Institution: University of Dundee		
Unit of Assessment: UoA5 Biological Sciences		
Title of case study: PROTACs: a revolutionary technology underpinning spin-out Amphista Therapeutics, and attracting inward investment, industry partnerships, and reagent sales		
Period when the underpinning research was undertaken: 2013-present		
Details of staff conducting the underpinning research from the submitting unit:		
Name(s): Prof. Alessio Ciulli	**Role(s) (e.g. job title):** Reader/Professor of Chemical and Structural Biology	**Period(s) employed by submitting HEI:** 2013-present
Period when the claimed impact occurred: 2013-present		
Is this case study continued from a case study submitted in 2014? N		

1. Summary of the impact

Professor Alessio Ciulli FRSC is one of the pioneers of the development of a new class of drugs, the proteolysis-targeting chimeras (PROTACs), that target disease-causing proteins for degradation. Amphista Therapeutics, a University of Dundee 2017 spin-out, is based on his technology. PROTACs have changed the direction of commercial drug discovery programmes. Most global pharmaceutical companies today work on this revolutionary approach, prompting over USD3.5 billion investment in the targeted protein degradation sector. Ciulli's structure-based design accelerates PROTAC deployment against previously undruggable disease proteins, as validated in a multi-million partnership with Boehringer Ingelheim. Benchmark PROTACs developed by Ciulli are marketed by several companies or freely-provided by Boehringer to speed innovation.

2. Underpinning research

Research led by Ciulli focussing on targeted protein degradation has driven the development of proteolysis-targeting chimeras (PROTACs). PROTACs are designed to harness the cell's natural disposal system to specifically remove disease-causing proteins. A PROTAC is a two-headed molecule, where one end binds an enzyme (an E3 ligase) and the other binds the disease protein, bringing the two into close proximity. The ligase can then label the disease protein for degradation by the cell's disposal system. Whereas conventional drugs only temporarily inhibit disease proteins by binding to their most important functional parts, PROTACs can bind at many positions and ensure the protein's destruction. This revolutionary mode of action allows PROTACs to attack targets previously thought 'undruggable' and constitutes a platform technology applicable across diverse diseases.

The broad idea behind PROTACs was first suggested in 2001 by Crews (Yale) and Deshaies (California Institute of Technology) but early molecules did not attract drug companies as they relied on bulky peptides to bind the ligase, had low potency and could not easily enter cells. Collaboration between Ciulli (then at University of Cambridge) and Crews between 2010 and 2012 produced three publications identifying non-peptide molecules that bind the E3 ligase enzyme VHL (von Hippel-Lindau protein), albeit with only moderate potency, a limitation also in contemporary work with other ligases. In April 2013, **Ciulli** moved to the University of Dundee and in 2014 he published the structure-guided design and optimization of dramatically improved drug-like small molecule ligands that bind VHL with high potency **[R1].** These were subsequently refined into a ligand with high VHL-specificity and cellular activity **[R2].**

In 2015, Ciulli developed one of the first ever non-peptidic two-headed PROTAC approaches by connecting his VHL ligands to a molecule that targets and binds to the BET (Bromodomain and Extra-Terminal motif) proteins that are critical for the growth and survival of cancer cells. This breakthrough cell-penetrant PROTAC, called MZ1, showed potent and unexpectedly selective degradation of one BET protein (called BRD4) over other BETs in cancer cells, offering unprecedented advantages over non-selective conventional inhibitors **[R3]**. As the BETs play crucial roles in pathways relevant to health and disease, the potential to accelerate development of drugs with minimal side effects and toxicity was immediately recognised.

Ciulli suggested that the specificity of a PROTAC might be influenced by the structure and interactions within each 3-part complex of ligase-PROTAC-target and, in 2017, he solved the first crystal structure of such a complex **[R4]**. This major breakthrough gave the new field the first ever glimpse of how a PROTAC brings the E3 ligase and its target protein together. Using this knowledge and the crystal structure to guide design, the **Ciulli** group produced a new PROTAC that had improved selectivity at depleting BRD4 **[R4]**.

E3 ligases can themselves be disease-related proteins. **Ciulli** recently reported the first 'Homo-PROTAC' that can dimerize E3 ligase VHL, inducing self-destruction - a highly innovative strategy of 'degrading the degrader' **[R5]**. He has also designed novel small molecule VHL ligands **[R6]**.

3. References to the research

[R1] Galdeano, C, Gadd, MS, Soares, P**,** Scaffidi, S, Van Molle, I, Birced, I, Hewitt, S, Dias, DM & **Ciulli, A (**2014) 'Structure-guided design and optimization of small molecules targeting the protein-protein interaction between the von Hippel-Lindau (VHL) E3 ubiquitin ligase and the hypoxia inducible factor (HIF) alpha subunit with in vitro nanomolar affinities' *Journal of Medicinal Chemistry*, vol. 57, no. 20, pp. 8657-8663. DOI: 10.1021/jm5011258

[R2] Frost, J, **Galdeano, C,** Soares, P, **Gadd, MS**, Grzes, KM, **Ellis, L, Epemolu, O,** Shimamura, S, Bantscheff, M, Grandi, P, **Read, KD**, **Cantrell, DA**, **Rocha, S** & **Ciulli, A (**2016) 'Potent and selective chemical probe of hypoxic signaling downstream of HIF-α hydroxylation via VHL inhibition', *Nature Communications*, vol. 7, 13312, pp. 1-12. DOI: 10.1038/ncomms13312

[R3] Zengerle, M, Chan, K-H & **Ciulli, A (**2015) 'Selective small molecule induced degradation of the BET bromodomain protein BRD4', *ACS Chemical Biology*, vol. 10, no. 8, pp. 1770-1777. DOI: 10.1021/acschembio.5b00216

[R4] Gadd, M, Testa, A, **Lucas, X, Chan, KH, Chen, W, Lamont, D, Zengerle, M** & **Ciulli, A** (2017) 'Structural basis of PROTAC cooperative recognition for selective protein degradation', *Nature Chemical Biology*, vol. 13, no. 5, pp. 514-521. DOI: 10.1038/nchembio.2329

[R5] Maniaci, C, **Hughes, SJ, Testa, A, Chen, W, Lamont, DJ, Rocha, S, Alessi, DR**, Romeo, R & **Ciulli, A (**2017) 'Homo-PROTACs: bivalent small-molecule dimerizers of the VHL E3 ubiquitin ligase to induce self-degradation', *Nature Communications*, vol. 8, 830, pp. 1-14. DOI: 10.1038/s41467-017-00954-1

[R6] Testa, A, Lucas, X, Castro, G**, Chan, KH, Wright, J,** Runcie, A**, Gadd, M**, Harrison, WTA, **Ko, EJ, Fletcher, D** & **Ciulli, A (**2018) '3-Fluoro-4-hydroxyprolines: Synthesis, conformational analysis and stereoselective recognition by the VHL E3 ubiquitin ligase for targeted protein degradation', *Journal of the American Chemical Society*, vol. 140, no. 29, pp. 9299-9313. DOI: 10.1021/jacs.8b05807

Key research grants relevant to this case study:

1. **Ciulli, A.** A Systems Approach for the Fragment-Based Development of Selective Chemical Probes of Bromodomain Function', BBSRC (2013-2015). Award Value: GBP333,693

2. **Ciulli, A.** Dissecting and Exploiting Molecular Recognition at Protein-Protein Interfaces'. BBSRC (2013-2015). Award Value: GBP307,126
3. **Ciulli, A.** Probing Druggability of Multisubunit Complexes: E3 Cullin RING Ligases' European Research Council (2013 –2018). Award value: EUR1,499,904

4. Details of the impact

Reshaping drug discovery programmes and investment within Pharma
Ciulli's research helped transform a niche area of chemical biology into one of the most exciting new fields of drug discovery. An expert opinion piece in Nature **[E1]** cites his 2015 publication **[R3]** and work of two other groups as achieving the spectacular leap in performance that enabled PROTAC technology, which had not gained traction in a decade, to become the focus of a "*gold rush*". By 2019, PROTACs were "*driving billions of US dollars in investment from pharmaceutical companies such as Roche, Pfizer, Merck, Novartis and GlaxoSmithKline*" **[E1]**. Intellectual property underpins investments; **Ciulli** has filed 3 patents since 2015 and 34 other unique patent families from 15 companies cite his research (including Arvinas, C4 Therapeutics, AstaZeneca, GlaxoSmithKline and Boehringer Ingelheim) **[E2]**. A 2020 global market report by Roots Analysis indicates over USD3.5billion investment in targeted protein degrader drugs since 2014, with PROTACs accounting for over 30% of pipeline drugs in this class.

Enabling development and successful clinical trial of PROTAC drugs for cancer patients
These research advances and investments directly resulted in a first wave of PROTAC drugs entering clinical trials. Arvinas Inc. has PROTACs in expedited Phase 1/2 clinical trials for highly refractory metastatic castration-resistant prostate cancer (>250,000 new patients a year in the USA alone) and metastatic breast cancer (half a million global deaths annually) respectively. In their December 2020 Clinical Program Update, Arvinas report continued patient benefit and clear signs of efficacy. The development of these drugs was significantly dependent on the research contribution of Ciulli, with **R1-R6** variously cited in 11 patent families filed by Arvinas **[E2]**.

Creation of spin-out company Amphista Therapeutics
The 2017 publication of the ligase-PROTAC-target crystal structure **[R4]** generated enormous investor interest. Amphista Therapeutics was consequently spun-out in December 2017 from the University of Dundee with co-founding venture capital investor Advent Life Sciences. In 2020, Amphista raised GBP6million Series A financing from co-investors Advent, the Scottish Investment Bank, the European Investment Fund and US-based BioMotiv **[E3]**. Amphista has created jobs for 15 employees (headcount: 15, FTEs:15) and is expected to grow to 25-30 in 2021. A General Partner of Advent Life Sciences explains:

> "*Advent invests in companies with first or best in class approaches…Amphista is an ideal example of the UK emulating some of the best US academic–industry partnerships. A world leading scientist who has pioneered an important new approach to the discovery of new medicines, working with an experienced Venture Firm to form a University spinout which then raises international finance to build an internationally recognised independent company operating within the UK. While there are some such companies in the UK, they are all too few compared to the US."* **[E3]**.

Partnerships with Boehringer Ingelheim and others
In July 2016 Boehringer Ingelheim (BI) announced a significant (non-disclosed) investment in a collaboration to develop new classes of therapeutics based on PROTAC technology **[E4]**. The partnership, extended in 2018, created 12 (headcount: 12; FTEs: 12) research jobs in Dundee and approximately 30 (headcount 30: FTEs 30) at BI as part of a novel joint research team **[E4, E5]**. With an initial focus on oncology, the team progresses PROTACs against human targets suggested by BI, such as the BAF complex that is mutated in 20% of human cancers **[E5]**. Of the partnership, a Senior Vice President at BI said:

> *"Dundee University share our vision to transform cancer medicines with PROTACs. We extended our multi-million-pound investment in our alliance with Prof Ciulli to progress new PROTAC therapeutics and bring previously deemed undruggable targets within reach. Alessio Ciulli's ground-breaking work in defining one of the first ever non-peptidic PROTAC degrader and mechanistic insights into how PROTACs work was instrumental in BI's decision to collaborate with the University of Dundee"* **[E4]**.

The research has continued to attract interest, leading to GBP18million of collective investment since 2016 into Dundee from pharmaceutical companies BI, Nurix, Ono Pharma, Eisai and Almirall, and a total commercialisation income (royalties plus milestones) of GBP1,020,858 **[E6]**.

Sale and open-access use of tools/reagents enabling PROTAC research and innovation
In 2018, Boehringer released Ciulli's MZ1 on their Open Innovation OpnMe portal, as the first PROTAC freely-available to researchers **[E7]**, with five Ciulli PROTACs now offered **[E4]**. Of 133 orders in 2019/2020, 45% came from private companies, with orders going to 25 countries **[E4]**. Two Dundee PROTACs are in the top 5 requested OPnMe molecules **[E4]**. Several biotech companies, including Tocris, ProbeChem, Medchemexpress and Cayman, market larger quantities of Ciulli's PROTACs under license from the University of Dundee **[E8]** with current royalty income to the university of GBP200,000 **[E6]**. Life Sciences company Promega has also significantly benefited, as explained by their Group Leader of Research & Development:

> "*The early sharing with us by Alessio Ciulli of key PROTACs discovered in his lab, including but not limited to MZ1, was truly enabling to Promega. He provided fundamental tools to develop novel technologies to study targeted protein degradation and benchmark our efforts in this space, which is today one of the fastest-growing commercial areas for Promega*" [E9].

5. Sources to corroborate the impact

[E1] Expert opinion piece citing Ciulli's research [R3]: Scudellari, M. (2019) 'Protein-slaying drugs could be the next blockbuster therapies'. *Nature* vol. 567, pp. 298-300. DOI: 10.1038/d41586-019-00879-3

[E2] Ciulli patents since 2016 and list of pharmaceutical industry patents citing Ciulli underpinning research **R1-R6** and related patents.

[E3] Statement by General Partner of Advent Life Sciences Venture Capital Firm; plus article on Amphista Series A deal.

[E4] Testimonial by the Senior Vice President, Boehringer Ingelheim.

[E5] Evidence on the extension of the Boehringer Ingelheim partnership with **Ciulli**, and joint output evidencing the effectiveness of the partnership:

(i) Press release: 2019, 'Boehringer Ingelheim and University of Dundee Highlight Successful PROTAC Drug Discovery Program and Extend Their Ongoing Anti-Cancer Alliance' *Boehringer Ingelheim* 11th June 2019.

(ii) Farnaby, W., Koegl, M., Roy, M. J., Whitworth, C., Diers, E., Trainor, N., Zollman, D., Steurer, S., Karolyi-Oezguer, J., Riedmueller, C., Gmaschitz, T., Wachter, J., Dank, C., Galant, M., Sharps, B., Rumpel, K., Traxler, E., Gerstberger, T., Schnitzer, R., **Ciulli, A**. (2019) 'BAF complex vulnerabilities in cancer demonstrated via structure-based PROTAC design. *Nature Chemical Biology*, vo.*15*, pp672-680. DOI: 10.1038/s41589-019-0294-6

[E6] Statement by Research Innovation Services at the University of Dundee verifying total investment, commercialisation income, and reagents sales income.

[E7] Article on the release of PROTAC MZ1 by Boehringer Ingleheim on their Open Innovation OpnMe portal, plus video about MZ1: 2019 'University of Dundee and Boehringer Ingelheim collaborate for free access of PROTAC compound on opnMe.com' *DiscoveryToday.com* 9th Jan 2019; Video from Boehringer Ingelheim: 2019, 'The Journey of MZ1, a BRD4 PROTAC, Dundee, Scotland' *Boehringer Ingelheim* Jan 2019. Available at https://opnme.com/molecules/bet-mz-1

[E8] Web sales pages for University of Dundee PROTAC compounds sold by biotech companies:

(i) Tocris Bioscience: MZ1 Cat.6154; AT1 Cat. 6356; CM11 Cat. 6416
(ii) Probechem: AT1 Cat. PC-35351
(iii) Medchemexpress: MZ1 Cat.HY-107425; AT1 Cat. HY-111433
(iv) Caymanchem: MZ1 Cat. 21622
(v) Abcam: MZ1 Cat.ab230371
(vi) Lifesensors: MZ1 Cat. PC1001

[E9] Evidence of impact of Ciulli's collaboration with Promega:

(i) Statement by R&D Group Leader, Promega Corporation
(ii) Promega's PROTAC drug discovery application webpage illustrating use of Ciulli's MZ1 PROTAC: 'Targeted Protein Degradation' *Promega.* Available at https://bit.ly/2ZahIG4 (Accessed 16th Dec 2020)
(iii) Publication by Promega corroborating use of MZ1 in Promega technology development which cites R2-4: Riching, K., Mahan, S., Corona, C., McDougall, M., Vasta, J., Robers, M., Urh, M. and Daniels, D., (2018) Quantitative Live-Cell Kinetic Degradation and Mechanistic Profiling of PROTAC Mode of Action. *ACS Chemical Biology*, Vol. 13 No.9, pp.2758-2770. DOI: 10.1021/acschembio.8b00692