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Published in:
Current Opinion in Pharmacology

DOI:
[10.1016/j.coph.2021.02.009](https://doi.org/10.1016/j.coph.2021.02.009)

Publication date:
2021

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Document Version
Peer reviewed version

[Link to publication in Discovery Research Portal](#)

Citation for published version (APA):
Farnaby, W., Koegl, M., McConnell, D. B., & Ciulli, A. (2021). Transforming targeted cancer therapy with PROTACs: A forward-looking perspective. *Current Opinion in Pharmacology*, 57, 175-183.
<https://doi.org/10.1016/j.coph.2021.02.009>

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Transforming targeted cancer therapy with PROTACs: A forward-looking perspective

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Introduction

Recent years have brought a wave of pre-clinical and clinical studies utilising bifunctional degrader molecules, known as proteolysis targeting chimeras (PROTACs). PROTAC molecules induce proximity between a recruited E3 ubiquitin ligase on one end towards a target protein on the other end, leading to ubiquitylation of the target protein and subsequent degradation. The repertoire of proteins degraded by PROTACs is expanding continuously, despite the fact that only a very small proportion of available E3 ligases has been recruited to date by this approach. Given the rapid developments in this still young field, it has already become clear that the concept of protein degradation by PROTACs is not a one-hit-wonder, but a lasting addition to the arsenal of molecular reagents and therapeutics to downregulate protein levels. A remaining open question is if and how PROTACs can be employed to best benefit patients. We provide here a forward-looking perspective on how unique features of PROTAC degraders may address key challenges in cancer drug discovery and in particular how these contrast with classical inhibitor approaches. We discuss the unique opportunities that PROTACs may offer with respect to target scope, resistance and finally, selectivity; be it for or against specific protein targets, cell types or tissues.

Target scope

Within oncology, the predictive power of CRISPR screens has been highly illuminating for identifying specific vulnerabilities and synthetic lethality within a large array of cancers[1,2]. However, the effect of a genetic knock-out is not always phenocopied by a small molecule inhibitor, since this requires a binding site suitable for potent inhibition of protein activity (see **Figure 1** for a comparison of chemical and non-chemical options to interfere with a target). PROTACs do not have this limitation as they will degrade the whole protein and, in the process remove any hitherto unappreciated function of the target, such as scaffolding roles. Furthermore, a PROTAC could also impact other protein subunits present in the same multi-protein complex as the bound target, either via induced adventitious “bystander” ubiquitination and subsequent degradation[3-5], or via destabilization of adjacent complex subunits following target degradation[6]. Recent studies have shown that by forming high affinity ternary complexes through the induction of *de novo* protein-protein interactions between the E3 ligase and target protein, PROTACs may be less dependent on high affinity interactions with the POI themselves[6-8]. This has been exemplified by studies on the BAF chromatin remodelling complex ATPases SMARCA2 and SMARCA4 where degradation at low nanomolar concentrations was demonstrated with a PROTAC molecule that recruits the SMARCA2 bromodomain with a K_D of just 2 μ M[6]. Furthermore, PROTAC ACBI-1 was able to recruit the SMARCA ATPases for degradation *via* the bromodomain and induce apoptosis in cancer cells, despite the bromodomain itself not being required for cell survival [6,9]. The ability to target non-functional binding sites with low affinity opens the possibility of targeting binding sites not previously considered “druggable” in the context of an inhibitor and thus potentially expanding the druggable proteome.

In another BAF complex related example, BRD9 bromodomain inhibitor probe BI-7273 was used in two separate studies to yield CRBN-recruiting BRD9 degrader dBRD9[10] and VHL-recruiting dual BRD7/9 degrader VZ185[11]. Whilst inhibiting BRD9 yields mild phenotypic responses, mainly restricted to AML cells[12,13], these degrader probes both demonstrate rapid and potent degradation and as a result are capable of anti-proliferative effects not only in AML cells but also in rhabdoid tumour cells, at more than ten-fold lower concentrations than the analogous bromodomain inhibitor[10,11]. Similarly, synovial sarcomas and malignant rhabdoid tumours have been shown to depend on BRD9; again, degradation of BRD9 by PROTACs has a much stronger effect on cell proliferation than just the small-molecule inhibition of its bromodomain[14].

Targeted protein degradation approaches have also demonstrated value in the devalidation of high-profile cancer targets[15-17], an important and sometimes underappreciated goal to ensure the efficient focus of both pre-clinical and clinical resources. For example, whilst the overexpression of focal adhesion tyrosine kinase (PTK2, or FAK) is associated with a number of advanced-stage solid cancers[18], the outcomes of genetic knockdown and pharmacological inhibition of PTK2 and the link to a reduction in tumorigenicity in hepatocellular carcinoma (HCC) models has been unclear[17]. BI-3663 is a rapid and, based on unbiased whole cell proteomics profiling, highly selective degrader of PTK2 demonstrating more than 80% PTK2 degradation at less than 100 nM across a panel of HCC cell lines[17]. Despite this, in the same panel, anti-proliferative effects were generally not observed at > 10 μ M, suggesting that preventing neither scaffolding nor enzymatic function of PTK2 is therapeutically actionable for HCC. Cromm *et al* likewise reported a lack of effect on cancer cell viability with their own defactinib-based PTK2 degrader[16]. The kinase MELK provides a similar example. This kinase had been implicated in driving tumor cell proliferation based on RNA interference. Small molecule MELK kinase inhibitors indeed displayed the expected antiproliferative effects[19], encouraging the initiation of clinical trials[20]. However, these inhibitors were later shown to be active in cells in which MELK had been knocked out by CRISPR[21]. Using the chemical genetic system dTAG, targeted MELK degradation could not recapitulate the antiproliferative effects, nor could more selective MELK inhibitors, thus closing the case on MELK as a useful cancer drug target[15]. It should be noted that the use of unbiased whole cell proteomics profiling, including comparison to non-degrading control compounds, was important in these cases to reassure that any observed phenotype was related to PROTAC induced on-target degradation.

As the requirement for high affinity binders for functionally relevant binding sites is relaxed, PROTACs may hold an advantage when it comes to target scope. Yet, the field still needs to clinically deliver on this promise. An area of particularly high value in this context is the targeting of oncogenic RAS. Covalent inhibitors that target KRAS G12C in its 'off-state' (GDP-bound) have been successfully employed for PROTAC design[22]. With the recent publications of RAS binding small molecules with increasing affinities, broadening this approach to target other oncogenic RAS variants, including in their activated state, would seem within reach[23-25].

Resistance

With the identification of the major gain-of-function tumour driver genes in the past decades, much hope was put on inhibiting the respective proteins with small molecules. In many cases, inhibitors of these drivers indeed met with initial success in the clinic, as exemplified by the responses seen with antagonists of androgen receptor (AR) in prostate cancer, or inhibitors of mutated BRAF or EGFR kinases in melanoma and lung cancer, respectively. However, in each and every case, the initial responses are followed by the development of drug resistant disease, typically missense mutations on the target protein that weaken or abrogate inhibitor binding. PROTACs bring

a new dimension to the resistance arms race which could provide solutions to current resistance mechanisms but are also likely to be susceptible to resistance mechanisms of their own.

In the case of castration resistant prostate cancer, elevated expression of AR can lead to ineffectiveness of antiandrogens[26], as can mutations in AR which cause AR antagonists such as enzalutamide to have agonistic effects[27]. Both resistance mechanisms can be nullified by AR degradation. PROTACs based on enzalutamide, which exert their effects not by antagonizing the protein but by removing it, are not sensitive to agonism-promoting mutations and are expected to be less susceptible to AR amplification[28-30]. PROTACs targeting AR for degradation were the first ones to be advanced into clinical testing and are currently in late phase 1.

Inhibition of Bruton's tyrosine kinase BTK by the covalent inhibitor ibrutinib is an effective first line treatment for chronic lymphocytic leukaemia. Clinical resistance to ibrutinib can arise if the cysteine to which ibrutinib covalently attaches gets mutated[31]. Despite the associated loss in affinity, ibrutinib-based PROTACs can still degrade mutant BTK and retain antiproliferative effects on CLL cells *in vitro*[32,33]. A similar case can be made for BRAF-targeting PROTACs: BRAF is frequently activated by mutation of valine 600 to glutamine (V600E) in a variety of cancers, which can be blocked by the clinically approved BRAF inhibitors[34]. However, there are limitations to the use of these inhibitors: most oncogenic BRAF mutations other than V600E, including BRAF translocations[35], dimer inducing mutants[36], mutants with increased binding to Ras proteins[37] or mutations of upstream drivers such as KRAS[38], lead to insensitivity to BRAF inhibitors. Moreover, resistance upon treatment can be caused by amplifications causing elevated expression of BRAF[38-40] or by splice versions of BRAF that lead to the expression of a truncated protein. In this case, constitutive dimerization of BRAF leads to a loss of responsiveness to the clinically approved inhibitors[41], which are only effective in inhibiting monomeric BRAF[34,42]. The emerging picture is that resistance occurs within, not outside of the Ras-Raf pathway and reactivates signalling, and that this in many cases involves increased Raf dimerization, which are insensitive to currently available inhibitors. Degraders should be able to overcome most of these limitations, since a reduction in protein abundance will reduce the activity of both monomeric and dimeric RAF proteins equally, and thus not be affected by the level of dimer formation.

Three papers recently reported the generation of BRAF degrading PROTACs[43-45]. Unexpectedly, all of them show a clear preference for the degradation of activated BRAF, be it by the V600E mutation or by other mutations, or by activation of upstream signalling. The selective degradation of activated Raf will dampen degradation in normal tissues with a lower Raf activity and may provide the basis for a therapeutic window, since simultaneous loss of BRAF and CRAF is not tolerated[46]. It remains to be seen if an optimization of RAF-targeting PROTACs to allow for clinical use will be possible.

Will PROTACs be exempt from the rule of resistance to targeted therapy? That seems unlikely. Multiple studies have shown that resistance can develop at the E3-targeting side of PROTACs: mutational loss or reduced expression of components of the ubiquitination machinery recruited by the PROTAC prevent drug-induced degradation[47-49]. There is no reason to assume that such events, which have been observed using RNA interference, CRISPR/Cas driven mutations or spontaneous mutations as either primary or acquired resistance in tissue culture cells, should not take place in tumours as mechanisms of acquired resistance, too. E3s that are essential to cell viability might have an advantage here, since inactivating mutations would not be expected to lead to outgrowth of PROTAC-resistant clones. Of note, resistance mutations in cancer cells treated with VHL-based PROTACs have been primarily observed as loss of the CUL2 subunit[47]. Conversely, resistance mutations in cancer cells treated with CRBN-based PROTACs have been primarily

observed as loss of CRBN subunit (but notably not of CUL4)[48-50], consistent with the evidence from clinical resistance to IMiDs[51]. Crucially, these two mechanisms are mutually exclusive i.e. loss of CUL2 will not cause cross-resistance to CRBN-based PROTAC and *conversely* loss of CRBN will not either with VHL PROTACs[52]. Consequently, the use of PROTAC drugs employing different E3 ligases may provide alternatives for treatment once resistance has developed. This reasoning is similar to the idea of targeting two different pockets on the same protein, such as in the case of BCR-ABL inhibition by both a competitive and an allosteric inhibitor[53]. This outlook appears even more plausible in the face of the more than 600 E3 ligases available, and the notion that we are only at the beginning of exploiting this family for small-molecule ligand and drug discovery.

Target selectivity

Beside the increase in target scope for PROTACs, which had been one of the driving expectations of the field early on, PROTACs offer a second, probably less expected advantage: PROTACs can display exceptional selectivity for their targets, which far exceeds that of the binder molecules they are based on[54-56]. Bromodomain and extra-terminal (BET) proteins are promising targets in oncology drug discovery with human clinical trials for several inhibitors ongoing[57,58]. Nevertheless, maintaining a sufficient therapeutic window has been a challenge for BET inhibitors in the clinic, raising a need for paralogue selective agents that could specifically interrogate the role of individual BET proteins in disease while potentially sparing the well documented on-target dose-limiting toxicities, e.g. thrombocytopenia [59-61]. Surprisingly, BET degrader MZ1, consisting of pan-BET inhibitor JQ1, a PEG based linker and a VHL ligand was found to demonstrate degradation of BRD4 at concentrations more than ten-fold lower than for paralogues BRD2 and BRD3, despite no such selectivity being demonstrated by JQ1 alone[54]. It has been shown that this selectivity can be attributed to differences in molecular recognition within the PROTAC induced VHL:MZ1:BRD4^{BD2} ternary complex between the E3 ligase and the bromodomain because of the lower structural conservation of sites peripheral to the JQ1 binding site itself which are nonetheless involved in the PROTAC-mediated protein-protein interactions that drive ternary complex formation and MZ1 mode of action[62,63]. Subsequent structure guided optimisation within this work led to PROTAC degrader AT1 which showed no discernible knockdown of any protein other than BRD4 at 1 μ M in HeLa cells as evaluated *via* unbiased and quantitative isobaric tagging mass spectrometry proteomics[62]. A further example of selective BRD4 degradation has also been shown with CRBN recruiting bifunctional degraders[64].

Within the kinase field, Bondeson *et al.* used a promiscuous kinase inhibitor, Foretinib, to study which portion of the kinases that this inhibitor could bind were degraded when tethered to VHL or CRBN recruiting motifs[65]. By correlating kinase binding data and quantitative proteomics data, the authors show that despite binding over 50 kinases, these VHL or CRBN based PROTACs degraded just 9 or 14 of these kinases, respectively[65]. This study shows how promiscuity in kinase binding may be overcome to provide enhanced functional selectivity. The concept of isoform selective kinase degradation has been further explored in the context of p38 MAPK degraders, an extension of the aforementioned Foretinib study[66], as well as with PROTACs targeted at cyclin-dependent kinases (CDKs)[55,67-69] and serum and glucocorticoid-induced protein kinases (SGKs)[56].

Building on a demonstrated ability to find isoform or paralogue selectivity, allele selective degradation would also be of therapeutic interest[22]. Indeed, the history of kinase targeted small molecule therapy includes examples where multiple generations of molecules were required to address a lack of isoform or allele selectivity in first generation inhibitors[70,71]. The ability to take relatively unselective binders and achieve a high degree of specificity in the context of a degrader

argues for a “degrader first” rather than “inhibitor first, degrader later”, approach for kinase drug discovery.

Tissue specificity

A significant challenge in cancer drug discovery is to find therapeutics that kill cancer cells, while sparing other cells in the body. Whilst the pre-clinical and clinical toxicology profiles of advanced PROTACs have not yet been disclosed, PROTACs may conceptually offer advantages in this regard. PROTAC mechanism of action requires recruitment of an E3 ligase, which may be expressed and/or activated to a higher degree in a tumour than in other tissues wherein target protein degradation would cause toxicity. There are a number of scenarios that could facilitate such an approach; highly tumour-specific expression of a particular E3 ligase[72,73], cell-cycle phase specific E3 ligase availability[74] or simply a lack of E3 expression at the sub-cellular, cellular or organ level in tissues that would be afflicted by toxic side-effects of target protein perturbation[75].

Navitoclax is a potent anti-cancer drug that has not reached approval due to side effects. It blocks activity of both BCL-2 and BCL-XL, with inhibition of the latter causing thrombocytopenia. While inhibiting both BCL-2 and BCL-XL is likely to be beneficial for killing cancer cells, the dose-limiting toxicity of BCL-XL inhibition negates the compound’s potential. This story received a new twist when Khan *et al.* made a PROTAC based on Navitoclax and VHL[75]. The resulting compound turned out to be more selective and resulted in degradation of BCL-XL only, sparing BCL-2, which is reminiscent of the increased selectivity of PROTACs observed within BET and kinase families. Moreover, since it is more potent than the inhibitor and since VHL is only expressed at low levels in platelets, it was significantly less toxic. While this concept is still in a pre-clinical state, it demonstrates how differential expression profile of the E3 ligase employed by a PROTAC can be exploited to derive PROTACs that are active only in a subset of tissues and thus avoid some of the unwanted effects of the drug.

It is noteworthy that VHL is rather uniformly expressed, except for the low expression in platelets. However, other E3 ligases or their subunits are more selectively expressed, such as the melanoma antigen (MAGE) family of proteins, which are almost exclusively expressed in gonads and in cancer[72,73]. As the number of E3 ligases with small molecule binders rises, we can expect that other examples of tissue-selective degradation by PROTACs will emerge. In many cases of interesting cancer targets, degradation will have to be selective to avoid toxicity, e.g. in the case of MYC. This oncogenic driver protein is not only involved in promoting tumorigenesis but it is also essential to many if not most cells[76]. On a similar note, pan-body degradation of beta catenin, which like MYC is a major oncogenic driver, will interfere with regeneration of the gut epithelium[77] and is also expected to weaken the blood-brain-barrier due to the role of beta catenin in the maintenance of tight junctions[78]. With the recent advent of the first small molecules binding to beta-catenin[79], tumour-selective or at least tissue-restricted PROTACs may provide a path forward on this yet-undrugged target.

PROTACs add an additional parameter to the pharmacodynamic properties of a drug: their effect persists after the drug has left the body until sufficient amounts of the drug’s target have been resynthesized. In the case of the anti-inflammatory drug target RIPK2, this has been shown to dampen inflammatory reactions for 6-10 days following a single dose of the PROTAC[80]. Such extended pharmacodynamic properties may allow very infrequent drug administrations to remain effective. It remains to be seen if similar examples emerge for cancer drug discovery.

Antibody-drug conjugates have been developed with the original idea of bringing a highly toxic substance selectively to the tumor *via* linkage to an antibody that selectively binds to tumour

cells[81]. This concept has met with clinical success in the past years, but resistance to the conjugated poisons creates the need for new independently acting cargos[82]. Pillow *et al.* demonstrated that PROTACs lend themselves to antibody conjugation, improving both their tissue selectivity and pharmacokinetic properties. They report targeted delivery of BET protein degradation by linking a highly potent BET PROTAC to an antibody against an antigen primarily found on AML cells[83]. While the resulting antibody-PROTAC conjugate must be delivered by intravenous injection, the antibody imparts the low clearance of large proteins onto the PROTAC and restricts its activity on AML cells. They show that a single dose of the conjugate causes regressions of models of AML in mice, while equivalent doses of the non-coupled PROTAC has no effect. Successful conjugation of PROTACs to antibodies has also been reported for other PROTACs[84], corroborating the feasibility of such an approach. An alternative opportunity for tissue selective PROTAC induced degradation, also applied in the context of BET degradation, has been the use of photo-activated degraders[85,86]. Such molecules incorporate a photoswitchable component in the linker of the PROTAC, allowing the molecules to be switched from an inactive to active state when irradiated.

Concluding remarks and future outlook

Many informative studies over recent years have successfully demonstrated how bifunctional degraders may bring significantly greater maximal efficacy, potency and selectivity over classical inhibitor-based approaches in the clinic (see **Figure 2** for a summary). Furthermore, the emerging evidence of the extra layers of nuance (and complexity) of PROTAC degraders over and above their component ligands provide the opportunity for these to be conceptually and actionably employed to supersede conventional inhibitory approaches. Success will now depend on which specific therapeutic challenges the current PROTAC knowledge base is applied to and in what ways this knowledge base can be expanded upon. We point towards the potential for targeting hitherto undrugged oncogenic proteins, such as non-G12C KRAS variants, due to a lack of reliance on highly ligandable binding sites. To further capitalise on the potential of a bifunctional modality, it will be increasingly important to view PROTACs as a dependable first choice strategy, where the extra dimensionality of bifunctional modalities can be of particular impact, such as when allele or tissue specific effects are desired. Next generation degraders will likely require the ability to recruit a broader array of E3 ligases, such that drug discovery programs can target a ligase that best complements their specific therapeutic rationale or that provides an advantage over first generation degrader drugs. To succeed as a new modality for treating cancer, efforts should be focussed on using PROTACs to address the areas we have highlighted here. When used to best exploit their strengths, PROTACs have a very bright future in drug discovery.

Author Contributions: William Farnaby and Manfred Koegl: Writing – original draft, Darryl McConnell and Alessio Ciulli: Writing - provided review and editing.

Declarations of interest:

William Farnaby: Declarations of interest: none

Manfred Koegl and Darryl McConnell are employees of Boehringer Ingelheim

Alessio Ciulli: A.C. is a scientific founder, shareholder, non-executive director and consultant of Amphista Therapeutics, a company that is developing targeted protein degradation therapeutic platforms. The Ciulli laboratory receives or has received sponsored research support from Ammirall, Amphista Therapeutics, Boehringer Ingelheim, Eisai, Nurix Therapeutics, and Ono Pharmaceutical.

References

* Of special interest

** Of outstanding interest

1. Meyers RM, Bryan JG, McFarland JM, Weir BA, Sizemore AE, Xu H, Dharia NV, Montgomery PG, Cowley GS, Pantel S, et al.: **Computational correction of copy number effect improves specificity of CRISPR-Cas9 essentiality screens in cancer cells.** *Nat Genet* 2017, **49**:1779-1784.
2. Behan FM, Iorio F, Picco G, Goncalves E, Beaver CM, Migliardi G, Santos R, Rao Y, Sassi F, Pinnelli M, et al.: **Prioritization of cancer therapeutic targets using CRISPR-Cas9 screens.** *Nature* 2019, **568**:511-516.
3. Maniaci C, Hughes SJ, Testa A, Chen W, Lamont DJ, Rocha S, Alessi DR, Romeo R, Ciulli A: **Homo-PROTACs: Bivalent small-molecule dimerizers of the VHL E3 ubiquitin ligase to induce self-degradation.** *Nat Commun* 2017, **8**:830.
4. Potjewyd F, Turner AW, Beri J, Rectenwald JM, Norris-Drouin JL, Cholensky SH, Margolis DM, Pearce KH, Herring LE, James LI: **Degradation of Polycomb Repressive Complex 2 with an EED-Targeted Bivalent Chemical Degradator.** *Cell Chem Biol* 2020, **27**:47-56 e15.
5. Hsu JH, Rasmusson T, Robinson J, Pacht F, Read J, Kawatkar S, DH OD, Bagal S, Code E, Rawlins P, et al.: **EED-Targeted PROTACs Degrade EED, EZH2, and SUZ12 in the PRC2 Complex.** *Cell Chem Biol* 2020, **27**:41-46 e17.
6. Farnaby W, Koegl M, Roy MJ, Whitworth C, Diers E, Trainor N, Zollman D, Steurer S, Karolyi-Oezguer J, Riedmueller C, et al.: **BAF complex vulnerabilities in cancer demonstrated via structure-based PROTAC design.** *Nat Chem Biol* 2019, **15**:672-680.
7. Testa A, Lucas X, Castro GV, Chan KH, Wright JE, Runcie AC, Gadd MS, Harrison WTA, Ko EJ, Fletcher D, et al.: **3-Fluoro-4-hydroxyprolines: Synthesis, Conformational Analysis, and Stereoselective Recognition by the VHL E3 Ubiquitin Ligase for Targeted Protein Degradation.** *J Am Chem Soc* 2018, **140**:9299-9313.
8. Han X, Zhao L, Xiang W, Qin C, Miao B, Xu T, Wang M, Yang CY, Chinnaswamy K, Stuckey J, et al.: **Discovery of Highly Potent and Efficient PROTAC Degradators of Androgen Receptor (AR) by Employing Weak Binding Affinity VHL E3 Ligase Ligands.** *J Med Chem* 2019, **62**:11218-11231.
9. Vangamudi B, Paul TA, Shah PK, Kost-Alimova M, Nottebaum L, Shi X, Zhan Y, Leo E, Mahadeshwar HS, Protopopov A, et al.: **The SMARCA2/4 ATPase domain surpasses the bromodomain as a drug target in SWI/SNF-mutant cancers: Insights from cDNA rescue and PFI-3 inhibitor studies.** *Cancer Res* 2015, **75**:3865-3878.
10. Remillard D, Buckley DL, Paulk J, Brien GL, Sonnett M, Seo H-S, Dastjerdi S, Wuhr M, Dhe-Paganon S, Armstrong SA, et al.: **Degradation of the BAF complex factor BRD9 by heterobifunctional ligands.** *Angew Chem Int Ed* 2017, **56**:5738-5743.
11. Zoppi V, Hughes SJ, Maniaci C, Testa A, Gmaschitz T, Wieshofer C, Koegl M, Riching KM, Daniels DL, Spallarossa A, et al.: **Iterative Design and Optimization of Initially Inactive Proteolysis Targeting Chimeras (PROTACs) Identify VZ185 as a Potent, Fast, and Selective von Hippel-Lindau (VHL) Based Dual Degradator Probe of BRD9 and BRD7.** *J Med Chem* 2019, **62**:699-726.
12. Martin LJ, Koegl M, Bader G, Cockcroft X-L, Fedorov O, Fiegen D, Gerstberger T, Hofmann MH, Hohmann AF, Kessler D, et al.: **Structure-Based Design of an in Vivo Active Selective BRD9 Inhibitor.** *J Med Chem* 2016, **59**:4462-4475.
13. Krämer K, Moreno N, Frühwald M, Kerl K: **BRD9 Inhibition, Alone or in Combination with Cytostatic Compounds as a Therapeutic Approach in Rhabdoid Tumors.** *Int J Mol Sci* 2017, **18**:1537.

14. Brien GL, Remillard D, Shi J, Hemming ML, Chabon J, Wynne K, Dillon ET, Cagney G, Van Mierlo G, Baltissen MP, et al.: **Targeted degradation of BRD9 reverses oncogenic gene expression in synovial sarcoma.** *Elife* 2018 doi: 10.7554/eLife.41305.
15. Huang HT, Seo HS, Zhang T, Wang Y, Jiang B, Li Q, Buckley DL, Nabet B, Roberts JM, Paulk J, et al.: **MELK is not necessary for the proliferation of basal-like breast cancer cells.** *Elife* 2017 doi: 10.7554/eLife.26693.
16. Cromm PM, Samarasinghe KTG, Hines J, Crews CM: **Addressing Kinase-Independent Functions of Fak via PROTAC-Mediated Degradation.** *J Am Chem Soc* 2018, **140**:17019-17026.
17. Popow J, Arnhof H, Bader G, Berger H, Ciulli A, Covini D, Dank C, Gmaschitz T, Greb P, Karolyi-Ozguer J, et al.: **Highly Selective PTK2 Proteolysis Targeting Chimeras to Probe Focal Adhesion Kinase Scaffolding Functions.** *J Med Chem* 2019, **62**:2508-2520.
18. Sulzmaier FJ, Jean C, Schlaepfer DD: **FAK in cancer: mechanistic findings and clinical applications.** *Nat Rev Cancer* 2014, **14**:598-610.
19. Chung S, Suzuki H, Miyamoto T, Takamatsu N, Tatsuguchi A, Ueda K, Kijima K, Nakamura Y, Matsuo Y: **Development of an orally-administrative MELK-targeting inhibitor that suppresses the growth of various types of human cancer.** *Oncotarget* 2012, **3**:1629-1640.
20. Ganguly R, Hong CS, Smith LG, Kornblum HI, Nakano I: **Maternal embryonic leucine zipper kinase: key kinase for stem cell phenotype in glioma and other cancers.** *Mol Cancer Ther* 2014, **13**:1393-1398.
21. Lin A, Giuliano CJ, Sayles NM, Sheltzer JM: **CRISPR/Cas9 mutagenesis invalidates a putative cancer dependency targeted in on-going clinical trials.** *Elife* 2017 doi: 10.7554/eLife.24179.
22. Bond MJ, Chu L, Nalawansha DA, Li K, Crews CM: **Targeted Degradation of Oncogenic KRAS(G12C) by VHL-Recruiting PROTACs.** *ACS Cent Sci* 2020, **6**:1367-1375.
23. Maurer T, Garrenton LS, Oh A, Pitts K, Anderson DJ, Skelton NJ, Fauber BP, Pan B, Malek S, Stokoe D, et al.: **Small-molecule ligands bind to a distinct pocket in Ras and inhibit SOS-mediated nucleotide exchange activity.** *Proc Natl Acad Sci U S A* 2012, **109**:5299-5304.
24. Sun Q, Burke JP, Phan J, Burns MC, Olejniczak ET, Waterson AG, Lee T, Rossanese OW, Fesik SW: **Discovery of small molecules that bind to K-Ras and inhibit Sos-mediated activation.** *Angew Chem Int Ed* 2012, **51**:6140-6143.
25. Kessler D, Gmachl M, Mantoulidis A, Martin LJ, Zoephel A, Mayer M, Gollner A, Covini D, Fischer S, Gerstberger T, et al.: **Drugging an undruggable pocket on KRAS.** *Proc Natl Acad Sci U S A* 2019, **116**:15823-15829.
26. Edwards J, Krishna NS, Grigor KM, Bartlett JM: **Androgen receptor gene amplification and protein expression in hormone refractory prostate cancer.** *Br J Cancer* 2003, **89**:552-556.
27. Joseph JD, Lu N, Qian J, Sensintaffar J, Shao G, Brigham D, Moon M, Maneval EC, Chen I, Darimont B, et al.: **A clinically relevant androgen receptor mutation confers resistance to second-generation antiandrogens enzalutamide and ARN-509.** *Cancer Discov* 2013, **3**:1020-1029.
28. Salami J, Alabi S, Willard RR, Vitale NJ, Wang J, Dong H, Jin M, McDonnell DP, Crew AP, Neklesa TK, et al.: **Androgen receptor degradation by the proteolysis-targeting chimera ARCC-4 outperforms enzalutamide in cellular models of prostate cancer drug resistance.** *Commun Biol* 2018 doi: 10.1038/s42003-018-0105-8.
29. Han X, Wang C, Qin C, Xiang W, Fernandez-Salas E, Yang CY, Wang M, Zhao L, Xu T, Chinnaswamy K, et al.: **Discovery of ARD-69 as a Highly Potent Proteolysis Targeting Chimera (PROTAC) Degradator of Androgen Receptor (AR) for the Treatment of Prostate Cancer.** *J Med Chem* 2019, **62**:941-964.
30. Kregel S, Wang C, Han X, Xiao L, Fernandez-Salas E, Bawa P, McCollum BL, Wilder-Romans K, Apel IJ, Cao X, et al.: **Androgen receptor degraders overcome common resistance mechanisms developed during prostate cancer treatment.** *Neoplasia* 2020, **22**:111-119.
31. Pula B, Golos A, Gorniak P, Jamrozik K: **Overcoming Ibrutinib Resistance in Chronic Lymphocytic Leukemia.** *Cancers (Basel)* 2019, **11**.

32. Buhimschi AD, Armstrong HA, Toure M, Jaime-Figueroa S, Chen TL, Lehman AM, Woyach JA, Johnson AJ, Byrd JC, Crews CM: **Targeting the C481S Ibrutinib-Resistance Mutation in Bruton's Tyrosine Kinase Using PROTAC-Mediated Degradation.** *Biochemistry* 2018, **57**:3564-3575.
33. Sun Y, Zhao X, Ding N, Gao H, Wu Y, Yang Y, Zhao M, Hwang J, Song Y, Liu W, et al.: **PROTAC-induced BTK degradation as a novel therapy for mutated BTK C481S induced ibrutinib-resistant B-cell malignancies.** *Cell Res* 2018, **28**:779-781.
34. Karoulia Z, Gavathiotis E, Poulikakos PI: **New perspectives for targeting RAF kinase in human cancer.** *Nat Rev Cancer* 2017, **17**:676-691.
35. Botton T, Talevich E, Mishra VK, Zhang T, Shain AH, Berquet C, Gagnon A, Judson RL, Ballotti R, Ribas A, et al.: **Genetic Heterogeneity of BRAF Fusion Kinases in Melanoma Affects Drug Responses.** *Cell Rep* 2019, **29**:573-588 e577.
36. Yao Z, Torres NM, Tao A, Gao Y, Luo L, Li Q, de Stanchina E, Abdel-Wahab O, Solit DB, Poulikakos PI, et al.: **BRAF Mutants Evade ERK-Dependent Feedback by Different Mechanisms that Determine Their Sensitivity to Pharmacologic Inhibition.** *Cancer Cell* 2015, **28**:370-383.
37. Yao Z, Yaeger R, Rodrik-Outmezguine VS, Tao A, Torres NM, Chang MT, Drosten M, Zhao H, Cecchi F, Hembrough T, et al.: **Tumours with class 3 BRAF mutants are sensitive to the inhibition of activated RAS.** *Nature* 2017, **548**:234-238.
38. Oddo D, Sennott EM, Barault L, Valtorta E, Arena S, Cassingena A, Filiciotto G, Marzolla G, Elez E, van Geel RM, et al.: **Molecular Landscape of Acquired Resistance to Targeted Therapy Combinations in BRAF-Mutant Colorectal Cancer.** *Cancer Res* 2016, **76**:4504-4515.
39. Shi H, Moriceau G, Kong X, Lee MK, Lee H, Koya RC, Ng C, Chodon T, Scolyer RA, Dahlman KB, et al.: **Melanoma whole-exome sequencing identifies (V600E)B-RAF amplification-mediated acquired B-RAF inhibitor resistance.** *Nat Commun* 2012, **3**:724.
40. Ahronian LG, Sennott EM, Van Allen EM, Wagle N, Kwak EL, Faris JE, Godfrey JT, Nishimura K, Lynch KD, Mermel CH, et al.: **Clinical Acquired Resistance to RAF Inhibitor Combinations in BRAF-Mutant Colorectal Cancer through MAPK Pathway Alterations.** *Cancer Discov* 2015, **5**:358-367.
41. Vido MJ, Le K, Hartsough EJ, Aplin AE: **BRAF Splice Variant Resistance to RAF Inhibitor Requires Enhanced MEK Association.** *Cell Rep* 2018, **25**:1501-1510 e1503.
42. Poulikakos PI, Persaud Y, Janakiraman M, Kong X, Ng C, Moriceau G, Shi H, Atefi M, Titz B, Gabay MT, et al.: **RAF inhibitor resistance is mediated by dimerization of aberrantly spliced BRAF(V600E).** *Nature* 2011, **480**:387-390.
43. Posternak G, Tang X, Maisonneuve P, Jin T, Lavoie H, Daou S, Orlicky S, Goulet de Rugy T, Caldwell L, Chan K, et al.: **Functional characterization of a PROTAC directed against BRAF mutant V600E.** *Nat Chem Biol* 2020, **16**:1170-1178.
44. Han XR, Chen L, Wei Y, Yu W, Chen Y, Zhang C, Jiao B, Shi T, Sun L, Zhang C, et al.: **Discovery of Selective Small Molecule Degraders of BRAF-V600E.** *J Med Chem* 2020, **63**:4069-4080.
45. Alabi. S ea: **Mutant-selective Degradation by BRAF-targeting PROTACs.** *BioRxiv Preprint Server* 2020 doi: 10.1101/2020.08.10.245159.
46. Sanclemente M, Francoz S, Esteban-Burgos L, Bousquet-Mur E, Djurec M, Lopez-Casas PP, Hidalgo M, Guerra C, Drosten M, Musteanu M, et al.: **c-RAF Ablation Induces Regression of Advanced Kras/Trp53 Mutant Lung Adenocarcinomas by a Mechanism Independent of MAPK Signaling.** *Cancer Cell* 2018, **33**:217-228 e214.
47. Zhang L, Riley-Gillis B, Vijay P, Shen Y: **Acquired Resistance to BET-PROTACs (Proteolysis-Targeting Chimeras) Caused by Genomic Alterations in Core Components of E3 Ligase Complexes.** *Mol Cancer Ther* 2019, **18**:1302-1311.
48. Ottis P, Palladino C, Thienger P, Britschgi A, Heichinger C, Berrera M, Julien-Laferriere A, Roudnicky F, Kam-Thong T, Bischoff JR, et al.: **Cellular Resistance Mechanisms to Targeted Protein Degradation Converge Toward Impairment of the Engaged Ubiquitin Transfer Pathway.** *ACS Chem Biol* 2019, **14**:2215-2223.

49. Mayor-Ruiz C, Jaeger MG, Bauer S, Brand M, Sin C, Hanzl A, Mueller AC, Menche J, Winter GE: **Plasticity of the Cullin-RING Ligase Repertoire Shapes Sensitivity to Ligand-Induced Protein Degradation.** *Mol Cell* 2019, **75**:849-858 e848.
50. Sievers QL, Gasser JA, Cowley GS, Fischer ES, Ebert BL: **Genome-wide screen identifies cullin-RING ligase machinery required for lenalidomide-dependent CRL4(CRBN) activity.** *Blood* 2018, **132**:1293-1303.
51. Gooding S, Ansari-Pour N, Towfic F, Ortiz Estevez M, Chamberlain PP, Tsai KT, Flynt E, Hirst M, Rozelle D, Dhiman P, et al.: **Multiple Cereblon genetic changes associate with acquired resistance to Lenalidomide or Pomalidomide in Multiple Myeloma.** *Blood* 2020 doi: 10.1182/blood.2020007081.
52. Shirasaki R, Matthews GM, Gandolfi S, de Matos Simoes R, Buckley DL, Raja Vora J, Sievers QL, Bruggenthies JB, Dashevsky O, Poarch H, et al.: **Functional Genomics Identify Distinct and Overlapping Genes Mediating Resistance to Different Classes of Heterobifunctional Degradors of Oncoproteins.** *Cell Rep* 2021, **34**:108532.
53. Schoepfer J, Jahnke W, Berellini G, Buonamici S, Cotesta S, Cowan-Jacob SW, Dodd S, Drueckes P, Fabbro D, Gabriel T, et al.: **Discovery of Asciminib (ABL001), an Allosteric Inhibitor of the Tyrosine Kinase Activity of BCR-ABL1.** *J Med Chem* 2018, **61**:8120-8135.
54. Zengerle M, Chan K-H, Ciulli A: **Selective small molecule induced degradation of the BET bromodomain protein BRD4.** *ACS Chem Biol* 2015, **10**:1770-1777.
55. Jiang B, Wang ES, Donovan KA, Liang Y, Fischer ES, Zhang T, Gray NS: **Development of Dual and Selective Degradors of Cyclin-Dependent Kinases 4 and 6.** *Angew Chem Int Ed* 2019, **58**:6321-6326.
56. Tovell H, Testa A, Zhou H, Shpiro N, Crafter C, Ciulli A, Alessi DR: **Design and Characterization of SGK3-PROTAC1, an Isoform Specific SGK3 Kinase PROTAC Degradator.** *ACS Chem Biol* 2019, **14**:2024-2034.
57. Xu Y, Vakoc CR: **Targeting Cancer Cells with BET Bromodomain Inhibitors.** *Cold Spring Harb Perspect Med* 2017 doi: 10.1101/cshperspect.a026674.
58. Cochran AG, Conery AR, Sims RJ, 3rd: **Bromodomains: a new target class for drug development.** *Nat Rev Drug Discov* 2019, **18**:609-628.
59. Amorim S, Stathis A, Gleeson M, Iyengar S, Magarotto V, Leleu X, Morschhauser F, Karlin L, Broussais F, Rezai K, et al.: **Bromodomain inhibitor OTX015 in patients with lymphoma or multiple myeloma: a dose-escalation, open-label, pharmacokinetic, phase 1 study.** *Lancet Haematol* 2016, **3**:e196-204.
60. Postel-Vinay S, Herbschleb K, Massard C, Woodcock V, Soria JC, Walter AO, Ewerton F, Poelman M, Benson N, Ocker M, et al.: **First-in-human phase I study of the bromodomain and extraterminal motif inhibitor BAY 1238097: emerging pharmacokinetic/pharmacodynamic relationship and early termination due to unexpected toxicity.** *Eur J Cancer* 2019, **109**:103-110.
61. Piha-Paul SA, Sachdev JC, Barve M, LoRusso P, Szmulewitz R, Patel SP, Lara PN, Jr., Chen X, Hu B, Freise KJ, et al.: **First-in-Human Study of Mivebresib (ABBV-075), an Oral Pan-Inhibitor of Bromodomain and Extra Terminal Proteins, in Patients with Relapsed/Refractory Solid Tumors.** *Clin Cancer Res* 2019, **25**:6309-6319.
62. Gadd MS, Testa A, Lucas X, Chan K-H, Chen W, Lamont DJ, Zengerle M, Ciulli A: **Structural basis of PROTAC cooperative recognition for selective protein degradation.** *Nat Chem Biol* 2017, **13**:514-521.
63. Roy MJ, Winkler S, Hughes SJ, Whitworth C, Galant M, Farnaby W, Rumpel K, Ciulli A: **SPR-Measured Dissociation Kinetics of PROTAC Ternary Complexes Influence Target Degradation Rate.** *ACS Chem Biol* 2019, **14**:361-368.
64. Nowak RP, DeAngelo SL, Buckley D, He Z, Donovan KA, An J, Safaei N, Jedrychowski MP, Ponthier CM, Ishoey M, et al.: **Plasticity in binding confers selectivity in ligand-induced protein degradation.** *Nat Chem Biol* 2018:706-714.

65. Bondeson DP, Smith BE, Burslem GM, Buhimschi AD, Hines J, Jaime-Figueroa S, Wang J, Hamman BD, Ishchenko A, Crews CM: **Lessons in PROTAC design from selective degradation with a promiscuous warhead.** *Cell Chem Biol* 2018, **25**:78-87.e75.
66. Smith BE, Wang SL, Jaime-Figueroa S, Harbin A, Wang J, Hamman BD, Crews CM: **Differential PROTAC substrate specificity dictated by orientation of recruited E3 ligase.** *Nat Commun* 2019, **10**:131.
67. Robb CM, Contreras JI, Kour S, Taylor MA, Abid M, Sonawane YA, Zahid M, Murry DJ, Natarajan A, Rana S: **Chemically induced degradation of CDK9 by a proteolysis targeting chimera (PROTAC).** *Chem Commun (Camb)* 2017, **53**:7577-7580.
68. Olson CM, Jiang B, Erb MA, Liang Y, Doctor ZM, Zhang Z, Zhang T, Kwiatkowski N, Boukhali M, Green JL, et al.: **Pharmacological perturbation of CDK9 using selective CDK9 inhibition or degradation.** *Nat Chem Biol* 2018, **14**:163-170.
69. Zhou F, Chen L, Cao C, Yu J, Luo X, Zhou P, Zhao L, Du W, Cheng J, Xie Y, et al.: **Development of selective mono or dual PROTAC degrader probe of CDK isoforms.** *Eur J Med Chem* 2020, **187**:111952.
70. Yang J, Nie J, Ma X, Wei Y, Peng Y, Wei X: **Targeting PI3K in cancer: mechanisms and advances in clinical trials.** *Mol Cancer* 2019, **18**:26.
71. Ayati A, Moghimi S, Salarinejad S, Safavi M, Pouramiri B, Foroumadi A: **A review on progression of epidermal growth factor receptor (EGFR) inhibitors as an efficient approach in cancer targeted therapy.** *Bioorg Chem* 2020, **99**:103811.
72. Florke Gee RR, Chen H, Lee AK, Daly CA, Wilander BA, Fon Tacer K, Potts PR: **Emerging roles of the MAGE protein family in stress response pathways.** *J Biol Chem* 2020, **295**:16121-16155.
73. Fon Tacer K, Montoya MC, Oatley MJ, Lord T, Oatley JM, Klein J, Ravichandran R, Tillman H, Kim M, Connelly JP, et al.: **MAGE cancer-testis antigens protect the mammalian germline under environmental stress.** *Sci Adv* 2019, **5**:eaav4832.
74. Riching KM, Mahan S, Corona CR, McDougall M, Vasta JD, Robers MB, Urh M, Daniels DL: **Quantitative Live-Cell Kinetic Degradation and Mechanistic Profiling of PROTAC Mode of Action.** *ACS Chem Biol* 2018, **13**:2758-2770.
75. Khan S, Zhang X, Lv D, Zhang Q, He Y, Zhang P, Liu X, Thummuri D, Yuan Y, Wiegand JS, et al.: **A selective BCL-XL PROTAC degrader achieves safe and potent antitumor activity.** *Nat Med* 2019, **25**:1938-1947.
76. Wang T, Birsoy K, Hughes NW, Krupczak KM, Post Y, Wei JJ, Lander ES, Sabatini DM: **Identification and characterization of essential genes in the human genome.** *Science* 2015, **350**:1096-1101.
77. Fevr T, Robine S, Louvard D, Huelsken J: **Wnt/beta-catenin is essential for intestinal homeostasis and maintenance of intestinal stem cells.** *Mol Cell Biol* 2007, **27**:7551-7559.
78. Tran KA, Zhang X, Predescu D, Huang X, Machado RF, Gothert JR, Malik AB, Valyi-Nagy T, Zhao YY: **Endothelial beta-Catenin Signaling Is Required for Maintaining Adult Blood-Brain Barrier Integrity and Central Nervous System Homeostasis.** *Circulation* 2016, **133**:177-186.
79. Kessler D, Mayer M, Zahn S, Zeeb M, Wohrle S, Bergner A, Bruchhaus J, Ciftci T, Dahmann G, Dettling M, et al.: **Getting a Grip on the Undrugged: Targeting beta-Catenin with Fragment-Based Methods.** *ChemMedChem* 2020 doi: 10.1002/cmdc.202000839.
80. Mares A, Miah AH, Smith IED, Rackham M, Thawani AR, Cryan J, Haile PA, Votta BJ, Beal AM, Capriotti C, et al.: **Extended pharmacodynamic responses observed upon PROTAC-mediated degradation of RIPK2.** *Commun Biol* 2020, **3**:140.
81. Joubert N, Beck A, Dumontet C, Denevault-Sabourin C: **Antibody-Drug Conjugates: The Last Decade.** *Pharmaceuticals (Basel)* 2020 doi: 10.3390/ph13090245.
82. Garcia-Alonso S, Ocana A, Pandiella A: **Resistance to Antibody-Drug Conjugates.** *Cancer Res* 2018, **78**:2159-2165.

83. Pillow TH, Adhikari P, Blake RA, Chen J, Del Rosario G, Deshmukh G, Figueroa I, Gascoigne KE, Kamath AV, Kaufman S, et al.: **Antibody Conjugation of a Chimeric BET Degradator Enables in vivo Activity.** *ChemMedChem* 2020 doi: 10.1002/cmdc.201900497.
84. Maneiro MA, Forte N, Shchepinova MM, Kounde CS, Chudasama V, Baker JR, Tate EW: **Antibody-PROTAC Conjugates Enable HER2-Dependent Targeted Protein Degradation of BRD4.** *ACS Chem Biol* 2020, **15**:1306-1312.
85. Pfaff P, Samarasinghe KTG, Crews CM, Carreira EM: **Reversible Spatiotemporal Control of Induced Protein Degradation by Bistable PhotoPROTACs.** *ACS Cent Sci* 2019, **5**:1682-1690.
86. Reynders M, Matsuura BS, Berouti M, Simoneschi D, Marzio A, Pagano M, Trauner D: **PHOTACs enable optical control of protein degradation.** *Sci Adv* 2020, **6**:eaay5064.