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PERSPECTIVE

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Development of targeted protein degradation therapeutics

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Targeted protein degradation as a therapeutic modality has seen dramatic progress and massive investment in recent years because of the convergence of two key scientific breakthroughs: optimization of first-generation peptidic proteolysis-targeted chimeras (PROTACs) into more drug-like molecules able to support in vivo proof of concept and the discovery that clinical molecules function as degraders by binding and repurposing the proteins cereblon and DCAF15. This provided clinical validation for the general approach through the cereblon modulator class of drugs and provided highly drug-like and ligand-efficient E3 ligase binders upon which to tether target-binding moieties. Increasingly rational and systematic approaches including biophysical and structural studies on ternary complexes are being leveraged as the field advances. In this Perspective we summarize the discoveries that have laid the foundation for future degradation therapeutics, focusing on those classes of small molecules that redirect E3 ubiquitin ligases to non-native substrates.

Targeted protein degradation is an emerging therapeutic modality whereby small-molecule ligands are used to redirect the cellular protein homeostasis machinery. As part of cellular processes, proteins are specifically recruited to E3 ubiquitin ligases and tagged for destruction with chains of ubiquitin[1,2]. These ubiquitin chains are used to target proteins to the proteasome, where they are unfolded and digested. By using small molecules to redirect this process (Fig. 1), proteins linked to disease can be specifically targeted for destruction. As such, targeted protein degradation offers tremendous promise for future drug discovery for the reasons discussed below.

Compared to inhibition strategies, degradation offers numerous advantages, including the chance for removal of the target protein and consequent ablation of all associated functions. The unique properties of degraders present opportunities for differentiated therapeutics, as well as the chance to tackle pathologies driven by proteins that were previously considered inaccessible to small-molecule intervention—proteins described as 'undruggable'. Despite the conceptual advantages, the degradation approach brings an elevated level of technical challenge and complexity and in some implementations confronts historical wisdom regarding molecular properties. In this Perspective we will describe our view of the current state of understanding associated with targeted protein degraders that bind and repurpose E3 ubiquitin ligases.

Convergent discoveries in targeted protein degradation

Recent years have seen a global surge in interest and investment in the degradation approach, from both academic and industrial efforts. This surge in interest came at the convergence of key breakthroughs that rendered the approach both more tractable and credible. The first peer-reviewed description of heterobifunctional ligands able to drive protein degradation appeared in 2001, with the coining of the term PROTACs[3]. From the initial proof-of-principle experiments, researchers were able to use the peptidic degron (discussed below) from hypoxia-inducible factor 1α (HIF1α) to target the androgen receptor or estrogen receptor (ER) proteins to the Von Hippel Lindau factor (VHL), a component of the Cul2-Rbx1-EloB/C-VHL E3 ligase (Fig. 1d)[4–6]. These early experiments were valuable for in vitro validation and for generating chemical biology tools; however, these peptidic molecules were limited in their in vivo utility.

The development of non-peptidic heterobifunctional ligands was therefore critical to the advancement of the field. This proved possible using the small molecule nutlin, fused via a polyethylene glycol (PEG) linker to an androgen-receptor-binding moiety to recruit the androgen receptor to the MDM2 E3 ligase (Fig. 1e)[7]. Small-molecule binders to the ligase cIAP1 (inhibitor of apoptosis) have also proven to be useful as ligase-binding moieties. IAP-binding heterobifunctional molecules have been used to drive the degradation of multiple proteins including nuclear receptors (Fig. 1f)[8,9]. It was subsequently shown that it is possible to optimize the VHL-binding moiety to a more drug-like scaffold capable of achieving in vivo protein knockdown (Fig. 1d)[10]. In vivo knockdown was also shown to be achievable with IAP-targeted heterobifunctional ligands, with the authors referring to their degrader molecules as SNIPERs (specific and non-genetic IAP-dependent protein erasers)[11].

Despite the substantial progress made in the chemistry of heterobifunctional degraders, there remained a considerable degree of skepticism as to whether these advances would be translatable to the clinic. These concerns arose substantially because of the challenges in producing molecules with desirable physicochemical properties that also have sufficient architecture to comprise two distinct binding moieties in the same molecule, as discussed in detail below. Certainly, the heterobifunctional ligands reported thus far deviate substantially from the conventional 'rule-of-five' guidelines for drug-like molecular properties. Incredibly, clinical validation for targeted protein degradation became available from an entirely parallel avenue of discovery. Historically, many drugs have gained regulatory approval despite limited knowledge of the molecular mechanism of action and a lack of knowledge of direct molecular targets. In the last ten years, two such clinical classes have been discovered to operate via targeted protein degradation by binding to different components of CRL4 E3 ubiquitin ligases.

Thalidomide was marketed in the late 1950s as a treatment for morning sickness in pregnant women, but it was withdrawn from sale following the realization that it was a potent teratogen[12,13]. Years

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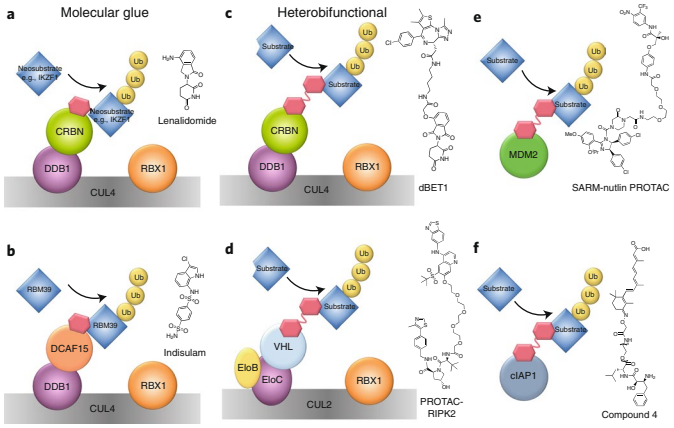

Fig. 1 | Examples of E3 ubiquitin ligases that have been repurposed with small molecules. a,**b**, Examples of 'molecular glue' represented by repurposing of $CRL4^{CRBN}$ by lenalidomide[20,21] (**a**) and $CRL4^{DCAF15}$ by indisulam[34,35] (**b**). **c**–**f**, examples of heterobifunctional ligands with representative ligases and ligands including $CRL4^{CRBN}$ and dBET1 (ref. [36]) (**c**), $CRL2^{VHL}$ and PROTAC-RIPK2 (ref. [10]) (**d**), MDM2 and SARM-nutlin[7] (**e**), and cIAP1 by 'Compound 4'[8] (**f**). Small molecules are shown as pink hexagons with a representative chemical structure shown to the right of each figure. Ubiquitin (Ub) is shown as yellow circles. E3 ligase components are shown as colored circles or ovals or grey boxes. Substrate proteins are shown as blue diamonds.

later, thalidomide was found to be efficacious in the treatment of several indications including erythema nodosum leprosum, a complication of leprosy, and multiple myeloma[14]. These discoveries enabled a return to clinical use, with a strict risk management strategy in place to prevent exposure during pregnancy. Thalidomide analogs with increased potency, including lenalidomide, pomalidomide, avadomide, and iberdomide, were subsequently discovered and developed at Celgene (Table 1)[15–17]. Preclinical and clinical development was performed without knowledge of the molecular mechanism until 2010, when the proteins cereblon and DNA-damage-binding protein 1 (DDB1) were co-purified using thalidomide-derived affinity beads and cereblon was shown to be the direct molecular target for thalidomide[18]. DDB1 is an adaptor protein within the CRL4 E3 ubiquitin ligase, and cereblon had been previously identified as a DDB1 and CUL4 associated factor (DCAF) (Fig. 1a)[19]. Subsequent work demonstrated that rather than acting as inhibitors, the thalidomide analogs bind to the surface of cereblon to create a hotspot for protein–protein interactions[20,21], imparting 'neomorphic' activities such as recruiting the transcription factors Ikaros and Aiolos for ubiquitination and degradation[22–24].

Mechanistic work on the cereblon E3 ligase modulating drugs (CELMoDs) such as thalidomide confirmed that they do not operate as heterobifunctional degraders. Indeed, it would be an incredible feat for molecules with the molecular weight of thalidomide, lenalidomide and pomalidomide (all <300 Da) to simultaneously engage the binding pockets on two separate proteins. Instead, these molecules are more analogous in mechanism to several natural products in their ability to scaffold protein–protein interactions[25]. Precedent already existed among the plant E3 ubiquitin ligases for ligase–substrate scaffolding by low-molecular-weight molecules, with the archetypal examples found in the plant signaling hormones auxin and jasmonate. These hormones were shown to scaffold protein–protein interactions between the cognate ligase–substrate pairs, prompting the authors to coin the term 'molecular glue' to describe this mode of action[26,27]. 'Molecular glue' remains a relevant term to capture the distinctions between the heterobifunctional degraders and the low-molecular-weight protein–protein inducers, which are applicable to different protein targets, are subject to different optimization strategies, and exhibit differences in pharmacology as described below.

Structural work subsequently showed that 'neosubstrates'—non-native substrates recruited by exogenous ligands—such as Ikaros, GSPT1, and CK1α, are all recruited via a common structural feature. The common feature by which substrates are recruited to an E3 ligase, known as a 'degron', is—in the case of cereblon neosubstrates—composed of a β-hairpin containing a glycine residue. This degron docks directly with the cereblon surface adjacent to the bound CELMoD (Fig. 2a–c)[28–30]. Ck1α, GSPT1, and Ikaros/Aiolos are unrelated in structure and function and share no obvious sequence homology within the cereblon interaction domains beyond the critical glycine residue. This lack of sequence specificity resulted in the interaction region being termed a 'structural degron'[28], as most studied degrons take the form of peptidic sequences. As a demonstration of the potential versatility of CELMoDs, GSPT1 recruitment is not mediated by lenalidomide or pomalidomide. Similarly, CK1α is a substrate for lenalidomide, but not for pomalidomide or thalidomide[15]. These findings are of great significance for the field of drug discovery, as they provide a mechanism to target proteins for destruction that are entirely deficient in ligand-binding sites. Work to catalog the occurrence of additional CELMoD neosubstrates continues[30–33], providing a new frontier for drug discovery.

Following the remarkable findings for the thalidomide analogs, it was also shown that indisulam, a small molecule under clinical investigation in myeloma, also functions to drive targeted protein degradation. In this case indisulam caused degradation of RBM39 (RNA Binding Motif Protein 39) by binding to the CRL4 component DCAF15 and redirecting the $CRL4^{DCAF15}$ E3 ligase[34,35]. Comparable to the CELMoDs, indisulam is a low-molecular-weight compound of the 'molecular glue' class without the structural architecture or pharmacology of a heterobifunctional molecule (Fig. 1c).

The mechanistic findings for CELMoDs helped advance the field of targeted protein degradation in several ways: they provided clinical validation for targeted protein degradation by demonstrating that efficacious drugs already operated through this mechanism; they proved that low-molecular-weight therapeutics with excellent drug-like properties could efficiently trigger assembly of the ligase-substrate-ligand ternary complex; they showed that it could be possible to degrade proteins from families assumed to be undruggable or even unligandable due to a lack of appropriate binding sites; and finally they provided highly efficient chemical scaffolds that could be used in a new generation of heterobifunctional ligands. Indeed, it was rapidly demonstrated that, with the appropriate linker, it is possible to link target-binding moieties to the thalidomide moiety to recruit the $CRL4^{CRBN}$ E3 ligase, resulting in robust protein degradation of BRD4 and FKBP12 (Fig. 1b)[36,37]. Subsequent efforts have demonstrated that this approach is broadly applicable with numerous proteins now reported to have been degraded in this manner, including BTK, BCR-Abl, FKBP12, BRD9, and CDK6 (refs. [36,38–41]).

The work on heterobifunctional degraders has therefore converged with studies on the clinical mechanism of action to provide conceptual validation and chemical tools to accelerate efforts in protein degradation. We anticipate many further clinical successes in both molecular glue and heterobifunctional space. In the following sections, we aim to outline some of the specific considerations for prosecuting drug discovery campaigns in the protein degradation arena, specifically focusing on the mechanisms that aim to bind and redirect E3 ubiquitin ligases for degradation of non-native substrates.

Pharmacological considerations for degraders

Protein degraders form an enzymatic complex in the cell that acts in a catalytic fashion to deplete the target protein. As such, there are

Table 1 | Clinical stage molecules that redirect E3 ubiquitin ligases for targeted protein degradation

Name	Ligase	Principle substrate	Principle indication	Regulatory stage	Class
Thalidomide	$CRL4^{CRBN}$	Ikaros, Aiolos	Myeloma	Approved	Molecular glue
Lenalidomide	$CRL4^{CRBN}$	Ikaros, Aiolos, CK1α	Myeloma, del(5q) MDS	Approved	Molecular glue
Pomalidomide	$CRL4^{CRBN}$	Ikaros, Aiolos	Relapsed refractory myeloma	Approved	Molecular glue
Avadomide (CC-122)	$CRL4^{CRBN}$	Ikaros, Aiolos	Lymphoma	Phase 2	Molecular glue
Indisulam	$CRL4^{DCAF15}$	RBM39	Myeloma	Phase 2	Molecular glue
Iberdomide (CC-220)	$CRL4^{CRBN}$	Ikaros, Aiolos	Relapsed refractory myeloma, Lupus	Phase 1b/2a	Molecular glue
CC-90009	$CRL4^{CRBN}$	To be disclosed	AML	Phase 1	Molecular glue
CC-92480	$CRL4^{CRBN}$	To be disclosed	Relapsed refractory myeloma	Phase 1	Molecular glue
ARV-110	Undisclosed	Androgen receptor	Prostate cancer	Phase 1	Heterobifunctional

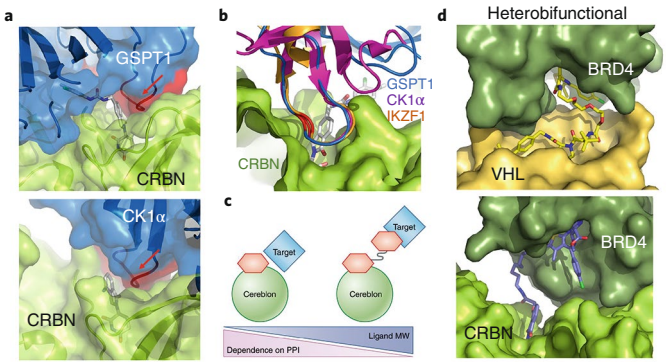

Fig. 2 | Crystal structures of E3 ligase-ligand-substrate ternary complexes. a,**b**, Representative molecular glue complexes with the cereblon neosubstrates GSPT1 (**a**) and CK1α (**b**). The neosubstrate degron is shown in red, and the conserved glycine residue marked by a red arrow. **b**, structural alignment of the degrons from GSPT1, CK1α, and IKZF1, with the glycine residue shown in red. **c**, Cartoon contrasting molecular glue and heterobifunctional substrate recruitment on a spectrum (MW, molecular weight; PPI, protein–protein interaction). Image reproduced from ref. [74]. **d**, Representative structures of heterobifunctional ternary complexes, with the structure of VHL in complex with BRD4 and MZ1 is shown in the top panel, and the structure of cereblon in complex with BRD4 and dBET23 shown below.

several aspects of the protein-degradation therapeutics that contrast with reversible drugs that have dominated pharmaceutical research for recent decades. There are many examples of disease-causing proteins in which inhibiting the catalytic activity offers no therapeutic benefit, and this has caused divergent results compared to genetic-knockdown approaches used to identify candidate therapeutic targets. CRISPR mutational scanning approaches have confirmed the functional differences of individual domains in multidomain proteins[42], which is of critical significance for inhibition strategies. In contrast, E3 ligase recruitment via any domain in a multidomain protein can lead to degradation of the entire protein. Furthermore, there should now be many opportunities to attack proteins in which noncatalytic functions drive disease, as degradation removes all functions of a protein.

Another powerful aspect of recruiting proteins of interest to E3 ligases is that the ubiquitin ligase is enzymatically active and has the ability to rapidly ubiquitinate target proteins with high substrate turnover. A single small-molecule ligase modulator can therefore drive the destruction of many molecules of a target protein. The capacity for catalytic amplification suggests great therapeutic potential in scenarios in which strong target suppression and long-lived pharmacology would be advantageous and may allow compounds with only moderate binding affinities to have potent cellular and therapeutic effects. As opposed to reversible small-molecule inhibitors that lose effectiveness as drug concentration drops, target destruction should be expected to drive long-lasting target suppression and strong biological effects. The kinetic aspects of heterobifunctional degraders have been discussed in detail[43].

An important consideration for targeted protein degraders is the rate of resynthesis of the target protein. A degrader must ultimately overcome the homeostatic regulation of the target to suppress its levels, and this is much more challenging for proteins that have high rates of synthesis and clearance. Resynthesis rates vary quite widely for different proteins, and some of the most therapeutically attractive targets are rapidly turned over in the cell.

Although there are common advantages to protein degradation, there are key pharmacological differences between molecular glue and heterobifunctional molecules. Heterobifunctional molecules have affinity for both ligase and substrate, which means that the ligand has the ability to bind to each protein target independently. The consequence of this is that at higher ligand concentrations, individual binary complexes become saturated and compete for the formation of the E3 ligase–substrate–ligand ternary complex. This phenomenon has been described as the 'hook' effect and manifests as a loss of degradation activity at higher ligand concentrations[44]. In contrast, because the CELMoDs have no measurable affinity for the free substrate[28,29], they do not exhibit this concentration-dependent phenomenon.

There are still very few reports of in vivo activity for heterobifunctional degraders, and few have entered clinical studies, so it remains unclear the extent to which the hook effect will manifest in vivo, wherein ligand concentrations will rise and fall following dosing according to the pharmacokinetic properties of the molecule. Clearance of circulating levels of ligand should prevent such a strong manifestation of the hook effect, as observed in vitro, wherein compound levels can remain, to some extent, constant for the duration of an experiment. Furthermore, in those instances in which the target-binding moiety is capable of effective inhibition of the target protein function, the pharmacological contribution to the overall efficacy of the molecule will persist during the 'hook' period.

This emergent modality of protein degradation presents opportunities for extremely robust and durable target suppression, which because of its catalytic nature may decouple pharmacokinetics from pharmacodynamics and efficacy. A typical inhibitor of a protein

target of interest generally will be expected to derive maximal efficacy (safety and tolerability considerations aside) whereby complete target engagement is maintained throughout the dosing cycle. This can require that protein binding adjusted C_{trough} concentrations (the lowest concentration of a pharmacological agent reached during a dosing cycle) are above the IC_{90} (the concentration at which 90% inhibition is achieved) for the target. Given that the protein target's concentration will remain roughly constant throughout the dosing cycle, clearance mechanisms that act upon the inhibitor and equilibria between plasma proteins and the target protein will work in concert to govern the relative stoichiometry to dictate target occupancy. A degrader for the same target, however, will experience a decline in target concentration resulting from the intended mechanism of action, and this decline in target concentration may intersect the clearance curve for the heterobifunctional degrader molecule, resulting in a favorable relative stoichiometry amplification, with declining concentrations of the degrader potentially able to still keep up with protein resynthesis rates.

Opportunities and risks using different ligases

There are estimated to be approximately 600 E3 ubiquitin ligases regulating the proteome, providing incredible opportunities for future therapeutic development. As the entire principle of targeted protein degradation relies on ligand-induced proximity, it is critical for there to be spatial and temporal colocalization of E3 ubiquitin ligases and the target protein. Differences in tissue expression, subcellular localization and regulation all offer potential pitfalls and opportunities. Cereblon, for example, has demonstrated clinically robust activity against nuclear proteins, such as Ikaros and Aiolos. A further important consideration is the effect on the biology of the ligase that is repurposed by ligand addition.

Early work on heterobifunctional degraders has repurposed literature binders for both substrate and ligase. Both IAP and MDM2 ligases have been associated with cancer, and, as such, small molecules have been discovered to alter their function in disease. The reported ligands are now available for use in heterobifunctional ligands, but they continue to retain the activities for which they were originally designed (Fig. 1). The SNIPER approach targeting IAP ligases has shown that engaging the ligase with small molecules can trigger degradation of the ligase[8]. This property is maintained in heterobifunctional ligands, although analogs have been reported that can bypass this phenomenon[11,45]. This effect offers the potential benefit of additional clinical activity, as cIAP1 has been linked to tumorigenesis, but there may be a potential cost in the efficiency of substrate degradation as ligase levels decrease. Similarly, MDM2 has a natural function in the ubiquitination of p53, and the MDM2 ligase binders were developed with the intention of stabilizing p53 protein[46]. Consequently, repurposing MDM2 with heterobifunctional molecules can bring the additional functional consequence of p53 stabilization[47]. VHL, in contrast, is a tumor suppressor with a native function in the ubiquitination of hypoxia-inducible factor 1 (HIF-1)[48,49]. It remains to be determined whether VHL-based degraders can function efficiently without inducing any of the clinical issues associated with VHL loss-of-function mutations.

In contrast, cereblon appears to be relatively silent when engaged by ligands or when genetically knocked down, although the thalidomide-binding pocket exhibits 100% amino acid sequence identity even in plants and insects, indicating strong functional constraint[20]. Despite attracting substantial research and clinical investments, cereblon biology remains incompletely understood, though there are links to the regulation of glutamine synthetase, AMPK (5' AMP-activated protein kinase), and MEIS2 (Meis Homeobox 2) (refs. [21,50,51]). However, despite the value in using cereblon modulators as ligase-binding moieties in heterobifunctional ligands, this strategy also brings risks. To date, all reported examples of cereblon-binding heterobifunctional ligands use ligase-binding motifs derived from the clinically approved CELMoDs. As a consequence, neosubstrate activity of cereblon can be retained in heterobifunctional ligands, leading to unintended 'off-target' activities. For example, degradation of the neosubstrates Ikaros and Aiolos has been shown in heterobifunctional molecules[38], and GSPT1 degradation has been reported as an off-target activity able to drive antiproliferative effects[52].

Among the risks associated with targeting cereblon is the potential to retain the teratogenic effects that caused the thalidomide birth-defect tragedy of the 1950s[12,13,53]. The ability to assign a molecular basis to such activities therefore presents substantial progress toward engineering safer drugs in the degradation space. Two independent studies have now established a plausible molecular explanation for the teratogenic effects by demonstrating that the embryonic transcription factor SALL4 is a robust neosubstrate for thalidomide-mediated degradation[32,33]. The link between SALL4 and embryopathy is supported both by human genetics and by experiments in animal models. Human mutations that reduce SALL4 activity have a clinical presentation that resembles thalidomide embryopathy so closely that it has led to misdiagnosis[54–56].

Haploinsufficiency in human SALL4 expression is sufficient to drive clinical embryopathy, indicating that a reasonable correlation can be made between genetic and pharmacological effects of SALL4 downregulation. In support of a major role in teratogenicity, it was found that SALL4 is robustly depleted by thalidomide in the fetuses of sensitive species such as rabbits, but not in resistant species such as mice. Furthermore, a transgenic mouse expressing human cereblon degrades other neosubstrates, but not murine SALL4, and remains resistant to teratogenic effects[33]. Critically, it is not only CELMoDs that exhibit degradation of SALL4; heterobifunctional molecules that work through cereblon have also been shown to degrade SALL4 to varying extents[32,33].

It remains to be determined whether SALL4 degradation is the only mechanism contributing to the clinical risk of teratogenicity for cereblon-binding drugs. Certainly, knowledge regarding the neosubstrate repertoire has dramatically increased over the last few years and has underscored the ability of cereblon to recruit many more neosubstrate proteins than may have been previously suspected, especially from the C2H2 zinc finger family[15,30–33]. This provides an incredible substrate for the identification of 'newly druggable' therapeutic targets. It will also be critical in future drug development to carefully monitor off-target activities in the discovery and development of both CELMoDs and cereblon-dependent heterobifunctional degraders.

With the first examples of E3 ligases successfully redirected by small molecules, it is anticipated that many additional ligases will be repurposed in this manner. The methods to identify ligases capable of performing by this modality remain empirical, but early efforts suggest that principles may be discovered that can accelerate the discovery process. The requirement to have efficient ligase-binding small molecules to investigate the possibility of coopting a ligase presents a considerable upfront resource investment. Using a fusion protein to probe the ability to recruit and ubiquitinate unnatural substrates, it has been reported that five out of six ligases could be repurposed in this way[57]. Covalent technologies developed for activity-based profiling also offer a relatively rapid path to identification of covalent ligase binders, and the first ligases have now been repurposed with heterobifunctional ligands discovered using this technique[58,59].

Differentiated therapeutics through degradation

Conventional reversible inhibitors must engage the target in a manner described by simple equations. In contrast, to achieve a consequential effect upon the target, degraders require a multistep process involving additional cellular factors beyond the simple ligand–target interaction. Key steps include formation of the

ternary complex between the target, the small-molecule ligand and the E3 ubiquitin ligase enzyme. This E3 ubiquitin ligase complex must result in efficient ubiquitin transfer and is preceded by a sequential cascade of ubiquitin-activating enzymes (E1) and ubiquitin-conjugating enzymes (E2) before substrates are labeled on surface lysine residues in a manner that can lead to proteasomal degradation. The ubiquitinated substrate must also be sufficiently resistant to ubiquitin removal catalyzed by deubiquitinating enzymes (DUBs). Teams from Harvard and Celgene found that the E2 ubiquitin-conjugating enzymes UBE2G1 and UBE2D3 act sequentially to enable efficient ubiquitin transfer to the known cereblon neosubstrates such as Ikaros and GSPT1 (refs. [60,61]). It has been shown that following ubiquitin transfer the protein P97 is required for the processing of cereblon neosubstrates before proteasomal degradation[62]. It is anticipated that as further degraders are developed, we will learn the extent to which the cellular machinery will be shared across molecules, ligases, tissues, organisms and patient populations.

The dependence on multiple cellular components adds complexity to the discovery process and may offer multiple paths to clinical resistance. The same complexity does, however, offer opportunities for therapeutic differentiation. For example, it has been demonstrated that selectivity in degradation can be achieved from promiscuous target binders. Key studies using promiscuous kinase inhibitors tethered to ligase-binding moieties in heterobifunctional molecules have shown that there is little correlation between binding affinity and extent of degradation[63,64], a phenomenon that was in some cases attributed to differential rates of ternary complex formation. It has also been shown that there can be strong preferences in ligase–substrate pairs, at least for the degrader chemistries examined, with some substrates appearing to be susceptible to degradation by particular ligases but not others.

One application for differentiated degradation therapeutics is to provide an opportunity to achieve selectivity in highly conserved protein families. Within the kinases, differential ternary complex formation has enabled the discovery of selective CDK6 degraders from CDK4–CDK6 dual inhibitors[38,65]. In those reports, the clinical CDK4/6 dual inhibitor palbociclib was used as the target-binding moiety, and a cereblon binder was used to recruit the E3 ligase. By varying the linker chemistry, the team was able to identify degraders with selectivity for CDK6. Selectivity between p38α and p38δ has also been demonstrated by varying linker chemistry[66].

The first crystal structure of a heterobifunctional ligand scaffolding a ternary complex was that of VHL bound to the tool compound MZ1 and a bromodomain of BRD4 (Fig. 2d)[67]. Along with structural studies, careful biophysical experiments were used to demonstrate the potential for some compounds to exhibit positive cooperativity in that system. In contrast to the work on VHL and BRD4, no evidence for positive cooperativity was detected in a report on the cereblon system[68]. In this study, selectivity could instead be driven by exploiting the negative cooperativity of the system to differentially reduce the ternary complex for proteins even when they are equally able to bind the heterobifunctional ligand. Similarly, little evidence for cooperativity was found in a report on a BTK–cereblon heterobifunctional system[40]. Of course, these reports do not preclude the discovery of positive cooperativity using cereblon with different heterobifunctional ligands or substrate systems.

It is clear that the multistep mechanism of action that occurs when E3 ligases are coopted for targeted protein degradation brings additional complexity that can be a major barrier to success, but it also presents opportunities for differentiation from classical inhibitors. In the appropriate scenario, it is possible that the selectivity profile that can be achieved via the degradation approach would be impossible to achieve via classical binding and inhibition alone. Similarly, with a dependence upon multiple cellular factors, there may be opportunities for tissue-specific degradation even where the ligase and target are ubiquitously expressed. As such, although protein–protein interactions do not appear to be strictly necessary for activity of heterobifunctional molecules, there have now been clear demonstrations of the potential benefits. Heterobifunctional molecules may therefore be considered to be on a spectrum of properties with molecular glue (Fig. 2c). The early examples of degradation selectivity were identified using empirical methods, but it is anticipated that with increased attention from the scientific community, and with the application of computational tools, additional rational approaches may be available to inform the discovery of next-generation therapeutics.

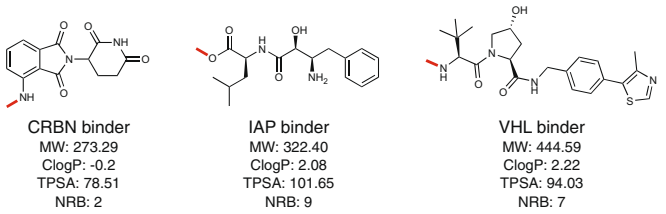

Fig. 3 | Comparison of commonly employed ligase binding moieties (LBMs) for frequently used E3 ligases in heterobifunctional degraders. Methyl groups (indicated in red) are added to the typical points of linker attachment. MW, molecular weight; ClogP, calculated logP; TPSA, total polar surface area; NRB, number of rotatable bonds, CRBN, cereblon; IAP, inhibitor of apoptosis.

Physicochemical properties and design considerations

Besides the biological differences in repurposing different ligases, there are also factors associated with the physicochemical differences between the ligase binders that are currently available (Fig. 3). The cereblon-binding moieties have substantial advantages, as they currently offer the lowest molecular weight and have been validated in the clinic as the molecular glue compounds listed in Table 1. The clinical molecular glue drugs conform to historical views with regards to desirable molecular weight and properties such as the 'rule of five'. However, the current literature on heterobifunctional molecules challenges these concepts and is anticipated to further push the boundaries of approved drug properties[69,70].

The challenges associated with attaining suitable physicochemical and absorption, distribution, metabolism, and excretion (ADME) properties adequate for oral dosing and/or even achieving central nervous system exposure in the heterobifunctional degrader class of molecules are not insignificant, though these larger and generally somewhat flexible molecules remain bound by the laws of physics, and a systematic approach to design and optimization can yield surprisingly favorable results. Unlike small molecules in a similar molecular-weight range (~700–900 Da) that exert their pharmacological effects by a unifunctional modality, the opportunities for molecular engineering afforded by the domain composition of heterobifunctional degraders (target binding moiety, linker, ligase binding moiety) allow some further element of control in the design of such molecules. Although these three domains do not necessarily have entirely discreet boundaries, and they must overall work in concert, it can be useful to consider them individually.

If one considers the design enterprise as operating on a molecular-weight budget, with the intent to keep the overall weight as low as possible to maximize the likelihood of favorable physicochemical properties, then it stands to reason that the less molecular weight that needs to be spent on the target-binding moiety (TBM) and ligase-binding moiety (LBM), the more that is available to be spent to impart favorable properties into the linker region. In this regard, the application of modern medicinal chemistry principles of ligand efficiency is an important aspect in design. Unlike small-molecule

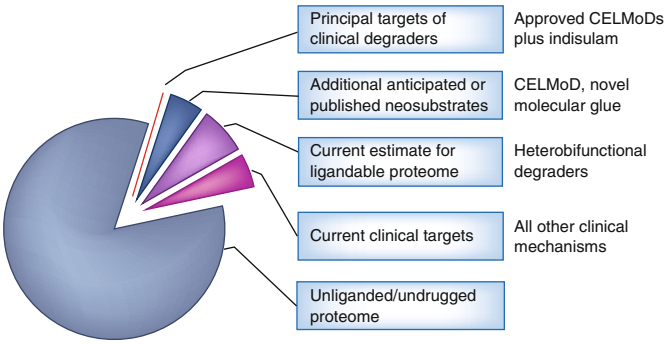

Fig. 4 | Promise for the expansion of the druggable proteome using both molecular glue and heterobifunctional degrader modalities. A pie chart representing the human proteome with potential opportunities in protein degradation alongside the current number of clincally established targets accessed by other modalities.

inhibitors of a given protein target of interest, whereby the typical default molecular profile targeted is one in which the highest affinity ligand is sought to maximize target engagement, the targeted protein degradation mechanism requires somewhat less affinity to the desired target to be competent as a recruiting moiety. As such, a more compact and highly efficient ligand as the TBM can be achieved at much lower cost to the molecular-weight budget. On a case by case basis, however, a somewhat larger and likely higher affinity TBM may be necessary to achieve the desired specificity versus the closest homologs.

With respect to the LBM, a number of considerations are highly relevant as well, notably the choice of ligase. The most common E3 ligases that have historically been used in the context of heterobifunctional degraders are cereblon and VHL. In addition to differences in cell biology between these ligases, the LBMs for each respective ligase that has been commonly employed in heterobifunctional degraders differ substantially in terms of molecular weight and intrinsic physicochemical properties (Fig. 3), which consequently impact the overall properties of the fully elaborated heterobifunctional degraders. There has yet to emerge a systematic molecular matched pair analysis attempting to quantitatively assess the overall impact on ultimate degrader physicochemical and ADME properties across many different contexts with differing TBMs and linkers so as to guide design principles to maximize pharmacokinetics and efficacy in vivo.

The linker region of these heterobifunctional degraders is seen by many in the field as the foremost critical domain for building in drug-like properties associated with amenability for oral delivery, and aspirationally, for crossing the blood-brain barrier. This is largely because this domain is perceived to be solvent exposed in the space spanning the gap between the protein of interest and the ligase upon ternary-complex formation; however dependent on the depth of the respective binding pockets, at least some portion of the linker may also be engaged in direct binding interactions with the target of interest and/or the ligase used. Early investigations into this modality largely used PEG derived linkers because of their synthetic accessibility, modularity for exploration of different lengths and flexibility to accommodate a range of geometric vectors to maximize the chances of observing some functional effects in cells[10,36]. The art of 'linkerology' has evolved substantially over the past few years in particular, with PEG being largely relegated to a research tool rather than a step along the path to developing a therapeutic for human medicine. Indeed, replacement of the PEG linker with an alkyl chain was used to optimize a heterobifunctional ligand to degrade BRD9 (ref. [39]). Emergent linker designs show evidence of efforts to optimize for reduced entropy, enhanced aqueous solubility and permeability, and reduced metabolic liability, all while concurrently giving some thought to the steric and electronic environments in the vicinity of exit vectors from the protein target and ligase, respectively.

Implications for the expansion of the druggable proteome

One of the most exciting and compelling aspects of unlocking the vast potential of heterobifunctional protein degraders is the opportunity to bring a wide variety of the historically undruggable proteome into play for therapeutic benefit. It is estimated that ~600 proteins have been targeted by clinical stage molecules (Fig. 4)[71]. Though the heterobifunctional and molecular glue classes of ubiquitin ligase modulators both share the advantages described above, the molecular differences between them means that there are significant differences in their scope of utility and in their chemical and pharmacological properties (Figs. 2 and 3). Molecular glue molecules can have much lower molecular weights than heterobifunctional molecules (Fig. 1), which is a substantial advantage in the development of therapeutics with good pharmacokinetic properties. This increased molecular efficiency is presumably possible because binding affinity is also contributed from the protein surfaces within the complex. This places a considerable restraint on the applicability of the 'molecular glue' approach: the protein surfaces must be able to complement each other and the ligand. For cereblon modulators, this protein–protein constraint is such that the substrates Ikaros, CK1α and GSPT1 all contain the structurally homologous neosubstrate degron (Fig. 2c).

Both small-molecule approaches to subverting E3 ligases offer an opportunity to expand the druggable proteome, but into different realms: heterobifunctional ligands still require a ligand with sufficient binding affinity for the target protein. Scaffolded protein–protein molecules do not necessarily require any ligand affinity for the target proteins outside of the E3 ligase–ligand–substrate complex. However, this approach is much more dependent upon specific surface features, restricting the utility to a subset of the proteome (Fig. 4). Both approaches offer the ability to tackle undruggable targets in scenarios in which protein removal offers a differentiated therapeutic outcome to binding alone.

As apparent from Table 1, the serendipitous discovery of the molecular glue mechanism in clinically approved drugs has meant that this class has dominated the clinical landscape to this point. The short-term clinical opportunities for targeted protein degraders are likely to come from a mix of molecular glue and heterobifunctional drugs. For the molecular glue class of molecules, cereblon is likely to remain a source of additional drugs despite the dependence on the key structural degron. There have now been several reports indicating that the degron occurs in multiple protein families, and a full systematic survey of the abundance of the degron may further help to establish the potential breadth of the mechanism.

For the heterobifunctional class, the availability of highly optimized binders to serve as target-binding moieties coupled to a well-defined clinical population means that the most rapid path to the clinic will be in situations in which reversible inhibitors have already been applied. As such, several well-established targets are likely to offer fast paths to additional clinical degraders. For example, the androgen receptor is an established clinical target with many options for potent binders and has been demonstrated to be vulnerable to degradation, and there are mechanisms of clinical resistance that would be anticipated to remain sensitive to the degradation mechanism. As such, the first announced heterobifunctional molecule to enter human clinical trials targets the androgen receptor[72]. The protein kinase family similarly contains examples of targets for which there is a clearly defined clinical need in the wake of reversible small-molecule drugs. BTK and IRAK4 have received early attention where factors such as robust and durable target suppression, the emergence of drug resistant mutations, or

the ability to target additional scaffolding functions may differentiate a degrader from clinical inhibitors. However, there are also signs of progress into undruggable space whereby degraders appear to offer unique opportunities. Degradation of pathological proteins in proteinopathies presents a clear opportunity for transformational therapies, and there are early signs of tractability. For example, a degrader of the protein tau, linked to neurodegeneration, has now been reported[73]. In this work, the target-binding moiety was derived from a positron emission tomography tracer.

Although much of the efforts to date in the field of heterobifunctional targeted protein degradation have, for reasons of expediency, focused on exploitation of the known ligandable proteome, the future prospects of this modality will undoubtedly venture into uncharted territory for the remaining ~18,000 protein targets, many of which are seen as high value drivers of serious diseases. Although the prospect for this remains intriguing, the additional front-loaded steps for targeting as yet unliganded proteins serve to protract the overall timelines for identification of a heterobifunctional degrader with which to achieve cellular proof of concept. The opportunities that may be brought by the chemical enabling of additional ligases are further expected to open up more parts of the proteome for intervention. Between these approaches to targeted protein degradation, a substantial horizon has opened for the development of new therapeutics.

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