1. From the Editors

From the Editors, with Dr Iain D.G. Watson, Editorial Board Member

Each quarter, the editorial board selects an area to highlight from the broad range of topics that fall under the umbrella of chemistry in cancer research. Our topic this quarter is *the ubiquitin proteasome system*. CICR editorial board member Iain Watson has taken the lead in assembling an overview of the topic.

The ubiquitin proteasome system (UPS)

In 2004 the Nobel Prize in Chemistry was awarded to Aaron Ciechanover, Avram Hershko and Irwin Rose for the discovery of ubiquitin-mediated proteolysis. These are the cellular processes by which proteins are identified for breakdown and elimination. Central to this system is the post-translational tagging of proteins with ubiquitin, a 76 amino-acid protein. Ubiquitin is activated, conjugated and ligated onto target proteins, most commonly between a lysine on the target protein and the C-terminal glycine of ubiquitin. This collaborative cascade is catalyzed by enzymes known as E1, E2 and E3 ligases, where an E1 enzyme may bind many E2s, which bind many E3s hierarchically, allowing regulation of the cellular machinery. Ubiquitination is reversible which is catalyzed by families of deubiquitinase enzymes (DUBs). Tagged proteins are transported to a large cylindrical multisubunit protease complex called the proteasome to be degraded. The common 26S proteasome is comprised of one 20S core particle and two regulatory 19S particles which recognize the ubiquitinated proteins, unfold them and feed them into the catalytic core. Numerous cellular processes are regulated by ubiquitin-mediated proteolysis including the cell cycle, DNA repair, transcription, protein quality control and the immune response. Defects in the UPS have a role in many human diseases, including a variety of cancers. By the turn of the current century, these fundamental discoveries were already being applied to oncology drug discovery, with FDA approval of bortezomib (Velcade) for multiple myeloma occurring in 2003. Covalently targeting the catalytic sites of the 26S proteasome, this compound was followed by the FDA approval of carfilzoib (Kyprolis) in 2012 also for multiple myeloma, which operates via a similar mechanism.

At this year's AACR Annual Meeting Dr. Craig M. Crews, Professor in the Departments of Chemistry and Pharmacology at Yale University, was awarded for outstanding achievement in chemistry in cancer research. Dr. Crews was honored for his work developing epoxyketone proteasome inhibitors, including carfilzomib. More recently, Dr. Crews has pioneered a new technology for co-opting the UPS system towards degradation of particular proteins of interest (POI). Proteolysis-targeting chimeras (PROTACs) are bifunctional molecules that contain an E3 ligase targeting ligand linked to a warhead targeting a POI. The induced proximity that occurs when a PROTAC binds both its partners causes the ubiquitination and subsequent degradation of the POI by the proteasome.

PROTACs are useful chemical tools for post-translational protein knockdown. The phenotypic effects are large, but also reversible, providing an alternative approach to genetic knockdowns when studying disease targets. Degradation is mechanistically distinct from the inhibition of a single protein domain, resulting in a closer phenocopy of genetic down-regulation. Several PROTACs have recently shown dramatic effects both *in vitro* and *in vivo* and the excitement in this area is apparent in the increasing number of development deals that are being signed in this space with startup companies such as Arvinas LLC and C4 Therapeutics.

Other components of the UPS have also been targeted for inhibition. The E3 ubiquitin ligase MDM2 is involved in the degradation of p53, an important tumor suppressor which prevents carcinogenesis by promoting cell cycle arrest and apoptosis. The discovery of the small molecule Nutlin showed that preventing the protein-protein interaction between wild-type p53 and MDM2 stabilized the tumor suppressor, increasing apoptosis and providing therapeutic benefit in treating human cancers. Some small molecules are known to inhibit protein activity by inducing the degradation of their targets. As an example, fulvestrant inhibits the signaling of estrogen receptor alpha (ERα) by destabilizing its structure, resulting in its degradation by normal UPS processes. The unique mechanism of immunomodulatory agents involves the co-opting of the E3 ligase cereblon towards the degradation of the transcription factors Ikaros and Aiolos, important targets in the treatment of multiple myeloma.

In this issue of the Newsletter, we give recent examples of some of the many areas in which chemical research is impacting our understanding of the ubiquitin proteasome system (UPS) and its effect on cancer. Articles highlighting the use of fragment screening, biophysical methods and medicinal chemistry approaches are presented, clearly showing the power of these methods for the discovery and development of probe and drug-like compounds. Potent and selective BET targeting PROTAC degraders and inhibitors of cereblon, RBM39 (CAPERα) and VHL are described. These chemical compounds are shown engaging and effecting cancer related pathways of the UPS and have the potential for significant disease effects. We are also pleased to showcase the work of Dr. Alessio Ciulli, a Professor of Chemical and Structural Biology in the School of Life Sciences at the University of Dundee. Dr. Ciulli's group is at the forefront of research in the UPS area and his work is highlighted in this issue's Profile of an Early-Career Researcher.

Changes to the Newsletter Editorial Board for 2018

The CICR Steering Committee has selected the Editor-Elect for 2018 and several new members for the Newsletter Editorial Board to replace members whose term expires at the end of the current year. Current Editorial Board member Dr. Alex G. Watterson, Research Associate Professor of Pharmacology and Chemistry at Vanderbilt University will be Editor-Elect in 2018 and become Editor in 2019. Replacing Alex for the remainder of his 2017-2020 term will be Dr. Daniel A.

Heller, Assistant Member in Molecular Pharmacy and Chemistry at Memorial Sloan Kettering Cancer Center. The Steering Committee has selected three members for 2018-2021 terms: Dr. Martin Swarbrick, Senior Group Leader, Discovery Chemistry at the Institute of Cancer Research UK, Dr. Zhao-Kui Wan, Head of Chemistry at Janssen Pharmaceutical Companies in Shanghai, and current board member Dr. Jordan L. Meier, Investigator in the Chemical Biology Laboratory at the National Cancer Institute. Thanks to all of them for their willingness to step up and serve.

CICR activities at upcoming conferences

As part of the mission to showcase the role of chemistry in cancer research, CICR plans events at the upcoming AACR-NCI-EORTC Molecular Targets and Cancer Therapeutics and New Horizons in Cancer Research: Research Propelling Cancer Prevention and Cures conferences later this year. For details see our News from the CICR Steering Committee section, below. It isn't too early to think about the AACR 2018 Annual Meeting. Many CICR members have commented that the number of presentations featuring chemistry and preclinical drug discovery at the Annual Meeting seems to be in decline. The CICR leadership makes every effort to create opportunities for these types of talks in the program, and all of us can help by submitting abstracts and sharing our science. The Program Committee chooses symposium and mini-symposium topics for the Annual Meeting based in part on the number of abstracts submitted in various areas, so please consider submitting an abstract this fall for next year's annual meeting, the regular deadline is December 1, 2017.

Chemical Probes

The value of chemical probes to the cancer research community has been highlighted by Dr David Uehling on behalf of the CICR Working Group in the Cancer Research Catalyst blog (http://blog.aacr.org/how-chemical-probes-can-boost-cancer-research/). Now, Prof. Julian Blagg [Chair Elect of the CICR and Deputy Director of the Cancer Research UK Cancer Therapeutics Unit at The Institute of Cancer Research (ICR)] and Prof. Paul Workman (Chief Executive and President of the ICR) have published a high profile Perspective in *Cancer Cell* that further highlights the importance of choosing and using small molecule chemical tools wisely (http://dx.doi.org/10.1016/j.ccell.2017.06.005). An impetus for the Perspective arose from CICR-sponsored sessions on chemical probes at recent AACR Annual Meetings where, despite high attendance, there was a feeling among attendees and speakers that key learnings on the use and abuse of chemical probes were failing to reach and influence many of the cancer biology user community.

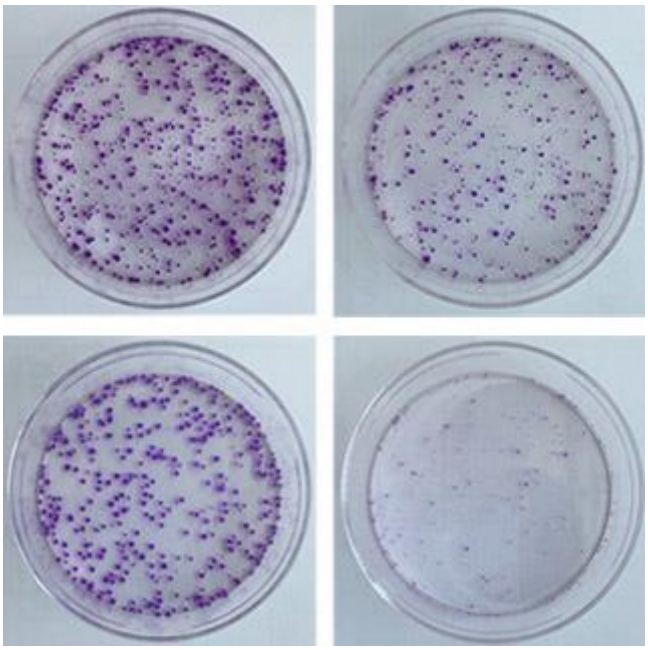
The Blagg and Workman Perspective outlines both best practices and notable cautions when considering the use of chemical probes to interrogate complex biological systems. The use of biology-friendly language and glossaries aims to

attract a biology readership that, hitherto, may not have considered “kicking the tires” on chemical tools in the same way that biological tools such as antibodies, RNAis or CRISPR reagents are ideally scrutinised. A key tenet of the article is that small molecules should be expected to be promiscuous in their interactions with complex biological systems, such as a cancer cell or *in vivo* animal model, unless they have been subjected to significant medicinal chemistry design and optimisation. Even optimized molecules may exhibit an “active night life” that can confound interpretation of the cell-based pharmacology they elicit. Whilst drugs may benefit from affinity for multiple biological targets, for example to counteract resistance mechanisms, potent and selective chemical probes are commonly required to interrogate mechanism-specific biological hypotheses; furthermore, structurally-matched inactive control compounds also add significant value to the interpretation of biological results.

Part of the CICR Working Group mission is to increase chemistry awareness and knowledge of those invested in cancer research. The Blagg and Workman Perspective aims to further connect the chemistry and bioscience cancer research communities by highlighting the need for expert review of chemical tools prior to their use. The perspective also highlights the Chemical Probes portal (www.chemicalprobes.org) which provides peer-reviewed expert guidance in the selection and use of chemical probes for specific protein targets, including the nomination of recommended probes and inactive controls. They also urge the bioscience community to engage and collaborate with chemistry colleagues in the selection and verification of chemical probes to reduce the risk of investing significant time and effort developing potentially erroneous biological hypothesis based upon flawed chemical tools – akin to a defective GPS system. Please mention this article to your colleagues, or refer to it in referee reports if you are called upon to review articles that use inappropriate molecules in biological studies.

From *Molecular Cancer Therapeutics*:

In this issue, we highlight the *Molecular Cancer Therapeutics* (*MCT*) article ‘Nuclear Export of Ubiquitinated Proteins Determines the Sensitivity of Colorectal Cancer to Proteasome Inhibitor’ by Wu, Chen, and Zhong, et al. Proteasome inhibitors such as bortezomib alone exhibited minimal clinical activity in solid tumors. In this study, the authors found that proteasome inhibition induced a remarkable nuclear exportation of ubiquitinated proteins. Inhibition of CRM1, the nuclear export carrier protein, hampered protein export and synergistically enhanced the cytotoxic action of bortezomib on colon cancer cells containing wild-type p53. In mice, the CRM1 inhibitor KPT330 markedly augmented the antitumor action of bortezomib against colon cancer xenografts that harbored functional p53. These results indicate that targeting nuclear exportation may serve as a novel strategy to overcome resistance and enhance the efficacy of chemotherapeutics, especially for the drugs that activate the p53 system.



Learn more about MCT and how to submit to the Journal.

In this issue's Career Forum section, we feature a Q&A with Prof. Ian Collins, one of MCT's Senior Editors. See this section to learn about his career path, research interests, role with MCT and more!

2. Selected Research Highlights

"Induced Protein Degradation: An Emerging Drug Discovery Paradigm"
Ashton C. Lai and Craig M. Crews
Nat. Rev. Drug Discov., **2017**, *16* (2), 101–114.
DOI: 10.1038/nrd.2016.211.

This review provides an overview of the current state of technologies that exploit the ubiquitin proteosome system (UPS) system to selectively degrade target proteins. In particular, technologies based on proteolysis-targeting chimaeras (PROTACs) are discussed along with their evolution and development over the last decade.

"Regulating the Master Regulator: Controlling Ubiquitination by Thinking Outside the Active Site"

Stacey-Lynn Paiva, Sara R. da Silva, Elvin D. de Araujo and Patrick T.Gunning
J. Med. Chem., **2017**, ASAP.
DOI: 10.1021/acs.jmedchem.6b01346.

This Perspectives Article prepared for a dedicated issue on inducing protein degradation provides an overview of compounds which exert control on

ubiquitination processes, focusing on allosteric modulation. In particular, allosteric inhibition of E1, E2 and E3 enzymes, deubiquitinating enzymes (DUB) and ubiquitin-like proteases (ULPs) are discussed with particular targets and compounds described and profiled.

“Impact of Target Warhead and Linkage Vector on Inducing Protein Degradation: Comparison of Bromodomain and Extra-Terminal (BET) Degraders Derived from Triazolodiazepine (JQ1) and Tetrahydroquinoline (I-BET726) BET Inhibitor Scaffolds”

Kwok-Ho Chan, Michael Zengerle, AndreaTesta and Alessio Ciulli
J. Med. Chem. **2017**, ASAP.
DOI: 10.1021/acs.jmedchem.6b01912.

Structural optimization of proteolysis-targeting chimaeras (PROTACs) can take place in one of three general regions, at the E3 ligase ligand, the linker or the targeting warhead ligand. In this article, the authors focus primarily on examining the effect of using two distinct chemotypes for the warhead ligand, targeting bromodomains for degradation. In particular, the authors synthesized novel VHL-recruiting PROTACs based on triazolodiazepine BET inhibitor JQ1 and the more potent tetrahydroquinoline BET inhibitor I-BET726. Counterintuitively, the less potent BET inhibitor provided the more potent PROTAC degrader. Although the attachment point to these ligands extends into the solvent, attachment of a linker results in distinct exit vectors for the E3-ligase ligands and creates the potential for different cooperative formation of ternary complexes that form the basis for target degradation. The authors used an isothermal calorimetry (ITC) assay to measure the cooperativity of ternary complex formation. In contrast to the JQ1 based PROTACs, the tetrahydroquinoline series showed negative cooperativity. This is likely the result of the exit vector for the linker resulting in an unfavorable orientation between the E3 ligase and the bromodomain. The article shows that more potent ligands will not necessarily result in more potent PROTACs, emphasizing the importance of measuring the cooperativity of the ligand in ternary complex formation for optimizing PROTAC activity.

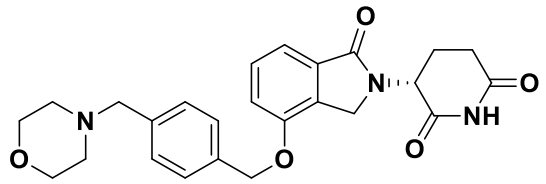
“Protein Degradation via $CRL4^{CRBN}$ Ubiquitin Ligase: Discovery and Structure-Activity Relationships of Novel Glutarimide Analogs That Promote Degradation of Aiolos and/or GSPT1”

Joshua D. Hansen, Kevin Condroski, Matthew Correa, George Muller, Hon-Wah Man, Alexander Ruchelman, Weihong Zhang, Fan Vocanson, Tom Crea, Wei Liu, Gang Lu, Frans Baculi, Laurie LeBrun, Afshin Mahmoudi, Gilles Carmel, Matt Hickman and Chin-Chun Lu
J. Med. Chem. **2017**, ASAP.
DOI: 10.1021/acs.jmedchem.6b01911.

“A Cereblon Modulator (CC-220) with Improved Degradation of Ikaros and Aiolos”

Mary E. Matyskiela, Weihong Zhang, Hon-Wah Man, George Muller, Godrej Khambatta, Frans Baculi, Matthew Hickman, Laurie LeBrun, Barbra Pagarigan, Gilles Carmel, Chin-Chun Lu, Gang Lu, Mariko Riley, Yoshitaka Satoh, Peter Schafer, Thomas O. Daniel, James Carmichael, Brian E. Cathers and Philip P. Chamberlain
J. Med. Chem. **2017**, ASAP.
DOI: 10.1021/acs.jmedchem.6b01921.

The return of thalidomide to the clinic as a treatment for multiple myeloma and the development of lenalidomide and pomalidomide as improved immunomodulatory (IMiDs) agents, have greatly improved patient outcomes. The drugs target cereblon (CRBN), the substrate receptor for the $CRL4^{CRBN}$ E3 ubiquitin ligase complex. However, instead of inhibiting cereblon, the ligand alters the substrate specificity of the ligase, promoting binding of different target substrates and their subsequent degradation by the proteasome. This mechanism contrasts to PROTAC approaches where the linked ligands recruit substrates to the ligase by distinct binding events. In particular, IMiDs target the transcription factors Ikaros (IKZF1) and Aiolos (IKZF3) for degradation, with reduction of their target genes resulting in antiproliferative and immunomodulatory effects. However, thalidomide analogues can be designed to target other proteins for degradation such as casein kinase 1α (CK1α) and G1 to S phase transition 1 protein (GSPT1). In the first article, Hansen et al describe the SAR surrounding a chemotype that focuses degradation on the GSPT1α translation termination factor. The compounds concentrated on 5-substituted analogues of the general thalidomide chemotype and the resultant compounds display a range of selectivity for degradation of GSPT1α versus Aiolos. Structural changes resulted in compounds with potency and selectivity for either target. The authors describe the challenges in compound progression arising from disconnects between compound potency and the efficiency of protein degradation. However, the results show advances for the progression towards more rational development approaches for compound with this unique mode of action, introducing elements of selectivity, efficiency and potency. In the second article, Matyskiela et al describe compound CC-220 which binds cereblon with higher affinity then lenalidomide or pomalidomide, degrading Ikaros and Aiolos to a greater extent. X-Ray analysis of the compound shows greater contacts with cereblon resulting from the addition of an extended chemical moiety which is positioned inside a groove on the protein surface. Furthermore, CC-220 has enhanced specificity as it does not target CK1α and GSPT1 for degradation. The compound is currently in phase 2 clinical trials for treatment of systematic lupus erythematosus (SLE) and multiple myeloma.



CC-220

“Anticancer Sulfonamides Target Splicing by Inducing RBM39 Degradation via Recruitment to DCAF15”

Ting Han, Maria Goralski, Nicholas Gaskill, Emanuela Capota, Jiwoong Kim, Tabitha C. Ting, Yang Xie, Noelle S. Williams and Deepak Nijhawan
Science, **2017**, *356* (6336), ASAP.
DOI: 10.1126/science.aal3755.

“Selective Degradation of Splicing Factor CAPERα by Anticancer Sulfonamides”
Taisuke Uehara, Yukinori Minoshima, Koji Sagane, Naoko Hata Sugi, Kaoru Ogawa Mitsuhashi, Noboru Yamamoto, Hiroshi Kamiyama, Kentaro Takahashi, Yoshihiko Kotake, Mai Uesugi, Akira Yokoi, Atsushi Inoue, Taku Yoshida, Miyuki Mabuchi, AkitoTanaka and Takashi Owa
Nat. Chem. Biol. **2017**, *13* (6), 675–680.
DOI: 10.1038/nchembio.2363.

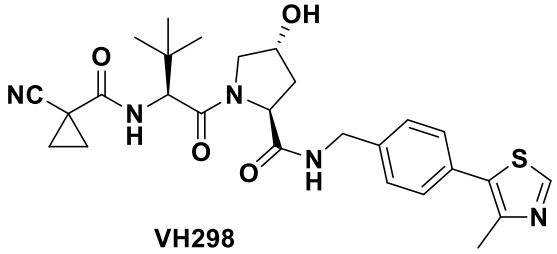
The interaction of immunomodulatory agents (IMiDs) with cereblon leads to the reprogramming of the $CRL4^{CRBN}$ E3 ubiquitin ligase complex towards the recruitment and degradation of transcription factors Ikaros and Aiolos. The unique mechanism was thought to be exceptional and the idea of similar small molecule E3 ligase modulators unlikely. However, in the first paper Han et al report the discovery that indisulam reprograms the $CRL4^{DCAF15}$ E3 ligase complex in a similar manner to IMiDs. Although the antiproliferative effects of indilsulam and other sulfonamides was known, the mechanism of action of these compounds was unknown. The authors set out by studying the genetic mutations that confer resistance to indisulam. A number of point mutations in nuclear protein RBM39 (CAPERα) conferred resistance to indisulam in cultured cells and mouse xenografts. The compound was found to bind to DCAF15, the substrate receptor for the $CRL4^{DCAF15}$ E3 ligase complex, causing ligand mediated recruitment of RBM39 and resultant ubiquitination and degradation of the nuclear protein. In experiments with purified proteins, indisulam formed a tertiary complex with both DCAF15 and RBM39, but had no detectable affinity for either protein alone. Remarkably, other clinically tested sulfonamide ligands share the same mechanism of action. The point mutations in RBM39 that were found to confer clinical resistance also prevent recruitment of $CRL4^{DCAF15}$, increasing RBM39 stability. Patients with higher gene expression of DCAF15 are more susceptible to treatment, providing a biomarker for treatment. In the second paper, Uehera et al similarly identified sulfonamides as modulating the $CRL4^{DCAF15}$ dependant degradation of RBM39 (CAPERα). Based on expression proteomics, the authors used a target identification campaign to discover the mechanism of action. A biotinylated probe was made for photoaffinity labeling studies, capturing and enriching DCAF15, with a number of sulfonamide ligands competing for binding. The biotinylated sulfonamide retained target engagement suggesting that these compounds may serve as the basis for DCAF15 recruiting PROTAC type molecules in the future. As with immunomodulatory agents, the reveal of this as

the mechanism of action creates opportunities for the development of more potent, selective and effective drugs and also a greater understanding of the fundamental biology of these processes within the UPS.

“Potent and Selective Chemical Probe of Hypoxic Signaling Downstream of HIF-α Hydroxylation via VHL Inhibition”

Julianty Frost, Carles Galdeano, Pedro Soares, Morgan S. Gadd, Katarzyna M. Grzes, Lucy Ellis, Ola Epemolu, Satoko Shimamura, Marcus Bantscheff, Paola Grandi, Kevin D. Read, Doreen A. Cantrell, Sonia Rocha and Alessio Ciulli
Nat. Commun. **2016**, *7*, 13312.
DOI: 10.1038/ncomms13312.

Hypoxia inducible factors (HIFs) are a family of transcription factors that are master regulators of hypoxic signaling. These comprise an oxygen labile α-subunit HIF-α and a stable β-subunit (HIF-β). HIF-α levels are maintained at low levels under normoxia due to efficient ubiquitination by the VHL Cullin RING E3 ubiquitin ligase complex ($CRL2^{VHL}$) followed by proteasomal degradation. Key to this recognition is the post-translational hydroxylation of key proline residues by the oxygen dependent activity of prolyl hydroxylase domain (PHD) enzymes. Under low oxygen levels HIF-α is not hydroxylated by PHD, escaping recognition by VHL inducing the expression of a number of genes linked to hypoxic response. The article describes the development of VH298, a potent inhibitor of the von Hippel-Lindau (VHL) E3 ligase. As a result, the compound stabilizes HIF-α by blocking its interaction with VHL, its E3 ligase. Optimization of the inhibitor from previous leads had the major addition of the cyanocyclopropyl group with a consequent improvement of the K_d = 90 nM, affording the most potent VHL inhibitor to date. The inhibitor’s increased logD improved its cell permeability resulting in observable cellular engagement shown using the cellular shift assay (CETSA) along with stabilization of HIF-α at 10 μM in HeLa cells. The compound represents a high quality selective chemical probe for the HIF signaling cascade. It has been reviewed on ChemicalProbes.org and is available commercially. Use of this general chemotype is widespread in PROTACs, which often target VHL engagement when targeting proteins for degradation by the UPS. While VH298 maximizes HIF stability, the opposite is desirable for PROTACs where such activity might interfere with the desired effects from protein knockdown. However, these results show that there is an exploitable window between usual PROTAC activity which is observed at nM concentrations and the μM concentrations for HIF-α stabilizing activity observed with VH298.



VH298

“Allosteric Targeting of the Fanconi Anemia Ubiquitin-Conjugating Enzyme Ube2T by Fragment Screening”

Francesca E. Morreale, Alessio Bortoluzzi, Viduth K. Chaugule, Connor Arkinson, Helen Walden and Alessio Ciulli
J. Med. Chem., **2017**, *60* (9), 4093–4098.
DOI: 10.1021/acs.jmedchem.7b00147.

There are 40 known E2 enzymes in humans, but the deep active site clefts on the proteins have made drug discovery challenging and consequently are few known inhibitors. Ube2T is the E2 enzyme that is associated with the DNA repair pathway and has been found overexpressed in several tumor types. The authors have identified an allosteric pocket on Ube2T using a fragment screen and biophysical methods of measuring binding. Protein-observed NMR was used to identify the region of binding for the top hits. This identified a pocket adjacent to the catalytic cysteine which was confirmed by a crystal structure with one of the hits. Although the fragments were weak binders, they reduced substrate ubiquitination in an assay of enzymatic activity. These fragments represent starting points for further development into high affinity inhibitors.

“Degradation of the BAF Complex Factor BRD9 by Heterobifunctional Ligands”

David Remillard, Dennis Buckley, Joshiawa Paulk, Gerard L. Brien, Matthew Sonnett, Hyuk-Soo Seo, Shiva Dastjerdi, Martin Wuhr, Sirano Dhe-Paganon, Scott A. Armstrong and James E. Bradner.
Angewandte Chemie, **2017,** *56,* 5738–5743.
doi: 10.1002/anie.201611281

SWI/SNF is a multiprotein chromatin remodeling complex that is dysregulated in many cancers. In this report, Remillard et al. describe a recent effort to trigger the proteasome-dependent degradation of BRD9, a member of SWI/SNF which has been identified as critical dependency in acute myeloid leukemia (AML). Using a diverse panel BRD9 inhibitors from both academic and industry groups, the authors first convert these molecules into bifunctional “degraders” by tethering them to E3 ligase ligands, and then use systematic structural, biophysical, and cellular studies to define the key determinants for BRD9 degradation. This leads to the discovery of a new chemical probe, dBRD9, which can be used to interrogate the role of BRD9-containing SWI/SNF complexes in rapid biological processes such as transcription and nucleosome positioning. More broadly, due to its comprehensive nature, this study offers several general insights that will be useful for researchers interested in addressing therapeutic targets using proteasome-dependent degradation strategies.

“BET Bromodomain Proteins Function as Master Transcription Elongation Factors Independent of CDK9 Recruitent”

Georg E. Winter, Andreas Mayer, Dennis L. Buckley, Michael A. Erb, Justine E. Roderick, Sarah Vittori, Jaime M. Reyes, Julia di Iulio, Amanda Souza, Christopher J. Ott, Justin M. Roberts, Rhamy Zeid, Thomas G. Scott, Joshiawa Paulk, Kate Lachance, Calla M. Olson, Shiva Dastjerdi, Sophie Bauer, Charles Y. Lin, Nathaniel S. Gray, Michelle A. Kelliher, L. Stirling Churchman and James E. Bradner
Mol. Cell **2017** *67*
doi.org/10.1016/j.molcel.2017.06.004

This paper describes an in-depth mechanistic investigation into differences in the biological effects between small molecule inhibitors of BET-family bromodomains and protein degraders of the BET-family proteins in T-ALL cells. The tool molecules JQ-1, a well characterized small molecule binder of BET family bromodomains and dBET6, a degrader based on JQ-1 linked to pomalidomide which engages cereblon to degrade BET-family proteins, were used in the study. While JQ-1 displaces BRD4 from superenhancers to downregulate a specific group of genes, dBET6 induces a general disruption of transcriptional elongation through ablation of BRD4, which is a key component of a multiprotein complex regulating elongation. This results in a more robust cytotoxic effect *in vitro*. Furthermore, PROTAC dBET6 was more effective than JQ-1 in a mouse model of T-ALL. These findings emphasize the potential of protein degradation to access differential effects from the traditional small molecule antagonist or enzyme inhibitor approaches.

“Quantitative mass spectrometry analysis of PD-L1 protein expression, N-glycosylation and expression stoichiometry with PD-1 and PD-L2 in human melanoma.”

Carlos A. Morales-Betanzos, Hyoungjoo Lee, Paula I. Gonzalez Ericsson, Justin M. Balko, Douglas B. Johnson, Lisa J. Zimmerman and Daniel C. Lieber
Mol. Cell. Proteomics. **2017** in press.
doi: 10.1074/mcp.RA117.000037.

Immunotherapy has emerged as a promising and effective strategy for cancer treatment. However, there remains a relatively poor understanding of which patients will benefit from therapies that target immune checkpoints and better diagnostics are needed to identify patients that will respond to therapy. Morales-Betanzos *et al.* recently published a study describing the development of a targeted mass spectrometry assay to quantify established immunotherapy protein targets: programmed cell death-1 (PD-1), PD-1 ligand (PD-L1), and PD-L2. These assays were demonstrated in formalin fixed, paraffin-embedded (FFPE) tissue sections from 22 human melanomas. PD-L1 measurements were found to be concordant with IHC data. PD-1 measurements were not concordant with measured levels of PD-1, but showed weak correlation with levels of lymphocytes and histiocytes. PD-L2 measurements suggested that PD-L2 is a higher affinity ligand for PD-1

compared to PD-L1 and could contribute to downregulation of T-cells. Interestingly, five glycoforms of PD-L1 at N192 were measured in all samples, and the extent of the modification varied by 10-fold. Correlations between IHC and LC-MS measurements of PD-L1 suggested that N-glycosylation of PD-L1 may have an effect on IHC measurements and PD-L1 function. Additional targeted LC-MS assays were performed on a variety of other immune checkpoint and co-regulator proteins and no correlation was observed with PD-1, PD-L1, and PD-L2. These studies suggest that targeted LC-MS assay may provide valuable information in cancer immunotherapy.

“Bioorthogonal Labeling of Human Prostate Cancer Tissue Slice Cultures for Glycoproteomics”

David R. Spiciarich, Rosalie Nolley, Sophia L. Maund, Sean C. Purcell, Jason Herschel, Anthony T. Iavarone, Donna M. Peehl and Carolyn R. Bertozzi
Angew Chem Int. Ed. Engl. **2017,** *56* (31), 8992-8997.
doi: 10.1002/anie.201701424.

Increased levels of sialic acid on the cell surface is a well-established feature of cancer and has been associated with a variety of processes such as tumorigenesis, immune evasion, and metastasis. Despite this well-known hallmark of cancer, there are relatively few systematic studies to understand which proteins have elevated levels of sialylation on the cell surface of cancer cells in human tissue. In recent years, various metabolic labeling techniques have been developed to allow the enrichment of sialylated proteins in cultured cells. Spiciarich et al. have reported the adaptation of a metabolic labeling approach to human tissues in ex vivo culture conditions. Slices of prostate cancer and normal tissues were cultured with an azido sugar (N-azidoacetylmannosamine-tetraacylated, Ac4 ManNAz) labeled mtetra-acetylated which was incorporated as azidosialic acid into cell surface and secreted sialic acid-containing glycoproteins. Subsequent biotinylation, enrichment, and mass spectrometry analysis resulted in the identification of sialylated glycoproteins that were elevated in or unique to prostate cancer tissue samples. This work demonstrates the utility of a metabolic biorthogonal labeling strategy to systematically study sialylated proteins in prostate cancer. Such studies have the potential to identify targets for cancer diagnosis and treatment.

“Quantitative proteomics identify Tenascin-C as a promoter of lung cancer progression and contributor to a signature prognostic of patient survival”

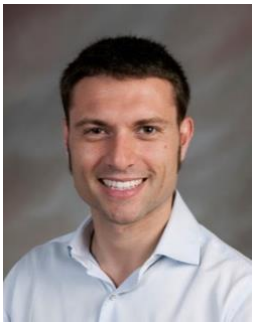
Vasilena Gocheva, Alexandra Naba, Arjun Bhutkar, Talia Guardia, Kathryn M. Miller, Carman Man-Chung Li, Talya L. Dayton, Francisco J. Sanchez-Rivera, Caroline Kim-Kiselak, Noor Jailhani, Monte M. Winslow, Amanda Del Rosario, Richard O. Hynes and Tyler Jacks
Proc Natl Acad Sci U S A. **2017,** *114* (28) E5625-E5644..
doi: 10.1073/pnas.1707054114.

The extracellular matrix (ECM) is comprised of a variety of cells and molecules that provide structural support and a local environment to normal and malignant tissue. Despite its importance, the extracellular matrix remains relatively poorly understood. In a recent report, Gocheva et al. investigated the contribution of the extracellular microenvironment to lung fibrosis and cancer progression. Excessive stromal expansion is observed in both pathologies and mouse models were utilized to characterize the ECM in normal, fibrotic, and malignant lung tumors, as well as metastases. Quantitative proteomics was used to measure the levels of ECM proteins and determine signatures of the different tissue types. Increased levels of S100 proteins were observed in lung tumors and metastasis versus normal tissues. The authors further demonstrated that the expression of the ECM protein tenascin C (Tnc) was repressed by Nkx2-1 (suppressor of metastatic progression). Further studies were performed with Tnc and CRISPR-mediated activation of the Tnc gene resulted in enhanced metastatic dissemination of lung cancer cells. Gene expression studies also showed that high levels of TNC expression were correlated with worse lung cancer prognosis. A signature of three ECM proteins was also reported to be a predictor of various important clinical features. These finding suggest that the ECM plays an important role in lung cancer and novel therapeutic and diagnostic targets may be gleaned by further studies of the ECM.

3. Profile of an Early-career Researcher:

Profile of an Early-Career Researcher: Prof. Alessio Ciulli

Alessio Ciulli, PhD
Professor of Chemical and Structural Biology
School of Life Sciences, University of Dundee
Research Group Site



Alessio Ciulli is a Professor at the University of Dundee, whose research focuses on the understanding of protein-protein interactions, with particular emphasis on E3 ubiquitin ligases and chromatin reader domains. During his research career he has made significant contributions to our understanding and small-molecule targeting of the ubiquitin proteosome system (UPS). His research group is involved with fragment-based discovery approaches, medicinal chemistry optimization for probe compound development and proteolysis targeting chimera

(PROTAC) technologies. The group's discoveries are rooted in creating greater understanding of fundamental biological systems and proteins with which their chemical ligands interact. New structural discoveries are advanced through medicinal chemistry, biophysical, computational, X-ray and NMR techniques and cellular studies. In particular, the lab's work on the von Hippel-Lindau E3 ligase complex ($CRL2^{VHL}$) has resulted in improved ligands for targeting VHL, application in PROTAC technologies and an understanding of the structural requirements for cooperative recognition and selective degradation of target proteins.

Prof. Ciulli graduated in Chemistry from the University of Florence in 2002. He was a Gates Cambridge Scholar during his PhD studies in Cambridge UK, under the supervision of Professor Chris Abell and in collaboration with Dr Glyn Williams' biophysics team at Astex Pharmaceuticals. After completing his PhD in 2006, Prof. Ciulli was awarded a College Research Fellowship to conduct research on biophysical fragment screening and fragment based drug discovery. The research was conducted within the framework of two international consortia funded by the Bill & Melinda Gates Foundation and the European Union FP6, jointly directed by Professor Abell and Professor Sir Tom Blundell. During this time Prof. Ciulli was awarded a Human Frontier Science Program (HFSP) Fellowship which enabled him to visit Yale University to start a collaboration with Professor Craig Crews laboratory on the design of small molecule ligands targeting VHL. Prof Ciulli returned to Cambridge UK to start his independent career in 2009 as a Fellow in the Department of Chemistry before moving to Dundee in 2013. He was promoted to Professor in 2016.

This issue of the Newsletter features a number of recent papers from the Ciulli group, including a fragment screen against the E2 conjugating enzyme Ube2T, the development of a potent and selective VHL inhibitor as chemical probe for the VHL E3 ligase, and a study of structural factors surrounding the efficacy of a BET targeting inducer of targeted protein degradation (PROTAC). Our last Newsletter (May 2017) included a recent article from the Ciulli group describing the structural basis for PROTAC cooperative binding. This article has been well received and widely covered by the scientific press.

The output and quality of research from the Ciulli group has greatly impacted the field and is increasingly recognized by the scientific community. As a result, Prof. Ciulli has been recently awarded the 2015 EFMC Prize for Young Medicinal Chemist in Academia, the 2015 ICBS Young Chemical Biologist Award, the 2016 RSC Capps Green Zomaya Award and is a Fellow of the Royal Society of Chemistry (FRSC, 2016). We are excited to highlight the excellent work of Professor Alessio Ciulli and his research group.

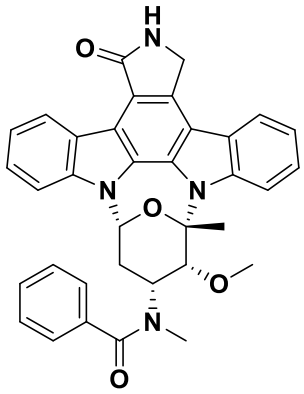
4. Spotlight on World News

Dr. Norman Sharpless named as director of the National Cancer Institute

In June Dr. Norman Sharpless, Director of the University of North Carolina at Chapel Hill's Lineberger Comprehensive Cancer Center was selected to become the Director of the National Cancer Institute. In addition to his previous role as Director of an NCI-designated Comrehensive Cancer Center, Dr. Sharpless is known for his research in the area of the Cell Cycle and being a co-founder of G1 Therapeutics, a clinical-stage biopharmaceutical company.
Source: National Institutes of Health (https://www.nih.gov/about-nih/who-we-are/nih-director/statements/selection-dr-norman-ned-sharpless-director-national-cancer-institute)

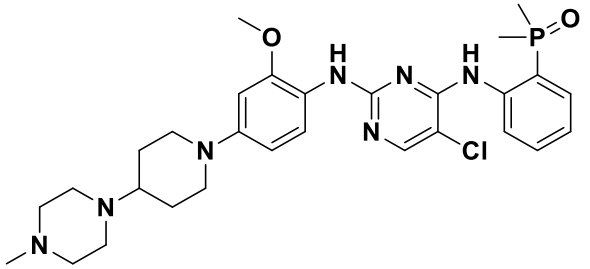
FDA Approval of Durvalumab (Imfinzi)
FDA-approval of PD-L1 immune checkpoint inhibitor durvalumab (AstraZeneca) occurred on May 1, 2017, just over a month after the previously approved inhibitor. The approval, for locally advanced or metastatic urothelial carcinoma, marks the fifth FDA-approval of a PD/PD-L1 antibody after pembrolizumab (Keytruda - Merck, 2014), nivolumab (Opdivo - Bristol-Myers Squibb, 2014), atezolizumab (Tecentriq - Roche Genentech, 2016) and avelumab (Bavencio - EMD Serono, 2017). Approval was based on a phase II trial which included an assay for the assessment of the PD-L1 protein in urothelial carcinoma tissue. Among patents whose tumors had high levels of the PD-L1 protein measured by this assay, the overall response rate was 26%, while the overall response rate was only 4% among patents with low levels. See related blogpost.

FDA Approval of Midostaurin (Rydapt)
The FLT3 inhibitor midostaurin (Novartis) was approved on April 28, 2017 for adults with newly diagnosed acute myeloid leukemia (AML) who are FLT3 mutation positive, in combination with chemotherapy. Midostaurin was awarded breakthrough designation for this indication and the approval occurred with a companion diagnostic to check patient FLT3 status. Midostaurin is a multikinase inhibitor and also has some activity against FLT3 and KIT. The inhibitor represents the first targeted therapy to treat patients with AML where about a third are FLT3 mutation positive, a form of the disease associated with faster disease progression, higher relapse and lower survival. See related blogpost.



FDA Approval of Brigatinib (Alunbrig)

The ALK inhibitor brigatinib (ARIAD Pharma) was approved on April 28, 2017 to treat patients with ALK+ metastatic non-small cell lung cancer (NSCLC) who have progressed or are intolerant to crizotinib (Xalkori). This is the fourth ALK inhibitor approval, following crizotinib (Pfizer, 2011), ceritinib (Novartis, 2014) and alectinib (Chugai/Roche, 2015). Approval was based on objective response rate data from the ALTA trial. Clinical work continues in the space with several trials ongoing using next generation ALK inhibitors. In addition to higher potency and activity against gatekeeper-mutant ALK, brigatinib is brain penetrant allowing activity against ALK+ brain metastasis in NSCLC. The compound also contains an unusual structural feature in its phosphine oxide functional group, which behaves as a hydrogen-bond acceptor driving the potency and selectivity of the inhibitor. See related blogpost and article.



5. News from the CICR Steering Committee, contributed by Dr. Melissa Vasbinder, Chairperson

The CICR Steering Committee cordially invites you to join us during two upcoming meetings this Fall where representatives from our CICR Steering Committee will be present to host a Town Hall and Networking Session. These events provide an opportunity to hear more about our ongoing CICR activities and connect with other researchers attending the meeting. The Town Hall in Shanghai represents our first gathering during the New Horizons in Cancer Research Conference, expanding our CICR footprint into the Asia Pacific community of cancer researchers. Refreshments will be served in Philadelphia and luncheon in Shanghai. We look forward to seeing you there!

AACR-NCI-EORTC International Conference on Molecular Targets and Cancer Therapeutics: Discovery, Biology, and Clinical Applications
Friday, October 27, 2017
12:30-1:30 p.m.
Pennsylvania Convention Center
Philadelphia, PA
Room 121C
Refreshments will be served

New Horizons in Cancer Research Conference
Thursday, November 9, 2017

1:15-3:00 p.m.
Shanghai Marriott Parkview Hotel
(room to be announced)
Shanghai, China
Lunch will be served (requires advance reservation, provided with Conference registration confirmation)

6. Career Forum

Career Forum Q&A with Ian Collins, PhD
Professor of Medicinal Chemistry
Cancer Research UK Cancer Therapeutics Unit at The Institute of Cancer Research
Division of Cancer Therapeutics
15 Cotswold Road
Sutton
London
SM2 5NG
ian.collins@icr.ac.uk
www.icr.ac.uk

1) What is your current professional position?

Professor of Medicinal Chemistry and team leader in the Cancer Research UK Cancer Therapeutics Unit at The Institute of Cancer Research, London, UK.

2) What is your area of research?

My work at The Institute of Cancer Research aims to discover and develop new small molecule drugs to treat cancer. My team applies medicinal and synthetic chemistry skills to design and synthesize potential drug molecules, and to

interpret their pharmacology. As well as the optimization of compounds to give clinical candidates, we are involved in discovering potent and selective chemical probes for new target proteins. We collaborate extensively with our colleagues in The Institute of Cancer Research and other organizations to use the compounds we make as tools to understand cancer biology. Our current research covers many different proteins involved in cancer biology, including kinases, kinesins, chaperones, helicases and ubiquitin ligases.

3) What influenced you to follow this career path?

I have been fascinated by how to discover new compounds with useful biological activities since I started my education as a synthetic chemist, and a career in drug discovery was an early goal. Figuring out how to manipulate the structure of small molecules to satisfy the complex physicochemical and biological requirements of a safe, effective medicine is perpetually challenging, but deeply rewarding when progress is made. I started my medicinal chemistry research career in the pharmaceutical industry in the area of neurological disease. After several productive and enjoyable years, I wanted to broaden my experience to encompass different research themes and environments. Having close family members living with cancer certainly influenced me at that time too. Moving to The Institute of Cancer Research was a great opportunity to contribute to drug discovery research and patient benefit in a fast moving and important field.

4) How long have you been involved with *MCT*? How did you become involved?

I've been a Senior Editor at *MCT* since January 2017. Having been approached by Editor-in-Chief Napoleone Ferrara and the team during 2016, as they sought new editorial colleagues to support the journal's activities.

5) What is your area of focus at the journal?

As one of several Senior Editors for *MCT*, I handle manuscripts that include research within my expertise, particularly the discovery and pharmacological characterization of small molecule tools and drugs. I also support the editorial team with chemical structure reviews of manuscripts. *MCT* policy on the publication of chemical structures and synthesis procedures aims to provide clarity for readers on the structures and properties of molecules used in studies and how to reliably reproduce these. Of course, authors also benefit if full details of their research are easily accessible to colleagues, to encourage further use and study.

6) What do you enjoy about working on MCT?

Contributing to the chemical structure reviews for *MCT* puts me in a privileged position of being exposed to the wide range of research and approaches published in the journal. As a Senior Editor, reading the perceptive comments and feedback from peer reviewers and authors is certainly helping me refine my own critical skills. The support from editorial colleagues at *MCT* is excellent, which makes handling the manuscripts much easier.

7) Why do you feel *MCT* is the right journal for medicinal chemists to publish their work?

I feel that medicinal chemists achieve the most effective therapeutic discoveries working together with scientists from other disciplines. *MCT* is an excellent place for medicinal chemists and colleagues to describe the discovery and study of new molecules in the context of the biology they elicit and the disease they affect. The discovery of new, high-quality chemical probes, and their application in thoughtful biological studies to interrogate new cancer targets, is essential for continued progress in discovering new cancer therapies. By sharing the discovery of exciting new molecules with the multidisciplinary readership of *MCT*, medicinal chemists can promote rapid adoption of new tools by the cancer research community.

Resources to assist you in your job search are provided below:

https://cancercareers.org/Pages/default.aspx

http://www.nature.com/naturejobs/science/jobs

http://jobs.rsc.org/

http://chemistryjobs.acs.org/

7. Conferences

2017 Chinese Academic Conference on Medicinal Chemistry
August 27-30, 2017. Beijing, China
Web Site

26th International Society of Heterocyclic Chemistry Congress
September 3-8, 2017, Regensburg, Germany
http://www-oc.chemie.uni-regensburg.de/ISHC2017/

AACR-NCI-EORTC Molecular Targets and Cancer Therapeutics
October 26-30, 2017, Philadelphia, Pennsylvania
Web Site

New Horizons in Cancer Research: Research Propelling Cancer Prevention and Cures
November 6-9, 2017, Shanghai, China
http://nhicr.aacr.asia/

American Association of Pharmaceutical Scientists Annual Meeting and Exposition
November 12-15, 2017, San Diego, CA
https://www.aaps.org/annualmeeting/

Atlantic Basin Conference on Chemistry
January 23-26, 2018, Cancun, Mexico
http://abcchem.org/

First Alpine Winter Conference on Medicinal and Synthetic Chemistry
January 28-February 1, 2018, St. Anton am Alberg, Austria
http://www.rsc.org/events/detail/27198/1st%20Alpine%20Winter%20Conference%20on%20Medicinal%20and%20Synthetic%20Chemistry

255th ACS National Meeting & Exposition
March 18-22, 2018. New Orleans, Louisiana

CHI 13th Annual Fragment Based Drug Discovery
April 2-6, 2018, San Diego, CA

AACR Annual Meeting 2018
April 14-18, 2018. Chicago, IL.
Web Site

ACS 36th National Medicinal Chemistry Symposium
April 29-May 2, 2018, Nashville, TN
http://www.nmcs.info/

FLDB (Fragment Based Lead Discovery) 2018
October 7-10, 2018, San Diego, CA
http://www.ysbl.york.ac.uk/fbld/2018/