Institution: University of Kent

Unit of Assessment: 3: Allied Health Professions, Dentistry, Nursing and Pharmacy

Title of case study: Commercial and Clinical Applications for Novel Activators of Potassium Channels

Period when the underpinning research was undertaken: 2009–2020

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:
Professor Alistair Mathie	Professor of Pharmacology	2007-2020
Dr Emma Veale	Senior Research Fellow	2007-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 (indicative maximum 100 words)

Professor Mathie and Dr Veale's research on the identification and characterisation of novel potassium channel activators has been adopted by LifeArc's Centre for Therapeutic Discovery. As a result, LifeArc has improved its internal ion flux assay systems and identified 'first in class' potential novel therapeutic agents to treat pain. From this work, as well as further studies in collaboration with Pfizer and Icagen, new tool compounds have been identified and are now sold commercially by Biotechne and other companies for fundamental research by the academic community and the pharmaceutical industry. One family of activators has been adopted, initially in the USA and then in other countries, and shown to be effective as the only current treatment for Birk Barel Mental Retardation Syndrome (BBMRDS).

2. Underpinning research (indicative maximum 500 words)

A number of currently used therapeutic agents target ion channels for a variety of different conditions. In the last two decades, significant progress has been made in the understanding of ion channel structure, function, and pharmacology; however, there remains a paucity of potent and selective agents, particularly agents that enhance ion channel activity. One particular family of ion channels in humans (the two-pore domain potassium (K2P) channels) lacks selective pharmacological activators. This is despite growing evidence that activation of such channels would be a useful therapeutic strategy for a number of diseases, including, but not limited to, chronic and neuropathic pain, pulmonary hypertension, and Birk Barel Mental Retardation Syndrome (BBMRDS; a rare but debilitating disease, also known as KCNK9 Imprinting Syndrome).

Over the last decade, research led by Mathie and Veale at the University of Kent has characterised the functional properties and regulation of these human K2P channels. Their initial work in this area was supported by the award of a Royal Society Industry Fellowship to Mathie (2009-13), in collaboration with Pfizer; a BBSRC Industrial Partnership Award (2012-15), in collaboration with colleagues at the University of Oxford and Pfizer, supported by additional financial support from Pfizer; and a BBSRC CASE Partnership Award with Pfizer (2009-13).

In 2014, Mathie and Veale observed that a genetic mutation on the K2P channel TASK-3 (KCNK9), which gives rise to BBMRDS, did not abolish channel function, as had been previously suggested in the literature, but instead resulted in functional channels with very small but still detectable currents. Importantly, they showed that current through these mutated channels could be restored

by pharmacological activators such as flufenamic acid and mefenamic acid **[R1]**, suggesting a potential new therapeutic approach to this disease. Subsequently they have worked with industrial collaborators at LifeArc to identify a novel, more effective activator of TASK-3 channels **[R2]**.

They extended the usefulness of pharmacological activation to K2P channels in other clinical situations, by demonstrating that pharmacological activation of the TREK family of K2P channels would be a plausible therapeutic strategy to treat certain forms of pain such as chronic and neuropathic pain **[R3, R4].** Subsequent collaboration with industrial partners (Pfizer, Icagen, and LifeArc), who have redesigned their research strategy to reflect Mathie and Veale's findings, has enabled them to develop more selective and effective activators of TREK K2P channels **[R5, R6]** that reduce the firing frequency of pain-signalling nerve cells **[R5]**. These results, and the novel compounds generated, are shaping the direction of future research in this area, where there is a pressing need to develop alternative therapeutic strategies in the context of the emerging worldwide opioid crisis.

In a press release in April 2019 **[a]**, announcing adoption of Mathie and Veale's joint research project on novel TREK channel activators, LifeArc stated: 'Through the work of Professor Alistair Mathie and Dr Emma Veale, the University of Kent has developed considerable expertise in the characterisation of potassium ion channels.'

3. References to the research (indicative maximum of six references)

[R1] Veale, E. L., Hassan, M., Walsh, Y., Al Moubarak, E., and **Mathie, A.** (2014a). 'Recovery of current through mutated TASK3 potassium channels underlying Birk Barel syndrome'. *Molecular Pharmacology* 85:397-407. http://www.scopus.com/inward/record.url?eid=2-s2.0-84894030346&partnerID=MN8TOARS

[R2] Wright P. D., **Veale, E. L.**, McCoull, D., Large, J., Tickle, D., Gothard, G., Ococks, E., Kettleborough, C., **Mathie, A.**, and Jerman, J. (2017). 'Terbinafine is a novel and selective activator of the two-pore domain potassium channel TASK3'. *Biochemical and Biophysical Research Communications* 493: 444-450. doi: https://doi.org/10.1016/j.bbrc.2017.09.002

[R3] Veale, E. L., Al Moubarak, E., Bajaria, N., Omoto, K., Cao, L., Tucker, S. J., Stevens, E. B., and **Mathie, A.** (2014b). 'Influence of the N-terminus on the Biophysical Properties and Pharmacology of TREK1 Potassium Channels'. *Molecular Pharmacology* 85: 671-681. http://www.scopus.com/inward/record.url?eid=2-s2.0-84897439035&partnerID=MN8TOARS

[R4] Veale, E. L., and **Mathie, A.** (2016). 'Aristolochic acid, a plant extract used in the treatment of pain and linked to Balkan Endemic Nephropathy, is a regulator of K2P channels'. *British Journal of Pharmacology* 173: 1639-1652. doi: https://doi.org/10.1111/bph.13465

[R5] Loucif, A., Saintot, P.-P., Liu, J., Antonio, B. M., Zellmer, S. G., Yoger, K., **Veale, E. L.**, Wilbrey, A., Omoto, K., Cao, L., Gutteridge, A., Castle, N.A., Stevens, E. B., and **Mathie, A.** (2018). 'GI-530159, a novel, selective, mechano-sensitive two-pore-domain potassium (K2P) channel opener, reduces rat dorsal root ganglion (DRG) neuron excitability'. *British Journal of Pharmacology* 175: 2272-2283. doi: https://doi.org/10.1111/bph.14098

[R6] Wright, P. D., McCoull, D., Walsh, Y., Large, J. M., Hadrys, B. W., Gaurilcikaite, E., Byrom, L., **Veale, E. L.**, Jerman, J., and **Mathie, A.** (2019). 'Pranlukast is a novel small molecule activator of the two-pore domain potassium channel TREK2'. *Biochemical and Biophysical Research Communications* 520: 35-40. doi: https://doi.org/10.1016/j.bbrc.2019.09.093

Grants

[G1] Royal Society Industry Fellowship (IF080012/AM). 'The role of two pore domain potassium channels in primary sensory neurons' (2009-13). PI: Alistair Mathie. Value: £158,697.

[G2] BBSRC Industrial Partnership Award with Pfizer (BBJ000930/1). 'The structural mechanism of K2P channel gating' (2012-15). PI: Alistair Mathie. Value: £200,000 (+ £25,000 from Pfizer).

[G3] BBSRC CASE Studentship with Pfizer (BB/H530603/01). 'K2P channels and pain pathways' (2009-13). PI: Alistair Mathie. Value: £100,170.

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

1) Stimulation of innovation and entrepreneurial activity by industry

Following a competitive process, a collaborative project on 'Therapeutic activators of TREK-2 K2P channels' was adopted by LifeArc's Centre for Therapeutic Discovery in March 2019 **[a]**. LifeArc stated: 'University of Kent and LifeArc have entered into a collaboration agreement on 6 March 2019 with respect to activators of TREK-2 potassium channels for the treatment of chronic pain under the direction of Prof Mathie and Dr Paul Wright' **[b]**. Furthermore, LifeArc 'will conduct target validation, high throughput screening and will further develop any "hits" identified through that screening' **[a].** This comprised an initial commitment from LifeArc of around £600k internal expenditure and £200k external expenditure **[b, c]**. In accordance with the agreed plan of work, LifeArc had, by October 2020, 'carried out thallium flux screens of the LifeArc Diversity Set (100K compounds) and identified a number of hit compounds for further analysis' **[c]**. They 'studied the detailed chemical and biological properties of these compounds and commissioned pharmacokinetic profiling of them'. 'The "in vivo" efficacy of a representative lead compound in models of pain has been confirmed through investment in studies by an independent external investigator.' Through this collaboration, LifeArc 'have already identified at least one family of novel TREK-2 channel activators as potential therapeutic agents to treat pain' **[d]**.

This project has also directly led to the 'refinement of existing assays used within LifeArc to optimise identification of K2P channel activator compounds' **[d]**. These developments have been published for the benefit of other research organisations working in this area **[R2, R6, d]**. In September 2020, LifeArc (with the University of Kent) were awarded an Industrial Fellowship from the 1851 Royal Commission of the Exhibition linked to this project **[e]**. The award covers salary costs for the fellow and other expenses and 'plays a crucial role in facilitating the relationship between institutions and industry in the UK, offering highly valued funding for research and development'.

2) New products recommended, repurposed, and adopted for use

Two compounds identified through the University of Kent team's collaborative research with LifeArc (terbinafine, since 2017; and pranlukast, since 2019) have been repurposed for use and are now sold by Biotechne and associated companies (2,200+ employees worldwide) as TASK-3 and TREK-2 channel activators, respectively. For both compounds, Biotechne cite the Kent team's original research with LifeArc **[R2, R6]** in their catalogue and product datasheets **[f],** as sole primary evidence for K2P channel activation by these compounds.

A related collaboration with Pfizer and Icagen identified GI-53059 **[R5]**, which has been recommended by the International Union of Basic and Clinical Pharmacology's (IUPHAR) authoritative *Guide to Pharmacology* as a selective activator of TREK K2P channels. Since 2018, GI-53059 has been sold commercially as a TREK channel activator by several international pharmaceutical companies (Biotechne, Glixx Biologicals, Probechem, and MedKoo). In catalogues and product datasheets **[g]**, the University of Kent team's original research results **[R5]** are cited as sole, primary evidence for the mechanism of action of GI-53059. Since 2019, GI-53059 and pranlukast have been included in Biotechne's Tocriscreen 2.0 compound library as 'best in class' biologically active chemical tools **[f, g]**.

3) Transformation in patient treatment

The Kent team's research has led to a new treatment for patients suffering from BBMRDS. Fenamate compounds, which the team identified as activators of the mutated TASK-3 K2P channels seen in BBMRDS **[R1]**, have been repurposed, as a direct result of the research, for use in this condition. This is the only treatment used for BBMRDS to date. In a 2016 publication, Dr John Graham and colleagues stated: 'Treatment of one Brazilian child with FFA ointment has been attempted without any obvious complications. Treatment of other patients with the drug MFA (Rx Ponstel) is currently underway in the USA with no apparent adverse effects. Patient 1 has been on MFA for one year, and his development and responsiveness is clearly better while on MFA than while off it during treatment rest periods. Patient 3 has been on MFA for 6 months, and he experienced improved developmental milestones about 4 months after starting the drug' **[h]**.

Since taking MFA, a patient in the UK has 'made remarkable progress and now reached the ceiling of the Hammersmith motor scale' **[i]**. The Director of Personalised Care and Professor of Clinical Pathology and Pediatrics at the Children's Hospital, Los Angeles, states: 'Four years of data is available for one patient. MFA was started at 15 months of age. Like other treated patients, he had an increase in energy and stamina while on MFA. At 24 months of treatment, he had significant improvement [in tone and motor outcomes] and has continued to improve by 5.5y.' The Director also confirms that 'without the pioneering experiments of Dr Veale and Prof Mathie, we would never have had the insight to test this promising treatment for Birk-Barel patients. Both clinicians and families are in their debt.' **[j]**.

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

[a] Life Arc press release confirming that LifeArc and the University of Kent team are collaborating to develop potential pain medicines.

[b] LifeArc award letter (March 2019), confirming the terms of the collaborative agreement between LifeArc and the University of Kent team.

[c] LifeArc/Kent Programme of Work, following adoption of the TREK-2 activator project by LifeArc's Centre for Therapeutic Discovery.

[d] Letter of support from the Principal Scientist at LifeArc, detailing progress since the March 2019 agreement.

[e] News article from the 1851 Royal Commission for the Exhibition, detailing the award of an Industrial Fellowship to LifeArc and the University of Kent.

[f] Collated links confirming that terbinafine and pranlukast are sold by Biotechne (Tocris), citing the University of Kent team's research as evidence of action**.**

[g] GI-530159 in *Guide to Pharmacology*, citing the University of Kent team's research as evidence of action; and GI 530159 for sale by Biotechne (Tocris), Medkoo Biosciences, ProbeChem, and several other companies, citing the Kent team's research as evidence of action (collated links).

[h] Article: Graham, J. M. Jr, et al. (2016). 'KCNK9 imprinting syndrome-further delineation of a possible treatable disorder'. *American Journal of Medical Genetics* 170(10): 2632-7. This publication cites and builds on **R1**, describing the importance of fenamates as a potential treatment for BBMRDS (KCNK9 imprinting syndrome). It offers a description of several cases of BBMRDS and suggest Mefenamic acid for treatment. For the quotation used in this impact case study, see p. 2637. https://doi.org/10.1002/ajmg.a.37740

[i] Letter co-signed by a Consultant Paediatric Neurologist at the Royal Preston Hospital and a Consultant Clinical Geneticist at the Royal Manchester Children's Hospital, testifying that the

hospital is using Mefenamic acid as a therapy for BBMRDS because of research results, and that the patient being treated with Mefenamic acid has made excellent progress.

[j] Letter from the Director of Personalised Care and Professor of Clinical Pathology and Pediatrics at the Children's Hospital, Los Angeles, confirming a long-term case-control study of BBMRDS patients treated with MFA, based on the results in **R1**.