Targeted protein degradation, medicinal chemistry & chemical structural biology literature highlights

Ciulli Lab
Targeted Protein Degradation

University of Dundee

Journal Club

May 2021

Content

Feature of the Month

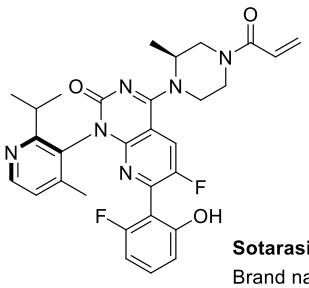
Contributor: Will and Tasuku

Sotorasib approval draws first blood in the fight to break cancer's beating heart

History and Importance

For nearly forty years the small GTPase, KRAS, which is mutated in approximately one quarter of all tumors, has been known to play a major role in cancer. In parallel to its rising profile as a molecular switch that drives cancer growth, it has become a poster child for the so-called 'undruggable'. On the 28th May 2021 the U.S. Food and Drug Administration (FDA) granted accelerated approval to Sotorasib, the first drug targeting a KRAS mutation, for adults with $KRAS^{G12C}$ mutated locally advanced or metastatic non-small cell lung cancer (NSCLC). This means that patients with $KRAS^{G12C}$ mutated NSCLC whose disease has progressed on or after at least one prior treatment now have a new option and new hope.

Whilst any drug approval is momentous, this one is particularly special because of the breadth of scientific, healthcare and business communities it will having meaning to. It is just 8 years since the ground-breaking study by Jonathan Ostrem and Ulf Peters in the lab of Kevan Shokat that showed an allosteric pocket in $KRAS^{G12C}$ could be targeted with electrophilic small molecule fragments. They showed that these molecules could lock $KRAS^{G12C}$ in its 'off' state and through structure-based design could replace the disulfide electrophiles initially used with acrylamides, conceived to be more 'developable' and which can be seen in use in Sotorasib itself. Within just 5 years of the publication of this study AMG510, later to be known as Sotorasib, was taken into Phase I clinical trials by Amgen. The discovery of this molecule is described by Brian Lanman and colleagues at Amgen in a 2020 publication. This 5-year journey itself was catalysed by discoveries made by multiple groups across the academia-industry spectra (including but not limited to the Shokat co-founded Wellspring Biosciences, Carmot Therapeutics and Amgen). Amgen have now successfully progressed Sotorasib from Phase I to approval in just three years, almost unprecedented speed for a small molecule to gain approval, with others also moving quickly with their own $KRAS^{G12C}$ inhibitors. This approval therefore represents a first blow to address KRAS as a drug target and a huge step forward for patients with $KRAS^{G12C}$ driven cancer. It is an inspiring tale that represents the importance of unconventional breakthrough academic science, pioneering drug discovery to translate that science and organisational agility.



Sotarasib (AMG510)
Brand name: LUMAKRAS®

Facts and Figures

In the Phase I clinical trial, 129 patients with advanced solid tumours harbouring the $KRAS^{G12C}$ mutation received Sotorasib once daily. No dose-limiting toxicity was observed in this study and the major adverse effects (>20%) were diarrhoea, fatigue, and nausea. In the patients with non-small cell lung cancer (NSCLC), >30% patients had a partial response and ~90% patients had disease control, and the median progression-free survival (PFS) was 6.3 months. Amgen was encouraged by these positive results and moved forward with the Phase II trial for patients with NSCLC harbouring the $KRAS^{G12C}$ mutation. 126 patients were treated with 960 mg of Sotorasib once daily and it was found that the observed results were consistent with the previous Phase I study. Amgen then submitted the Sotorasib New Drug Application (NDA) in December 2020. After granting Priority Review Designation in February 2021, the FDA approved Sotorasib for the treatment of patients with $KRAS^{G12C}$ mutated locally advanced or metastatic 2nd line NSCLC on May 28th, 2021. The original Prescription Drug User Fee Action (PDUFA) date (targeted response date) was August 16th, 2021, so the FDA approved Sotorasib only 6 months after the application – this highlights the clinical robustness of Sotorasib for the treatment of $KRAS^{G12C}$ mutated NSCLC. Currently several clinical studies of combination treatments (e.g. anti-PD-1 antibody or MEK inhibitor) and other solid tumours with $KRAS^{G12C}$ mutation (e.g. colorectal cancers) are in progress so it is likely that Sotorasib will be a great therapeutic option for patients with $KRAS^{G12C}$ mutated cancers.

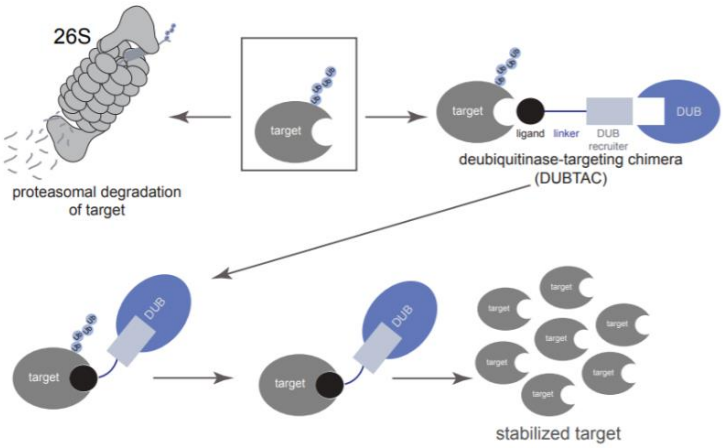
Targeted Protein Degradation

Contributor: Tom

Deubiquitinase-Targeting Chimeras for Targeted Protein Stabilization

Nathaniel J. Henning[§], Lydia Boike[§], Jessica N. Spradlin[§], Carl C. Ward[§], …, Daniel K. Nomura[§]*

bioRxiv **2021**, DOI: 10.1101/2021.04.30.441959



The authors present a noteworthy proof-of-concept study into the development of chimeric small molecules which induce targeted protein stabilisation (TPS). It is well known that the advent of targeted protein degradation as a therapeutic approach has expanded the 'druggable' proteome. TPS presents another forward step in the quest to access a greater range of disease-relevant targets.

A chemoproteomic-enabled covalent ligand discovery approach (activity-based protein profiling, ABPP) was used to discover a novel covalent ligand, acrylamide EN253, that targets a non-catalytic allosteric cysteine C23 in the K48 ubiquitin-specific deubiquitinase OTUB1. EN253 was linked covalently to lumacaftor (cystic fibrosis treatment which binds ΔF508-CFTR) to generate a small series of heterobifunctional deubiquitinase targeting chimeras (DUBTACs). The authors demonstrated stabilisation of ΔF508-CFTR in human cystic fibrosis bronchial epithelial cells by one of the DUBTACs, NJH-2-057, in a dose-responsive and time-dependent manner. This was also shown to be OTUB1 dependent. To further validate Western blotting data for CFTR stabilisation, a TMT-based quantitative proteomic analysis of NJH-2-057 treated cells was performed. The proteomic analysis showed CFTR among the most robustly stabilised proteins.

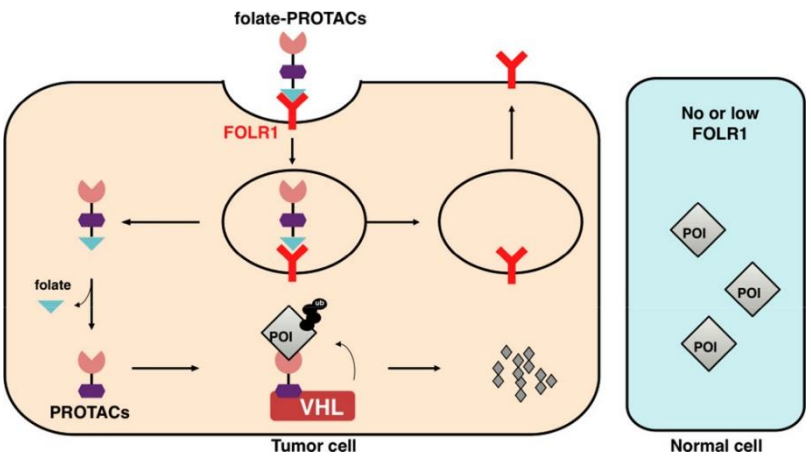
Interestingly, in an isoTOP-ABPP experiment, OTUB1 C23 was captured but only showed a ratio of 1:6 (corresponding to ~60% target occupancy). The authors note the parallels with E3 ligase recruitment for targeted protein degradation: minimal target occupancy of E3 ligases can still lead to robust degradation of target proteins due to the catalytic nature of the PROTAC mechanism. It will be exciting to keep an eye on the TPS space and watch how it develops!

Contributor: Xingui

Cancer Selective Target Degradation by Folate-Caged PROTACs

Jing Liu[§], He Chen[§], …, Jian Jin*, Wenyi Wei*

J. Am. Chem. Soc. **2021**, *143*, 7380. DOI: 10.1021/jacs.1c00451



Following light-controllable PROTACs and antibody-based PROTACs, the authors in this paper present folate-caged PROTACs for cancer selective target degradation. The folate-caged PROTAC is constructed by conjugating a folate receptor α (FOLR1) ligand to the OH group of the hydroxyl prolyl moiety of the VHL-based PROTAC molecule through an ester bond. Therefore, it is a combination of PROTAC pro-drug and PROTAC carrier. Folate-caged PROTACs achieve cancer selective target degradation in four consecutive steps: firstly, the FOLR1 ligand moiety of folate-caged PROTACs recognize and bind to cells with FOLR1, which is highly expressed in cancer cells; followed with endocytosis

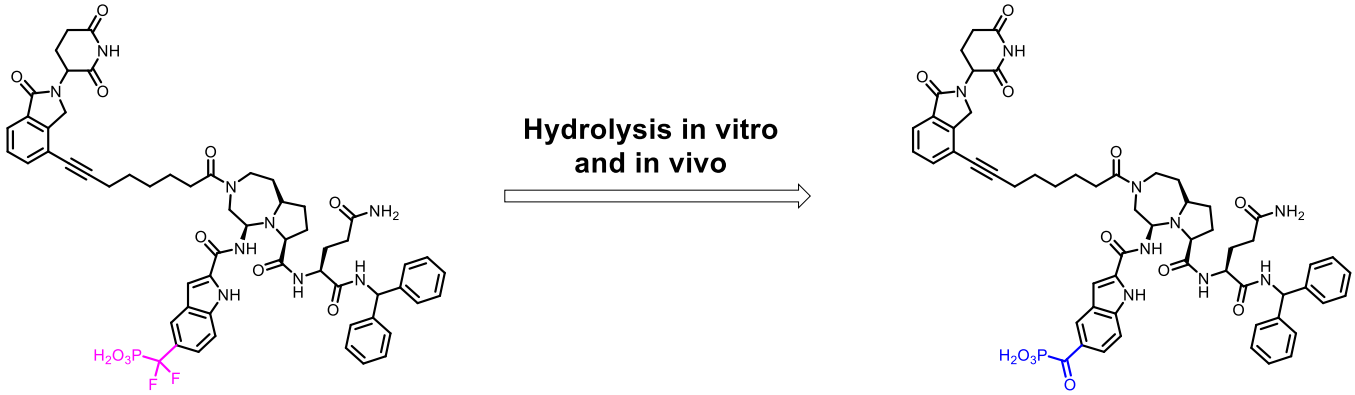
of FOLR1, together with folate-caged PROTACs; the folate-caged PROTACs then release the active PROTACs in the presence of the intracellular hydrolase, while the FOLR1 is dissociated and recycled to the cell surface; the active PROTACs then recruit VHL ligase to induce target protein degradation via the proteasome.
The authors have successfully applied this folate-caged PROTAC concept to several targets, including BRDs, ALK and MEK. These folate-caged PROTACs degrade target proteins in FOLR1- and VHL-dependent manners and achieved selectivity between cancer cells and normal cells. However, the degradation capabilities of the folate-caged PROTACs are not better than their parent PROTAC counterparts when compared side-by-side in cancer cells. It would be interesting to see if the potencies of the folate-caged PROTACs can be improved either through increasing endocytosis efficiency (e.g. improving FOLR1 affinity) or hydrolysis efficiency (e.g. reducing steric hinderance).

Contributor: Xingui

SD-91 as A Potent and Selective STAT3 Degrader Capable of Achieving Complete and Long-Lasting Tumor Regression

Haibin Zhou[§], …, Shaomeng Wang*

ACS Med. Chem. Lett. **2021**, DOI: 10.1021/acsmedchemlett.1c00155



SD-36 (First potent, selective and highly efficacious STAT3 degrader)

SD-91(A highly potent, selective and efficacious STAT3 degrader with excellent chemical stability)

SD-36 is a potent, selective, and highly efficacious STAT3 degrader discovered by the Wang group. Its discovery provided a successful example of targeting undruggable targets via PROTAC mediated degradation. **SD-36** derives from a potent STAT3 inhibitor baring a $-CF_2PO_3H_2$ group. The fluorine atoms were introduced to increase the acidity of the $-PO_3H_2$ group and therefore the binding affinity to STAT3.
In this work, the authors found that the gem-difluoride of **SD-36** can be hydrolysed to the ketone to afford **SD-91** both *in vitro* and *in vivo*. Extensive study of **SD-91** revealed that it is also a potent, selective, and highly efficacious STAT3 degrader with excellent chemical and metabolic stability. The biological effects previously observed on **SD-36** was therefore a combination effect of **SD-36** and **SD91**.

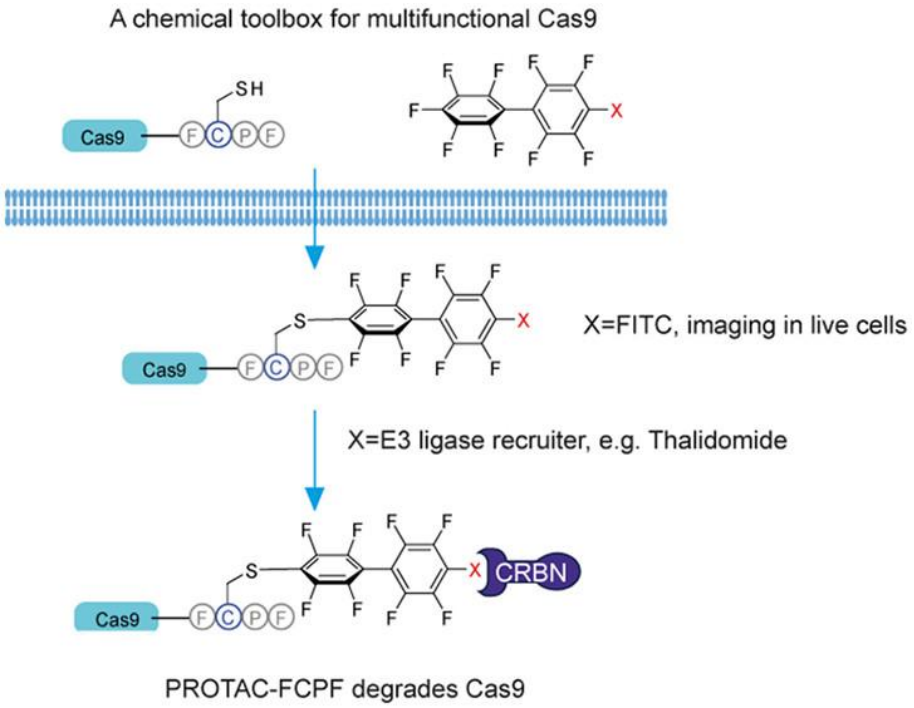
Fluorine atoms are constantly introduced into bioactive molecules to improve bioactivity, physicochemical or/and pharmacokinetic properties. The C-F bond is generally considered to be a strong single bond in organic chemistry; however, its stability tends to be environment-dependent. Although **SD-91** turned out to be a potent STAT3 degrader, the hydrolysis of **SD-36** *in vitro* and *in vivo* provides another cautionary tale about the potential liability of fluorine-containing molecules.

Contributor: Xingui

A Chemical Toolbox for Labeling and Degrading Engineered Cas Proteins

Rodrigo A. Gama-Brambila[§], …, Xinlai Cheng*

JACS Au **2021**, DOI: 10.1021/jacsau.1c00007



CRISPR/Cas system is a revolutionary gene editing technology. One major limitation associated with this technology is off-target mutation(s), which could be reduced by manipulating the enzymatic activity of Cas proteins.

By marrying CRISPR/Cas system with PROTAC technology, the authors developed PROTAC-FCPF as probes to regulate the activity of Cas proteins. In this new system, the Cas protein is modified by inserting a Phe-Cys-Pro-Phe (FCPF) amino acid sequence (known as π-clamp system) that can be recognized by perfluoaromatics. When perfluoaromatics are conjugated with a fluorophore, the Cas proteins can be labelled for live cell imaging. Conjugating perfluoaromatics with an E3 ligase ligand affords bifunctional PROTAC-FCPF. PROTAC-FCPF can recruit an E3 ligase to the engineered Cas protein, resulting in its degradation, and therefore regulate its intracellular activity.

The PROTAC-FCPF bares similarities to the Halo-PROTAC and dTAG systems. The advantage of PROTAC-FCPF over the other two is that the FCPF amino sequence is short and does not interfere with the activity of the engineered protein. However, like the Halo-PROTAC that forms covalent bond with halogenase, the perfluoaromatics moiety of PROTAC-FCPF recognizes FCPF sequence by forming sulfide bond with cysteine, which abolishes the catalytic nature of PROTAC molecules. Nevertheless, PROTAC-FCPF can still be of great value if it can reduce the off-target effect of CRISPR/Cas system in a non-catalytic manner. It would be interesting to see the application of PROTAC-FCPF in a real case and the relationship between the dosage of PROTAC-FCPF and the Cas protein activity.

Contributor: Tom

Haven't Got a Glue: Protein Surface Variation for the Design of Molecular Glue Degraders

Zuzanna Kozicka[§], Nicolas Holger Toma*

Cell. Chem. Biol, **2021**, *28*, 1. DOI: 10.1016/j.chembiol.2021.04.009

This publication is a comprehensive review detailing a range of target- and ligand-binding molecular glue degraders, primarily focusing on induction of *de novo* protein-protein interfaces for degradation via the CRL4 family. The authors describe examples which have been structurally characterised and offer thought-provoking strategies for rational design of such compounds.

Table 1. Comparison of structurally characterized drug-induced protein-protein interfaces for small-molecular degraders

Compound	Ligase	Target	PDB ID	Protein-protein interface (%)	Ligase-ligand interface (%)	Target-ligand interface (%)	Total interface area (Å^2)
Auxin	$SKP1^{TIR1}$	IAA7 peptide	2P1Q[a]	71	22	7	926
Lenalidomide	$CRL4^{CRBN}$	CK1α	F5QD[b]	68	23	9	1,052
Pomalidomide	$CRL4^{CRBN}$	SALL4 (ZF2)	6UML[c]	52	35	13	681
CC-885	$CRL4^{CRBN}$	GSPT1	5HXB[d]	52	28	20	1,164
CC-90009	$CRL4^{CRBN}$	GSPT1	6XK9[e]	54	27	19	1,221
Pomalidomide	$CRL4^{CRBN}$	IKZF1 (ZF2)	6H0F[f]	61	28	12	859
Pomalidomide	$CRL4^{CRBN}$	ZNF692 (ZF4)	6H0G[f]	61	28	10	880
Thalidomide	$CRL4^{CRBN}$	SALL4 (ZF2)	7BQU[g]	56	32	13	706
5-Hydroxythalidomide	$CRL4^{CRBN}$	SALL4 (ZF2)	7BQV[g]	60	29	12	802
E7820	$CRL4^{DCAF15}$	RBM39	6PAI[h], 6Q0R[i]	68	22	10	1,463
Indisulam	$CRL4^{DCAF15}$	RBM39	6UD7[j], 6Q0W[i]	72	19	9	1,665
CR8	$CRL4^{\Delta}$	cyclin K	6TD3[k]	79	6	16	2,672
NRX-103094	$SKP1^{\beta\text{-}TrCP}$	β-catenin peptide	6M91[l]	54	25	21	958
MZ1	$CRL2^{VHL}$	BRD4 (BD2)	5T35[m]	26	37	38	1,338
dBET23	$CRL4^{CRBN}$	BRD4 (BD1)	6BN7[n]	39	23	38	1,328

Interface areas were calculated with PISA (Krissinel and Henrick, 2007). For indisulam and E7820, average values from the two available structures are shown.

The authors note that in the case of the IMiD family, CRBN-neosubstrate ternary complex structures have revealed a common structural degron, comprising a β-hairpin loop and a key glycine residue. These features engage the otherwise solvent-exposed phthalimide moiety of IMiDs. In contrast, existing RBM39 degraders contain an aryl sulfonamide moiety; the sulfonyl oxygens have been shown to hydrogen-bond with backbone nitrogen atoms of DCAF15. The sulfonyl nitrogen engages RBM39 side chains through water-mediated hydrogen bonds. Aryl sulfonamides bind a shallow groove on DCAF15 – the buried nature and extensive protein-protein interaction interface has made it hard to fine-tune the degradation profile of these compounds thus far. The specificity of these *de novo* protein-protein interactions is highlighted by the thalidomide derivative example: in the case of 5-hydroxythalidomide, a thalidomide metabolite, the presence of a single hydroxyl induces a change in degradation selectivity from IKZF1 to SALL4. This point is further recapitulated by the interesting observation that 'using less efficient molecular glue compounds that give rise to 2- to 5-fold weaker neosubstrate recruitment *in vitro* can trigger steep transitions between degradation and non-degradation *in vivo*'. A key contrast between IMiDs and aryl sulfonamides is that the latter have low affinity for the DCAF15 receptor, demonstrating that molecular glues do not critically depend on high affinity interactions.

The cyclin K degrader story is also highlighted. A bioinformatic screen looking for correlation between cytotoxicity and significant ligase mRNA levels highlighted CR8, a kinase inhibitor, which binds CDK12-cyclin K. Significantly, CDK12 is not a component of the E3 ligase machinery but serves as a unique drug-induced substrate receptor. CDK12 links DDB1 to the ubiquitination target thus bypassing the need for a canonical DCAF substrate receptor. This shows that recruitment of a traditionally undruggable neosubstrate for degradation is a viable strategy. The authors mention the BCL6 degrader BI-3802 as another example of a target binding molecular glue.

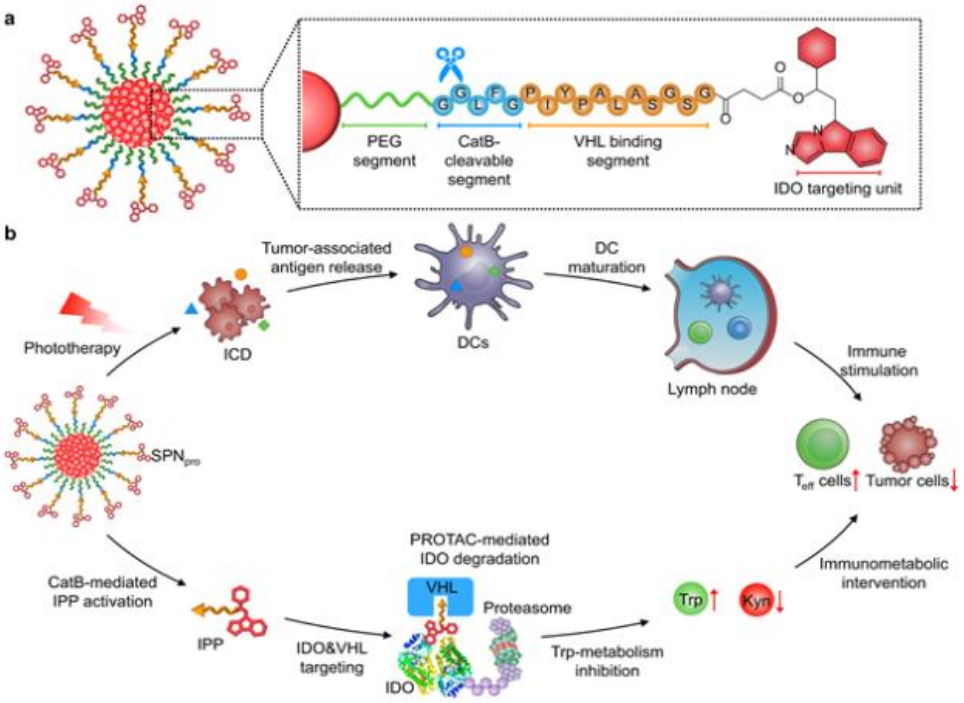
Other notable sections that are worth attention and further reading are 'Screening for Molecular Glues' and 'Gluing Weak Interactions'. These two sections nicely qualify the point that that the key to rational design of molecular glue degraders is developing a deeper understanding of protein-protein interfaces and how these can be manipulated with small molecules.

Contributor: Xingui

Semiconducting polymer nano-PROTACs for activatable photo-immunometabolic cancer therapy

Chi Zhang[§], …, Kanyi Pu*

Nat. Commun. **2021**, *12*, 2934. DOI: 10.1038/s41467-021-23194-w



The authors in this paper expanded the application of the PROTAC technology to cancer photo-immunometabolic therapy by developing a new kind of PROTAC, known as semiconducting polymer nano-PROTACs (SPN_{pro}). SPN_{pro} consists of three major components: the semi-conducting polymer core; the Cathepsin B (CatB) cleavable linker; and the indoleamine 2,3-dioxygenase (IDO) targeting PROTAC. It is an anti-cancer strategy that can kill two birds with one stone. On the one hand, the semi-conducting polymer core can accumulate in tumours through the enhanced permeability and retention effect (EPR) and generates $^1O_2$ upon near infrared (NIR) laser stimulation. The generated $^1O_2$ can induce a series of cancer immune responses and therefore enhance tumour immunogenicity. On the other hand, the highly expressed CatB in cancer cells can cleave the CatB-cleavable linker to release the PROTAC molecule which targets IDO for degradation. IDO is an enzyme involved in immunometabolism that converts tryptophan (Try) to kynurenine (Kyn). Overexpressed IDO in tumour tissue results in Trp overconsumption and Kyn accumulation, leading to immune suppression. Degradation of IDO leads to elevated Try and decreased Kyn levels, which in turn reverses immune suppression.

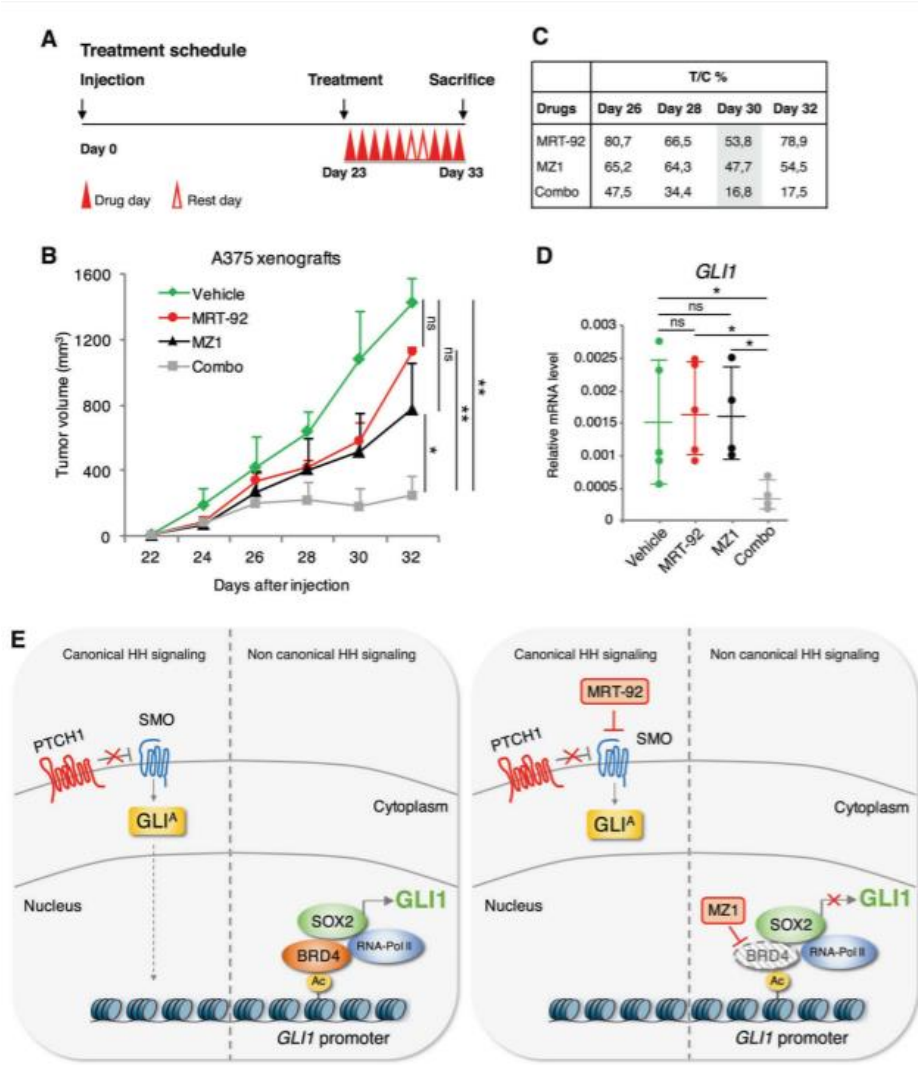
The SPN_{pro} strategy is superior to the IDO inhibitor encapsulated nanosystems due to the fact that it degrades IDO instead of inhibiting it. It is also superior to the PROTAC alone by being able to target tumour tissue and release the PROTAC selectively at the tumour tissue. However, the low penetrating ability of NIR may limit this strategy to tumours residing in subcutaneous tissue.

Contributor: Ollie

Targeting non-canonical activation of GLI1 by the SOX2-BRD4 transcriptional complex improves the efficacy of HEDGEHOG pathway inhibition in melanoma

Silvia Pietrobono*, …, Barbara Stecca*

Oncogene **2021**. DOI: 10.1038/s41388-021-01783-9



Melanoma is known for being one of the most aggressive forms of skin cancer, and its treatment is challenging due to the canonical and non-canonical Hedgehog/Glioma-associated oncogene (HH/GLI) pathways that promote survival, angiogenesis and metastasis. In canonical HH/GLI signalling, the GPCR Smoothened (SMO) becomes derepressed which initiates a complex intracellular signalling cascade culminating in activation of transcription factors which promote tumour growth. MRT-92 is an acyl-guanidine derivative which targets SMO and has shown promising results, decreasing human melanoma growth in xenograft models. However, this treatment alone may not be sufficient in melanoma treatment due to the non-canonical HH/GLI pathway being unaffected. The present study identifies a novel BRD4-SOX2 transcriptional complex involved in non-canonical GLI1 (transcription factor) activation in melanoma and they provide evidence for the synergistic effects of the BRD4-targeting PROTAC MZ1 in combination with MRT-92 for melanomas with an active SOX2-BRD4-GLI1 axis.

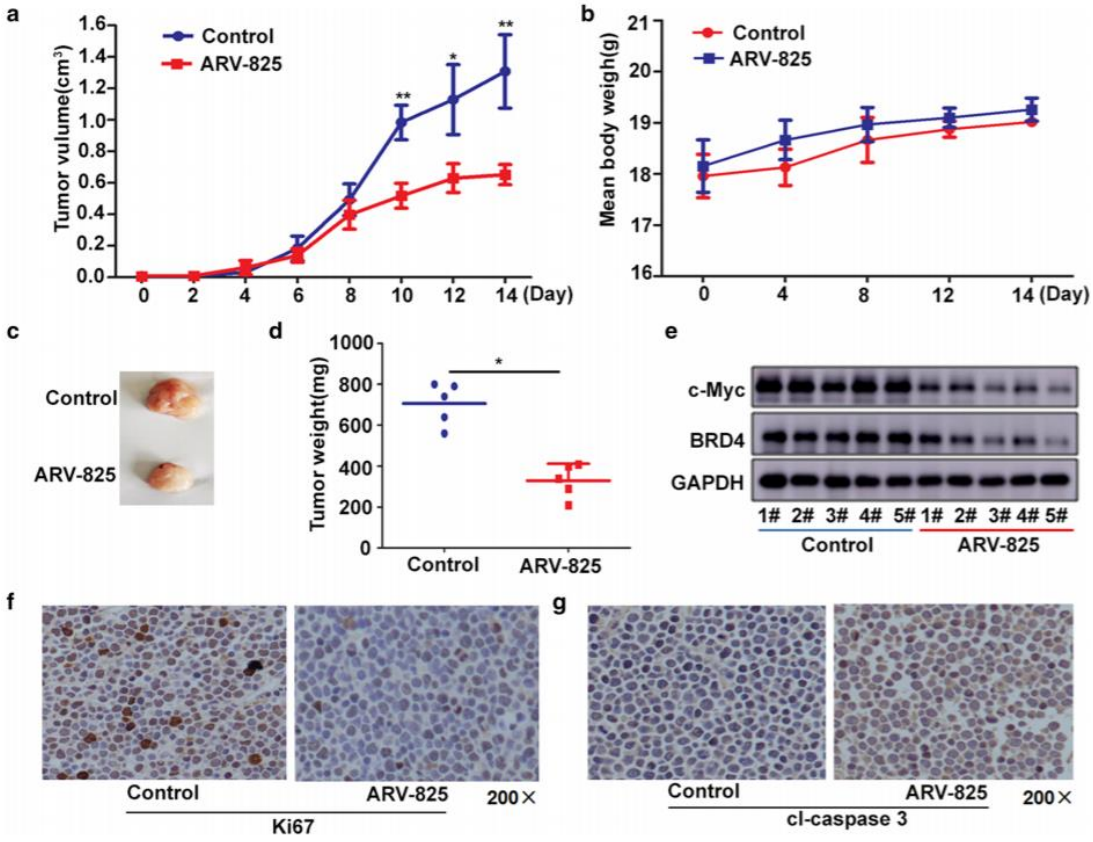
This work provides excellent data in the discovery and targeting of the SOX2/BRD4 complex involved in non-canonical HH/GLI signalling, using *in vitro* and *in vivo* methods. They even show that they can rescue the effects of the combination treatment of MRT-92 and MZ1 by overexpression of GLI1. This paper, while not focussed on PROTACs, does however provide strong evidence for the use of PROTACs not only as tools for research but as strong candidates for combination treatments with existing pre-clinical drugs.

Contributor: Ollie

BRD4 PROTAC degrader ARV-825 inhibits T-cell acute lymphoblastic leukemia by targeting 'Undruggable' Myc-pathway genes

Shuiyan Wu[§], You Jiang[§], Yi Hong[§], …, Jian Pan*, Shaoyan Hu*

Cancer Cell Int. **2021**, DOI: 10.1186/s12935-021-01908-w



T-cell acute lymphoblastic leukaemia (T-ALL) has a high rate of relapse after intensive combination chemotherapy, and relapsed T-ALL is associated with very poor outcomes. High expression of the transcriptional and epigenetic regulator BRD4 is associated with tumorigenesis and progression in many solid tumours and hematologic malignancies, making BRD4 a very important target. BET protein-targeting small molecule inhibitors such as OTX015 have been shown to inhibit growth and induce apoptosis in a host of cancers, however some studies demonstrated that these BET inhibitors lead to an accumulation of BRD4 in cells. Wu and colleagues thus set out to characterise the use of the OTX015-based CRBN-recruiting PROTAC ARV-825 in the treatment of T-ALL. ARV-825 has previously been shown to have long-lasting effects on BRD4 degradation, however the functional and molecular mechanisms governing ARV-825's activity have remained unclear. Wu and colleagues demonstrate in the present work that not only is BRD4 a suitable target in T-ALL, but that ARV-825 has superior toxicity in T-ALL cell lines compared with JQ1, OTX015 and the PROTAC dBET1.

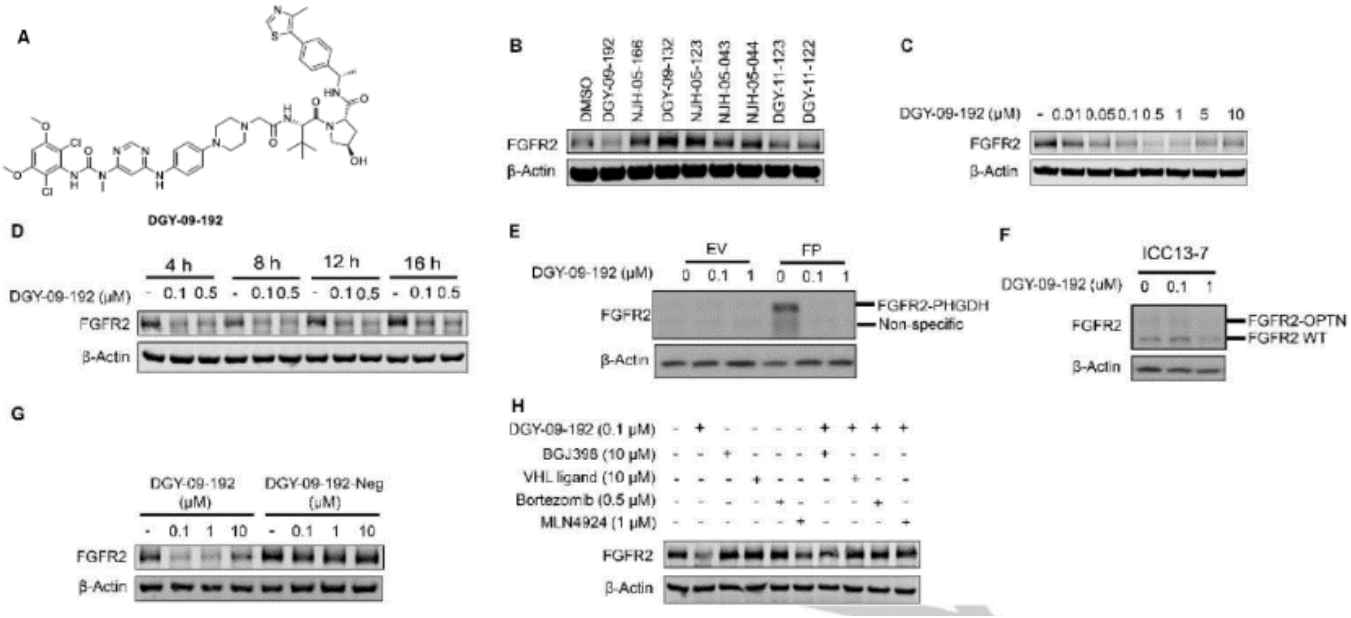
Despite being limited by patient samples for T-ALL and healthy donors, the present study provides compelling evidence for further studies and preclinical evaluation for ARV-825 in the treatment of T-ALL. The authors use a good variety of techniques to probe the mechanisms of ARV-825, including flow cytometry, western blotting using both immortalized cell lines and primary cells from healthy and T-ALL patients, RNA-seq, ChIP and a T-ALL xenograft model. Wu and colleagues have provided evidence that ARV-825 suppresses the proliferation and promotes the programmed cell death of T-ALL cells via BET protein depletion and concomitant c-Myc inhibition, which should open the door for this new treatment strategy for T-ALL.

Contributor: Ollie

Discovery of a Potent Degrader for Fibroblast Growth Factor Receptor 1/2

Guangyan Du[§], Jie Jiang[§], Qibiao Wu[§], …, Nabeel Bardeesy*, Tinghu Zhang*, Nathaneal S. Gray*

Angew. Chem. Int. Ed. **2021**, DOI: 10.1002/anie.202101328



Du and colleagues describe here the first PROTAC targeting fibroblast growth factor receptor (FGFR) family members using a pan-FGFR inhibitor BGJ398. They couple this inhibitor to a $CRL2^{VHL}$-recruiting ligand after finding CBRN-recruiting ligands showed no degradation activity. The resulting VHL-based PROTAC, named DGY-09-192, induced degradation of FGFR1 and 2 while sparing FGFR3 and 4. The PROTAC exhibited antiproliferative activity in gastric cancer and cholangiocarcinoma cell lines (DC_{50} <100 nM for WT FGFR2 as well as for disease-relevant FGFR2 fusions). DGY-09-192-induced degradation of FGFR2 fusion protein was also observed *in vivo* in a xenograft model.

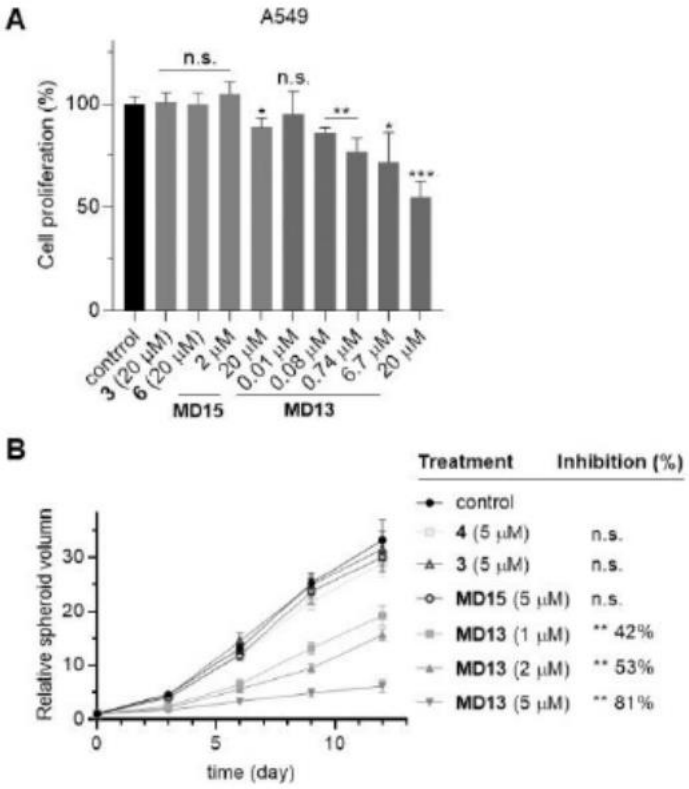
As has been shown previously in the design of heterobifunctional degraders, it is possible that not all E3 ligases will induce degradation of a particular target. Du and colleagues show here that their CRBN-based candidates exhibited no degradation activity for FGFR family members, however switching to a VHL-based ligand resulted in strong degradation. While this work is a strong proof-of-concept for FGFR-targeting degraders, the authors themselves highlight that further optimisation is required for the molecule. They show that FGFR1 and 2 are preferentially degraded over the other two family members however they also show that the molecule inhibits all isoforms, like the parent inhibitor BGJ398, and thus its antiproliferative activity cannot be solely attributed to the degradation of FGFR1 and 2 alone. They also are unable to improve upon the antiproliferative activity of the parent inhibitor, highlighting the difficulty of differentiating degradation from inhibition with kinase PROTACs made of potent inhibitors.

Contributor: Ollie

Proteolysis Targeting Chimera (PROTAC) for Macrophage Migration Inhibitory Factor (MIF) Has Anti-proliferative Activity in Lung Cancer Cells

Zhangping Xiao[§], Shanshan Song[§], …, Frank J. Dekker*

Angew. Chem. Int. Ed. **2021**, DOI: 10.1002/anie.202101864



The authors of this paper describe the first macrophage migration inhibitory factor (MIF)-targeting PROTAC molecule. MIF is an important regulator of innate immunity and has been shown to be involved in the pathogenesis of cancers. Overexpression is found in cancers including melanoma and neuroblastoma, while downregulation of MIF via genetic mechanisms reduced tumour progression while inducing antitumour immune pathways. MIF interacts with its cognate receptor CD74 which triggers the activation of the MAPK pathway resulting in p53 inhibition and cell growth, amongst other events. MIF is also known to possess enzymatic function via its proline-1, giving rise to tautomerase activity, however the role of this enzymatic activity remains largely unknown. Small molecule inhibitors targeting this enzymatic activity have been developed over the last 20 years, however despite improvements in the binding potency (now in the nanomolar range), the potency to inhibit the tautomerase activity does not always correlate with the ability to inhibit the MIF-CD74 interaction and subsequent downstream oncogenic signalling events. Xiao and colleagues therefore set about designing PROTAC molecules targeting MIF using MIF-binding molecules coupled to pomalidomide for CRBN recruitment. Their most potent molecule, MD13, exhibited a DC_{50} of <100 nM and a D_{max} of >90% in A549 cells, while the PROTAC was able to inhibit the growth of both 2D and 3D cancer cell line systems.

Xiao and colleagues present here a well-rounded study detailing the first MIF-targeting PROTAC. MIF inhibitors while potent in their binding to MIF, have been unable to target the MIF-CD74 interaction. One of the key advantages of PROTACs is their ability to target proteins involved in multi-protein complexes such as scaffolds, or in this case the interaction between a protein and its receptor. They demonstrate that MD13 triggers a reduction in MAPK pathway activation which results in G2/M phase arrest and the inhibition of proliferation of A549 cells. What is particularly nice about this work is the use of a 3D spheroid model which is a good mimic for solid human tumours, and this model showed that MD13 treatment reduced the volume by up to 81% at a concentration of 5 μM.

Contributor: Tom

Design, Synthesis and Evaluation of WD-Repeat Containing Protein 5 (WDR5) Degraders

Anja Dölle[§], Bikash Adhikari[§],…, Elmar Wolf*, Stefan Knapp*

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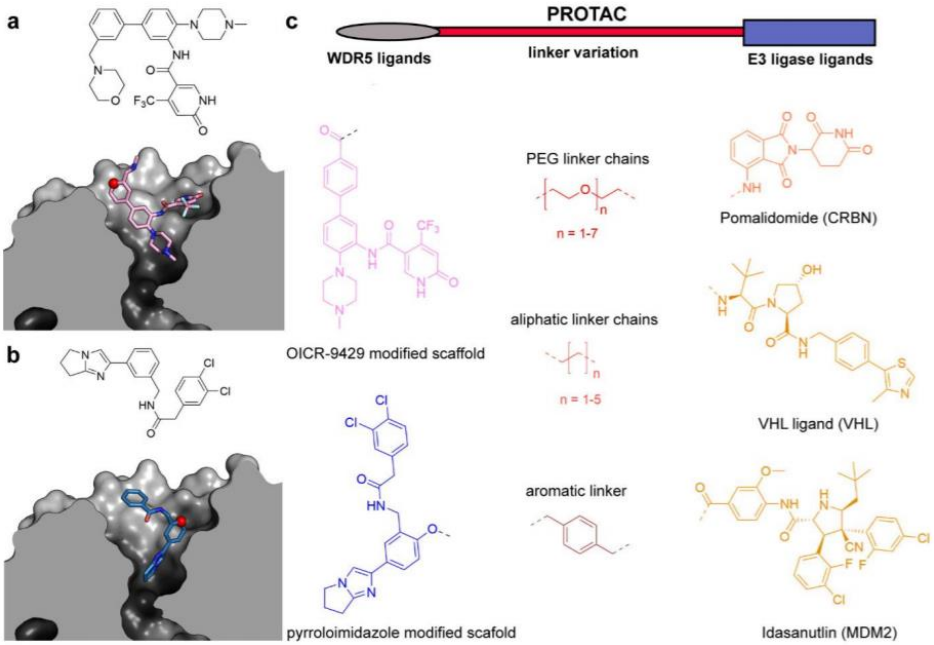

This study details a medicinal chemistry campaign followed by *in vitro* and *in cellular* evaluation of two diverse WDR5 degrader series.

WDR5 is a histone methyltransferase (HMT) regulatory scaffolding protein which is associated with controlling transcription factors such as MYC, as well as long non-coding RNAs. The authors note that dysfunctional HMTs complexes and upregulated MYC levels in a range of tumours make WDR5 an attractive target for PROTAC development. Like many other epigenetic targets, small molecule protein-protein interface inhibitors have been developed. These suffer from the caveat that only a subset of WDR5 interactions are inhibited, and targeted protein degradation may offer a way to attenuate all oncogenic function.

Two established WDR5 antagonists (the (trifluoromethyl)-pyridine-2-one OICR-9429 as well as the pyrroloimidazole-based inhibitor published by Wang and co-workers) which enter the WDR5 binding pocket at different angles were chosen as the appropriate POI ligands (as shown by overlays of available crystal structures). The authors hypothesised that the use of ligands which have distinct linker attachment geometries may increase the possibility of achieving productive ternary complex formation and therefore degradation of WDR5. These ligands were covalently linked to established E3 ligands for CRBN, VHL and MDM2 via PEG or aliphatic chains of varying length, as well as one example of an aromatic linker. An interesting note on the initial biophysical analysis performed was that the DSF ΔT_m shifts for binary affinity of the PROTACs vs WDR5 did not necessarily correlate with binding affinities obtained by ITC, and with the degradation profile.

The authors also investigated the behaviour of their degraders in cells using NanoBRET, looking at cellular permeability and target engagement. Most CRBN targeting degraders had similar cellular potency and low µM affinities for WDR5. VHL recruiting degraders showed weaker cellular activity which the authors attribute to the peptidic nature of the VHL ligand, though further investigation is certainly needed to qualify that statement. With MDM2 PROTACs, weak *in vitro* activity translated to weak cellular activity. Building on the cellular permeability and target engagement data, PROTAC-mediated cellular degradation of WDR5 was also assessed using WDR5-HiBit fusion protein. Cells were treated with different concentrations of PROTAC for 24h and WDR5 levels were determined based on luciferase measurements. Interestingly, none of the CRBN based PROTACs showed significant depletion, but many of the VHL based PROTACs demonstrated cellular degradation efficacy. Differences in degradation efficacy were also observed with varying linker lengths and types. These data nicely highlight the need to assess a thorough SAR around linker length, type and which E3 ligase gives the best degradation profile. This also highlights the fact that good binary affinity for the target does not necessarily constitute productive degradation.

Contributor: Tom

In Vivo Synthetic Chemistry of Proteolysis Targeting Chimeras (PROTACs)

Shusuke Tomoshige[§]*, Minoru Ishikawa

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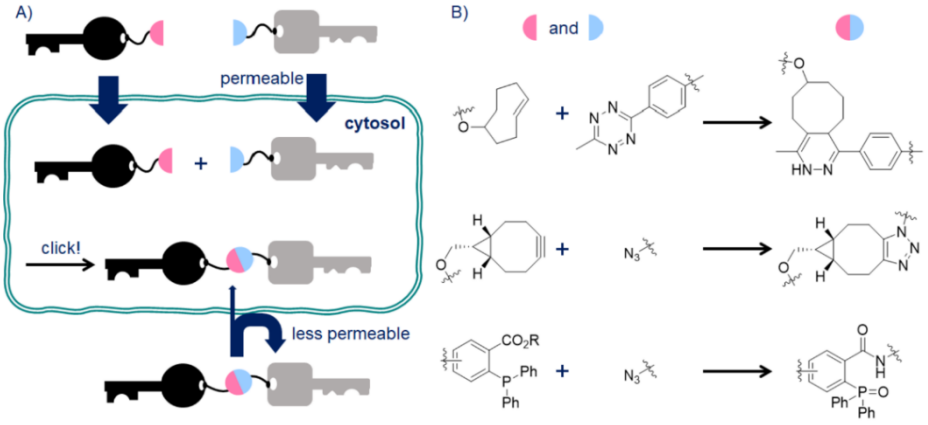

This review covers recent advances in *in vivo* synthesis of PROTAC degraders as a tool for the development of next-generation degraders, and the exploitation of PROTACs as chemical tools for *in vivo* synthesis of ubiquitinated proteins.

The hetero-bifunctional nature of PROTACs generally means that their drug-like properties deviate from Lipinski's rule of 5, with poor pharmacokinetic and pharmacodynamic properties therefore providing one of the greatest challenges to overcome in the rational design of PROTAC degraders. It was therefore hypothesised by a group at Astex Pharmaceuticals, that the *in vivo* synthesis of PROTAC molecules from separately administered small-molecule warheads could overcome some of these caveats. In this work, tetrazine or trans-cyclooctene-tagged ligands for both the E3 and POI are used and are attached in the cell via inverse electron demand Diels-Alder cycloaddition (IEDDA) to yield bifunctional PROTACs (CLIPTACs). The described technology was used to demonstrate chemically induced knockdown of BRD4 and ERK1/2, and the pre-clicked CLIPTAC system showed no activity on these targets. This shows that this technology can induce protein degradation even if the parent CLIPTAC compound lacks cell permeability.

The authors offer notable opinions on the future perspective of the CLIPTAC approach. They suggest that this should be expandable to other biorthogonal click reactions which are also cell-friendly, such as strain-promoted azide-alkyne cycloaddition and Staudinger-Bertozzi ligation. They note that a disadvantage of the CLIPTAC technology is that the product of these click reactions may give rise to undesirable structural features incorporated into the linker, and this can be extremely influential in governing productive ternary complex formation. The fact that this approach has been validated by several groups suggests it is a viable strategy for the development of next-generation PROTACs.

Other Paper Highlights

Contributor: Will

Skeletal editing through direct nitrogen deletion of secondary amines

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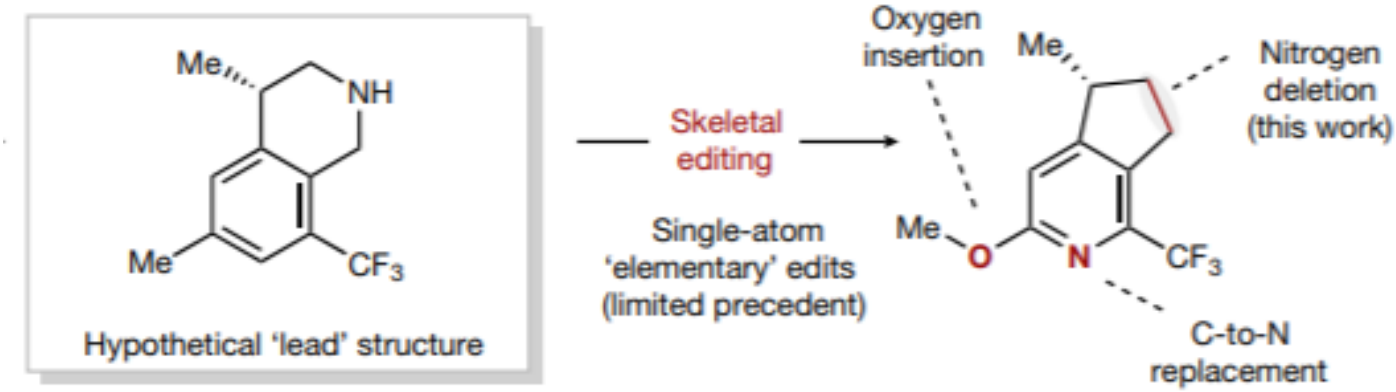

In this study the authors report a method for removing a nitrogen atom from a secondary amine and in the process forming a new C-C bond. They achieve this by using an electrophilic amide reagent, which converts secondary amines into isodiazenes. In turn, the targeted nitrogen atom is extruded as dinitrogen, producing short lived radical species that recombine to produce the new C-C bond. The 'anomeric amide' reagent used has undergone some optimization to avoid unwanted side reactivity and to be easily accessible in multi-gram quantities in three steps. A broad array of functional group tolerance is exemplified including compatibility with protic groups and heterocyclic nitrogens. A conjugated radical on at least one reaction partner is a requirement for productive C-C bond formation.

This study promises to have significant impact across many organic chemistry applications. The method is simple, the reagent is accessible and the scope, whilst not without limitations, is very broad. Forming secondary amines is generally considered facile with many commercially available reagents for, for example, reductive aminations. This method therefore provides an incredibly exciting path from a readily accessible feedstock to access carbon-carbon bonds. Whilst it may find many applications, the authors highlight it may be of particular use to access carbon based cyclic structures that would otherwise require significantly more complex routes.

Contributor: Tasuku

_N_-Ammonium Ylide Mediators for Electrochemical C–H Oxidation

Masato Saito[§], Yu Kawamata[§]*, …, Matthew Neurock*, and Phil S. Baran*

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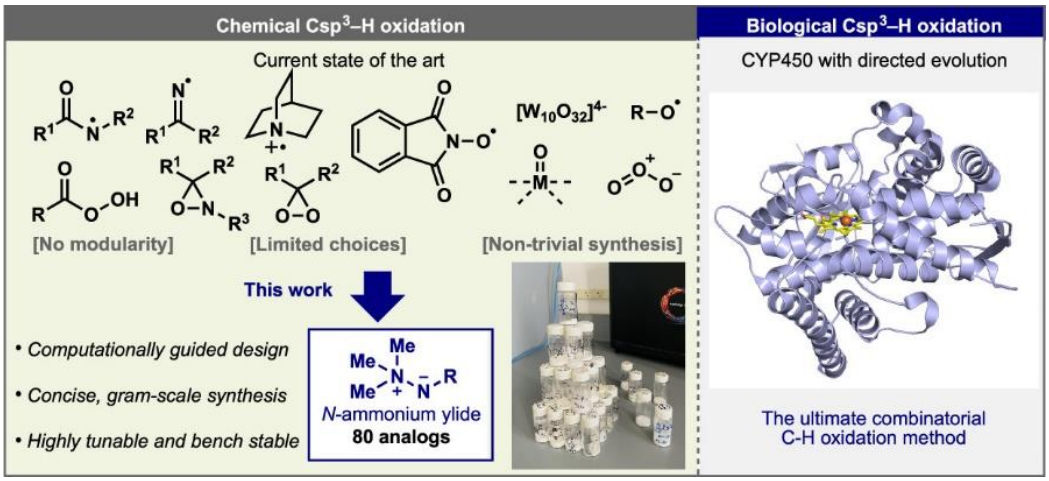

Identification and preparation of metabolites of active pharmaceutical ingredients (APIs) is an essential part of ongoing clinical studies for evaluating potential toxicological risks. However, chemical syntheses of some metabolites are a challenge for medicinal chemists because the oxidation sometimes occurs at chemically unusual positions. Late-stage selective C-H oxidation is one of the most attractive approaches for solving these issues, but convenient and tuneable methodologies have not been established yet. In this paper, the authors conducted DFT calculations against potential HAT-based mediators and identified that *N*-ammonium radicals have desired chemical profiles and excellent tunability. They assessed *N*-ammonium ylides (precursors of the radical species) in an electrochemical setting to obtain the scope of C-H oxidation with a broad range of substrates and found that those ylides have superior reactivity for many substrates than other reported mediators. In addition, the regioselectivity of ammonium ylides differs from other mediators in some cases. One of the advantages of this technology is that the ylide precursors are easily synthesized (only 2 steps) and the reactivity and regioselectivity are tuneable by using different ylides. I think this new late-stage C-H oxidation reaction would be a great 'painkiller' for medicinal chemists who need to synthesize metabolites.

Contributor: Alessio

Expression and purification of functional recombinant CUL2•RBX1 from *E. coli*

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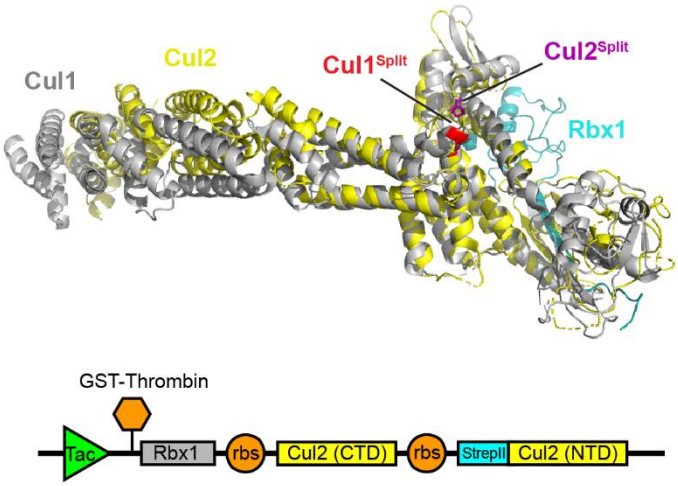
Sci. Rep. **2021**, *11*, 11224



Cullin RING ligases (CRLs) are an important class of E3 ligases because of their biological roles and growing interest in modulating them with small molecules inhibitors as well as degraders. Cullin2 is a prominent member of the family, most notably recruited as scaffold subunit within the von Hippel-Lindau (VHL) ligase, which targets substrate HIF-1α for ubiquitination and degradation. VHL-CRL2 is also one of the two ligases most widely recruited by PROTACs for targeted degradation, the other one being CRBN-CRL4. Here, the authors report a first expression and purification of Cullin2-Rbx1 complexes from *E. coli*. This is important and significant because, to date, only expression from insect cells was reported. While the insect cell expression works well and produces active and pure complexes suitable for crystallographic studies, as shown by us in 2017, expressing a protein from *E. coli* can have significant advantages, including ease of scale-up and cost-effectiveness. The authors obtained soluble, monomeric and mostly functional protein via using two clever tricks: 1) the fusion with the acidic solubilizing tag MsyB; 2) the "split-and-coexpress" approach, first shown by Zheng and colleagues with Cul1. They show that a range of constructs yield soluble pure protein, monodisperse in gel filtration, and provide evidence for functionality via pull-down experiments, VHL-CRL2 complex assembly via gel filtration, and HIF-1alpha ubiquitination assay. They also show that the purified Cullin2 can be neddylated in vitro.

This is an excellent piece of biochemistry and protein engineering that provides an important step forward for working with this challenging protein. I welcome this paper and congratulate the authors on this achievement. Producing suitably functional protein in *E. coli* is no small feat. To put this advance into context, many labs, including our own, had previously tried to express Cul2 in *E. coli*. A former PhD student in the lab, Teresa Cardote, worked hard to achieve a functional construct of *E. coli*-expressed Cul2 to no avail, despite intensive efforts going through many different constructs and truncates, as documented in her PhD thesis. It was nice to see the thesis referenced in the article. Take-home message for students: "negative results" are not always wasted efforts, so should not be perceived as failures. Lessons can be learnt, others can build upon them, and so can contribute to advance science.

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