Institution: University of Glasgow (UofG)		
Unit of Assessment: UoA5 (Biological Sciences)		
Title of case study: Establishing Caldan Therapeutics Ltd: a spin-out company to exploit the first selective agonists of Free Fatty Acid Receptors		
Period when the underpinning research was undertaken: 2008–2015		
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:**
Prof. Graeme Milligan Dr Brian Hudson	Gardiner Professor of Biochemistry Research Associate; Research Fellow; Lecturer	1986–present 2010–2014; 2014–2018; 2018–present
Period when the claimed impact occurred: 2015–2020		
Is this case study continued from a case study submitted in 2014? No		

1. Summary of the impact

A UofG research collaboration with University of Southern Denmark (USD) led to the creation of the spin-out company Caldan Therapeutics in 2015. The company exploits UofG-USD discoveries that made Free Fatty Acid Receptors (FFAR) tractable as a therapeutic target for type-2 diabetes, inflammatory conditions and non-alcoholic steatohepatitis. Between 2015 and 2020, Caldan Therapeutics attracted GBP8.15 million in multiple rounds of funding; patent-protected its central chemistry programmes; and employed up to 17 staff in the company and three partner contract research organisations. Independently, the core therapeutic chemical series in the research has formed the basis for several pharmaceutical drug discovery programmes for new FFAR therapeutics.

2. Underpinning research

Prof. Milligan is a global opinion leader on the therapeutic potential of poorly characterised G protein-coupled receptors (GPCRs) and how to identify ligands that modify them, and has developed and licenced technologies to the biopharmaceutical sector to do so. In recent years it has become clear that metabolic products, including free fatty acids (FFAs), derived from ingested foodstuffs are not only sources of energy; they also act as homeostatic regulators of tissue function—thus act in a hormone-like manner. Activation of specific GPCRs lead to many such effects. In particular, GPCRs responsive to long chain FFAs are expressed by many cells and tissues that control the disposition and use of glucose and, therefore, have potential to positively influence metabolic disorders including type-2 diabetes and obesity. However, to date, clinical trials of synthetic drugs that target FFA receptors (FFARs), e.g. fasiglafam, have failed because the drugs have been too lipophilic, resulting in liver toxicity, and because the biological regulation of these GPCRs has been inadequately understood.

In initial studies (2008–2011) funded by BBSRC, Milligan employed combinations of mutagenesis and homology modelling to understand how fatty acids bind to Free Fatty Acid Receptor 1 (FFAR1) and developed insights into how to produce novel ligands with improved drug-like properties. Partnering with Professor Trond Ulven (USD) rapidly allowed synthesis of novel ligands, with UofG work revealing that these were effective in reducing elevated blood glucose levels without inducing hypoglycaemia—a danger with a number of anti-diabetic medicines that, unlike FFAR1 agonists, function whether blood glucose is elevated or not—and were able to do so effectively over at least 28 days of treatment [1,2].

In parallel Milligan and Ulven developed the first potent selective agonists of Free Fatty Acid Receptor 4 (FFAR4, known previously as GPR120 in the literature). Progress in drug development towards establishing FFAR4 as a drug target had been limited by a lack of effective and selective receptor modulators. In 2012, publication of the pioneering prototype ligand, TUG-

891 [3], galvanised the field, resulting in more than 250 papers that have since highlighted the therapeutic potential of this receptor. Biology studies led by Glasgow (2011-2017), funded by BBSRC, illustrated the opportunities, but also challenges, of developing medicines that target this receptor [4,5]. For example, a key challenge is that the FFA4 receptor rapidly becomes desensitised and effectiveness is lost when an agonist is added and stays around too long [4]. As such, the focus is to develop compounds that can be used once a day, but then are rapidly cleared so that response does not decline over time.

Despite a plethora of medicines being used in the treatment of type-2 diabetes this remains a global epidemic and new treatments that offer more than simple reduction in blood glucose levels are a key aspiration. It is widely accepted that new medicines to treat diabetes must show 'glucose PLUS'—delivering benefits on multiple aspects of type-2 diabetes pathophysiology—to provide effective treatment and competitor discrimination. UofG research provided evidence that targeting FFAR4, by providing additional anti-inflammatory effects, can do so [6].

3. References to the research

1. Christiansen E, Due-Hansen ME, Urban C, Grundmann M, Schmidt J, Hansen SV, **Hudson BD**, Zaibi M, Markussen SB, Hagesaether E, **Milligan G**, Cawthorne MA, Kostenis E, Kassack MU, Ulven T. (2013) Discovery of a potent and selective free fatty acid receptor 1 agonist with low lipophilicity and high oral bioavailability. *J Med Chem*. 56(3):982-92. (doi: 10.1021/jm301470a)
2. Christiansen E, Hansen SV, Urban C, **Hudson BD**, Wargent ET, Grundmann M, Jenkins L, Zaibi M, Stocker CJ, Ullrich S, Kostenis E, Kassack MU, **Milligan G**, Cawthorne MA, Ulven T. (2013) Discovery of TUG-770: A Highly Potent Free Fatty Acid Receptor 1 (FFA1/GPR40) Agonist for Treatment of Type 2 Diabetes. *ACS Med Chem Lett*. 4(5):441-445. (doi: 10.1021/ml4000673)
3. Shimpukade B, **Hudson BD**, Hovgaard CK, **Milligan G** and Ulven T. (2012) Discovery of a potent and selective GPR120 agonist. *J Med Chem*. 55: 4511-4515 (doi:10.1021/jm300215x).
4. **Hudson BD**, Shimpukade B, Mackenzie AE, Butcher AJ, Pediani, JD, Heathcote H, Tobin AB, Ulven T and **Milligan, G**. (2013) The pharmacology of TUG-891, a potent and selective agonist of the Free Fatty Acid Receptor 4 (FFA4/GPR120), demonstrates both potential opportunity and possible challenges to therapeutic agonism. *Mol Pharmacol*. 84, 710-725 (doi: 10.1124/mol.113.087783)
5. **Hudson BD**, Shimpukade B, **Milligan G** and Ulven T. (2014) The molecular basis of ligand interaction at Free Fatty Acid Receptor 4 (FFA4/GPR120). *J Biol Chem*. 289: 20345-20358. (doi:10.1074/jbc.M114.561449)
6. Christiansen E, Watterson KR, Stocker CJ, Sokol E, Jenkins L, Simon K, Grundmann M, Petersen RK, Wargent ET, **Hudson BD**, Kostenis E, Ejsing CS, Cawthorne MA, **Milligan G**, Ulven T. (2015) Activity of dietary fatty acids on FFA1 and FFA4 and characterisation of pinolenic acid as a dual FFA1/FFA4 agonist with potential effect against metabolic diseases. *Br J Nutr*. 113(11):1677-88 (doi: 10.1017/S000711451500118X)

Grants:

- Uncovering the pharmacology of the G-protein coupled receptor GPR40 (BB/E019455/1), BBSRC, 2008–2011, GBP360,791 (PI: Milligan)
- GPR120: a G protein-coupled receptor with the potential to regulate insulin secretion and inflammation (BB/K019864/1), BBSRC, 2013–2018, GBP490,847 (PI: Milligan)
- Danish Agency for Science, Technology and Innovation (2012–2016). FFARMED–The molecular effects of food on metabolic diseases through nutrient sensing free fatty acid receptors. (GBP190,345 to UofG). With Professor Trond Ulven, University of Southern Denmark. Total value DKK17,351,016

4. Details of the impact

Research at the UofG has provided key insights, direction and proofs of principle towards the synthesis and assessment of novel molecules that selectively target Free Fatty Acid receptors FFAR1 and FFAR4. In doing so, UofG research has provided a platform for the design of novel therapeutic molecules with the potential to treat type-2 diabetes, obesity and other aspects of 'metabolic syndrome' such as the fatty liver disease non-alcoholic steatohepatitis (NASH).

To help realise this potential, a spin-out company has been founded with significant financial investment, creating new jobs; money invested in the spin-out has funded research and development, with 80% spent within UK scientific services—benefitting the operations of partner contract research organisations; the spin-out has generated new patented intellectual property and identified late stage drug candidates for progression (impact 1). The UofG research has also influenced the wider thinking and direction of independent pharmaceutical research and development (impact 2).

Impact 1: Caldan established to exploit FFAR agonists

On the basis of this research and collaboration between Professor Graeme Milligan (UofG) and Professor Trond Ulven (USD), in 2015 the UofG and USD spun out **Caldan Therapeutics Ltd.** (Caldan, https://www.caldantherapeutics.com/). Caldan's core business is developing novel, small molecule therapeutic agents to treat type-2 diabetes as well as NASH by targeting either FFAR4 selectively or activating both this receptor and FFAR1.

Caldan has attracted GBP8.15 million in funding. This comprises an initial GBP4.45 million in Series A funding (2015) from Epidarex Capital (a leading international early-stage life science venture capital fund) and the Scottish Investment Bank [5.A]. It has gone on to receive two further rounds of funding, of GBP2.1 million (2019) and GBP1.5 million (2020), reflecting their progress and investor belief in Caldan's potential. "*Epidarex had identified Professor Graeme Milligan as an eminent researcher working in an area of high strategic interest. Subsequent discussions between Epidarex and Professor Milligan, his collaborator Professor Ulven, and the commercialisation offices at UoG and USD, led to the initiation of the Caldan investment*" – Partner, Epidarex [5.A]. The initial investment package was supported specifically by:

- a portfolio of patented FFAR1-activating molecules developed through a multidisciplinary collaboration between USD and UofG [3.1, 3.2]—validating the therapeutic potential of these ligands;
- a portfolio of selective FFAR4-activating molecules, which together with the underpinning portfolio of UofG research on FFAR4 [3.3–3.5], has established know-how to identify molecules and dosing regimens that will not desensitise FFAR4—making them suitable for long-term conditions;
- establishing know-how on molecules that can dually target both FFAR1 and FFAR4—offering added value as potential therapeutics [3.6].

"The research collaboration between UofG and USD offered compelling preclinical evidence that Caldan's FFAR programmes could address unmet needs in treatments for type-2 diabetes and NASH" [5.A]. The investment decision also followed successful independent due diligence with consultants in type-2 diabetes drug discovery and medicinal chemistry validating the strength and rationale of the UofG/USD technology and IP, and pharmaceutical interest in targeting FFARs [5.A].

Caldan has created new jobs with this investment and 80% of funds are spent directly on scientific efforts within the UK [5.B]. The company employs a highly experienced management team: Chief Executive Officer, Chief Operating Officer, Chief Financial Officer and a Vice-President Drug Discovery, with an office based in Nottingham. Caldan operates a lean in-house

model using a variety of contract research organisations to go through Lead Generation and Lead Optimisation phases to compete with the large Pharma groups also investigating FFA4 agonists [5.B]. The workforce has largely been employed at three companies (Sygnature Discovery, RenaSci and XenoGesis) based at BioCity in Nottingham and varies from 8–13 according to whether the research is in a 'discovery chemistry' mode, or a 'deep *in vivo* biological testing' phase but has included: 4–6 medicinal chemists, 1–2 computational chemists, 1–2 biological screening, 1–2 *in vivo* testing and 1 drug metabolism pharmokineticist [5.B]. Following his research characterising FFAR, Dr Brian Hudson has, since 2019, also acted as a consultant to Caldan and previously was bought out by Caldan at a 20% level [5.B].

Building on the multidisciplinary partnership between USD and UofG, Caldan made rapid progress on a selective FFAR4 agonist programme, which is now supported by a patent 'Tetrahydro-benzo[d]azepine derivatives as GPR120 [FFAR4] modulators' (WO/2018/172727, international publication date 27.09.2018) [5.C]. Caldan has developed several series of molecules which have shown activity in animal models of metabolic dysfunction, notably type-2 diabetes and NASH. In particular, these have shown excellent efficacy in models of diet-induced obesity using a novel dosing regimen that was predicted by understanding the mechanisms of regulation of FFAR4, uncovered by Milligan and Hudson [3.5], and were integral to Caldan's foundation. The company now has late-stage compounds with appropriate properties to be candidates for progression into development as novel treatments for these diseases.

In August 2019, Caldan secured a further GBP2.2 million in Series A investment, primarily from LifeArc Seed Funds [5.A, 5.D]. "*Attracting LifeArc as new investors into Caldan is a reflection of the great progress that Caldan has made and the company's potential to become the leading company developing GPR120 [FFAR4] agonists for metabolic disease*" – Partner, Epidarex [5.A]. This funding has allowed the company to identify preclinical candidates from late stage FFAR4 agonist compounds and demonstrate efficacy in models of NASH. NASH is a severe form of fatty liver disease that causes inflammation of the liver, affecting 5% of the UK population, and can lead to liver damage and is associated with increased risk of diabetes and high blood pressure. With no drugs to treat the underlying condition, current treatment is through lifestyle change and palliative care. Takeda Pharmaceutical Company (Japan) has estimated (p.13) that clinically useful treatments for NASH is a market expected to be worth USD3.8 billion. In October 2020, Caldan's investors provided additional funding of GBP1.5 million; Epidarex stated that this will "*equip the company with a clear runway for the company to declare a clinical candidate molecule for NASH, a key milestone for the company*" [5.A].

Impact 2: wider influence on pharmaceutical drug discovery research

The phenyl-propionic acid backbone of the TUG-891 ligand, identified through the UofG-USD collaboration [3.3, 3.4] has been instrumental to the chemistry and design of chemical series to target FFAR4 in the pharmaceutical industry. This molecule was the first potent and selective agonist of FFAR4 and has been used as a probe compound by almost all groups involved in the free fatty acid biological and drug discovery arena.

"*The structure of TUG-891 has had a huge influence on the chemistry of FFAR4 agonists. … As evidenced by literature publications and patents several large and small pharma groups have taken the phenylpropionic acid group as the basis for their drug discovery programs. These include BMS* [Bristol Myers Squibb]*, Cymabay, Janssen and Piramal who elaborate the appendage in their individual, novel way. Others such as LG Life Sciences and Merck start with a phenylpropionic acid but introduce novelty by adding ring constraints or scaffold-hopping to related but rearranged frameworks. It is fair to say that the phenylpropionic acid grouping underlies a great deal of the current medicinal chemistry research looking for drug-like agonists of FFA4*" – VP Drug Discovery, Caldan [5.B].

Several academic and industry-published reviews and research articles have similarly highlighted the broad stimulus to industrial drug discovery derived from TUG-891 chemistry and biological characterisation within the REF2021 period. For example, a team at Janssen Research & Development LLC cite Shimpukade *et al*., 2012 [3.3], describing TUG-891 and its biological properties, and state that they '*sought ways to modify the structure to improve pharmaceutical properties*' [5.E1]. In a 2020 review of the FFAR4 patent literature the same author identified how Janssen lead compounds are structurally related to TUG-891 [5.E2]. They also state, "*TUG-891 has been an important tool compound, as a selective GPR120* [FFAR4] *agonist, for validating the effects of GPR120 activation on insulin sensitization, dietary fat intake and obesity. The ortho-biphenyl of the [1,1'-biphenyl]-2-yl-methoxyphenyl core, which is a distinguishing structural feature of TUG-891, has been applied to other chemical scaffolds in the patent literature*." They go on to highlight screening activities at Piramal Enterprises Ltd (Mumbai, India) that has also used this chemistry as a driver [5.E2].

Between 2017–2019, both Janssen [5.F] and Piramal [5.G] were granted patents that cite Shimpukade *et al*. [3.3]. These were also reported by Li *et al*. (2016) in a review of FFAR drug development: with regard to Janssen they stated: "*patent applications by Janssen claimed a series of heterocyclic compounds as FFAR4 agonists which are bioisosteres of TUG-891 series"*. With regard to Piramal they stated, "*based on the structure of TUG-891, Piramal has disclosed a series of substituted phenyl alkanoic acid as FFAR4 agonists in 2016"* [5.H].

In 2016, the company was named 'Early Stage/Risk Capital Deal of the Year' at the 2016 'Scottish Business Insider Deals and Dealmakers' awards [5.I]. Prof. Milligan was also named one of nine finalists, from 50 entries, in the BBSRC's 2016 Innovator of the Year awards for his work linked to Caldan [5.J].

5. Sources to corroborate the impact

A. Statement from Partner, Epidarex Capital
B. Testimony from Vice-President of Discovery, Caldan Therapeutics Ltd.
C. Tetrahydro-benzo[d]azepine derivatives as GPR120 modulators (WO/2018/172727, international publication date 27.09.2018)
D. Caldan Therapeutics news announcement
E. Janssen articles: (1) Zhang *et al*. (2017a) Design, synthesis and SAR of a novel series of heterocyclic phenylpropanoic acids as GPR120 agonists. *Bioorg Med Chem Lett*. 27:3272-3278 (doi:10.1016/j.bmcl.2017.06.028) [See article p.3272]; (2) Zhang & Macielag (2020) GPR120 agonists for the treatment of diabetes: a patent review (2014-present). *Expert Opin Ther Pat*. 30: 729–742 (doi: 10.1080/13543776.2020.1811852) [See article p.5 – Janssen R&D; p.8 – Piramal]
F. Janssen patents: Sui *et al*. US9562053B2 (2017), US10155737B2 (2018)
G. Piramal patents: Kumar *et al*. (2019) US10214521B2, US10273230B2, US10227360B2
H. Li *et al*. (2016) Free fatty acid receptor agonists for the treatment of type 2 diabetes: drugs in preclinical to phase II clinical development. *Expert Opin Investig Drugs*, 25: 871–890 (doi: 10.1080/13543784.2016.1189530)
I. Early Stage/Risk Capital Deal of the Year' at the 2016 'Scottish Business Insider Deals and Dealmakers' awards
J. BBSRC Innovator of the Year announcement and correspondence with BBSRC