

# Pharmacological targets in the ubiquitin system offer new ways of treating cancer, neurodegenerative disorders and infectious diseases

Mariola J. Edelman<sup>1,\*</sup>, Benjamin Nicholson<sup>2</sup> and Benedikt M. Kessler<sup>3,\*</sup>

Recent advances in the development and discovery of pharmacological interventions within the ubiquitin–proteasome system (UPS) have uncovered an enormous potential for possible novel treatments of neurodegenerative disease, cancer, immunological disorder and microbial infection. Interference with proteasome activity, although initially considered unlikely to be exploitable clinically, has already proved to be very effective against haematological malignancies, and more specific derivatives that target subsets of proteasomes are emerging. Recent small-molecule screens have revealed inhibitors against ubiquitin-conjugating and -deconjugating enzymes, many of which have been evaluated for their potential use as therapeutics, either as single agents or in synergy with other drugs. Here, we discuss recent advances in the characterisation of novel UPS modulators (in particular, inhibitors of ubiquitin-conjugating and -deconjugating enzymes) and how they pave the way towards new therapeutic approaches for the treatment of proteotoxic disease, cancer and microbial infection.

<sup>1</sup>Institute of Genomics, Biocomputing and Biotechnology, Mississippi Agricultural and Forestry Experimental Station, Mississippi State University, Mississippi State, MS 39762, USA

<sup>2</sup>Progenra Inc., Malvern, PA 19355, USA

<sup>3</sup>Henry Wellcome Building for Molecular Physiology, Nuffield Department of Medicine, University of Oxford, Oxford, OX3 7BN, UK

\*Corresponding authors: Benedikt M. Kessler, Henry Wellcome Building for Molecular Physiology, Nuffield Department of Medicine, University of Oxford, Roosevelt Drive, Oxford OX3 7BN, UK. E-mail: bmk@ccmp.ox.ac.uk and Mariola J. Edelman, Institute for Genomics, Biocomputing and Biotechnology, Mississippi Agricultural and Forestry, Experimental Station, Pace Seed Lab, Rm 115, Mississippi State University, 650 Stone Boulevard, Mississippi State, MS 39762, USA. E-mail: mje100@mafes.msstate.edu

Pharmacological targets in the ubiquitin system offer new ways of treating cancer, neurodegenerative disorders and infectious diseases

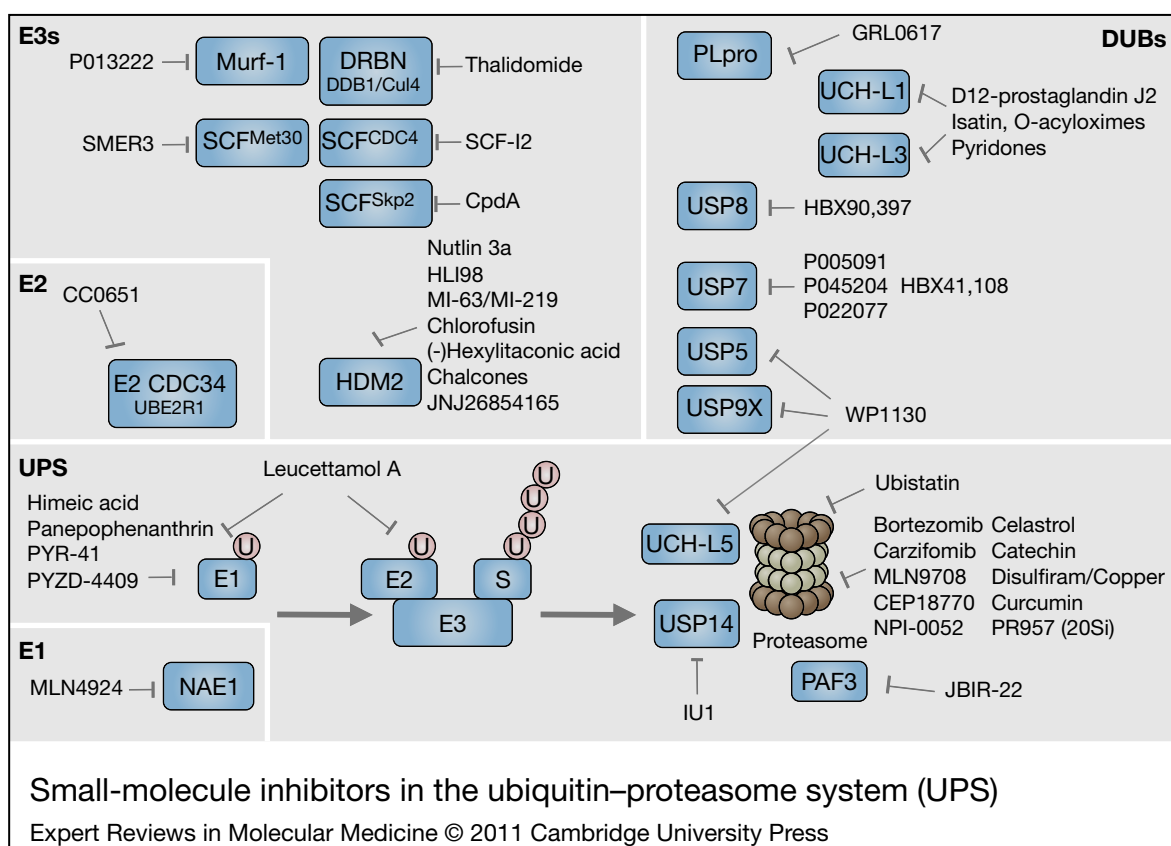
The ubiquitin–proteasome system (UPS) controls the turnover and biological function of most proteins within the cell, and alterations in this process can contribute to cancer progression, neurodegenerative disorders and pathogenicity associated with microbes. Therefore, pharmacological targeting of the UPS can potentially provide chemotherapeutics for the treatment of tumours, neurodegenerative conditions and infectious diseases. The widespread involvement of components of the UPS in many biological processes is reflected by the fact that several hundred genes have now been associated with this pathway (Refs 1, 2). Ubiquitin is a protein with 76 amino acids that can be covalently attached to other proteins, thereby influencing their fate and function. Protein ubiquitylation has numerous physiological functions. It can act as a recognition signal for proteasomal degradation (polyubiquitylation), serve as a signalling scaffold for protein–protein interactions (Lys63-poly- or monoubiquitylation) or represent a targeting signal for the lysosomal pathway or other cellular compartments (mostly monoubiquitylation). The ability of the ubiquitylation machinery to selectively target substrates is mediated by the specificity of ubiquitin ligation (E2 and E3 enzymes) and deconjugation, promoted by deubiquitylating enzymes (DUBs). Interference with either arm of this pathway should allow highly targeted pharmacological intervention, provided that compounds with sufficient selectivity can be identified (Refs 3, 4, 5, 6, 7, 8, 9) (Fig. 1). Additional opportunities are provided by the discovery of pathogen-encoded factors that evolved to target the UPS of the host cell, representing attractive targets for treatments against infectious diseases (Refs 10, 11, 12). Therefore, the UPS offers a source of novel pharmacological targets as the basis for the successful development of drugs to treat human diseases. However, the complexity of the ubiquitin system causes considerable challenges for high-throughput drug discovery because of extensive structural similarities. The generation of selective inhibitors is also impeded by the large number of DUBs (Refs 13, 14), ubiquitin-conjugating enzymes (E2s) and ubiquitin ligases (E3s) (Ref. 15) that might have redundancies in their biological functions. All these enzymes possess affinity for ubiquitin and various

ubiquitin conjugates. Therefore, their specificity is dependent on other structural subtleties and differences in protein–protein interactions unique to each enzyme species. To address this problem, an array of methodologies is used, such as the identification of ‘hits’ by high-throughput screening (HTS), the development of suitable assays for functional screening in vitro and in cells, and the use of protein structures to aid rational drug design. These approaches have already resulted in the discovery of a panel of inhibitory compounds against the proteasome, several ubiquitin-conjugating enzymes and DUBs, all of which have potential for further specific drug development, as discussed here.

### Targeting proteasome subsets for inhibition – reducing overall toxicity and overcoming drug resistance

Protein degradation by the proteasome, a multicatalytic proteinase complex, is at the centre of the UPS pathway (Fig. 1), and its pharmacological inhibition was originally considered lethal for all cell types. It was therefore rather surprising that bortezomib (Velcade) was approved as treatment for multiple myeloma in 2003 (Ref. 16). Since then, bortezomib has also been approved for the treatment of mantle cell lymphoma (Ref. 17). More recently, other derivatives have been developed that are at various stages of clinical trials, such as carfizomib (Phase III against relapsed multiple myeloma), MLN9708 (Phase I), CEP18770 (Phase I) and the natural product NPI-0052 (Phase I) (Ref. 3) (Fig. 1). Ubistatins were also discovered to inhibit proteasomal proteolysis by interfering with the recognition of polyubiquitin chains by the proteasome (Ref. 18). In addition to NPI-0052, further natural products with potential anticancer properties have been characterised to interfere with proteasomal proteolysis (reviewed in Ref. 19), such as celastrol (Ref. 20), catechin(–), the component of green tea (Ref. 21), disulfiram in combination with copper (Ref. 22), a triterpenoid inhibitor (Ref. 23), curcumin (Ref. 24) and JBIR-22, which inhibits homodimer formation of proteasome assembly factor 3 (Ref. 25). Many of these natural products have intrinsic antitumour properties, although it is not clear whether this is solely attributable to their proteasome inhibitory capacities. For instance, statins have pleiotropic effects and are

Pharmacological targets in the ubiquitin system offer new ways of treating cancer, neurodegenerative disorders and infectious diseases



**Figure 1. Small-molecule inhibitors in the ubiquitin–proteasome system (UPS).** Schematic representation of components of the UPS including E1, E2–E3 ligases, DUBs and the proteasome complex (20Si: immunoproteasome). Ubiquitin is indicated as pink circle labelled U. The UPS pathway and different examples of E1, E2, E3s and DUBs are highlighted in blue boxes. Increasing numbers of small-molecule inhibitors that interfere at various steps of the UPS cascade are being discovered.

used to treat a variety of different diseases, including prevention of cardiovascular events, although it is not entirely clear whether this is due to direct or indirect interference with proteasomal proteolysis (Ref. 26). As expected, proteasome inhibition causes side effects such as peripheral neuropathy, myelosuppression, nausea, hypersensitivity and increased susceptibility to infection (Ref. 27). One problem with proteasome inhibitors is the emergence of resistance; however, the combination of several proteasome inhibitors that exert complementary specificities appears to overcome the problem of resistance and might have the added benefit of enabling reduced dosing of the individual drugs (Refs 28, 29). An alternative way of circumventing the general toxicity of these compounds is to develop inhibitors selective for immunoproteasomes (Refs 30, 31). Immunoproteasomes are predominantly

expressed in B-cells, T-cells, macrophages, dendritic cells and other cell types of the haematopoietic lineage, and can be induced by cell exposure to interferon- $\gamma$  and tumour necrosis factor - $\alpha$ . This leads to an exchange of the catalytic  $\beta$ -subunits, thereby enhancing antigen presentation and preserving cell viability during inflammation-induced oxidative stress (Refs 32, 33). Based on the observation that the immunoproteasome promotes enhanced antigen processing and presentation, it is predicted that immunoproteasome inhibitors may have immunomodulatory effects, such as attenuating autoimmune-related pathologies. Indeed, a selective immunoproteasome inhibitor PR957 was shown to prevent experimental colitis (Ref. 34) and interfere with arthritis in a mouse model (Ref. 35). This strategy appears to be less toxic and particularly promising for treating autoimmune disorders, and might be

Pharmacological targets in the ubiquitin system offer new ways of treating cancer, neurodegenerative disorders and infectious diseases

extended to targeting the thymoproteasome, a subform expressed in the thymus (Ref. 36).

### **Inhibition of DUBs as a novel approach to treat cancer, neurodegenerative diseases and viral infection**

DUBs have also been molecular targets for inhibitor development in recent years (Fig. 1). Members of the DUB family known to contribute to neoplastic transformation include USP1 (Fanconi anaemia), USP2 (prostate cancer), DUB3 (breast cancer), USP4 (adenocarcinoma), USP7 (prostate cancer, non-small-cell lung adenocarcinoma), USP9X (leukaemias and myelomas) and BRCC36 (breast cancer) (Refs 13, 14, 37, 38, 39, 40). Mutations in the gene encoding the ubiquitin-specific protease CYLD can lead to the neoplastic condition cylindromatosis, and other DUBs are also expressed at lower levels in cancer, including A20 (B-cell and T-cell lymphomas), USP10 (carcinomas) (Ref. 41) and BAP1 (brain, lung and testicular cancers) (Ref. 37).

#### **USP7 and cancer**

USP7, also known as Herpes virus associated USP (HAUSP), is critical in cancer progression because of its destabilising effect on the tumour suppressor p53 (Refs 8, 42, 43). USP7 preferentially deubiquitylates the E3 ligase HDM2 and its binding partner HDMX, resulting in the destabilisation of p53 and the repression of p53 transactivation activity (Refs 43, 44, 45). Additional substrates of USP7 include claspin, FOXO4 and PTEN (Refs 40, 46, 47). Thus USP7 exerts both p53-dependent and p53-independent effects on the control of cell proliferation and apoptosis, making USP7 an attractive target for pharmaceutical intervention (Refs 48, 49). Recently, a high-throughput screen identified the small-molecule compound HBX 41,108 as an uncompetitive and reversible inhibitor of USP7 (Figs 1 and 2). HBX 41,108 (Fig. 2, compound 1) is a functionalised cyano-indenopyrazine derivative that inhibits several DUBs, including USP7, and stabilises p53 in a nongenotoxic manner, resulting in the induction of apoptosis (Refs 5, 50). An independent screen recently identified compound P005091 and analogues such as P045204 and P022077 (Fig. 2, compound 2) as USP7 inhibitors in vitro and in living cells (Refs 51, 59). Proof of concept in cells was demonstrated by the stabilisation of p53 and the

induction of p21 by a representative compound from the P005091 series (Ref. 49).

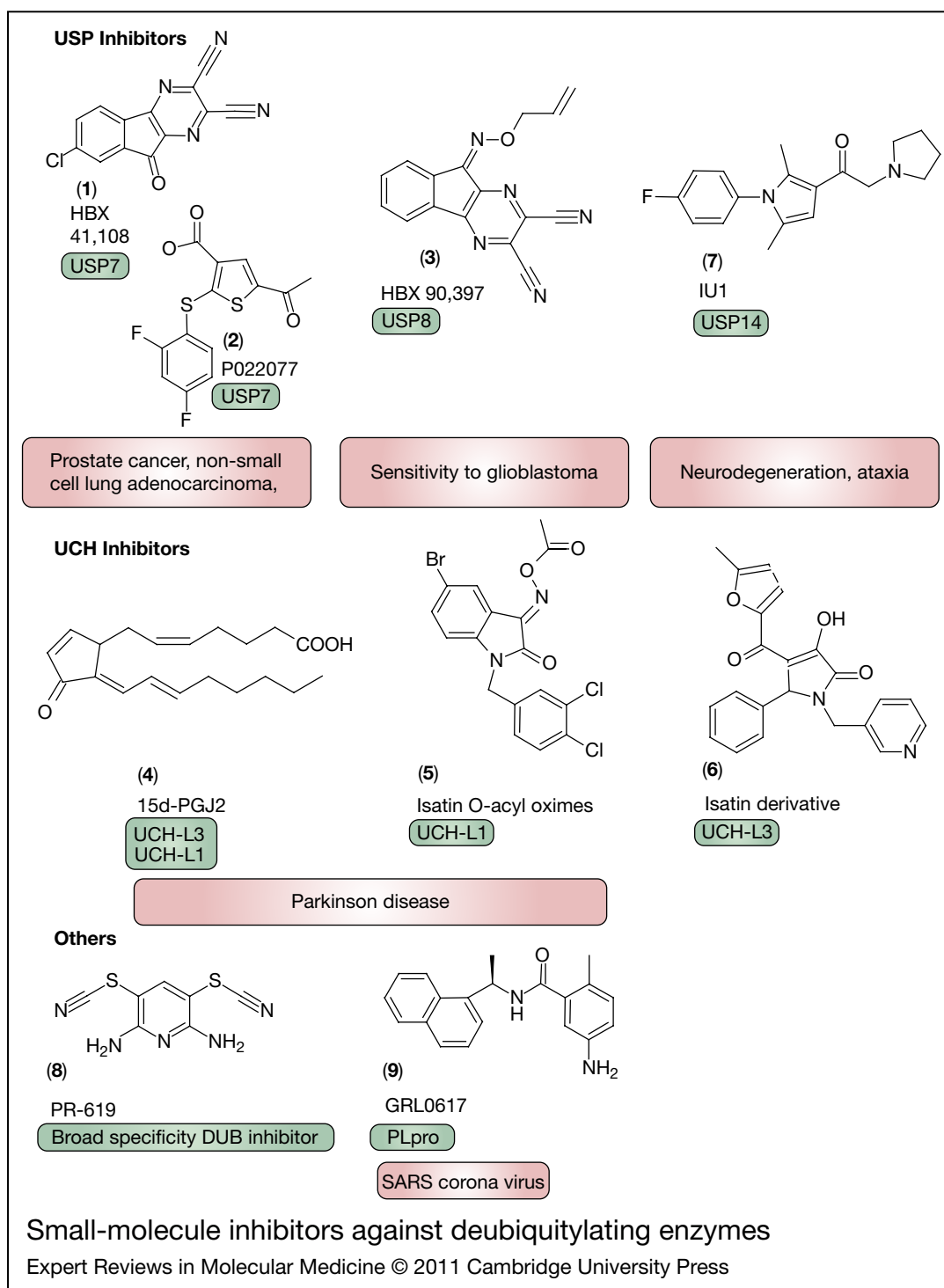
#### **USP8 (UBPY) and endocytosis**

As a function of its key role in the regulation of receptor endocytosis and trafficking, USP8 (UBPY) interacts with a number of clinically relevant cancer targets, including the epidermal growth factor receptor (EGFR) (Ref. 60). Knockdown of USP8 results in the accumulation of ubiquitylated EGFR in endosomes (Ref. 61). Hybrigenics reported the identification of the USP8 inhibitor HBX 90,397 (Fig. 2, compound 3). However, the clinical usefulness of USP8 inhibitors is questionable following the report that conditional knockout of USP8 in adult mice resulted in liver failure, probably as a result of pronounced decreases in receptor tyrosine kinases such as EGFR (Refs 62, 63).

#### **Prostaglandins as DUB inhibitors**

Prostaglandins are also reported to have inhibitory activity against DUBs in cellular assays. This family of compounds form a group of lipid derivatives that serve as signalling molecules that affect diverse protein functions, depending on their localisation and physiological context. Prostaglandins have been characterised as important messengers in inflammation (Ref. 64) and immune responses (Refs 65, 66), with emerging roles in cancer. The J-series prostaglandins are known to promote apoptosis in a p53-independent fashion (Refs 67, 68). D12-prostaglandin J2 was shown to inhibit ubiquitin isopeptidase activity in cell lysates, owing to the presence of the cross-conjugated  $\alpha,\beta$ -unsaturated ketones in their structure (Fig. 1). Similarly, compounds unrelated to prostaglandins, but also containing cross-conjugated  $\alpha,\beta$ -unsaturated ketones and accessible  $\beta$ -carbons, also inhibit isopeptidase activity. By contrast, the A-series prostaglandins, which contain a single  $\alpha,\beta$ -unsaturated ketone, are less efficacious as ubiquitin isopeptidase inhibitors (Refs 68, 69). Hence, the mechanism of inhibition by J-series prostaglandins is most likely based on the nucleophilic addition of a DUB thiol to the endocyclic  $\beta$ -carbon of a compound and the electrophilic accessibility of prostaglandin resulting from olefin–ketone conjugation. The inhibitory effect of prostaglandins is exemplified by 15-deoxy- $\Delta$ 12,14-prostaglandin J2 (15d-PGJ2) (Fig. 2, compound 4), which inhibits the activities





**Figure 2. Small-molecule inhibitors against deubiquitylating enzymes.** Examples of DUB inhibitors characterised in the literature targeting the USP family: HBX41,108 (1) (Ref. 50) and P022077 (2) (Ref. 51) specific for USP7, HBX 90,397 inhibits USP8 (3) (Ref. 52) and IU1 (7) inhibits USP14 (Ref. 53). Inhibitors targeting the UCH family include 15d-PGJ2 (Ref. 54) (4) and isatin O-acyl oximes (5) (Ref. 55) specific against UCH-L3 and other isatin derivatives (6) specific against UCH-L1 (Refs 56, 57). PR-619 (6) targets a broad range of DUBs (Ref. 51) and GRL0617 (9) inhibits SARS virus encoded papain-like protease (PLpro) (Ref. 58).

Pharmacological targets in the ubiquitin system offer new ways of treating cancer, neurodegenerative disorders and infectious diseases

of UCHL-3 (Ref. 54) and UCH-L1 (Refs 56, 57) (Fig. 1). 15d-PGJ2 has a detrimental effect on UCH-L1 structure and therefore perturbs its activity, offering possibilities of interfering with progression of Parkinson disease associated with UCH-L1 mutations (Ref. 57).

Punaglandins are cyclopentadienone and cyclopentenone prostaglandins chlorinated at the endocyclic  $\alpha$ -carbon position. These compounds, originally isolated from *Telesto riisei* coral, exhibit anti-inflammatory and antitumour activities (Ref. 70), with higher cytotoxic effects on cells than the J-series prostaglandins. Interestingly, their effect on apoptosis is independent of p53 (Ref. 71). Consistent with this, punaglandins, such as punaglandin 4, are also more potent inhibitors of cellular DUB activity than the J-series prostaglandins. The proposed mechanism for this enhanced inhibitor activity is the presence of an electronegative Cl substituent at the  $\alpha$ -position of  $\alpha,\beta$ -unsaturated carbonyls that increases the reactivity towards the nucleophilic addition of the DUB catalytic cysteine thiol group. These chlorinated lipids could therefore represent a new class of cancer therapeutics (Ref. 71).

Inhibitors based on other molecular scaffolds with preferential specificity towards UCHs have also been reported. For instance, Stein and colleagues have reported the discovery of isatin *O*-acyl oximes (Fig. 2, compounds 5 and 6) with inhibitory activity against UCH-L1 and UCH-L3 (Ref. 55). More recently, the same group reported a second series of UCH-L1 inhibitors based on a pyridone scaffold (Ref. 72). The authors speculate that these compounds might have potential in the treatment of neurodegenerative conditions.

### USP14 and neurodegeneration

In HTS, the small-molecule compound IU1 was found to be a selective inhibitor of USP14. USP14 is a DUB associated with the proteasome that blocks the degradation of ubiquitylated substrates. Cellular data confirmed the hypothesis that IU1-mediated inhibition of USP14 indirectly accelerates proteasomal degradation of proteins, such as tau and ataxin-3, both of which are involved in neurodegenerative diseases (Ref. 53). Because many neurodegenerative disorders such as Parkinson disease and Creutzfeldt–Jakob disease are associated with the accumulation of

misfolded proteins, IU1 (Fig. 2, compound 7) or other USP14-directed small-molecule inhibitors could potentially be used to eliminate these toxic proteins and improve the prognosis in neurodegenerative diseases. In support of this, USP14 has been previously linked to neurodegenerative disease, and loss of USP14 leads to an ataxic neurological phenotype in mice (Refs 73, 74).

### General DUB inhibitors

WP1130 (degrasyn) is a derivative of AG490, a small-molecule compound that blocks Janus-activated kinase 2 (JAK2) activity. In cells, WP1130 treatment induces accumulation of polyubiquitylated conjugates. This phenomenon has been attributed to the inhibitory activity of WP1130 towards several DUBs, including USP9x, USP5, USP14 and UCH-L5 (Ref. 75). WP1130 might be of therapeutic value, and its proapoptotic properties have been recently described (Ref. 76). In support of this, treatment of cells with WP1130 results in the modulation of anti- and proapoptotic proteins, such as MCL-1 and p53 (Ref. 75). Further work suggests that WP1130 could be administered with bortezomib because the combination resulted in synergistic inhibition of tumour cells, regulation of apoptosis and prolonged survival of the animals (Ref. 77).

Recently, an additional broad-specificity DUB inhibitor, PR-619 (Fig. 2, compound 8), was discovered using ubiquitin–CHOP reporter technology (Refs 51, 78). Given its broad specificity, the utility of PR-619 probably lies in its role as a tool for recovering ubiquitylated proteins from both cell-free and cellular experimental systems.

### Inhibitors of viral DUBs

Viruses also encode DUBs, and these can be targeted to block viral infection. Papain-like protease (PLpro) is a DUB encoded by severe acute respiratory syndrome coronavirus (SARS-CoV) (Refs 12, 79, 80). PLpro blocks IRF3-dependent antiviral responses, indicating its relevance to key infectious processes and viral evasion of host innate immune responses. GRL0617 is a noncovalent inhibitor of PLpro (Fig. 2, compound 9), which blocks SARS-CoV viral replication without measurable cytotoxic effects and hence is a promising antiviral drug candidate. GRL0617 induces a conformational

change in PLpro, which effectively inactivates this DUB. Profiling of DUB activity in cells exposed to GRL0617 using ubiquitin-specific active-site probes demonstrated that this inhibitor is selective for PLpro, which might explain its low cytotoxicity (Ref. 58).

In summary, these studies illustrate the potential of many DUBs as suitable drug targets. Several challenges remain, but more recent developments in novel discovery platforms and enzyme substrates such as multi-ubiquitin chains of different type and length, CHOP reporter technology and other isopeptide-bond-based assays are now being used to identify novel DUB inhibitors with greater specificity and sensitivity, providing the framework for optimisation of more suitable drugs.

### Antagonists of E3 ubiquitin ligases

E3 ubiquitin ligases represent a diverse group of proteins with significant roles in ubiquitin conjugation. First, the E3 ligases catalyse the covalent transfer of ubiquitin to a lysyl side chain of a substrate (Ref. 81). Second, the specificity of E3 ligases determines which substrates become ubiquitylated, based on the recognition signals on target proteins (Refs 82, 83). Unsurprisingly, numerous functions in neurodegenerative disorders, autoimmune diseases, inflammation and cancer have been ascribed to E3 ligases (Refs 84, 85, 86). One of the most crucial E3 ligases in cancer cell physiology is the proto-oncogene HDM2 (or its murine homologue Mdm2), which has been reported to be amplified in many tumour cells (Ref. 87). The best characterised substrate of HDM2 is p53, which is targeted to the proteasome by HDM2. Thus, inhibition of HDM2 leads to activation of the p53 pathway, providing an attractive therapeutic strategy for cancers that retain wild-type p53 expression. The crystal structure of HDM2 bound to p53-derived peptide reveals a deep hydrophobic cleft in HDM2 necessary for p53 binding. This feature can be exploited for anticancer treatment by a rational design of peptide- and nonpeptide-based antagonists of the HDM2–p53 interaction by targeting the HDM2 cleft.

### HDM2 E3 ligase inhibitors in cancer

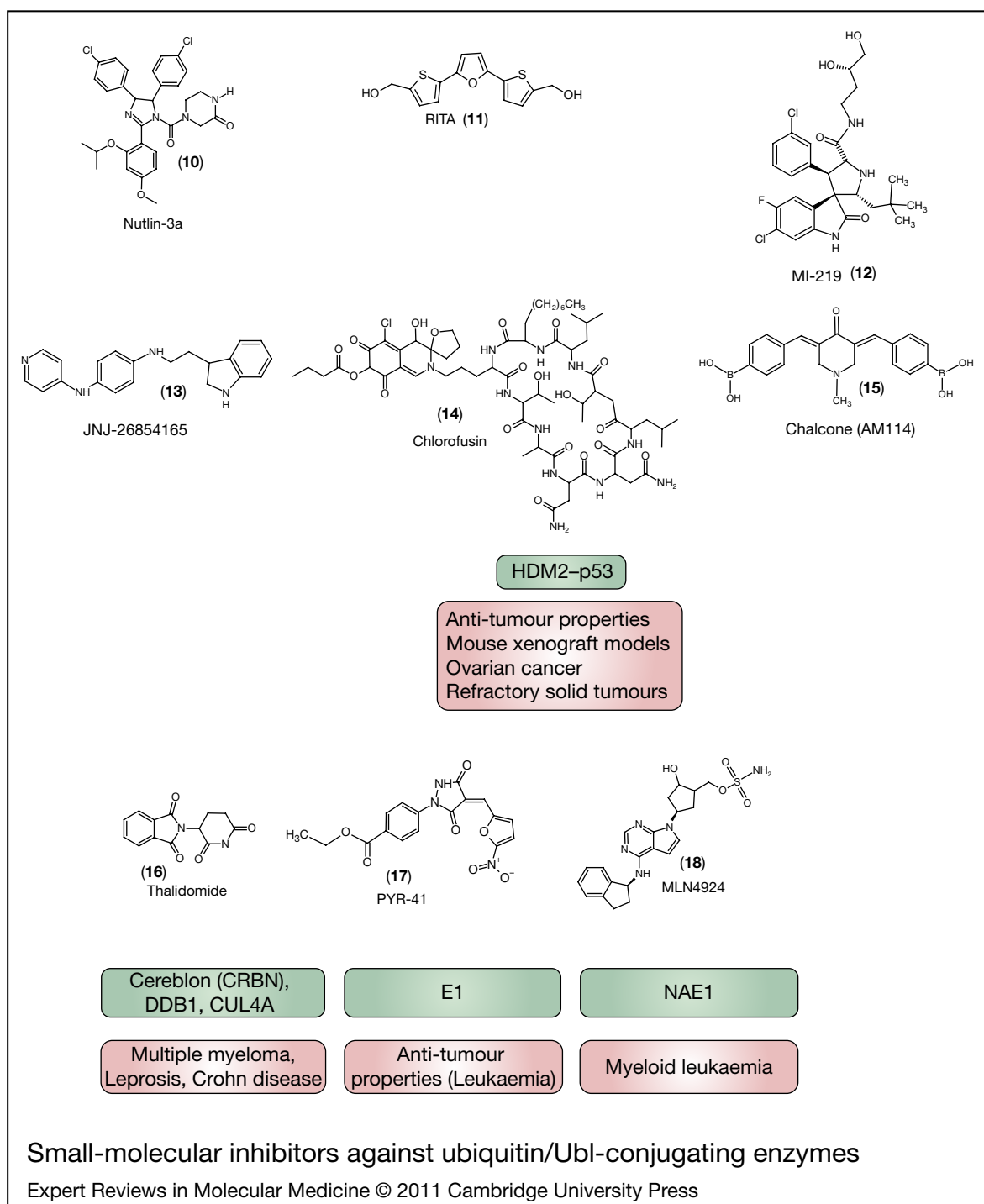
Nutlin-3a (Fig. 3, compound 10) belongs to a class of tetrasubstituted imidazolines and is a potent nonpeptide HDM2 antagonist. It acts as a

competitive inhibitor, blocking the interaction of p53 with HDM2 (Ref. 88). Consequently, Nutlin-3a stabilises p53 and its substrates p21 and Noxa, contributing to increased apoptosis and cell cycle arrest in the G1 phase. Its antitumour properties have been demonstrated for cancer cells expressing wild-type p53 (Refs 97, 98). Nutlin-3a efficiently eliminates tumour xenografts in mice, causing no measurable abnormalities in animals (Ref. 88). In other preclinical studies, the combination of Nutlin-3a and the proteasome inhibitor bortezomib induced additive cytotoxicity in malignant multiple myeloma cells. These synergistic antitumour activities might extend the clinical applications of bortezomib to neoplasias exhibiting reduced sensitivity to this proteasome inhibitor (Ref. 99). R7112, an analogue of Nutlin-3a, is currently in Phase I clinical trials for the treatment of solid tumours and haematological malignancies.

The proof-of-concept data provided by the discovery of the nutlins spurred additional research in this area, including the identification of the HLI98 family of compounds (7-nitro-5-deazaflavin) and RITA (Fig. 3, compound 11) that also target the ubiquitin-ligase activity of HDM2, resulting in the activation of p53-dependent apoptosis (Refs 100, 101). In addition, the spiro-oxindoles exemplified by MI-63 and MI-219 (Fig. 3, compound 12) and the chromenotriazolopyrimidines were also reported to be effective nonpeptidomimetic small-molecule inhibitors of the HDM2–p53 interaction (Refs 102, 103, 104).

Another mode of inhibition of HDM2 is by blocking its association with the proteasome. JNJ-26854165 (Fig. 3, compound 13) is one of the first compounds found to induce p53 levels in tumour cell lines and activate p53 transcriptional activity (Ref. 89). JNJ-26854165 is currently being investigated as an oral agent for the treatment of refractory solid tumours in clinical trials.

Natural products are also an important source of novel E3 ligase inhibitors. For example, 53 000 microbial extracts derived from fermented microorganisms, such as actinomycete bacteria and fungi, were screened to discover new compounds that antagonise the HDM2–p53 interaction. Among these, chlorofusin (Fig. 3, compound 14), a fungal metabolite isolated from *Fusarium* sp., was found to have the highest



**Figure 3. Small-molecule inhibitors against ubiquitin/Ubl-conjugating enzymes.** Examples of inhibitors against E3 ligases include Nutlin-3a (10), RITA (11), MI-219 (12), JNJ-26854165 (13), chlorofusin (14) and chalcone (AM114) (15), which specifically interfere with the HDM2-p53 or HDM2-proteasome interactions (Refs 88, 89, 90, 91, 92, 93), and thalidomide (16), which inhibits CRBN (Ref. 94). The ubiquitin-activating enzyme E1 is targeted by PYR-41 (Ref. 95) (17), and the NEDD8-activating enzyme NAE1 is inhibited by MLN4924 (18) (Ref. 96). All molecular targets are associated with disease pathologies, in particular cancer. See text for further details. Abbreviations: CRBN, cereblon; NEDD8, neural precursor cell expressed developmentally down-regulated 8; Ubl, ubiquitin-like protein.

Pharmacological targets in the ubiquitin system offer new ways of treating cancer, neurodegenerative disorders and infectious diseases



inhibitory activity towards HDM2 (Ref. 90). Chlorofusin has a nine-residue cyclic peptide containing an *l*-ornithine side chain linked to a highly functionalised tricyclic chromophore. Further studies established that neither the cyclic peptide, the chromophore of chlorofusin alone or simple derivatives thereof account for inhibition of the HDM2–p53 interaction, but that the whole structure of chlorofusin is required (Ref. 105).

The second example of a natural product that exhibits inhibition of the p53–HDM2 interaction is the (–) enantiomer of hexylitaconic acid isolated from a culture of marine-sponge-derived fungus *Arthrimum* sp. (Ref. 91). The (–) hexylitaconic acid impairs p53–HDM2 interactions in a dose-dependent manner, but its derivatives, including a monomethyl ester, a dihydro derivative and a dihydro derivative monomethyl ester, showed no inhibitory activity.

Chalcones are aromatic ketones previously characterised as potential antitumourigenic therapeutics in ovarian cancer (Ref. 92), gastric cancer and other tumours (Ref. 106). Chalcone derivatives interfere with p53–HDM2 interactions by binding near the tryptophan-binding pocket of the HDM2 hydrophobic cleft (Ref. 93). Molecular modelling studies indicate that boronic acid binds to lysine residues Lys51 and Lys94 of HDM2 (Ref. 107). The detailed mechanism of the cytotoxic activity of chalcones remains to be determined. In some cases, the enhanced apoptosis is related to inhibition of the 20S proteasome and thus stabilisation of p53, as exemplified by boronic chalcone derivative AM114 (Fig. 3, compound 15) (Ref. 108). Regardless, it seems likely that chalcone-mediated inhibition of the p53–HDM2 interaction is a contributory mechanism to their reported antitumour properties (Ref. 93).

### SCF<sup>Skp2</sup> E3 ligase inhibitors and cancer

HDM2 is not the only ubiquitin E3 ligase that constitutes a potential therapeutic target. SCF<sup>Skp2</sup> is an SCF (S-phase kinase-associated protein 1–cullin–F-box) ubiquitin E3 ligase containing Skp2, an F-box protein that determines substrate specificity. Upregulation of SCF<sup>Skp2</sup> is associated with decreased p27Kip1 levels and is negatively correlated with a good prognosis in cancer (Ref. 109); therefore, compounds directly targeting SCF<sup>Skp2</sup> represent potential drugs for cancer therapy. Using HTS, Chen and colleagues identified compound A

(CpdA) as a promising SCF<sup>Skp2</sup> inhibitor that prevents incorporation of Skp2 F-box protein into the SCF<sup>Skp2</sup> ligase complex. CpdA thus leads to ubiquitin-dependent accumulation of substrates for SCF<sup>Skp2</sup> E3 ligase activity, such as p27, and consequently induces G1–S cell cycle arrest and apoptosis. Notably, CpdA works synergistically with the proteasome inhibitor bortezomib (Ref. 110), probably by interfering with Cul1 neddylation.

Recently, two additional examples of E3 inhibitors were reported. First, using a fluorescence polarisation screen, the biplanar dicarboxylic acid compound SCF-I2 was shown to be an allosteric inhibitor of substrate recognition by the yeast F-box protein SCF<sup>Cdc4</sup>. SCF<sup>Cdc4</sup> degrades many substrates, such as SIC1, in a phosphorylation-dependent manner, and the SCF-I2 inhibitor perturbs the phosphodegron binding pocket of SCF<sup>Cdc4</sup> (Ref. 111). A second group used a yeast-based chemical genetics screen to identify modulators of SCF<sup>Met30</sup> activity (Ref. 112). Biochemical studies confirmed that SMER3 specifically inhibits SCF<sup>Met30</sup>-dependent ubiquitylation of the transcription factor Met4 by reducing the binding of Met30 to Skp1, which is probably due to its direct binding to Met30.

### CRBN E3 ligase targeted by the teratogenic agent thalidomide

Cereblon (CRBN), damaged DNA-binding protein 1 (DDB1) and Cul4A form an E3 ligase complex that is important for embryonic development. This complex is targeted by thalidomide (Fig. 3, compound 16), a clinically approved drug for the treatment of multiple myeloma, leprosy and inflammatory bowel disease (Crohn disease) (Ref. 113). One of the enantiomers of thalidomide was found to have teratogenic side effects. Binding of thalidomide to the CRBN complex and inhibition of CRBN E3 ligase activity appear to be the underlying molecular mechanisms for thalidomide-induced teratogenicity by the perturbation of embryonic development (Ref. 94).

### MuRF1 E3 ligase inhibition and muscular atrophy

MuRF1 is a key effector enzyme of muscular atrophy, an area of unmet medical need for several different pathologies (Ref. 114). Using an ELISA-based HTS platform, Progenra

identified a novel modulator of the E3 ubiquitin ligase, P013222, which inhibited MuRF1 autoubiquitylation and myosin heavy-chain ubiquitylation and protected myotubes from dexamethasone-induced muscle wasting (Ref. 115).

As outlined above, most of the identified E3 ligase inhibitors are directed towards protein–protein interactions, and their ‘druggability’ is therefore challenging. The complexity of this enzyme family, the lack of details on their precise molecular mechanism and the fact that most E3s rely on protein–protein interactions to mediate their activity makes the design of E3 ligase inhibitors difficult (Ref. 4), but potentially offers the framework for translational applications by interfering with many different biological processes in a highly specific manner. Recent reports of the identification of specific SCF inhibitors increase our confidence that it will be possible to develop inhibitors of this emerging class of important drug targets.

### Inhibition of ubiquitin-activating enzymes

Ubiquitin conjugation requires initial activation of ubiquitin by E1 enzyme, which adenylates the C-terminal carboxyl group of ubiquitin, forming a high-energy ubiquitin adenylate intermediate, followed by the formation of a thiol ester between the carboxyl group of Gly76 of ubiquitin and a thiol group of E1. This series of reactions activates the C-terminus of ubiquitin for a subsequent nucleophilic attack (Ref. 116). Blocking this reaction could therefore be used to inhibit ubiquitin conjugation. In vitro studies suggest that knockdown of E1 ligase results in lower levels of protein ubiquitylation and eventually induces cell death in malignant cells (Ref. 117). To identify novel E1 inhibitors, Yang and colleagues screened a library of small compounds and identified 4[4-(5-nitro-furan-2-ylmethylene)-3,5-dioxo-pyrazolidin-1-yl]-benzoic acid ethyl ester (PYR-41) as the first cell-permeable E1 inhibitor. PYR-41 efficiently reduces bulk protein ubiquitylation and sumoylation, and prevents degradation of p53, contributing to enhanced apoptosis. PYR-41 also attenuates cytokine-mediated nuclear factor- $\kappa$ B (NF- $\kappa$ B) activation by regulating proteasomal degradation of I $\kappa$ B $\alpha$ , an inhibitory subunit of NF- $\kappa$ B. Functionally, PYR-41 probably binds irreversibly to the active-site cysteine in E1

ligase, therefore preventing ubiquitin transfer (Ref. 95). However, this compound also targets several DUBs, including USP5, and crosslinks to kinases and has antitumour activity in animals (Ref. 118). PYZD-4409 (Fig. 3, compound 17), another small-molecule inhibitor, has been shown to interfere with the activity of E1 ligase, preferentially inducing tumour cell death in primary acute myeloid leukaemia cells. The effects of PYZD-4409 have also been studied in a mouse model of leukaemia, where it reduced tumour weight and volume. This study underlines the importance of E1 as a potential drug target in leukaemia and possibly other cancers, especially in cases where neoplastic cells are resistant to treatment with proteasome inhibitors such as bortezomib (Ref. 117). Furthermore, two natural products have been identified to inhibit E1, panepophenanthrin isolated from the mushroom *Panus rudis* (Refs 119, 120) and himeic acid A derived from the fungus *Aspergillus* sp. (Ref. 121), both of which inhibit the E1-catalysed ubiquitin activation in vitro, but with unknown mechanisms.

Recently, small-molecule inhibitors of E2 enzymes were also discovered. Leucettamol A, a compound isolated from a marine sponge *Leucetta aff. Microrhaphis*, was identified as a novel inhibitor of the Ubc13–Uev1A interaction, thereby blocking the formation of the E1–E2 complex (Ref. 122). Also, an allosteric inhibitor of the human Cdc34 E2 ligase, CC0651, was found through a small-molecule screen for inhibitors of SCF<sup>Skp2</sup>-dependent ubiquitylation of p27Kip1, and was shown to interfere with the proliferation of human cancer cell lines (Ref. 123).

Generally, when targeting E1–E2 conjugating enzymes, several pathways that are dependent on ubiquitylation, such as DNA repair or endocytosis, are inhibited at the same time, potentially contributing to increased nonspecific cytotoxicity. Therefore, from the therapeutic standpoint, the use of ubiquitin E1 (or E2)-specific inhibitors is currently awaiting additional preclinical validation before advancing to clinical studies.

### Targeted inhibition of NEDD8-activating enzymes

Neural precursor cell-expressed developmentally downregulated-8 (NEDD8) is a ubiquitin-like protein with the highest homology to ubiquitin. Its conjugation to substrates (neddylation)

requires activation by the E1 APPBP1-UBA3 and transfer by the E2 UBC12 (Ref. 124). NEDD8 primarily functions in the regulation of E3 ubiquitin ligases, modifying most members of the cullin family. Cullins are scaffold components of the SCF E3 ubiquitin ligases that control the proteasomal degradation of proteins involved in the cell cycle, transcriptional regulation or signal transduction (Refs 124, 125). Neddylation of cullins results in increased ubiquitylation of the SCF substrate proteins and their subsequent proteasomal degradation. SCF E3 ligases promote the ubiquitylation of proteins involved in inflammation and tumourigenesis (Ref. 126), such as HIF- $\alpha$  and I $\kappa$ B $\alpha$  (Refs 127, 96); therefore, specific inhibition of NEDD8-activating enzymes (E1) and other components of the neddylation pathway represents an alternative approach to targeting the UPS for cancer treatment. MLN4924 (Fig. 3, compound 18) is a small-molecule inhibitor of the NEDD8-activating enzyme and is presently being evaluated in Phase I clinical trials. MLN4924 increases the apoptosis of several tumour cell lines and murine tumour xenografts and is considered a promising drug candidate for myeloid leukaemia (Refs 96, 128, 129). In contrast to the proteasome inhibitor bortezomib, MLN4924 is more specific because it does not inhibit bulk proteasomal degradation (Ref. 96). The functional mechanism of MLN4924 involves formation of the MLN4924–NEDD8 covalent adduct, which is similar to the first intermediate of the reaction catalysed by the NEDD8-activating enzyme, thus efficiently inhibiting the NEDD8 E1 enzyme (Ref. 130).

Given that SCF E3 ligases represent several hundred of all known E3 ubiquitin ligases, there is concern that inhibition of ~300 E3 ligases might lead to serious side effects in a clinical setting. However, the impact of side effects must be taken in the context of proteasome inhibitors, which have been shown to exhibit acceptable clinical profiles for the treatment of cancer, yet modulate the stability of many more proteins (relative to SCF E3s). Ultimately, the evaluation of MLN4924 and any successor compounds in a clinical setting will determine whether this strategy is a therapeutically acceptable approach.

### Conclusions and perspectives

The ubiquitin system or ‘ubiquitome’ has been compared with the well-characterised ‘kinome’

and has spurred an entire array of novel inhibitors against molecular targets that manipulate ubiquitin and ubiquitin-like molecules. Similarly to the kinase field, functional redundancy, the structural similarities of active sites (DUBs) and the diversity of protein–protein interaction domains (conjugating enzymes) render the discovery of specific inhibitors challenging. Several novel compounds are promising results in clinical trials, such as the proteasome inhibitors carfizomib (Phase III), MLN2238 (Phase I) and NPI-0052 (Phase I) or the NAE inhibitor MLN4924 (Phase I against AML and solid tumours). Thalidomide, which has been in clinical use for many years, has been recently identified as an E3 ligase inhibitor. Also, several E1 and E3 ligase inhibitors such as PYR-41, Nutlin-3a, Compound A, P013222 and SCF-I2 have proved successful in the preclinical stage. Generally, it remains to be determined whether small molecules are required to specifically target only one molecule to be clinically useful or whether, in a manner similar to medically relevant kinase inhibitors, molecules with broader specificities against subfamilies of enzymes might exhibit clinical efficacy. Clearly, the emergence of new inhibitors directed against UPS components supplements the activities of kinase inhibitors, cytotoxic agents and other compounds, and it is predicted that ultimately the combinatorial use of these drugs holds the greatest promise for future therapies against cancer, neurodegenerative disorders and infectious disease. These disease pathologies are highly complex, and substantial differences can occur between individuals. A broadened arsenal of small-molecule compounds, including drugs targeting components of the UPS, might provide the framework for individualised drug regimens as part of a trend towards personalised medicine.

### Outstanding research questions

- Generate further understanding of the precise role of E1/E2/E3 ligases and DUBs and other components in the UPS in disease processes, to establish correlations between their dysfunction and properties of disease pathology.
- Develop promising lead compounds into more effective inhibitors with greater potency.
- Whereas many small-molecule compounds show antiproliferative activities in tumour cell



lines, it is less clear how this can be translated into inhibiting tumour growth in vivo without affecting normal cells. Distinguishing between these two scenarios should drive the selection and development of more effective compounds in the future.

- Is it really necessary to chemically target one enzyme for optimal interference with disease progression? Many diseases, in particular cancer, exert aberrations in several biochemical pathways. The discovery and pharmacological targeting of all abnormally functioning networks will be necessary for better treatments in the future.

### Acknowledgements

M.J.E. is supported by a USDA NIFA grant (Nr. 2009-34609-20222). B.N. is supported by grants DK07139 from NIDDK/NIH and CA115205 from NCI/NIH to Progenra Inc. B.M.K. is supported by the NIHR Biomedical Research Centre, Oxford, UK and by an Action Medical Research Grant (Charity Nr. 208701 and SC039284). We thank members of the Kessler group for helpful discussions and also the peer reviewers for useful comments on the manuscript.

### References

- 1 Behrends, C. and Harper, J.W. (2011) Constructing and decoding unconventional ubiquitin chains. *Nature Structural and Molecular Biology* 18, 520-528
- 2 Clague, M.J. and Urbe, S. (2010) Ubiquitin: same molecule, different degradation pathways. *Cell* 143, 682-685
- 3 Bedford, L. et al. (2011) Ubiquitin-like protein conjugation and the ubiquitin-proteasome system as drug targets. *Nature Reviews. Drug Discovery* 10, 29-46
- 4 Eldridge, A.G. and O'Brien, T. (2010) Therapeutic strategies within the ubiquitin proteasome system. *Cell Death and Differentiation* 17, 4-13
- 5 Guedat, P. and Colland, F. (2007) Patented small molecule inhibitors in the ubiquitin proteasome system. *BMC Biochemistry* 8 (Suppl 1), S14
- 6 Kirkin, V. and Dikic, I. (2011) Ubiquitin networks in cancer. *Current Opinion in Genetics and Development* 21, 21-28
- 7 Nalepa, G., Rolfe, M. and Harper, J.W. (2006) Drug discovery in the ubiquitin-proteasome system. *Nature Reviews. Drug Discovery* 5, 596-613
- 8 Nicholson, B. et al. (2007) Deubiquitinating enzymes as novel anticancer targets. *Future Oncology* 3, 191-199
- 9 Sippl, W., Collura, V. and Colland, F. (2011) Ubiquitin-specific proteases as cancer drug targets. *Future Oncology* 7, 619-632
- 10 Edelmann, M.J. and Kessler, B.M. (2008) Ubiquitin and ubiquitin-like specific proteases targeted by infectious pathogens: emerging patterns and molecular principles. *Biochimica Biophysica et Acta* 1782, 809-816
- 11 Isaacson, M.K. and Ploegh, H.L. (2009) Ubiquitination, ubiquitin-like modifiers, and deubiquitination in viral infection. *Cell Host and Microbe* 5, 559-570
- 12 Lindner, H.A. (2007) Deubiquitination in virus infection. *Virology* 362, 245-256
- 13 Nijman, S.M. et al. (2005) A genomic and functional inventory of deubiquitinating enzymes. *Cell* 123, 773-786
- 14 Reyes-Turcu, F.E., Ventii, K.H. and Wilkinson, K.D. (2009) Regulation and cellular roles of ubiquitin-specific deubiquitinating enzymes. *Annual Review of Biochemistry* 78, 363-397
- 15 Pickart, C.M. and Eddins, M.J. (2004) Ubiquitin: structures, functions, mechanisms. *Biochimica Biophysica et Acta* 1695, 55-72
- 16 Chen, D. et al. (2011) Bortezomib as the first proteasome inhibitor anticancer drug: current status and future perspectives. *Current Cancer Drug Targets* 11, 239-53
- 17 Kouroukis, C.T. et al. (2011) A phase II study of bortezomib and gemcitabine in relapsed mantle cell lymphoma from the National Cancer Institute of Canada Clinical Trials Group (IND 172). *Leukemia and Lymphoma* 52, 394-399
- 18 Verma, R. et al. (2004) Ubistatins inhibit proteasome-dependent degradation by binding the ubiquitin chain. *Science* 306, 117-120
- 19 Schneekloth, J.S., Jr, and Crews, C.M. (2011) Natural product inhibitors of the ubiquitin-proteasome pathway. *Current Drug Targets* 12, 1581-94
- 20 Yang, H. et al. (2006) Celastrol, a triterpene extracted from the Chinese "Thunder of God Vine," is a potent proteasome inhibitor and suppresses human prostate cancer growth in nude mice. *Cancer Research* 66, 4758-4765
- 21 Landis-Piwowar, K.R. et al. (2007) A novel prodrug of the green tea polyphenol (-)-epigallocatechin-3-gallate as a potential anticancer agent. *Cancer Research* 67, 4303-4310



- 22 Chen, D. et al. (2006) Disulfiram, a clinically used anti-alcoholism drug and copper-binding agent, induces apoptotic cell death in breast cancer cultures and xenografts via inhibition of the proteasome activity. *Cancer Research* 66, 10425-10433
- 23 Tiedemann, R.E. et al. (2009) Identification of a potent natural triterpenoid inhibitor of proteasome chymotrypsin-like activity and NF-kappaB with antimyeloma activity in vitro and in vivo. *Blood* 113, 4027-4037
- 24 Jana, N.R. et al. (2004) Inhibition of proteasomal function by curcumin induces apoptosis through mitochondrial pathway. *Journal of Biological Chemistry* 279, 11680-11685
- 25 Izumikawa, M. et al. (2011) JBIR-22, an inhibitor for protein-protein interaction of the homodimer of proteasome assembly factor 3. *Journal of Natural Products* 73, 628-631
- 26 Ludwig, A. et al. (2005) Effect of statins on the proteasomal activity in mammalian endothelial and vascular smooth muscle cells. *Biochemical Pharmacology* 70, 520-526
- 27 Basler, M. et al. (2009) The proteasome inhibitor bortezomib enhances the susceptibility to viral infection. *Journal Immunology* 183, 6145-6150
- 28 Mirabella, A.C. et al. (2011) Specific cell-permeable inhibitor of proteasome trypsin-like sites selectively sensitizes myeloma cells to bortezomib and Carfilzomib. *Chemistry and Biology* 18, 608-618
- 29 Ruschak, A.M. et al. (2011) Novel proteasome inhibitors to overcome bortezomib resistance. *Journal of the National Cancer Institute* 103, 1007-1017
- 30 Ho, Y.K. et al. (2007) LMP2-specific inhibitors: chemical genetic tools for proteasome biology. *Chemistry and Biology* 14, 419-430
- 31 Singh, A.V. et al. (2011) PR-924, a selective inhibitor of the immunoproteasome subunit LMP-7, blocks multiple myeloma cell growth both in vitro and in vivo. *British Journal of Haematology* 152, 155-163
- 32 Kloetzel, P.M. and Ossendorp, F. (2004) Proteasome and peptidase function in MHC-class-I-mediated antigen presentation. *Current Opinion in Immunology* 16, 76-81
- 33 Seifert, U. et al. (2010) Immunoproteasomes preserve protein homeostasis upon interferon-induced oxidative stress. *Cell* 142, 613-624
- 34 Basler, M. et al. (2010) Prevention of experimental colitis by a selective inhibitor of the immunoproteasome. *Journal Immunology* 185, 634-641
- 35 Muchamuel, T. et al. (2009) A selective inhibitor of the immunoproteasome subunit LMP7 blocks cytokine production and attenuates progression of experimental arthritis. *Nature Medicine* 15, 781-787
- 36 Groettrup, M., Kirk, C.J. and Basler, M. (2010) Proteasomes in immune cells: more than peptide producers? *Nature Reviews. Immunology* 10, 73-78
- 37 Hussain, S., Zhang, Y. and Galardy, P.J. (2009) DUBs and cancer: the role of deubiquitinating enzymes as oncogenes, non-oncogenes and tumor suppressors. *Cell Cycle* 8, 1688-1697
- 38 Pereg, Y. et al. (2010) Ubiquitin hydrolase Dub3 promotes oncogenic transformation by stabilizing Cdc25A. *Nature Cell Biology* 12, 400-406
- 39 Schwickart, M. et al. (2010) Deubiquitinase USP9X stabilizes MCL1 and promotes tumour cell survival. *Nature* 463, 103-107
- 40 Song, M.S. et al. (2008) The deubiquitylation and localization of PTEN are regulated by a HAUSP-PML network. *Nature* 455, 813-817
- 41 Yuan, J. et al. (2010) USP10 regulates p53 localization and stability by deubiquitinating p53. *Cell* 140, 384-396
- 42 Cummins, J.M. and Vogelstein, B. (2004) HAUSP is required for p53 destabilization. *Cell Cycle* 3, 689-692
- 43 Li, M. et al. (2004) A dynamic role of HAUSP in the p53-Mdm2 pathway. *Molecular Cell* 13, 879-886
- 44 Cummins, J.M. et al. (2004) Tumour suppression: disruption of HAUSP gene stabilizes p53. *Nature* 428, 1 p following 486
- 45 Meulmeester, E. et al. (2005) Loss of HAUSP-mediated deubiquitination contributes to DNA damage-induced destabilization of Hdmx and Hdm2. *Molecular Cell* 18, 565-576
- 46 Faustrup, H. et al. (2009) USP7 counteracts SCFbetaTrCP- but not APCCdh1-mediated proteolysis of Claspin. *Journal of Cell Biology* 184, 13-19
- 47 van der Horst, A. et al. (2006) FOXO4 transcriptional activity is regulated by monoubiquitination and USP7/HAUSP. *Nature Cell Biology* 8, 1064-1073
- 48 Cheon, K.W. and Baek, K.H. (2006) HAUSP as a therapeutic target for hematopoietic tumors (review). *International Journal of Oncology* 28, 1209-1215
- 49 Nicholson, B. and Suresh Kumar, K.G. (2011) The multifaceted roles of USP7; new therapeutic opportunities. *Cell Biochemistry and Biophysics* 60, 61-68
- 50 Colland, F. et al. (2009) Small-molecule inhibitor of USP7/HAUSP ubiquitin protease stabilizes and

Pharmacological targets in the ubiquitin system offer new ways of treating cancer, neurodegenerative disorders and infectious diseases

- activates p53 in cells. *Molecular Cancer Therapeutics* 8, 2286-2295
- 51 Tian, X. et al. (2011) Characterization of selective ubiquitin and ubiquitin-like protease inhibitors using a fluorescence-based multiplex assay format. *Assay and Drug Development Technologies* 9, 165-173
- 52 Colland, F. (2010) The therapeutic potential of deubiquitinating enzyme inhibitors. *Biochemical Society Transactions* 38, 137-143
- 53 Lee, B.H. et al. (2010) Enhancement of proteasome activity by a small-molecule inhibitor of USP14. *Nature* 467, 179-184
- 54 Li, Z. et al. (2004) Delta12-prostaglandin J2 inhibits the ubiquitin hydrolase UCH-L1 and elicits ubiquitin-protein aggregation without proteasome inhibition. *Biochemical and Biophysical Research Communications* 319, 1171-1180
- 55 Liu, Y. et al. (2003) Discovery of inhibitors that elucidate the role of UCH-L1 activity in the H1299 lung cancer cell line. *Chemistry and Biology* 10, 837-846
- 56 Liu, H. et al. (2010) Modification of ubiquitin-C-terminal hydrolase-L1 by cyclopentenone prostaglandins exacerbates hypoxic injury. *Neurobiology of Disease* 41, 318-28
- 57 Koharudin, L.M. et al. (2010) Cyclopentenone prostaglandin-induced unfolding and aggregation of the Parkinson disease-associated UCH-L1. *Proceedings of the National Academy of Sciences of the United States of America* 107, 6835-6840
- 58 Ratia, K. et al. (2008) A noncovalent class of papain-like protease/deubiquitinase inhibitors blocks SARS virus replication. *Proceedings of the National Academy of Sciences of the United States of America* 105, 16119-16124
- 59 Cao, P. et al. (2011) Anti-neoplastic compounds, compositions and methods. WIPO Patent Application WO/2010/114881 <http://www.sumobrain.com/patents/wipo/Anti-neoplastic-compounds-compositions-methods/WO2010114881.html>
- 60 Williams, R.L. and Urbe, S. (2007) The emerging shape of the ESCRT machinery. *Nature Reviews. Molecular Cell Biology* 8, 355-368
- 61 Mizuno, E. et al. (2006) A deubiquitinating enzyme UBPY regulates the level of protein ubiquitination on endosomes. *Traffic* 7, 1017-1031
- 62 Colombo, M. et al. (2010) Synthesis and biological evaluation of 9-oxo-9H-indeno[1,2-b]pyrazine-2,3-dicarbonitrile analogues as potential inhibitors of deubiquitinating enzymes. *ChemMedChem* 5, 552-558
- 63 Niendorf, S. et al. (2007) Essential role of ubiquitin-specific protease 8 for receptor tyrosine kinase stability and endocytic trafficking in vivo. *Molecular and Cellular Biology* 27, 5029-5039
- 64 Scher, J.U. and Pillinger, M.H. (2009) The anti-inflammatory effects of prostaglandins. *Journal of Investigative Medicine* 57, 703-708
- 65 Rocca, B. and FitzGerald, G.A. (2002) Cyclooxygenases and prostaglandins: shaping up the immune response. *International Immunopharmacology* 2, 603-630
- 66 Harris, S.G. et al. (2002) Prostaglandins as modulators of immunity. *Trends in Immunology* 23, 144-150
- 67 Clay, C.E. et al. (1999) Influence of J series prostaglandins on apoptosis and tumorigenesis of breast cancer cells. *Carcinogenesis* 20, 1905-1911
- 68 Mullally, J.E. et al. (2001) Cyclopentenone prostaglandins of the J series inhibit the ubiquitin isopeptidase activity of the proteasome pathway. *Journal of Biological Chemistry* 276, 30366-30373
- 69 Mullally, J.E. and Fitzpatrick, F.A. (2002) Pharmacophore model for novel inhibitors of ubiquitin isopeptidases that induce p53-independent cell death. *Molecular Pharmacology* 62, 351-358
- 70 Baker, B.J. and Scheuer, P.J. (1994) The punaglandins: 10-chloroprostanoids from the octocoral *Teleso riisei*. *Journal of Natural Products* 57, 1346-1353
- 71 Verbitski, S.M. et al. (2004) Punaglandins, chlorinated prostaglandins, function as potent Michael receptors to inhibit ubiquitin isopeptidase activity. *Journal of Medicinal Chemistry* 47, 2062-2070
- 72 Mermerian, A.H. et al. (2007) Structure-activity relationship, kinetic mechanism, and selectivity for a new class of ubiquitin C-terminal hydrolase-L1 (UCH-L1) inhibitors. *Bioorganic and Medicinal Chemistry Letters* 17, 3729-3732
- 73 Wilson, S.M. et al. (2002) Synaptic defects in ataxia mice result from a mutation in Usp14, encoding a ubiquitin-specific protease. *Nature Genetics* 32, 420-425
- 74 Anderson, C. et al. (2005) Loss of Usp14 results in reduced levels of ubiquitin in ataxia mice. *Journal of Neurochemistry* 95, 724-731
- 75 Kapuria, V. et al. (2010) Deubiquitinase inhibition by small-molecule WP1130 triggers aggresome

- formation and tumor cell apoptosis. *Cancer Research* 70, 9265-9276
- 76 Bartholomeusz, G.A. et al. (2007) Activation of a novel Bcr/Abl destruction pathway by WP1130 induces apoptosis of chronic myelogenous leukemia cells. *Blood* 109, 3470-3478
- 77 Pham, L.V. et al. (2010) Degrasyn potentiates the antitumor effects of bortezomib in mantle cell lymphoma cells in vitro and in vivo: therapeutic implications. *Molecular Cancer Therapeutics* 9, 2026-2036
- 78 Nicholson, B. et al. (2008) Characterization of ubiquitin and ubiquitin-like-protein isopeptidase activities. *Protein Science* 17, 1035-1043
- 79 Lindner, H.A. et al. (2005) The papain-like protease from the severe acute respiratory syndrome coronavirus is a deubiquitinating enzyme. *Journal of Virology* 79, 15199-15208
- 80 Barretto, N. et al. (2005) The papain-like protease of severe acute respiratory syndrome coronavirus has deubiquitinating activity. *Journal of Virology* 79, 15189-15198
- 81 Hershko, A. et al. (1983) Components of ubiquitin-protein ligase system. Resolution, affinity purification, and role in protein breakdown. *Journal of Biological Chemistry* 258, 8206-8214
- 82 Hershko, A. and Ciechanover, A. (1998) The ubiquitin system. *Annual Review of Biochemistry* 67, 425-479
- 83 Yaron, A. et al. (1997) Inhibition of NF-kappa-B cellular function via specific targeting of the I-kappa-B-ubiquitin ligase. *EMBO Journal* 16, 6486-6494
- 84 Melino, G. et al. (2008) Itch: a HECT-type E3 ligase regulating immunity, skin and cancer. *Cell Death and Differentiation* 15, 1103-1112
- 85 Gomez-Martin, D., Diaz-Zamudio, M. and Alcocer-Varela, J. (2008) Ubiquitination system and autoimmunity: the bridge towards the modulation of the immune response. *Autoimmunity Reviews* 7, 284-290
- 86 Nyhan, M.J., O'Sullivan, G.C. and McKenna, S.L. (2008) Role of the VHL (von Hippel-Lindau) gene in renal cancer: a multifunctional tumour suppressor. *Biochemical Society Transactions* 36, 472-478
- 87 Momand, J. et al. (1998) The MDM2 gene amplification database. *Nucleic Acids Research* 26, 3453-3459
- 88 Vassilev, L.T. et al. (2004) In vivo activation of the p53 pathway by small-molecule antagonists of MDM2. *Science* 303, 844-848
- 89 Dickens, M.P., Fitzgerald, R. and Fischer, P.M. (2010) Small-molecule inhibitors of MDM2 as new anticancer therapeutics. *Seminars in Cancer Biology* 20, 10-18
- 90 Duncan, S.J. et al. (2001) Isolation and structure elucidation of Chlorofusin, a novel p53-MDM2 antagonist from a *Fusarium* sp. *Journal of the American Chemical Society* 123, 554-560
- 91 Tsukamoto, S. et al. (2006) Hexylitaconic acid: a new inhibitor of p53-HDM2 interaction isolated from a marine-derived fungus, *Arthrinium* sp. *Bioorganic and Medicinal Chemistry Letters* 16, 69-71
- 92 De Vincenzo, R. et al. (1995) Effect of synthetic and naturally occurring chalcones on ovarian cancer cell growth: structure-activity relationships. *Anticancer Drug Design* 10, 481-490
- 93 Stoll, R. et al. (2001) Chalcone derivatives antagonize interactions between the human oncoprotein MDM2 and p53. *Biochemistry* 40, 336-344
- 94 Ito, T. et al. (2010) Identification of a primary target of thalidomide teratogenicity. *Science* 327, 1345-1350
- 95 Yang, Y. et al. (2007) Inhibitors of ubiquitin-activating enzyme (E1), a new class of potential cancer therapeutics. *Cancer Research* 67, 9472-9481
- 96 Soucy, T.A. et al. (2009) An inhibitor of NEDD8-activating enzyme as a new approach to treat cancer. *Nature* 458, 732-736
- 97 Endo, S. et al. (2010) Potent in vitro and in vivo antitumor effects of MDM2 inhibitor nutlin-3 in gastric cancer cells. *Cancer Science* 102, 605-13
- 98 Tabe, Y. et al. (2009) MDM2 antagonist nutlin-3 displays antiproliferative and proapoptotic activity in mantle cell lymphoma. *Clinical Cancer Research* 15, 933-942
- 99 Ooi, M.G. et al. (2009) Interactions of the Hdm2/p53 and proteasome pathways may enhance the antitumor activity of bortezomib. *Clinical Cancer Research* 15, 7153-7160
- 100 Issaeva, N. et al. (2004) Small molecule RITA binds to p53, blocks p53-HDM-2 interaction and activates p53 function in tumors. *Nature Medicine* 10, 1321-1328
- 101 Yang, Y. et al. (2005) Small molecule inhibitors of HDM2 ubiquitin ligase activity stabilize and activate p53 in cells. *Cancer Cell* 7, 547-559
- 102 Allen, J.G. et al. (2009) Discovery and optimization of chromenotriazolopyrimidines as potent inhibitors of the mouse double minute 2-tumor

- protein 53 protein-protein interaction. *Journal of Medicinal Chemistry* 52, 7044-7053
- 103 Ding, K. et al. (2006) Structure-based design of spiro-oxindoles as potent, specific small-molecule inhibitors of the MDM2-p53 interaction. *Journal of Medicinal Chemistry* 49, 3432-3435
- 104 Shangary, S. et al. (2008) Temporal activation of p53 by a specific MDM2 inhibitor is selectively toxic to tumors and leads to complete tumor growth inhibition. *Proceedings of the National Academy of Sciences of the United States of America* 105, 3933-3938
- 105 Clark, R.C. et al. (2009) Evaluation of chlorofusin, its seven chromophore diastereomers, and key analogues. *Tetrahedron Letters* 50, 3151-3153
- 106 Shibata, S. (1994) Anti-tumorigenic chalcones. *Stem Cells* 12, 44-52
- 107 Modzelewska, A. et al. (2006) Anticancer activities of novel chalcone and bis-chalcone derivatives. *Bioorganic and Medicinal Chemistry* 14, 3491-3495
- 108 Achanta, G. et al. (2006) A boronic-chalcone derivative exhibits potent anticancer activity through inhibition of the proteasome. *Molecular Pharmacology* 70, 426-433
- 109 Nakayama, K.I. and Nakayama, K. (2006) Ubiquitin ligases: cell-cycle control and cancer. *Nature Reviews. Cancer* 6, 369-381
- 110 Chen, Q. et al. (2008) Targeting the p27 E3 ligase SCFSkp2 results in p27-and Skp2-mediated cell-cycle arrest and activation of autophagy. *Blood* 111, 4690-4699
- 111 Orlicky, S. et al. (2010) An allosteric inhibitor of substrate recognition by the SCFCdc4 ubiquitin ligase. *Nature Biotechnology* 28, 733, U1743
- 112 Aghajan, M. et al. (2010) Chemical genetics screen for enhancers of rapamycin identifies a specific inhibitor of an SCF family E3 ubiquitin ligase. *Nature Biotechnology* 28, 738-742
- 113 Bosch, M.E. et al. (2008) Recent advances in analytical determination of thalidomide and its metabolites. *Journal of Pharmaceutical and Biomedical Analysis* 46, 9-17
- 114 Bodine, S.C. et al. (2001) Identification of ubiquitin ligases required for skeletal muscle atrophy. *Science* 294, 1704-1708
- 115 Eddins, M.J. et al. (2011) Targeting the ubiquitin E3 ligase MuRF1 to inhibit muscle atrophy. *Cell Biochemistry and Biophysics* 60, 113-118
- 116 Ciechanover, A. et al. (1982) "Covalent affinity" purification of ubiquitin-activating enzyme. *Journal of Biological Chemistry* 257, 2537-2542
- 117 Xu, G.W. et al. (2010) The ubiquitin-activating enzyme E1 as a therapeutic target for the treatment of leukemia and multiple myeloma. *Blood* 115, 2251-2259
- 118 Kapuria, V. et al. (2011) Protein cross-linking as a novel mechanism of action of a ubiquitin-activating enzyme inhibitor with anti-tumor activity. *Biochemical Pharmacology* 82, 341-9
- 119 Sekizawa, R. et al. (2002) Panepophenanthrin, from a mushroom strain, a novel inhibitor of the ubiquitin-activating enzyme. *Journal of Natural Products* 65, 1491-1493
- 120 Matsuzawa, M. et al. (2006) Enantio- and diastereoselective total synthesis of (+)-panepophenanthrin, a ubiquitin-activating enzyme inhibitor, and biological properties of its new derivatives. *Chemistry, an Asian Journal* 1, 845-851
- 121 Tsukamoto, S. et al. (2005) Himeic acid A: a new ubiquitin-activating enzyme inhibitor isolated from a marine-derived fungus, *Aspergillus* sp. *Bioorganic and Medicinal Chemistry Letters* 15, 191-194
- 122 Tsukamoto, S. et al. (2008) Leucettamol A: a new inhibitor of Ubc13-Uev1A interaction isolated from a marine sponge, *Leucetta aff. microrhaphis*. *Bioorganic and Medicinal Chemistry Letters* 18, 6319-6320
- 123 Ceccarelli, D.F. et al. (2011) An allosteric inhibitor of the human cdc34 ubiquitin-conjugating enzyme. *Cell* 145, 1075-1087
- 124 Xirodimas, D.P. (2008) Novel substrates and functions for the ubiquitin-like molecule NEDD8. *Biochemical Society Transactions* 36, 802-806
- 125 Herrmann, J., Lerman, L.O. and Lerman, A. (2007) Ubiquitin and ubiquitin-like proteins in protein regulation. *Circulation Research* 100, 1276-1291
- 126 Pan, Z.Q. et al. (2004) Nedd8 on cullin: building an expressway to protein destruction. *Oncogene* 23, 1985-1997
- 127 Clifford, S.C. et al. (2001) The pVHL-associated SCF ubiquitin ligase complex: molecular genetic analysis of elongin B and C, Rbx1 and HIF-1alpha in renal cell carcinoma. *Oncogene* 20, 5067-5074
- 128 Soucy, T.A., Smith, P.G. and Rolfe, M. (2009) Targeting NEDD8-activated cullin-RING ligases



- for the treatment of cancer. *Clinical Cancer Research* 15, 3912-3916
- 129 Swords, R.T. et al. (2010) Inhibition of NEDD8-activating enzyme: a novel approach for the treatment of acute myeloid leukemia. *Blood* 115, 3796-3800
- 130 Brownell, J.E. et al. (2010) Substrate-assisted inhibition of ubiquitin-like protein-activating enzymes: the NEDD8 E1 inhibitor MLN4924 forms a NEDD8-AMP mimetic in situ. *Molecular Cell* 37, 102-111

### Further reading, resources and contacts

- Bedford, L. et al. (2011) Ubiquitin-like protein conjugation and the ubiquitin–proteasome system as drug targets. *Nature Reviews. Drug Discovery* 10, 29-46
- Nalepa, G., Rolfe, M. and Harper, J.W. (2006) Drug discovery in the ubiquitin–proteasome system. *Nature Reviews. Drug Discovery* 5, 596-613
- Eldridge, A.G. and O'Brien, T. (2010) Therapeutic strategies within the ubiquitin proteasome system. *Cell Death and Differentiation* 17, 4-13
- Nicholson, B. et al. (2007) Deubiquitinating enzymes as novel anticancer targets. *Future Oncology* 3, 191-199
- Dickens, M.P., Fitzgerald, R. and Fischer, P.M. (2010) Small-molecule inhibitors of MDM2 as new anticancer therapeutics. *Seminars in Cancer Biology* 20, 10-18

### Features associated with this article

#### Figures

- Figure 1. Small-molecule inhibitors in the ubiquitin–proteasome system (UPS).
- Figure 2. Small-molecule inhibitors against deubiquitylating enzymes.
- Figure 3. Small-molecule inhibitors against ubiquitin/Ubl-conjugating enzymes.

### Citation details for this article

Mariola J. Edelmann, Benjamin Nicholson and Benedikt M. Kessler (2011) Pharmacological targets in the ubiquitin system offer new ways of treating cancer, neurodegenerative disorders and infectious diseases. *Expert Rev. Mol. Med.* Vol. 13, e35, November 2011, doi:10.1017/S1462399411002031