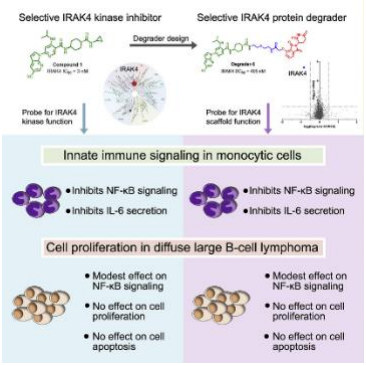
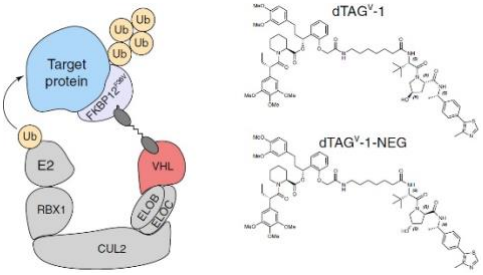
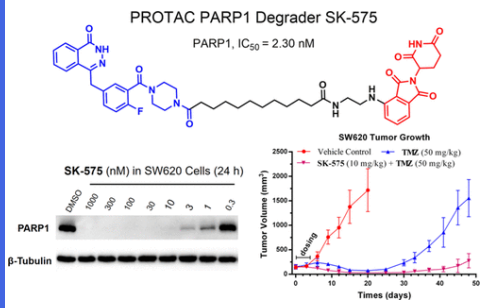
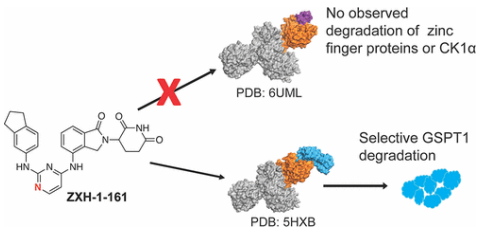
Ciulli Group Journal Club

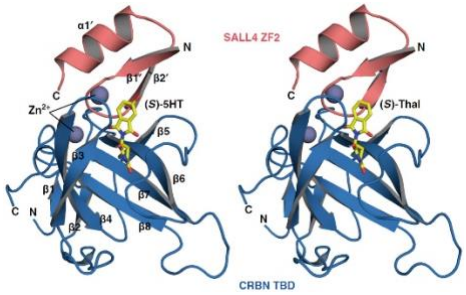


Targeted protein degradation and Other literature highlights

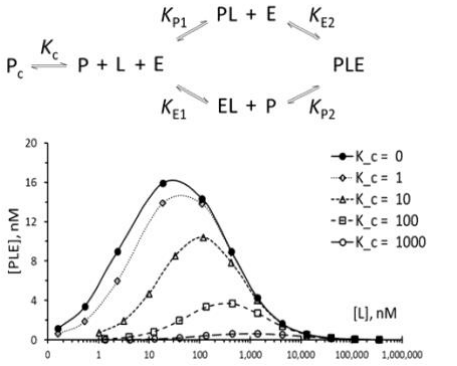




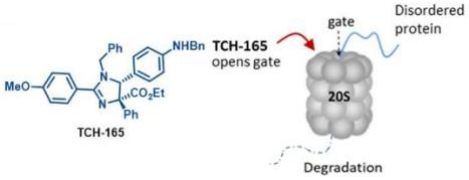




Edited by
Conner Craigon,
Tasuku Ishida
and Oliver Hsia



September 2020 Edition



University of Dundee

Ciulli Journal Club Contents

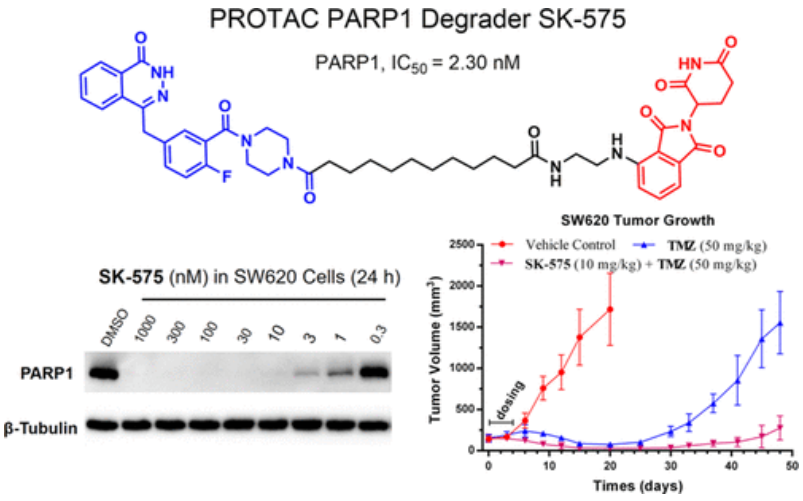
Targeted Protein Degradation

Contributor: Oliver

Discovery of SK-575 as a Highly Potent and Efficacious Proteolysis Targeting Chimera (PROTAC) Degrader of PARP1 for Treating Cancers

ChaoGuo Cao[§], Jie Yang, Yong Chen[§], ….. , Lifeng Zhao,* Yuanwei Chen*

J. Med. Chem. **2020**, DOI: 10.1021/acs.jmedchem.0c00821



This 'Just Accepted' manuscript presents interesting findings for the discovery of a series of degraders which target the nuclear protein poly(ADP-ribose) polymerase-1 (PARP1), which plays a well-studied role in DNA repair as well as a role in the differentiation and proliferation of tumour cells. PARP1 knockout cancer cells are known to exhibit an increased sensitivity to cytotoxic agents and there has been significant interest in developing PARP inhibitors to complement traditional chemo and radio-therapy measures. In addition, PARP inhibitors have been shown to be effective in treating non-oncogenic conditions where over-active PARP1 leads to depletion of ATP levels and thus results in necrotic cell death, while high levels of PARP1 also result in accumulation of PAR polymers which trigger the release of apoptosis inducing factor (AIF) from mitochondria to cause a form of cell death termed parthanatos. Mechanistically, PARP inhibitors work by trapping PARP1 protein while it is bound to DNA in an inhibitor-PARP1-DNA complex which blocks and damages DNA replication. The present work details the development of a series of PROTACs based on the PARP1/2 inhibitor Olaparib, combining this with thalidomide/lenalidomide-based warheads for recruiting CRBN/Cul4A. One of the compounds, SK-575, was shown to trigger PARP1 degradation with a DC_{50} in the picomolar range in MDA-MB436, SW620 and Capan-1 cell lines. Interestingly, use of a VHL ligand resulted in significantly lower potency of the candidate PROTAC compounds and much less PARP1 degradation, however PARP1 inhibition was detected with IC_{50} values of 54nM and 87nM in MAD-MB-436 and Capan-1 cells respectively, suggesting that the VHL-based compounds function as inhibitors rather than degraders. SK-575 was shown to inhibit tumour growth *in vivo* as a single agent in homologous-repair deficient (containing mutant BRCA1/2) xenograft models and to synergise with cytotoxic agents (such as cisplatin) at well-tolerated dose schedules.

This work represents compelling proof-of-concept for the development of PARP1-targeting degraders and provides preliminary evidence for the higher effectiveness of CRBN-based degraders compared to VHL-based ones when used in conjugation with this particular target-ligand. This is the most potent and efficacious PARP1 degrader to date, and this work may provide a strong platform for the treatment of diseases caused by overactivation of PARP1.

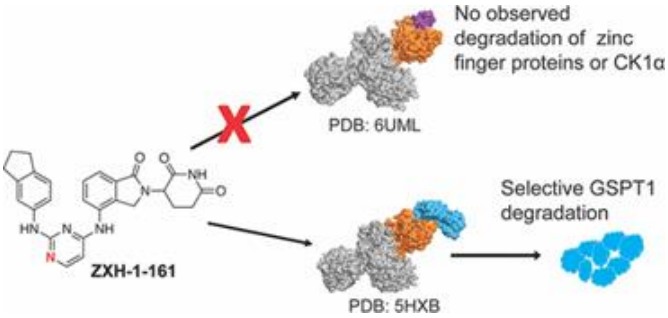
Contributor: Tasuku

Selective Degradation of GSPT1 by Cereblon Modulators Identified via a Focused Combinatorial Library

Chelsea E. Powell,[§] Guangyan Du,[§] …, Nathanael S. Gray*

ACS Chem. Biol. **2020**, DOI: 10.1021/acschembio.0c00520

The authors developed a lenalidomide-based combinatorial library and screened it against multiple myeloma cell line MM1.S WT and CRBN$^{-/-}$ to identify compounds to induce degradation of new neo-substrates. Five compounds were selected as hit compounds and found that they have a similar chemical motif and are supposed to fill the same binding pocket. The whole proteomics analysis of three compounds showed that they degrade GSPT1 and GSPT2 selectively and not degrade other proteins, like IKZFs. The computational analysis of ZXH-1-161 to the CRBN-GSPT1 complex indicated that the nitrogen atom on the pyrimidine ring forms a hydrogen bond to Lys628 on GSPT1, and that was supported by the result of the corresponding derivative from nitrogen to carbon at the same position. The compounds they found degraded GSPT1 more selectively than compounds previously reported, therefore, they should be a good tool for exploring the therapeutic potential of GSPT1 degradation.



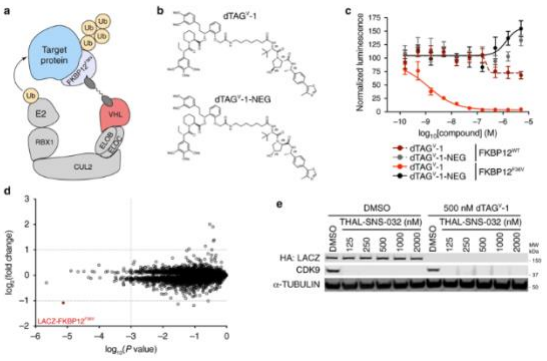
Contributor: Conner

Rapid and direct control of target protein levels with VHL-recruiting dTAG molecules

Behnam Nabet[§]*, Fleur M. Ferguson[§], ……, Kimberly Stegmaier & Nathanael S. Gray*

Nat. Commun. **2020**, *11*, 4687

This research describes the development of dTAG^{V}-1, a VHL-recruiting dTAG PROTAC that rapidly degrades FKBP12^{F36V}-tagged proteins. This research paper is an update to their previous work which demonstrated the application of the dTAG degron degrading FKBP12^{F36V}-tagged proteins using a CRBN-based PROTAC termed dTAG-13 (*https://doi.org/10.1038/s41589-018-0021-8*). dTAG-13 has shown limitation in that it is ineffective at degrading oncogenes that are recalcitrant to CRBN-mediated degradation.



Nabet et al. hypothesized that a VHL-recruiting dTAG PROTAC could mitigate this issue and therefore, in this research they demonstrate the synthesis, characterization and utility of a VHL-recruiting dTAG molecule termed dTAG^{V}-1. In this paper they established the functionality of dTAG^{V}-1 using compound titrations assays on 293FT FKBP12^{WT}-Nluc and FKBP12^{F36V}-Nluc dual luciferase systems. Following this they demonstrate in-vivo applicability of dTAG^{V}-1 through injection of MV4;11 luciferase-FKBP12^{F36V} (luc-FKBP12^{F36V}) cells into mice for non-invasive bioluminescent monitoring of leukemic burden pre- and post- dTAG^{V}-1 exposure. Loss of bioluminescent signal was identified 4 h after the first

administration of dTAG^{V}-1. Importantly they go on to demonstrate dTAG^{V}-1 improved ability to degrade an EWS/FLI conjugate protein that is recalcitrant to degradation by CRBN-based PROTACs. EWS/FLI is a chimeric oncoprotein responsible for Ewing sarcoma and child neuroectodermal tumours. They demonstrate that EWS/FLI can be rapidly degraded by dTAG^{V}-1 but not dTAG-13, thereby signifying the utility of developing degraders that utilise different E3 ligases.

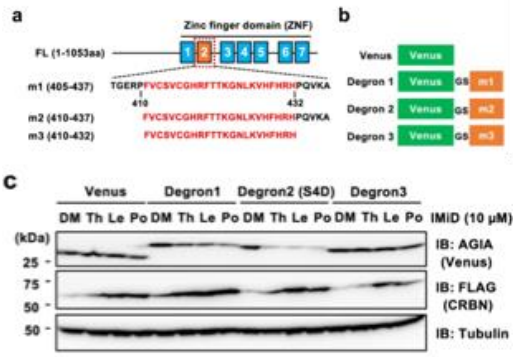
This paper harbours a great selection of cell-based assays for analysing PROTAC function in in-cellulo and in-vivo contexts. More importantly this paper adds to the arsenal of inducible degron technologies for targeted protein degradation.

Contributor: Conner

An IMiD-induced SALL4 degron system for selective degradation of target proteins

Satoshi Yamanaka, … , Tatsuya Sawasaki*

Commun. Biol., **2020**, *11*, 515



In this investigation Yamanaka et al., develops an IMiD-dependent Sal-like protein 4 (SALL4) C2H2 zinc finger nuclease (S4D) system for targeted protein knockdown. They demonstrate that the S4D tag is able to degrade ectopically overexpressed S4D-tagged AGIA polypeptide using 10 μM IMiD compounds thalidomide (Th), lenalidomide (Le), or pomalidomide over a 16-hour treatment.

They later go on to demonstrate using ectopically expressed N- and C-terminal p53 tagged with S4D that they can induce degradation of S4D-p53 conjugates in the presences of 10 μM pomalidomide over a 16-hour treatment. It was also shown that the S4D degron could induce degradation of transiently expressed S4D-tagged proteins localised to different specific regions within the cell such as the nucleus (p53), the ER membrane (STING), the plasma membrane (DRD1), the mitochondrial outer membrane (MAVS) and the Golgi (GOLGA2/GM130) in the presence of IMiD compounds. After establishing S4D knock-in cell lines for the proteins RelA and IκB, Yamanaka et al. demonstrate significant degradation of S4D-fused RelA and IκB after a 3-hour treatment of pomalidomide. This research goes on to highlight the possibility of an unknown mechanism for RelA translocation to the nucleus as they demonstrate that IκBα degradation is insufficient for RelA nuclear translocation. They then go on to show that 5-hydroxythalidomide – a thalidomide metabolite - can more readily induce degradation of S4D-tagged proteins compared to proteins tagged with another IMiD-based degron called I3D.

This paper highlights a novel degron system based upon IMID's affinity for SALL4 C2H2 zinc finger domain to selectively target proteins for CRBN-mediated degradation through the use of the IMID degraders thalidomide, lenalidomide, pomalidomide, and 5-hydroxythalidomide. Importantly, this research adds to a growing collection of different inducible degron systems that enable a more cost effective, and time saving approach for the induced degradation of therapeutic

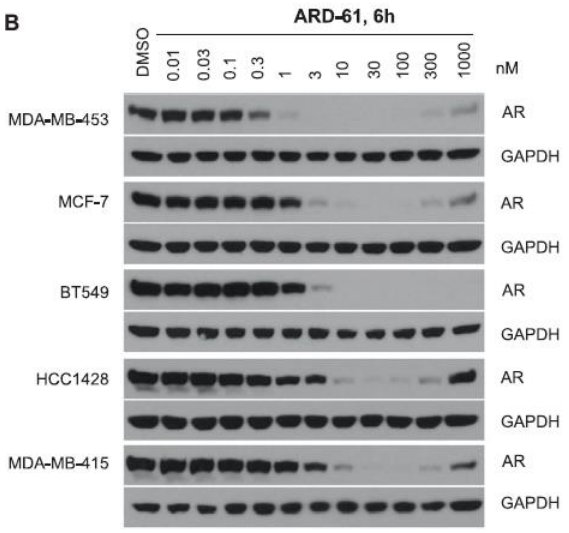
targets. Since each inducible degron technology has limitations and advantages, having the S4D degron as a novel degron system broadens the potential applicability of degron technology as a whole.

Contributor: Oliver

A highly potent PROTAC androgen receptor (AR) degrader ARD-61 effectively inhibits AR-positive breast cancer cell growth in vitro and tumor growth in vivo

Lijie Zhao[§], Xin Han[§], ….. , Shaomeng Wang*

*Neoplasia **2020**, 22*, 522. DOI: 10.1016/j.neo.2020.07.002



Targeting of the androgen receptor (AR) using PROTACs has been shown to be effective *in vitro* and *in vivo* and the drug ARV-110 from Arvinas is in phase I clinical trials for the treatment of metastatic castration resistant prostate cancer (mCRPC) in patients who have also been treated with abiraterone or enzalutamide.

Zhao and colleagues at the University of Michigan have shown in their latest work that their degrader called ARD-61 is highly potent in depleting AR levels in breast cancer models in vitro and in vivo. AD-61 is composed of the AR antagonist ARi-16 and the VHL ligand HXD079 and the researchers have shown that this PROTAC is more potent than enzalutamide and ARi-16 in inhibiting AR+ cell growth via apoptosis induction. Furthermore AD-61 was shown to block AR signalling and inhibit AR-target gene expression in MDA-MB-453 cells (breast cancer).

They also showed using a mouse xenograft model of the same tumour cells that AD-61 was effective in degrading AR protein and was more effective in promoting long-term tumour inhibition in mice than enzalutamide. This work provides compelling evidence for the development and evaluation of AR degraders for the treatment of AR+ breast cancer in addition to the existing PROTAC treatment of prostate cancer using ARV-110.

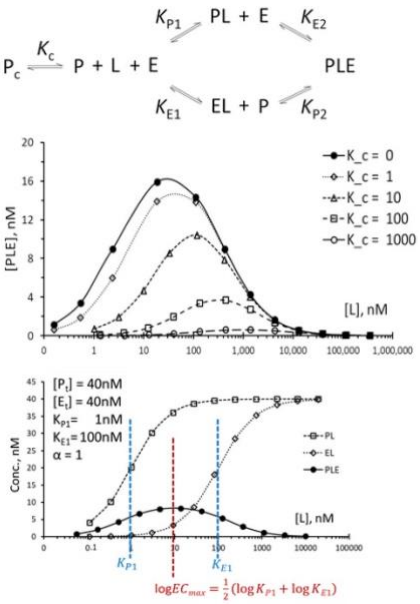
AD-61 appears to be an excellent candidate for clinical development based on the safety profile and effectiveness demonstrated in this work. The researchers expand upon their previous discovery that AD-61 is effective in inducing degradation of AR protein in human prostate cancer cell lines in vitro as well as in AR+ prostate cancer tumour tissue, and effective in inhibiting tumour growth in prostate cancer xenograft models in vivo including models that are resistant to enzalutamide. This PROTAC may have an important use in the treatment of multiple cancers.

Contributor: Tasuku

A suite of mathematical solutions to describe ternary complex formation and their application to targeted protein degradation by hetero-bifunctional ligands

Bomie Han*

J. Biol. Chem. **2020**, DOI: 10.1074/jbc.RA120.014715



In the case of traditional drugs that form a binary complex with the target proteins, their behaviour is described by a simple mathematical equation. But there is no mathematical equation to describe the unique characters of PROTACs, like the hook effect and full equilibrium binding characteristics of the ternary complexes. In this paper, the author showed a full, exact, and universal mathematical description of the ternary complex system at equilibrium. The author then investigated the effect of each parameter and revealed that internal ligands that compete with the PROTACs and/or the ratio between the active and inactive form effect not only for the effective concentration but also the maximal concentration of the ternary complexes ($[PLE]_{max}$). The author also noted that the cooperativity value α affects $[PLE]_{max}$ but not the ligand concentration that shows maximal $[PLE]_{max}$ (EC_{max}). In addition, it was shown that EC_{max} is described as the equation below:

$$\log EC_{max} = 1/2(\log K_{P1} + \log K_{E1})$$

That equation means the EC_{max} is located between K_{P1} and K_{E1} in the logarithmic scale. The mathematical descriptions and findings should be helpful to understand the experimental results of PROTACs and could guide selection of suitable combinations of proteins of interest and E3 ligases.

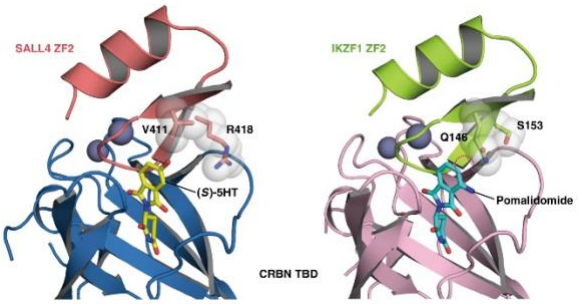
The author provided Excel calculation programs of the descriptions. You can find them on the web site of the supporting information; *https://www.jbc.org/content/early/2020/08/28/jbc.RA120.014715/suppl/DC1*

Contributor: Tasuku

Structural bases of IMiD selectivity that emerges by 5-hydroxythalidomide

Hirotake Furihata,[§] Satoshi Yamanaka,[§]..., Masaru Tanokura*, Tatsuya Sawasaki*, and Takuya Miyakawa*

Nat. Commun. **2020***,* 4578*.* DOI: 10.1038/s41467-020-18488



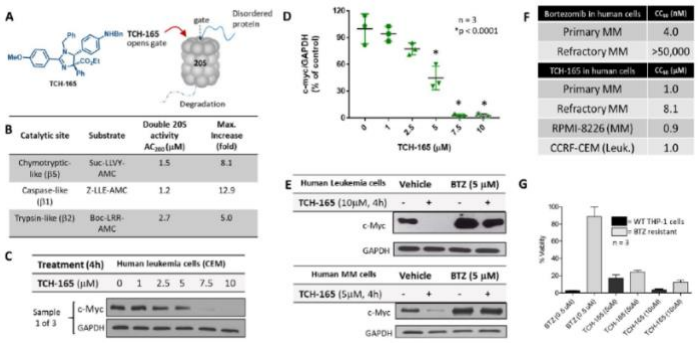
Recent studies have suggested SALL4 as one of the proteins potentially linked to the teratogenicity of IMiDs. 5-hydroxythalidomide (5-HT), which is one of the metabolites of thalidomide, induces degradation of SALL4 strongly but not IKZFs. On the other hand, IMiDs induce degradation of IKZFs but not SALL4. The authors obtained the cocrystal structure of a SALL4:5-HT:CRBN ternary complex to clarify the mechanism of such selectivity. The importance of steric differences was confirmed by mutation analysis of SALL4 and IKZF. Interestingly, the 5-hydroxyl group of 5-HT formed a hydrogen bond to H358 on CRBN and that contributes to improving the binding affinity to CRBN. Their findings provide a guide to design iMiDs molecular glues to degrade novel neo-substrates. In addition, information on the hydrogen bond between the 5-hydroxyl group and H358 on CRBN could be used to improve degradation activities of CRBN-based PROTACs.

Contributor: Oliver

Enhancing c-MYC degradation via 20S proteasome activation induces *in vivo* anti-tumor efficacy

Evert Njomen, ….. , Jetze J. Tepe*

bioRxiv **2020**, DOI: 10.1101/2020.08.24.265470



This preprint research investigates the enhancement of protein degradation via activation of the 20S proteosome using imidazoline-based compounds. Previous work (*https://dx.doi.org/10.1021%2Facs.biochem.8b00579*) has shown that one of these compounds, named TCH-165, enhances 20S-mediated degradation of peptides by inducing an open-gate conformation. This was expanded upon by showing that TCH-165 is able to increase the degradation of intrinsically disordered proteins (IDPs) in cell cultures.

The present paper demonstrates that this compound can enhance the degradation of the oncoprotein c-MYC, which for its large part is an IDP and is found expressed in more than half of all human cancers. TCH-165 was shown to sensitise bortezomib-resistant human acute monocytic leukaemia THP-1 cells following 72h pre-treatment with the compound. *In vivo* mouse xenograft models showed that TCH-165 was able to block tumour growth with minimal concomitant loss of body weight, indicating acceptable tolerance, and oral treatment in dogs was shown to have little toxicity and significantly increase substrate proteolysis compared to untreated canines.

Overall, this paper makes a compelling case for proteosome enhancers as potential candidates for the treatment of diseases which are driven by disordered proteins, and the fact that there are few changes to the cellular proteome is an important finding in the context of potential therapeutic use with minimal off-target effects.

This paper makes good use of *in vitro*, *ex vitro* and *in vivo* models to demonstrate the efficacy and tolerance of TCH-165 in reducing the levels of the oncoprotein c-MYC. The specificity of the 20S proteosome for IDPs coupled with the ubiquitin-independent nature of this degradation makes this therapy an interesting and different approach to protein degradation compared to proteolysis targeting chimeras (PROTACs). These two approaches to protein degradation may be able to act synergistically for the treatment of some cancers further down the line.

Contributor: Conner

Assessing IRAK4 Functions in ABC DLBCL by IRAK4 Kinase Inhibition and Protein Degradation

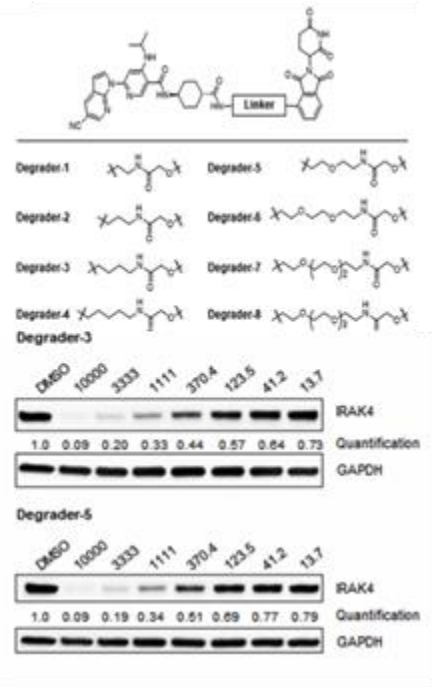
Jing Zhang, [§] Liqiang Fu, [§] … , Xuedong Dai*

Cell Chem. Biol. **2020,** *DOI*: 10.1016/j.chembiol.2020.08.010

This research paper investigates what role the kinase-independent scaffolding function of IRAK4 - a central kinase governing myddosome-mediated expression of inflammatory molecules - might have on endogenous myddosome signalling and lymphoma cell growth as evaluated in activated B-cell-like diffuse large B cell lymphoma (ABC DLBCL) cancer cell survival. ABC DLBCL harbour mutations in MyD88 an adapter protein that, like IRAK4, forms part of the myddosome complex making it an ideal system for exploring IRAK4 role in cancer cell survival.

In this paper, Zhang et al. take a potent and selective inhibitor of the IRAK4 kinase, that harbours suboptimal anti-proliferative activity in ABC DLBCL cells, and produce several degrader compounds that harbour <1μM DC50 degradation capacity and IRAK4 selectivity.

They go on to demonstrate that neither inhibition of IRAK4 kinase activity nor IRAK4 protein degradation leads to significant cell apoptosis or growth inhibition, suggesting that IRAK4 plays a dispensable role in ABC DLBCL cell survival.



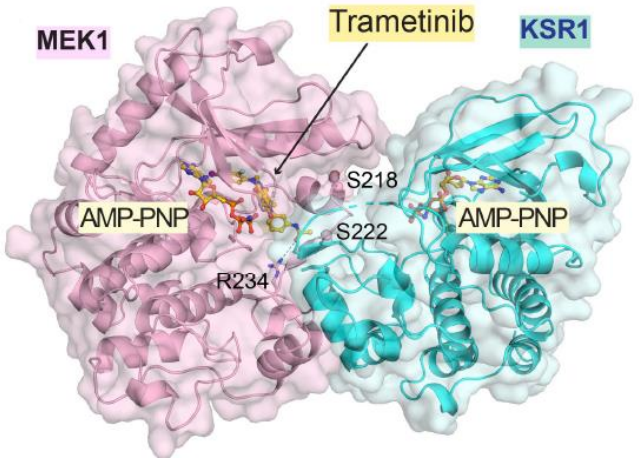
This paper demonstrates clearly that IRAK4 plays a dispensable role in the survival of myddosome implicated ABC DLBCL cells. Thereby, demonstrating that IRAK4 is less well understood than initially thought and further study of IRAK4 is needed. The degraders that Zheng and Fu have developed for IRAK4 can be used as chemical tools to further evaluate the biological roles of IRAK4.

Contributor: Will

Structural basis for the action of the drug trametinib at KSR-bound MEK

Khan, Z.M, ..., Arvin C. Dar*

Nature **2020**, DOI: 10.1038/s41586-020-2760-4



The RAS/RAF/MEK/ERK signal transduction pathway is notoriously overactivated and a driver of tumour growth in many cancers. Despite there being clinically approved inhibitors of the MEK kinase, their precise mechanisms of action are not well understood and adaptive forms of drug-resistance limit their use. Susceptibility to this adaptive response has been partly attributed to the inability of current MEK inhibitors to trap RAF in its inactive state in MEK:RAF complexes, indeed current inhibitors are believed to function *via* inhibition of active RAF with MEK. Furthermore, the adaptive response is dependent on another RAF family member, the scaffolding protein kinase suppressor of RAS 2 (KSR2). In this study Khan et al. report crystal structures of MEK:KSR complexes in the presence of a number of MEK inhibitors. They observe that the FDA approved MEK inhibitor Trametinib, unlike the other inhibitors investigated, is able to form contacts with KSR whilst bound to MEK, due to its unique 3-substituted phenyl acetamide group. They also show that the MEK inhibitor allosteric pocket is markedly different when in complex with KSR compared with isolated MEK. Whilst Trametinib is shown to stabilise MEK:KSR complexes, it inhibits RAF:MEK interactions. Through structure-based approaches the authors are able to design a novel molecule, Trametiglue, that via the introduction of a sulfamide in place of the acetamide is capable of stabilising both RAF:MEK and KSR:MEK complexes. Through co-immunoprecipitation experiments for binding to endogenous MEK they show that Trametiglue, but not Trametinib, is capable of enhancing capture of BRAF. This is thus suggested to be representing a novel approach and mechanism of action that holds potential for antagonising RAS-RAF-MEK driven cancers.

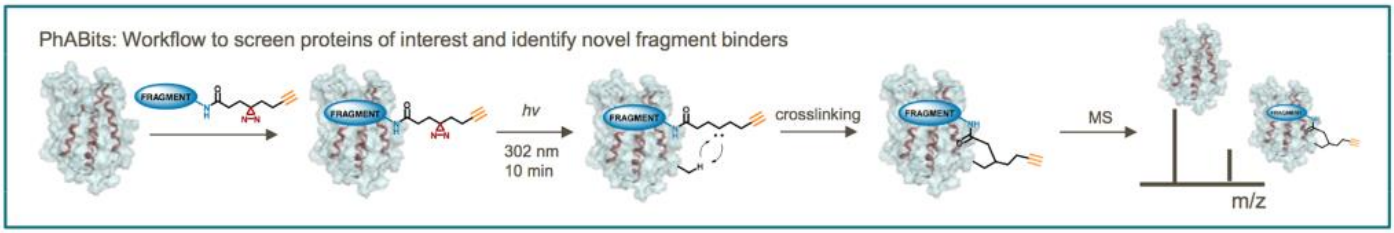
The authors nicely describe how their data support trametinib acting as a 'bumped' MEK inhibitor able to induce interactions with KSR proteins via a conserved 'hole'. The study is a fantastic example of how clear structural elucidation can drive not only understanding of drug function but also the design of a molecule with potentially novel modes of action. In future studies it would be particularly interesting to see how molecules such as trametiglue function in trametinib resistant cell lines and/or cell models capable of adaptive responses following increased dosing of trametiglue over time.

Contributor: Alessio

A Photoaffinity-Based Fragment-Screening Platform for Efficient Identification of Protein Ligands

Grant, E.K, ..., Jacob T. Bush*

Angew. Chem. Int. Ed. **2020**, DOI: 10.1002/anie.202008361



Novel targets are increasingly being selected for drug discovery based on genomic and genetic data, e.g. from gene knock-out and knock-down studies, but triaging which of those targets are most viable for intervention by small molecules (i.e. ligandable) can be challenging. Ligandable targets possess greater chances to become eventually druggable, so target ligandability becomes an essential pre-requisite to establish at the outset of a small-molecule drug discovery project if resources are to be prioritized on screening efforts most likely to yield positive outcomes.

Folks at GSK have a long history of developing ideas and strategies to better assess target ligandability and tractability in the early stages of drug discovery campaigns. The tradition continues with this paper that presents a powerful new method that they called Photo Activatable Bits (PhABits). A library of fragments functionalized with a photoreactive diaziridine group is screened against a purified target protein upon short UV irradiation, with the goal to photocapture protein-fragment covalent adducts, detected by RapidFire LC-MS, as reporters of weak non-covalent protein-fragment interactions. [Note: a terminal alkyne tag (not exploited here) was included in the fragments to enable potential screen of the same library in living cells, as previously shown by the Cravatt group, see Parker et al. *Cell* 2017 Jan 26; 168(3): 527–541.e29]. First, proof-of-concept library screens were conducted against a set of model target proteins. These screens identified known as well as novel binding chemotypes, exhibiting a range of crosslinking yields up to 53%, though the majority had moderate to low crosslinking (up to 5%). Specific interactions where demonstrated via competition (displacement) assays e.g. for the BET Brd4 bromodomain using a known BET inhibitor. Dose response curves allowed measurements of binding affinities for PhABits as well as their competitive ligands, over a wide range of K_ds. Tryptic digest of crosslinked samples allowed identification of the protein surface residue(s) being modified by the PhABit, which they could support by a co-crystal structure. Some of the PhABits for the Brd4 bromodomain included the previously well-characterized dimethylisoxazole fragment (see Hewings et al. *J. Med. Chem.* 2011, 54, 19, 6761–6770) and were conjugated to the VHL-ligand to yield functional, albeit weak, PROTAC degraders. Finally, PhABits library screen against a non-G12C mutant KRAS enabled identification of weak-binding fragment hits covering both novel as well as known chemotypes, likely targeting the switch I/II pocket previously targeted independently by Fesik, Genentech and Boehringer Ingelheim using more conventional protein-observed HSQC NMR fragment screens (references therein). Together, this excellent piece of work qualifies a new fragment screening approach that aids identification of valuable hits and binding sites on targets that might prove otherwise difficult to identify using more conventional methods.

I enjoyed hearing Mike Hann presenting this story at a few meetings where our paths crossed last year (pre-COVID..). I have since looked forward to seeing this coming out, so nice to see the full story published after an early preprint in ChemRxiv earlier this year. The paper provides strong evidence to support the claims and includes a good balance of chemical biology combined with robust biophysical and structural validation – which is what we like! Kudos to the authors on introducing PhABits as a new fragment-based approach to identify ligandable binding pockets on target proteins, shining new light on target tractability. It will be interesting to learn in future how readily progressable into high-quality chemical probes will PhABits hits be, particularly in the quest to de-orphanize some of the most challenging targets we so urgently need to find good ligands for!
