HHS Public Access

Author manuscript

Nat Rev Cancer. Author manuscript; available in PMC 2022 April 01.

Published in final edited form as:

Nat Rev Cancer. 2021 October ; 21(10): 638–654. doi:10.1038/s41568-021-00365-x.

Advancing targeted protein degradation for cancer therapy

Brandon Dale[#1,2,3,4], **Meng Cheng**[#5,6], **Kwang-Su Park**[1,2,3,4], **H. Ümit Kaniskan**[1,2,3,4], **Yue Xiong**[5,6,7,8,✉], **Jian Jin**[1,2,3,4,✉]

[1]Mount Sinai Center for Therapeutics Discovery, Icahn School of Medicine at Mount Sinai, New York, NY, USA

[2]Department of Pharmacological Sciences, Icahn School of Medicine at Mount Sinai, New York, NY, USA

[3]Department of Oncological Sciences, Icahn School of Medicine at Mount Sinai, New York, NY, USA

[4]Tisch Cancer Institute, Icahn School of Medicine at Mount Sinai, New York, NY, USA

[5]Lineberger Comprehensive Cancer Center, University of North Carolina at Chapel Hill, Chapel Hill, NC, USA

[6]Curriculum in Genetics and Molecular Biology, University of North Carolina at Chapel Hill, Chapel Hill, NC, USA

[7]Department of Biochemistry and Biophysics, University of North Carolina at Chapel Hill, Chapel Hill, NC, USA

[8]Cullgen Inc., San Diego, CA, USA

[#] These authors contributed equally to this work.

Abstract

The human proteome contains approximately 20,000 proteins, and it is estimated that more than 600 of them are functionally important for various types of cancers, including nearly 400 non-enzyme proteins that are challenging to target by traditional occupancy-driven pharmacology. Recent advances in the development of small-molecule degraders, including molecular glues

[✉] yue.xiong@cullgen.com; jian.jin@mssm.edu.

Author contributions

All authors researched data for the manuscript and contributed to discussion of the content and writing and editing of the manuscript.

Peer review information

Nature Reviews Cancer thanks R. Deshaies, F. Ferguson, and the other, anonymous, reviewer(s) for their contribution to the peer review of this work.

Supplementary information

The online version contains supplementary material available at https://doi.org/10.1038/s41568-021-00365-x.

Related Links

DRIVE Data Portal: https://oncologynibr.shinyapps.io/drive/
Expression Atlas Database: https://www.ebi.ac.uk/gxa/experiments/E-PROT-29/Results
Hugo Gene Nomenclature Committee: https://www.genenames.org/
National Cancer institute Genomic Data Commons: https://portal.gdc.cancer.gov/projects
RCSB Protein Data Bank: https://www.rcsb.org/
The Human Protein Atlas: https://www.proteinatlas.org/
UniProt Knowledgebase: https://www.uniprot.org/help/uniprotkb

and heterobifunctional degraders such as proteolysis-targeting chimeras (PROTACs), have made it possible to target many proteins that were previously considered undruggable. In particular, PROTACs form a ternary complex with a hijacked E3 ubiguitin ligase and a target protein, leading to polyubiguitination and degradation of the target protein. The broad applicability of this approach is facilitated by the flexibility of individual E3 ligases to recognize different substrates. The vast majority of the approximately 600 human E3 ligases have not been explored, thus presenting enormous opportunities to develop degraders that target oncoproteins with tissue, tumour and subcellular selectivity. In this Review, we first discuss the molecular basis of targeted protein degradation. We then offer a comprehensive account of the most promising degraders in development as cancer therapies to date. Lastly, we provide an overview of opportunities and challenges in this exciting field.

Extensive efforts have been made to map cancer dependency by identifying genes that, when functionally inactivated, inhibit the growth of cancer cells and are thus potential therapeutic targets[1]. These efforts have resulted in the identification of many protein-coding genes, both mutated and non-mutated in human cancers, which are potential cancer targets[1–4]. The Sanger Institute identified 627 priority targets, of which 232 are enzymes (37%)[4], and these have been the focus of developing small-molecule anticancer drugs. However, the 395 non-enzyme targets, which include proteins such as transcription factors, generally do not have an active site that is functionally critical and amendable to targeting by occupancy-driven small-molecule inhibitors. Thus, they are often referred to as ‘undruggable’, and the vast majority of them have not been therapeutically targeted thus far.

Targeted protein degradation (TPD), the process of eliminating a protein of interest (POI), holds great promise for the development of cancer therapeutics[5]. A key focus of TPD is the development of heterobifunctional small-molecule degraders, including proteolysis-targeting chimeras (PROTACs), which contain two linked moieties with one binding the POI and the other binding an E3 ligase. The formation of an E3–degrader–POI ternary complex results in polyubiquitination of the POI and its subsequent degradation by the 26S proteasome. Since the PROTAC concept was first published in 2001 (REF[6]), a number of critical discoveries have been made (FIG. 1). The pursuit of improved prototype peptide-derived PROTACs together with the discovery of immunomodulatory imide drugs (IMiDs) acting as molecular glues to connect neosubstrates to an E3 ligase led to the development of highly effective PROTACs in 2015 (REFS[7–12]). The field of TPD has seen explosive growth over the past 5 years. Remarkably, the first two PROTACs entered clinical development as cancer therapies in 2019 (REFS[13,14]).

In this Review, we discuss the molecular basis of TPD by the ubiquitin–proteasome system, plasticity of E3 ligases in targeting neosubstrates and considerations for developing novel E3 ligands. We also review the advancement of heterobifunctional small-molecule degraders as cancer therapeutics, with emphasis on those that are first-in-class, in or soon to be in clinical development, or efficacious in in vivo preclinical studies. Lastly, we outline key considerations for developing heterobifunctional degraders as cancer therapeutics, including limitations and potential opportunities. For non-cancer-related heterobifunctional and other degrader-related chronology, we refer readers to previous reviews[5,15–17].

The ubiquitin–proteasome system

The ubiquitin–proteasome system regulates most, if not all, cellular processes[18]. It covalently attaches ubiquitin, a 76-amino-acid protein that is highly conserved and ubiquitously expressed in all eukaryotic species and tissues, to a lysine residue in substrate proteins via an enzyme cascade involving a ubiquitin-activating enzyme (E1), a ubiquitin-conjugating enzyme (E2) and a ubiquitin ligase (E3)[19,20] (FIG. 2a). Ubiquitin can be ligated either as a single moiety to one lysine residue or multiple lysine residues or as a polyubiquitin chain where successive ubiquitin molecules are connected[21]. One of the well-known consequences of polyubiquitination is the proteolytic degradation of substrate proteins by the 26S proteasome[22]. The ubiquitin–proteasome system manages immense complexity and, at the same time, achieves a high degree of specificity of protein ubiquitination through a large repertoire of more than 600 E3 ubiquitin ligases in human cells[23] (FIG. 2b). Recurrent alterations of many E3 ligases have been found in numerous human diseases, including cancers[24]. However, only three E3-targeting small-molecule drugs, thalidomide, lenalidomide and pomalidomide, which bind a substrate receptor of the E3 ligase cereblon (CRBN)[25], have been approved by the US Food and Drug Administration. This is largely due to the lack of an active site in the RING-type E3 ligases, which account for more than 95% of E3 ligases (FIG. 2b). While challenges in targeting E3 ligases remain, PROTAC technology has now made it possible to exploit the vast repository of E3 ligases and the sophisticated ubiquitin–proteasome system to target oncoproteins.

On the basis of their catalytic mechanism, the E3 family of ubiquitin ligases can be divided into three types: the homologous to E6AP carboxy terminus (HECT) type, the RING type and the RING1-in-between-RING2 (RBR) type (FIG. 2b). The human genome encodes 305 RING finger proteins according to the HUGO Gene Nomenclature Committee (HGNC) database, most of which are believed to act as E3 ligases[26]. Although not containing a RING finger domain themselves, members of the evolutionarily conserved cullin family of proteins bind to a small RING protein, either RING box protein 1 (RBX1; also known as ROC1) or RBX2 (also known as ROC2), to assemble as many as 300 distinct, multi-subunit cullin-RING E3 ligases (CRLs; BOX 1). All PROTACs reported to date hijack a RING-type E3 ligase, including both single-subunit and multi-subunit CRLs (FIG. 2c; TABLE 1). The single-subunit E3 ligases typically ubiquitinate very few substrates. For example, the single-subunit E3 ligase MDM2 primarily regulates p53, and BIRC2 (also known as cIAP1) controls CRABP2 and DDIT3 (also known as CHOP)[27,28]. By contrast, the multi-subunit CRLs often target multiple substrates. Twenty-six substates have been reported for $CRL1^{SKP2}$ (the superscript denotes the substrate receptor within the complex), 28 have been reported for $CRL1^{\beta\text{-}TRCP}$ (REF.[29]), 27 have been reported for $CRL3^{SPOP}$ (REF[30]), 14 have been reported for $CRL4^{DTL}$ (REF[31]) and 9 have been reported for $CRL5^{SOCS1}$ (REF[32]). This high degree of plasticity of CRLs in targeting multiple substrates is achieved by the combination of the intrinsic flexibility of each individual subunit within the CRL complexes (BOX 1). Such plasticity is a requisite for TPD, which requires the E3 ligase to ubiquitinate the POI or neosubstrate. The two most commonly used E3 ligases for TPD are members of the CRL subfamily, namely $CRL2^{VHL}$ and $CRL4^{CRBN}$ (FIG. 2c).

Heterobifunctional small-molecule degraders

To date, ten E3 ligases have been exploited for TPD in the form of either molecular glues or PROTACs (TABLE 1). Most reported PROTACs (Supplementary Table 1) were developed with use of two types of E3 small-molecule ligands, namely IMiDs that bind CRBN[7–9] and von Hippel–Lindau tumour suppressor (VHL) ligands that bind VHL[12,33,34] within the CRL complexes (TABLE 1; Supplementary Figs. 1,2). It is not yet clear why other E3 ligands have not been more widely used, and many E3 ligases are yet to be explored. However, the plasticity of the CRL complexes may greatly facilitate ternary complex formation and subsequent ubiquitination of the target protein. Key elements of the PROTAC and other TPD approaches are discussed in the following sections.

General principles of PROTAC design

The basic chemical moieties of PROTACs include (1) an E3 ligand that binds and recruits an E3 ligase, (2) a ligand that binds the POI and (3) a linker that connects the E3 and POI ligands (FIG. 3a). PROTACs are catalytic in nature, in contrast to traditional small-molecule inhibitors, which are non-catalytic and rely on occupancy-driven pharmacology[5,12]. This important feature of PROTACs minimizes the need for a long residence time and continuous drug exposure. Thus, PROTACs have the potential to deliver robust therapeutic effects at low doses and in infrequent dosing regimens with fewer side effects[35].

A unique feature of PROTACs as therapeutic modalities is that they rely on the expression of the corresponding E3 ligase in target cells. This raises the possibility for control of PROTAC activity in selected tissues, organs, tumour types or subcellular compartments. Expression levels and subcellular localization of E3 ligases in targeted cellular systems are therefore important considerations in PROTAC design (BOX 2).

To design an effective PROTAC, it is critical to select an E3 ligand that has sufficient binding affinity for the desired E3 ligase and, importantly, recruits the functionally active E3 ligase complex without compromising its ubiquitination activity. The availability of such ligands is limited (TABLE 1). The POI ligand selection is also crucial for successful PROTAC design. Usually, a solvent-exposed region of the POI ligand is selected as a linker attachment point to minimize interference of the linker and E3 ligand with PROTAC–POI binding. Additionally, the linker type and length are critical, and it is challenging to predict computationally which linkers are optimal in the absence of ternary complex structures. In general, exploration of multiple E3 ligands, POI ligands, linker attachments, and linker types and lengths is needed to generate effective PROTACs.

Ternary complex assembly

For a PROTAC to effectively degrade the POI, a sufficiently stable POI–PROTAC–E3 ternary complex generally needs to be formed[36], although exceptions have been reported[37,38]. Several technologies have been developed to study the ternary complex formation, intracellular target engagement and E3 ligase recruitment, which have been reviewed elsewhere[39,40]. The linker type and length are critical elements for the formation of sufficiently stable ternary complexes. An optimal linker is one that contributes to stabilizing

the ternary complex by enhancing protein-protein interactions between the POI and the E3 ligase. Multiple linker types can adequately facilitate ternary complex formation. PROTACs with an optimal linker can induce cooperative binding. For example, it was reported that for the VHL-recruiting BRD4-targeting PROTAC known as MZ1, there is positive binding cooperativity in the BRD4–MZ1–VHL ternary complex compared with binary complexes (BRD4–MZ1 and VHL–MZ1), leading to effective degradation of BRD4 in vitro[41].

To date, several POI–PROTAC–VHL ternary complex[41–44] (RCSB Protein Data Bank (PDB) IDs 6SIS[45], 5T35 (REF[46]), 6HR2 (REF[47]), 6HAX[48], 6HAY[49] and 6ZHC[50]), POI–PROTAC–CRBN ternary complex[51] (PDB IDs 6BN7 (REF.[52]), 6BN8 (REF.[53]), 6BN9 (REF.[54]), 6BNB[55] and 6BOY[56]) and POI–PROTAC–BIRC2 ternary complex[38] (PDB IDs 6W7O[57] and 6W8I[58]) crystal structures have been reported. Within these structures, new interactions between the POI and the E3 ligase have been identified (FIG. 3b). These PROTAC-induced protein–protein interactions provide a structural basis for the observed cooperative binding. The protein–protein interactions revealed by these ternary complex structures also enable structure-based design to generate more effective PROTACs, an opportunity barely explored in the PROTAC field to date.

An important advantage of PROTACs over traditional small-molecule inhibitors is that PROTACs can be selective for POI isoforms or subtypes even though the POI ligands used in the PROTACs are not. For example, PROTACs have been developed that are selective for HER2 (also known as ERBB2) over EGFR and vice versa[59], for CDK4 over CDK6 and vice versa[60], for signal transducer and activator of transcription 3 (STAT3) over other STAT isoforms[61] and for BCL-XL over BCL-2 (REF.[62]) even though the POI ligands used are not isoform selective. The ability of these PROTACs to induce isoform-selective degradation is likely due to a preference for ternary complex formation with one particular POI isoform over others. Additionally, PROTACs incorporating pan-kinase inhibitors such as foretinib as POI ligands demonstrated selective degradation of only a subset of the kinases that the POI ligands can bind[63]. This selectivity could be due to the ability of the PROTACs to induce ternary complex formation for only a subset of the kinases, leading to polyubiquitination and degradation of these kinases only. Although ternary complex formation is necessary, this alone is not sufficient for PROTACs to induce POI degradation. For example, a VHL-recruiting PROTAC based on foretinib can form ternary complexes with two isoforms of the mitogen-activated protein kinase p38, but promotes degradation of only one of them[64]. Other factors such as the half-life of the PROTAC, the synthesis rate of the POI and the abundance of the POI and the E3 ligase in cells are important for effective degradation of the POI.

Other approaches

Hydrophobic tag-mediated degradation.—While PROTACs have generated much of the excitement surrounding degrader-mediated therapies, other TPD approaches using heterobifunctional small molecules have been developed. One of these approaches is hydrophobic tagging, which links a POI ligand with a highly lipophilic moiety that activates the unfolded protein response, thereby inducing degradation of the POI[65]. Fulvestrant is a selective oestrogen receptor (ER) degrader that first realized the concept of hydrophobic

tagging[66]. Fulvestrant stabilizes a conformation of ER that exposes the hydrophobic tag region, leading to its subsequent proteasomal degradation[66,67]. This finding generated interest in creating compounds with a similar mechanism of action, such as selective androgen receptor (AR) degraders[68]. While the mechanism of action has not been fully elucidated, it is postulated that hydrophobic tagging co-opts and triggers the function of chaperones. Typically, chaperone proteins assist in the refolding of the proteins that have been misfolded into a conformation that exposes hydrophobic, non-polar regions of the proteins. When the misfolded proteins cannot be ameliorated by chaperone proteins, they are marked for degradation by the proteasome[69].

Hydrophobic tag-mediated degradation starts in the endoplasmic reticulum and differs from PROTAC-mediated degradation[70]. The interplay between hydrophobic tag-mediated degradation and the 26S proteasome was explored following the discovery that *tert*-butyl carbamate Boc_3-protected arginine (B_3A) acts as a hydrophobic tag to elicit degradation of its POI (a tagged fusion protein dihydrofolate reductase) in a B_3A-dependent, non-covalent manner (Boc3, three *tert*-butoxycarbonyl protecting groups)[71]. Additionally, haemagglutinin-tagged glutathione *S*-transferase A1 (GSTA1), a potential target for sensitizing chemoresistant cells to chemotherapy[72], was degraded with an ethacrynic acid–B_3A compound in HEK293T cells[71]. Another hydrophobic tag, an adamantyl group, has also been used to induce TPD; the adamantyl-tagged compound HyT13 was shown to degrade a HaloTag-fused HRAS-G12V protein (a HaloTag is a self-labeling fusion-protein tag that can bind a ligand with a halogenated alkyl group), resulting in marked tumour reductions in a transformed mouse fibroblast NIH-3T3 HaloTag–HRAS–G12V mouse model[73]. Another adamantyl-based degrader, derived from a covalent inhibitor of the pseudokinase HER3 (also known as ERBB3), was capable of degrading HER3 in vitro[74]. In both cases, covalent POI ligands were used to generate the heterobifunctional degraders.

Molecular glues.—Molecular glues are not heterobifunctional molecules. Instead, they are simple small molecules that simultaneously bind an E3 ligase and a neosubstrate, leading to degradation of the neosubstrate. Of the ten E3 ligases that have been exploited for TPD, molecular glues have been identified for four of them (TABLE 1). IMiDs are the most well-studied molecular glues; they bind CRBN and create a new binding interface for as many as 13 reported neosubstrates[17,75]. A glycine-containing β-hairpin motif in these otherwise diverse neosubstrates appears to be critical for their binding with CRBN[75–78]. Sulfonamides such as indisulam and E7820 stabilize binding of the E3 ligase DDB1- and CUL4-associated factor 15 (DCAF15) with its neosubstrate, mRNA splicing factor RBM39, through non-polar interactions at a specific helical site found in RBM39 (REFS[79–82]). Most recently, several CDK12 inhibitors (including CR8, HQ461, dCeMM2, dCeMM3 and dCeMM4) were identified as molecular glues of DDB1 to promote degradation of cyclin K[83–85]. CR8 occupies the ATP-binding pocket of CDK12 and interacts with three residues in DDB1 through its hydrophobic phenylpyridine ring[83], effectively converting CDK12 into a substrate receptor of DDB1 and recruiting the CDK12 binding partner cyclin K as a neosubstrate for polyubiquitination and degradation[82]. In contrast to degradation, members of the manumycin family of polyketide metabolites act as molecular glues by covalently linking to a cysteine residue in the RING E3 ligase UBR7 and binding with the neosubstrate

p53, resulting in non-proteolytic ubiquitination and functional activation of the p53 tumour suppressor in MDA-MB-231 breast cancer cells[86]. A significant advantage of molecular glues is their low molecular weight, which more likely results in favourable pharmacokinetic properties such as oral bioavailability[87]. However, a key issue is the lack of a rational design approach for discovering novel molecular glues. Nevertheless, molecular glues provide an attractive approach for advancing the TPD field.

First-in-class TPD cancer therapies

A large variety of heterobifunctional small-molecule degraders have been developed for many protein targets since 2015. While we do not discuss all of them here, we summarize those reported to date in the context of cancer in Supplementary Table 1. A subset of these degraders, which have established in vivo efficacy or sufficient in vivo pharmacokinetic properties, are summarized in TABLE 2. In the following section, we highlight key heterobifunctional degraders that are first-in-class and in or soon to be in clinical development or that are efficacious in vivo.

AR degraders

The first report of a heterobifunctional degrader as a potential cancer therapeutic was the AR-targeting Protac-3 (REF.[88]). Protac-3 was constructed by linking an IκBα (also known as NFKBIA) phosphopeptide that binds to β-TRCP (also known as BRTC), the substrate receptor of the $CRL1^{\beta\text{-}TRCP}$ complex, with an AR ligand, dihydrotestosterone; the compound was shown to rapidly reduce the GFP signal in cells expressing a GFP–AR fusion protein in vitro[88]. More recently, potent AR PROTACs such as ARD-69 (FIG. 3c; TABLE 2) have been developed. ARD-69, a VHL-recruiting PROTAC, achieved complete degradation of AR in AR^+ prostate cancer cell lines and effectively reduced the levels of both AR and prostate-specific antigen for 48 hours in xenograft models[89]. ARD-61 (TABLE 2), another VHL-recruiting PROTAC and a close analogue of ARD-69, was subsequently developed[90]. ARD-61 is the most potent AR PROTAC degrader to date, and was efficacious at inhibiting tumour growth in vivo in both castration-resistant prostate cancer and AR^+ breast cancer xenograft models[90,91]. These preclinical findings suggest that AR degradation may provide a potential therapeutic strategy for overcoming the AR amplification or overexpression seen in castration-resistant prostate cancer that small-molecule antagonists such as enzalutamide cannot overcome.

Importantly, the AR-targeting PROTAC ARV-110 (TABLE 2), which is unrelated to ARD-69 and ARD-61, has entered clinical development and has shown promising preliminary clinical results[92–94]. This orally bioavailable compound showed AR degradation in a variety of AR^+ tumour cells with various point mutations in preclinical studies, and acceptable safety profiles in a phase I/II trial[92,93]. The phase I/II dose escalation clinical trial (NCT03888612) included 18 patients, and only one patient experienced adverse events (grade 1) with treatment. Additionally, ARV-110 dosing at 420 mg orally showed evidence of antitumour activity in patients with T878A or H875Y mutations in AR. Notably, one patient demonstrated a confirmed partial response by RECIST (Response Evaluation Criteria in Solid Tumors), with a 97% decline in prostate-specific antigen level and 80%

reduction in tumour size[94]. Although preliminary, the results from this phase I/II trial suggest that ARV-110 has an acceptable safety profile and shows promise as a treatment for enzalutamide-resistant prostate cancer. This advancement into clinical development marks a significant milestone in the field of PROTAC therapeutics, and additional results from this ongoing clinical trial are keenly awaited.

ER degraders

ER acts as a master regulator of gene expression and is involved in the pathogenesis of breast cancer[95]. The discovery that fulvestrant acts as a hydrophobic tag to mediate degradation of ER (see the section Hydrophobic tag-mediated degradation) has prompted further research into therapeutic ER degradation. Recently, ERD-308 (Supplementary Table 1) was developed as a VHL-recruiting ER-targeting PROTAC through successive rounds of structure–activity relationship studies and optimizations[96], and it was found to completely degrade ER in ER^+ breast cancer cells[96]. Importantly, ARV-471 (TABLE 2), an orally bioavailable ER-targeting PROTAC with potent in vivo activity, is now in clinical development[13]. ARV-471 is only the second PROTAC to enter clinical development (AR-targeting ARV-110 being the first). On the basis of a preliminary report, ARV-471 displayed potent ER degradation in cells and significant reduction of tumour burden in xenograft models[97]. A recent congress abstract describing a phase I/II clinical trial in 21 patients with treatment-refractory, advanced ER^+ and $HER2^-$ breast cancer reported no dose-limiting toxicities and no grade 3 or grade 4 treatment-related adverse events in patients who received ARV-471 up to a 360-mg dose[13,98]. Plasma exposures of ARV-471 were dose proportional up to a once-daily oral dose of 360 mg, and the half-life was 28 hours. Notably, one patient demonstrated a confirmed partial response (51% reduction in lesion size) per RECIST[98]. These preliminary clinical results suggest that ARV-471 has a tolerable safety profile and promising antitumour activity.

STAT3 degraders

A significant breakthrough in therapeutic targeting of STAT3 was recently achieved via development of SD-36 (FIG. 3c; TABLE 2), a potent and selective STAT3 PROTAC[61]. SD-36 incorporates an analogue of the CRBN ligand lenalidomide and a STAT3 inhibitor (SI-109)[61,99], and was found to potently and rapidly degrade STAT3 in leukaemia and lymphoma cell lines[61]. Interestingly, SD-36 was highly selective for STAT3 degradation over other STAT isoforms even though SI-109 itself is not isoform selective. SD-36 showed a robust and long-lasting STAT3 degradation response in vivo, and treatment with SD-36 resulted in complete tumour regression in several xenograft mouse models, which lasted for 2 weeks after treatment termination[61]. SD-36 was also well tolerated in immunocompetent mice, with no toxic effects even though it induced similarly sustained degradation of STAT3 (REF.[61]). Collectively, these results suggest that pharmacological degradation of STAT3 is a promising therapeutic strategy for treatment of STAT3-driven malignancies. These encouraging results also highlight the potential of using degraders to target transcription factors that where once deemed ‘undruggable’.

BCL-XL degraders

BCL-XL, a member of the BCL-2 protein family, protects cells from undergoing programmed cell death[100] and underlies the ability of many malignancies to escape normal cell cycle regulation[101]. Many solid and haematological tumours that display BCL-2-independent survival show increased BCL-XL expression, suggesting a hierarchy of anti-apoptotic proteins necessary for survival[102,103]. BCL-XL, as a key regulator of cell death, is therefore a promising therapeutic target for BCL-2-independent cancers. Small-molecule inhibitors have been developed to target the BCL-2 class of proteins, such as ABT263 (BCL-2 and BCL-XL dual inhibitor) and ABT199 (BCL-2-selective inhibitor)[62,104,105]. However, significant side effects, including severe thrombocytopenia, have limited the utility of these compounds[106,107]. Recently, DT2216 (FIG. 3c; TABLE 2), generated by linking ABT263 with a VHL ligand, was developed as a potent BCL-XL PROTAC with much reduced thrombocytopenic side effects in vivo relative to ABT263 due to the very low expression of VHL in platelets[62]. DT2216 displayed potent BCL-XL degradation in tumour cells, while inducing little BCL-XL degradation in platelets. Interestingly, DT2216 did not induce BCL-2 degradation even through it binds BCL-XL and BCL-2 with similar affinities. Furthermore, DT2216 completely inhibited MOLT-4 T cell acute lymphoblastic leukaemia xenograft growth in vivo, with only mild reductions in platelet count and no change in body weight[62], in contrast to ABT263 treatment, which resulted in significant tumour burden reductions but also severe thrombocytopenia[62]. This elegant study provides support for advancing BCL-XL PROTACs to the clinic. It also exploited the differential tissue expression of the E3 ligase VHL; as additional E3 ligands are being developed, it is expected that PROTACs will increasingly exploit this selective expression of E3 ligases.

EZH2 degraders

Enhancer of Zeste homologue 2 (EZH2) is the main catalytic subunit of Polycomb repressive complex 2 (PRC2), which catalyses histone H3 lysine 27 methylation, and elevated EZH2 activity is a feature of many cancers[108]. As one of the most prominent epigenetic drug targets, EZH2 has been intensively targeted, and multiple EZH2 small-molecule inhibitors have entered clinical developoment[109]. Among them, tazemetostat has been approved for clinical use for the treatment of epithelioid sarcoma and follicular lymphoma[110]. However, EZH2 also has functions independent of its catalytic activity in lung, prostate and breast cancers[111–114], which cannot be effectively targeted by traditional inhibitors. MS1943 (FIG. 3c; TABLE 2) is the first EZH2-selective degrader and uses a hydrophobic tagging approach similar to the mechanism of action of fulvestrant[66]. MS1943, derived from an EZH2 non-covalent inhibitor linked to an adamantyl group, effectively degraded EZH2 and suppressed proliferation in multiple EZH2-dependent triple-negative breast cancer and other cancer cell lines, while EZH2 inhibitors, including tazemetostat, did not[65]. MS1943 was orally bioavailable in mice and completely inhibited tumour growth, with no weight loss or other toxicities in xenograft models[65]. Additionally, the development of a CRBN-recruiting EZH2 PROTAC and the development of two PROTACs that target Polycomb protein EED (Supplementary Table 1), a core component of PRC2, was reported recently[115–117]. Overall, these results provide evidence that pharmacological degradation of PRC2 components may be a promising therapeutic strategy, and EZH2 degradation may offer advantages over EZH2 inhibition for treatment of EZH2-dependent tumours.

BRAF degraders

BRAF, a component of the RAS–RAF–MEK–ERK signalling pathway, is frequently mutated in a variety of cancers, making it a desirable therapeutic target[118]. The BRAF V600E mutation accounts for more than 80% of the activation mutations in BRAF. Vemurafenib, a BRAF small-molecule inhibitor that inhibits both wild-type (WT) BRAF and BRAF-V600E, is used in the clinic for treatment of advanced melanoma as it was shown to prolong progression-free survival in patients with advanced melanoma[119]. However, high rates of resistance to vemurafenib and other BRAF inhibitors have resulted in the development of new therapeutic strageies[120]. The first BRAF-V600E-selective PROTACs were reported recently[121]. CRBN-recruiting degraders 12 and 23 (Supplementary Table 1), which are based on the BRAF inhibitors vemurafenib and BI882370, respectively, potently induced degradation of BRAF-V600E in melanoma cells[121]. Notably, the degradation was selective for BRAF-V600E over WT BRAF, even though both degraders bind WT BRAF and BRAF-V600E equally well[121].

Plausible mechanisms for this BRAF mutant selectivity were subsequently explored in two subsequent studies by Posternak et al. and Alabi et al.[122,123] Another BRAF PROTAC, P4B (TABLE 2), was reported by Posternak et al.[122]. P4B, generated by linking the BRAF inhibitor BI882370 to pomalidomide, an IMiD-based CRBN ligand, displayed potent degradation of BRAF-V600E but not WT BRAF. Interestingly, P4B was capable of degrading other BRAF mutants besides BRAF-V600E[122]. This was posited to result from the fact that WT BRAF exists in an inactive state that resisted degradation due to blockade of the ternary complex formation, while BRAF mutants exist in an active form that more easily undergoes ternary complex formation, thereby facilitating degradation. Additionally, while P4B displayed poor in vivo exposure, it degraded BRAF-V600E in malignant melanoma xenograft models[122].

More recently, Alabi et al. reported that the PROTAC SJF-0628 (FiG. 3c; TABLE 2) induced degradation of mutant BRAF while sparing WT BRAF, and displayed antitumour activity in an A375 melanoma xenograft model[123]. SJF-0628 was generated by linking vemurafenib with a VHL ligand. It potently degraded mutated BRAF proteins and effectively inhibited growth in cells with mutant BRAF but not in WT BRAF cells. It also induced interactions of all three BRAF mutant classes, but not WT BRAF, with the CUL2 E3 ligase complex. Interestingly, WT BRAF degradation could be triggered by promoting it to an active state that more closely resembles mutated BRAF. For example, activation of WT BRAF by pretreatment with the MEK inhibitor trametinib made it susceptible to SJF-0628-mediated degradation. Notably, SJF-0628 induced marked degradation of BRAF-V600E and inhibited tumour growth in vivo in an A375 melanoma xenograft model, with no significant body weight loss[123].

In summary, the ability of PROTAC technology to selectively target mutant BRAF over WT BRAF highlights another advantage of PROTACs over traditional enzymatic inhibitors. Mechanistic understanding of this selectivity for mutant proteins will aid in future development of mutant-selective degraders for other notoriously challenging targets.

HPK1 degraders

Modulation of the proteins that control T cell function holds great promise as a potential strategy for enhancing the antitumour effect of T cell-mediated therapy; one such regulator is the serine/threonine STE20-related protein kinase HPK1 (also known as MAP4K1)[124]. HPK1 is an intracellular negative regulator of T cells that phosphorylates lymphocyte cytosolic protein 2, which in turn destabilizes the T cell receptor complex, leading to inhibition of T cell proliferation and activation[125,126]. Recently, the first HPK1-targeting PROTAC, SS47 (FIG. 3c; TABLE 2), was generated by linking the HPK1 inhibitor ZYF0033 to the CRBN ligand thalidomide[127]. SS47 induced degradation of HPK1 in cells and in an ex vivo model. Treatment of bone marrow-derived dendritic cells with SS47 resulted in significantly increased proliferation of $CD4^+$ and $CD8^+$ T cells upon antigen presentation. In addition, treatment with SS47 in a 4T-1 breast cancer mouse model significantly reduced tumour growth and synergized with an anti-PD1 antibody[127]. While these results are encouraging, it is worth noting that selectivity of SS47 was not assessed. In particular, it is unknown whether this CRBN-recruiting PROTAC degrades CRBN neosubstrates, including GSPT1.

Design and development considerations

Developing novel and functional E3 ligands

Identifying a functional E3 ligand that can be used for the development of degraders is challenging, mainly because only a limited number of binding sites in an E3 ligase are suitable for productive polyubiquitination. As a result, binding of a protein substrate to a random site in an E3 ligase is unlikely to lead to productive ubiquitination. Not only does the ‘right binding site’ need to accommodate interactions with a protein substrate but it also needs to have sufficient space to allow processive ubiquitin chain elongation. The following approaches should be considered when one is attempting to identify functional E3 ligands.

First, converting substrate peptides into drug-like small-molecule E3 ligands is an approach that was successfully used in the development of VHL ligands[128]. A major advantage of this strategy is that increasing numbers of E3 ligase–substrate crystal structures are available to enable structure-based design. However, this approach is challenging and laborious, as protein–protein interactions often involve large, flat and hydrophobic binding interfaces, lacking deep binding pockets necessary for small molecules to achieve sufficient affinity.

Another approach involves designing E3 ligands from molecular glues. The major advantage of using molecular glues as starting points is that they occupy the ‘right binding site’ where the neosubstrate is recruited and ubiquitinated. The feasibility of developing PROTACs based on molecule glues is expected to vary on the basis of each molecular glue’s affinity for the E3 ligase in the absence of a neosubstrate. In addition to the IMiD class of molecular glues, a PROTAC based on a DCAF15 molecular glue was recently developed[129] (TABLE 1). Generally, molecular glues with high E3 ligase affinity are preferred for developing PROTACs.

A third approach involves identification of covalent ligands. A recently developed chemoproteomics approach has enabled discovery of cysteine-reactive covalent ligands for

undruggable proteins[130], and several covalent E3 ligands have been identified and can be developed into PROTACs to degrade a POI[86,131,132]. Many E3 ligases are postulated to contain at least one reactive cysteine residue that could be modified by a covalent ligand[131]. Identification of covalent E3 ligands that have sufficient selectivity and that can efficiently hijack the ubiquitin–proteasome system would be highly valuable.

Finally, there are lessons to be learned from viral hijacking. Many viruses have evolved a common mechanism to counter host defence by encoding proteins that hijack host E3 ligases, especially the CRLs, to degrade antiviral factors that are otherwise not degraded in uninfected cells[133]. Human papillomavirus protein E6 hijacks human E6-associated protein (E6AP; also known as ubiquitin-protein ligase E3A), a member of the HECT E3 ligases, to degrade p53 (REF.[134]). Human and woodchuck hepatitis virus protein X (HBx and WHx, respectively) and paramyxovirus SV5-V protein bind to DDB1 to promote degradation of different host proteins, using a shared α-helical motif, H-box, that was also found in multiple cellular DCAF proteins, and can bind a deep pocket in DDB1 (REF.[135]). Primate lentiviruses produce small proteins that hijack three different host E3 ligases, all CRLs, to degrade multiple host antiviral restriction factors[136]. These small viral proteins can be viewed as prototypical PROTACs as they contain two functional domains, one for binding the E3 ligase and the other for binding the neosubstrate antiviral factor. These viruses identify the ‘right site’ in the E3 ligases that can interact with and support the polyubiquitination of neosubstrate antiviral factors. Importantly, the same E3 ligase binding site can be hijacked by different viral proteins[133], suggesting that this binding site may be targeted by distinct E3 ligand scaffolds. Thus, targeting these viral binding sites to discover novel E3 ligands offers a unique approach that is worth consideration.

Pharmacokinetics and oral bioavailability

The catalytic nature of heterobifunctional degraders is a key advantage of these compounds. Because these degraders do not rely on occupancy-driven pharmacology, the need for a long residence time and continuous drug exposure is significantly reduced. However, the high molecular weight, high lipophilicity and more rotatable bonds of most degraders present significant challenges for achieving adequate cell permeation ability, aqueous solubility and oral bioavailability[137–139]. Despite these obstacles, effective protein knockdown by heterobifunctional degraders has been achieved in many preclinical species. For example, RC32, an FKBP12 degrader, showed essentially complete degradation of FKBP12 across heart, liver, kidney, skin and adipose tissues in mice, Bama pigs and rhesus monkeys[140]. Furthermore, orally bioavailable and in vivo efficacious heterobifunctional degraders such as ARV-110, ARV-471 and MS1943 have been generated[65,94,98].

An extensive analysis of 38 PROTACs was conducted to predict which would have sufficient oral bioavailability using physicochemical properties, including the calculated distribution coefficient (log *D*), the molecular weight, the number of hydrogen bond donors and hydrogen bond acceptors and the number of rotatable bonds[141]. MDM2-recruiting and BIRC2-recruiting PROTACs exhibit high molecular weights, lipophilicities and numbers of rotatable bonds, suggesting that it would be very challenging to achieve sufficient oral bioavailability for these types of degraders. VHL-recruiting PROTACs fared better than

MDM2-recruiting and BIRC2-recruiting PROTACs in the analysis. However, they still have high molecular weights and numbers of rotatable bonds, suggesting a big hurdle for achieving good oral bioavailability. On the other hand, CRBN-recruiting PROTACs have the lowest molecular weights, moderate lipophilicities and reduced hydrogen bond donor/rotatable bond counts, and are predicted to be more likely to be orally bioavailable[141]. Analyses of a diverse set of PROTACs generated by AstraZeneca's internal programmes revealed that acceptable oral bioavailability (more than 30%) can be regularly achieved for CRBN-recruiting PROTACs but not for VHL-recruiting PROTACs[142]. While there will be exceptions to these generalizations, it is important to pay keen attention to minimizing the molecular weight, lipophilicity, number of hydrogen bond donors and number of rotatable bonds, and to using rigid linkers when one is optimizing oral bioavailability of heterobifunctional degraders.

Consideration of drug metabolism properties of heterobifunctional degraders is also worth noting[143]. For example, while degraders generally display acceptable metabolic profiles, issues surrounding potentially active metabolites exist. A recent study reported the observation of potentially active metabolites, including both POI and E3 ligand derivatives[142]. Therefore, careful consideration should be given when one is evaluating in vivo stability and toxicity of heterobifunctional degraders.

Covalent reversible and irreversible POI binders

Three major classes of POI binders have been used in the design of PROTACs: non-covalent, covalent reversible and covalent irreversible POI binders. The vast majority of reported PROTACs use non-covalent POI binders, however, PROTACs using covalent reversible or irreversible POI binders were reported recently[144,145]. While PROTACs bearing covalent irreversible POI binders are not catalytic, those based on covalent reversible POI binders can maintain the catalytic nature[144]. In an elegant study, three BTK degraders using a reversible covalent POI binder (RC-1), a reversible non-covalent POI binder (RNC-1) and an irreversible covalent POI binder (IRC-1) were designed and compared[144]. RC-1 induced potent degradation of BTK, while RNC-1 was less potent, and not surprisingly, IRC-1 was least effective and showed no degradation even at high concentrations. RC-1 also induced greater inhibition of cell proliferation than RNC-1 in vitro. These results suggest that reversible covalent POI binders offer an advantage over reversible non-covalent POI binders in PROTAC design. Additional studies to further validate these findings would be valuable.

Limitations of current TPD technology

Limitations of current E3 ligands

In addition to the paucity of E3 ligands, several aspects of current E3 ligands likely limit their broad applicability for therapeutic development, such as toxicity and acquired resistance resulting from functional non-essentiality of the E3 gene. The dependency of degraders on E3 ligases provides a new avenue for tumour cells to develop resistance through impairing the function of E3 ligase components. Indeed, it was demonstrated that tumour cells acquired resistance to both VHL-recruiting and CRBN-recruiting PROTACs following prolonged treatment[146,147]. Acquired resistance to the PROTACs was primarily

caused by genomic alterations or significantly reduced expression of the *CRBN* gene or the *CUL2* gene instead of point mutations targeting the residues involved in binding the POI and E3 ligase[146,147]. Genomic deletion of *CRBN* was also found as the primary cause of resistance to IMiDs in myeloma cells[148]. Consistently, genome-wide CRISPR–Cas9 knockout screens in the leukaemia cell line KBM7 treated with five degraders based on three different E3 ligases, $CRL2^{VHL}$, $CRL4^{CRBN}$ and $CRL4^{DCAF15}$, showed that deletion of *CRBN*, *VHL* and *DCAF15* conferred resistance to these degraders[149]. These findings highlight a new and more likely route for tumour cells to develop resistance to degraders based on functionally non-essential E3 ligases. Of the eight E3 ligases that have been exploited for PROTAC development, data from deep RNAi interrogation of viability effects in cancer are available for five of them, all of which are either functionally non-essential or essential in only a small fraction of cancer cells[3] (TABLE 1). Interestingly, there was no overlap in genes whose alteration conferred resistance to VHL-recruiting or CRBN-recruiting PROTACs after prolonged treatment in vitro[146,147]. This observation suggests that alternating use of degraders recruiting different E3 ligases may provide a viable strategy to achieve durable responses.

Drug toxicity is a major factor limiting the usefulness of therapeutic agents. In the TPD field, the toxicity of IMiDs is an issue worthy of attention. Thalidomide was approved in the late 1950s but was banned after the discovery that it caused widespread (more than 10,000) severe birth defects[150]. It was repurposed for treating multiple myeloma[151]. Toxic effects including hypersensitivity rash, neutropenia and cardiac dysrhythmia were observed for IMiDs[152]. Some of these toxic effects are believed to be caused by IMiD-mediated degradation of CRBN neosubstrates. MDM2 ligands such as nutlin 3 were originally discovered as MDM2 antagonists and p53 activators by inhibiting MDM2–p53 interactions[153]. While preclinical studies and early phase clinical trials showed the efficacy of nutlin 3 for treating cancers, its dose-limiting on-target toxicities resulting from p53 activation in normal cells remain a significant challenge to advance nutlin 3 as a monotherapy agent[154,155] and will likely limit its use in TPD. Sulfonamides, which are DCAF15 ligands, induce degradation of RNA splicing factors such as RBM39, which is functionally essential in many cell types; in line with this, dose-limiting toxicities have been observed in clinical trials[156]. However, the BIRC2 ligand bestatin[157] and the RNF114 ligand nimbolide[158] both appear to have low toxicity and relatively positive safety profiles in clinical use. Given the paucity of current E3 ligands and their limitations, the TPD field has an urgent need and plentiful opportunities to develop novel E3 ligands with low toxicity.

Degradation of E3 ligase neosubstrates by PROTACs

While no neosubstrates have been reported for VHL, numerous neosubstrates of the IMiD–CRBN complex have been reported (TABLE 1), and many of them contain a C2H2 zinc-finger degron motif[77,159]. Among these, GSPT1, a translation termination factor, is probably the most concerning neosubstrate, which may be degraded by CRBN-recruiting PROTACs because of the critical role of GSPT1 in most cells and potential toxic effects resulting from its degradation. It was first reported that GSPT1 was unintentionally degraded by MI-389, a CRBN-recruiting PROTAC that uses the pan-kinase inhibitor sunitinib as the POI binder, while the intended target, KIT, was not degraded[160]. However, degradation of

GSPT1 by a CRBN-recruiting PROTAC may lead to degradation of the POI as a secondary effect due to the critical role of GSPT1 in translation[77,161]. Degradation of other CRBN neosubstrates by CRBN-recruiting PROTACs has been reported[36]. For example, CDK4 and CDK6 PROTACs were found to degrade the lymphoid transcription factors IKZF1 and IKZF3, two neosubstrates of CRBN-recruiting IMiDs, with notably higher efficacies in killing mantle cell lymphoma cells over CDK4 and CDK6 degradation alone[60]. Therefore, it is critical to determine neosubstrate degradation profiles of CRBN-recruiting PROTACs to rule out 'secondary' pharmacology, which may contribute to the observed phenotypes. Furthermore, both RBM39 and cyclin K, the neosubstrates of DCAF15 and DDB1-CDK12, respectively, are functionally essential in many cell types[84]; therefore, it will be important to determine neosubstrate degradation profiles of DCAF15-recruiting and DDB1–CDK12-recruiting PROTACs.

Recent advances and future directions

To gain temporal control and avoid side effects, optically controlled small-molecule degraders have recently been developed[162–166]. For example, pomalidomide, and BET and ALK PROTACs were caged using a photolabile group, which can be activated (uncaged) with short ultraviolet light irradiation, leading to spatio-temporally controlled degradation of their target proteins[166].

Another advancement in the field has been the creation of the dTAG system, which allows degradation of protein targets in cells and mice, and serves as an excellent research tool[167]. This technology uses a fusion protein (POI fused with FKBP12-F36V) paired with a PROTAC derived by linking an FKBP12-F36V binder to a CRBN ligand, enabling rapid and effective degradation of the POI fusion protein by the PROTAC[167]. The dTAG system proved fundamental for validating that transmembrane solute carrier protein can be degraded using the PROTAC approach, leading to development of the first solute carrier protein PROTAC that effectively degraded solute carrier protein transporters[168].

Lastly, LYTAC (lysosome-targeting chimera) technology was recently developed to degrade extracellular and membrane-associated proteins[169]. The LYTAC system uses a small-molecule or antibody binder of the POI that is linked to a glycopeptide ligand, which binds to the mannose 6-phosphate receptor, a cell-surface lysosome-shuttling receptor, resulting in lysosomal degradation of the POI. Several extracellular and membrane-associated proteins were successfully degraded with use of this technology[169]. Autophagy-mediated degradation technologies, such as autophagy-targeting chimeras (AUTACs) and autophagosome-tethering compounds (ATTECs), and chemical genetic degradation systems such as HaloPROTAC have also been developed[170]. Collectively, these latest developments have laid a solid foundation for further advancing the heterobifunctional molecule field.

Conclusions

Following the development of the first small-molecule CRBN-recruiting and VHL-recruiting PROTACs in 2015, the pace of discovery has accelerated exponentially. Numerous first-in-class heterobifunctional degraders with robust in vivo efficacy have been developed.

Additional neosubstrates of CRBN and new molecular glues and E3 ligands have been identified. Remarkably, two PROTACs have entered clinical development as cancer therapies[13,14].

Tremendous opportunities exist for further advancing the TPD field. First, ‘undruggable’ targets such as transcription factors that lack an enzymatic active site can be targeted. Transforming low-affinity, functionally inactive binders into effective PROTACs is a significant opportunity. Second, enormous opportunities exist for the development of novel and functional small-molecule ligands for E3 ligases. Third, several isoform-selective PROTACs based on isoform non-selective POI ligands have been generated. The field should further exploit this unique dimension to achieve isoform selectivity for suitable targets. Fourth, advancements towards generating mutant-selective degraders for oncoproteins are a unique opportunity. Fifth, exploiting differential E3 ligase expression levels in tumour and normal cells to rationally develop PROTACs with reduced side effects and an improved therapeutic window is another significant opportunity. Sixth, hydrophobic tag-based degraders are currently underexplored, and opportunities exist in this area as in vivo efficacious, orally bioavailable degraders can be developed[65]. Lastly, structure-based rational design of effective PROTACs is another aspiration for the field as more POI–PROTAC–E3 ternary complex structures are solved.

Overall, the TPD field has seen exciting advancements. While challenges exist, we expect that the breath-taking pace of discovery in recent years will continue to accelerate and more heterobifunctional degraders will enter clinical development, some of which will likely become effective cancer therapeutics for patients.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgements

The authors thank J. Na for helping with DDB1 and CUL5 structural analysis and K. Lu for helping search for references. J.J. acknowledges support from the US National Institutes of Health (grants R01CA218600, R01CA230854, R01CA260666, R01GM122749, R01HD088626 and P30CA196521 from) and an endowed professorship from the Icahn School of Medicine at Mount Sinai. Y.X. acknowledges support from the US National Institutes of Health (grant R01GM067113) and an endowed professorship from the University of North Carolina at Chapel Hill. B.D. acknowledges support from the Medical Scientist Training Program (training grant T32GM007280) at the Icahn School of Medicine at Mount Sinai and the US National Institutes of Health (grant 3R01CA230854-03S1).

Competing interests

J.J., Y.X., H.Ü.K. and M.C. are inventors named on patent applications filed by the Icahn School of Medicine at Mount Sinai and the University of North Carolina at Chapel Hill. The Jin laboratory has received research funds from Celgene Corporation, Levo Therapeutics and Cullgen, Inc. The Xiong laboratory has received research funds from Cullgen Inc. J.J. is an equity shareholder in and consultant for Cullgen Inc. Y.X. is an equity shareholder in and currently an employee of Cullgen, Inc. B.D. and K-S.P declare no competing interests.

Glossary

26S proteasome

A 2.5-MDa proteolytic protein complex that controls protein homeostasis and specific cellular process in all eukaryotes.

Immunomodulatory imide drugs (IMiDs)
A class of small molecules that have been used in the clinic to modulate the immune system via binding the E3 ligase cereblon (CRBN). IMiDs recruit neosubstrates to CRBN for ubiquitination and subsequent degradation and have often been used as E3 ligands in proteolysis-targeting chimeras (PROTACs).

Molecular glues
Small molecules that act like adhesives to induce or stabilize protein–protein interactions between an E3 ligase and a neosubstrate, leading to degradation of the neosubstrate.

Neosubstrates
Substrates of an E3 ligase that are not recognized by the E3 ligase under physiological conditions but interact with the E3 ligase in the presence of a molecular glue.

HECT
The HECT domain, which is approximately 350 amino acids long and homologous to the E6AP carboxy terminus (HECT), contains an evolutionarily conserved cysteine residue that forms a thioester linkage with ubiquitin.

RBR
A tripartite domain of approximately 140 amino acid in length, consisting of three zinc-binding domains, RING1–IBR–RICN2. RING1-in-between-RiGN2 (RBR) ligases combine mechanistic features of RING-type and homologous to the E6AP carboxy terminus (HECT)-type ligases by using RING1 to recognize the E2 ~ ubiquitin complex (the tilde denotes a high-energy thioester bond) and RING2 to form the thioester intermediate with ubiquitin.

RING finger
First identified as a novel cysteine-rich sequence motif present in the ’really interesting new gene’ (RING 1). RING fingers promote the transfer of ubiquitin directly from E2 to the substrate by locking the E2 ~ ubiqution conjugate (the tilde denotes a high-energy thioester bond) in a closed conformation.

SKP1
S-phase kinase associated protein 1 (SKP1) binds CUL1 and functions as the adaptor protein for CRL1 complexes.

Elongin B–elongin C complex
A heterodimer that functions as the adaptor protein complex for both CRL2 and CRL5.

F-box
A domain, first identified in cyclin F, approximately 40 amino acids long that binds the adaptor protein SKP1 and functions as the substrate receptors for CRL1 complexes.

SOCS box

An approximately 40 amino acid region originally identified in members of suppressors of cytokine signalling proteins that consists of two separate sequences, one for binding the elongin B–elongin C heterodimer (BC box) and one for binding CUL5 (CUL5 box).

WD40 repeat
A domain defined at the primary sequence level by a Gly-His dipeptide and a Trp-Asp (WD) dipeptide separated by 20–30 residues that is commonly found in many proteins of diverse function and typically forms β-propeller structures.

BTB domain
Also known as the POZ domain, a conserved domain of 115–130 residues that consists of five α-helices and binds the amino-terminal domain of CUL3 and functions as the substrate receptor for cullin 3-RING ligase (CRL3) complexes.

Cooperative binding
The enhanced binding of a proteolysis-targeting chimera (PROTAC) to both the protein of interest and the E3 ligase compared with the binding of the PROTAC to the protein of interest or the E3 ligase alone.

Unfolded protein response
A cellular stress response that is activated by high levels of misfolded or unfolded proteins in the endoplasmic reticulum. The unfolded protein response aims to decrease the amount of unfolded proteins to maintain cellular function or induce apoptosis when this cannot be achieved.

DDB1- and CUL4-associated factor (DCAF)
A member of a family of proteins also known as DDB1-binding WD40 (DWD) proteins that bind DDB1 and function as substrate receptors for CRL4 complexes.

DDB1
Damaged DNA-binding protein complex subunit 1 (DDB1) binds CUL4 and functions as an adaptor protein for cullin 4-RING ligase (CRL4) complexes.

Degron motif
A specific molecular feature that is recognized by E3 ligases.

References

1. Tsherniak A, et al.Defining a cancer dependency map. Cell170, 564–576 e16 (2017). [PubMed: 28753430]
2. Hart T, et al.High-resolution CRISPR screens reveal fitness genes and genotype-specific cancer liabilities. Cell163, 1515–1526 (2015). [PubMed: 26627737]
3. McDonald III ER, et al.Project DRIVE: a compendium of cancer dependencies and synthetic lethal relationships uncovered by large-scale, deep RNAi screening. Cell170, 577–592 e10 (2017). [PubMed: 28753431]
4. Behan FM, et al.Prioritization of cancer therapeutic targets using CRISPR-Cas9 screens. Nature568, 511–516 (2019). [PubMed: 30971826]
5. Lai AC, & Crews CM Induced protein degradation: an emerging drug discovery paradigm. Nat. Rev. Drug Discov 16, 101–114 (2017). [PubMed: 27885283]

6. Sakamoto KM, et al.Protacs: chimeric molecules that target proteins to the Skp1–cullin–F box complex for ubiquitination and degradation. Proc. Natl Acad. Sci. USA98, 8554–8559 (2001). [PubMed: 11438690] This is the first publication of the PROTAC concept.
7. Ito T, et al.Identification of a primary target of thalidomide teratogenicity. Science327, 1345–1350 (2010). [PubMed: 20223979] This is the first report that the E3 ligase CRBN is a molecular target of thalidomide.
8. Kronke J, et al.Lenalidomide causes selective degradation of IKZF1 and IKZF3 in multiple myeloma cells. Science343, 301–305 (2014). [PubMed: 24292625] This is one of the first articles reporting the mechanism underlying the antitumour activity of IMiDs: binding of lenalidomide to CRBN induces degradation of IKZF1 and IKZF3.
9. Lu G, et al.The myeloma drug lenalidomide promotes the cereblon-dependent destruction of ikaros proteins. Science343, 305–309 (2014). [PubMed: 24292623] This is one of the first articles reporting the mechanism underlying the antitumour activity of IMiDs: binding of lenalidomide to CRBN induces degradation of IKZF1 and IKZF3.
10. Gandhi AK, et al.Immunomodulatory agents lenalidomide and pomalidomide co-stimulate T cells by inducing degradation of T cell repressors Ikaros and Aiolos via modulation of the E3 ubiquitin ligase complex CRL4 CRBN. Br. J. Haematol164, 811–821 (2014). [PubMed: 24328678]
11. Winter GE, et al.Phthalimide conjugation as a strategy for in vivo target protein degradation. Science348, 1376–1381 (2015). [PubMed: 25999370] This article reports the discovery of the first CRBN-recruiting PROTACs.
12. Bondeson DP, et al.Catalytic in vivo protein knockdown by small-molecule PROTACs. Nat. Chem. Biol11, 611–617 (2015). [PubMed: 26075522] This article reports the discovery of the first VHL-recruiting small-molecule PROTACs.
13. US National Library of Medicine. ClinicalTrials.govhttps://clinicaltrials.gov/ct2/show/NCT04072952 (2019).This trial relates to the first PROTAC targeting ER to enter clinical trials for breast cancer.
14. US National Library of Medicine. ClinicalTrials.govhttps://clinicaltrials.gov/ct2/show/NCT03888612 (2019).
15. Schapira M, Calabrese MF, Bullock AN, & Crews CM, Targeted protein degradation: expanding the toolbox. Nat. Rev. Drug Discov 18, 949–963 (2019). [PubMed: 31666732]
16. Sun X, et al.PROTACs: great opportunities for academia and industry. Signal. Transduct. Target. Ther4, 64 (2019). [PubMed: 31885879]
17. Chamberlain PP, & Hamann LG, Development of targeted protein degradation therapeutics. Nat. Chem. Biol 15, 937–944 (2019). [PubMed: 31527835]
18. Nalepa G, Rolfe M, & Harper JW, Drug discovery in the ubiquitin–proteasome system. Nat. Rev. Drug Discov 5, 596–613 (2006). [PubMed: 16816840]
19. Hershko A, & Ciechanover A, The ubiquitin system. Annu. Rev. Biochem 67, 425–479 (1998). [PubMed: 9759494]
20. Pickart CM, Mechanisms underlying ubiquitination. Annu. Rev. Biochem70, 503–533 (2001). [PubMed: 11395416]
21. Oh E, Akopian D, & Rape M, Principles of ubiquitin-dependent signaling. Annu. Rev. Cell. Dev. Biol 34, 137–162 (2018). [PubMed: 30110556]
22. Bard JAM, et al.Structure and function of the 26S proteasome. Annu. Rev. Biochem87, 697–724 (2018). [PubMed: 29652515]
23. Li W, et al.Genome-wide and functional annotation of human E3 ubiquitin ligases identifies MULAN, a mitochondrial E3 that regulates the organelle's dynamics and signaling. PLoS ONE3, e1487 (2008). [PubMed: 18213395]
24. Popovic D, Vucic D, & Dikic I, Ubiquitination in disease pathogenesis and treatment. Nat. Med 20, 1242–1253 (2014). [PubMed: 25375928]
25. Holstein SA, & McCarthy PL, Immunomodulatory drugs in multiple myeloma: mechanisms of action and clinical experience. Drugs 77, 505–520 (2017). [PubMed: 28205024]
26. Liu L, et al.UbiHub: a data hub for the explorers of ubiquitination pathways. Bioinformatics35, 2882–2884 (2019). [PubMed: 30601939]

27. Okuhira K, et al.Specific degradation of CRABP-II via cIAP1-mediated ubiquitylation induced by hybrid molecules that crosslink cIAP1 and the target protein. FEBS Lett. 585, 1147–1152 (2011). [PubMed: 21414315]
28. Qi Y, & Xia P, Cellular inhibitor of apoptosis protein-1 (cIAP1) plays a critical role in beta-cell survival under endoplasmic reticulum stress: promoting ubiquitination and degradation of C/EBP homologous protein (CHOP). J. Biol. Chem 287, 32236–32245 (2012). [PubMed: 22815481]
29. Frescas D, & Pagano M, Deregulated proteolysis by the F-box proteins SKP2 and beta-TrCP: tipping the scales of cancer. Nat. Rev. Cancer 8, 438–449 (2008). [PubMed: 18500245]
30. Wang Z, et al.The diverse roles of SPOP in prostate cancer and kidney cancer. Nat. Rev. Urol17, 339–350 (2020). [PubMed: 32355326]
31. Panagopoulos A, Taraviras S, Nishitani H, & Lygerou Z, $CRL4^{Cdt2}$: coupling genome stability to ubiquitination. Trends Cell Biol. 30, 290–302 (2020). [PubMed: 32044173]
32. Okumura F, Joo-Okumura A, Nakatsukasa K, & Kamura T, The role of cullin 5-containing ubiquitin ligases. Cell Div. 11, 1 (2016). [PubMed: 27030794]
33. Buckley DL, et al.Targeting the von Hippel-Lindau E3 ubiquitin ligase using small molecules to disrupt the VHL/HIF-1alpha interaction. J. Am. Chem. Soc134, 4465–4468 (2012). [PubMed: 22369643]
34. Buckley DL, et al.Small-molecule inhibitors of the interaction between the E3 ligase VHL and HIF1alpha. Angew. Chem. Int. Ed51, 11463–11467 (2012).
35. Mares A, et al.Extended pharmacodynamic responses observed upon PROTAC-mediated degradation of RIPK2. Commun. Biol3, 1–13 (2020). [PubMed: 31925316]
36. Donovan KA, et al.Mapping the degradable kinome provides a resource for expedited degrader development. Cell183, 1714–1731.e10 (2020). [PubMed: 33275901]
37. Zorba A, et al.Delineating the role of cooperativity in the design of potent PROTACs for BTK. Proc. Natl Acad. Sci. USA115, E7285–E7292 (2018). [PubMed: 30012605]
38. Schiemer J, et al.Snapshots and ensembles of BTK and cIAP1 protein degrader ternary complexes. Nat. Chem. Biol17, 152–160 (2021). [PubMed: 33199914]
39. Daniels DL, Riching KM, & Urh M, Monitoring and deciphering protein degradation pathways inside cells. Drug Discov. Today Technol 31, 61–68 (2019). [PubMed: 31200861]
40. Liu X, et al.Assays and technologies for developing proteolysis targeting chimera degraders. Future Med. Chem12, 1155–1179 (2020). [PubMed: 32431173]
41. Gadd MS, et al.Structural basis of PROTAC cooperative recognition for selective protein degradation. Nat. Chem. Biol13, 514–521 (2017). [PubMed: 28288108] This article reports the first crystal structure of a POI–PROTAC–VHL ternary complex.
42. Farnaby W, et al.BAF complex vulnerabilities in cancer demonstrated via structure-based PROTAC design. Nat. Chem. Biol15, 672–680 (2019). [PubMed: 31178587]
43. Chung C, et al.Structural insights into PROTAC-mediated degradation of Bcl-xL. ACS Chem. Biol15, 2316–2323 (2020). [PubMed: 32697072]
44. Testa A, Hughes SJ, Lucas X, Wright JE, & Ciulli A, Structure-based design of a macrocyclic PROTAC. Angew. Chem. Int. Ed 59, 1727–1734 (2020).
45. Hughes SJ, Testa A, & Ciulli A, Crystal structure of macrocyclic PROTAC 1 in complex with the second bromodomain of human Brd4 and pVHL:elonginC:elonginB. PDB 10.2210/pdb6SIS/pdb (2019).
46. Gadd MS, Zengerle M, & Ciulli A, The PROTAC MZ1 in complex with the second bromodomain of Brd4 and pVHL:elonginC:elonginB. PDB 10.2210/pdb5T35/pdb (2016).
47. Roy M, Bader G, Diers E, Trainor N, Farnaby W, & Ciulli A, Crystal structure of PROTAC 2 in complex with the bromodomain of human SMARCA4 and pVHL:elonginC:elonginB. PDB 10.2210/pdb6HR2/pdb (2018).
48. Roy M, Bader G, Diers E, Trainor N, Farnaby W, & Ciulli A, Crystal structure of PROTAC 2 in complex with the bromodomain of human SMARCA2 and pVHL:elonginC:elonginB. PDB 10.2210/pdb6HAX/pdb (2018).

49. Roy M, Bader G, Diers E, Trainor N, Farnaby W, & Ciulli A, Crystal structure of PROTAC 1 in complex with the bromodomain of human SMARCA2 and pVHL:elonginC:elonginB. PDB 10.2210/pdb6HAY/pdb (2018).
50. Chung C, PROTAC6 mediated complex of VHL:EloB:EloC and Bcl-xL. PDB10.2210/pdb6ZHC/pdb (2020).
51. Nowak RP, et al.Plasticity in binding confers selectivity in ligand-induced protein degradation. Nat. Chem. Biol14, 706–714 (2018). [PubMed: 29892083] This article reports the first crystal structures of POI–PROTAC–CRBN ternary complexes.
52. Nowak RP, DeAngelo SL, Buckley D, Bradner JE, & Fischer ES, Crystal structure of DDB1-CRBN-BRD4(BD1) complex bound to dBET23 PROTAC. PDB 10.2210/pdb6BN7/pdb (2017).
53. Nowak RP, DeAngelo SL, Buckley D, Bradner JE, & Fischer ES, Crystal structure of DDB1-CRBN-BRD4(BD1) complex bound to dBET55 PROTAC. PDB 10.2210/pdb6BN8/pdb (2017).
54. Nowak RP, DeAngelo SL, Buckley D, Bradner JE, & Fischer E, Crystal structure of DDB1-CRBN-BRD4(BD1) complex bound to dBET70 PROTAC. PDB 10.2210/pdb6BN9/pdb (2017).
55. Nowak RP, DeAngelo SL, Buckley D, Ishoey M, He Z, Zhang T, Bradner JE, & Fischer ES, Crystal structure of DDB1-CRBN-BRD4(BD1) complex bound to dBET57 PROTAC. PDB 10.2210/pdb6BNB/pdb (2017).
56. Nowak RP, DeAngelo SL, Buckley D, Bradner JE, Fischer ES, (2017) Crystal structure of DDB1-CRBN-BRD4(BD1) complex bound to dBET6 PROTAC. PDB10.2210/pdb6BOY/pdb (2017)
57. Calabrese MF, Schiemer JS, Ternary complex structure - BTK cIAP compound 17. PDB10.2210/pdb6W7O/pdb (2020)
58. Calabrese MF, Schiemer JS, Ternary complex structure - BTK cIAP compound 15. PDB10.2210/pdb6W8I/pdb (2020)
59. Burslem GM, et al.The advantages of targeted protein degradation over inhibition: an RTK case study. Cell Chem. Biol25, 67–77.e3 (2018). [PubMed: 29129716]
60. Jiang B, et al.Development of dual and selective degraders of cyclin-dependent kinases 4 and 6. Angew. Chem. Int. Ed58, 6321–6326 (2019).
61. Bai L, et al.A potent and selective small-molecule degrader of STAT3 achieves complete tumor regression in vivo. Cancer Cell36, 498–511.e17 (2019). [PubMed: 31715132] This article reports the development of the first STAT3 PROTAC with robust in vivo efficacy.
62. Khan S, et al.A selective BCL-XL PROTAC degrader achieves safe and potent antitumor activity. Nat. Med25, 1938–1947 (2019). [PubMed: 31792461] This article reports the development of the first BCL-XL PROTAC that uses E3 expression to abrogate toxic side effects of the parental inhibitor.
63. Bondeson DP, et al.Lessons in PROTAC design from selective degradation with a promiscuous warhead. Cell Chem. Biol25, 78–87.e5 (2018). [PubMed: 29129718]
64. Smith BE, et al.Differential PROTAC substrate specificity dictated by orientation of recruited E3 ligase. Nat. Commun10, 131 (2019). [PubMed: 30631068]
65. Ma A, et al.Discovery of a first-in-class EZH2 selective degrader. Nat. Chem. Biol16, 214–222 (2020). [PubMed: 31819273] This article reports the discovery of the first EZH2-selective degrader, which is a hydrophobic tag-based heterobifunctional small-molecule degrader.
66. Osborne CK, Wakeling A, & Nicholson RI, Fulvestrant: an oestrogen receptor antagonist with a novel mechanism of action. Br. J. Cancer 90, S2–S6 (2004). [PubMed: 15094757]
67. Guan J, et al.Therapeutic ligands antagonize estrogen receptor function by impairing its mobility. Cell178, 949–963.e18 (2019). [PubMed: 31353221]
68. Bradbury RH, et al.Small-molecule androgen receptor downregulators as an approach to treatment of advanced prostate cancer. Bioorg. Med. Chem. Lett21, 5442–5445 (2011). [PubMed: 21782422]
69. Neklesa TK, & Crews CM, Greasy tags for protein removal. Nature 487, 308–309 (2012). [PubMed: 22810693]
70. Long MJC, Gollapalli DR, & Hedstrom L, Inhibitor mediated protein degradation. Chem. Biol 19, 629–637 (2012). [PubMed: 22633414]
71. Shi Y, et al.Boc3Arg-linked ligands induce degradation by localizing target proteins to the 20S proteasome. ACS Chem. Biol11, 3328–3337 (2016). [PubMed: 27704767]

72. Allocati N, Masulli M, Di Ilio C, & Federici L, Glutathione transferases: substrates, inihibitors and pro-drugs in cancer and neurodegenerative diseases. Oncogenesis 7, 8 (2018). [PubMed: 29362397]
73. Neklesa TK, et al.Small-molecule hydrophobic tagging–induced degradation of HaloTag fusion proteins. Nat. Chem. Biol7, 538–543 (2011). [PubMed: 21725302]
74. Xie T, et al.Pharmacological targeting of the pseudokinase Her3. Nat. Chem. Biol10, 1006–1012 (2014). [PubMed: 25326665]
75. Sievers QL, et al.Defining the human C2H2 zinc finger degrome targeted by thalidomide analogs through CRBN. Science362, eaat0572 (2018). [PubMed: 30385546]
76. Petzold G, Fischer ES, & Thoma NH, Structural basis of lenalidomide-induced CK1alpha degradation by the $CRL4^{CRBN}$ ubiquitin ligase. Nature 532, 127–130 (2016). [PubMed: 26909574]
77. Matyskiela ME, et al.A novel cereblon modulator recruits GSPT1 to the $CRL4^{CRBN}$ ubiquitin ligase. Nature535, 252–257 (2016). [PubMed: 27338790]
78. Matyskiela ME, et al.SALL4 mediates teratogenicity as a thalidomide-dependent cereblon substrate. Nat. Chem. Biol14, 981–987 (2018). [PubMed: 30190590]
79. Han T, et al.Anticancer sulfonamides target splicing by inducing RBM39 degradation via recruitment to DCAF15. Science356, eaal3755 (2017). [PubMed: 28302793] This is one of the first articles reporting sulfonamides acting as molecular glue degraders of RBM39 via binding the E3 ligase DCAF15.
80. Uehara T, et al.Selective degradation of splicing factor CAPERα by anticancer sulfonamides. Nat. Chem. Biol13, 675–680 (2017). [PubMed: 28437394] This is one of the first articles reporting sulfonamides acting as molecular glue degraders of RBM39 via binding the E3 ligase DCAF15.
81. Faust TB, et al.Structural complementarity facilitates E7820-mediated degradation of RBM39 by DCAF15. Nat. Chem. Biol16, 7–14 (2020). [PubMed: 31686031] This is one of the first articles reporting the crystal structure of a DCAF15–molecular glue–RBM39 ternary complex.
82. Bussiere DE, et al.Structural basis of indisulam-mediated RBM39 recruitment to DCAF15 E3 ligase complex. Nat. Chem. Biol16, 15–23 (2020). [PubMed: 31819272] This is one of the first articles reporting the crystal structure of a DCAF15-molecular glue-RBM39 ternary complex.
83. Słabicki M, et al.The CDK inhibitor CR8 acts as a molecular glue degrader that depletes cyclin K. Nature585, 293–297 (2020). [PubMed: 32494016] This is the first report that a CDK12 inhibitor can act as a moleculat glue degrader of cyclin K and it also reports the first crystal structure of the DDB1–molecular glue–CDK12–cyclin K complex.
84. Mayor-Ruiz C, et al.Rational discovery of molecular glue degraders via scalable chemical profiling. Nat. Chem. Biol16, 1199–1207 (2020). [PubMed: 32747809]
85. Lv L, et al.Discovery of a molecular glue promoting CDK12-DDB1 interaction to trigger cyclin K degradation. eLife9, e59994 (2020). [PubMed: 32804079]
86. Isobe Y, et al.Manumycin polyketides act as molecular glues between UBR7 and P53. Nat. Chem. Biol16, 1189–1198 (2020). [PubMed: 32572277]
87. Che Y, Gilbert AM, Shanmugasundaram V, & Noe MC, Inducing protein-protein interactions with molecular glues. Bioorg. Med. Chem. Lett 28, 2585–2592 (2018). [PubMed: 29980357]
88. Sakamoto KM, et al.Development of Protacs to target cancer-promoting proteins for ubiquitination and degradation. Mol. Cell. Proteom2, 1350–1358 (2003).
89. Han X, et al.Discovery of ARD-69 as a highly potent proteolysis targeting chimera (PROTAC) degrader of androgen receptor (AR) for the treatment of prostate cancer. J. Med. Chem62, 941–964 (2019). [PubMed: 30629437]
90. Kregel S, et al.Androgen receptor degraders overcome common resistance mechanisms developed during prostate cancer treatment. Neoplasia22, 111–119 (2020). [PubMed: 31931431]
91. Zhao L, Han X, Lu J, McEachern D, & Wang S, A highly potent PROTAC androgen receptor (AR) degrader ARD-61 effectively inhibits AR-positive breast cancer cell growth in vitro and tumor growth in vivo. Neoplasia 22, 522–532 (2020). [PubMed: 32928363]
92. Neklesa TK, et al.An oral androgen receptor PROTAC degrader for prostate cancer. J Clin Oncol. 36 (Suppl. 6), 381 (2018).

93. Petrylak DP, et al.First-in-human phase I study of ARV-110, an androgen receptor (AR) PROTAC degrader in patients (pts) with metastatic castrate-resistant prostate cancer (mCRPC) following enzalutamide (ENZ) and/or abiraterone (ABI). J. Clin. Oncol38, 3500–3500 (2020).This abstract reports clinical results of the first PROTAC that has entered clinical development.
94. Lawrence S, et al.Discovery of ARV-110, a first in class androgen receptor degrading PROTAC for the treatment of men with metastatic castration resistant prostate cancer [abstract 43]. in Proceedings of the 112th Annual Meeting of the American Association for Cancer Research (American Association for Cancer Research, 2021).
95. Carroll JS, et al.Genome-wide analysis of estrogen receptor binding sites. Nat. Genet38, 1289–1297 (2006). [PubMed: 17013392]
96. Hu J, et al.Discovery of ERD-308 as a highly potent proteolysis targeting chimera (PROTAC) degrader of estrogen receptor (ER). J. Med. Chem62, 1420–1442 (2019). [PubMed: 30990042]
97. Flanagan J, et al.ARV-471, an oral estrogen receptor PROTAC degrader for breast cancer [abstract]. Cancer Res. 79 (Suppl. 4), P5-04-18 (2018).
98. Lawrence S, et al.The discovery of ARV-471, an orally bioavailable estrogen receptor degrading PROTAC for the treatment of patients with breast cancer [abstract 44]. in Proceedings of the 112th Annual Meeting of the American Association for Cancer Research (American Association for Cancer Research, 2021).
99. Chen J, et al.Structure-based design of conformationally constrained, cell-permeable STAT3 inhibitors. ACS Med. Chem. Lett1, 85–89 (2010). [PubMed: 20596242]
100. Pentimalli F, BCL2: A 30-year tale of life, death and much more to come. Cell Death Differ25, 7–9 (2018). [PubMed: 29125600]
101. Hanahan D, & Weinberg RA, Hallmarks of cancer: the next generation. Cell 144, 646–674 (2011). [PubMed: 21376230]
102. Leverson JD, et al.Exploiting selective BCL-2 family inhibitors to dissect cell survival dependencies and define improved strategies for cancer therapy. Sci. Transl. Med7, 279ra40 (2015).
103. Perini GF, Ribeiro GN, Pinto Neto JV, Campos LT, & Hamerschlak N, BCL-2 as therapeutic target for hematological malignancies. J. Hematol. Oncol 11, 65 (2018). [PubMed: 29747654]
104. Tse C, et al.ABT-263: a potent and orally bioavailable Bcl-2 family inhibitor. Cancer Res. 68, 3421–3428 (2008). [PubMed: 18451170]
105. Souers AJ, et al.ABT-199, a potent and selective BCL-2 inhibitor, achieves antitumor activity while sparing platelets. Nat. Med19, 202–208 (2013). [PubMed: 23291630]
106. Schoenwaelder SM, et al.Bcl-xL-inhibitory BH3 mimetics can induce a transient thrombocytopathy that undermines the hemostatic function of platelets. Blood118, 1663–1674 (2011). [PubMed: 21673344]
107. Kaefer A, et al.Mechanism-based pharmacokinetic/pharmacodynamic meta-analysis of navitoclax (ABT-263) induced thrombocytopenia. Cancer Chemother. Pharmacol74, 593–602 (2014). [PubMed: 25053389]
108. Kim KH, & Roberts CWM, Targeting EZH2 in cancer. Nat. Med 22, 128–134 (2016). [PubMed: 26845405]
109. Kaniskan HÜ, Martini ML, & Jin J, Inhibitors of protein methyltransferases and demethylases. Chem. Rev 118, 989–1068 (2018). [PubMed: 28338320]
110. February PO, et al.First EZH2 inhibitor approved-for rare sarcoma. Cancer Discov. 10, 333–334 (2020). [PubMed: 32041739]
111. Kim KH, et al.SWI/SNF-mutant cancers depend on catalytic and non-catalytic activity of EZH2. Nat. Med21, 1491–1496 (2015). [PubMed: 26552009]
112. Lawrence CL, & Baldwin AS, Non-canonical EZH2 transcriptionally activates RelB in triple negative breast cancer. PLoS ONE 11, e0165005 (2016). [PubMed: 27764181]
113. Kim J, et al.Polycomb- and methylation-independent roles of EZH2 as a transcription activator. Cell Rep. 25, 2808–2820.e4 (2018). [PubMed: 30517868]
114. Zhao Y, et al.EZH2 cooperates with gain-of-function p53 mutants to promote cancer growth and metastasis. EMBO Rep. 38, e99599 (2019).

115. Potjewyd F, et al.Degradation of polycomb repressive complex 2 with an EED-targeted bivalent chemical degrader. Cell Chem. Biol27, 47–56.e15 (2020). [PubMed: 31831267]
116. Hsu JHR, et al.EED-targeted PROTACs degrade EED, EZH2, and SUZ12 in the PRC2 complex. Cell Chem. Biol27, 41–46.e17 (2020). [PubMed: 31786184]
117. Liu Z, et al.Design and synthesis of EZH2-based PROTACs to degrade the PRC2 complex for targeting the noncatalytic activity of EZH2. J. Med. Chem10.1021/acs.jmedchem.0c02234 (2021).
118. Davies H, et al.Mutations of the BRAF gene in human cancer. Nature417, 949–954 (2002). [PubMed: 12068308]
119. Shelledy L, & Roman D, Vemurafenib: first-in-class BRAF-mutated inhibitor for the treatment of unresectable or metastatic melanoma. J. Adv. Pract. Oncol 6, 361–365 (2015). [PubMed: 26705496]
120. Lovly CM, & Shaw AT, Molecular pathways: resistance to kinase inhibitors and implications for therapeutic strategies. Clin. Cancer Res 20, 2249–2256 (2014). [PubMed: 24789032]
121. Han XR, et al.Discovery of selective small molecule degraders of BRAF-V600E. J. Med. Chem63, 4069–4080 (2020). [PubMed: 32223235] This is one of the first articles reporting mutant-selective BRAF PROTAC degraders.
122. Posternak G, et al.Functional characterization of a PROTAC directed against BRAF mutant V600E. Nat. Chem. Biol16, 1170–1178 (2020). [PubMed: 32778845] This is one of the first articles reporting mutant-selective BRAF PROTAC degraders.
123. Alabi S, et al.Mutant-selective degradation by BRAF-targeting PROTACs. Nat. Commun12, 920 (2021). [PubMed: 33568647] This is one of the first articles reporting mutant-selective BRAF PROTAC degraders.
124. Hu MCT, Qiu WR, Wang X, Meyer CF, & Tan TH, Human HPK1, a novel human hematopoietic progenitor kinase that activates the JNK/SAPK kinase cascade. Genes Dev 10, 2251–2264 (1996). [PubMed: 8824585]
125. Lasserre R, et al.Release of serine/threonine-phosphorylated adaptors from signaling microclusters down-regulates T cell activation. J. Cell Biol195, 839–853 (2011). [PubMed: 22105350]
126. Hernandez S, et al.The kinase activity of hematopoietic progenitor kinase 1 is essential for the regulation of T cell function. Cell Rep. 25, 80–94 (2018). [PubMed: 30282040]
127. Si J, et al.Hematopoietic progenitor kinase1 (HPK1) mediates T cell dysfunction and is a druggable target for T cell-based immunotherapies. Cancer Cell38, 551–566.e11 (2020). [PubMed: 32860752] This article reports the discovery of the first HPK1 PROTAC degrader.
128. Galdeano C, et al.Structure-guided design and optimization of small molecules targeting the protein-protein interaction between the von Hippel-Lindau (VHL) E3 ubiquitin ligase and the hypoxia inducible factor (HIF) alpha subunit with in vitro nanomolar affinities. J. Med. Chem57, 8657–8663 (2014). [PubMed: 25166285] This article reports the discovery of the first widely used ligand of the E3 ligase VHL.
129. Li L, et al.In vivo target protein degradation induced by PROTACs based on E3 ligase DCAF15. Signal. Transduct. Target. Ther5, 129 (2020). [PubMed: 32713946]
130. Backus KM, et al.Proteome-wide covalent ligand discovery in native biological systems. Nature534, 570–574 (2016). [PubMed: 27309814]
131. Zhang X, Crowley VM, Wucherpfennig TG, Dix MM, & Cravatt BF, Electrophilic PROTACs that degrade nuclear proteins by engaging DCAF16. Nat. Chem. Biol 15, 737–746 (2019). [PubMed: 31209349] This article reports the discovery of the first DCAF16 covalent ligand and DCAF16-recruiting PROTAC degrader.
132. Ward CC, et al.Covalent ligand screening uncovers a RNF4 E3 ligase recruiter for targeted protein degradation applications. ACS Chem. Biol14, 2430–2440 (2019). [PubMed: 31059647]
133. Mahon C, Krogan NJ, Craik CS, & Pick E, Cullin E3 ligases and their rewiring by viral factors. Biomolecules 4, 897–930 (2014). [PubMed: 25314029]
134. Howley PM, Munger K, Romanczuk H, Scheffner M, & Huibregtse JM, Cellular targets of the oncoproteins encoded by the cancer associated human papillomaviruses. Princess Tak. Symp 22, 239–248 (1991).

135. Li T, Robert EI, van Breugel PC, Strubin M, & Zheng N, A promiscuous alpha-helical motif anchors viral hijackers and substrate receptors to the CUL4-DDB1 ubiquitin ligase machinery. Nat. Struct. Mol. Biol 17, 105–111 (2010). [PubMed: 19966799]
136. Simon V, Bloch N, & Landau NR, Intrinsic host restrictions to HIV-1 and mechanisms of viral escape. Nat. Immunol 16, 546–553 (2015). [PubMed: 25988886]
137. Matsson P, Doak BC, Over B, & Kihlberg J, Cell permeability beyond the rule of 5. Adv. Drug Deliv. Rev 101, 42–61 (2016). [PubMed: 27067608]
138. Matsson P, & Kihlberg J, How big is too big for cell permeability? J. Med. Chem 60, 1662–1664 (2017). [PubMed: 28234469]
139. Foley CA, Potjewyd F, Lamb KN, James LI, & Frye SV, Assessing the cell permeability of bivalent chemical degraders using the chloroalkane penetration assay. ACS Chem. Biol. 15, 290–295 (2020). [PubMed: 31846298]
140. Sun X, et al.A chemical approach for global protein knockdown from mice to non-human primates. Cell Discov. 5, 1–13 (2019). [PubMed: 30603102]
141. Edmondson SD, Yang B, & Fallan C, Proteolysis targeting chimeras (PROTACs) in 'beyond rule-of-five' chemical space: recent progress and future challenges. Bioorg. Med. Chem. Lett 29, 1555–1564 (2019). [PubMed: 31047748]
142. Pike A, Williamson B, Harlfinger S, Martin S, & McGinnity DF, Optimising proteolysis-targeting chimeras (PROTACs) for oral drug delivery: a drug metabolism and pharmacokinetics perspective. Drug Discov. Today 25, 1793–1800 (2020).
143. Goracci L, et al.Understanding the metabolism of proteolysis targeting chimeras (PROTACs): the next step toward pharmaceutical applications. J. Med. Chem63, 11615–11638 (2020). [PubMed: 33026811]
144. Guo W-H, et al.Enhancing intracellular accumulation and target engagement of PROTACs with reversible covalent chemistry. Nat. Commun11, 4268 (2020). [PubMed: 32848159]
145. Gabizon R, et al.Efficient targeted degradation via reversible and irreversible covalent PROTACs. J. Am. Chem. Soc142, 11734–11742 (2020). [PubMed: 32369353]
146. Zhang L, Riley-Gillis B, Vijay P, & Shen Y, Acquired resistance to BET-PROTACs (proteolysis-targeting chimeras) caused by genomic alterations in core components of E3 ligase complexes. Mol. Cancer Ther 18, 1302–1311 (2019). [PubMed: 31064868]
147. Ottis P, et al.Cellular resistance mechanisms to targeted protein degradation converge toward impairment of the engaged ubiquitin transfer pathway. ACS Chem. Biol14, 2215–2223 (2019). [PubMed: 31553577]
148. Zhu YX, et al.Cereblon expression is required for the antimyeloma activity of lenalidomide and pomalidomide. Blood118, 4771–4779 (2011). [PubMed: 21860026]
149. Mayor-Ruiz C, et al.Plasticity of the cullin-RING ligase repertoire shapes sensitivity to ligand-induced protein degradation. Mol. Cell75, 849–858 e8 (2019). [PubMed: 31442425]
150. Vargesson N, Thalidomide-induced teratogenesis: history and mechanisms. Birth Defects Res. C. Embryo Today105, 140–156 (2015). [PubMed: 26043938]
151. Singhal S, et al.Antitumor activity of thalidomide in refractory multiple myeloma. N. Engl. J. Med341 , 1565–1571 (1999). [PubMed: 10564685]
152. Franks ME, Macpherson GR, & Figg WD, Thalidomide. Lancet 363, 1802–1811 (2004). [PubMed: 15172781]
153. Vassilev LT, et al.In vivo activation of the p53 pathway by small-molecule antagonists of MDM2. Science303, 844–848 (2004). [PubMed: 14704432]
154. Zanjirband M, & Rahgozar S, Targeting p53-MDM2 interaction using small molecule inhibitors and the challenges needed to be addressed. Curr. Drug Targets 20, 1091–1111 (2019). [PubMed: 30947669]
155. Khurana A, & Shafer DA, MDM2 antagonists as a novel treatment option for acute myeloid leukemia: perspectives on the therapeutic potential of idasanutlin (RG7388). OncoTargets Ther. 12, 2903–2910 (2019).
156. Assi R, et al.Final results of a phase 2, open-label study of indisulam, idarubicin, and cytarabine in patients with relapsed or refractory acute myeloid leukemia and high-risk myelodysplastic syndrome. Cancer124, 2758–2765 (2018). [PubMed: 29660836]

157. Ota K, & Uzuka Y, Clinical trials of bestatin for leukemia and solid tumors. Biotherapy 4, 205–214 (1992). [PubMed: 1599804]

158. Bodduluru LN, Kasala ER, Thota N, Barua CC, & Sistla R, Chemopreventive and therapeutic effects of nimbolide in cancer: the underlying mechanisms. Toxicol. Vitr 28, 1026–1035 (2014).

159. Gao S, Wang S, & Song Y, Novel immunomodulatory drugs and neo-substrates. Biomark. Res 8, 2 (2020). [PubMed: 31938543]

160. Ishoey M, et al.Translation termination factor GSPT1 is a phenotypically relevant off-target of heterobifunctional phthalimide degraders. ACS Chem. Biol13, 553–560 (2018). [PubMed: 29356495]

161. Buhimschi AD, & Crews CM, Evolving rules for protein degradation? Insights from the zinc finger degrome. Biochemistry 58, 861–864 (2019). [PubMed: 30681837]

162. Xue G, Wang K, Zhou D, Zhong H, & Pan Z, Light-induced protein degradation with photocaged PROTACs. J. Am. Chem. Soc 141, 18370–18374 (2019). [PubMed: 31566962]

163. Reynders M, et al.PHOTACs enable optical control of protein degradation. Sci. Adv6, eaay5064 (2020). [PubMed: 32128406]

164. Pfaff P, Samarasinghe KTG, Crews CM, & Carreira EM, Reversible spatiotemporal control of induced protein degradation by bistable photoPROTACs. ACS Cent. Sci 5, 1682–1690 (2019). [PubMed: 31660436]

165. Naro Y, Darrah K, & Deiters A, Optical control of small molecule-induced protein degradation. J. Am. Chem. Soc 142, 2193–2197 (2020). [PubMed: 31927988]

166. Liu J, et al.Light-induced control of protein destruction by opto-PROTAC. Sci. Adv6, eaay5154 (2020). [PubMed: 32128407]

167. Nabet B, et al.The dTAG system for immediate and target-specific protein degradation. Nat. Chem. Biol14, 431–441 (2018). [PubMed: 29581585]

168. Bensimon A, et al.Targeted degradation of SLC transporters reveals amenability of multi-pass transmembrane proteins to ligand-induced proteolysis. Cell Chem. Biol27, 728–739 (2020). [PubMed: 32386596]

169. Banik SM, et al.Lysosome-targeting chimaeras for degradation of extracellular proteins. Nature584, 291–297 (2020). [PubMed: 32728216]

170. Ding Y, Fei Y, & Lu B, Emerging new concepts of degrader technologies. Trends Pharmacol. Sci 41 , 464–474 (2020). [PubMed: 32416934]

171. Schneekloth AR, Pucheault M, Tae HS, & Crews CM, Targeted intracellular protein degradation induced by a small molecule: en route to chemical proteomics. Bioorg. Med. Chem. Lett 18, 5904–5908 (2008). [PubMed: 18752944] This is the first report of an MDM2-recruiting small-molecule PROTAC degrader of AR.

172. Hines J, Lartigue S, Dong H, Qian Y, & Crews CM, MDM2-recruiting PROTAC offers superior, synergistic antiproliferative activity via simultaneous degradation of BRD4 and stabilization of p53. Cancer Res. 79, 251–262 (2019). [PubMed: 30385614]

173. Zhao Q, Lan T, Su S, & Rao Y, Induction of apoptosis in MDA-MB-231 breast cancer cells by a PARP1-targeting PROTAC small molecule. Chem. Commun 55, 369–372 (2019).

174. Buckley DL, et al.HaloPROTACS: use of small molecule PROTACs to induce degradation of HaloTag fusion proteins. ACS Chem. Biol10, 1831–1837 (2015). [PubMed: 26070106]

175. Raina K, et al.PROTAC-induced BET protein degradation as a therapy for castration-resistant prostate cancer. Proc. Natl Acad. Sci. USA113, 7124–7129 (2016). [PubMed: 27274052]

176. Soares P, et al.Group-based optimization of potent and cell-active inhibitors of the von Hippel-Lindau (VHL) E3 ubiquitin ligase: structure-activity relationships leading to the chemical probe (2S,4R)-1-((S)-2-(1-cyanocyclopropanecarboxamido)-3,3-dimethylbutanoyl)-4-hydr. J. Med. Chem61, 599–618 (2018). [PubMed: 28853884]

177. Krönke J, et al.Lenalidomide induces ubiquitination and degradation of CK1α in del(5q) MDS. Nature523, 183–188 (2015). [PubMed: 26131937]

178. Matyskiela ME, et al.A cereblon modulator (CC-220) with improved degradation of Ikaros and Aiolos. J. Med. Chem61, 535–542 (2018). [PubMed: 28425720]

179. Donovan KA, et al.Thalidomide promotes degradation of SALL4, a transcription factor implicated in Duane radial ray syndrome. eLife7, e38430 (2018). [PubMed: 30067223]
180. Asatsuma-Okumura T, et al.P63 is a cereblon substrate involved in thalidomide teratogenicity. Nat. Chem. Biol15, 1077–1084 (2019). [PubMed: 31591562]
181. Yamamoto J, et al.ARID2 is a pomalidomide-dependent CRL4CRBN substrate in multiple myeloma cells. Nat. Chem. Biol16, 1208–1217 (2020). [PubMed: 32958952]
182. Umezawa H, Aoyagi T, Suda H, Hamada M, & Takeuchi T, Bestatin, an inhibitor of aminopeptidase B, produced by actinomycetes. J. Antibiot 29, 97–99 (1976).
183. Itoh Y, Ishikawa M, Naito M, & Hashimoto Y, Protein knockdown using methyl bestatin–ligand hybrid molecules: design and synthesis of inducers of ubiquitination-mediated degradation of cellular retinoic acid-binding proteins. J. Am. Chem. Soc 132, 5820–5826 (2010). [PubMed: 20369832] This article reports the discovery of the first BIRC2-recruiting small-molecule PROTAC degrader.
184. Okuhira K, et al.Development of hybrid small molecules that induce degradation of estrogen receptor-alpha and necrotic cell death in breast cancer cells. Cancer Sci. 104, 1492–1498 (2013). [PubMed: 23992566]
185. Ohoka N, et al.Cancer cell death induced by novel small molecules degrading the TACC3 protein via the ubiquitin-proteasome pathway. Cell Death Dis. 5, e1513 (2014). [PubMed: 25375378]
186. Okuhira K, et al.Molecular design, synthesis, and evaluation of SNIPER(ER) That induces proteasomal degradation of ERalpha. Methods Mol. Biol1366, 549–560 (2016). [PubMed: 26585163]
187. Shibata N, et al.Development of protein degradation inducers of oncogenic BCR-ABL protein by conjugation of ABL kinase inhibitors and IAP ligands. Cancer Sci. 108, 1657–1666 (2017). [PubMed: 28556300]
188. Shibata N, et al.Development of protein degradation inducers of androgen receptor by conjugation of androgen receptor ligands and inhibitor of apoptosis protein ligands. J. Med. Chem61, 543–575 (2018). [PubMed: 28594553]
189. Ohoka N, et al.Derivatization of inhibitor of apoptosis protein (IAP) ligands yields improved inducers of estrogen receptor alpha degradation. J. Biol. Chem293, 6776–6790 (2018). [PubMed: 29545311]
190. Zandvliet AS, Schellens JH, Copalu W, Beijnen JH, & Huitema AD, Covariate-based dose individualization of the cytotoxic drug indisulam to reduce the risk of severe myelosuppression. J. Pharmacokinet. Pharmacodyn 36, 39–62 (2009). [PubMed: 19199010]
191. Kirkwood JM, et al.A phase 2 study of tasisulam sodium (LY573636 sodium) as second-line treatment for patients with unresectable or metastatic melanoma. Cancer117, 4732–4739 (2011). [PubMed: 21456002]
192. Ting TC, et al.Aryl sulfonamides degrade RBM39 and RBM23 by recruitment to CRL4-DCAF15. Cell Rep. 29, 1499–1510 e6 (2019). [PubMed: 31693891]
193. Spradlin JN, et al.Harnessing the anti-cancer natural product nimbolide for targeted protein degradation. Nat. Chem. Biol15, 747–755 (2019). [PubMed: 31209351] This article reports the discovery of the first RNF114 covalent ligand and RNF114-recruiting PROTAC degrader.
194. Zhang C, et al.Proteolysis targeting chimeras (PROTACs) of anaplastic lymphoma kinase (ALK). Eur. J. Med. Chem151, 304–314 (2018). [PubMed: 29627725]
195. Kang CH, et al.Induced protein degradation of anaplastic lymphoma kinase (ALK) by proteolysis targeting chimera (PROTAC). Biochem. Biophys. Res. Commun505, 542–547 (2018). [PubMed: 30274779]
196. Zhao Q, et al.Discovery of SIAIS178 as an effective BCR-ABL degrader by recruiting von Hippel-Lindau (VHL) E3 ubiquitin ligase. J. Med. Chem62, 9281–9298 (2019). [PubMed: 31539241]
197. Bai L, et al.Targeted degradation of BET proteins in triple-negative breast cancer. Cancer Res. 77, 2476–2487 (2017). [PubMed: 28209615]
198. Sun Y, et al.Degradation of Bruton's tyrosine kinase mutants by PROTACs for potential treatment of ibrutinib-resistant non-Hodgkin lymphomas. Leukemia33, 2105–2110 (2019). [PubMed: 30858551]

199. Dobrovolsky D, et al.Bruton tyrosine kinase degradation as a therapeutic strategy for cancer. Blood133, 952–961 (2019). [PubMed: 30545835]
200. Chi JJ, et al.A novel strategy to block mitotic progression for targeted therapy. EBioMedicine49, 40–54 (2019). [PubMed: 31669221]
201. Wu X, et al.Distinct CDK6 complexes determine tumor cell response to CDK4/6 inhibitors and degraders.Nat. Cancer138, S5 (2021).
202. Cheng M, et al.Discovery of potent and selective epidermal growth factor receptor (EGFR) bifunctional small-molecule degraders. J. Med. Chem63, 1216–1232 (2020). [PubMed: 31895569]
203. Burslem GM, Song J, Chen X, Hines J, & Crews CM, Enhancing antiproliferative activity and selectivity of a FLT-3 inhibitor by proteolysis targeting chimera conversion. J. Am. Chem. Soc 140, 16428–16432 (2018). [PubMed: 30427680]
204. Li Y, et al.Discovery of MD-224 as a first-in-class, highly potent, and efficacious proteolysis targeting chimera murine double minute 2 degrader capable of achieving complete and durable tumor regression. J. Med. Chem62, 448–466 (2019). [PubMed: 30525597]
205. Wei J, et al.Discovery of a first-in-class mitogen-activated protein kinase kinase 1/2 degrader. J. Med. Chem62, 10897–10911 (2019). [PubMed: 31730343]
206. Hu J, et al.Potent and selective mitogen-activated protein kinase kinase 1/2 (MEK1/2) heterobifunctional small-molecule degraders. J. Med. Chem63, 15883–15905 (2020). [PubMed: 33284613]
207. Shen Y, et al.Discovery of first-in-class protein arginine methyltransferase 5 (PRMT5) degraders. J. Med. Chem63, 9977–9989 (2020). [PubMed: 32787082]
208. Song Y, et al.Development and preclinical validation of a novel covalent ubiquitin receptor Rpn13 degrader in multiple myeloma. Leukemia33, 2685–2694 (2019). [PubMed: 30962579]
209. Chen L, et al.Discovery of first-in-class potent and selective tropomyosin receptor kinase degraders. J. Med. Chem63, 14562–14575 (2020). [PubMed: 33058680]
210. Scheffner M, Nuber U, & Huibregtse JM, Protein ubiquitination involving an E1-E2-E3 enzyme ubiquitin thioester cascade. Nature 373, 81–83 (1995). [PubMed: 7800044]
211. Ozkan E, Yu H, & Deisenhofer J, Mechanistic insight into the allosteric activation of a ubiquitin-conjugating enzyme by RING-type ubiquitin ligases. Proc. Natl Acad. Sci. USA 102, 18890–18895 (2005). [PubMed: 16365295]
212. Plechanovova A, Jaffray EG, Tatham MH, Naismith JH, & Hay RT, Structure of a RING E3 ligase and ubiquitin-loaded E2 primed for catalysis. Nature 489, 115–120 (2012). [PubMed: 22842904]
213. Pruneda JN, et al.Structure of an E3:E2 ~ Ub complex reveals an allosteric mechanism shared among RING/U-box ligases. Mol. Cell47, 933–942 (2012). [PubMed: 22885007]
214. Dou H, Buetow L, Sibbet GJ, Cameron K, & Huang DT, BIRC7-E2 ubiquitin conjugate structure reveals the mechanism of ubiquitin transfer by a RING dimer. Nat. Struct. Mol. Biol 19, 876–883 (2012). [PubMed: 22902369]
215. Berndsen CE, & Wolberger C, New insights into ubiquitin E3 ligase mechanism. Nat. Struct. Mol. Biol 21, 301–307 (2014). [PubMed: 24699078]
216. Zheng N, et al.Structure of the Cul1-Rbx1-Skp1-F boxSkp2 SCF ubiquitin ligase complex. Nature416, 703–709 (2002). [PubMed: 11961546]
217. Zimmerman ES, Schulman BA, & Zheng N, Structural assembly of cullin-RING ubiquitin ligase complexes. Curr. Opin. Struct. Biol 20, 714–721 (2010). [PubMed: 20880695]
218. Skowyra D, Craig KL, Tyers M, Elledge SJ, & Harper JW, F-box proteins are receptors that recruit phosphorylated substrates to the SCF ubiquitin-ligase complex. Cell 91, 209–219 (1997). [PubMed: 9346238]
219. Feldman RM, Correll CC, Kaplan KB, & Deshaies RJ, A complex of Cdc4p, Skp1p, and Cdc53p/cullin catalyzes ubiquitination of the phosphorylated CDK inhibitor Sic1p. Cell 91, 221–230 (1997). [PubMed: 9346239]
220. Pause A, et al.The von Hippel-Lindau tumor-suppressor gene product forms a stable complex with human CUL-2, a member of the Cdc53 family of proteins. Proc. Natl Acad. Sci. USA94, 2156–2161 (1997). [PubMed: 9122164]

221. Lisztwan J, Imbert G, Wirbelauer C, Gstaiger M, & Krek W, The von Hippel-Lindau tumor suppressor protein is a component of an E3 ubiquitin-protein ligase activity. Genes Dev. 13, 1822–1833 (1999). [PubMed: 10421634]
222. Kamura T, et al.Muf1, a novel elongin BC-interacting leucine-rich repeat protein that can assemble with Cul5 and Rbx1 to reconstitute a ubiquitin ligase. J. Biol. Chem276, 29748–29753 (2001). [PubMed: 11384984]
223. Shiyanov P, Nag A, & Raychaudhuri P, Cullin 4A associates with the UV-damaged DNA-binding protein DDB. J. Biol. Chem 274, 35309–35312 (1999). [PubMed: 10585395]
224. Kamura T, et al.The elongin BC complex interacts with the conserved SOCS-box motif present in members of the SOCS, ras, WD-40 repeat, and ankyrin repeat families. Genes. Dev12, 3872–3881 (1998). [PubMed: 9869640]
225. Kamura T, et al.VHL-box and SOCS-box domains determine binding specificity for Cul2-Rbx1 and Cul5-Rbx2 modules of ubiquitin ligases. Genes Dev. 18, 3055–3065 (2004). [PubMed: 15601820]
226. Jin J, Arias EE, Chen J, Harper JW, & Walter JC, A family of diverse Cul4-Ddb1-interacting proteins includes Cdt2, which is required for S phase destruction of the replication factor Cdt1. Mol. Cell 23, 709–721 (2006). [PubMed: 16949367]
227. Angers S, et al.Molecular architecture and assembly of the DDB1-CUL4A ubiquitin ligase machinery. Nature443, 590–593 (2006). [PubMed: 16964240]
228. Higa LA, et al.CUL4-DDB1 ubiquitin ligase interacts with multiple WD40-repeat proteins and regulates histone methylation. Nat. Cell Biol8, 1277–1283 (2006). [PubMed: 17041588]
229. He YJ, McCall CM, Hu J, Zeng Y, & Xiong Y, DDB1 functions as a linker to recruit receptor WD40 proteins to CUL4-ROC1 ubiquitin ligases. Genes Dev. 20, 2949–2954 (2006). [PubMed: 17079684]
230. Pintard L, et al.The BTB protein MEL-26 is a substrate-specific adaptor of the CUL-3 ubiquitin-ligase. Nature425, 311–316 (2003). [PubMed: 13679921]
231. Xu L, et al.BTB proteins are substrate-specific adaptors in an SCF-like modular ubiquitin ligase containing CUL-3. Nature425, 316–321 (2003). [PubMed: 13679922]
232. Geyer R, Wee S, Anderson S, Yates J, & Wolf DA, BTB/POZ domain proteins are putative substrate adaptors for cullin 3 ubiquitin ligases. Mol. Cell 12, 783–790 (2003). [PubMed: 14527422]
233. Furukawa M, He YJ, Borchers C, & Xiong Y, Targeting of protein ubiquitination by BTB-cullin 3-Roc1 ubiquitin ligases. Nat. Cell Biol 5, 1001–1007 (2003). [PubMed: 14528312]
234. Wang Z, Liu P, Inuzuka H, & Wei W, Roles of F-box proteins in cancer. Nat. Rev. Cancer 14, 233–247 (2014). [PubMed: 24658274]
235. Linossi EM, & Nicholson SE, The SOCS box-adapting proteins for ubiquitination and proteasomal degradation. IUBMB Life 64, 316–323 (2012). [PubMed: 22362562]
236. Wang P, Song J, & Ye D CRL3s: the BTB-CUL3-RING E3 ubiquitin ligases in Cullin-RING Ligases and Protein Neddylation: Biology and Therapeutics (eds Sun Y, Wei W, & Jin J) 211–223 (Springer, 2020).
237. Braschi B, et al.RING finger proteins. HGNC Database, HUGO Gene Nomenclature Committee, European Molecular Biology Laboratory, European Bioinformatics Institute, Wellcome Genome Campus, Hinxton. https://www.genenames.org/data/genegroup/#!/group/58%0A (2020).
238. Sarikas A, Hartmann T, & Pan Z-Q, The cullin protein family. Genome Biol. 12, 220 (2011). [PubMed: 21554755]
239. Goldenberg SJ, et al.Structure of the Cand1-Cul1-Roc1 complex reveals regulatory mechanisms for the assembly of the multisubunit cullin-dependent ubiquitin ligases. Cell119, 517–528 (2004). [PubMed: 15537541]
240. Muniz JR, et al.Molecular architecture of the ankyrin SOCS box family of Cul5-dependent E3 ubiquitin ligases. J. Mol. Biol425, 3166–3177 (2013). [PubMed: 23806657]
241. Fischer ES, et al.Structure of the DDB1–CRBN E3 ubiquitin ligase in complex with thalidomide. Nature512, 49–53 (2014). [PubMed: 25043012] This article reports one of the first crystal structures of DDB1–CRBN–IMiD complexes.

242. Fischer ES, et al.The molecular basis of CRL4DDB2/CSA ubiquitin ligase architecture, targeting, and activation. Cell147, 1024–1039 (2011). [PubMed: 22118460]

243. Liu J, & Nussinov R, Rbx1 flexible linker facilitates cullin-RING ligase function before neddylation and after deneddylation. Biophys. J 99, 736–744 (2010). [PubMed: 20682250]

244. Scott DC, et al.Structure of a RING E3 trapped in action reveals ligation mechanism for the ubiquitin-like protein NEDD8. Cell157, 1671–1684 (2014). [PubMed: 24949976]

245. Baek K, et al.NEDD8 nucleates a multivalent cullin-RING-UBE2D ubiquitin ligation assembly. Nature578, 461–466 (2020). [PubMed: 32051583]

246. Grossman RL, et al.Toward a shared vision for cancer genomic data. N. Engl. J. Med375, 1109–1112 (2016). [PubMed: 27653561]

247. Oliner JD, Saiki AY, & Caenepeel S, The role of MDM2 amplification and overexpression in tumorigenesis. Cold Spring Harb. Perspect. Med 6, a026336 (2016). [PubMed: 27194168]

248. Pi L, et al.Evaluating dose-limiting toxicities of MDM2 inhibitors in patients with solid organ and hematologic malignancies: a systematic review of the literature. Leuk. Res86, 106222 (2019). [PubMed: 31522038]

249. Liu Y, & Mallampalli RK, Small molecule therapeutics targeting F-box proteins in cancer. Semin. Cancer Biol 36, 105–119 (2016). [PubMed: 26427329]

250. Simonetta KR, et al.Prospective discovery of small molecule enhancers of an E3 ligase-substrate interaction. Nat. Commun10, 1402 (2019). [PubMed: 30926793]

251. Zhuang M, et al.Structures of SPOP-substrate complexes: insights into molecular architectures of BTB-Cul3 ubiquitin ligases. Mol. Cell36, 39–50 (2009). [PubMed: 19818708]

252. Guo ZQ, et al.Small-molecule targeting of E3 ligase adaptor SPOP in kidney cancer. Cancer Cell30, 474–484 (2016). [PubMed: 27622336]

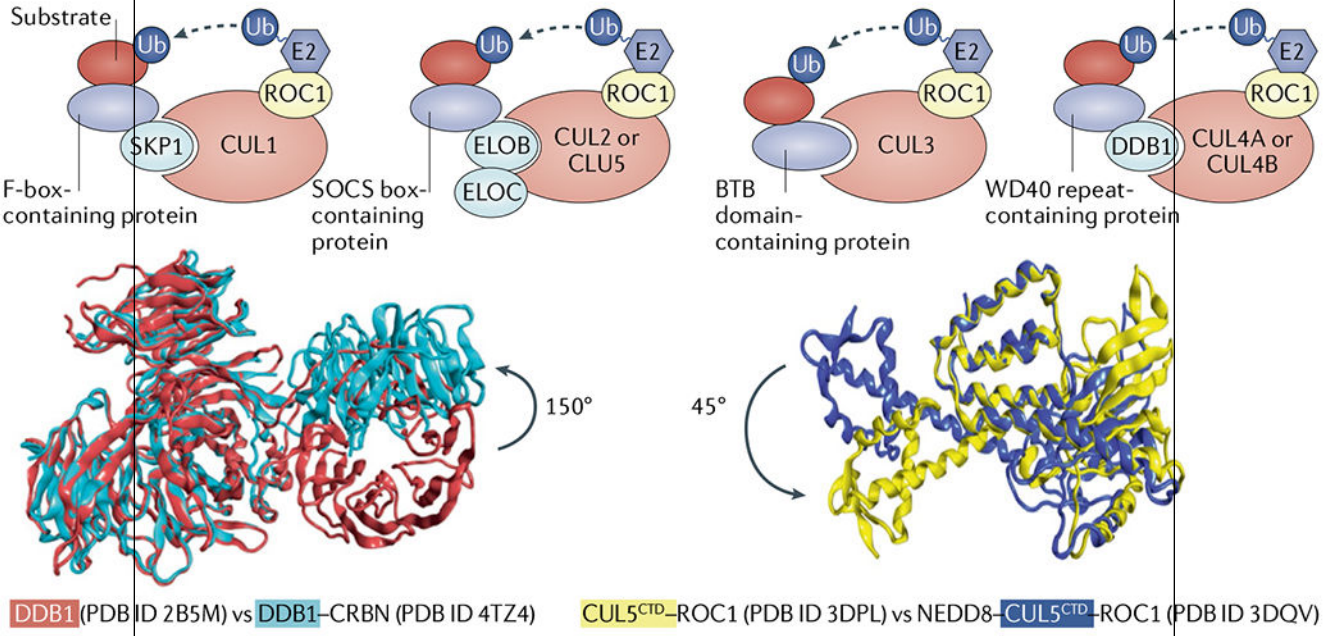
253. Wang D, et al.A deep proteome and transcriptome abundance atlas of 29 healthy human tissues. Mol. Syst. Biol15, 1–16 (2019).

254. Chamberlain PP, et al.Structure of the human cereblon–DDB1–lenalidomide complex reveals basis for responsiveness to thalidomide analogs. Nat. Struct. Mol. Biol21, 803–809 (2014). [PubMed: 25108355] This article reports one of the first crystal structures of DDB1–CRBN–IMiD complexes.

Box 1 |

Assembly and plasticity of cullin-RING E3 ligases

Cullins adopt an overall elongated crescent-shaped structure consisting of a stalk-like amino-terminal domain (NTD) that comprises three cullin repeats and a globular carboxy-terminal domain (CTD) that binds RING box protein 1 (ROC1) or ROC2 (REFS[216,217]). The NTD in different cullins binds a different adaptor protein: CUL1 binds with SKP1 (REFS[218,219]), CUL2 (REFS[220,221]) and CUL5 (REF.[222]) bind with the elongin B–elongin C complex, and CUL4A and CUL4B bind with DDB1 (REFS[217,223]). Each adaptor protein interacts with a different protein motif: SKP1 interacts with F-box[218,219], elongin B–elongin C interacts with SOCS box[220,224,225] and DDB1 interacts with WD40 repeat[226–229] (see the figure). Distinct from other cullins, CUL3 integrates the functions of an adaptor and a substrate receptor into one protein by using its NTD to bind a BTB domain[230–233]. Human cells express an estimated 69 proteins containing an F-box[234], 32 containing a SOCS box[235], 188 containing a BTB domain[236] and 18 containing a DDB1- and CUL4-associated factor (DCAF) (HUGO Gene Nomenclature Committee (HGNC) database)[237]. These proteins also contain additional domains for interacting with their substrate proteins and function as the substrate receptors, suggesting the potential assembly of more than 200 distinct cullin–RING E3 ligase (CRL) complexes[238]. The intrinsic flexibility of the cullin scaffold, adaptor, substrate receptor and ROC1 collectively contributes to the high degree of plasticity of CRL complexes. In CUL1, cullin repeat 3 can be twisted by 18° with respect to repeats 1 and 2 (REFS[216,239]). A similarly flexible rotation within the CTD was also observed for CUL5 (REF,[240]). The adaptor proteins can be flexible as seen in DDB1, which contains three seven-bladed β-propellers, BPA, BPB and BPC. BPB binds CUL4 and is loosely attached to BPC, allowing it to rotate up to 150° before and after binding to its substrate receptor cereblon (CRBN) or SV5-V (see the figure)[227,241,242,254]. ROC1 contains a flexible linker between the β-strand that binds the cullin CTD and the RING finger domain[216]. The flexible linker allows the RING domain to reorient with respect to the CTD and form multiple potential catalytic geometries with E2 enzyme[243–245].

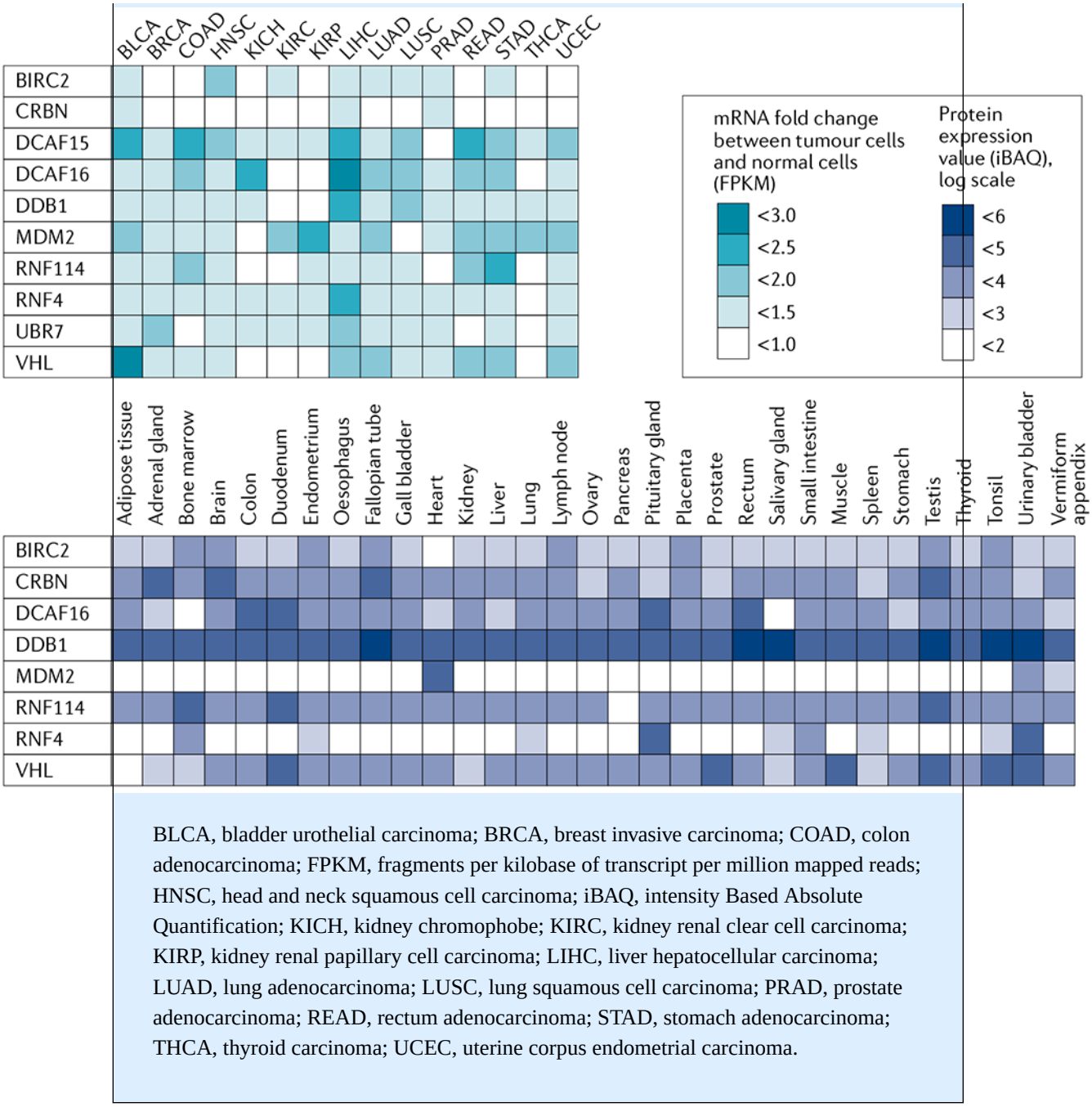


PDB, Protein Data Bank; ub, ubiquitin.

Box 2 |

Selective expression of E3 ligase genes for targeted protein degradation

Of ten E3 ligases that have been exploited for TPD, some localize predominantly in one specific subcellular compartment, such as MDM2 and UBR7 in the nucleus, and DDB1- and CUL4-associated factor 15 (DCAF15) in mitochondria, while others, such as cereblon (CRBN), von Hippel–Lindau tumour suppressor (VHL) and BIRC2, localize across the cell (UniProt Knowledgebase). DCAF16 is a nuclear protein and forms the substrate receptor subunit of cullin 4-RING ligase (CRL4). A proteolysis-targeting chimera (PROTAC) based on a DCAF16 covalent ligand was found to promote nuclear-restricted degradation of the protein of interest[131], illustrating the potential for subcellular-specific degradation of a protein of interest in the context of selective localization of an E3 ligase. The expression of the ten E3 ligases currently used in the PROTAC field differs significantly in different tumours, based on data from the National Cancer Institute's Genomic Data Commons[246] (see the figure, top). One interesting possibility is to exploit oncogenic E3 ligases that are expressed at a very low level in normal tissues or only in a few normal cells but are amplified or overexpressed in tumours. For example, the *MDM2* gene is frequently amplified in numerous cancers, resulting in p53 inactivation and contributing to tumorigenesis[247]. Currently available MDM2 antagonists are associated with high toxicity[248]; if the toxicity issue can be resolved, MDM2-recruiting PROTACs could be particularly efficacious in tumours with elevated MDM2 expression such as sarcoma. F-box proteins SKP2 and β-TRCP[29] are frequently overexpressed in many cancer cells, and small molecules binding to both proteins have been reported[249,250]. SPOP, a BTB domain-containing protein and substrate receptor of CRL3 that is overexpressed in clear cell renal carcinoma[30], contains a well-defined substrate-binding groove[251], and small molecules binding to this groove have also been identified[252]. The expression of the E3 ligases used in PROTACs also varies significantly in different tissues and organs, according to data from the Expression Atlas Database[253] (see the figure, bottom).



BLCA, bladder urothelial carcinoma; BRCA, breast invasive carcinoma; COAD, colon adenocarcinoma; FPKM, fragments per kilobase of transcript per million mapped reads; HNSC, head and neck squamous cell carcinoma; iBAQ, intensity Based Absolute Quantification; KICH, kidney chromophobe; KIRC, kidney renal clear cell carcinoma; KIRP, kidney renal papillary cell carcinoma; LIHC, liver hepatocellular carcinoma; LUAD, lung adenocarcinoma; LUSC, lung squamous cell carcinoma; PRAD, prostate adenocarcinoma; READ, rectum adenocarcinoma; STAD, stomach adenocarcinoma; THCA, thyroid carcinoma; UCEC, uterine corpus endometrial carcinoma.

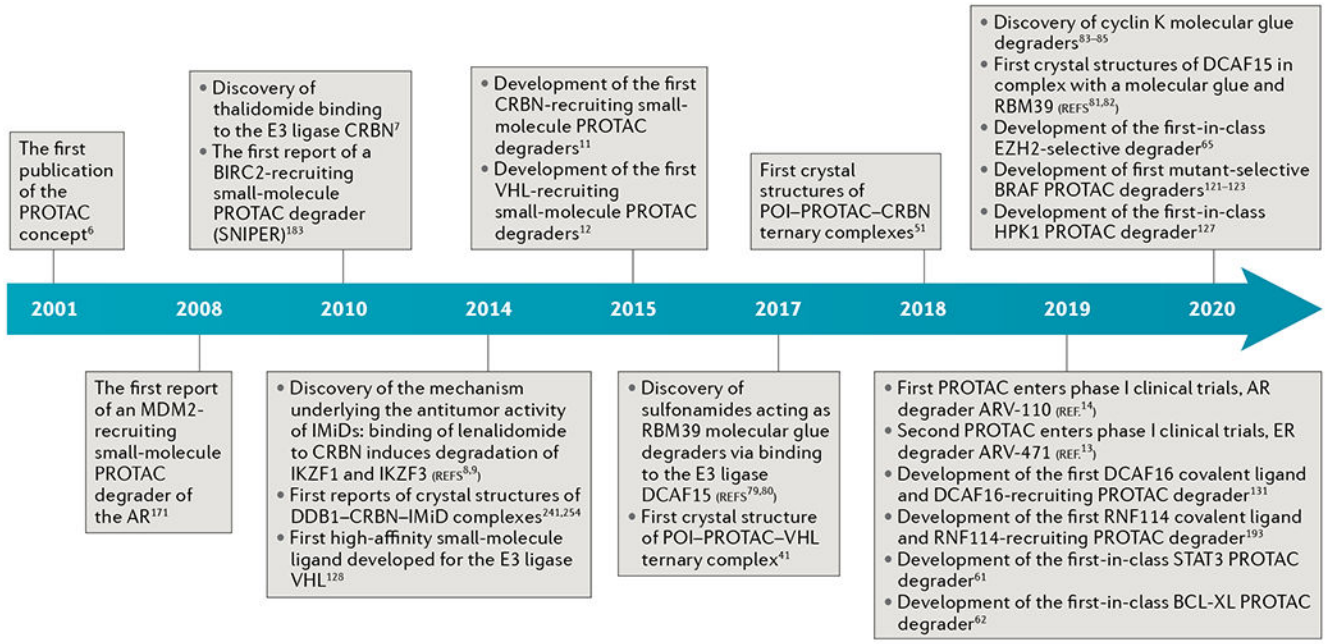


Fig. 1 |. Key discoveries and developments in the targeted protein degradation field.

The figure shows a timeline beginning from the first publication of the proteolysis-targeting chimera (PROTAC) concept up to the most recent discoveries. AR, androgen receptor; CRBN, cereblon; DCAF15, DDB1- and CUL4-associated factor 15; ER, oestrogen receptor; EZH2, enhancer of Zeste homologue 2; IMiD, immunomodulatory imide drug; POI, protein of interest; STAT3, signal transducer and activator of transcription 3; VHL, von Elippel–Lindau tumour suppressor.

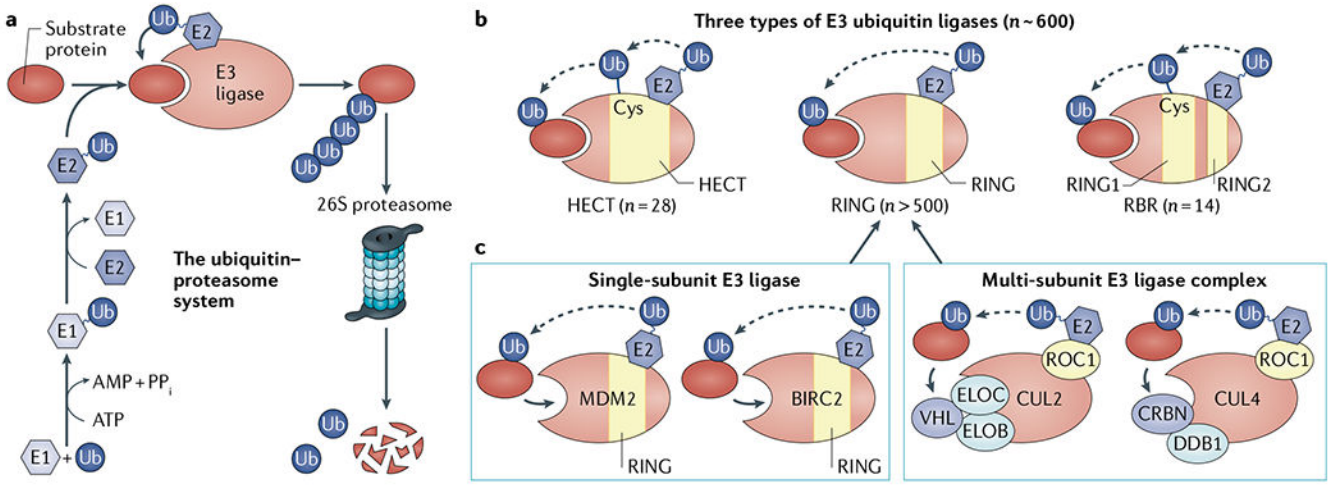


Fig. 2 |. The ubiquitin–proteasome system and classification of E3 ubiquitin ligases.

a | Ubiquitination begins with the ATP-dependent activation of ubiquitin (Ub) by the E1 ubiquitin-activating enzyme (UBA), resulting in Ub~E1 thioester bond formation (the tilde denotes a high-energy thioester bond), followed by the transfer of ubiquitin to an E2 ubiquitin-conjugating enzyme. Finally, an E3 ubiquitin ligase brings the substrate and the E2~Ub conjugate together and promotes ubiquitin ligation to a lysine residue in the substrate protein. Ubiquitin can be ligated either as a single moiety to one lysine residue or multiple lysine residues or as a polyubiquitin chain where successive ubiquitin molecules are connected. The consequences of ubiquitination for the substrate protein depend not only on whether it is monoubiquitinated or polyubiquitinated but also on the topology of the polyubiquitin chain[21]. One of the well-known consequences of polyubiquitination is the proteolytic degradation of substrate proteins by the 26S proteasome[22]. **b** | The three types of E3 ubiquitin ligases. Traditionally, E3 ubiquitin ligases are separated into homologous to E6AP carboxy terminus (HECT) and RING types. Both types interact with E2~Ub and recognize substrate proteins, but differ in their catalytic mechanisms. While the classical HECT E3 ligases form a thioester intermediate with ubiquitin on an active-site cysteine before transferring it onto its substrates[210], RING finger proteins do not form an intermediate with ubiquitin[211]. Instead, they promote the direct transfer of ubiquitin from E2 to the substrate, in part by immobilizing the carboxy-terminal glycine of the ubiquitin in an otherwise highly flexible E2~Ub, for attack by the acceptor lysine[212–215]. More recently, a third class of E3 ligases was identified that contains a tripartite RING1-in-between-RING2 (RBR) domain arrangement and catalyses ubiquitination through a RING–HECT hybrid catalytic mechanism. RING1 in the RBR domain forms a typical cross-brace RING structure to interact with E2~Ub. The ubiquitin is then transferred to, and forms a thioester intermediate with, an active-site cysteine in RING2, followed by the transfer of ubiquitin to a substrate. **c** | Classification of RING-type E3 ligases. RING type E3 ligases can be either a single subunit, which contain an intrinsic RING finger domain, or a multi-subunit complex assembled on the cullin scaffold that does not contain a RING finger domain but instead binds in *trans* a small RING finger protein, either RING box protein 1 (ROC1) or ROC2. See BOX 1 for details of the cullin–RING E3 ubiquitin ligase assembly. CRBN, cereblon; VHL, von Hippel–Lindau tumour suppressor.

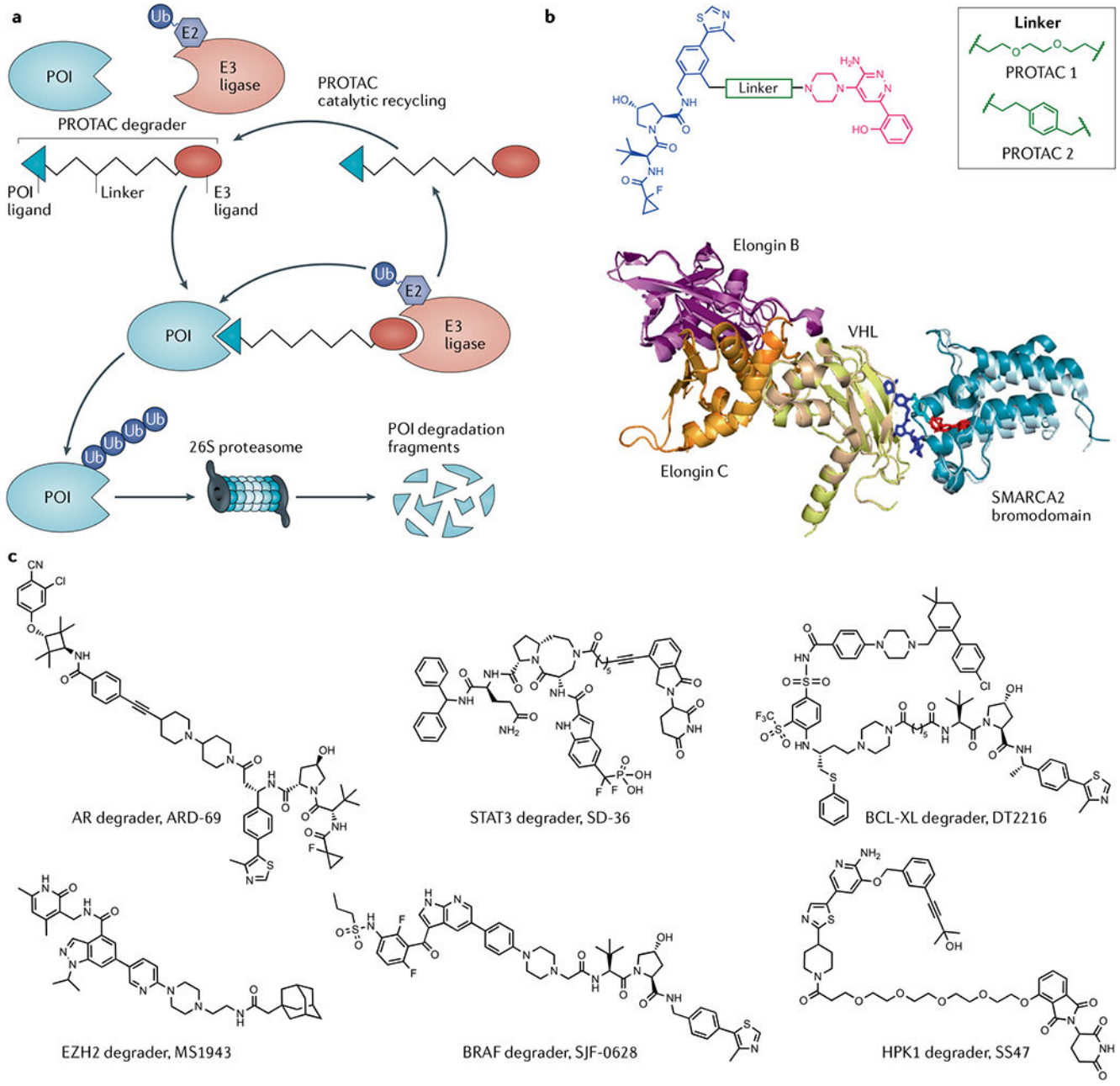


Fig. 3 |. PROTAC degraders.

a | General schematic of proteolysis-targeting chimera (PROTAC) degraders. A protein of interest (POI) can be degraded by the ubiquitin-proteasome system mediated by a PROTAC, which consists of a POI ligand connected to an E3 ligand via a linker. PROTACs are catalytic. The tilde denotes a high-energy thioester bond. **b** | PROTACs induce new protein–protein interactions between the POI and the E3 ligase. The image shows an overlay of two SMARCA2 bromodomain–PROTAC–von Hippel–Lindau tumour suppressor (VHL)–elongin C–elongin B complex crystal structures (RCSB Protein Data Bank IDs 6HAX[48] and 6HAY[49]) in a ribbon representation. PROTAC 1 and PROTAC 2 share the same VHL

ligand and POI ligand but have different linkers, which are specified in the box. The colours within the chemical structure (PROTAC 1 and PROTAC 2) correspond to the colours of the compounds in the crystal (ribbon) structures, with the VHL ligand in blue, the linker in green and the SMARCA2 bromodomain ligand in magenta. **c** | Chemical structures of several key heterobifunctional degraders highlighted in this Review. ARD-69, SJF-0628 and DT2216 are VHL-based PROTAC degraders, SS47 and SD-36 are cereblon (CRBN)-based PROTAC degraders and MS1943 is a hydrophobic tag-based degrader. AR, androgen receptor; EZH2, enhancer of Zeste homologue 2; STAT3, signal transducer and activator of transcription 3; Ub, ubiquitin.

Table 1 |

E3 ligands used for targeted protein degradation

E3 ligand	Binding E3 subunit	E3 ligase complex[a]	Neosubstrates of the E3 ligand	Toxicity of E3 ligand	Mechanism of action	Functional essentiality of the E3 subunit		Refs
						In cells[b]	In mice	
Nutlin 3, idasanutlin	MDM2	MDM2	Not reported	High	PROTAC	5.2%	Embryonic lethal	171–173
VHL ligands	VHL	$CRL2^{VHL}$	Not reported	NA	PROTAC	0.5%	Embryonic lethal	12,33,89,128,174–176
IMiDs	CRBN	$CRL4^{CRBN}$	ARID2, CK1α, GSPT1, IKZF1, IKZF3, p63, ZBTB16, SALL4, ZFP91, ZNF276, ZNF653, ZNF692, ZNF827	High	Molecular glue and PROTAC	0%	Viable	7,9,75,77,78,177–181
Bestatin, LCL161, MV-1	BIRC2	BIRC2	Not reported	Low	PROTAC	2.3%	Viable	182–189
Sulfonamides	DCAF15	$CRL4^{DCAF1}$	RBM39, RBM23	Medium	Molecular glue and PROTAC	NA	NA	79–82,129,190–192
CCW16	RNF4	RNF4	Not reported	NA	PROTAC	2.3%	Embryonic lethal	132
KB02	DCAF16	$CRL4^{DCAF1}$	Not reported	NA	PROTAC	NA	NA	131
Nimbolide	RNF114	RNF14	Not reported	None	PROTAC	NA	Viable	193
CR8, HQ461, dCeMM2, dCeMM3, dCeMM4	DDB1	$CRL4^{CDK12}$	Cyclin K	NA	Molecular glue	67.2%	Embryonic lethal	83–85
Manumycin polyketides	UBR7	UBR7	p53	NA	Molecular glue	0%	NA	86

CRBN, cereblon; CRL, cullin–RING E3 ligase; DCAF, DDB1- and CUL4-associated factor; IMiD, immunomodulatory imide drug; NA, not available; PROTAC, proteolysis-targeting chimera; VHL, von Hippel–Lindau tumour suppressor.

[a] The superscript denotes the substrate receptor within the complex.

[b] Calculated with use of the DRIVE (deep RNAi interrogation of viability effects in cancer) data portal[3]. A gene with a redundant small inferring RNA activity value of −3 or less is considered to be essential in that cell line. The total number of cell lines analysed is between 384 and 387, and the percentage represents the proportion of cell lines in which the gene is essential.

Table 2 |

Cancer-related heterobifunctional degraders with in vivo efficacy or pharmacokinetics (PK) data

Compound	Disease	Target (POI)	MOA	Key bioassay data	Refs
MS4078	NSCLC, DLBCL	ALK	CRBN	In vivo PK and cellular efficacy	194
TD-004	NSCLC, DLBCL	ALK	VHL	In vivo efficacy	195
ARV-110	CRPC	AR	CRBN	Phase I/II clinical trial	14,93,94
ARD-69	CRPC	AR	VHL	In vivo efficacy	89
ARD-61	CRPC	AR	VHL	In vivo efficacy	90,91
DT2216	T-ALL, TCL	BCL-XL	VHL	In vivo efficacy	62
SIAIS178	CML	BCR–ABL	VHL	In vivo efficacy	196
BETd-246	TNBC	BET family	CRBN	In vivo efficacy	197
P4B	Melanoma	BRAF-V600E	CRBN	In vivo PD	122
SJF-0628	Melanoma	BRAF-V600E	VHL	In vivo efficacy	123
DP1	DLBCL	BRD4	DCAF15	In vivo efficacy	129
RC-1	AML	BTK	CRBN	In vivo efficacy	144
L18I	DLBCL	BTK	CRBN, VHL	In vivo efficacy	198
DD-03-171	MCL	BTK	CRBN	In vivo efficacy	199
CP5V	BC	CDC20	VHL	In vivo efficacy	200
MS140	MCL, CRC	CDK4, CDK6	CRBN	In vivo efficacy	201
MS39	NSCLC	EGFR	VHL	In vivo PK and cellular efficacy	202
Fulvestrant	BC	ER	Hydrophobic tag	FDA approved	66
ARV-471	BC	ER	CRBN	Phase I/II clinical trial	13,97,98
MS1943	TNBC	EZH2	Hydrophobic tag	In vivo efficacy	65
PROTAC FLT3	AML	FLT3	VHL	In vivo efficacy	203
SS47	MM	HPK1	CRBN	In vivo efficacy	127
MD-224	ALL	MDM2	CRBN	In vivo efficacy	204
MS432	MM, CRC	MEK1, MEK2	VHL	In vivo PK and cellular efficacy	205
MS934	MM, CRC	MEK1, MEK2	VHL	In vivo PK and cellular efficacy	206

Compound	Disease	Target (POI)	MOA	Key bioassay data	Refs
MS4322	ER^+ BC, NSCLC	PRMT5	VHL	In vivo PK and cellular efficacy	207
WL-40	MM	Rpn13 homologue	CRBN	In vivo efficacy	208
SD-36	AML, ALL	STAT3	CRBN	In vivo efficacy	61
CG428	CC	TRK	CRBN	In vivo PK and cellular efficacy	209

Entries are given in alphabetical order of the target. A comprehensive list of all cancer-related heterobifunctional degraders can be found in Supplementary Table 1. ALL, acute lymphoblastic leukaemia; AML, acute myeloid leukaemia; AR, androgen receptor; BC, breast cancer; BET, bromodomain and extra terminal; CC, colon cancer; CML, chronic myeloid leukaemia; CRBN, cereblon; CRC, colorectal cancer; CRPC, castration-resistant prostrate cancer; DCAF15, DDB1- and CUL4-associated factor 15; DLBCL, diffuse large B cell lymphoma; ER, oestrogen receptor; EZH2, enhancer of Zeste homologue 2; MCL, mantle cell lymphoma; MM, multiple myeloma; MOA, mechanism of action; NSCLC, non-small-cell lung cancer; PD, pharmacodynamics; POI, protein of interest; PROTAC, proteolysis-targeting chimera; Rpn13 homologue, 26S proteasome ubiguitin receptor (also known as ADRM1); STAT3, signal transducer and activator of transcription 3; T-ALL, T cell acute lymphoblastic leukaemia; TCL, T cell lymphoma; TNBC, triple-negative beast cancer; TRK, tropomyosin receptor family kinase; VHL, von Hippel–Lindau tumour suppressor.