Institution: University of Oxford		
Unit of Assessment: 8 – Chemistry		
Title of case study: Oxygenases – from Chemistry to Medicine		
Period when the underpinning research was undertaken: 2001 - 2017		
Details of staff conducting the underpinning research from the submitting unit:		
Name(s):	**Role(s) (e.g. job title):**	**Period(s) employed by submitting HEI:**
Christopher J. Schofield	Professor of Chemistry	1985 – present
Akane Kawamura	Professor of Chemistry	17/8/2009 – present
Kirsty Hewitson	Postdoctoral researcher	1/4/2001 – 30/4/2008
Luke McNeill	Postdoctoral researcher	9/8/1999 – 14/3/2005
Imre Schlemminger	DAAD Postdoctoral fellow	2002 – 2004
Period when the claimed impact occurred: 1st Aug 2013 – 31st Dec 2020		
Is this case study continued from a case study submitted in 2014? Yes		

1. Summary of the impact (indicative maximum 100 words)

The University of Oxford's research on mechanisms, structures and inhibition of oxygenases has had major impact in medicine. Collaborative work between the Department of Chemistry and the Nuffield Department of Clinical Medicine has revealed how humans adapt to altered dioxygen availability, e.g. at altitude or in disease. Oxford's work has identified and validated unforeseen drug targets for diseases, including anaemia and cancer. These new targets are being pursued by large pharmaceutical companies (e.g. GSK, Bayer, Astellas, Merck) and smaller companies (e.g. Fibrogen, Akebia), with two compounds (Daprodustat and Vadadustat) approved for treatment of anaemia in kidney disease in Japan, and another (Roxadustat) in China and Japan. Oxford's research has also enabled the development of products for use in biomedicinal/medicinal chemistry research, including in the rapidly growing field of epigenetics. The work has also been important in collaborative efforts to repurpose an existing drug (Almitrine) for COVID-19 treatment (MHRA approved in 2020).

2. Underpinning research (indicative maximum 500 words)

Collaborative structural and mechanistic work by Professor Chris Schofield and researchers in Chemistry and Clinical Medicine at the University of Oxford, concerning oxygenases that catalyse reactions currently impossible for non-biological catalysts, has had major impact in medicine and biomedical research. Crystallographic studies on microbial oxygenases led to prediction of 60-70 human oxygenases, most with unassigned functions. Subsequent interdisciplinary work concerning functional assignments enabled identification of unprecedented cell signalling pathways and new medicinal chemistry targets [**1,2**]. The discovery in 2000 (with Professor Peter Ratcliffe, Clinical Medicine) that 2-oxoglutarate (2OG) dependent prolyl-hydroxylases play a sensing role in the response of animals to hypoxia (limiting dioxygen availability) enabled development of a new class of medicines. Subsequent to the discovery that levels of the hypoxia inducible transcription factors (HIFs) are regulated by O_2 (by Semenza), a key goal was to identify the mechanism(s) of O_2 dependent regulation of HIF. Prolyl hydroxylation was shown to decrease HIF-α levels by promoting binding to a targeting component of a ubiquitin E3 ligase (the von Hippel Lindau protein). Structural and mechanistic insights from Oxford led to proposed candidate sequences for the HIF prolyl hydroxylases, leading to the assignments of PHD1-3 as human hypoxia sensors, of which (PHD2) is highly conserved in animals [**1**-**4**].

A crucial translational aspect of the Oxford Chemistry work was the realisation that small-molecules that mimic binding of 2OG to the PHD active sites inhibit their hydroxylase activity and so elevate HIF target genes, including that of erythropoietin (EPO), a protein widely used for anaemia treatment [**2**,**3**]. The Oxford chemistry work on PHD inhibition demonstrated the therapeutic viability of this approach [**4**], which has subsequently been pursued by multiple large and small pharmaceutical companies.

In a second important line of investigation Schofield pursued another type of HIF-α hydroxylase, factor inhibiting HIF (FIH), the activity of which decreases HIF transcriptional activity (as shown by the Whitelaw laboratory). Structural studies in Oxford Chemistry revealed FIH as the first

identified JmjC protein to have oxygenase activity [**5**]; other JmjC members were subsequently shown to have roles in modifying chromatin (N^{ε}-methyl lysine histone demethylases) and other elements of machinery involved in protein biosynthesis. Along with other functional assignments and structural work on oxygenases, these results have led to the development of selective PHD inhibitors [**4**] and revealed other potential therapeutic targets (e.g. histone demethylases for cancer treatment) [**6**].

3. References to the research - all references are journal articles:

1. P Jaakkola, DR Mole, Y Tian, MI Wilson, J Gielbert, SJ Gaskell, A von Kriegsheim, HF Hebestreit, M Mukherji, CJ Schofield, PH Maxwell, CW Pugh and PJ Ratcliffe; Targeting of HIF-alpha to the von Hippel-Lindau ubiquitylation complex by O-2-regulated prolyl hydroxylation, Science, 2001, 292:468-72. DOI: 10.1126/science.1059796. *Identification of the role of prolyl hydroxylation in hypoxia sensing: Citations: >3,790 (Web of Science, 11/12/2020)*
2. ACR Epstein, JM Gleadle, LA McNeill, KS Hewitson, J O'Rourke, DR Mole, M Mukherji, E Metzen, MI Wilson, A Dhanda, Y Tian, N Masson, DL Hamilton, P Jaakkola, R Barstead, J Hodgkin, PH Maxwell, CW Pugh, CJ Schofield and PJ Ratcliffe; C. elegans EGL-9 and Mammalian Homologs Define a Family of Dioxygenases that Regulate HIF by Prolyl Hydroxylation, Cell, 2001, 107:43-54. DOI: 10.1016/S0092-8674(01)00507-4. *Identification of the HIF prolyl hydroxylase sequences; Citations: >2,300 (WoS, 11/12/2020)*
3. DR Mole, I Schlemminger, LA McNeill, KS Hewitson, CW Pugh, PJ Ratcliffe, and CJ Schofield; 2-Oxoglutarate analogue inhibitors of hif prolyl hydroxylase, Bioorganic & Medicinal Chemistry Letters, 2003, 13:2677-80. DOI: 10.1016/S0960-894X(03)00539-0. *Demonstration (with [1]) 2OG analogues inhibit the PHDs; Citations: 115 (WoS, 11/12/2020)*
4. Y Yeh, TM Leissing, MI Abboud, CC Thinnes, O Atasoylu, JP Holt-Martyn, D Zhang, A Tumber, K Lippl, CT Lohans, IKH Leung, H Morcrette, IJ Clifton, TDW Claridge, A Kawamura, E Flashman, X Lu, PJ Ratcliffe, R Chowdhury, CW Pugh and CJ Schofield; Molecular and cellular mechanisms of HIF prolyl hydroxylase inhibitors in clinical trials, Chemical Science, 2017, 8: 7651-68. DOI: 10.1039/C7SC02103H. *Characterizes mechanisms of action of PHD inhibitors in clinical trials; Citations: >55 (WoS, 11/12/20)*
5. KS Hewitson, LA McNeill, MV Riordan, Y Tian, AN Bullock, RW Welford, JM Elkins, NJ Oldham, S Bhattacharya, JM Gleadle, PJ Ratcliffe, CW Pugh and CJ Schofield; Hypoxia Inducible Factor (HIF) asparagine hydroxylase is identical to Factor Inhibiting HIF(FIH) and is related to the cupin structural family, Journal of Biological Chemistry, 2002, 277: 26351-5. DOI: 10.1074/jbc.C200273200. *Identification of a FIH, as the HIF Asn-hydroxylase, and consequently of the family of JmjC proteins/enzymes as oxygenases, opening up a new field in transcriptional regulation; Citations: >520 (WoS, 11/12/20)*
6. Kruidenier L, Chung C-W, Cheng Z, Liddle J, Che K, Joberty G, Bantscheff M, Bountra C, Bridges A, Diallo H, Eberhard D, Hutchinson S, Jones E, Katso R, Leveridge M, Mander PK, Mosley J, Ramirez-Molina C, Rowland P, Schofield CJ, Sheppard RJ, Smith JE, Swales C, Tanner R, Thomas P, Tumber A, Drewes G, Oppermann U, Patel DJ, Lee K, Wilson DM; A selective jumonji H3K27 demethylase inhibitor modulates the proinflammatory macrophage response. Nature, 2012, 488, 404-408. DOI: 10.1038/nature11262. *Links inflammation to JmjC demethylases via use of selective inhibitors*; *Citations: >540 (WoS, 11/12/20)*

Evidence of the quality of the research
Research [**1**] cited in the 2019 Nobel Prize for Physiology and Medicine

4. Details of the impact (indicative maximum 750 words)

Identification/validation of new drug targets for diseases ranging from anaemia to cancer that are being pursued by multiple companies
Enabling work in Oxford laboratories from 2000 onwards demonstrated that small-molecule mediated inhibition of the HIF prolyl-hydroxylases (PHDs) upregulates proteins of major medicinal relevance (including erythropoietin, EPO), validating the HIF hydroxylases as viable drug targets (see Section 2). PHD inhibitors (PHDi) manipulate the natural mechanisms of adaptation to hypoxia, by promoting bone-marrow to make more red blood cells. PHDi were thus proposed to benefit patients with anaemia, which is common in patients with severe chronic kidney disease (CKD), including those maintained by dialysis because the kidneys no longer produce sufficient

erythropoietin. The current treatment is injection with recombinant erythropoietin (Epoetin) and generates large spikes in EPO levels requiring intravenous iron that is associated with increased mortality. One aim of the Schofield research was to enable replacement of EPO protein with an oral small-molecule PHDi. This work led to the filing in 2002 of patent WO2002074981A2, *Assays, methods and means relating to hypoxia inducible factor (HIF) hydroxylases*.

Based on the pioneering Oxford PHDi work, pharmaceutical companies including Amgen, GSK, Merck, Bayer, Fibrogen/Astellas, Akebia and Johnson & Johnson pursued PHDi. **[Text removed for publication]**

At least 4 companies have progressed their PHDi to late-stage clinical trials for anaemia treatment (worldwide market >USD20,000,000,000 as of September 2020), including:

- **Roxadustat (FG-4592):** Following Phase I and II clinical trials demonstrating FG-4592 correcting and maintaining hemoglobin levels in multiple subpopulations of CKD anaemia patients, FibroGen, Astellas and AstraZeneca collaborated on Phase III clinical trials leading to approval of Roxadustat for oral treatment of anaemia in CKD in China (2018, licensed to FibroGen and AstraZeneca) and Japan (2019, licensed to FibroGen and Astellas). Roxadustat is under review by the US Food and Drug Administration (FDA) for approval in CKD anaemia patients. In the US and Europe it is in Phase III trials on over 180 patients with anaemia associated with myelodysplastic syndromes, a type of rare blood cancer, and is in US Phase II trials on 100 patients with chemotherapy-induced anemia [**A**, **H**].
- **Daprodustat (GSK1278863):** Phase III trials of GSK's oral agent Daprodustat on 598 patients in Japan showed that it met its primary endpoint of non-inferiority to Epoetin. In June 2020 GSK received regulatory approval in Japan for Daprodustat (Duvroq) for patients with renal anaemia due to CKD [**B, H**]. Daprodustat is in Phase III trials in Europe and the US, which should declare key results in early 2021.
- **Molidustat (BAY 85-3934):** Bayer developed Molidustat and commenced Phase III trials on more than 300 patients with renal anaemia in Japan in 2018 [**C**].
- **Vadadustat (AKB-6548):** Akebia progressed Vadadustat to Phase III clinical trials and in June 2020 received regulatory approval to market it in Japan (as VAFSEO™). In August 2020 Akebia, in collaboration with physicians at the University of Texas Health Science Center, were granted an Investigational New Drug Application by the US FDA and began Phase II clinical trials investigating Vadadustat's effectiveness in preventing or lessening severity of acute respiratory distress syndrome in 300 COVID-19 patients [**D**, **H, I**].
- **MK-8617:** Merck has reported extensive medical chemistry studies on PHDi leading to the identification of MK-8617 that has advanced to clinical trials for the treatment of anaemia [**I**, **J**].

The above pharmaceutical developments of PHDi are described in the patent literature and are extensively recognized in peer-reviewed academic literature [**E**]. The medical importance of the PHDi has been recognized by the scientific community, e.g. the scientific background to the 2019 Nobel Prize for Physiology and Medicine cites Schofield and Ratcliffe's collaborative work [**1**] and states '*The identification of these hydroxylases gave rise to the possibility of creating specific PHD inhibitors to increase HIF activity; e.g., to increase EPO levels in patients with anemia*' [**F**]. Although they have been optimized for medicinal application, it is notable that all of the aforementioned PHDi in clinical use and development work by the same mechanism as the pioneering Oxford Chemistry compounds (i.e. they inhibit the PHDs by competition with 2OG), and have the same outcome (i.e. upregulation of one or more HIF target genes, including that encoding for EPO – see Figure 1 below).

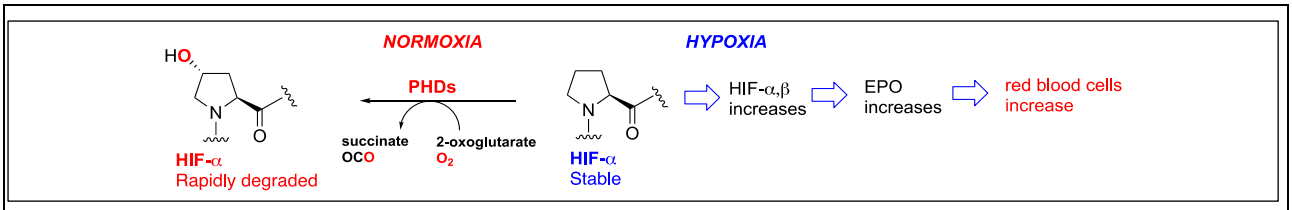

***Figure 1**. Role of the PHDs in regulating EPO levels and hence red blood cell levels. Inhibition of the PHDs hinders HIF-α degradation and hence raises EPO then red blood cell levels.*

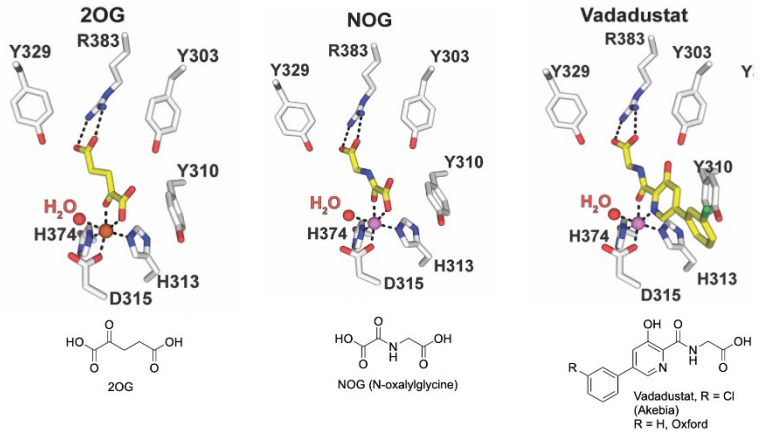

***Figure 2**. Comparison of binding modes of PHD cosubstrate (2OG), the pioneering Oxford PHDi N-oxalylglycine (NOG), and a clinically applied PHDi (Vadadustat) to the active site of human PHD2 (Note the similarity of the binding mode of Vadadustat to 2OG/NOG and of its structure to compounds prepared in Oxford).*

Oxford's functional assignment work on oxygenases beyond the PHDs is enabling better PHDi and revealing new targets

Oxford Chemistry's identification of one of the HIF hydroxylases (FIH) as a JmjC protein [**5**] stimulated work leading to the assignment of JmjC proteins as histone/chromatin demethylases acting on all 3 *N*-epsilon methylation/hydroxylation states of lysine. Because of the fundamental role of histone methylation in the regulation of gene expression, sometimes in an epigenetic manner, the JmjC histone demethylases have attracted considerable attention as potential targets for diseases ranging from cancer to genetic diseases (by several companies including GSK [**H**], Genentech and Epitherapeutics). Oxford Chemistry's research on functional assignment of human 2OG oxygenases is also enabling improved PHDi by providing assays leading to the development of highly selective PHDi – this is important given that PHDi will be will be a long-term treatment for anaemia and many human 2OG oxygenases have key roles in embryonic development and are critical for life in adults.

[Text removed for publication]

Commercial sale of products including small-molecules therapeutics, including PHDi arising directly from Oxford research

Oxford Chemistry's research and that of its collaborators has directly and indirectly resulted in multiple commercially available products for biomedical research. With respect to 2OG oxygenase/demethylase inhibitors, the Schofield group initiated a joint Wellcome Trust-funded project (2009-15) with the Structural Genomics Consortium, the NIH and (ultimately) more than 10 industrial partners and the National Institutes of Health, USA, to identify and distribute small molecule inhibitors of 'epigenetic' enzymes/proteins for use in probing biological function. Some of the oxygenase/demethylase inhibitors arising from work in the Oxford Chemistry laboratories (in most cases in collaboration) are commercially available, for example, Daminozide, IOX1, IOX2, JQ1, DMOG [**2,3**], GSK J1, GSK J4 (arising from collaboration with GSK [**6**]), and (*R*)-2-hydroxyglutaric acid, are sold by companies including Tocris Biosciences, Cayman Chemical Company, Selleck Chemicals, MilliporeSigma (Merck), BioVision Inc., Axon Medchem, and Enzo Life Sciences [**G**].

Repurposing Almitrine for treatment of COVID-19

Schofield's expertise on hypoxia/PHDi has helped repurpose an existing drug not previously licensed in the UK (Almitrine) to clinical trials (MHRA approval granted; in part funded by medical

research charity LifeArc) for treatment of severe COVID-19, symptoms of which are a reduction in oxygen circulating in blood (hypoxemia) that can lead to organ failure or death. During COVID-19 infection, patients can suffer from 'shunt flow', where circulating blood passes through the lungs without receiving sufficient oxygen from the alveoli. While treatment options beyond supplementary dioxygen exist, they can only be delivered in specialist centres and these may not have the capacity to treat the number of patients who could potentially benefit. Almitrine is a respiratory stimulant (previously used for treatment of chronic obstructive pulmonary lung disease) which, when used to treat patients with acute respiratory distress syndrome, reduces shunt flow; diverting blood to healthier regions of the lungs, allowing more oxygen to pass into blood. Guided by the Oxford University team (led by Schofield and Professor Peter Robbins (Department of Physiology, Anatomy and Genetics), who provided (bio)chemical/synthetic and physiology expertise, respectively), Almitrine capsules have been manufactured by two UK pharmaceutical companies, Concept Life Sciences and CustomPharma, and approved by the MHRA for use. Clinical trials for treatment of COVID-19 patients needing respiratory support will begin in early 2021 at three UK hospitals [**K**].

5. Sources to corroborate the impact (indicative maximum of 10 references)

A. Roxadustat**:** Press releases from FibroGen on success of clinical trials using HIF prolyl hydroxylases as targets and regulatory approval of Roxadustat in China (2018) & Japan (2019); details of clinical trials from US National Library of Medicine on patients with myelodysplastic syndromes & chemotherapy-induced anemia (2017-20)
B. Daprodustat**:** GSK press releases on positive Phase III trial results (2018) and being granted regulatory approval to market drug in Japan (2020)
C. Molidustat**:** Details of Bayer's Phase III clinical trials from US National Library of Medicine on patients with renal anaemia on dialysis (2018-19)
D. Vadadustat**:** Akebia press releases on commercial launch of Vadadustat in Japan (2020) and study on effect of drug on ARDS in patients with COVID-19 (2020); clinical trial details from US National Library of Medicine on the Phase II COVID-19 study (2020)
E. Published peer-reviewed results concerning clinical applications of PHDi (Roxadustat, Daprodustat, Molidustat and Vadadustat)
F. 2019 Nobel Prize for Physiology and Medicine: Scientific background by the Professor of Hypoxia Biology at the Karolinska Institutet citing Schofield & Ratcliffe's collaborative research [**1**] (page 13)
G. Catalogue entries of reagents arising from this oxygenase work by Tocris Biosciences, Cayman Chemical Company, Selleck Chemicals, MilliporeSigma (Merck), BioVision Inc, Axon Medchem and Enzo Life Sciences
H. Letter of support from a Senior Scientific Director of Medicinal Chemistry, GSK (Jan 2021) corroborating Schofield's pioneering research opening up the HIF-prolyl hydroxylases as new drug targets
I. Letter of support from Founder of Bohicket Pharma Consulting LLC (Jan 2021) corroborating Schofield's work on the design and synthesis of inhibitors as an approach to the treatment of anemia and cancer by several large pharmaceutical companies
J. Publication reporting on Merck's orally active PHD inhibitor which was advanced to the clinic: John S. Debenham et al, *Discovery of N-[Bis(4-methoxyphenyl)methyl]-4-hydroxy-2-(pyridazin-3-yl)pyrimidine-5-carboxamide (MK-8617), an Orally Active Pan-Inhibitor of Hypoxia-Inducible Factor Prolyl Hydroxylase 1–3 (HIF PHD1–3) for the Treatment of Anemia*, J. Med. Chem. 2016. DOI: 10.1021/acs.jmedchem.6b01242
K. Letter of support from a Consultant Respiratory Physician at University of Oxford NHS Foundation Trust (Feb 2021) on the Almitrine COVID-19 clinical trials and Prof Schofield's enabling hypoxia and oxygen sensing work