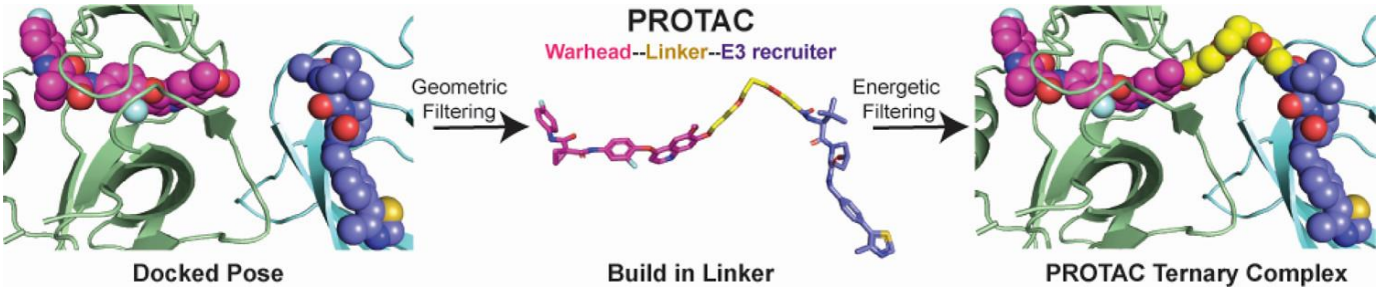
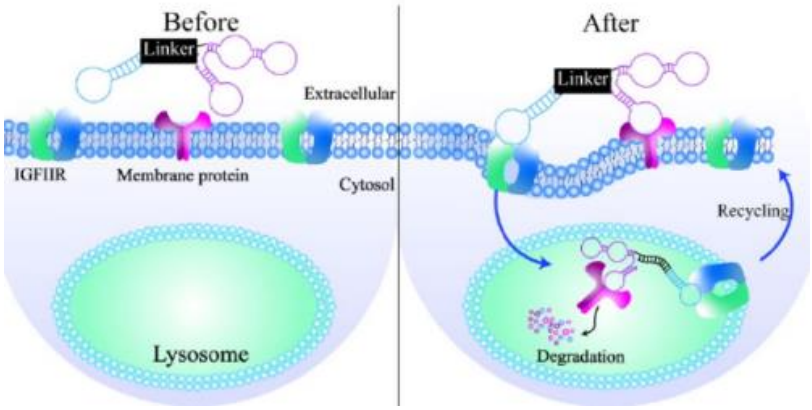
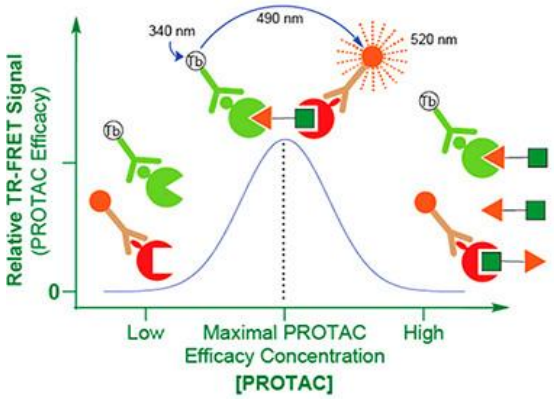
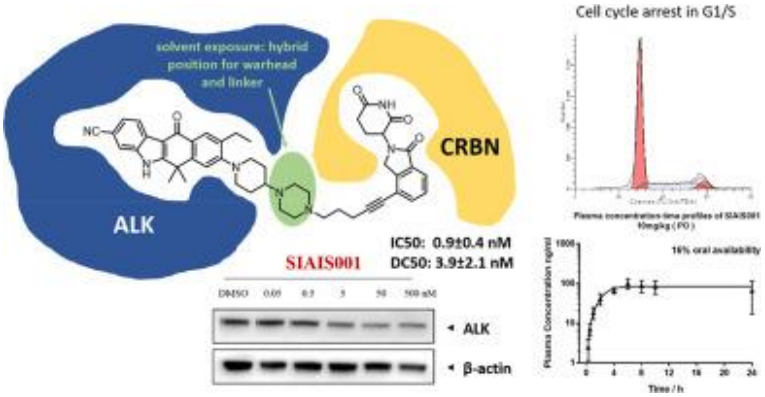
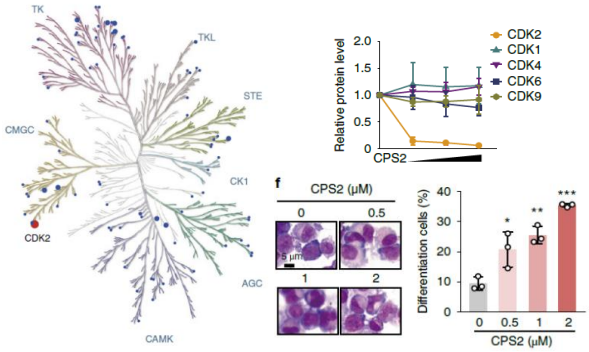
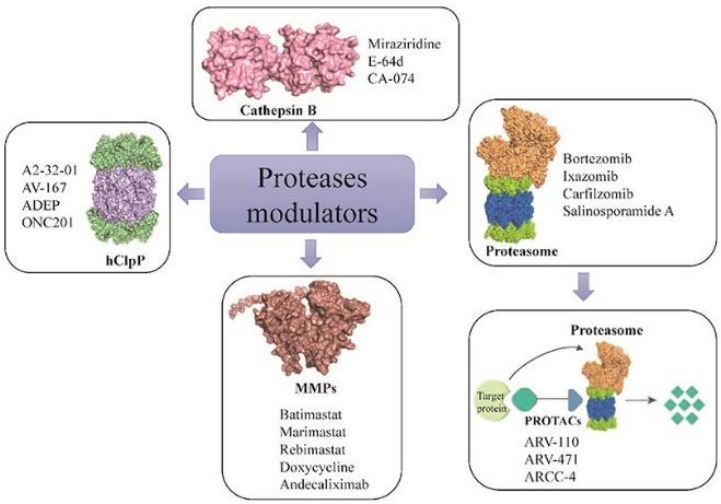












Ciulli Group Journal Club

Targeted Protein Degradation, Medicinal Chemistry and Chemical Structural Biology Literature Highlights

March 2021 Edition

Contents

Feature of the Month

Contributor: Charlotte

As TPD literature explodes and degraders continue to approach and progress through the clinic, it is more important than ever to keep track of what is happening in the field.

March saw the release of the Degrader Digest, a new resource for learning about TPD, created by Marc Cohen (C4 Therapeutics). The website has already been populated with a number of video animations about TPD, webinars, industry events and media articles, as well as listing companies working on TPD.

The Degrader Digest is a timely resource which will undoubtedly be of great value to new scientists entering the field as well as current researchers for finding useful TPD updates.

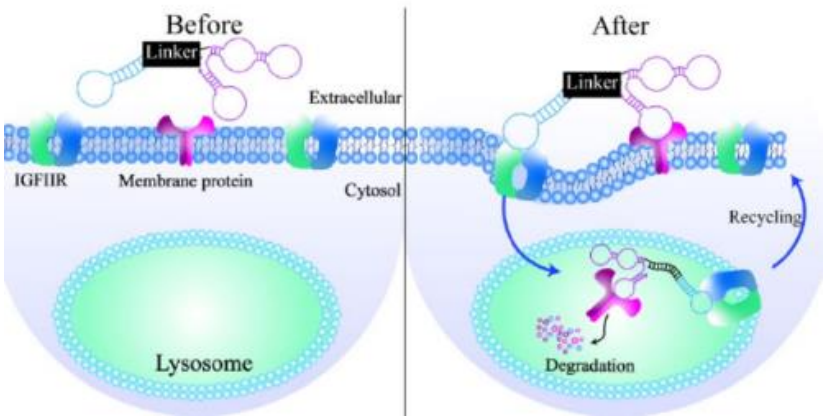
Targeted Protein Degradation

Contributor: Aileen

Bispecific Aptamer Chimeras Enable Targeted Protein Degradation on Cell Membranes

Da Han[§]*, …, Yang Yang

Angew. Chem. Int. Ed. **2021**, DOI: 10.1002/anie.202102170



Aptamers are oligonucleotides which can bind to proteins specifically, including those on the cell membrane. This has been exploited in this report, which uses bispecific aptamer chimeras to affect degradation of extracellular proteins via the lysosome. Herein, bispecific aptamer chimeras are designed which bind to both a target protein on the cell membrane and to the IGFIIR (cell-surface lysosome-shuttling receptor). This enables shuttling of the protein of interest to the lysosome and results in lysosome-mediated degradation.

The authors demonstrate application of this methodology to both Met receptor and PTK-7, membrane proteins that are potential targets for cancer therapy. Treatment of HeLa and CEM cells with bispecific aptamer chimeras designed to target Met and PTK-7, respectively, resulted in reduction of fluorescence signals indicating on-target protein degradation (greater than 65% for Met after 24 h). Lower levels of cellular viability and mobility were shown after targeted degradation of Met, however it would be interesting to see if these were sufficient to halt disease progression.

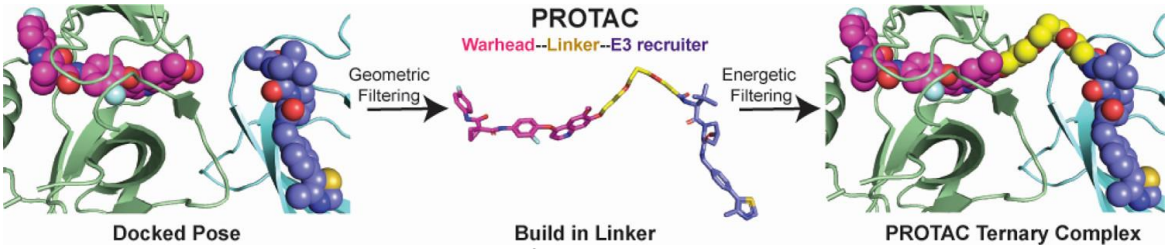
The authors highlight that aptamers can be identified via an in vitro selection strategy known as "systematic evolution of ligands by exponential enrichment (SELEX)", which potentially expedites application of this technique to many membrane-bound proteins. It will be interesting to see if this indeed proves to be the case, and if the in vivo stability of the chimeras can be attuned so that this methodology can make an impact on drug discovery.

Contributor: Kevin

Rationalizing PROTAC-Mediated Ternary Complex Formation Using Rosetta

Nan Bai[§], …, John Karanicolas*

J. Chem. Inf. Model. **2021**, *61*, 1368



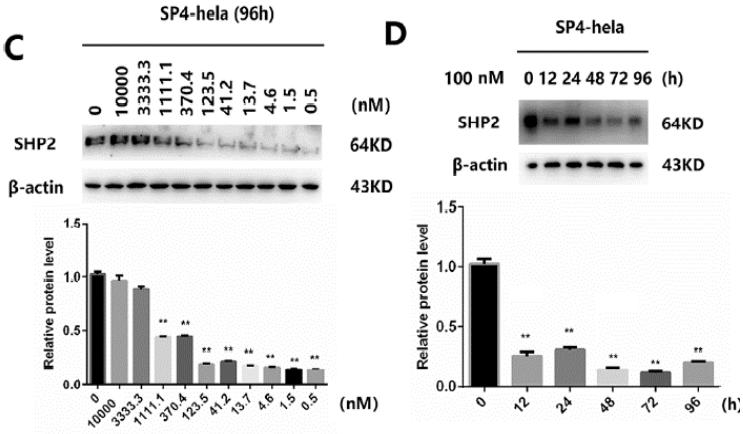
Rational design of PROTAC linkers remains an unsolved challenge. In this paper, the authors use a computational approach to model the structure of PROTAC-mediated ternary complexes using Rosetta. They first separately create ensembles reflecting the possible protein/protein interactions (PPIs) in the absence of the PROTAC linker using a docking procedure and the full conformational space of the linker using OMEGA and then score what proportion of the linker conformations is compatible with the predicted PPIs. The fraction fully compatible conformations (FFC) obtained this way correlated very well with observed degradation efficiency for multiple series of PROTACs and reliably reflected preferences for favourable linker length and E3 ligase choice. Several of the characterized ternary complexes show more than one binding mode with several structurally distinct conformers fulfilling the geometric and energetic requirements equally well. This makes a strong case for future studies on the solution structure of PROTAC-induced ternary complexes. Until this data will become available the computational approach presented here will be valuable to streamline linker design for novel PROTACs.

Contributor: Claire

Novel PROTACs for degradation of SHP2 protein

Mengzhu Zheng[§], Yang Liu[§], Canrong Wu[§], …, Yirong Zhou*, Lixia Chen*, Hua Li*

Bioorg. Chem. **2021**, *110*, 104788



Protein tyrosine phosphatase SHP2 is a promising anticancer drug target as it is involved in several key cell signalling pathways. Thus far, catalytic inhibition of SHP2 has lacked the selectivity required for an effective anticancer therapy. Allosteric SHP2 inhibitors have therefore gained traction, and one such potent and selective orally bio-available inhibitor, SHP099, was utilised in this paper in a bid to more efficiently inhibit SHP2 activity in cancer cells. CRBN-recruiting SHP2 PROTACs, **SP2**-**SP5**, were made by connecting SHP099 to pomalidomide using varying linker length. All four SHP2 PROTACs inhibited SHP2 with sub micro-molar efficacy however only **SP3** and **SP4** (mid-length linkers) were shown to degrade SHP2 and induce an antiproliferative effect in HeLa cells. **SP4** PROTAC was found to induce a greater apoptotic effect in HeLa cells than SHP099 inhibitor as well as induce cell cycle arrest and inhibit RAS/MAPK signalling.

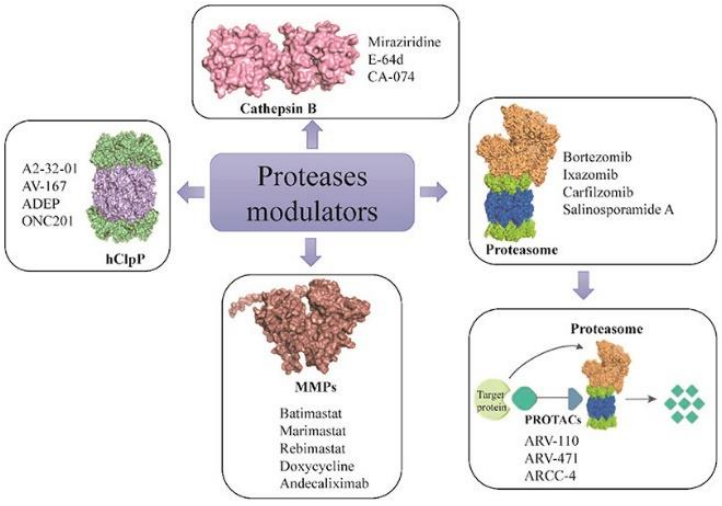
Initial profiling of the SHP2 PROTACs highlighted in this paper demonstrates therapeutic promise in targeting SHP2 for degradation. It would be interesting to see more extensive profiling of direct binding affinities and characterisation of the ternary complex as well as the effects of these SHP2 PROTACS in more disease-relevant cell models such as leukaemia cell lines. Demonstrating that these SHP2 PROTACs are able to induce degradation at therapeutically relevant time points will also be important. Indeed, the authors highlight the recent development of alternative VHL-based SHP2 PROTACs that also utilise the SHP099 inhibitor and have shown an effect in AML and esophageal cancer cell lines (covered in our June 2020 issue).

Contributor: Aileen

Proteases and Their Modulators in Cancer Therapy: Challenges and Opportunities

Rao Song[§], Wenliang Qiau[§], Jun He[§], …, Youfu Luo*, Tao Yang*

J. Med. Chem. **2021**, *64*, 2851



This perspective describes a number of proteases, giving an overview of their structure, function, and attempts at targeting in cancer therapy. The Proteasome, CathepsinB, hClpP and MMPs are featured. In addition to outlining known inhibitors, the section on the proteasome also contains a concise summary of the PROTAC field including MDM2, cIAP1, VHL and CRBN-based degraders. This would provide a nice introduction for anyone new to the field.

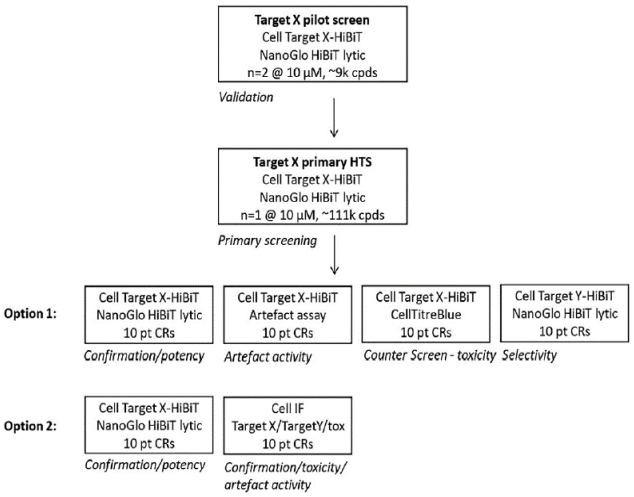
The authors provide a critical overview of methods by which proteases have been targeted for cancer therapy, with limitations highlighted. It is interesting to see a discussion of TPD in the context of inhibition of proteases.

Contributor: Claire

Small-Molecule Degraders beyond PROTACs—Challenges and Opportunities

Johanna M. Kastl[§*], Gareth Davies, Eleanor Godsman, and Geoffrey A. Holdgate

SLAS Discov. **2021**, DOI: 10.1177/2472555221991104



This perspectives piece gives a MoA overview for the various types of heterobifunctional degraders (PROTACs, AUTACs, LYTACs) and small-molecule degraders (molecular glues, SERDs, HyTs). A need within the TPD field for unbiased high-throughput screening methods to identify potential degrader modalities is discussed and proposed to be implemented at the beginning of a TPD hit finding campaign to complement a PROTAC approach. A cellular HiBiT screen of a 111,000 compound library was utilised to identify alternative small-molecule degraders of an undisclosed oncology target, impressively taking less than 3 days. The library consisted of a diversity set as well as confirmed target binders identified from a previous competitive binding biochemical screen (focused set) whilst target-specific PROTACs were used as positive controls for degradation. The assay was shown to reliably identify known degraders and produce hits from both the diversity and focused set. The inclusion of known cytotoxic compounds for assay validation highlighted cytotoxicity as a source of false positives and the need for a cell viability assay to be incorporated into the screen upfront. The authors go on to discuss the caveats surrounding the need to follow up identified hits with mechanistic characterisation which remains challenging to apply a single assay to assess across TPD modalities whilst maintaining high throughput capacity.

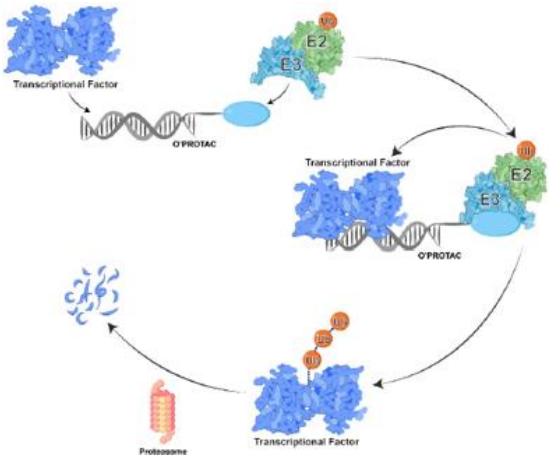
The power of the HiBiT degradation assay strikes again! It is great to see this valuable technology so widely used within the TPD field and applied here in the context of high throughput screening to identify hits and triage not only PROTACs but a range of degrader modalities.

Contributor: Kevin

Destruction of DNA-binding proteins by programmable O'PROTAC: Oligonucleotide-based PROTAC

Jingwei Shao[§], Yuqian Yan[§], Donglin Ding[§], …, Hong-yu Li*, Haojie Huang*

bioRxiv **2021**, DOI: 10.1101/2021.03.08.434493



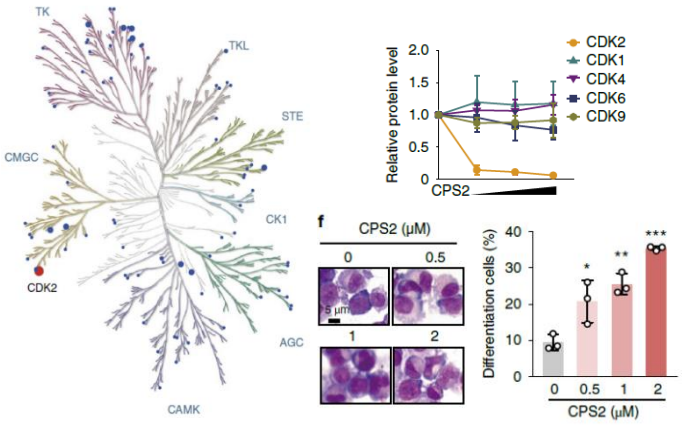
Transcription factors are important factors in cancer and other diseases, but generally lack ligand binding sites that would make them attractive drug targets. Here the authors exploit their ability to specifically bind well defined DNA sequence motifs to design Oligonucleotide-based PROTACs (O'PROTACs). They fuse the DNA-binding motifs of ERG and LEF1 transcription factors as short oligonucleotide-duplexes to VHL or Cereblon-recruiting moieties. After transfection using lipofectamine into cells they observe targeted degradation of the transcription factors in cell. Due to the simple synthesis and straight-forward design of a warhead the authors envision a high-throughput approach for O'PROTAC library design to target a variety of transcription factors. While delivery for oligonucleotide-based therapeutics may be challenging, the recent success in mRNA vaccines suggests that these issues can be overcome, especially considering the simpler structure and higher stability of the DNA warheads compared to more traditional RNA-based therapeutics. Given these advances O'PROTACs may be a valuable strategy to target previously undruggable transcription factors by combining the catalytic mechanism of PROTACs with well-established DNA synthesis.

Contributor: Claire

Discovery of a first-in-class CDK2 selective degrader for AML differentiation therapy

Liguo Wang[§], Xuejing Shao[§], Tianbai Zhong[§], Yue Wu[§], Aixiao Xu[§], …, Meidan Ying*, Yu Rao*

Nat. Chem. Biol. **2021**, DOI: 10.1038/s41589-021-00742-5



CDK2 is an attractive target for the treatment of AML through differentiation, rather than antiproliferation. Targeting CDK2 with PROTACs offers a means for this as inhibiting the enzymatic function of CDK2 does not promote AML differentiation and there are currently no selective CDK2 inhibitors available. From a range of VHL- and CRBN-recruiting PROTACs utilising nonselective CDK2 ligands, the CRBN-recruiting PROTAC CPS2 was identified as the most potent CDK2 degrader in initial screens in Ramos lymphoma cells. CPS2 was therefore taken forward for further profiling and was found to improve selectivity for CDK2 over the parent inhibitor J2, a derivative of the CDK binder JNJ-7706621. CPS2 was found to induce rapid, potent and selective CDK2 degradation and antiproliferation in a range of blood cancer cell lines. The CDK2 PROTAC offered improved safety compared to the CDK inhibitor and induced AML cell differentiation akin to shCDK2. The antileukemic and AML differentiation effects of CPS2 were confirmed to be CDK2 degradation specific. This differentiation effect was also observed in primary HSC and AML cells treated with CPS2.

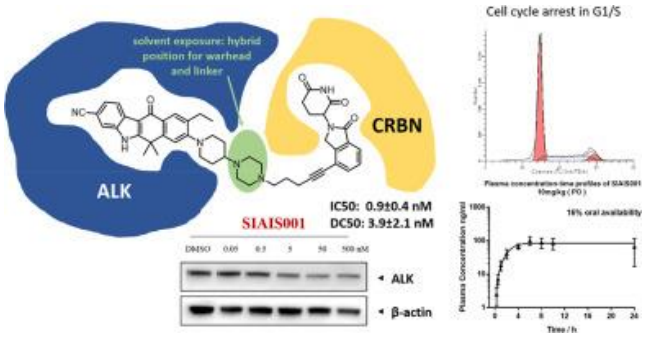
Following the recent successes of CDK4, CDK6 and CDK9 degraders, CDK2 PROTACs can be added to the expanding list of selective CDK degraders. Characterising the ternary complex of CPS2 could enable optimisation towards a CDK2 PROTAC with a further improved selectivity window and enhanced differentiation-inducing effect. Additionally, determining cell permeability and pharmacokinetics of CDK2 PROTAC versus inhibitor would help with understanding the nature of the apparent improved safety profile of CPS2.

Contributor: Aileen

Structure-based discovery of SIAS001 as an oral bioavailability ALK degrader constructed from Alectinib

Chaowei Ren[§], Ning Sun[§], …, Xiaoling Song*, Xiaobao Yang*, Biao Jiang*

Eur. J. Med. Chem. **2021**, DOI: 10.1016/j.ejmech.2021.113335



This publication explores the use of Alectinib, an inhibitor used to treat ALK positive non-small cell lung cancer, to construct PROTACs with the aim of degrading ALK. The binary crystal structure of Alectinib with ALK is used to identify a suitable solvent exposed site for linker attachment, and a number of PROTACs are made. The PROTACs are evaluated via antiproliferation and degradation assays in SR cells, with two compounds, SIAS001 and SIAS091, identified as degraders (DC_{50} = 3.9 and 6.1 nM and D_{max} = 70 and 89%, respectively).

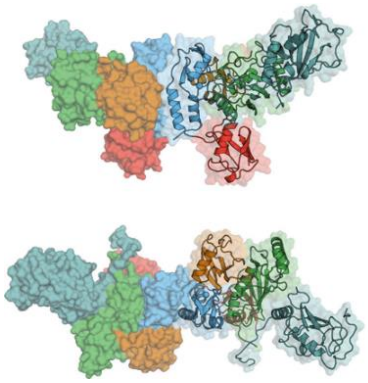
Further profiling of the two lead compounds includes a pharmacokinetic study in rats, in which it is found that SIAS001 shows 16% oral bioavailability (10 mg/kg dose). The authors state that the plasma concentration in this case is above the cellular IC_{50} value. As an example of a PROTAC which achieves oral bioavailability, this study is worthy of note. However, it would be valuable to examine the ALK degradation approach in the context of disease progression, particularly in comparison to inhibition by Alectinib.

Contributor: Kevin

RING domains act as both substrate and enzyme in a catalytic arrangement to drive self-anchored ubiquitination

Leo Kiss[§]*, Dean Clift, Nadine Renner, David Neuhaus, Leo C. James*

Nat. Commun. **2021**, DOI: 10.1038/s41467-021-21443-6



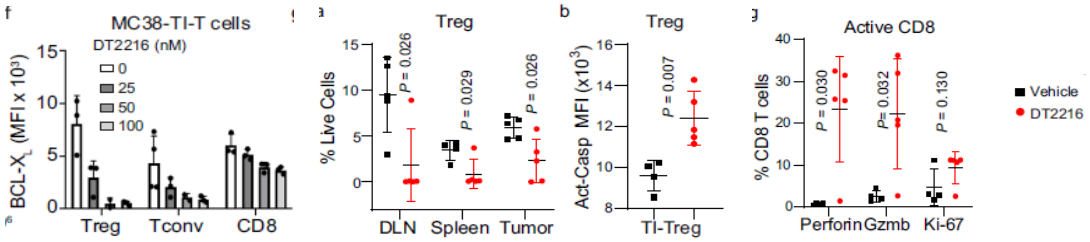
Ubiquitination is the central process in targeted protein degradation, but although recruitment of E2~Ub conjugates and chain elongation by RING domains are structurally characterized, the production of specific ubiquitin chains anchored to substrates is not well understood. In this paper, Kiss et al. present structural snapshots of chain elongation through the Ube2N/Ube2V2 E2 on mono-ubiquitinated RING of TRIM21, the E3 ligase exploited by Trim-away for targeted protein degradation. They find a crucial role of D113 of Ube2N in interacting with ubiquitin-K63 and selectively lowering its pk_a, explaining the linkage specificity of this E3 ligase. The presented structure explains the need for RING dimerization commonly observed in TRIMs as geometric restrains only allow ubiquitination in trans until the chain reaches a length of at least four ubiquitins after which chain elongation can occur in cis with no need for dimerization and a significant acceleration is observed. The authors demonstrate the necessity for the formation of the described TRIM21/Ube2N/Ube2V2 topology leading to K63-linked ubiquitin chains in targeted degradation of GFP using Trim-away. Although it remains unexplained how K63-linked ubiquitin chains lead to protein degradation in this system, the paper gives detailed insight into the mechanism of linkage specific substrate ubiquitination.

Contributor: Claire

Proteolysis-targeting chimera against BCL-X_L destroys tumor-infiltrating regulatory T cells

Ryan Kolb[§], Umasankar De[§], …, Daohong Zhou*, Weizhou Zhang*

Nat. Commun. **2021**, DOI: 10.1038/s41467-021-21573-x



Tumour-infiltrating regulatory T cells (TI-Tregs) can be exploited by cancer cells to inhibit antitumour immune responses within the tumour microenvironment. TI-Tregs are able to survive the pro-apoptotic nature of the tumour microenvironment by upregulating anti-apoptotic proteins of the BCL-2 family, which includes BCL-X_L, as shown in this paper through single-cell RNA sequencing and flow cytometric characterisation of immune cells from renal cell carcinomas and breast cancers. The on-target and dose-limiting toxicity demonstrated by BCL-X_L inhibitors has been overcome by BCL-XL PROTACs, previously developed by the same groups (and mentioned in our August 2020 issue), that are either VHL-recruiting (DT2216) or CRBN-recruiting (PZ15227). The authors now use DT2216- and PZ15227-induced BCL-X_L degradation to define the pro-survival function of BCL-X_L within TI-Tregs of renal cell carcinoma and other cancers, demonstrating the potential of BCL-X_L-PROTACs as a means to deplete TI-Tregs for cancer immunotherapy. PROTAC-induced degradation of BCL-X_L was achieved in syngeneic tumour models and TI-Tregs derived from renal and breast cancers. Potent DT2216-mediated degradation of BCL-X_L within the tumour microenvironment led to apoptosis-driven TI-Treg depletion in MC38 tumour-bearing mice alongside an increase in CD8+ T cell activation. This response was associated with inhibition of tumour growth without significant induction of detectable tissue damage and demonstrates that DT2216 leads to an immune active tumour microenvironment.

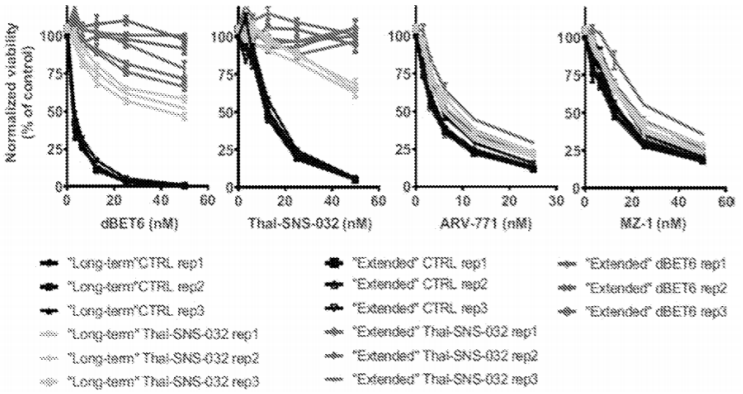
As mentioned by the authors, it will be critical to determine the subset of patients for which BCL-X_L-PROTACs could be effective, for example with a tumour reliance on TI-Tregs, and it would be worthwhile to ensure that BCL-X_L-PROTAC treatment does not induce any other organ-specific autoimmune pathology.

Contributor: Aileen

Methods for treating cancer using serial administration of E3 ubiquitin ligase degraders

Constantine S. Mitsiades, Ryosuke Shirasaki, Geoffrey M. Matthews, Sara Gandolfi, Ricardo De Matos Simoes

WO2021050832



This patent describes studies into elucidating the mechanism of resistance of cancer cells to PROTAC-mediated protein degradation and attempts to overcome this. The targets concerned are CDK9 and BRD2, BRD3 and BRD4, with published PROTACs dBET6, Thal-SNS-032, ARV-771 and MZ1 utilised. Whole genome CRISPR/Cas9-based gene editing LOF screens are undertaken to investigate which genes are implicated in resistance in both VHL and CRBN mediated degradation. It is found that dysregulation of the CUL2-VHL RING ligase complex and its interactors mediates resistance to VHL-based degraders. Dysregulation of CRBN and its interactors results in resistance to CRBN-mediated degradation. It is thus proposed that PROTACs which target the same oncoprotein but promote degradation via different E3 ligases could be used sequentially in cases where resistance arises. An experiment is performed in which MM cells, which have developed resistance to a CRBN-based degrader via long-term treatment, are then treated with CRBN and VHL-based degraders in a cell viability assay. The results of dose-dependent cell viability studies suggest a reduction in cell viability after treatment with VHL-recruiting PROTACs ARV-771 and MZ1, whereas cell viability levels seem to be maintained treatment with CRBN-recruiting PROTACs dBET6 and Thal-SNS-032.

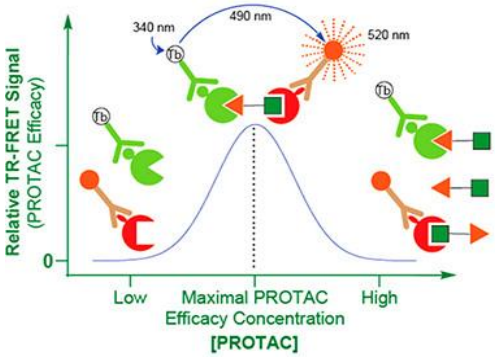
The findings within this patent suggest that resistance to TPD therapy, at least for the targets studied, is mediated by prevention of degradation rather than adaptation to loss of the POI. This provides an opportunity to overcome observed resistance by switching the E3 ligase recruited. Bearing in mind that re-optimisation of a PROTAC would be likely upon switching E3 ligase due to the formation of a different ternary complex, it would be interesting to see how easily this concept could be applied to a novel target for which probe compounds are not already available.

Contributor: Kevin

General Stepwise Approach to Optimize a TR-FRET Assay for Characterizing the BRD/PROTAC/CRBN Ternary Complex

Wenwei Lin[§], Taosheng Chen*

ACS Pharmacol. Transl. Sci. **2021**, DOI: 10.1021/acsptsci.1c00032



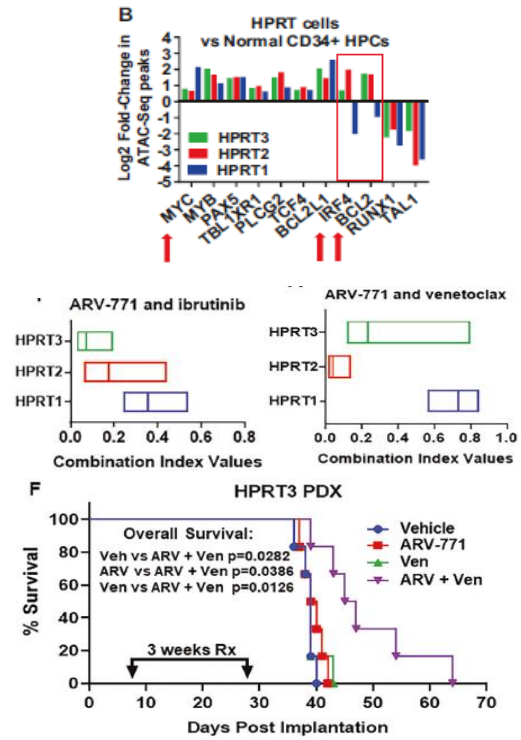
Ternary complex formation is a critical step in PROTAC-mediated protein degradation and an excellent predictor of degradation efficiency. There is therefore a need for assays to measure ternary complex affinity and several techniques have been employed to that end. In this paper an assay based on time-resolved Förster resonance energy transfer (TR-FRET) with a lanthanide donor is presented. TR-FRET is advantageous here as it allows to specifically assess the formation of only the ternary complex without interference from subcomplexes. The presented protocol is modular due to the use of fluorescently labelled antibodies specific for the affinity tag (His or GST) rather than for the protein of interest, allowing for easy substitution between proteins of interest. The system is extensively validated for PROTAC-induced BRD/CRBN complexes and yields the expected bell-shaped response curves, while replicating literature reported compound ranks. While the use of TR-FRET for PROTAC-induced complexes is not new, the modular nature of this assay and the detailed description of assay validation make this paper valuable for anyone trying to establish TR-FRET assays on their own system.

Contributor: Claire

BET proteolysis targeted chimera-based therapy of novel models of Richter Transformation-diffuse large B-cell lymphoma

Warren Fiskus[§], Christopher P. Mill[§], …, Kapil N. Bhalla*

Leukemia **2021**, DOI: 10.1038/s41375-021-01181-w



Richter Transformation develops as aggressive, therapy-resistant, diffuse large B cell lymphoma (RT-DLBCL) that can be clonally related (CLR) or unrelated (CLUR) to underlying chronic lymphocytic leukaemia. Given the lack of pre-clinical human models and successful therapies, the authors have established and characterised three patient-derived xenograft models of CLR- and CLUR-RT-DLBCL that demonstrated differential sensitivities to a range of targeted agents tested. These sensitivities correlated with the expression levels of genes involved in regulating sensitivity to the targeted agents. This included increased expression of BRD4 and TCF4 and depletion of IRF4 that were associated with increased sensitivity to the BET-PROTAC ARV-771 and BET inhibitors OTX015 and ABBV-075. Additionally, higher levels of BCL2 and Bcl-xL, were associated with an enhanced response to the BCL2 inhibitor venetoclax and the Bcl-xL inhibitor A-1155463. Synergistic lethality was observed with co-treatment of either BET-inhibitor or BET-PROTAC alongside venetoclax or the BTK inhibitor ibrutinib. Compared to monotherapy, combination therapy with BET-PROTAC and venetoclax significantly reduced lymphoma burden and improved survival of immune-depleted mice engrafted with CLR-RT-DLBCL.

Further studies that utilise degraders in combination therapies will certainly be one to watch out for and another example is covered in the entry below!

Contributor: Kevin

MZ1 co-operates with trastuzumab in HER2 positive breast cancer

María del Mar Noblejas-López[§], Cristina Nieto-Jiménez[§], …, Miguel Burgos*and Alberto Ocaña*

J. Exp. Clin. Cancer Res. **2021**, DOI: 10.1186/s13046-021-01907-9

Trastuzumab is a monoclonal antibody targeting HER2, a transmembrane tyrosine kinase commonly overexpressed in breast cancer. It has antitumoral activity and improves clinical outcome for HER2+ tumor patients, but resistance to the treatment develops quickly. Therefore, novel combination therapies are required to suppress resistance. In this study, the authors explored the use of BET-targeting PROTACs in combination with trastuzumab and found that the BRD4-specific PROTAC MZ1 originally developed in the Ciulli lab in combination with trastuzumab had a stronger antiproliferative effect in HER2-overexpressing cell lines than either of the compounds alone. They show efficient degradation of BRD4 and a minor downregulation for ERBB2 for the combination as well as induction of apoptosis. In xenograft models the combination therapy, but not the single compounds reduced tumor progression and lower BRD4 expression was observed for the combination compared to the single therapies. In summary, the finding of synergistic effects between the PROTAC MZ1 and established therapeutics in the treatment of HER2+ cancer further highlights the potential of PROTACs in a clinical setting.

Other Paper Highlights

Contributor: Tasuku

One Atom Makes All the Difference: Getting a Foot in the Door between SOS1 and KRAS

Juergen Ramharter[§]*, Dirk Kessler, …, Darryl B. McConnell*

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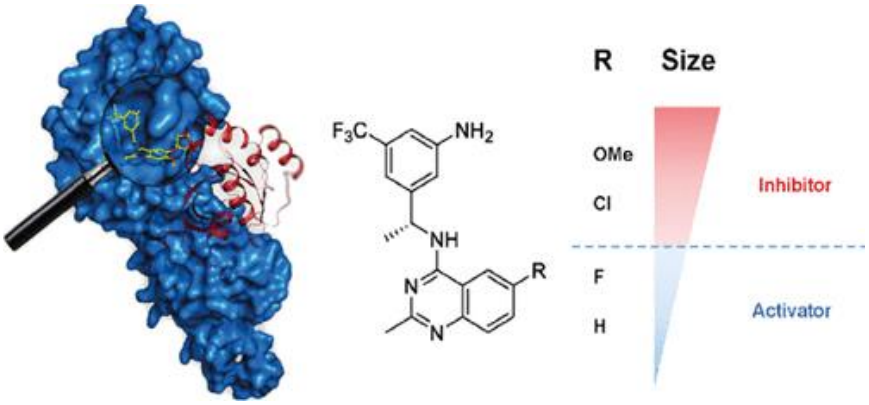

KRAS mutations are one of the major and most notorious drivers of cancer progression. KRAS is also well-known as an "undruggable" target because of its strong binding affinity to GTP, its natural cofactor. In this paper, the authors focused on KRAS:SOS1 protein-protein interaction inhibition to disrupt the feedback activation of SOS1 following reduction of the active form of KRAS. In their HTS campaign and subsequent confirmation assays, they identified 18 hit compounds that included quinazoline-type EGFR inhibitors. To exclude kinase inhibitory activities, they introduced a simple methyl group at the 2-position of the quinazoline ring and found a significant decrease in kinase inhibition while maintaining the desired on-target activity. Surprisingly, the substituents at the 6-position of the quinazoline ring showed drastic effect. The hydrogen and fluorine derivatives displayed SOS1 activation activity whereas chlorine and methoxy derivatives showed SOS1 inhibitory activity. In crystal structural analyses, they observed that minor changes affect the key interaction between Y884 on SOS1 and R73 on KRAS. Their findings revealed the power of structural analysis to rationalize drug design and the massive impact of simple substituent changes not only to exclude undesired activity but also to switch between activator and inhibitor.