
86. Almond, J. B. & Cohen, G. M. The proteasome: a novel target for cancer chemotherapy. *Leukemia* **16**, 433–443 (2002).
87. Figueiredo-Pereira, M. E., Chen, W. E., Li, J. & Johdo, O. The antitumor drug aclacinomycin A, which inhibits the degradation of ubiquitinated proteins, shows selectivity for the chymotrypsin-like activity of the bovine pituitary 20 S proteasome. *J. Biol. Chem.* **271**, 16455–16459 (1996).
88. Lum, R. T. *et al.* A new structural class of proteasome inhibitors that prevent NF-κB activation. *Biochem. Pharmacol.* **55**, 1391–1397 (1998).
89. Lum, R. T. *et al.* Selective inhibition of the chymotrypsin-like activity of the 20S proteasome by 5-methoxy-1-indanone dipeptide benzamides. *Bioorg. Med. Chem. Lett.* **8**, 209–214 (1998).
90. Wilk, S., Pereira, M. & Yu, B. Probing the specificity of the bovine pituitary multicatalytic proteinase complex by inhibitors, activators, and by chemical modification. *Biomed. Biochim. Acta* **50**, 471–478 (1991).
91. Orlowski, M., Cardozo, C. & Michaud, C. Evidence for the presence of five distinct proteolytic components in the pituitary multicatalytic proteinase complex. Properties of two components cleaving bonds on the carboxyl side of branched chain and small neutral amino acids. *Biochemistry* **32**, 1563–1572 (1993).
92. Meng, L., Kwok, B. H., Sin, N. & Crews, C. M. Eponemycin exerts its antitumor effect through the inhibition of proteasome function. *Cancer Res.* **59**, 2798–2801 (1999).
93. Kim, K. B., Myung, J., Sin, N. & Crews, C. M. Proteasome inhibition by the natural products epoxomicin and dihydroeponemycin: insights into specificity and potency. *Bioorg. Med. Chem. Lett.* **9**, 3335–3340 (1999).
94. Bogyo, M. *et al.* Covalent modification of the active site threonine of proteasomal β subunits and the *Escherichia coli* homolog HslV by a new class of inhibitors. *Proc. Natl Acad. Sci. USA* **94**, 6629–6634 (1997).
95. Elliott, P. J., Zollner, T. M. & Bochncke, W.-H. Proteasome inhibition: a new anti-inflammatory strategy. *J. Mol. Med.* **81**, 235–245 (2003).
96. An, B., Goldfarb, R. H., Siman, R. & Dou, Q. P. Novel dipeptidyl proteasome inhibitors overcome Bcl-2 protective function and selectively accumulate the cyclin-dependent kinase inhibitor p27 and induce apoptosis in transformed, but not normal, human fibroblasts. *Cell Death Differ.* **5**, 1062–1075 (1998).
97. Adams, J. *et al.* Potent and selective inhibitors of the proteasome: dipeptidyl boronic acids. *Bioorg. Med. Chem. Lett.* **8**, 333–338 (1998).
98. Shah, I. M., Lees, K. R., Pien, C. P. & Elliott, P. J. Early clinical experience with the novel proteasome inhibitor PS-519. *Br. J. Clin. Pharmacol.* **54**, 269–276 (2002).
99. Andre, P. *et al.* An inhibitor of HIV-1 protease modulates proteasome activity, antigen presentation, and T cell responses. *Proc. Natl Acad. Sci. USA* **95**, 13120–13124 (1998).
100. Adams, J. *et al.* Proteasome inhibitors: a novel class of potent and effective antitumor agents. *Cancer Res.* **59**, 2615–2622 (1999).
A key paper describing the selection, characteristics, and early *in vitro* and *in vivo* studies of PS-341, later named bortezomib.
101. Dietrich, C., Bartsch, T., Schanz, F., Oesch, F. & Wieser, R. J. p53-dependent cell cycle arrest induced by *N*-acetyl-L-leucinyl-L-leucinyl-L-norleucinal in platelet-derived growth factor-stimulated human fibroblasts. *Proc. Natl Acad. Sci. USA* **93**, 10815–10819 (1996).
102. Lopes, U. G., Erhardt, P., Yao, R. & Cooper, G. M. p53-dependent induction of apoptosis by proteasome inhibitors. *J. Biol. Chem.* **272**, 12893–12896 (1997).
103. Ling, Y. H. *et al.* PS-341, a novel proteasome inhibitor, induces Bcl-2 phosphorylation and cleavage in association with G2-M phase arrest and apoptosis. *Mol. Cancer Ther.* **1**, 841–849 (2002).
104. Dou, Q. P., McGuire, T. F., Peng, Y. & An, B. Proteasome inhibition leads to significant reduction of Bcr–Abl expression and subsequent induction of apoptosis in K562 human chronic myelogenous leukemia cells. *J. Pharmacol. Exp. Ther.* **289**, 781–790 (1999).
105. Yu, C. L. & Burakoff, S. J. Involvement of proteasomes in regulating Jak–STAT pathways upon interleukin-2 stimulation. *J. Biol. Chem.* **272**, 14017–14020 (1997).
106. Callus, B. A. & Mathey-Prevot, B. Interleukin-3-induced activation of the JAK/STAT pathway is prolonged by proteasome inhibitors. *Blood* **91**, 3182–3192 (1998).
107. Pasquini, L. A., Paez, P. M., Moreno, M. A., Pasquini, J. M. & Soto, E. F. Inhibition of the proteasome by lactacystin enhances oligodendroglial cell differentiation. *J. Neurosci.* **23**, 4635–4644 (2003).
108. Shah, S. A. *et al.* 26S proteasome inhibition induces apoptosis and limits growth of human pancreatic cancer. *J. Cell Biochem.* **82**, 110–122 (2001).
109. Russo, S. M. *et al.* Enhancement of radiosensitivity by proteasome inhibition: implications for a role of NF-κB. *Int. J. Radiat. Oncol. Biol. Phys.* **50**, 183–193 (2001).
This paper demonstrates the ability of bortezomib to enhance the sensitivity of neoplastic cells to radiation therapy *in vitro* and *in vivo* in a manner that is associated with the inhibition of radiation-induced NF-κB activation.
110. Cusack, J. C. Jr. *et al.* Enhanced chemosensitivity to CPT-11 with proteasome inhibitor PS-341: implications for systemic nuclear factor-kappaB inhibition. *Cancer Res.* **61**, 3535–3540 (2001).
A demonstration of the ability of bortezomib to act in combination with conventional chemotherapy in solid tumour cells *in vitro* and *in vivo*. This paper also demonstrates the ability of bortezomib to inhibit the NF-κB activation induced by SN-38.
111. Ma, M. H. *et al.* The proteasome inhibitor PS-341 markedly enhances sensitivity of multiple myeloma tumor cells to chemotherapeutic agents. *Clin. Cancer Res.* **9**, 1136–1144 (2003).
112. Lenz, H. J. Clinical update: proteasome inhibitors in solid tumors. *Cancer Treat. Rev.* **29**(Suppl 1), 41–48 (2003).
113. Chauhan, D. *et al.* Blockade of Hsp27 overcomes bortezomib/proteasome inhibitor PS-341 resistance in lymphoma cells. *Cancer Res.* **63**, 6174–6177 (2003).
114. Chandra, J. *et al.* Proteasome inhibitors induce apoptosis in glucocorticoid-resistant chronic lymphocytic leukemic lymphocytes. *Blood* **92**, 4220–4229 (1998).
115. Gatto, S. *et al.* The proteasome inhibitor PS-341 inhibits growth and induces apoptosis in Bcr/Abl-positive cell lines sensitive and resistant to imatinib mesylate. *Haematologica* **88**, 853–863 (2003).
116. Hideshima, T. *et al*. NF-kB as a therapeutic target in multiple myeloma. *J. Biol. Chem.* **277**, 16639–16647 (2002).
117. Mitsiades, N. *et al.* Molecular sequelae of proteasome inhibition in human multiple myeloma cells. *Proc. Natl Acad. Sci. USA* **99**, 14374–14379 (2002).
118. Hideshima, T. *et al.* Molecular mechanisms mediating antimyeloma activity of proteasome inhibitor PS-341. *Blood* **101**, 1530–1534 (2003).
119. LeBlanc, R. *et al.* Proteasome inhibitor PS-341 inhibits human myeloma cell growth *in vivo* and prolongs survival in a murine model. *Cancer Res.* **62**, 4996–5000 (2002).
120. Sunwoo, J. B. *et al.* Novel proteasome inhibitor PS-341 inhibits activation of nuclear factor-κB, cell survival, tumor growth, and angiogenesis in squamous cell carcinoma. *Clin. Cancer Res.* **7**, 1419–1428 (2001).
121. Teicher, B. A., Ara, G., Herbst, R., Palombella, V. J. & Adams, J. The proteasome inhibitor PS-341 in cancer therapy. *Clin. Cancer Res.* **5**, 2638–2645 (1999).
122. Wang, C. Y., Cusack, J. C. J., Liu, R. & Baldwin, A. S. Jr. Control of inducible chemoresistance: enhanced anti-tumor therapy through increased apoptosis by inhibition of NF-κB. *Nature Med.* **5**, 412–417 (1999).
123. Mitsiades, N. *et al.* The proteasome inhibitor PS-341 potentiates sensitivity of multiple myeloma cells to conventional chemotherapeutic agents: therapeutic applications. *Blood* **101**, 2377–2380 (2003).

This paper demonstrates the synergistic activity of bortezomib used in combination with DNA-damaging agents in multiple myeloma cells, as well as the ability of bortezomib to remove cell-adhesion-mediated drug resistance. Bortezomib is also shown to downregulate multiple DNA-repair proteins.
124. Bold, R. J., Virudachalam, S. & McConkey, D. J. Chemosensitization of pancreatic cancer by inhibition of the 26S proteasome. *J. Surg. Res.* **100**, 11–17 (2001).
125. Aghajanian, C. *et al.* A phase I trial of the novel proteasome inhibitor PS341 in advanced solid tumor malignancies. *Clin. Cancer Res.* **8**, 2505–2511 (2002).
126. Orlowski, R. Z. *et al.* Phase I trial of the proteasome inhibitor PS-341 in patients with refractory hematologic malignancies. *J. Clin. Oncol.* **20**, 4420–4427 (2002).
One of two key Phase I clinical trials of bortezomib. This was a dose-ranging study in patients with advanced haematological malignancies, including multiple myeloma. The study demonstrated patient responses and acceptable toxicities and, in conjunction with reference 125, helped define the dose for the Phase II trials.
127. Richardson, P. G. *et al.* A phase 2 study of bortezomib in relapsed, refractory myeloma. *N. Engl. J. Med.* **348**, 2609–2617 (2003).
The key Phase II trial that led to the approval of the first proteasome inhibitor for the treatment of patients with multiple myeloma.
128. Read, M. A. *et al.* The proteasome pathway is required for cytokine-induced endothelial-leukocyte adhesion molecule expression. *Immunity* **2**, 493–506 (1995).
129. Nefedova, Y., Landowski, T. H. & Dalton, W. S. Bone marrow stromal-derived soluble factors and direct cell contact contribute to *de novo* drug resistance of myeloma cells by distinct mechanisms. *Leukemia* **17**, 1175–1182 (2003).
130. Damiano, J. S., Cress, A. E., Hazlehurst, L. A., Shtil, A. A. & Dalton, W. S. Cell adhesion mediated drug resistance (CAM-DR): role of integrins and resistance to apoptosis in human myeloma cell lines. *Blood* **93**, 1658–1667 (1999).
131. Grigorieva, I., Thomas, X. & Epstein, J. The bone marrow stromal environment is a major factor in myeloma cell resistance to dexamethasone. *Exp. Hematol.* **26**, 597–603 (1998).
132. Vacca, A. *et al.* Bone marrow neovascularization, plasma cell angiogenic potential, and matrix metalloproteinase-2 secretion parallel progression of human multiple myeloma. *Blood* **93**, 3064–3073 (1999).
133. Oikawa,T. *et al.* The proteasome is involved in angiogenesis. *Biochem. Biophys. Res. Commun.* **246**, 243–248 (1998).
134. Mezquita, J., Mezquita, B., Pau, M. & Mezquita, C. Down-regulation of *Flt-1* gene expression by the proteasome inhibitor MG262. *J. Cell Biochem.* **89**, 1138–1147 (2003).
135. Bladé, J. *et al.* Criteria for evaluating disease response and progression in patients with multiple myeloma treated by high-dose therapy and haemopoietic stem cell transplantation. Myeloma Subcommittee of the EBMT. European Group for Blood and Marrow Transplant. *Br. J. Haematol.* **102**, 1115–1123 (1998).
136. Berenson, J. R. *et al.* Experience with long–term therapy using the proteasome inhibitor, bortezomib, in advanced multiple myeloma (MM). *Proc. Am. Soc. Clin. Oncol.* **22**, 710 (2003).
137. Orlowski, R. Z. *et al.* Phase I study of the proteasome inhibitor bortezomib and pegylated liposomal doxorubicin in patients with refractory hematologic malignancies. *Blood* **102**, 449a (2003).
138. Yang, H. H. *et al.* A phase I/II trial of VELCADE™ and melphalan combination therapy (Vc+M) for patients with relapsed or refractory multiple myeloma (MM). *Blood* **102**, 235a (2004).
139. Richardson, P. G. *et al.* Peripheral neuropathy following bortezomib (VELCADE™, formerly PS–341) therapy in patients with advanced multiple myeloma (MM): characterization and reversibility. *Blood* **102**, 149a (2003).

Acknowledgements
Preparation of the manuscript was supported in part by an unrestricted grant from Millennium Pharmaceuticals.

Competing interests statement
The author declares competing financial interests: see web version for details.

Online links

DATABASES
The following terms in this article are linked online to:
Cancer.gov: http://cancer.gov/
acute lymphoblastic leukaemia | acute myelogenous leukaemia | breast cancer | colorectal cancer | multiple-myeloma | non-Hodgkin's lymphoma | non-small-cell lung cancer | ovarian cancer | pancreatic cancer | prostate cancer
LocusLink: http://www.ncbi.nlm.nih.gov/LocusLink/
BCL2 | caspase-3 | caspase-8 | CDC25A | CDC25B | CDC25C | E1 | KIP1 | *MDR1* | p53 | WAF1

FURTHER INFORMATION
Cell alive!: http://www.cellsalive.com/
Genome Knowledge Base: http://www.genome knowledge.org
International Myeloma Foundation: http://www.myeloma.org
Multiple Myeloma Research Foundation: http://www.multiplemyeloma.org/
NCI Cancer.gov Search for Clinical Trials: http://www.cancer.gov/search/clinical_trials/
Proteasome Inhibitors in Cancer by Millennium Pharmaceuticals: http://www.mlnm.com/clinicians/oncology/proteasome/index.asp
FDA Center for Drug Evaluation and Research: http://www.fda.gov/cder/index.html
United States Department of Energy Genome Image Library: http://www.ornl.gov/TechResources/Human_Genome/graphics/slides/sciproteasome. html
Velcade full prescribing information: http://www.millennium.com/products/velcade/full_prescrib_velcade.pdf
Access to this interactive links box is free online.