Targeted protein degradation (TPD) is attracting substantial interest owing to its potential to therapeutically modulate proteins that have proved difficult to target with conventional small molecules. Some have been intractable because their active sites are broad, shallow pockets that are difficult to bridge with small molecules; others have ‘smooth’ surfaces that offer few sites for a small molecule to bind. Many of these targets have key roles in cancer and other diseases, and so have remained of great therapeutic interest, despite their intractability because their active sites are broad, shallow pockets that are difficult to bind. Many of these targets have key roles in cancer and other diseases, and so have remained of great therapeutic interest, despite their recalcitrance to small-molecule inhibitors.

A major class of molecules that may enable such proteins to be modulated through TPD are known as proteolysis-targeting chimera (PROTAC) protein degraders. These are heterobifunctional small molecules consisting of two ligands joined by a linker: one ligand recruits and binds a protein of interest (POI) while the other recruits and binds an E3 ubiquitin ligase. Simultaneous binding of the POI and ligase by the PROTAC induces ubiquitylation of the POI and its subsequent degradation by the ubiquitin–proteasome system (UPS), after which the PROTAC is recycled to target another copy of the POI (Fig. 1). It is this catalytic-type mechanism of action (MoA) and event-driven pharmacology that distinguishes PROTACs from classical inhibitors, which have a one-to-one relationship with the POI and whose pharmacology is driven by stoichiometry and, usually, by interactions with a catalytic site.

There are also several other types of targeted protein degrader. Molecular glues, based on the serendipitous discovery that thalidomide and its analogues act as degrader molecules, constitute another important therapeutic class (Fig. 2; Box 1). Although not heterobifunctional in the manner of PROTAC molecules, molecular glues promote ubiquitylation of a POI by enhancing a protein–protein interaction (PPI) between a ligase and a potential substrate (Fig. 2; Box 1).

In the 20 years since the first small-molecule PROTAC was reported in the literature, the technology has moved from academia to industry, where several biotech and pharmaceutical companies have disclosed programmes in preclinical and early clinical development (Fig. 3). In 2019, the first PROTAC molecules entered clinical testing; in 2020, these trials provided the first clinical proof-of-concept for the modality against two well-established cancer targets: the oestrogen receptor (ER) and the androgen receptor (AR). With this success in hand, the TPD field is now poised to tackle ‘undruggable’ targets and other classes of difficult protein target.

In this Review, we briefly summarize the first two decades of PROTAC development, as it has been extensively reviewed elsewhere (for example10–13) and the current status of clinical translation of TPD. We then focus on discussing key questions relevant to what TPD could achieve therapeutically and what is needed to move the field forwards over the next 20 years.
The concept of harnessing this natural degradation system for therapeutic purposes was inspired by early studies of viruses and plants (Box 2), which highlighted the possibility of deliberately designing small molecules that co-opt E3 ligases and recruit them for degradation of a POI. The initial work to co-opt E3 ligases focused on two areas of research. One involved using ligase-recruiting ligands to probe the biology and phenotype of a POI as an alternative to targeted inhibitors, such as the small GTPase KRAS or the transcription factor MYC.

In vitro proof of concept for the first fully synthetic PROTAC, dubbed Protac-1, was reported in 2001 (Ref. 1). Protac-1 was designed to target methionyl aminopeptidase 2 (METAP2), the putative target of the potent angiogenesis inhibitors ovalicin and fumagillin, and consisted of two domains: ovalicin, and a 10-amino acid phosphopeptide from nuclear factor-κB inhibitor-α (NF-κBIα; also known as IκBα) that is recognized by the E3 ligase β-transducin repeat-containing E3 ubiquitin–protein ligase (β-TRCP). Protac-1 acted as a tether between METAP2 and β-TRCP, enabling the ligase to ubiquitylate METAP2 in extracts from unfertilized Xenopus laevis eggs.

The subsequent discovery of a peptide from hypoxia-inducible factor 1 subunit-α (HIF1α) that bound the E3 ligase von Hippel–Lindau tumour suppressor (VHL) led to the design of cell-penetrating PROTACs that degraded a range of POIs. Technically, these early PROTACs are now considered ‘bioPROTACs’ because they are not fully small-molecule structures but instead contain peptide ligands for the E3 ligase. The discovery of small-molecule mimetics of the HIF1α peptide opened the door to the rational design of PROTACs based wholly on small molecular structures — for example, the first PROTACs based on the bromodomain protein inhibitor JQ1 that recruited VHL to degrade bromodomain-containing protein 4 (BRD4).

In parallel with the development of these early PROTAC molecules, the E3 ligase cereblon (CRBN) was identified as the target of thalidomide and its analogues lenalidomide and pomalidomide, which are known as immunomodulatory inside drugs (IMiDs) in the context of cancer therapy. These drugs co-opt CRBN to target IKAROS family zinc finger 1 (IKZF1) and IKZF3 for degradation, and are now considered as pioneering examples of molecular glues.

Additionally, the sulfonamide indisulam, a small-molecule cell-cycle inhibitor used to treat some solid tumours and leukemias, was shown to function in a similar manner by facilitating the interaction between the E3 ligase damage-specific DNA damage-binding protein 1 (DDB1) and CUL4-associated factor 15 (DCAF15) and the pre-mRNA splicing factor RNA-binding motif protein 39 (RBM39) and the related splicing factor RBM23.

Over the past two decades, evidence for the broad therapeutic potential of PROTACs and other TPD molecules has moved from studies in cell lysates and cell culture to studies in animals and animal models of disease. In the same time frame, TPD molecules have progressed from being based on peptides to fully synthetic, rationally designed small molecules. Although the exploration...
of TPD as a therapeutic modality is still in its early days, the realization that TPD as a therapeutic small-molecule paradigm may offer advantages over target inhibition and genomic targeted approaches (for example, small interfering RNAs (siRNAs), antisense oligonucleotides (ASOs), short hairpin RNAs (shRNAs) and CRISPR-based agents) has spurred widespread interest in academia and industry to explore its full potential30.

Status of clinical translation of TPD

The era of rationally designed targeted protein degraders as potential human therapeutics began in 2019 with the entry of two heterobifunctional degraders into first-in-human trials (Fig. 3): the PROTACs ARV-110 (NCT03888612) and ARV-471 (NCT04072952), targeting the AR and the ER, respectively. Both of these degraders have now moved on to phase II trials (TABLE 1), and have been followed into the clinic by degraders from Bristol Myers Squibb (BMS), Nurix Therapeutics, Kymera Therapeutics, Dialectic Therapeutics, Foghorn Therapeutics and others.

Additionally, the clinical development of molecular glue compounds such as CC-90009 and CC-92480, built around the IMiD platform at Celgene (now BMS), as well as molecular glue compounds from C4 Therapeutics and Novartis (TABLE 2), underscores the advances made in understanding and exploiting protein degradation as a therapeutic modality. Although not heterobifunctional, these new-generation CRBN modulators have been engineered with therapeutic targets in mind (IKZF1/IKZF3 and G1 to S phase transition 1 (GSPT1)), which are recruited by the compounds in a molecular glue mechanism to target them for degradation (BOX 1).

The two foundational TPD modalities (FIG. 2) — one based on the discovery of IMiDs pioneered at Celgene/BMS (reviewed extensively elsewhere31,32), and the other based on the conceptualization and development of heterobifunctional PROTACs based on rational pairings of an E3 ligase recruiter with a POI-targeting warhead pioneered at Arvinas33–35 — have been catalysts in taking TPD from the bench to the clinic.

Target selection for degraders

Novel CRBN modulators are built on the basic understanding of the mechanism of action of thalidomide analogues, which primarily target the IKZF family of transcription factors, and potentially other C2H2 zinc finger transcription factors, as well as proteins that contain the Gly-β-hairpin loop structural motifs36,37. By contrast, the establishment of rationally designed, heterobifunctional PROTAC degraders started with...
specific POI targets in mind, which has driven the rapid expansion of this TPD approach across diverse therapeutic targets and disease indications.

For any new therapeutic modality, the selection of well-known targets is important for establishing proof of concept because it introduces just one variable (the modality), not two (the modality and a novel target) into the equation. So, the initial wave of heterobifunctional degraders in the clinic (Table 1) has focused on proteins with a combination of well-characterized biological and biochemical properties, clinically validated roles in diseases with clear unmet medical need, and previously established clinical efficacy for their inhibition. The ER in breast cancer,

Clinical proof of concept for PROTACs

Before 2020, four key questions remained unanswered about heterobifunctional degrader molecules. Would they have drug-like properties? Would they be safe in humans? Would they work against the target protein as expected? Would they have a therapeutic effect?

In 2020, initial positive data reported from the phase I trials of ARV-110 and ARV-471 answered all four foundational questions in the affirmative—not just for the compounds themselves but for the entire TPD field. Given the importance of these findings for the TPD modality, we briefly summarize them here.

The AR degrader ARV-110 was evaluated in heavily pretreated patients with metastatic castration-resistant prostate cancer (mCRPC). The phase I trial (NCT03888612) was an excellent test case for PROTAC degraders because AR is a well-known driver of prostate cancer, and patients with mCRPC, especially this heavily pretreated subset, have limited therapeutic options owing to insensitivity or resistance to anti-androgenic therapies that are the mainstays of prostate cancer treatment. Initial trial data showed that ARV-110 was well tolerated at doses up to 420 mg. The data also demonstrated ARV-110-mediated degradation of the protein target in tumours—the first such evidence for a PROTAC molecule in humans—and revealed early signs of antitumour activity, as measured by reductions in levels of prostate specific antigen (PSA) and/or by RECIST (Response Evaluation Criteria in Solid Tumors). These data support the continued development of ARV-110, and the phase II ARDENT trial began in October 2020 with a dose of 420 mg (REF 40).

ARV-471, an ER degrader, entered clinical trials as a monotherapy for patients with ER+/HER2- locally advanced or metastatic breast cancer. The interim data for ARV-471 revealed a manageable tolerability profile with robust signals of clinical efficacy 41, a 42% clinical benefit rate in a heavily pretreated population and evidence of better ER degradation than fulvestrant and other clinical-stage selective ER degraders (SERDs) when compared at the same stage of development. ARV-471 has now progressed to phase II (VERITAC; NCT0472952) in metastatic breast cancer as a single agent, and a phase 1b study evaluating ARV-471 in combination with the cyclin-dependent kinase 4/cyclin-dependent kinase 6 (CDK4/CDK6) inhibitor palbociclib has also commenced. ARV-471 is now being co-developed by Arvinas and Pfizer to treat ER+/HER2- metastatic breast cancer.

The early but strong clinical profiles for these two PROTAC degraders, in two very different populations, in which they demonstrated desirable safety, efficacious exposure and signs of clinical efficacy with meaningful benefits for patients, are solidifying the therapeutic viability of the modality. Twenty years after its theoretical conception, at least 15 targeted protein degraders, among them heterobifunctional PROTACs and molecular glues, had entered the clinic by the end of 2021 (REF 39) (Tables 1, 2), and many more are expected to follow.

Outlook for the next 20 years of TPD

Beyond the current slate of compounds in clinical testing and others in the development pipelines of multiple companies, where can—and even should—the TPD field aim to go in the next 20 years? What challenges and possibilities remain for the field, and what might be accomplished when those are explored and addressed? What additional tools—E3 ligases, ligands and targeted protein degrader classes—might be useful in which diseases?

The basic science behind TPD has grown exponentially and matured substantially in the past few years (for example, as reviewed in Refs 42–44). In our opinion, the next milestones in this ‘new era’ of TPD will focus on four fundamental translation inflexion points, namely, defining and clinically demonstrating the target classes best served by degradation over inhibition; expanding the scope of E3 ubiquitin ligases employed clinically in a

Box 1 | Strategies for discovery of molecular glues

Rational degrader discovery using the modular proteolysis-targeting chimera (PROTAC) strategy (Fig. 2) can be complemented by the purposeful discovery of degrader compounds known as molecular glues. Molecular glues can enhance complex formation between an E3 ligase and a target by wedging between protein–protein interfaces. Although the best-known examples of molecular glues today are compounds that induce protein degradation, there are many examples of additional intramolecular and intermolecular glues that function outside the ubiquitin–proteasome system (UPS), such as the allosteric protein tyrosine phosphatase non-receptor type 11 (PTPN11; also known as SHP2) inhibitor SHP099 (REF 36), which stabilizes a closed conformation of SHP2 (intramolecular); and cyclosporin 37, which induces the proximity of calcineurin and cyclophilin (intermolecular). Two excellent recent reviews describe a plethora of molecular glue approaches, ranging from small molecules to multi-specific antibodies, and paint a promising picture for proximity-induced pharmacology in modern medicine 38,39.

Although historically, molecular glue degraders were discovered retrospectively—that is, the mechanism of action of cytotoxic compounds such as thalidomide were determined after their FDA approval—the recent rise in interest in targeted protein degradation as a therapeutic modality has led to a focus on identifying compounds with this mode of action from the beginning of the drug discovery process. The first rational discovery of molecular glues between a ligase and a substrate involved a series of compounds that restored binding affinity between the β-TrCP ligase and its mutated phospho-degron, discovered in a biochemical screen 40. More recently, targeted discovery of degrader compounds against oncproteins in a cell-based system has enabled the discovery of novel cereblon (CRBN)-dependent and CRBN-independent IKAROS family zinc finger 1 (IKZF1) degraders 41, suggesting that screening for degraders against a target of choice is a strategy that can yield a bounty of phenotypically relevant degrader molecules. Lastly, multiple recent papers have described agnostic screening approaches for degrader molecules 42–46 (as described in more detail in the main text) that were also highlighted by a comprehensive review, which offers guidelines for discovering E3 ligase modulators by phenotypic screening strategies 39.

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targeted fashion to enable precision medicine; extending the clinical reach of the modality beyond oncology; and validating TPD modalities beyond IMiDs and PROTACs (Box 3) in clinical settings. In this section, we discuss each of these points and what excites us for the next 20 years of degrader discovery and development.

**Targets best suited for degradation**

As described above, the first wave of clinical-stage protein degraders is aimed at classically drugged targets that have clinically validated roles in disease and readily available chemical matter. Success against these targets has begun to solidify PROTACs as a therapeutic modality and underscores the potential of these molecules to become best-in-class medicines by way of degrading a target instead of inhibiting it. However, the true promise of the modality is reaching targets that are currently difficult to drug with existing modalities or have not yet been drugged at all.

To date, traditional small-molecule drug discovery research for intracellular targets has often focused on developing high-affinity inhibitors that target either the active site or an allosteric site on an enzyme to shut down the function of the POI (occupancy-driven pharmacology). Although this has been a highly effective approach, it has left potential drug targets undrugged or underdrugged45. PROTACs bring the degradation function to the target (event-driven pharmacology), negating the need for an active site and redefining undruggable targets as simply undrugged.

The optimal targets for PROTAC therapy, which we have dubbed ‘Tenets of PROTAC targets’ (Fig. 4) can have several common characteristics, including: a change away from the natural state, via overexpression, mutation, aggregation, isoform expression or localization, that results in a disease-causing gain of function; a binding surface that is approachable by an E3 ligase; and ideally, an unstructured region to thread into the proteasome46,47.
Proteins that have evolved resistance mutations to targeted therapies, proteins with scaffolding functions and proteins that are considered ‘undruggable’ with other modalities can also be highly suitable PROTAC targets. The early PROTAC targets focused on POIs that had existing ligands, in the form of available inhibitors, but were still associated with clear unmet medical need. There are several reasons that existing ligands failed to fully benefit patients, such as incomplete inhibition, narrow therapeutic window and partial selectivity. By incorporating the ligand into a PROTAC, which often acts in an iterative or ‘pseudo-catalytic’ fashion by binding and facilitating the interaction of its ligase and POI targets, the POI can be degraded without requiring a large excess of drug, thereby resulting in more complete target blockade and a wider therapeutic window. PROTACs may also benefit from cooperative PPIs between the E3 ligase and the POI. This can have the advantages of improved potency and selectivity.

Although ‘PROTACable’ POIs do not need an enzyme active site, they do need a small-molecule binding site that is approachable by an E3 ligase. Using these sites does not require a high-affinity ligand if coupled to the right E3 ligand, but moderate affinity (2–500 nM) is typically needed, and access to the POI surface near the binding site by a recruited E3 ligase is essential. Achieving such binding affinities can often be challenging and has promoted research into alternative degraders (Box 3). Selection of the ligand-binding site is particularly important in the case of scaffolding proteins, where the POI may only be partially exposed within a given complex. It may be possible to degrade a POI by targeting a neighbouring protein within a protein complex (the bystander effect). This approach may prove useful in degrading scaffolding proteins in which the surface of the POI is mostly buried within the complex or the POI is a membrane-associated protein.

Cell-surface proteins are currently not considered optimal targets for PROTAC therapy as the UPS resides inside the cell. However, Bensimon et al. challenged this view by showing that multi-transmembrane proteins from the solute carrier (SLC) transporter family could be degraded using the dTAG technology. Furthermore, they developed 99A-2, a pomalidomide-based PROTAC that degraded SLC family 9 member A1 (SLC9A1) and, to a lesser extent, other SLC9 family members — leading to impaired pH homeostasis and cytotoxicity in multiple cancer cell lines, via a mechanism consistent with UPS-dependent TPD. More recently, Wang et al. showed that programmed cell death ligand 1 (PD-L1) — an immune checkpoint protein that resides on the cell surface to carry out its natural function but circulates between the surface and the cytoplasm — could be degraded using a CRBN-based PROTAC linked to the BMS-37 PDL1 warhead in MC-38 cells.

Although the ligand affinity for the POI can be low, an ideal E3 ligase ligand would be a potent binder with a slow off-rate. PROTACs that covalently bind the POI lose the ability to turn over and act iteratively, whereas PROTACs that covalently bind an E3 ligase would reduce the three-body assembly kinetics into a two-body problem and enhance the catalytic efficiency. Currently there are limited methods to selectively covalently bind to a protein, but advancements in bio-orthogonal chemistry could result in longer-lasting PROTACs with higher catalytic efficiencies.

In general, PROTAC physicochemical property space falls beyond the ‘rule of 5’ (REFS[9,4]) owing to the bifunctional nature of the molecules. The rule of 5 was developed in the 1990s as a guideline for assessing high-throughput screening (HTS) libraries. However, several orally bioavailable compounds have been developed that fall outside the rule of 5 parameters. These compounds typically have higher molecular weights and a higher number of hydrogen-bond acceptors than the rule dictates, but particular attention must also be paid to hydrogen-bond donors, topological polar surface area and cLogP, as well as lipophilic efficiency and other physicochemical parameters, including those summarized in a recent review. Short linkers can be used to modulate PROTAC molecular weights and properties, but ‘linker-ology’ does more than link the POI and E3 warheads together and influence properties. The linker can contribute to the PROTAC solution conformation

Box 2 | Targeted protein degradation examples from viruses and plants

At least two dozen viruses are known to hijack the human ubiquitin–proteasome system (UPS) to promote their own survival and replication. For example, the E6 protein of human papillomavirus type 16 (HPV-16) and type 18 (HPV-18) recruits the human E3 ligase, ubiquitin–protein ligase E6A (UBE3A; also known as E6AP) to ubiquitylate p53, resulting in its degradation. Human immunodeficiency virus 1 (HIV-1) deploys its viral proteins Vpr and Vpx, to recruit DDB1 and CUL4-associated factor 1 (DCAF1) to target several different human proteins, including DNA repair proteins, for ubiquitylation. Plant studies revealed that, in addition to proteins, small molecules are capable of inducing UPS-mediated protein degradation. In many species of plants, auxin (indole-3-acetic acid; IAA) functions as a hormone that promotes degradation of the Aux/IAA family of transcriptional repressors, which are involved in regulating plant development. Auxin acts by stabilizing the interaction between Aux/IAA proteins and the plant E3 ligase transport inhibitor response 1 (Tir1)239,240. Another plant hormone, jasmonate, employs the same mechanism and many other classes of small molecule in plants promote protein degradation.

These early examples from viruses and plants highlighted the possibility of deliberately designing small molecules that co-opt E3 ligases and recruit them for degradation of a protein of interest. More recently, it has been appreciated that viruses provide several examples of the fusion protein and antibody mimic subgroups of bioPROTACs (defined as proteolysis-targeting chimeras (PROTACs) composed of peptide ligands; see main text). For instance, the Vif protein of HIV-1 can be viewed as a fusion protein between an F-box domain and target recognition domain. The F-box domains from hIv-1 vif, von Hippel–Lindau (vhl) and suppressor of cytokine signalling 2 (SocS2) have high catalytic efficiencies. In general, PROTAC physicochemical property space falls beyond the ‘rule of 5’ owing to the bifunctional nature of the molecules. The rule of 5 was developed in the 1990s as a guideline for assessing high-throughput screening (HTS) libraries. However, several orally bioavailable compounds have been developed that fall outside the rule of 5 parameters. These compounds typically have higher molecular weights and a higher number of hydrogen-bond acceptors than the rule dictates, but particular attention must also be paid to hydrogen-bond donors, topological polar surface area and cLogP, as well as lipophilic efficiency and other physicochemical parameters, including those summarized in a recent review. Short linkers can be used to modulate PROTAC molecular weights and properties, but ‘linker-ology’ does more than link the POI and E3 warheads together and influence properties. The linker can contribute to the PROTAC solution conformation.
and degradation efficiency (DCₜ₀/Dₘₐₓ)³⁵, and influence the E3 ligase–POI interactions and presentation of the POI within the zone of ubiquitylation (Fig. 5).

However, short linkers limit the number of ways the E3 ligase and POI come together and, ultimately, how the POI fits into the full E3–E2–ubiquitin complex. To take full advantage of favourable PROTAC-induced E3–POI interactions a wider set of PROTACable E3 ligases will be needed, as discussed in the next section.

Overall, understanding the PROTACability of a target is key, especially as emerging, and already validated targets, are considered for classical small-molecule inhibition or targeted protein degradation as a therapeutic modality. To assess the PROTACable genome, which can be defined as the share of druggable targets that could be targeted by the PROTAC modality, Schneider et al.³⁵ have provided the first systematic assessment of drug targets according to their ability to be degraded by PROTAC molecules. They based their analysis on several aspects of protein degradation mentioned here, such as availability of a small-molecule binder against the target, information on the endogenous ubiquitylation status of the target and target protein half-life. Their perspective offers the first comprehensive assessment of future potential targets with an eye towards protein degradation and can serve as a valuable resource to researchers in the protein degradation field.

Expanding the ligase landscape

Recently, it was disclosed that both ARV-110 and ARV-471 use CRBN as the E3 ligase that is recruited to their respective targets, AR and ER, to catalyse their ubiquitylation and proteasomal degradation.⁴²,⁴³ Taken together with established CRBN modulators, such as the IMiD class that targets IKZF1/IKZF3 (REFS ⁴⁴,⁴⁵), and a new generation of CRBN modulators that target GSPT1 (REFS ⁴⁶,⁴⁷) (reviewed elsewhere ⁴⁸,⁴⁹), CRBN appears to be emerging as a preferred E3 ligase for the first wave of TPD therapeutics in clinical trials. A notable exception is a BCL-ₓ ligase, degrader known as DT2216 developed by Dialectic Therapeutics that uses VHL as the recruiting mechanism; sulfonamides previously in clinical trials⁸¹,⁸² may well see CRBN and VHL supplanted as the initial ‘workhorses’ of TPD. With more than 600 human ubiquitin E3 ligases⁸³,⁸⁴ to potentially explore, the question becomes not ‘if’ new E3 ligase-based TPD therapeutics will reach patients, but ‘when’ — and for what diseases?

As noted earlier, degrader discovery has converged upon two main paths for discovering molecules that result in specific target degradation (FIG. 2; BOX 1). The first stems from an opportunistic approach, in which a particular compound is shown to cause degradation of a target, leading to retrospective discovery of the E3 ligase responsible for its degradation. Examples here include the approved IMiD class, which relies on CRBN to degrade IKZF1/IKZF3 (REFS ⁴⁴,⁴⁵) via a molecular glue mechanism; sulfonamides previously in clinical trials ¹¹,¹² that have since been shown to use DCAF15 to degrade RBM39 (REFS ¹³,¹⁴), also via a molecular glue mechanism elucidated by elegant structural biology in recent work.

### Table 1 | Heterobifunctional PROTAC targeted protein degraders in clinical development

| Company       | Degrader | Target        | Indications                          | E3 ligase | ROA      | Highest phase | Clinical trial no. (if applicable) |
|---------------|----------|---------------|--------------------------------------|-----------|----------|---------------|-----------------------------------|
| Arvinas       | ARV-110  | AR            | Prostate cancer                      | CRBN      | Oral     | Phase II      | NCT03888612                      |
| Arvinas/Pfizer| ARV-471  | ER            | Breast cancer                        | CRBN      | Oral     | Phase II      | NCT04072952                      |
| Accutar Biotech| AC682    | ER            | Breast cancer                        | CRBN      | Oral     | Phase I       | NCT05080842                      |
| Arvinas       | ARV-766  | AR            | Prostate cancer                      | CRBN      | Oral     | Phase I       | NCT05067140                      |
| Bristol Myers Squibb| CC-94676 | AR            | Prostate cancer                      | CRBN      | Oral     | Phase I       | NCT04428788                      |
| Dialectic Therapeutics| DT2216 | BCL-ₓ         | Liquid and solid tumours             | VHL       | I.v.     | Phase I       | NCT04886622                      |
| Foghorn Therapeutics| FHD-609 | BRD9         | Synovial sarcoma                     | Undisclosed| I.v.     | Phase I       | NCT04965753                      |
| Kymera/Sanofi | KT-474   | IRAK4         | Autoimmune diseases (e.g., AD, HS, RA)| Undisclosed | Oral     | Phase I       | NCT04772885                      |
| Kymera        | KT-413   | IRAK4         | Diffuse large B cell lymphoma (MYD88-mutant)| CRBN     | I.v.     | Phase I       |                                  |
| Kymera        | KT-333   | STAT3         | Liquid and solid tumours             | Undisclosed| Undisclosed| Phase I       |                                  |
| Nurix Therapeutics| NX-2127 | BTK          | B cell malignancies                  | CRBN      | Oral     | Phase I       | NCT04830137                      |
| Nurix Therapeutics| NX-5948 | BTK          | B cell malignancies and autoimmune diseases | CRBN     | Oral     | Phase I       | NCT05131022                      |
| C4 Therapeutics| CFT8634 | BRD9         | Synovial sarcoma                     | CRBN      | Oral     | IND-e         |                                  |
| C4 Therapeutics| CFT8919 | EGFR-L858R    | Non-small-cell lung cancer           | CRBN      | Oral     | IND-e         |                                  |
| Cullagen      | CG001419 | TRK           | Cancer and other indications         | CRBN      | Oral     | IND-e         |                                  |

AD, atopic dermatitis; AR, androgen receptor; BCL-ₓ, B cell lymphoma-extra large; BRD9, bromodomain-containing protein 9; BTK, Bruton’s tyrosine kinase; CRBN, cereblon; EGFR, epidermal growth factor receptor; ER, oestrogen receptor; HS, hidradenitis suppurativa; IND-e, in IND-enabling preclinical studies; IRAK4, interleukin-1 receptor-associated kinase 4; i.v., intravenous; PROTAC, proteolysis-targeting chimera; RA, rheumatoid arthritis; ROA, route of administration; STAT3, signal transducer and activator of transcription 3; TRK, tropomyosin receptor kinase; VHL, von Hippel–Lindau.

**Bio-orthogonal chemistry**

A chemical reaction that occurs inside a living organism without altering its biology.

**Rule of 5**

A set of physicochemical property guidelines for small molecules that indicate the likelihood of a small molecule being orally bioavailable in humans. It is more of a rule than an absolute rule, and many approved drugs fall outside the rule of 5.

**Protein half-life**

The time required for the amount or concentration of a protein to be reduced by 50% under physiological conditions. It is a measure of the propensity of a protein to be degraded by the ubiquitin–proteasome system (UPS). Proteins with short half-lives are rapidly degraded by the UPS (constantly being turned over), whereas proteins with long half-lives are more stable.
by multiple groups; and, most recently, a unique class of cyclin-dependent kinase 12 (CDK12) inhibitors, exemplified by CR8, which, unexpectedly, has been shown to directly co-opt DDB1 as an E3 ligase for the degradation of cyclin K (CCNK) in fact, not only CR8, but four separate classes of compounds, each discovered by a different screening method, were found to have molecular glue-like properties in potentiating the interaction of DDB1 and CDK12, resulting in CCNK degradation. These findings suggest that many more compounds, including those approved or already in the clinic, could have a hitherto overlooked degrader contribution to their mechanism of action.

All these molecular glue compounds had shown to be efficacious without a priori knowledge that they were, in fact, protein degraders. Their intricate mechanisms of action — in which the ligase and target become glued together — have been reviewed extensively, most recently by Chamberlain and colleagues.

The other path to degrader discovery is rationally driven by the selected therapeutic target, affording the ability to tailor the ligase to the POI by using an inhibitor of choice against the POI and a ligase-recruiting molecule that are connected by a linker. This approach to PROTAC discovery is exemplified by VHL-linked, MDM2-linked and inhibitor of apoptosis (IAP)-linked degraders in preclinical discovery settings, and by ARV-110 and ARV-471 and other clinical compounds that recruit CRBN. The modular nature of such PROTACs could allow ‘ligase-hopping’ — the ability to swap ligase ‘handles’ that offer the most potent degradation and the best physicochemical properties — and can potentially enable the employment of E3 ligases with unique characteristics that could offer advantages against a particular target or in a given therapeutic setting.

This wave of rational TPD therapeutics will probably continue to use E3 ligases that have been evaluated and targeted with degraders over the past decade — namely, VHL, CRBN and potentially IAPs. The reach afforded by just these few ligases, coupled with clinically validated inhibitors that could be converted into degrader warheads, already appears large.

Accordingly, it has become routine to generate and characterize a new degrader in cell culture experiments and animal models of disease, using either VHL or CRBN as a recruiting ligase. The modular nature of the modality and its ability to achieve near-immediate degradation of a wide range of target proteins allows rapid evaluation of such basic degraders and characterization of their functions in cells through acute protein loss. Although use of small-molecule PROTACs in an endogenous setting is limited to protein targets that have ligands available, Buckley et al. have developed a tagging system based on HaloTag fusions that can be targeted by HaloPROTACs, and Nabet et al. have developed a heterologous tagging platform, dTAG, that allows validation of unliganded or yet-to-be-ligated proteins as degradation targets and is enabled for both VHL and CRBN. HaloPROTACs, dTAGs and other degradation proof-of-concept strategies, such as the AID system, have been reviewed extensively elsewhere and now serve as crucial target validation tools when approaching a new therapeutic POI.

### Table 2: Molecular glue targeted protein degraders in clinical development

| Company          | Degrader | Target | Indications                      | E3 ligase | Highest phase | Clinical trial no. (if applicable) |
|------------------|----------|--------|----------------------------------|-----------|---------------|-----------------------------------|
| Bristol Myers Squibb | CC-220   | IKZF1/3 | MM                              | CRBN      | Phase II      | NCT02773030                       |
| Bristol Myers Squibb | CC-92480 | IKZF1/3 | MM                              | CRBN      | Phase II      | NCT03989414                       |
| Bristol Myers Squibb | CC-90009 | GSPT1  | Acute myeloid leukaemia         | CRBN      | Phase II      | NCT02848001/NCT04336982           |
| Bristol Myers Squibb | CC-99282 | IKZF1/3 | Chronic myeloid leukaemia, non-Hodgkin lymphoma | CRBN | Phase I | NCT04434906/NCT03930953 |
| C4 Therapeutics   | CFT7455  | IKZF1/3 | MM                              | CRBN      | Phase I       | NCT04756726                       |
| Novartis          | DKY709   | Helios | Solid tumours (NSCLC)           | CRBN      | Phase I       | NCT03891953                       |

CRBN, cereblon; GSPT1, G1 to S phase transition 1; IKZF1, IKAROS family zinc finger 1; MM, multiple myeloma; NSCLC, non-small-cell lung carcinoma.
The success of proteolysis-targeting chimeras (PROTACs) in hijacking the ubiquitin–proteasome system (UPS) for targeted protein degradation (TPD) has motivated research into other classes of heterobifunctional molecules that depend on non-UPS pathways to degrade a protein of interest (POI)\textsuperscript{114,115}. These classes include autophagy-targeting chimeras (AUTACs), autophagosome-tethering compounds (ATTECs), lysosome-targeting chimeras (LYTACs) and antibody-based PROTACs (AbTACs). Collectively, AUTACs and ATTECs have also been categorized as macroautophagy degradation-targeting chimeras (MADTACs)\textsuperscript{116}. Like PROTACs, ATTECs and ATTECs focus on intracellular triggering of TPD, while LYTACs and AbTACs trigger intercellular TPD via an extracellular process.

AUTACs link a warhead for the POI to a guanidine derivative that tags the protein for degradation by the autophagy machinery. Takahashi et al.\textsuperscript{116} have demonstrated an autophagy-dependent mechanism for an AUTAC that S-quantylated enhanced-GRP (EGFP) in mouse embryonic fibroblasts. Their study also explored the generality of directed autophagic degradation by making AUTACs against methionyl aminopeptidase 2 (METAP2), FKBP prolyl isomerase 1A (FKBP1A; also known as FKBP12) and BET family proteins, all of which were found to be effective degraders. ATTECs link a POI warhead to a ligand that binds to the autophagy protease LC3 (microtubule-associated protein 1 light chain 3a) glue, thereby bypassing the ubiquitin pathways by directly tethering the POI to the autophagosome. Li et al.\textsuperscript{117} have shown that ATTECs targeting mutant huntingtin (mHTT) directed the protein to the autophagosome for degradation in both cells and animals, and rescued phenotypes relevant to Huntington disease.

LYTACs bind a membrane-bound POI and the extracellular domains of a lysosome-shuttling receptor, which then drags the POI into the lysosome for degradation. The first LYTACs used a monoclonal antibody (mAb) conjugate: the mAb was directed against the POI, while the conjugated glycoprotein, which contained multiple serine-O-mannose-6-phosphonate (M6PN) residues, interacted with the cation-independent M6P receptor (CI-M6PR) for internalization and lysosomal degradation of the POI. LYTACs against the APOE4 allele of apolipoprotein E (APOE), epidermal growth factor receptor (EGFR), CD71 and PDL1 all degraded their respective targets in various cell lines\textsuperscript{118}.

AbTACs are bispecific antibodies that recruit membrane-bound E3 ligases to a membrane POI for degradation by the lysosome degradation pathway. Cotton et al.\textsuperscript{119} recently debuted AbTACs by describing AC-1, consisting of a recombinant antibody (R3) to the extracellular domain of the E3 ligase RING finger protein 43 (RNF43) and the PDL1-binding antibody atezolizumab. In MDA-MB-231 cells, AC-1 induced the degradation of PDL1 via an RNF43- and lysosomal-dependent mechanism.

ubiquitin E2 conjugating enzyme charged with a ubiquitin molecule (E2–Ub), by binding both molecules, then catalysing the transfer of ubiquitin from the active site cysteine of the E2 onto a lysine side chain of a target protein\textsuperscript{119–122}. Ubiquitin ligases achieve this catalysis via multiple mechanisms; for this reason, the more than 600 E3 ligases in the human genome fall into different classes, depending on which ubiquitin ligation strategy they use (reviewed in Refs\textsuperscript{123–126}). As a handy online tool, the Structural Genomics Consortium (SGC) has created UbiHub, a database of E3 ligases and other protein classes in the UPS\textsuperscript{127}.

To recap briefly: the largest class of ubiquitin ligases, RING ligases, bind to the target protein and E2–Ub simultaneously and transfer ubiquitin directly onto a lysine residue of the target protein. The second class, HECT ligases, bind the target then use an intermediate strategy of transferring ubiquitin onto themselves before transferring ubiquitin to the target\textsuperscript{117,118}. RBR ligases use a hybrid between the RING and HECT ligation strategies\textsuperscript{114,115}. To add to the complexity of ubiquitin ligation to substrates, RING and RBR ligases have recently been shown to work in unison to mediate substrate ubiquitylation in a site-specific manner\textsuperscript{114,115}.

Furthermore, RING ligases are classified as Cullin–RING ligases (CRLs) and non-CRL ligases. CRLs are multi-protein complexes in which E2–Ub binding is performed by the RING domain proteins, RING-box protein 1 (RBX1) and RBX2, and target binding is performed by other subunits in the complex, held together by Cullin scaffold proteins (see Fig. 5). In such CRLs, target binding happens via substrate receptor proteins that can pair with different CRLs, as reviewed extensively elsewhere\textsuperscript{119}. CRBN and VHL, for example, are substrate receptor proteins that pair with CRL4 and CRL2, respectively.

**The search for new E3 ligases.** The diversity and complexity of E3 ligases raises two key questions for TPD: where to start looking for new ligases, and what characteristics would make them ideal for novel PROTAC development? In our opinion, there are several exciting avenues to consider when seeking and developing a novel ligase for PROTAC development.

One practical and valuable avenue is to pursue widely applicable, ubiquitously present ligases, similar to CRBN and VHL, that could be paired with any target of choice and applied across many different therapeutic indications without limitations. Currently, all PROTACs to date use just a handful of these non-specialized ligases, collated in an online database: PROTAC-DB\textsuperscript{128}.

Interestingly, however, the pairing of VHL versus CRBN for a given target can result in different degradation efficiencies\textsuperscript{129}, as was first observed when targeting the fusion oncogene BCR–ABL by PROTACs\textsuperscript{130}. It is therefore possible that one ligase in particular may be better than others for degrading a certain target. A recent good example, and cautionary tale, of focusing on a single ligase when targeting endogenous proteins is the development of KRAS PROTAC degraders targeting the KRAS\textsuperscript{G12C} variant, enabled by the revolutionary work in developing covalent KRAS(G12C) inhibitors\textsuperscript{131–133}, including Amgen’s recently approved sotorasib and others that are currently in phase II trials\textsuperscript{134,135}. Zeng et al.\textsuperscript{136} initially developed a covalent PROTAC (XY-4-88) based on ARS-1620, a KRAS(G12C)-binding covalent warhead, and thalidomide as the CRBN-recruiting ligand\textsuperscript{137}. Although XY-4-88 was able to degrade a GFP–KRAS(G12C) fusion protein, albeit in a non-catalytic manner because it covalently bound the target, it could not degrade untagged, endogenous KRAS(G12C). Subsequent studies revealed that XY-4-88 induced the CRBN–KRAS(G12C) complex, but not its poly-ubiquitylation. By contrast, Bond et al.\textsuperscript{138} developed a covalent VHL–KRAS(G12C) PROTAC (LC-2), based on the MRTX849 KRAS(G12C)-targeting warhead and a VHL ligand. LC-2 was able to engage endogenous KRAS(G12C), induce its poly-ubiquitylation and degrade it via the 20S proteasome. The different outcomes of these two studies could arise from the different KRAS warheads, the linker between the POI and E3 ligase warheads, and/or the choice of E3 ligase. KRAS is a small protein (189 amino acids) and, when farnesylated, it is localized to the plasma membrane. Its proximity to the membrane and the large size of the Cullin 4A (CUL4A)–DDB1–CRBN–E2–Ub complex...
relative to the smaller CUL2–elongin B (ELOB)–ELOC–VHL–E2–Ub complex (FIG. 5) could be a contributing factor. Collectively, these findings demonstrate that KRAS(G12C) is specifically targetable via the PROTAC mechanism, but that targetability depends on the choice of E3 ligase; the findings could be extended to non-covalent PROTACs targeting other KRAS variants (for example, KRAS(G12D) or KRAS(G12D/G12V)) using non-covalent warheads and thus represent an alternative therapeutic modality for targeting KRAS-driven cancers in the future.

Similarly, building on earlier work on the degradability of kinases, a recent massive kinase degradation study using various kinase inhibitors as warheads has revealed the ‘degradable kinome’ and a diversity of kinase-degrading profiles among E3 ligases — highlighting the importance that ligase choice can make in targeting kinases for degradation. The differences in degradation profiles conferred by different ligases can be driven by several factors. One is shape complementarity between the ligase and the target. The second is the ability of the ligase to form degradation-competent ternary complexes between the ligase and POI. Ternary complex formation is a necessary step in the action of a PROTAC molecule, but the degree of ternary complex formation may differ from substrate to substrate; that is, a ternary complex that forms too efficiently and remains tightly bound will not lead to efficient degradation. Cooperativity plays a role in the efficiency of the VHL–BRD4 degrader MZ1 (REF 139), but is dispensable for CRBN–BRD4 degraders140. A third factor is differential subcellular localization of ligase and target; for example, a nuclear E3 ligase, DCAF16, has been shown to restrict target degradation to the nucleus141. A fourth factor is cell-type-specific expression profiles of ligase and target, which we discuss in greater detail in the next section.

Accordingly, besides the aforementioned ligases (CRBN, VHL, MDM2, IAPs, DCAF15 and DCAF16), a few additional ‘generic’ E3 ligases have been shown to be exploitable by PROTACs, using small-molecule covalent tools that specifically recruit the ligases to degrade the prototypical TPD substrate, BRD4; these ligases include ring finger protein 4 (RNF4)142, RNF114 (REFS 133–135), KEAP1 (REFS 136,137) and most recently, fem-1 homologue B (FEM1B)143. How far initial chemical matter will be developed for these newly established degrader ligases, and how widely they can and will be adopted as in vivo-compatible ligases with a broad target reach, will be the subject of intense experimentation over the next few years.

Beyond the ligases already vetted for TPD development, there are several other ubiquitin ligases, enabled by structural and/or validated substrate information, that present a path for the development of small molecules that can ultimately be converted into PROTACs, yet await development as potential PROTACable ligases. Most of these ‘low-hanging fruit’ ligases with structural data have been summarized by two excellent reviews144,145. However, it must also be mentioned that the definition of low-hanging fruit with respect to structural enablement of ligases has recently been redefined with the ground-breaking publication of artificial intelligence (AI)-driven tertiary structure prediction models from Google/DeepMind146 and RoseTTAFold147. Alphafold from DeepMind has made available all their high-quality predicted models across the proteome (https://alphafold.ebi.ac.uk/) and now serves as a major stepping stone in potentially enabling computer-aided drug discovery for many hitherto unreachable targets and ligases.

Tissue- and cell-specific E3 ligases. The discovery and development of novel E3 ligases for TPD clearly involve practical considerations, such as structural enablement, druggability and mechanistic understanding of the ligase. For example, is a validated substrate known, can that ligase–substrate relationship be exploited in development of a biochemical or cell-based binding assay to enable a screening paradigm, and is the ligase always ‘on’ or does it need to be activated by a physiological stimulus? Several ligases exist in an ‘off’ state and are auto-inhibited in the absence of an activating post-translational modification or presence of a binding partner. Co-opting such auto-inhibited ligases may...
Fig. 5 | Example Cullin–RING ligases and their substrate adaptors. Examples of Cullin–RING ligases (CRLs) and a U-box E3 ligase modelled as complete E3–E2–ubiquitin (Ub) complexes: CRL4–cereblon (CRBN) (panel a); CRL2–von Hippel–Lindau (VHL) (panel b); CRL1–β-transducin repeat-containing E3 ubiquitin–protein ligase (β-TRCP) (panel c); CHIP (STIP1 homology and U-box-containing protein 1) (panel d). The assemblies demonstrate the different size, shape and electrostatic potential surface of each ligase, all potentially enabling degradation of a wide variety of proteins of interest when co-opted as ligases for degradation (Cullin/RING-box protein 1 (RBX1) subunits are coloured orange/purple, respectively; adaptor proteins are green; substrate receptors are blue; ligands are sea green). The surface-rendered substrate receptors in the lower images show the electrostatic potential coloured surface of the binding protein/domain of the substrate as viewed from the E2 (indicated by (>). The U-box CHIP E3 ligase is a single protein and is coloured by domain in panel d (U-box: orange; TRP, tetratricopeptide repeat: blue). The zone of ubiquitylation is defined by the reach of E2–Ub and is shown for CRL2–VHL in panel b. The high-probability ubiquitylation sites on the protein of interest are lysine residues that can be positioned within the zone and within ~4 Å of the reactive thioester between E2 and ubiquitin. The crystal structure protein database (PDB) codes used to generate the models are: CRBN (2HYE, 3UGB, 6BN7 [REF. 130]), VHL (6R7F, 3UGB, 5T35 [REF. 129]), β-TRCP (6TTU, 3UGB) and CHIP (2C2L, 6S53 [REF. 227]). ELOB/ELOC, elongin B–elongin C.
pose additional challenges when considering them for degrader discovery.

However, ligases have other key characteristics, such as tissue and cell-type specificity, tumour enrichment and tumour essentiality (FIG. 6), that can offer opportunities for ‘niche’ ligase degrader development. The intersection between the practical and aspirational attributes of these niche ligases with respect to novel degrader development is often minimal at best, thus offering high risk/high reward scenarios for PROTAC discovery and development of degraders that recruit such specialized ligases. Ultimately, the wider UPS field will benefit from the intense industry interest in characterizing novel ligases for PROTACs, and from the tool molecules that will be developed.

Fig. 6 | Specialized E3 ligases for potential PROTAC applications. a | Schematic representation of the human body, highlighting E3 ligases with increased tissue specificities that may be harnessed by targeted protein degradation (TPD) modalities to enable tissue- and cell-type-specific targeting of disease-causing proteins. b | Table showing representative E3 ligase examples from different mechanistic classes of E3 ligase, highlighting their various (but not exhaustive) attributes to be considered for proteolysis-targeting chimera (PROTAC) development, including tumour and tissue enrichment, tumour dependence (CERES/DepMap profile) and mechanistic understanding of ubiquitin ligation onto their substrates. The E3 examples were chosen for each E3 class from multiple E3-centric review articles to highlight various characteristics and the expansive choice in ligase selection for novel PROTACs. One article is referenced for each E3, but there is considerable overlap between the content of the cited references. There is no single attribute that makes or breaks an E3 ligase for PROTAC development; all attributes should be considered in totality, including the target that is being considered for degradation. CNS, central nervous system; CRBN, cereblon; DCAF2, DDB1- and CUL4- associated factor 2; ELOB, elongin B; GID4, glucose-induced degradation protein 4 homologue; KLHL40, kelch-like family member 40; MAGEs, melanoma antigen genes; RNF182, RING finger protein 4 homologue; TRIM9, tripartite motif-containing protein 9; VHL, von Hippel–Lindau.

| E3 ligase family | Example member | Tumour-enriched | Tumour-dependent | Tissue-enriched | Insights into ubiquitylation mechanism exist | Highlighted in reference |
|-----------------|----------------|----------------|-----------------|----------------|---------------------------------------------|--------------------------|
| ELOB/ ELOC      | VHL            | No             | No              | No             | Yes                                        | Workhorse TPD E3         |
| DCAF            | CRBN           | No             | No              | No             | Yes                                        | Workhorse TPD E3         |
| CTLH            | GID4           | No             | No              | No             | Yes                                        | Ishida & Ciulli (2021)29  |
| DCAF            | DCAF2          | Yes            | Yes             | No             | Yes                                        | Shirasaki et al. (2021)21 |
| BTB             | KLHL41         | No             | No              | Yes            | Yes                                        | Jevtic et al. (2021)29    |
| RNF             | RNF182         | Yes            | No              | Yes            | Unclear                                    | Jevtic et al. (2021)29    |
| TRIM            | TRIM71         | Yes            | No              | No             | Yes                                        | Jevtic et al. (2021)29    |
| TRIM            | TRIM9          | No             | No              | Yes            | Yes                                        | Jevtic et al. (2021)29    |
| SPRY            | SPSB4          | Yes            | No              | No             | Yes                                        | Ishida & Ciulli (2021)29  |
specific to any. Despite the lack of clear tissue- or cell-specific expression, FBXO44 and other tissue- or cell-enriched ligases nonetheless have exciting potential for TPD, because the affinity of their ligands could be tuned to engineer in the specificity towards the tissue of choice, even though some basal level of ligase expression exists ubiquitously.

Interestingly, some ligases exhibit ‘reverse specificity’ — low expression in some tissue or cell types — that may also be advantageous for protein degraders. A remarkable example is the low expression of VHL in platelets: degradation of BCL-x, by the VHL-recruiting PROTAC DT2216 was spared in platelets, thus resulting in reduced platelet-driven toxicity and an improved therapeutic index compared with a BCL-x, inhibitor. DT2216 is in phase I trials and represents an exciting step towards pTPD.

E3 ligase enrichment in diseased versus healthy tissues and cells. Another new frontier to explore for pTPD is specific targeting of a PROTAC molecule by tumour cells over adjacent, non-cancerous tissue or uptake by a given type of tumour-initiating cell, which may be achieved by targeting tumour-specific or tumour-enriched E3 ligases. Often, but not always, tumour enrichment of an E3 ligase coincides with the dependence of the tumour on expression of that ligase. These correlations can be drawn and have been shown by analysing CERES scores in DepMap of E3 ligases across multiple tumour cell lines. This has allowed the identification of E3 ligases and other UPS genes — some better-characterized than others, such as cell division cycle 20 (CDC20), cytosolic iron–sulfur assembly component 1 (CIAO1) and WD repeat-containing protein 82 (WDR82) — that exhibit high tumour essentiality across many different cancer cell types. The advantage of ligases with these profiles is that tumour cells would be less able to develop ligase-based resistance to PROTACs, which has been shown to occur for some CRBN- and VHL-recruiting PROTACs, as discussed previously.

One caveat for this group of ligases is that tumour enrichment provides only a potential window of opportunity for PROTACs, which may not translate to a greatly expanded therapeutic index for pleiotropically toxic inhibitors that are converted into tumour-specific PROTACs. A second caveat is that highly tumour-enriched and tumour-dependent E3 ligases and ligase adaptors tend to be associated with the cell cycle — for example, CDC20 or S-phase kinase-associated protein 2 (SKP2) and others — and are likely to have similar profiles in tumours and rapidly dividing non-cancerous cells in the human body, such as in the bone marrow. PROTACs based on these ligases might therefore exhibit toxicities associated with classical chemotherapeutic agents, but this remains to be tested.

Finally, cancer-testis antigens (CTAs) also comprise ubiquitin ligases that have restricted expression in the normal testis but are highly overexpressed across multiple cancer types. An example of such a family of ligases are the MAGE (melanoma antigen genes) family of ubiquitin E3 ligases, often referred to as the MAGE-RING ligases (MRLs), which comprise proteins that serve as substrate-recruitment modules for other RING E3 ligases, adding to and/or altering the substrate specificity of their co-E3 ligases (reviewed elsewhere). The exact mechanism of ubiquitylation for MRLs is not as well understood as the mechanism for CRLs, for which substrate recruitment by multiple adaptors and the molecular details of Ub transfer from E2–Ub to the target has been extensively characterized at the atomic level of an entire CRL1 complex. However, MAGEs have recently received attention owing to their unique expression profiles. Although not all MAGE E3 ligases are tumour specific and it may not be possible to co-opt all of them for PROTACs, as some do not degrade their targets, testis-specific, tumour-enriched E3 ligases are nevertheless a space to watch for increased understanding of MRL function, biology and more.

Identifying, characterizing and developing tumour-specific E3 ligases for PROTACs by identifying small molecules that can co-opt them for TPD is only one way to potentially achieve tumour specificity and greater therapeutic indexes with PROTACs compared with classic pleiotropically toxic agents. An alternative approach is antibody-mediated tumour specificity using antibody–drug conjugates (ADCs), whereby a degrader molecule is coupled to a tumour-specific antibody. This technology has recently emerged as an exciting avenue in delivering PROTAC molecules in a tumour-directed fashion. Dragovic et al. at Genentech have pushed this modality forward and successfully demonstrated tumour-specific targeting in two different cancer types with ADCs involving VHL–BRD4 PROTACs. Although these antibody–PROTAC conjugates obviously lose the clear advantages of oral bioavailability and tunable dosing schedule of small-molecule PROTACs, the ADC approach can be valuable for potent and robust degraders that have poor physicochemical properties for parenteral administration.

Therapeutic areas beyond oncology

Degrader drugs currently on the market (the IMiD class) and the overwhelming majority of newly developed PROTACs and molecular glue compounds currently in clinical trials target various types of cancer. However, given its potential to degrade any target of choice, the reach of TPD is extending further than oncology.

Inflammation, immunity and immuno-oncology.

Already in this first wave of clinical degraders, two compounds entering clinical trials could test the PROTAC modality in non-oncology indications. A BTK degrader (NX-5948) and an IRAK4 degrader (KT-474) in phase I clinical trials target various types of cancer. However, given its potential to degrade any target of choice, the reach of TPD is extending further than oncology.
resistance mutation in BTK that renders first-generation BTK inhibitors less effective\textsuperscript{166,170}. Herein lies one of the great advantages of PROTACs: owing to their event-driven (versus occupancy-driven) pharmacology, PROTACs may overcome insensitivity to such resistance mutations through catalytic degradation of the target. A BTK PROTAC from Nurix Therapeutics that targets haematological malignancies (NX-2127) is currently in a phase I trial (TABLE 1).

By contrast, there are currently no approved IRAK4-targeting therapies, but the role of IRAK4 in inflammatory conditions, as well as in B cell lymphomas, is well established, and several inhibitors are currently in clinical trials in autoimmune indications\textsuperscript{165,172}. Here, the potential scaffolding role of IRAK4 is especially intriguing as a target, as inhibitors of its kinase function may not fully address the role of IRAK4 in these conditions. Another advantage of PROTAC degraders is their ability to degrade the target completely, instead of only inhibiting its enzymatic function, which would not affect any scaffolding role that a target may have. Particularly for IRAK4, its scaffolding role in Toll-like receptor (TLR) signalling around the myddosome is established\textsuperscript{165,172}, and may only be addressable by PROTAC degraders, many of which have been described to date\textsuperscript{172-173}. Of these, KT-474 from Kymera Therapeutics is the first to enter clinical trials (TABLE 1).

Following in the footsteps of immunotherapy, small-molecule-mediated activation of the immune response against tumours is a rich area of drug development\textsuperscript{166,177}. PROTACs have the potential to be first-in-class medicines in immuno-oncology as small-molecule drugs that target immune cell activation to phenocopy PD1/PDL1-directed agents\textsuperscript{166,177}. Most recently, PROTACs that target mitogen-activated protein kinase kinase kinase kinase 1 (MAP4K1; also known as HPK1) have been described with promising preclinical activity\textsuperscript{180}, although HPK1 inhibitors were shown to be equally effective. Here, the use of a PROTAC over an inhibitor may not be necessary or obvious, but given that PROTACs can comparatively achieve greater specificity with respect to off-target effects of some kinase inhibitors\textsuperscript{126,181}, a PROTAC approach may result in a cleaner inhibitory phenotype and better tolerability as a therapeutic agent.

**Neurology and neurodegeneration.** A key PROTAC property is the ability to degrade proteins that are not classically targetable by small-molecule inhibitors because they lack active sites. This attribute makes targets for several neurodegenerative diseases involving toxic build-up of proteins, such as tau, α-synuclein, mutant huntingtin (mHTT), TAR RNA-binding protein (TARDBP; also known as TDP43) and FUS RNA-binding protein (FUS), potentially addressable with the PROTAC modality.

Tauopathies are good examples of diseases in which small-molecule inhibitors have disappointed but PROTACs could succeed. Tauopathies include neurological disorders, such as Alzheimer disease, frontotemporal dementia (FTD) and progressive supranuclear palsy (PSP)\textsuperscript{191}, in which pathology is heavily associated with toxic accumulation of aberrant tau species, leading tau to aggregate into paired helical filaments and ultimately into neurofibrillary tangles that result in neuronal cell death\textsuperscript{192}. Therefore, tau downregulation — at any node of the dynamics of tau’s transitions from monomer to oligomer to aggregate\textsuperscript{193} — is a desired therapeutic strategy. In fact, in preclinical models, decreasing tau protein levels via ASO strategies\textsuperscript{185,186} and recently by engineered zinc-finger transcription factors\textsuperscript{194} was shown to be durable and to reverse tau pathology. Importantly, induced turnover of tau by the UPS, and the degradability of tau species, has been demonstrated by a tau–kelch-like ECH-associated protein 1 (KEAP1) chimeric peptide PROTAC molecule\textsuperscript{187}, as well as by a VHL–tau PROTAC\textsuperscript{188}. The most advantageous point at which to interfere with the dynamics of tau aggregation and co-opt the protein for degradation, and whether degradation of various tau species is shared by the UPS and by lysosomal pathways, are areas of active investigation\textsuperscript{184}.

Developing small-molecule tau-targeting PROTACs and other degraders that would combine the phenotype of knocking down tau — as ASOs do — with systemic administration and blood–brain barrier penetration, is a particularly active avenue. Tau in its monomeric form is an intrinsically disordered protein without clearly defined small-molecule binding pockets, which has made development of small-molecule inhibitors challenging. However, small-molecule probes against tau oligomeric species, such as \textsuperscript{18}F-T807, have been developed and are currently in use as clinical tau PET tracers\textsuperscript{189}. In fact, one strategy has utilized such PET tracers by turning them into tool PROTAC molecules that target tau by recruiting CRBN, and these were shown to cause degradation of tau species in cell-based models of tauopathy\textsuperscript{190}.

Key questions remain as to what the pathologically relevant tau species may be, and whether it is more advantageous to degrade tau generally, irrespective of its oligomeric state. Given the clear unmet medical needs represented by tauopathies and the suitability of the PROTAC approach for tackling neurodegenerative diseases, this area will see key milestones tackled over the next decade, with the potential to deliver breakthrough medicines.

Other targets in neurology where a PROTAC modality may offer clear advantages over current therapeutic agents include α-synuclein, a protein that can accumulate in the neurons of patients with Parkinson disease\textsuperscript{192}. α-Synuclein and tau are both intrinsically disordered proteins with distinct conformations that probably drive their respective pathologies and for which induced proteolytic degradation could be disease-modifying\textsuperscript{184}. A peptide-based α-synuclein degrader (see below) has been reported and was shown to protect neurons from α-synuclein overexpression-induced toxicity\textsuperscript{193}.

In Huntington disease, poly-glutamine-expanded mHTT aggregates and accumulates\textsuperscript{184}, presenting another difficult drug target that may be addressable by a PROTAC, as clearance of mHTT via the UPS pathway is thought to be beneficial for disease progression\textsuperscript{195}. In fact, two small-molecule TPD strategies have been reported to lower mHTT levels: a small-molecule glue
Table 3 | Characteristics of TPD approaches as therapeutic modalities

| Therapeutic modality                        | PROTACs/ molecular glues | Small-molecule inhibitors | Gene-based strategies | bioPROTAC | oligoTAC | LYTAC | ATTEC | AUTAC | abTAC |
|---------------------------------------------|--------------------------|---------------------------|-----------------------|-----------|---------|-------|-------|-------|-------|
| Target scaffolding functions                | ✓                        | ✓                         | ✓                     | ✓         | ✓       | ✓     | ✓     | ✓     | ✓     |
| Potential to treat undruggable proteins     | ✓                        | ✓                         | ✓                     | ✓         | ✓       | ✓     | ✓     | ✓     | ✓     |
| Iterative mechanism of action               | ✓                        | ✓                         | ✓                     | ✓         | ✓       | ✓     | ✓     | ✓     | ✓     |
| Broad tissue penetration                     | ✓                        | ✓                         | ✓                     | ✓         | ✓       | ✓     | ✓     | ✓     | ✓     |
| Orally bioavailable                         | ✓                        | ✓                         | ✓                     | ✓         | ✓       | ✓     | ✓     | ✓     | ✓     |
| Ease of manufacturing                       | ✓                        | ✓                         | ✓                     | ✓         | ✓       | ✓     | ✓     | ✓     | ✓     |
| Preclinical validation, proof-of-concept established | ✓ (Phase II for PROTACs) | ✓                         | ✓                     | ✓         | ✓       | ✓     | ✓     | ✓     | ✓     |
| Clinical validation                         | ✓                        | ✓                         | ✓                     | ✓         | ✓       |       |       |       |       |

PROTAC, proteolysis-targeting chimera; TPD, targeted protein degradation.

approach inducing the proximity between mHTT and microtubule-associated protein 1 light chain 3a (MAP1LC3a; also known as LC3) to target it to the autophagy pathway for degradation [201] and small-molecule PROTACs that recruit mHTT aggregates to E3 ligases from the IAP family, causing the proteasomal degradation of the mutant protein [197]. With the recent discontinuation of the phase III trial of tominersen, an HTT-lowering ASO that it was hoped could become the first disease-modifying therapy for Huntington disease, the PROTAC modality could provide a useful alternative route to pursue this key target.

Antiviral PROTACs. Interestingly, PROTACs could emerge as potential antiviral agents as well, essentially bringing the field full circle from the viral hijacking of the UPS that inspired the TPD concept (Box 2), to turning that hijacking strategy upon viruses themselves and targeting their proteins for degradation. The feasibility of antiviral PROTACs was first established with the degradation of the hepatitis C virus (HCV) NS3/4A protease in cell-based assays by an HCV inhibitor linked to CRBN[198], but has been brought to the forefront with the emergence of SARS-CoV-2, the virus responsible for the COVID-19 pandemic. A large PPI study in SARS-CoV-2-infected cells revealed several potential druggable pathways and proteins that may be exploited by repurposing inhibitors[199], and that could potentially be addressed by SARS-CoV-2-targeting PROTAC molecules. One provocative paper raised the possibility of targeting the viral envelope protein [200], but SARS-CoV-2 catalytic virulence factors, such as its two proteases, Mpro and PLpro[201], and the RNA-dependent RNA polymerase (Rdrp), which is the target of remdesivir[202], are potentially targetable by a PROTAC. Importantly, Pfizer initiated a phase I trial of its orally bioavailable SARS-CoV-2 Mpro inhibitor, PF-07321332 (NCT04756531) in healthy individuals in February 2021, followed by a phase II/III study, in which the first patient was dosed in September 2021 (NCT04960202). Pfizer has also developed an Mpro inhibitor prodrug, PF-07304814 [REF. 203], which is given intravenously and which entered clinical trials in hospitalized patients with COVID-19 in 2020 (NCT04535167). These antiviral inhibitors could provide starting points for the development of antiviral PROTACs that target SARS-CoV-2.

Additionally, Nurix Therapeutics has announced that it is in the discovery phase for three protein degradation chimeric targeting molecules (CTMs) against SARS-CoV-2, dubbed COVID-CTMs.

Alternative PROTAC modalities

Although PROTACs are poised to become an important modality for certain target classes, there are POIs that do not have the small-molecule binding sites needed for this approach. Even so, these targets are still susceptible to degradation by alternative PROTAC approaches, including bioPROTACs and hybrid PROTACs. Additionally, PROTACs have inspired the exploration of other classes of heterobifunctional molecules that harness non-UPS machinery to degrade POIs (Box 3), each of which offer features that set it apart from the other TPD modalities and from small-molecule inhibitors (TABLE 3).

For this section, we broadly define three PROTAC categories as follows: PROTACs are composed of traditional small-molecule ligands; bioPROTACs are composed of peptide ligands; and hybrid PROTACs contain both a peptide and a traditional small-molecule warhead. This categorization redefines the original PROTAC reported in 2001[REF. 9] as a hybrid-PROTAC, as it is composed of a small-molecule warhead that interacts with METAP2 linked to a phosphopeptide that interacts with the E3 ligase β-TRCP. Each of these three forms offers powerful tool molecules for driving drug discovery, and each form has unique challenges and considerations for development as therapeutic agents. As ARV-110 and ARV-471 have demonstrated, PROTACs can be developed as oral therapeutics, but bioPROTACs and hybrid PROTACs are unlikely to be oral drugs owing to their peptide components. Indeed, bioPROTACs would most likely be delivered as mRNA or DNA constructs via a viral vector gene delivery system[204–206] or in a nanoparticle[207–209].
**bioPROTACs and pepTACs.** The bioPROTAC category can be further divided into three subgroups: bifunctional peptides (pepTACs), fusion proteins, and bispecific antibody mimics (of which several examples are provided by viruses (Box 2)). Of the three subgroups of bioPROTAC, pepTACs are the most closely related to PROTACs, in that they are composed of a peptide that binds to an E3 ligase and a peptide that binds to the POI. The two peptide warheads are typically connected with a peptide linker, and the N or C terminus typically has attached a cell-penetrating peptide (CPP). However, pepTACs composed of natural amino acids may dispense with the CPP and be delivered by transfecting cells with a DNA or mRNA transcript to synthesize the degrader in situ.

Zhou et al. described some of the earliest chimeric bioPROTACs, including a fusion consisting of full-length β-TRCP protein and an N-terminal peptide of the E7 protein of human papillomavirus type 16 (HPV-16) that degraded retinoblastoma protein (RB1) in a human osteosarcoma cell line. These studies demonstrated that the SCF E3 ligase could be redirected to degrade non-SCF targets.

Since then, other groups have reported fusion bio-PROTACs that degraded the cancer targets human epidermal growth factor receptor 2 (HER2), MYC and KRAS and demonstrated therapeutically relevant effects in human cancer cell lines and mouse xenograft tumour models overexpressing these POIs. The reported fusions involved replacing the substrate-binding domain of an E3 ligase, such as the Cbl proto- oncogene (CBL), U-box-type E3 ligase STIP1 homology and U-box-containing protein 1 (STUB1; also known as CHIP), VHL, E6AP and HIV-1 Vif, with a domain specific for the POI — or, in the case of MYC, with the MYC-associated factor X (MAX), a small protein that forms a heterodimer with MYC. Interestingly, Pan et al. reported a Vif-based bioPROTAC as their best KRAS degrader. However, in mice bearing xenograft pancreatic tumours, orthotopic injection of the Vif-based degrader was more efficacious than intraperitoneal injection, highlighting the need for better delivery methods to advance bioPROTACs from laboratory tools to therapeutics.

Additionally, multiple groups have described a range of pepTACs that harness SKP1/SCF F-box, VHL, KEAP1 and other E3 ligases to degrade tau or α-synuclein in primary neurons and other cell lines, further supporting the potential of TPD to tackle Parkinson disease, Alzheimer disease and other tauopathies.

### The anti-GFP bioPROTAC platform.

Another approach for exploring the degradation efficiency and respective targets of chimeric E3 ligases from the CRL family is the anti-green fluorescent protein (GFP) bioPROTAC platform (anti-GFP bioPROTAC platform), developed by Partridge and colleagues. In brief, the platform constructs bioPROTACs by replacing the natural substrate recognition domain of an adaptor protein with a GFP-binding domain, such as the anti-GFP nanobody vhhGFP4 (REFS 217,218); then, a doxycycline-inducible system is used to drive co-expression of the bioPROTAC and a reporter in cells constitutively expressing H2B–GFP.

The platform has been used to generate GFP-degrading bioPROTACs based on various E3 ligases: the CUL1 ligases β-TRCP, F-box and WD repeat domain-containing 7 (FBW7) and SKP2; the CUL2 ligase VHL; the CUL3 ligase speckle type BTB/POZ protein (SPOP); the CUL5 ligases suppressor of cytokine signalling 2 (SOCS2) and ankyrin repeat and SOCS box-containing 1 (ASB1); and the U-box E3 ligase CHIP.

The platform also enables translation of GFP-degrading constructs into bioPROTACs that degrade a POI. For example, after demonstrating that their GFP-degrading bioPROTACs also degraded a GFP-tagged proliferating cell nuclear antigen (PCNA), Partridge and colleagues replaced the GFP-binding domain in one of those bioPROTACs with the 16-amino-acid Con1 peptide, which contains the conserved PIP sequence common to PCNA binding partners, to generate a Con1–SPOP bioPROTAC. This construct rapidly degraded PCNA and maintained low levels of PCNA for the 10 days of doxycycline dosing. Additionally, the platform has also been applied to the discovery of KRAS-targeting bioPROTACs.

### Oligonucleotide-based PROTACs.

Transcription factors and RNA-binding proteins (RBPs) are two classes of protein that are required for DNA repair, replication, transcription and many RNA-dependent processes. However, when mutated, transcription factors and RBPs give rise to multiple types of cancer, obesity and cardiovascular and neurological diseases. Despite much effort, these POIs have largely remained undrugged as many of them lack ligand-binding sites.

To address these POIs, RNA-PROTACs that target RBPs, and oligonucleotide-based PROTACs (O’PROTACs) and transcription factor-targeting chimereas (TRAFTACs) that target transcription factors have been developed.

RNA-PROTACs use an RNA consensus sequence as the RBP-binding warhead, linked to an E3 ligase-binding element. Similarly, O’PROTACs and transcription factor PROTACs use a double-stranded DNA (dsDNA) consensus sequence as the transcription factor-binding warhead, linked to an E3 ligase-recruiting ligand. Such constructs have been shown to reduce levels of the RBP LIN28A and the transcription factors ETS transcription factor (ERG) and lymphoid enhancer-binding factor 1 (LEF1) in various human cancer cell lines.

TRAFTACs consist of a HaloPROTAC that bridges an E3 ligase and a dCas9–HT7 fusion protein, then binds a bifunctional dsDNA–CRISPRRNA to form a transcription factor-recruiting complex. The TRAFTAC system has been used to degrade NF-κB and brachyury (T-box transcription factor T; TBXT) in human cell lines in a UPS-dependent manner that was specific to the dsDNA sequence and E3 ligase.

### The future of bioPROTACs.

Although bioPROTACs, O’PROTACs and TRAFTACs have the potential to become therapeutic agents, delivery and dosing are key hurdles to overcome. Small-molecule PROTACs have
Conclusion
Given the progress in the past two decades and the recent level of interest and investment from both academia and industry, it is clear that targeted protein degradation could become a key therapeutic modality. Witnessing its transition from intriguing idea to clinically proven concept, first via IMiDs and molecular glues and, most recently, via heterobifunctional PROTACs in phase I and phase II clinical trials, has been truly gratifying. We are excited about the discoveries in basic biology and chemistry that will further advance targeted protein degrada-
tion in the decades to come, and we believe this modality has the potential to offer patients new treatment options across diverse indications.

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