Published in final edited form as:

Curr Opin Chem Biol. 2017 August ; 39: 46–53. doi:10.1016/j.cbpa.2017.05.016.

Targeted Protein Knockdown using Small Molecule Degraders

Kanak Raina^a and Craig M. Crews^{b,c,d}

^aArvinas LLC, New Haven, CT 06511

^bDepartment of Molecular, Cellular, and Developmental Biology, Yale University, New Haven, CT 06520

^cDepartment of Chemistry, Yale University, New Haven, CT 06520

^dDepartment of Pharmacology, Yale University, New Haven, CT 06520

Abstract

Small molecule probes of biological systems have traditionally been designed to bind to and inhibit the active sites of their protein targets. While this class of pharmacological agents has been broadened by the development of a small number of allosteric and protein-protein interaction (PPI) inhibitors, conventional drug design still excludes ‘undruggable’ proteins that are neither enzymes nor receptors. Recent years have seen the emergence of new classes of small molecules that can target hitherto undruggable proteins by recruiting the cellular proteostasis machinery to selectively tag them for degradation. These molecules, especially the class known as Proteolysis Targeting Chimera (PROTACs), represent a paradigm shift in chemical genetics, but their most tantalizing potential is as novel therapeutic agents. This review briefly summarizes the preclinical development of small molecule-based protein degraders, and describes the recent improvements in the technology that have positioned PROTACs on the cusp of entering the clinic.

Graphical Abstract

Corresponding Author: Crews, CM (craig.crews@yale.edu) Ph: +1(203) 432-9364.

Conflict of interest statement: C.M.C. is the founder and Chief Scientific Advisor of Arvinas, LLC. Both authors possess shares in, Arvinas LLC.

Publisher's Disclaimer: This is a PDF file of an unedited manuscript that has been accepted for publication. As a service to our customers we are providing this early version of the manuscript. The manuscript will undergo copyediting, typesetting, and review of the resulting proof before it is published in its final citable form. Please note that during the production process errors may be discovered which could affect the content, and all legal disclaimers that apply to the journal pertain.

Introduction

The post-genomic era has enabled the identification of molecular bases underlying various pathologies. However, drug discovery has failed to keep pace with the genomics revolution, in part because it has proven extremely hard to develop therapeutic modalities beyond traditional small-molecule approaches targeting the enzymatic activity of proteins. While the advent of biologics has generated optimism, especially given their higher success rate in getting from Phase I to launch[1], the lack of cellular penetration with typical biomacromolecules is a severely limiting feature of these drugs. Improvements in drug delivery technologies have allowed for some progress in this regard[2], but their applicability is limited. Nucleic-acid based intracellular target knockdown has had success[3], but this too are hampered by numerous challenges, such as chemical instability, poor tissue distribution, inadequate specificity, and potential immunogenicity[4]. Meanwhile, exciting advances like CRISPR-Cas9 remain untested in patients[5]. Therefore, small molecules are still the most thoroughly validated therapeutic modality, comprising >60% of agents entering Phase I trials since 2006[6]. However, conventional small molecule based drugs typically require the presence of a pocket or active site within the protein target, binding to which directly alters its enzymatic function. This excludes the array of potential drug targets that do not possess enzymatic activity and are thus deemed 'undruggable'[7], such as transcription factors and scaffolding proteins. Moreover, the 'occupancy-based' paradigm of enzyme inhibition *in vivo* often requires maintenance of high systemic drug levels, resulting in off-target effects and toxicity[8,9]. In this context, several novel classes of small molecules that induce the specific degradation of pathogenic intracellular proteins have been developed. Aside from summarizing the early versions of small molecular protein degradation technologies, we focus here on the recent activity around PROTACs, and their clinical path forward.

PROTACs

The most advanced means of targeted protein degradation, PROTACs are heterobifunctional molecules consisting of a ligand that binds the protein of interest (POI) connected via a linker domain to a recruitment moiety for an E3 ubiquitin ligase (Fig. 1). More than 600 E3 ligases are known[10], most of which function as recognition modules that bring substrates into proximity with E2 ubiquitin-conjugating enzymes, enabling ubiquitin transfer on to surface lysines on the substrate. Lysine polyubiquitination acts as a signal for the cell to degrade the substrate protein via the proteasome. PROTACs facilitate this process by forming a ternary complex with the E3 ligase and the POI, thereby catalyzing the selective removal of the latter by the UPS machinery[11]. Unlike inhibitors, PROTACs function like true catalysts, and survive the polyubiquitination event intact to carry out successive rounds of ternary complex formation (For a summary of the key pharmacological features of PROTACs, see Box 1).

Box 1**Key advantages of PROTACs over conventional small molecule inhibitors**

Feature	Explanation
No target active site requirement	Binding anywhere on target protein is sufficient for activity
Added layer of specificity over inhibitors	Ternary complex and ubiquitin transfer-dependent mechanism of action
Sub-stoichiometric drug requirement	Catalytic mechanism of action
Increased potency compared to component inhibitor	Catalytic mechanism of action and PPI during ternary complex formation
Prolonged pharmacodynamic suppression <i>in vivo</i>	Restoration of target activity requires protein re-synthesis

First generation PROTACs were greatly hampered by a paucity of small molecular ligands for E3 ligases (recent advances reviewed here[12,13]). The first attempts utilized a peptidic recognition motif for the SKP-Cullin-FBox E3 ligase β -TRCP. This approach resulted in membrane impermeable PROTACs that were nevertheless successfully used to target MetAP-2, ER, and AR *in vitro*, or via cell microinjection[14,15] (Fig. 2a). Conjugation of the poly-Arg cell-permeability motif to a HIF1 α -derived von Hippel Lindau (VHL) E3 ligase binding peptide sequence allowed for the generation of the first cell-permeable PROTAC, used to target a GFP-FKBP12 (F36V) fusion protein using a bump-hole mutant FKBP12 ligand[16]. Similar PROTACs were reported to target the androgen receptor (AR) [17] and the X-protein of the hepatitis B virus[18]. VHL-binding peptide based PROTACs targeting the aryl hydrocarbon receptor[19,20] or ER α [21,22] were subsequently shown to be efficacious even without the poly-Arg sequence. A variation on the PROTAC theme was provided by the receptor tyrosine kinase (RTK) activity-mediated phosphorylation-dependent, VHL-based, conditional PROTAC used to target PI3K for degradation in cell culture and in tumor xenografts[23]. This ‘phosphoPROTAC’ was the first such molecule to demonstrate *in vivo* activity.

Early PROTACs described above were good *in vitro* biological probes, and even showed activity in mouse models. However, they were high molecular weight compounds, with a number of chemically labile and metabolically susceptible peptide bonds, and compromised by low cell permeability and relatively low potency. For these reasons, they were poor pharmaceutical candidates, and it was not until the development of low molecular weight ligands for E3 ligases, that PROTACs crossed the threshold between chemical biology curiosity and potential drug.

MDM2 and IAP

The first E3 ligase to be targeted into a small molecule PROTAC paradigm was MDM2, through the use of a nutlin ligand, which, when conjugated to a selective androgen receptor modulator (SARM), was able to degrade the AR[24] (Fig. 2b). More recently, the E3 ligases cIAP1 has been recruited into the PROTAC paradigm (Fig. 2c), through the use of the ligand bestatin. cIAP1 based PROTACs have been used to selectively degrade a number of proteins,

such as CRABP-II[25], ER α [26,27], and TACC3[28]. Unfortunately, these bestatin-based PROTACs, like the parent ligand[29], induced autoubiquitination and degradation of the E3 ligase itself, a potential explanation for their relatively low potency. This limitation was recently circumvented by the use of the high-affinity IAP antagonist LCL161, resulting in PROTACs that primarily recruited XIAP instead of cIAP1 to target ER α , PDE4, BRD4, and BCR-Abl[30] (Fig. 2d). These PROTACs were active at low nanomolar concentrations while resulting in minimal loss of XIAP itself, and demonstrated potent *in vivo* activity.

VHL and Cereblon

The first E3 ligase used widely for small-molecule based selective target protein degradation, was the cullin-RING ubiquitin ligase (CRL) component VHL. This breakthrough was enabled by the development of small molecule mimetics of a hydroxyproline-based HIF1 α -derived VHL-binding peptide via a classical fragment-based approach[31–33]. These VHL ligands were soon incorporated into highly active PROTACs by linking them to a chloroalkane moiety targeting an ectopically expressed EGFP-Halotag7 fusion protein in a proof-of-concept study[34]. Following closely on the heels of VHL, immunomodulatory drugs belonging to the phthalimide family, which had recently been discovered to bind the CRL family E3 component cereblon (CRBN)[35,36], were utilized in the PROTAC paradigm. In a flurry of papers that appeared almost simultaneously, both VHL and CRBN based PROTACs were used target the bromo- and extra-terminal (BET) proteins. First, phthalimide analogs were conjugated to a triazolo-diazepine-acetamide BET-binding moiety derived from BET inhibitors in clinical development, and shown to degrade BRD2/3/4 with low nanomolar potency in several different disease contexts[37,38]. In parallel, a different group reported similarly highly active VHL-based BET PROTACs[39]. More recently, another publication by the same group revealed the crystal structure of a BRD4 bromodomain (BRD4^{BD2}) in a ternary complex with a BRD4-targeting PROTAC and the VHL E3 ligase[40]. These publications taken together revealed a number of intriguing features of the pharmacology of PROTACs. In particular, one recurrent observation with PROTACs spanning multiple E3 ligases and protein targets is the so-called ‘hook effect’, whereby these heterobifunctional molecules begin to lose efficacy at concentrations greatly exceeding their DC₅₀, due to competition between the formation of degradation-inducing ternary E3::PROTAC::Target complex, and ineffective dimeric PROTAC::E3 and PROTAC::Target complexes. Another key feature of PROTACs appears to be their catalytic property, established biochemically, and evidenced by DC₅₀ values that routinely indicate far greater potency than the binding affinity of constituent ligands for the E3 or the target. This observation is likely also rooted in the discovery of extensive PPIs between the target and the E3 ligase upon ternary complex formation, as exemplified in the BRD4^{BD2} case[40]. This feature of PROTACs has great potential clinical utility, due to reduced amount of drug-on-board systemically needed to exert pharmacodynamic effects *in vivo*, in a manner analogous to an irreversible inhibitor. Finally, by carefully modulating the chemical structure of the linker region connecting the BET inhibitor warhead to the VHL ligand and by an analysis of PPIs formed during ternary complex formation, PROTACs capable of degrading BRD4 with >10-fold selectivity over BRD2 and BRD3 could be designed[39,40]. Given that the warhead binds all three proteins with comparable affinity, this additional layer of

selectivity was attributable to the mechanism of action of PROTACs. Similarly, an earlier report on the degradation of c-Abl and the oncogenic fusion protein BCR-Abl demonstrated that by altering the choice of the warhead against the target (bosutinib/dasatinib) and/or the E3 ligase being recruited (VHL/CRBN), PROTACs could be made to selectively target one or both proteins[41]. Soon thereafter, VHL-based and CRBN-based BET PROTACs (Fig. 3a, 3b) were shown to be highly active against models of metastatic castration resistant prostate cancer (CRPC)[42] and triple-negative breast cancer (TNBC)[43], respectively, both *in vitro* and via intravenous administration in tumor xenograft models. These reports made an intriguing case for PROTACs in the clinic, by demonstrating that degradation of BET proteins generated a biological response distinct from blocking BET function using conventional small-molecule inhibitors. In both cases, BET PROTACs were shown to be 50–500 fold more antiproliferative than BET inhibitors, with far greater induction of apoptosis. In addition, BET degradation was shown to directly modulate AR protein levels in CRPC, while BET inhibitors merely blocked AR-mediated transcription. Similarly, BET proteins have recently been shown to have bromodomain-independent transcriptional and proliferative activity[44] in TNBC, that would be unaffected by inhibitors, possibly explaining the superior efficacy of BET PROTACs in this context.

Ligand-Induced Degradation and Hydrophobic Tagging

Unlike PROTACs, which recruit an E3 ligase to degrade a POI, some examples of small molecules that bind their target protein and cause its degradation have also been reported. The most notable of these is the FDA approved drug Fulvestrant, which binds and downregulates the ER by inducing conformational instability[45]. ER appears to be susceptible to this technique, and other selective ER downregulators (SERDs) have also been identified[46]. Other nuclear hormone receptors are thought to be possible candidates, and selective AR downregulators (SARDs) have even made it into the clinic[47,48], albeit unsuccessfully thus far[49]. CI-1033, an ErbB2 degrader, is thought to function in an analogous manner[50]. Attempts to broaden the scope of these ligand-mediated strategies have been made through ‘hydrophobic tagging’, whereby a known ligand against a POI is conjugated to a bulky hydrophobic tag (HyT), such as an adamantyl group. Upon addition to cells, this molecule functions as a small molecule degron, putatively by causing localized conformational instability or/and by mimicking an unfolded state of the POI by the display of a hydrophobic patch on the protein surface, and results in POI downregulation via the proteasome (Fig. 4). This technology, which was invented using ectopically expressed fusion proteins, has subsequently been extended to more disease relevant proteins such as AR[51], and the conventionally undruggable pseudokinase Her3[52]. An analogous approach, developed in parallel, uses a Boc₃Arg group[53] instead of the adamantyl containing HyT. The mechanism of Boc₃Arg tagging-based degradation is under investigation, and appears to involve docking of the POI at the 20S proteasome[54].

Conclusion

While ligand-induced degradation and hydrophobic tagging approaches have seen some success, the specific characteristics of the ligand/hydrophobic tag and the target protein which enable degradation are not yet predictable and need to be separately explored in each

case. PROTACs, on the other hand, have been shown to be much more generalizable, due to their modular construction and well-understood mechanism of action. The main challenge facing this modality is the absence of ligands known to bind many tantalizing drug targets. Although PROTACs described in the literature have thus far relied on known ligands for target proteins, this approach could potentially be extended to thus-far undruggable proteins, using target-recruiting ligands that currently tend to be discarded due to the absence of a functional outcome. Screening small molecule libraries against proteins lacking any active sites would be a major step in extending this technology toward fulfilling the vast promise of the post-genomics era, by yielding drugs that attack protein targets inaccessible to conventional small molecule-based therapeutic intervention.

Acknowledgments

C.M.C. gratefully acknowledges support from the NIH (R35CA197589).

References

1. Smietana K, Siatkowski M, Møller M. Trends in clinical success rates. *Nat Rev Drug Discov.* 2016; 15:379–380. [PubMed: 27199245]
2. Mitragotri S, Burke PA, Langer R. Overcoming the challenges in administering biopharmaceuticals: formulation and delivery strategies. *Nat Rev Drug Discov.* 2014; 13:655–72. [PubMed: 25103255]
3. Lundin KE, Gissberg O, Smith CIE. Oligonucleotide Therapies: The Past and the Present. *Hum Gene Ther.* 2015; 26:475–85. [PubMed: 26160334]
4. Burnett JC, Rossi JJ. RNA-Based Therapeutics: Current Progress and Future Prospects. *Chem Biol.* 2012; 19:60–71. [PubMed: 22284355]
5. Fellmann C, Gowen BG, Lin P-C, Doudna JA, Corn JE. Cornerstones of CRISPR–Cas in drug discovery and therapy. *Nat Rev Drug Discov.* 2016; doi: 10.1038/nrd.2016.238
6. BIO. Clinical development success rates 2006–2015. Report. 2016; The authors compare the performances of all therapeutic modalities in the clinic over a 10-year period. doi: 10.1038/nrd.2016.85
7. Lazo JS, Sharlow ER. Drugging Undruggable Molecular Cancer Targets. *Annu Rev Pharmacol Toxicol.* 2016; 56:23–40. [PubMed: 26527069]
8. Adjei AA. What is the right dose? The elusive optimal biologic dose in phase I clinical trials. *J Clin Oncol.* 2006; 24:4054–4055. [PubMed: 16943522]
9. Schall TJ, Proudfoot AEI. Overcoming hurdles in developing successful drugs targeting chemokine receptors. *Nat Rev Immunol.* 2011; 11:355–363. [PubMed: 21494268]
10. Li W, Bengtson MH, Ulbrich A, Matsuda A, Reddy VA, Orth A, Chanda SK, Batalov S, Joazeiro CAP. Genome-wide and functional annotation of human E3 ubiquitin ligases identifies MULAN, a mitochondrial E3 that regulates the organelle's dynamics and signaling. *PLoS One.* 2008; 3:e1487. [PubMed: 18213395]
11. Bondeson DP, Mares A, Smith IED, Ko E, Campos S, Miah AH, Mulholland KE, Routly N, Buckley DL, Gustafson JL, et al. Catalytic in vivo protein knockdown by small-molecule PROTACs. *Nat Chem Biol.* 2015; 11:611–617. The authors conduct a biochemical investigation of the catalytic property of PROTACs. [PubMed: 26075522]
12. Skaar JR, Pagan JK, Pagano M. SCF ubiquitin ligase-targeted therapies. *Nat Rev Drug Discov.* 2014; 13:889–903. [PubMed: 25394868]
13. Lucas X, Ciulli A. Recognition of substrate degrons by E3 ubiquitin ligases and modulation by small-molecule mimicry strategies. *Curr Opin Struct Biol.* 2017; 44:101–110. [PubMed: 28130986]
14. Sakamoto KM, Kim KB, Kumagai A, Mercurio F, Crews CM, Deshaies RJ. Protacs: chimeric molecules that target proteins to the Skp1-Cullin-F box complex for ubiquitination and degradation. *Proc Natl Acad Sci U S A.* 2001; 98:8554–9. [PubMed: 11438690]

15. Sakamoto KM. Development of PROTacs to Target Cancer-promoting Proteins for Ubiquitination and Degradation. *Mol Cell Proteomics*. 2003; 2:1350–1358. [PubMed: 14525958]
16. Schneekloth JS, Fonseca FN, Koldobskiy M, Mandal A, Deshaies R, Sakamoto K, Crews CM. Chemical Genetic Control of Protein Levels: Selective in Vivo Targeted Degradation. *J Am Chem Soc*. 2004; 126:3748–3754. The first description of an 'all small molecule' PROTAC. [PubMed: 15038727]
17. Tang Y-Q, Han B-M, Yao X-Q, Hong Y, Wang Y, Zhao F-J, Yu S-Q, Sun X-W, Xia S-J. Chimeric molecules facilitate the degradation of androgen receptors and repress the growth of LNCaP cells. *Asian J Androl*. 2009; 11:119–126. [PubMed: 19050678]
18. Montrose K, Krissansen GW. Design of a PROTAC that antagonizes and destroys the cancer-forming X-protein of the hepatitis B virus. *Biochem Biophys Res Commun*. 2014; 453:735–740. [PubMed: 25305486]
19. Lee H, Puppala D, Choi EY, Swanson H, Kim KB. Targeted degradation of the aryl hydrocarbon receptor by the PROTAC approach: A useful chemical genetic tool. *ChemBioChem*. 2007; 8:2058–2062. [PubMed: 17907127]
20. Puppala D, Lee H, Kim KB, Swanson HI. Development of an Aryl Hydrocarbon Receptor Antagonist Using the Proteolysis-Targeting Chimeric Molecules Approach: A Potential Tool for Chemoprevention. *Mol Pharmacol*. 2008; 73:1064–1071. [PubMed: 18178667]
21. Bargagna-Mohan P, Baek SH, Lee H, Kim K, Mohan R. Use of PROTACS as molecular probes of angiogenesis. *Bioorganic Med Chem Lett*. 2005; 15:2724–2727.
22. Cyrus K, Wehenkel M, Choi EY, Lee H, Swanson H, Kim KB. Jostling for position: Optimizing linker location in the design of estrogen receptor-targeting PROTACs. *ChemMedChem*. 2010; 5:979–985. [PubMed: 20512796]
23. Hines J, Gough JD, Corson TW, Crews CM. Posttranslational protein knockdown coupled to receptor tyrosine kinase activation with phosphoPROTACs. *Proc Natl Acad Sci U S A*. 2013; 110:8942–7. [PubMed: 23674677]
24. Schneekloth AR, Pucheault M, Tae HS, Crews CM. Targeted intracellular protein degradation induced by a small molecule: En route to chemical proteomics. *Bioorg Med Chem Lett*. 2008; 18:5904–5908. [PubMed: 18752944]
25. Okuhira K, Ohoka N, Sai K, Nishimaki-Mogami T, Itoh Y, Ishikawa M, Hashimoto Y, Naito M. Specific degradation of CRABP-II via cIAP1-mediated ubiquitylation induced by hybrid molecules that crosslink cIAP1 and the target protein. *FEBS Lett*. 2011; 585:1147–1152. [PubMed: 21414315]
26. Demizu Y, Okuhira K, Motoi H, Ohno A, Shoda T, Fukuhara K, Okuda H, Naito M, Kurihara M. Design and synthesis of estrogen receptor degradation inducer based on a protein knockdown strategy. 2012
27. Okuhira K, Demizu Y, Hattori T, Ohoka N, Shibata N, Nishimaki-Mogami T, Okuda H, Kurihara M, Naito M. Development of hybrid small molecules that induce degradation of estrogen receptor- α and necrotic cell death in breast cancer cells. *Cancer Sci*. 2013; 104:1492–1498. [PubMed: 23992566]
28. Ohoka N, Nagai K, Hattori T, Okuhira K, Shibata N, Cho N, Naito M. Cancer cell death induced by novel small molecules degrading the TACC3 protein via the ubiquitin–proteasome pathway. *Cell Death Dis*. 2014; 5:e1513. [PubMed: 25375378]
29. Sekine K, Takubo K, Kikuchi R, Nishimoto M, Kitagawa M, Abe F, Nishikawa K, Tsuruo T, Naito M. Small Molecules Destabilize cIAP1 by Activating Auto-ubiquitylation. *J Biol Chem*. 2008; 283:8961–8968. [PubMed: 18230607]
30. Ohoka N, Okuhira K, Ito M, Nagai K, Shibata N, Hattori T, Ujikawa O, Shimokawa K, Sano O, Koyama R, et al. In Vivo Knockdown of Pathogenic Proteins via Specific and Nongenetic IAP-dependent Protein Erasers (SNIPERs). *J Biol Chem*. 2017; The authors describe highly optimized IAP based PROTACs with in vivo activity. doi: 10.1074/jbc.M116.768853
31. Buckley DL, Van Molle I, Gareiss PC, Tae HS, Michel J, Noblin DJ, Jorgensen WL, Ciulli A, Crews CM. Targeting the von Hippel–Lindau E3 Ubiquitin Ligase Using Small Molecules To Disrupt the VHL/HIF-1 α Interaction. *J Am Chem Soc*. 2012; 134:4465–4468. [PubMed: 22369643]

32. Buckley DL, Gustafson JL, Van Molle I, Roth AG, Tae HS, Gareiss PC, Jorgensen WL, Ciulli A, Crews CM. Small-Molecule Inhibitors of the Interaction between the E3 Ligase VHL and HIF1 α . *Angew Chemie Int Ed*. 2012; 51:11463–11467.
33. Frost J, Galdeano C, Soares P, Gadd MS, Grzes KM, Ellis L, Epemolu O, Shimamura S, Bantscheff M, Grandi P, et al. Potent and selective chemical probe of hypoxic signalling downstream of HIF- α hydroxylation via VHL inhibition. *Nat Commun*. 2016; 7:13312. [PubMed: 27811928]
34. Buckley DL, Raina K, Darricarrere N, Hines J, Gustafson JL, Smith IE, Miah AH, Harling JD, Crews CM. HaloPROTACS: Use of Small Molecule PROTACs to Induce Degradation of HaloTag Fusion Proteins. *ACS Chem Biol*. 2015; 10:1831–7. [PubMed: 26070106]
35. Ito T, Ando H, Suzuki T, Ogura T, Hotta K, Imamura Y, Yamaguchi Y, Handa H. Identification of a Primary Target of Thalidomide Teratogenicity. *Science*. 2010; 327:1345–1350. [PubMed: 20223979]
36. Lu G, Middleton RE, Sun H, Naniong M, Ott CJ, Mitsiades CS, Wong K-K, Bradner JE, Kaelin WG. The myeloma drug lenalidomide promotes the cereblon-dependent destruction of Ikaros proteins. *Science*. 2014; 343:305–9. [PubMed: 24292623]
37. Lu J, Qian Y, Altieri M, Dong H, Wang J, Raina K, Hines J, Winkler JD, Crew AP, Coleman K, et al. Hijacking the E3 Ubiquitin Ligase Cereblon to Efficiently Target BRD4. *Chem Biol*. 2015; 22:755–763. [PubMed: 26051217]
38. Winter GE, Buckley DL, Paulk J, Roberts JM, Souza A, Dhe-Paganon S, Bradner JE. DRUG DEVELOPMENT. Phthalimide conjugation as a strategy for in vivo target protein degradation. *Science*. 2015; 348:1376–81. [PubMed: 25999370]
39. Zengerle M, Chan K-H, Ciulli A. Selective Small Molecule Induced Degradation of the BET Bromodomain Protein BRD4. *ACS Chem Biol*. 2015; 10:1770–7. [PubMed: 26035625]
- 40■. Gadd M, Testa A, Lucas X, Chan K, Chen W, Lamont D, Zengerle M, Ciulli A. Structural basis of PROTAC cooperative recognition for selective protein degradation. *NatChemBiol*. 2017; Armed with a E3::PROTAC::POI trimer crystal structure, the authors provide a biophysical explanation for the exquisite selectivity and potency of PROTACs. doi: 10.2210/PDB5T35/PDB
41. Lai AC, Toure M, Hellerschmied D, Salami J, Jaime-Figueroa S, Ko E, Hines J, Crews CM. Modular PROTAC Design for the Degradation of Oncogenic BCR-ABL. *Angew Chemie - Int Ed*. 2016; 55:807–810.
- 42■. Raina K, Lu J, Qian Y, Altieri M, Gordon D, Rossi AM, Wang J, Chen X, Dong H, Siu K, et al. PROTAC-induced BET protein degradation as a therapy for castration-resistant prostate cancer. *Proc Natl Acad Sci U S A*. 2016; 113:7124–7129. The authors provide the first instance of a PROTAC active in vivo via a clinically viable route of administration. [PubMed: 27274052]
43. Bai L, Zhou B, Yang C-Y, Ji J, McEachern D, Przybranowski S, Jiang H, Hu J, Xu F, Zhao Y, et al. Targeted degradation of BET proteins in triple-negative breast cancer. *Cancer Res*. 2017; doi: 10.1158/0008-5472.CAN-16-2622
- 44■. Shu S, Lin CY, He HH, Witwicki RM, Tabassum DP, Roberts JM, Janiszewska M, Jin Huh S, Liang Y, Ryan J, et al. Response and resistance to BET bromodomain inhibitors in triple-negative breast cancer. *Nature*. 2016; The authors propose a bromodomain independent and inhibitor resistant activity for BET proteins. doi: 10.1038/nature16508
45. Wittmann BM, Sherk A, McDonnell DP. Definition of Functionally Important Mechanistic Differences among Selective Estrogen Receptor Down-regulators. *Cancer Res*. 2007; 67
46. McDonnell DP, Wardell SE, Norris JD. Oral Selective Estrogen Receptor Downregulators (SERDs), a Breakthrough Endocrine Therapy for Breast Cancer. *J Med Chem*. 2015; 58:4883–7. [PubMed: 26039356]
47. Loddick SA, Ross SJ, Thomason AG, Robinson DM, Walker GE, Dunkley TPJ, Brave SR, Broadbent N, Stratton NC, Trueman D, et al. AZD3514: A Small Molecule That Modulates Androgen Receptor Signaling and Function In Vitro and In Vivo. *Mol Cancer Ther*. 2013; 12
48. Omlin A, Jones RJ, van der Noll R, Satoh T, Niwakawa M, Smith SA, Graham J, Ong M, Finkelman RD, Schellens JHM, et al. AZD3514, an oral selective androgen receptor down-regulator in patients with castration-resistant prostate cancer – results of two parallel first-in-human phase I studies. *Invest New Drugs*. 2015; 33:679–690. [PubMed: 25920479]

49. Williams R. Discontinued drugs in 2012: oncology drugs. *Expert Opin Investig Drugs*. 2013; 22:1627–1644.
50. Citri A, Alroy I, Lavi S, Rubin C, Xu W, Grammatikakis N, Patterson C, Neckers L, Fry DW, Yarden Y. Drug-induced ubiquitylation and degradation of ErbB receptor tyrosine kinases: implications for cancer therapy. *EMBO J*. 2002; 21:2407–17. [PubMed: 12006493]
51. Gustafson JL, Neklesa TK, Cox CS, Roth AG, Buckley DL, Tae HS, Sundberg TB, Stagg DB, Hines J, McDonnell DP, et al. Small-Molecule-Mediated Degradation of the Androgen Receptor through Hydrophobic Tagging. *Angew Chemie Int Ed*. 2015; 54:9659–9662.
52. Xie T, Lim SM, Westover KD, Dodge ME, Ercan D, Ficarro SB, Udayakumar D, Gurbani D, Tae HS, Riddle SM, et al. Pharmacological targeting of the pseudokinase Her3. *Nat Chem Biol*. 2014; 10:1006–1012. [PubMed: 25326665]
53. Long MJC, Gollapalli DR, Hedstrom L. Inhibitor mediated protein degradation. *Chem Biol*. 2012; 19:629–37. [PubMed: 22633414]
54. Shi Y, Long MJC, Rosenberg MM, Li S, Kobjack A, Lessans P, Coffey RT, Hedstrom L. Boc3Arg-linked ligands induce degradation by localizing target proteins to the 20S proteasome. *ACS Chem Biol*. 2016; The authors describe the unique mechanism of Boc3Arg mediated degradation in cells. doi: 10.1021/acscchembio.6b00656

Highlights

- Small molecule protein degraders (PROTACs) can target the ‘undruggable’ proteome.
- Ligands that bind the target anywhere on its surface can be used to make PROTACs that degrade it.
- PROTACs act in a catalytic manner and can be made more selective than inhibitors.
- PROTACs have been shown to be highly active in numerous preclinical *in vivo* models.
- Advanced PROTACs are poised to enter the clinic as investigational therapeutics.

Fig. 1. PROTAC mechanism of action

PROTACs bind a POI (not necessarily at an active site) and enforce proximity with an E3 ligase. The ternary complex thus formed is favored by PPIs between the POI and the E3. Ubiquitin transfer follows, enabling the formation of the polyubiquitin signal on the POI. Degradation occurs via the proteasome, and the unharmed PROTAC is regenerated to carry out successive rounds of ubiquitination.

Fig. 2. Incremental progress in PROTAC development

a) Early peptidic PROTAC (named PROTAC-1) targeted MetAP-2 by recruiting it to the β -TRCP SCF E3 ligase. b) SARM-nutlin PROTAC that used the MDM2 E3 ligase for degradation of AR. c) SNIPER(TACC-3)-1, a PROTAC targeting TACC3 using the cIAP E3 ligase ligand bestatin. d) An IAP based PROTAC (SNIPER(ER)-87) that preferentially recruited XIAP over cIAP to target ER α for degradation.

Fig. 3. VHL- and CRBN-based PROTACs with *in vivo* activity

a) ARV-771, a JQ-1 based VHL recruiting BET PROTAC, that was shown to be active in CRPC models *in vivo*. b) BETd-246, a PROTAC that used a novel BET bromodomain binding chemotype conjugated to thalidomide to degrade BRD2/3/4 and was shown to have activity against a patient-derived xenograft (PDX) model of treatment resistant breast cancer.

Fig. 4. Hydrophobic tagging mechanism of action

Adamantyl moiety containing hydrophobic tags bind a POI (not necessarily at an active site), and result in protein degradation through one or both of two possible mechanisms: recruitment of protein quality control chaperones for which the hydrophobic adamantyl group signals protein unfolding, or by bona fide protein unfolding local to the POI ligand binding site due to the bulky nature of the large hydrocarbon moiety. Similar to PROTACs, hydrophobic tags are liberated intact subsequent to protein degradation, and can effect successive rounds of POI destruction.