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Review article

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Protein degraders enter the clinic — a new approach to cancer therapy

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Abstract

Heterobifunctional protein degraders, such as PROteolysis TArgeting Chimera (PROTAC) protein degraders, constitute a novel therapeutic modality that harnesses the cell's natural protein-degradation machinery — that is, the ubiquitin–proteasome system — to selectively target proteins involved in disease pathogenesis for elimination. Protein degraders have several potential advantages over small-molecule inhibitors that have traditionally been used for cancer treatment, including their event-driven (rather than occupancy-driven) pharmacology, which permits sub-stoichiometric drug concentrations for activity, their capacity to act iteratively and target multiple copies of a protein of interest, and their potential to target nonenzymatic proteins that were previously considered 'undruggable'. Following numerous innovations in protein degrader design and rigorous evaluation in preclinical models, protein degraders entered clinical testing in 2019. Currently, 18 protein degraders are in phase I or phase I/II clinical trials that involve patients with various tumour types, with a phase III trial of one initiated in 2022. The first safety, efficacy and pharmacokinetic data from these studies are now materializing and, although considerably more evidence is needed, protein degraders are showing promising activity as cancer therapies. Herein, we review advances in protein degrader development, the preclinical research that supported their entry into clinical studies, the available data for protein degraders in patients and future directions for this new class of drugs.

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Key points

- The concept of harnessing the natural, intracellular protein-degradation machinery (that is, the ubiquitin–proteasome system) to eliminate disease-causing proteins was proposed more than two decades ago.
- Since then, numerous primary papers and review articles have described the mechanistic development of protein degraders and their potential as a new therapeutic approach, including for patients with cancer.
- As of 8 January 2023, 18 heterobifunctional protein degraders are under evaluation in clinical trials in patients with various solid tumours and haematological cancers, and the first clinical data on these molecules are now emerging.
- Preclinical data that have been disclosed for the protein degraders currently in clinical development support their target specificity and their potency in inhibiting tumour growth compared with small-molecule inhibitors.
- Preliminary data for protein degraders that target the androgen receptor, the oestrogen receptor and BTK have shown encouraging clinical activity in patients with prostate cancer, breast cancer and chronic lymphocytic leukaemia, respectively, and results from additional ongoing clinical studies are anticipated.

Introduction

Before the turn of the twenty-first century, the mainstays of treatment for patients with cancer were chemotherapy, radiotherapy and surgery. Although all three modalities remain key pillars of cancer therapy, cytotoxic chemotherapy and radiotherapy have well-recognized limitations relating to toxicities, morbidities and important long-term adverse effects that result from nonspecific targeting of nonmalignant cells in addition to malignant cells. The discovery of small-molecule inhibitors that target the active sites of specific proteins involved in the pathogenesis of cancer and leave noncancerous cells largely untouched ushered in a new era of precision medicine. Imatinib, a small-molecule inhibitor of the constitutively active BCR–ABL1 tyrosine kinase fusion protein, which is the hallmark of chronic myeloid leukaemia, was the first of these agents to be approved by the FDA in 2001 (ref. [1]). Since then, scores of small-molecule inhibitors have been approved by the FDA and globally as treatments for solid tumours and haematological cancers[2,3].

Despite the promise of small-molecule inhibitors, their current widespread use and continued development, these treatments have limitations as cancer therapies. A primary shortcoming is that inhibition by these drugs requires that the target protein has a suitable binding pocket, rendering 85% of the proteome (including transcription factors and scaffolding proteins) 'undruggable' by small-molecule inhibitors[4]. In addition, high local concentrations of small-molecule inhibitors must be continuously present for these drugs to exert their therapeutic effects (occupancy-driven pharmacology)[5]. Chronic, elevated drug exposure might increase the risk of certain adverse effects as well as cause cumulative toxicities[6]. Continual treatment with small-molecule inhibitors might also select for mutations in their target proteins or induce activation of compensatory mechanisms that lead to drug resistance, which has been reviewed in detail elsewhere[3,7–13].

Heterobifunctional protein degraders, including PROteolysis TArgeting Chimera (PROTAC) protein degraders, are a new class of agents that eliminate, rather than just inhibit, their target proteins. The protein degrader mechanism of action, first proposed more than two decades ago[14], is anticipated to ameliorate some of the drawbacks associated with small-molecule inhibitors. After substantial efforts to optimize these drugs in the laboratory, heterobifunctional protein degraders first entered clinical testing in 2019, and initial safety, efficacy and pharmacokinetic results in patients with cancer are now emerging. Herein, we provide an overview of the progress of heterobifunctional protein degrader development, review the preclinical and clinical data for protein degraders currently in clinical trials for patients with cancer, and consider prospects and potential challenges for these agents.

Protein degrader mechanism of action

The ubiquitin–proteasome system is a principal cellular pathway for protein homeostasis. In brief, unneeded or misfolded proteins are tagged with multiple units of ubiquitin and thus marked for degradation by the 26S proteasome. This tagging function is carried out through the concerted actions of several enzymes: an E1 ubiquitin-activating enzyme, an E2 ubiquitin-conjugating enzyme and an E3 ubiquitin ligase. The E3 subset is involved in the recognition of protein substrates to be degraded. The detailed structure and function of ubiquitin ligases and the proteasome have been reviewed elsewhere[15–19].

More than 20 years have elapsed since the first report to describe the use of fully synthetic chemical biology tools to leverage an E3 ubiquitin ligase to trigger the degradation of a target protein that is not its natural substrate[14]. Heterobifunctional protein degrader molecules are tripartite, bivalent molecules consisting of a target-protein binder, an E3 ligase binder and a linker that joins the two binders. By bringing the target protein and E3 ligase into close physical proximity, the ubiquitin ligase machinery can be co-opted to transfer ubiquitin to the target protein, leading to its degradation by the proteasome (Fig. 1).

Heterobifunctional protein degraders, which contain separate moieties to engage both the target protein and E3 ligase, share many similarities, and some important differences, with the related molecular glue degraders[20,21] (Box 1). The heterobifunctional proximity-inducing concept pioneered with protein degraders has also been shown to extend to other modes of degradation, as well as to post-translational modifications beyond ubiquitination[22] (Box 2); however, this article focuses on heterobifunctional protein degraders.

Heterobifunctional protein degraders have features that differentiate their pharmacology from that of traditional small-molecule inhibitors (Fig. 1). First, because protein degraders orchestrate the formation of a transient and reversible ternary complex (consisting of the target protein, E3 ligase and the protein degrader) and because the subsequent proteasomal degradation is kinetically irreversible, protein degraders can promote the degradation of multiple target molecules in a sub-stoichiometric manner. Protein degraders are thus freed from the occupancy-driven paradigm of traditional pharmacology and are instead best described as having event-driven pharmacology. Second, protein degrader ternary complexes can be positively or negatively cooperative owing to induced protein–protein interactions between the target protein and E3 ligase[23]. Therefore, limited direct relationships might exist between the binary binding affinity of the protein degrader for the target or E3 ligase and the overall binding affinity of the ternary

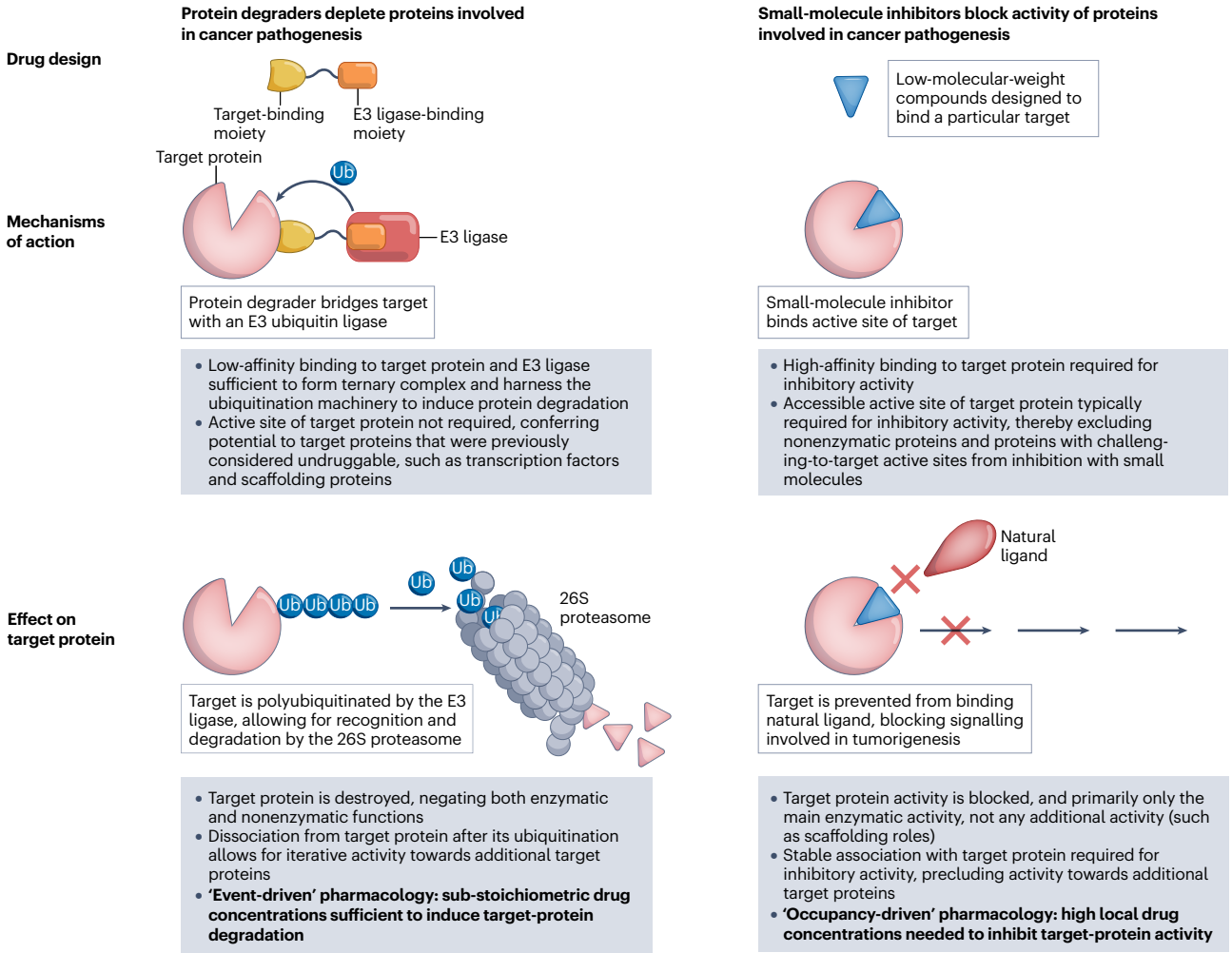

Fig. 1 | Targeting proteins involved in cancer pathogenesis with protein degraders versus small-molecule inhibitors. Heterobifunctional protein degraders mediate a transient interaction between an E3 ligase and a target protein, leading to ubiquitin (Ub) tagging of the target protein and its subsequent degradation by the proteasome. Small-molecule inhibitors bind to an active site or allosteric site on a target protein to block its activity.

complex and/or the subsequent degradation efficiency[24]. The lack of these direct relationships, as discussed in further detail below, might lead to unexpected and counterintuitive phenomena, including the generation of potent degraders from target-protein binders with low affinity for the protein of interest and dramatic improvements in the selectivity of target degradation compared with inhibition. The requisite ternary complex might also lead to a bell-shaped dose–response curve (termed the 'hook effect') with protein degraders, resulting from binary complex formation outcompeting ternary complex formation at high degrader concentrations[25]. Therefore, new terminology has arisen to describe protein degraders, notably the concentration to achieve half-maximal degradation (DC_{50}) of target proteins and the maximal degradation achieved (D_{max})[26]. Attention has also been dedicated to understanding the kinetics that underlie the mechanism of protein degrader action, the time dependence of DC_{50} and D_{max}, and additional descriptors beyond DC_{50} and D_{max}[27].

The earliest examples of protein degraders were peptidic in nature[14]. Protein degraders composed entirely of small molecules were first described in 2008 (ref. [28]) (Fig. 2). The discovery of 'all-small-molecule' protein degraders, together with the identification of high-quality, small-molecule E3 ligase ligands, has triggered a landslide of academic and industrial research activity (Fig. 2), culminating in the founding of multiple protein degrader-focused companies to explore the human therapeutic potential of the modality.

Protein degrader design and advances

Target selection

When considering the therapeutic potential of protein degraders, a crucial early decision related to which targets to pursue. Given the pharmacokinetic burden of relatively large, 'beyond rule of 5' molecules such as heterobifunctional protein degraders (see 'Pharmacokinetic considerations' section), the logical targets to pursue were those for which other therapeutic modalities had been tried and failed or could not be tried at all. A framework, the tenets of protein degrader targets, was proposed to inform target selection[29]. This framework consists of six general areas poised to take advantage of the unique, event-driven

Box 1

Molecular glues versus heterobifunctional protein degraders

Molecular glues are monovalent small molecules that induce the degradation of a target protein[20,21]. They contrast chemically with heterobifunctional protein degraders, which are bivalent, but share similarities in their overall mechanism of action. Classic molecular glues bind to and alter the substrate preference of an E3 ligase towards the target protein of interest. Other glues have been identified that mediate degradation by simultaneously binding to both an E3 ligase and the protein of interest in an induced pocket, or that induce degradation by destabilizing the protein of interest through aggregation[20,21]. Given the spectrum of possible binding events leading to degradation, molecular glues and protein degraders are best described as being on a mechanism of action continuum. Molecular glues that are being tested in clinical trials in patients with cancer include CC-220 (NCT02773030), CC-92480 (NCT03989414), CC-90009 (NCT02848001 and NCT04336982), CC-99282 (NCT04434196 and NCT03930953), CFT7455 (NCT04756726) and DKY709 (NCT03891953).

pharmacology of protein degraders compared with other therapeutic modalities: tenet 1, classically undruggable targets; tenet 2, resistance mutations; tenet 3, gene amplification and/or protein overexpression; tenet 4, differential isoform expression or localization; tenet 5, proteins with scaffolding function; and tenet 6, protein aggregates[29]. Classically undruggable oncology targets (tenet 1), such as KRAS[30] and the transcription factor signal transducer and activator of transcription 3 (STAT3)[31], have now been successfully degraded using protein degraders. The potential to degrade proteins without directly targeting an active site has been further demonstrated with protein degraders that target the myristate-binding site of the oncogenic fusion protein BCR–ABL1 (ref. [32]). Resistance to targeted oncology therapies is often driven by the emergence of mutations that impair inhibitor binding (tenet 2) and/or overexpression of the target (tenet 3) to the point that the drug can no longer be dosed to achieve sufficient occupancy for efficacy. Protein degraders have been described that can target the clinically relevant C481S mutant of BTK[33]. The C481S mutation in BTK precludes the covalent binding that underlies the activity of clinical BTK inhibitors, but the residual weak non-covalent binding affinity of those inhibitors is sufficient to be derivatized into active protein degraders. The continued dependence of metastatic castration-resistant prostate cancer (mCRPC) on the androgen receptor (AR), driven by mutations and overexpression in response to anti-androgen therapies[34–37], coupled with the known clinical validity of this target, was a primary driver in the selection of AR as the target for multiple protein degraders[38–41]. Single protein isoforms are often disease drivers, but achieving isoform selectivity with small-molecule inhibitors is challenging owing to a high degree of binding-site homology (tenet 4). Protein degraders can demonstrate emergent isoform degradation specificity, even when designed using non-selective target-protein binders, as in the case of the development of selective CDK4 or CDK6 degraders from dual CDK4/6 inhibitors[42]. Emergent selectivity of degraders, even within families of highly homologous proteins, is commonly observed and has been demonstrated to be a consequence of differential cooperativity in ternary complex formation[23,43]. Scaffolding proteins, which exert their function in complex with other proteins instead of through catalytic activity of their own, are difficult to directly target with traditional small molecules (tenet 5). Such proteins can, however, be targeted using protein degraders, as in the case of degraders of the IRAK3 pseudokinase[44]. Protein aggregates (tenet 6) are implicated in neurodegenerative disorders, including Alzheimer disease and Parkinson disease, and protein degrader approaches are also being explored in this area[45].

Choice of E3 ligase

More than 600 E3 ligases are known to be encoded in the human genome and are potentially available for recruitment by heterobifunctional protein degraders, although only about ten of these have been successfully used to date for targeted protein degradation. The first all-small-molecule protein degrader leveraged nutlin-based binders of the MDM2 ligase[28]. Degraders based on binders of the cellular inhibitor of apoptosis (cIAP) ligase, termed SNIPERs (specific and non-genetic inhibitor of apoptosis-dependent protein erasers), have also been developed[46]. However, the two E3 ligases that have become the workhorses of targeted protein degradation are von Hippel–Lindau (VHL) and cereblon (CRBN). The development of VHL-based protein degraders was first driven by the discovery of a potent and specific small-molecule VHL binder[47,48] that exploits the unique hydroxyproline recognition element in the HIF1α-binding site of VHL[49,50]. CRBN-based protein degraders, in turn, were enabled by the discovery that the so-called immunomodulatory imide drugs (IMiDs), a class of molecular glue degraders, bind to and modulate the substrate recognition function of CRBN[51–53]. These two ligases have achieved a privileged status because of the following characteristics: first, they can be engaged using existing, readily available, small-molecule binders with structural enablement for identification of linker attachment points; second, they have flexibility to robustly degrade a wide variety of targets; and third, their relatively ubiquitous expression enables high levels of systemic degradation to be achieved. However, exploitable exceptions to this ubiquitous expression exist; for example, low expression of VHL in platelets has been leveraged to deliver the clinical stage BCL-X_L degrader DT2216 with reduced potential for thrombocytopenia compared with small-molecule inhibitors of this anti-apoptotic protein[54].

Beyond CRBN and VHL is a vast open frontier of new ligand discovery for E3 ligases[55]. Increasing interest is being focused on the development of ligands for tissue-specific or tumour-specific E3 ligases, which might be of benefit when indiscriminate systemic target degradation could lead to unacceptable toxicities and a narrow therapeutic index. Common pan-essential gene products targeted in cancer with inhibitors include those involved in the cell cycle, epigenetic regulation, the DNA damage response or protein homeostasis and are frequently associated with narrow therapeutic indices[56]. Systemic degradation of such a pan-essential gene product would probably also cause substantial systemic toxicity, but restricted degradation in cancers via the engagement of tumour-specific E3 ligases could in principle provide a well-tolerated therapeutic approach. New discovery platforms based on covalent ligand screening have shown promise for the rapid identification of new E3 ligase binders, and several covalent tool ligands have now been identified for RNF4, RNF114, DCAF16, KEAP1, DCAF11 and FEM1B[57].

Target ligand design

Historically, heterobifunctional protein degraders have leveraged 'off-the-shelf' target-protein binders, originally designed as inhibitors without foresight towards evolution into degraders. As novel targets are explored for degradation, particularly classically undruggable targets, the need to identify new target-protein binders has intensified. DNA-encoded library (DEL) technology[58], a complementary approach for ligand identification for use in protein degraders, is agnostic to the binding site and functional activity of the ligand, and provides a potential linker attachment vector (at the point of DNA barcode attachment) in the absence of any further structural enablement. A proof-of-principle study using DEL for the discovery of new oestrogen receptor-α (ERα) ligands, which were subsequently turned into active protein degraders, was reported in 2021 (ref. [59]). DEL technology is also potentially useful for the discovery of new molecular glue degraders, E3 ligase ligands and whole-protein degraders[55,60,61].

Linker design

The chemical linker between the target protein and E3 ligase binders is an area of increasing medicinal chemistry focus in protein degrader design, a field of study colloquially termed linkerology. Linker length is routinely surveyed using simple alkyl or polyethylene glycol (PEG) linkers to find the optimal spacing between the target protein and E3 ligase. Too short a linker prevents the formation of a productive ternary complex owing to steric clash between the proteins; too long a linker might miss the opportunity to capitalize on positive cooperativity in the ternary complex[62]. In some cases, linkers are not bystanders in the ternary complex and can form their own contacts with protein surfaces[23]. Several studies have now demonstrated that conformational constraint of the linker can further enhance potency via reduction in degrees of freedom or locking in of a bioactive conformation[41,63–65]. Conformationally restricted linkers have been used as a potency driver in a series of SMARCA2/4 degraders[63], AR degraders[41,64] and the ER degrader ARV-471 (ref. [65]). Linker attachment points and the distance between the target-protein and E3 ligase binders can profoundly influence degradation selectivity, as demonstrated in the case of tuning p38 isoform degradation selectivity, and more broadly overall kinase degradation selectivity, starting from a relatively promiscuous target-protein binder[66]. Specifically with regard to the use of CRBN as the E3 ligase, a study showed that variation of the linker attachment point to the E3 ligase binder influenced both overall protein degrader aqueous stability and CRBN neosubstrate degradation[67]. Furthermore, linkers present an opportunity to tune the pharmacokinetic properties of a protein degrader (see 'Pharmacokinetic considerations' below).

Structure-aided design

The rational design of protein degraders informed by structural biology remains in its infancy. This challenge is compounded by difficulty in obtaining 3D structural images of the ternary complexes that are crucial to the protein degrader mechanism of action, whether via X-ray crystallography, cryo-electron microscopy or modelling. In a landmark study, an X-ray crystal structure obtained between a protein target of interest (BRD4) and an E3 ligase (VHL) mediated by a protein degrader (MZ1) demonstrated extensive protein–protein contacts in the ternary interface that drive ternary binding cooperativity, involvement of the linker in protein–ligand interactions and, thus, a potential explanation for degradation selectivity within the BRD family[23]. This structure also revealed a path to linker optimization that involves a different attachment vector, constituting the first reported example of structure-based linker design reported for a protein degrader. Structural biology has subsequently been successfully used in the case of SMARCA2/4–VHL ternary complexes to optimize a protein degrader linker for improved degradation potency[63]. In another study, a set of X-ray crystal structures of protein degrader-mediated ternary complexes between BTK and cIAP indicated a high degree of conformational plasticity, dependent on the degrader linker length[68], implying difficulty in subsequent compound optimization based on such structures. On the computational side, a suite of in silico tools from various groups has emerged to predict docking poses of protein degraders[69–72].

Pharmacokinetic considerations

As a class, heterobifunctional protein degraders lie predominantly in the 'beyond the rule of 5' space[73], meaning that they possess physicochemical properties (molecular weight, hydrogen bond donor and/or acceptor counts, and octanol–water partition coefficient, among others) outside the ranges commonly associated with probable oral absorption[74]. Nonetheless, the presence of multiple orally bioavailable protein degraders in clinical development speaks to the need for a reconsideration of the chemical properties associated with oral absorption. Although the rule of 5 presents a window of probable oral absorption, several reports have attempted to describe the boundaries of a possible oral absorption space beyond the rule of 5 space with physicochemical descriptors, both generally[75] and for protein degraders specifically[76,77]. A clear set of physicochemical property descriptors specifically for orally bioavailable protein degraders has not yet been reported, although Pike et al.[77] noted that IMiD-based, CRBN-recruiting degraders are overall more drug-like than those that recruit other E3 ligases based on their lower molecular weight, hydrogen bond donor and/or acceptor counts and lipophilicity. Only a few systematic studies have reported on the pharmacokinetics of heterobifunctional protein

Box 2

Alternative chimeric molecule approaches as cancer treatment

The concept of proximity-induced pharmacology with heterobifunctional small molecules, pioneered with protein degraders for targeted protein degradation, has been shown to be extensible to other modes of degradation, other post-translational modifications and other types of target molecule (such as RNA)[22]. Other examples of small-molecule proximity-induced pharmacology include:

- Lysosomal and autophagosomal degradation: lysosome-targeting chimeras (LYTACs); macroautophagy degradation-targeting chimeras (MADTACs); autophagy-targeting chimeras (AUTACs); and autophagosome-tethering compounds (ATTECs)
- RNA degradation: ribosomal targeting chimeras (RIBOTACs)
- Protein phosphorylation and dephosphorylation: phosphorylation-inducing chimeric small molecules (PHICS); phosphorylation targeting chimeras (PhosTACs); and phosphatase recruiting chimeras (PHORCs)
- Protein acetylation: acetylation tagging system (AceTAG)
- Protein deubiquitination: deubiquitinase-targeting chimeras (DUBTACs)

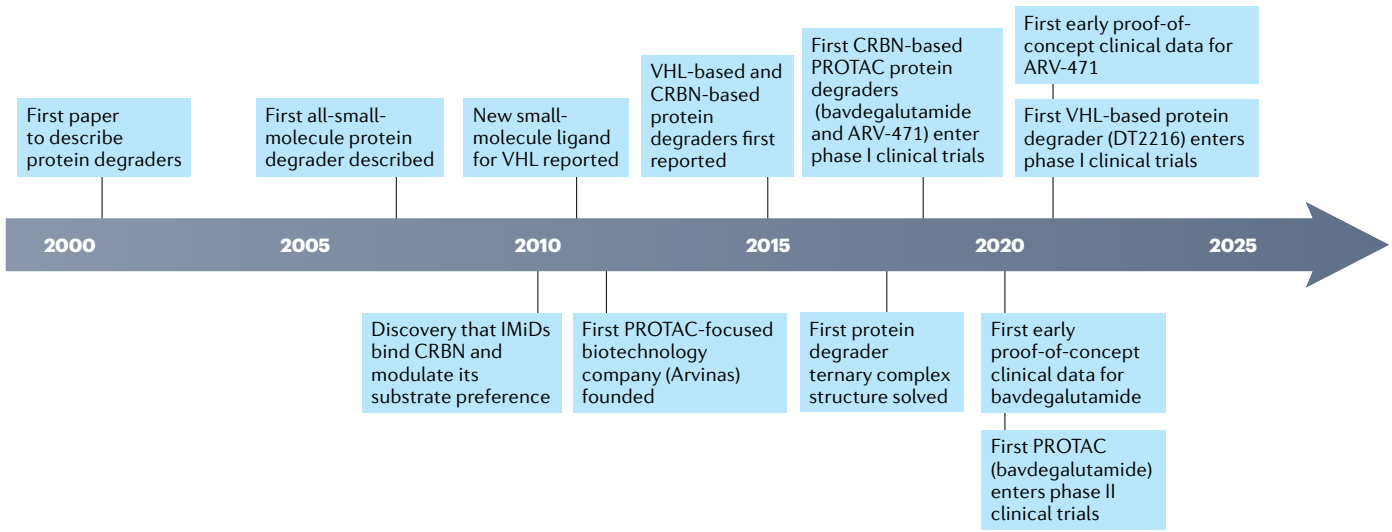

Fig. 2 | Timeline of key advances in protein degrader development. Extraordinary progress has been made since the first publication on heterobifunctional protein degrader technology in 2001, including novel designs and refinement of the molecules through medicinal chemistry that ultimately led to initiation of clinical trials of protein degraders in patients with cancer. CRBN, cereblon; IMiD, immunomodulatory imide drug; PROTAC, PROteolysis TArgeting Chimera; VHL, von Hippel–Lindau.

degraders within a given chemical series, but the available reports demonstrate that optimization of degraders to achieve adequate levels of oral absorption is possible[64,78]. However, data from a small number of more-comprehensive studies have increased awareness that simple alkyl and PEG linkers might have metabolic liabilities and are associated with a tendency for high protein degrader clearance rates[79]. More conformationally constrained, bespoke linker designs afford the potential to reduce metabolism and/or enhance oral absorption, for example, via the introduction of solubilizing groups such as basic amines[41,64,65].

Protein degraders are anticipated to show nonlinear pharmacodynamics based on the irreversible and time-dependent step (proteasomal degradation) integral to their mechanism of action. Once a protein has been degraded, the recovery of the protein is dictated by its intrinsic resynthesis rate. For proteins with long resynthesis rates, this latency can lead to large disconnects between protein degrader concentrations at a point in time and the degradation observed at the same point in time. An extreme case of this phenomenon has been demonstrated, whereby a single subcutaneous administration of an RIPK2-targeting protein degrader in rats is sufficient to suppress RIPK2 protein levels for >168 h, well beyond the ~72 h duration of the degrader concentration being maintained above the whole-blood IC_{90} (ref. [80]). Owing to slow subcutaneous release of the protein degrader, suppression of RIPK2 can persist for longer than 1 month after a single dose[81]. Beyond experimental studies, additional efforts have been directed towards prospective modelling of protein degrader pharmacokinetic–pharmacodynamic relationships[82,83].

Preclinical evidence

The breakthroughs in protein degrader design described above have paved the way for early phase clinical trials of these agents. To our knowledge, 18 heterobifunctional protein degraders are now undergoing evaluation in phase I–III clinical trials in patients with cancer as of 8 January 2023 (Supplementary Table 1). Here, we review the preclinical data that supported the move of these drugs from laboratory to clinical studies. The following summary relies on data that have been published or presented at scientific meetings; data that have been disclosed only on corporate websites or industry events are not included.

AR-targeting protein degraders

The key role of androgen signalling in driving prostate cancer progression[34–37] made the AR an appealing protein degrader target. Moreover, several drugs that impede the AR pathway are standard treatments for patients with mCRPC[84] and provide benchmarks for new AR-targeting agents. Accordingly, five protein degraders that target the AR (bavdegalutamide (previously known as ARV-110), CC-94676, AC176, HP518 and ARV-766) are in clinical trials in patients with metastatic prostate cancer. Preclinical data for bavdegalutamide have been reported at several scientific meetings, whereas data for the remaining AR degraders have not been presented or published to date and, therefore, are not discussed here.

Bavdegalutamide, a PROTAC protein degrader, contains a cyclohexyl moiety that binds to the AR ligand-binding domain and engages the CRBN-containing E3 ubiquitin ligase via an IMiD-based moiety to enable AR polyubiquitination[41]. Bavdegalutamide induced potent degradation of the AR in both vertebral cancer of the prostate (VCaP) and lymph-node carcinoma of the prostate (LNCaP) cell lines, with a DC_{50} of ~1 nM[39,41]. In a proteomic screen of nearly 4,000 detectable proteins in VCaP cells, treatment with 10 nM bavdegalutamide for 8 h led to selective AR degradation with a D_{max} of 85%[39,41]. In addition to wild-type AR, bavdegalutamide degraded certain clinically relevant AR mutants (specifically, T878A, H875Y, F877L and M896V variants) that are associated with resistance to novel hormonal agents, including abiraterone and enzalutamide[39].

In comparison with enzalutamide (which is an AR antagonist approved for the treatment of men with prostate cancer), bavdegalutamide resulted in greater inhibition of prostate-specific antigen (PSA) synthesis and cellular proliferation as well as greater induction of apoptosis in prostate cancer cell lines[39]. The activity of bavdegalutamide

has been tested in various animal models of prostate cancer. In both castrated and non-castrated mice harbouring VCaP tumours, oral bavdegalutamide demonstrated substantially greater tumour growth inhibition than enzalutamide, and in an AR-expressing patient-derived xenograft (PDX) model (TM00298), 100% inhibition of tumour growth by bavdegalutamide was accompanied by a >90% reduction in PSA levels[39,41].

Bavdegalutamide has also been tested in mouse models of tumours with resistance to approved AR-targeting agents, including enzalutamide and the androgen biosynthesis inhibitor abiraterone. Treatment with bavdegalutamide at doses of 3 mg/kg and 10 mg/kg once daily inhibited tumour growth by 60% and 70%, respectively, relative to vehicle alone in an enzalutamide-resistant VCaP model[39]. A three-phase preclinical study in castrated mice bearing VCaP tumour xenografts evaluated treatment with abiraterone alone, bavdegalutamide alone or the combination of abiraterone and bavdegalutamide (phase 1), followed by treatment with abiraterone alone until the development of resistance (phase 2) and then randomization to abiraterone or bavdegalutamide treatment (phase 3)[85]. In phase 1, the abiraterone–bavdegalutamide combination showed greater tumour growth inhibition than either agent alone, and in phase 3, bavdegalutamide reduced the volume of abiraterone-resistant tumours as compared with abiraterone retreatment. These data suggest the potential for enhanced clinical activity with an abiraterone–bavdegalutamide combination as well as for bavdegalutamide as an add-on therapy to abiraterone at the time of biochemical progression (rising PSA levels), to overcome abiraterone resistance; the latter hypothesis is being tested in a phase Ib clinical trial[85] (NCT05177042; Supplementary Table 1).

ER-targeting protein degraders

ER^+ breast cancer accounts for most breast cancer cases in women, and several endocrine therapies that block ER activity are approved to treat various stages of this disease[86,87]. The ER antagonist fulvestrant acts, in part, by inhibiting nuclear translocation of the ER, leading to proteasomal degradation of this protein[87]. Therefore, fulvestrant has confirmed the value of ER degradation as a treatment approach[88]; however, up to 50% of baseline ER levels remain after fulvestrant treatment[89,90]. In addition, patients can develop mutations in the gene that encodes the ER (*ESR1*) during treatment with endocrine therapy[86,87], and some of these alterations might reduce the sensitivity of cells to fulvestrant and other investigational selective ER degraders (SERDs)[91]. To address the deficiencies of fulvestrant as an ER degrader, two CRBN-based protein degraders that target the ER (ARV-471 and AC682) are in clinical development for the treatment of ER^+ breast cancer (Supplementary Table 1), and preclinical data have been reported at scientific meetings.

In preclinical analyses in numerous breast cancer cell lines, the PROTAC ARV-471 resulted in degradation of wild-type ER, with a DC_{50} of 1–2 nM; ER mutants, such as Y537S and D538G, were also degraded following exposure to ARV-471 (refs. [65,92]). Moreover, ARV-471 monotherapy has encouraging antitumour activity in several models of ER-dependent breast cancer[65,92]. In an orthotopic, oestradiol-dependent MCF7 xenograft model, treatment with ARV-471 at doses of 10 mg/kg or 30 mg/kg once daily resulted in tumour growth inhibition of >90% (with evidence of tumour regression, that is, >100% tumour growth inhibition, at the 30 mg/kg dose), compared with only 46% by fulvestrant, with concurrent reduction of tumoural ER levels by >90%[92]. Once-daily doses of 30 mg/kg ARV-471 also resulted in 65% tumour growth inhibition and a 73% decrease in tumoural ER levels in a tamoxifen-resistant MCF7 xenograft model[92]. Additionally, in an *ESR1*-mutant (ER^{Y537S}) PDX model, ARV-471 inhibited tumour growth by 99% and 106% at once-daily doses of 10 mg/kg and 30 mg/kg, respectively, compared with 62% with twice-weekly doses of 200 mg/kg fulvestrant; the associated levels of ER^{Y537S} degradation were 79% and 88%, respectively, with ARV-471 versus 63% with fulvestrant[65,92].

Combination of ARV-471 with the CDK4/6 inhibitor palbociclib has been tested in preclinical models. In the oestradiol-dependent MCF7 xenograft model, treatment with 30 mg/kg ARV-471 once daily plus 60 mg/kg palbociclib once daily resulted in greater tumour growth inhibition than either agent alone[92]. This combination yielded 131% tumour growth inhibition compared with 108% tumour growth inhibition with the combination of 200 mg/kg fulvestrant twice weekly plus 60 mg/kg palbociclib once daily[65,92]. These findings support the ARV-471–palbociclib combination cohort that is part of the first-in-human phase I/II trial of ARV-471 in patients with advanced-stage ER^+HER2^- breast cancer[93] (NCT04072952; Supplementary Table 1).

The second ER-targeting protein degrader, AC682, induces degradation of ER at a subnanomolar DC_{50} in several breast cancer cell lines, including those expressing the ER mutants Y537S and D538G, as well as in tamoxifen-resistant, oestrogen-deprived cells, with peak activity after a few hours of treatment[94]. Degradation of the ER by AC682 translates into reduced expression of ER-regulated genes and inhibition of cell proliferation[94]. AC682 results in tumour growth inhibition or regression with >90% decreases in tumoural ER levels in the oestradiol-dependent MCF7 xenograft model, with tumour stasis observed at a dose of 3 mg/kg daily[94]. In an *ESR1*-mutant (ER^{Y537S}) PDX model, AC682 resulted in substantially greater tumour growth inhibition than fulvestrant[94]. Administration of AC682 in combination with palbociclib in both oestradiol-dependent and tamoxifen-resistant MCF7 models suggested synergistic activity of these agents[94].

BTK-targeting protein degraders

BTK is a key component of signalling pathways that lead to the activation, proliferation and survival of B cells. Accordingly, several small-molecule inhibitors of BTK are approved (such as ibrutinib) or are in clinical development for the treatment of B cell malignancies; however, the efficacy of these agents is limited by the development of resistance mutations in *BTK*[95]. Four CRBN-based protein degraders that target BTK (NX-2127, NX-5948, BGB-16673 and HSK29116) are in clinical development for patients with B cell malignancies (Supplementary Table 1). Preclinical data for NX-2127 (refs. [96–98]) and NX-5948 (ref. [99]) have been disclosed at scientific meetings, but findings for BGB-16673 and HSK29116 are not yet available.

NX-2127 induces degradation of wild-type BTK in diffuse large B cell lymphoma (DLBCL) and mantle-cell lymphoma (MCL) cell lines, with DC_{50} values of 4 nM and 4–6 nM, respectively; additionally, NX-2127 results in degradation of the ibrutinib-resistant BTK^{C481S} mutant (DC_{50} 13 nM) and thus blocks proliferation of ibrutinib-resistant DLBCL cells[96]. NX-2127 has activity similar to IMiDs via CRBN-induced degradation of the neosubstrates Ikaros and Aiolos, resulting in T cell activation and IL-2 production[96]. NX-2127 at doses of 30 mg/kg and 90 mg/kg has been shown to inhibit tumour growth in mouse xenograft models of lymphoma expressing wild-type or C481S-mutant BTK[96]. Substantial degradation of BTK in B cells has been observed with oral administration of 1 mg/kg, 3 mg/kg and 10 mg/kg NX-2127 to cynomolgus monkeys[96]. In a subsequent presentation[97], NX-2127 was shown to more potently reduce the in vitro viability of DLBCL and MCL cells than the BTK inhibitors ibrutinib, acalabrutinib and pirtobrutinib or the IMiDs pomalidomide and lenalidomide. RNA sequencing revealed that

exposure to NX-2127 resulted in different gene-expression profiles in MCL cells from those associated with BTK inhibitor or IMiD exposure; in contrast to ibrutinib or pomalidomide, NX-2127 downregulated genes involved in DNA replication and repair, the cell cycle and survival signalling pathways[97]. In addition, NX-2127 was associated with increased expression of CD1c, which is involved in T cell recognition of cancer cells[97]. NX-2127 has been shown to bind to and induce degradation of multiple BTK inhibitor-resistant mutants in addition to the C481S variant (including the kinase-dead L528W and V416L mutants with a scaffold function), in several cases with comparable kinetics to wild-type BTK[98]. NX-2127 reduced expression of CD86, a marker for B cell activation, in cells expressing wild-type or mutant BTK and more potently induced killing of cells expressing wild-type or mutant BTK than small-molecule BTK inhibitors or IMiDs[98].

The protein degrader NX-5948 was designed to target BTK for degradation but, unlike NX-2127, lacks the ability to degrade Ikaros or Aiolos, thus precluding immunomodulatory effects associated with the degradation of these substrates[99]. NX-5948 results in degradation of both wild-type and C481S-mutant BTK in DLBCL cell lines with a DC_{50} of 0.32 nM and 1.0 nM, respectively, with reduced cell viability also observed[99]. Selective degradation of BTK by NX-5948 was confirmed in a proteomic analysis in DLBCL cells[99]. In mouse xenograft models of ibrutinib-resistant DLBCL, NX-5948 at doses of 3 mg/kg, 10 mg/kg and 30 mg/kg inhibited tumour growth by 36.3%, 99.0% and 99.7%, respectively[99]. Notably, NX-5948 was shown to permeate through the blood–brain barrier, inducing BTK degradation by >80% in implanted DLBCL cells and microglia in the brain[99]. Accordingly, NX-5948 reduced the tumour burden of intracranial DLBCL cells and prolonged survival compared with vehicle in a mouse model[99].

BRD9-targeting protein degraders

Bromodomain-containing protein 9 (BRD9) is a component of the aberrant BAF chromatin remodelling complex that also contains the oncogenic SS18–SSX fusion protein that is implicated in the development of synovial sarcoma[100]. Two protein degraders that target BRD9, CFT8634 and FHD-609, are being evaluated in patients with advanced-stage synovial sarcoma (Supplementary Table 1).

CFT8634 is a CRBN-based protein degrader that selectively degrades BRD9 with a DC_{50} of 2.7 nM after 2 h of treatment[101]. In a proteomic screen in the HSSYII synovial sarcoma cell line, treatment with 100 nM CFT8634 for 4 h yielded substantial degradation of only BRD9 among >9,000 quantified proteins[101]. CFT8634 induced degradation of BRD9 in both a synovial sarcoma (SS18–SSX1 fusion-positive) cell line and a soft-tissue sarcoma (BAF wild-type) cell line[101]. Dose-proportional exposure of CFT8634 was demonstrated in a synovial sarcoma cell line-derived xenograft model, and CFT8634 at doses ranging from 1 mg/kg to 50 mg/kg once daily induced robust tumour growth inhibition in two different PDX models of synovial sarcoma[101]. In one of these PDX models, tumour regression persisted after withdrawal of CFT8634 treatment[101].

FHD-609, the second protein degrader that targets BRD9, induced BRD9 degradation in tumour tissue of a xenograft model of synovial sarcoma after 3 h of treatment at a dose of 0.1 mg/kg or 3 mg/kg, although multiple doses of the 3 mg/kg dose were needed for complete degradation[102]. RNA sequencing of xenografts 24 h after treatment with FHD-609 showed decreased expression of MYC compared with vehicle treatment, as well as decreased expression of genes activated by MYC and increased expression of genes repressed by MYC; this effect was greater at the higher dose of FHD-609 and after multiple doses[102].

Protein degraders that target other proteins involved in cancer pathogenesis

DT2216 is a VHL-based protein degrader that targets the anti-apoptotic protein BCL-X_L. DT2216 was selected among other candidates for clinical development in patients with various solid tumours on the basis of its potent degradation of BCL-X_L (DC_{50} 63 nM after 16 h of treatment) and cytotoxic effects in T cell acute lymphoblastic leukaemia (T-ALL) cells but not in platelets[54]. DT2216 has reported binding affinity for other BCL-2 family members, but selectively degrades BCL-X_L without affecting protein levels of BCL-2, BCL-W or MCL-1 (ref. [54]). Once-weekly dosing of DT2216 led to substantial tumour growth inhibition in a mouse xenograft model of T-ALL[54]. A subsequent publication reported cytotoxic activity of DT2216 towards various BCL-X_L-dependent T cell lymphoma cell lines and inhibition of tumour growth in a mouse xenograft model of BCL-X_L-dependent T cell lymphoma[103]. Treatment of various solid tumour specimens with DT2216 depleted BCL-X_L from the tumour microenvironment, which led to elimination of regulatory T (T_{reg}) cells[104]. These findings suggest the therapeutic potential of DT2216 in cancers that are dependent on BCL-X_L for survival and those in which T_{reg} cells have a key role in maintaining an immunosuppressive microenvironment that enables tumour progression, which is the case in numerous solid tumours[105]. In a study reported in 2023, BCL-X_L levels and survival were evaluated in 13 T-ALL cell lines after treatment with DT2216 (ref. [106]). DT2216 potently inhibited cell growth in 12 of these cell lines regardless of pretreatment BCL-X_L expression; only one cell line required DT2216 concentrations that exceeded those reported in the mouse xenograft model of T-ALL and was associated with reduced efficiency of BCL-X_L degradation[54,106].

KT-413 (previously known as KTX-120) is a CRBN-based protein degrader that targets IL-1 receptor-associated kinase 4 (IRAK4), a protein involved in transmitting signals downstream of Toll-like receptors as part of the innate immune response to pathogens. Similar to NX-2127, KT-413 has degradation activity towards Ikaros and Aiolos in addition to its target protein[107]. KT-413 induces selective and potent degradation of IRAK4 (DC_{50} 8 nM after 16–24 h of treatment) and inhibition of cell growth in *MYD88*-mutant DLBCL cell lines (half-maximal inhibitory concentration 7–29 nM)[107]. Intermittent oral or intravenous dosing of KT-413 results in tumour growth inhibition, including regression, in several mouse *MYD88*-mutant xenograft models and PDX models of DLBCL[107]. KT-413 was subsequently shown to inhibit MYD88-dependent nuclear factor-κB (NF-κB) transcription and induce type 1 interferon signalling, based on downregulation of interferon-regulatory factor 4 (IRF4) and upregulation of IRF7 (ref. [108]). In addition, downregulation of DNA replication and cell cycle genes and activation of pro-apoptotic and antiproliferative genes was observed with KT-413 (ref. [108]).

KT-333 is a protein degrader that targets STAT3, a transcriptional activator involved in cell proliferation and apoptosis. KT-333 induces potent degradation of STAT3 in solid tumour cell lines, lymphoma cell lines and primary immune cells (DC_{50} < 10 nM in most cell types); degradation of STAT3 was highly selective in a lymphoma cell line[109]. Accordingly, KT-333 reduces the proliferation and induces apoptosis of lymphoma cells in vitro, and weekly intravenous KT-333 doses that range from 5 mg/kg to 45 mg/kg or biweekly intravenous doses that range from 10 mg/kg to 40 mg/kg inhibit tumour growth in mouse xenograft models of lymphoma[109]. On the basis of pharmacodynamic efficacy simulations, a dose of >1 mg/kg weekly of KT-333 is predicted to inhibit tumour growth in humans[109].

ASP3082 is a protein degrader that targets $KRAS^{G12D}$, a mutant form of a GTPase that regulates cell proliferation and survival via the

mitogen-activated protein kinase pathway, that has been associated with multiple solid tumours, including pancreatic cancer, colorectal cancer and lung cancer[110]. ASP3082 induced potent degradation of $KRAS^{G12D}$ in pancreatic cancer cells harbouring this mutation and inhibition of ERK phosphorylation, a downstream signal of KRAS[111]. A quantitative proteomics assay showed selective degradation of $KRAS^{G12D}$ among >9,000 proteins[111]. In a xenograft model of *KRAS*^{G12D}-mutant pancreatic ductal adenocarcinoma, ASP3082 administered intravenously once weekly resulted in dose-dependent tumour growth inhibition, including tumour regression[111]. A single intravenous dose of ASP3082 led to sustained drug concentrations[111].

CFT1946 is a CRBN-based protein degrader that targets $BRAF^{V600X}$, a mutant form of a serine/threonine protein kinase that is a downstream effector of RAS, that has been seen in various cancers including melanoma, colorectal cancer and lung cancer; several small-molecule $BRAF^{V600X}$ inhibitors have been approved for the treatment of patients with *BRAF*$^{V600E/K}$-mutant cancers[112], making $BRAF^{V600X}$ a rational target for protein degrader technology. CFT1946 selectively targeted $BRAF^{V600E}$ among nearly 9,000 proteins in a melanoma cell line, induced degradation of $BRAF^{V600E}$ (but not wild-type BRAF) in a dose-dependent manner with a DC_{50} of 14 nM at 24 h and inhibited ERK phosphorylation[113]. In a mouse xenograft model of *BRAF*^{V600E}-mutant melanoma, a twice-daily dose of 10 mg/kg CFT1946 led to tumour regression and was considered to be the minimum efficacious dose[113]. In a *BRAF*^{V600E}*NRAS*^{Q61K}-mutant xenograft model of resistance to BRAF inhibitors, CFT1946 showed stronger inhibition of ERK phosphorylation than the BRAF inhibitor encorafenib; in addition, the combination of CFT1946 with the MEK inhibitor trametinib reduced ERK phosphorylation to a greater extent than encorafenib plus trametinib and showed greater tumour growth inhibition than CFT1946, trametinib or encorafenib alone[113]. CFT1946 also demonstrated degradation of non-V600E BRAF mutants that were ectopically expressed in a human cell line[113].

Available clinical evidence

In 2019, the PROTAC AR degrader bavdegalutamide became the first drug of this class to enter clinical trials in a phase I study in patients with mCRPC (Fig. 3). The designs for several clinical trials of protein degraders as cancer treatment (Supplementary Table 1) were presented at scientific meetings in 2022 (refs. [85,93,114–118]). To date, however, clinical data from ongoing oncology studies have been disclosed only for the PROTAC AR degrader bavdegalutamide, the PROTAC ER degrader ARV-471 and the BTK degrader NX-2127.

Bavdegalutamide clinical data

The initial phase I/II trial of bavdegalutamide (NCT03888612) enrolled heavily pretreated patients with mCRPC who had exhausted approved treatment options and thus present an unmet need for novel therapies. As a first-in-human trial of a first-in-class therapy, the aims of the phase I portion of this trial were to evaluate safety and tolerability of bavdegalutamide to determine the maximum tolerated dose and identify a recommended phase II dose (RP2D). In this dose-escalation phase, bavdegalutamide at doses ranging from 35 mg to 700 mg once daily or 140 mg to 420 mg twice daily were orally administered to men with mCRPC who had received at least two prior therapies (including abiraterone and/or enzalutamide) and experienced disease progression on their most recent therapy[38,40,41].

The first presentation of data from this study reported results from the initial 22 patients treated with bavdegalutamide, of whom 77% had previously received both abiraterone and enzalutamide, and 77% had received prior chemotherapy[40]. Key findings included histological evidence of AR degradation with a decrease in AR staining after treatment with bavdegalutamide and further proof of concept with clinical responses[40]. Treatment-related adverse events (TRAEs) reported in ≥20% of these patients were nausea (27%), diarrhoea (27%) and fatigue (23%)[40]. At doses from 35 mg to 280 mg once daily, bavdegalutamide exposure was dose-proportional[40], and doses of ≥140 mg once daily yielded exposure above the efficacious threshold based on tumour growth inhibition in castrated and non-castrated VCaP mouse models[39]. Together, the safety and pharmacokinetic data supported further dose escalation. Decreased levels of AR protein were observed in tumour tissue of a patient after 6 weeks of bavdegalutamide treatment (Fig. 3a), providing clinical proof of concept for the protein degrader mechanism of action. In this highly refractory mCRPC population, two patients had clinically relevant responses with bavdegalutamide, with serum PSA reductions of 74% and 97%, the latter associated with a confirmed RECIST partial response (Fig. 3b). A subsequent analysis of the phase I dose-escalation cohort provided further support for an exposure–activity relationship for bavdegalutamide, and showed that with a total daily dose of 420 mg, exposure exceeded the predicted efficacious threshold based on tumour growth inhibition in a preclinical enzalutamide-resistant model[41]; on the basis of safety, pharmacokinetic and efficacy findings, 420 mg once daily was selected as the RP2D[41].

A notable finding from this phase I study was enhanced activity of bavdegalutamide in a biomarker-defined patient subset; in five patients with prior exposure to novel hormonal agents and tumours harbouring *AR* T878 and/or H875 mutations (AR 878/875 positive), which have previously been shown to confer resistance to novel hormonal agents[34,119–122], the rate of best serum PSA declines ≥ 50% (PSA_{50}) was 40%[38]. Bavdegalutamide has degradation kinetics towards AR^{T878A} and AR^{H875Y} similar to those towards wild-type AR in vitro — notably, evidence that other drugs can target altered versions of the AR is lacking[39]. Thus, preclinical and early clinical data obtained with bavdegalutamide spurred the design of the phase II expansion (ARDENT) cohort in patients with confirmed mCRPC. Patients who had received one or two prior novel hormonal agents (for example, abiraterone and/or enzalutamide) and no more than one chemotherapy regimen each for CRPC and castration-sensitive prostate cancer were enrolled in biomarker-defined subgroups based on tumour DNA sequencing: an AR 878/875 subgroup, a subgroup with tumours harbouring wild-type AR or other AR alterations (AR WT/Other) and a subgroup with tumours harbouring *AR*^{L702H} mutation or the AR-V7 splice variant (AR 702/V7); in preclinical studies, bavdegalutamide was a less potent degrader of AR^{L702H} and did not degrade AR-V7. Patients who had received only one prior novel hormonal agent and no prior chemotherapy were enrolled in a clinically defined, biomarker-agnostic subgroup ('Less Pretreated'). Patients in the AR 702/V7 and Less Pretreated subgroups could also have AR 878/875 mutations.

On the basis of the most recent presentation of data from this phase I/II trial of bavdegalutamide (as of the 20 December 2021 data cut-off), 195 patients had been enrolled (71 in phase I and 124 in phase II)[38]. The median number of prior lines of therapy was six in the phase I portion (69% with both prior abiraterone and enzalutamide and 75% with prior chemotherapy) and four in the phase II portion (39% with both prior abiraterone and enzalutamide and 31% with prior chemotherapy), noting that the phase II study included the Less Pretreated subgroup[38]. Across 152 patients in the phase I/II population who were biomarker-evaluable and PSA-evaluable, the PSA_{50} rate was 17% and the rate of best PSA

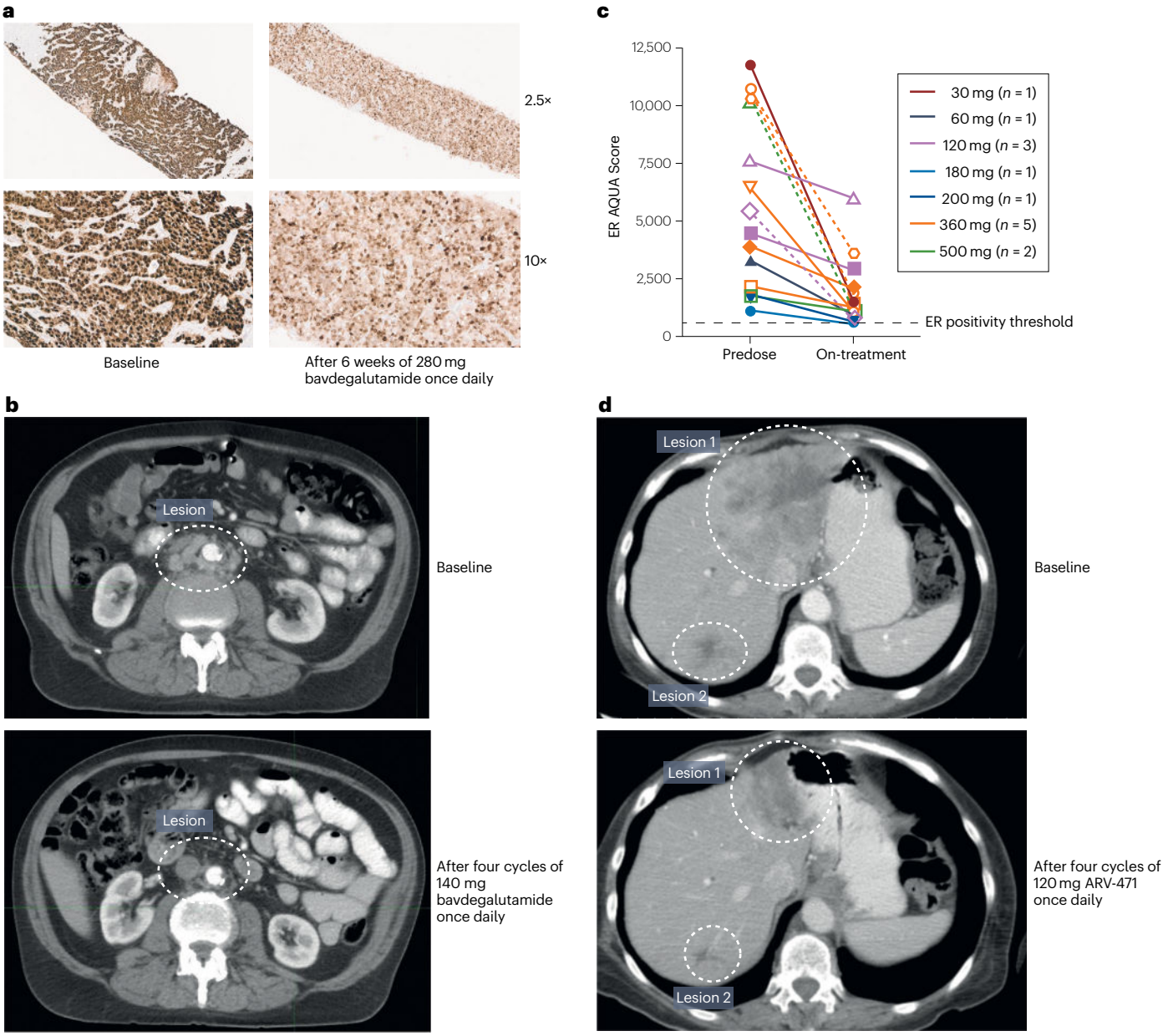

Fig. 3 | Clinical proof of concept for PROTAC protein degraders. **a**, Immunohistochemistry images demonstrating decreased androgen receptor (AR) protein levels in a wild-type-*AR*-amplified tumour from a patient with metastatic castration-resistant prostate cancer (mCRPC) after 6 weeks of 280 mg of bavdegalutamide once daily[40]. **b**, CT images showing near-complete resolution of retroperitoneal lymphadenopathy in a patient with $AR^{T878A/H875Y}$-mutant mCRPC after four 28-day cycles of 140 mg of bavdegalutamide once daily[40,41]. **c**, Quantitative immunofluorescence data demonstrating decreased oestrogen receptor (ER) protein levels in tumour biopsy samples from patients with breast cancer after a median of 31 days (range 16–77) of once-daily treatment with doses of ARV-471 used in phase I dose escalation[123]. Dashed lines, wild-type *ESR1*; solid lines, mutant *ESR1*. **d**, CT images showing reductions in size of target lesions in the liver of a patient with breast cancer harbouring an $ESR1^{D538G}$ mutation after four 28-day cycles of 120 mg of ARV-471 once daily[123]. AQUA, automated quantitative analysis; PROTAC, PROteolysis TArgeting Chimera.

declines ≥ 30% (PSA_{30}) was 31%[38]. In 28 patients with AR 878/875-positive tumours, regardless of study phase or subgroup, the PSA_{50} rate was 46% and the PSA_{30} rate was 57%[38]. The PSA_{50} rates in biomarker-evaluable and PSA-evaluable patients in the phase II ARDENT cohort were 75% in the AR 878/875 subgroup ($n = 8$), 11% in the AR WT/Other subgroup ($n = 44$), 4% in the AR 702/V7 subgroup ($n = 25$) and 22% in the Less Pretreated subgroup ($n = 27$)[38]. Together, these data demonstrate clinical activity of bavdegalutamide in patients with mCRPC, many of whom were heavily pretreated and all of whom had disease progression on prior AR-directed therapy. Moreover, the study helped to delineate the

biology of prostate cancer in patients who were previously exposed to novel hormonal agents, indicating that mCRPC might remain heavily AR dependent in the setting of *AR* mutations associated with drug resistance; patients with AR 878/875-positive tumours might constitute a population particularly sensitive to bavdegalutamide.

TRAEs reported in >20% of 138 patients treated at the RP2D across the phase I and II portions were nausea (48%), fatigue (36%), vomiting (26%), decreased appetite (25%) and diarrhoea (20%)[38]. TRAEs were generally grade 1 or 2, with no grade 4 or higher events, and infrequently led to bavdegalutamide dose reduction (in 8% of patients) or treatment discontinuation (9%)[38]. These results substantiate the tolerability profile seen in the earlier analysis of the phase I data[40], with no evidence of off-target effects of bavdegalutamide. Further investigation of bavdegalutamide in patients with mCRPC is planned[38].

ARV-471 clinical data

Protein degrader clinical activity was corroborated with early data from the first-in-human phase I/II trial (NCT04072952) of the PROTAC ER degrader ARV-471 in patients with locally advanced or metastatic ER^+HER2^- breast cancer. In the phase I dose-escalation portion of this study, patients had received at least one prior CDK4/6 inhibitor, at least two prior endocrine therapies and no more than three prior lines of chemotherapy; as of the 30 September 2021 data cut-off, 60 patients had been treated with total daily ARV-471 oral doses ranging from 30 mg to 700 mg in this portion of the study[123]. Patients had received a median of four prior lines of therapy (100% with prior CDK4/6 inhibitors, 80% with prior fulvestrant and 78% with prior chemotherapy)[123]. ARV-471 was well tolerated across dose levels, with nausea (28%) and fatigue (20%) being the only TRAEs reported in ≥20% of patients, and no dose-limiting toxicities or grade ≥4 TRAEs[123]. Preliminary data showed dose-related increases in pharmacokinetic parameters for total daily doses from 30 mg to 500 mg[123]. Clinical proof of concept for the mechanism of action of ARV-471 was confirmed by robust ER degradation (up to 89%) shown by quantitative ER immunofluorescence in post-treatment tumour biopsy samples; ER degradation occurred regardless of *ESR1* mutation status with a median value of 67% across dose levels (Fig. 3c). This initial dataset also revealed encouraging clinical activity in patients with ER^+ breast cancer. The clinical benefit rate (rate of confirmed complete or partial response or stable disease lasting at least 24 weeks; the primary end point of the study) in 47 evaluable patients was 40% (95% CI 26–56%)[123]. In all, three evaluable patients had confirmed partial responses (Fig. 3d), and tumour shrinkage was seen across dose cohorts.

The phase II cohort expansion portion (VERITAC) of this phase I/II trial is evaluating two doses of ARV-471 (200 mg and 500 mg once daily) based on the safety, pharmacokinetic and efficacy data from the phase I portion, and the first dataset was presented in December 2022 (ref. [124]). As of the 6 June 2022 data cut-off, 71 patients with advanced-stage ER^+HER2^- breast cancer and a median of four prior lines of therapy (100% with a prior CDK4/6 inhibitor, 79% with prior fulvestrant and 73% with prior chemotherapy (45% in the metastatic setting)) received oral ARV-471 (ref. [124]). The clinical benefit rate was 37.1% (95% CI 21–55%) in 35 evaluable patients at the 200 mg dose and 38.9% (95% CI 23–57%) in 36 evaluable patients at the 500 mg dose[124]. The clinical benefit rates among patients with *ESR1* mutations were 47.4% (95% CI 24–71%) in 19 evaluable patients in the 200 mg dose cohort and 54.5% (95% CI 32–76%) in 22 evaluable patients in the 500 mg dose cohort[124]. Two patients (one in each dose cohort) had a confirmed partial response[124]. The median progression-free survival duration was 3.5 months (95% CI 1.8–7.8) in the 200 mg dose cohort and 5.5 months (95% CI 1.8–8.5) in the *ESR1*-mutated subgroup treated at that dose level; progression-free survival data were not mature in the 500 mg dose cohort[124]. In VERITAC, ARV-471 had a manageable safety profile, and most TRAEs were grade 1/2 (ref. [124]). The most common TRAEs were similar between dose cohorts; the only TRAE that occurred in ≥20% of patients was fatigue (34% overall)[124]. In the 500 mg cohort, treatment-emergent adverse events (TEAEs) led to dose reductions in three patients and to treatment discontinuation in two patients; in the 200 mg cohort, TEAEs led to discontinuation in one patient, with no dose reductions required owing to TEAEs[124]. On the basis of comparable efficacy, favourable tolerability and robust ER degradation (median 69%, range 28–95%, in evaluable patients across the phase I/II study), ARV-471 200 mg once daily was selected as the phase III monotherapy dose and is being compared with fulvestrant in a randomized phase III trial in patients with advanced-stage ER^+HER2^- breast cancer who have received one line of prior CDK4/6 inhibitor therapy in combination with endocrine therapy (NCT05654623)[124].

NX-2127 clinical data

The first presentation of results from the phase I study of the BTK degrader NX-2127 reported data from 36 patients with relapsed and/or refractory B cell malignancies (of whom 23 had chronic lymphocytic leukaemia (CLL)) who were treated with oral doses of 100 mg, 200 mg or 300 mg of NX-2127 once daily, with a data cut-off date of 21 September 2022 (ref. [125]). The median number of lines of prior therapy was four in the overall population (five in the subgroup with CLL), 86% of patients had received a prior BTK inhibitor (100% in the subgroup with CLL) and 35% had a BTK inhibitor resistance mutation (48% in the subgroup with CLL). TEAEs reported in ≥20% of patients were fatigue (53%), neutropenia (39%), contusion (28%), thrombocytopenia (25%), hypertension (25%) and anaemia (22%)[125]. There was one dose-limiting toxicity of cognitive disturbance in a patient with CLL treated at the 300 mg once-daily dose, but a maximum tolerated dose of NX-2127 was not reached[125]. After a median follow-up duration of 5.6 months (range 0.3–15.7), 14 of 23 patients with CLL remained on NX-2127 treatment; in 15 evaluable patients with CLL, the objective response rate was 33% (95% CI 12–62%)[125]. Treatment with 100 mg of NX-2127 once daily in patients with CLL resulted in sustained reduction in BTK levels and decreased plasma levels of CCL4, a marker of B cell activation[125]. NX-2127 induced BTK degradation and led to clinical responses regardless of *BTK* mutation status[98,125].

Future directions for protein degraders

Protein degrader development has rapidly advanced since its inception[14] – from design and refinement of the molecules through medicinal chemistry to evaluation of activity in preclinical experiments to validation in clinical studies (Fig. 2). The reported preclinical data strongly support the specificity of protein degraders for their targets as well as their potency in inhibiting tumour growth compared with small-molecule inhibitors. Moreover, preclinical evidence indicates that protein degraders have activity against resistance mutants that develop after treatment with small-molecule inhibitors. Clinical data, albeit sparse, support the efficacy of protein degraders in patients with advanced-stage prostate cancer, breast cancer or CLL, including those with *AR*, *ER* and *BTK* resistance mutations, respectively. Particularly noteworthy is the tolerability of protein degraders in patients and the absence of any signal suggesting adverse events inherently associated with this technology, for example, owing to hijacking of the ubiquitin–proteasome

system in general. It will be instructive to see how efficacy and safety results bear out in larger patient populations.

Protein degraders that target the AR, ER or BTK were logical forerunners for development, given that these proteins have established roles in the pathogenesis of prostate cancer, breast cancer and CLL, respectively, and approved agents targeting them could serve as benchmarks for preclinical and clinical testing[34–37,84,86,87,95]. Now that protein degradation and therapeutic activity by the protein degrader modality has been clinically demonstrated with bavdegalutamide, ARV-471 and NX-2127, with other agents following close behind, a key next step is to determine whether this approach will meet the expectations of tenet 1 for protein degraders in the clinic, that is, degradation of classically undruggable targets. This will probably be addressed with data from the first-in-human phase I studies of KT-333, a STAT3 degrader, in patients with relapsed and/or refractory lymphomas and advanced-stage solid tumours[114] and of ASP3082, a $KRAS^{G12D}$ degrader, in patients with advanced-stage $KRAS^{G12D}$-mutated solid tumours[111].

The prospect of targeted protein degrader delivery to minimize potential toxicity from systemic delivery is on the horizon. Various targeted delivery systems have now been described, including antibody–protein degrader conjugates[126] and protein degraders that can be selectively activated in tumours by either light, folate or reactive oxygen species[127–131]. In addition, although this Review focuses on protein degraders in development for cancer, the technology might also be used to target proteins involved in the development of other diseases (for example, IRAK4 for autoimmune diseases[29]), thus delivering the promise of broad therapeutic potential.

Potential challenges for protein degraders

Although protein degraders have shown favourable attributes in both the preclinical and clinical settings, they might be met with certain hurdles as they are more widely used in patients with cancer. One question that has not yet been addressed with clinical data is whether patients might develop resistance to protein degraders. Preclinical reports on this phenomenon are limited, but to date, most instances of protein degrader resistance have occurred via alterations that affect the ubiquitin–proteasome system, for example, loss of function of E2 ubiquitin-conjugating enzymes, components of E3 ubiquitin ligases or regulators of the E3 ligase activity (such as the COP9 signalosome), rather than alterations in the target protein[132–135]. A preclinical study revealed upregulation of the drug efflux pump MDR1 as a mechanism of resistance to protein degraders and suggested that co-administration with MDR1 inhibitors might help to overcome this resistance[136]. Evaluation of patients treated with protein degraders for extended periods will be needed to confirm whether the mechanisms of resistance observed preclinically also occur in the clinical setting.

The ability to design novel protein degraders for other targets and cancer types has limitations inherent to the technology, including the requirement for them to bind to their target protein in a manner that permits them to simultaneously access the intracellular ubiquitin–proteasome machinery. For example, transmembrane protein targets with ligands that generally bind on the extracellular-facing surface, such as G-protein-coupled receptors, cannot physically access the cytosolic ubiquitin–proteasome machinery to drive the protein degrader mechanism of action. Additionally, although protein degraders that target classically undruggable proteins are an area of great interest and potential, the difficulty in identifying a binding site for the degrader should not be underestimated. Technologies such as DEL for protein degraders[58], as well as technologies that are complementary to protein degraders (Box 2), might overcome these obstacles.

Conclusions

Protein degraders have taken a bona fide bench-to-bedside odyssey over the past two decades. Much of this journey to date has been spent in the laboratory as protein degraders were devised, improved and rigorously tested using in vitro and in vivo systems. Protein degraders entered clinical development just 4 years ago, and the fruition of preliminary clinical data suggesting the potential for protein degraders as treatments for patients with cancer is encouraging and gratifying; additional data from clinical studies of protein degraders are eagerly awaited. Their bespoke design suggests that protein degraders might offer substantial promise as therapies for a spectrum of diseases.

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K.R.H. wrote the manuscript. All authors researched data for the article, made substantial contributions to discussion of content, and reviewed/edited the manuscript before submission.

Competing interests
D.C. and K.R.H. are employees and shareholders of Arvinas Operations. C.M.C. is a founder and shareholder of Arvinas Operations, as well as a founder, shareholder and consultant of Halda Therapeutics and Siduma Therapeutics, which support research in his lab.

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