Institution: University of Dundee		
Unit of Assessment: UoA 5 Biological Sciences		
Title of case study: Delivery of new and repurposed drugs for visceral leishmaniasis and transfer of revolutionary screening platforms to industry		
Period when the underpinning research was undertaken: 2007-present		
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:**
1. Prof. Alan Fairlamb CBE 2. Prof. Sir Mike Ferguson CBE 3. Prof. Ian Gilbert 4. Prof. David Gray 5. Prof. David Horn 6. Prof. Kevin Read 7. Dr. Manu De Rycker 8. Prof. Paul Wyatt 9. Dr. Susan Wyllie	1. Professor of Biochemistry 2. Regius Professor of Life Sciences 3. Head of the Division of Biological Chemistry and Drug Discovery. 4. Head of Biology, Drug Discovery Unit 5. Professor of Parasite Molecular Biology 6. Head of Drug Metabolism and Pharmacokinetics, Drug Discovery Unit 7. Head of Translational Parasitology, Drug Discovery Unit 8. Director of the Wellcome Centre for Anti-Infectives Research and Head of the Drug Discovery Unit 9. Head of Mode of Action Group	1. 1996- present 2. 1988-present 3. 2005-present 4. 2010-present 5. 2013-present 6. 2008-present 7. 2009- present 8. 2006-present 9. 2001-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

Visceral leishmaniasis is a risk to 600 million people with over 50,000 new cases annually, making it one of the world's major parasitic killers. Research at the University of Dundee resulted in Phase II clinical trials to determine the efficacy of repurposing the drug fexinidazole to treat visceral leishmaniasis. Dundee drug discovery programmes, in collaboration with GlaxoSmithKline, have delivered two further novel preclinical drug candidates with different modes of action that are in clinical trials. The efficiency and productivity of leishmaniasis drug discovery in industry has been revolutionised by adoption of Dundee's screening platforms and mode of action data.

2. Underpinning research

There are estimated to be 600 million people at risk of visceral leishmaniasis (VL) across the globe with 50,000 to 90,000 new cases every year, mainly among the poorest of the poor. The disease is caused by *Leishmania* parasites and is spread through the bite of infected sandflies. Limited therapeutic options make the treatment of this neglected disease very challenging. Each drug currently in use has serious drawbacks, such as difficulty in administration, length of treatment, toxicity, cost, and emerging drug resistance. For example, miltefosine is the only oral drug, but is contraindicated in women of childbearing age as it can cause birth defects.

In 2010, the global not-for-profit Drugs for Neglected Diseases initiative (DND*i*) reported 'rediscovery' of the abandoned drug fexinidazole for the treatment of *Trypanosoma brucei* (causing African sleeping sickness), a parasite related to *Leishmania*. The drug was thought to require activation by an enzyme in the parasite. Despite the prevailing consensus at DND*i* that fexinidazole was inactive against *Leishmania*, the Dundee team identified that *Leishmania* had a gene for a similar activating enzyme. In 2011, Prof Alan Fairlamb and Dr. Susan Wyllie in

collaboration with Prof Kevin Read and the University of Dundee Drug Discovery Unit (DDU) undertook a project to examine the efficacy of fexinidazole against *Leishmania donovani*. This culminated in 2012 with the demonstration that fexinidazole displayed excellent parasite killing activity *in vivo* **[R1]** with activity dependent on oral administration and fexinidazole transformation in the liver into its active metabolites. The work was reported pre-publication to DND*i* and in 2013 they instigated a Phase II proof-of-concept clinical trial for the treatment of adults with VL in East Africa.

In parallel, given the paucity of front-line treatments for VL, the DDU in 2011 initiated a major research programme to uncover new drugs for this disease, including developing novel assay platforms to revolutionise leishmaniasis drug discovery **[R2, R3]**. A 'Mode of Action' research team was also established to discover the molecular targets of drug candidates that were at an advanced stage of development **[R4-R6]**. The DDU and GlaxoSmithKline currently have a research team of >40 scientists working on this Wellcome-funded programme of translational research for VL. Success of the programme is evidenced by the discovery and development of two clinical drug candidates for VL, each with different mechanisms of action. The first candidate arose from a compound series previously discovered in Dundee that inhibited growth of the related *Trypanosoma brucei.* Through clever iterative changes to the chemical core scaffold, the Dundee team were able to produce a compound active against the *Leishmania* parasite. The work, published in 2018, also identified the target of the compound as cyclin-dependent related kinase 12 (CRK12), providing Pharma with a completely novel validated drug target for *Leishmania* **[R4]**. Discovery of the second clinical drug candidate also began with compounds discovered in Dundee to be active against a different related parasite, *Trypanosoma cruzi*, which causes Chagas disease. Repurposing and optimisation in Dundee **[R5, R6]** produced a compound with potent activity against *Leishmania* that was shown to act through inhibition of the proteasome **[R6]**.

3. References to the research

[R1] Wyllie, S, **Patterson, S, Stojanovski, L, Simeons, FRC, Norval, S, Kime, R**, **Read, KD** & **Fairlamb, AH** (2012) 'The Anti-Trypanosome Drug Fexinidazole Shows Potential for Treating Visceral Leishmaniasis', *Science Translational Medicine*, vol. 4, no. 119, pp119re1, DOI: 10.1126/scitranslmed.3003326

[R2] De Rycker, M, **Hallyburton, I, Thomas, J, Campbell, L, Wyllie, S, Joshi, D, Cameron, S, Gilbert, IH, Wyatt, PG, Frearson, JA**, **Fairlamb, AH** & **Gray, DW** (2013) 'Comparison of a high-throughput high-content intracellular *Leishmania donovani* assay with an axenic amastigote assay.', *Antimicrobial Agents and Chemotherapy*, vol. 57, no. 7, pp. 2913-2922. DOI: 10.1128/AAC.02398-12

[R3] Nühs, A, De Rycker, M, **Manthri, S,** Comer, E, Scherer, CA, Schreiber, SL, Ioset, J-R & **Gray, DW** (2015) 'Development and validation of a novel *Leishmania donovani* screening cascade for high-throughput screening using a novel axenic assay with high predictivity of Leishmanicidal intracellular activity', *PLoS Neglected Tropical Diseases*, vol. 9, no. 9, e0004094. DOI: 10.1371/journal.pntd.0004094

[R4] Wyllie, S, **Thomas, M, Patterson, S**, Crouch, S, De **Rycker, M**, Lowe, R, Gresham, S, Urbaniak, MD, Otto, TD, **Stojanovski, L, Simeons, FRC, Manthri, S, MacLean, LM**, **Zuccotto, F, Homeyer, N**, Pflaumer, H, Boesche, M, **Sastry, L**, Connolly, P, **Albrecht, S,** Berriman, M, Drewes, G, **Gray, DW**, Ghidelli-Disse, S, Dixon, S, Fiandor, JM, **Wyatt, PG**, **Ferguson, MAJ, Fairlamb, AH**, Miles, TJ, **Read, KD** & **Gilbert, IH** (2018) 'Cyclin-dependent kinase 12 is a drug target for visceral leishmaniasis', *Nature*, vol. 560, no. 7717, pp. 192-197. DOI: 10.1038/s41586-018-0356-z

[R5] Thomas, MG, **De Rycker, M**, Ajakane, M, **Albrecht, S**, Álvarez-Pedraglio, AI, Boesche, M, **Brand, S, Campbell, L**, Cantizani-Perez, J, **Cleghorn, LAT**, Copley, RCB, Crouch, SD, Daugan, A, Drewes, G, Ferrer, S, Ghidelli-Disse, S, Gonzalez, S, Gresham, SL, Hill, AP,

Hindley, SJ, Lowe, RM, **MacKenzie, CJ**, **MacLean, L, Manthri, S**, Martin, F, Miguel-Siles, J, Nguyen, VL, **Norval, S, Osuna-Cabello, M, Woodland, A**, **Patterson, S**, Pena, I, Quesada-Campos, MT, Reid, IH, **Revill, C, Riley, J**, Ruiz-Gomez, JR, **Shishikura, Y, Simeons, FRC**, **Smith, A, Smith, VC, Spinks, D, Stojanovski, L**, **Thomas, J, Thompson, S**, Underwood, T, Gray, DW, Fiandor, JM, **Gilbert, IH**, **Wyatt, PG**, **Read, KD** & Miles, TJ (2019) 'Identification of GSK3186899/DDD853651 as a Preclinical Development Candidate for the Treatment of Visceral Leishmaniasis', *Journal of Medicinal Chemistry*, vol. 62, no. 3, pp. 1180-1202. DOI: 10.1021/acs.jmedchem.8b01218

[R6] Wyllie, S, **Brand, S**, **Thomas, M, De Rycker, M**, Chung, C-W, Peña, I, Bingham, R, **Bueren-Calabuig, J**, Cantizani, J, Cebrian, D, Craggs, PD, **Ferguson, L**, Goswami, P, **Hobrath, J**, Howe, J, **Jeacock, L**, **Ko, EJ**, Korczynska, J, **MacLean, L**, **Manthri, S**, Santos Martinez, M, Mata-Cantero, L, **Moniz, S**, **Nuhs, A**, **Osuna-Cabello, M**, **Pinto, E, Riley, J,** Robinson, S, Rowland, P, **Simeons, F**, **Shishikura, Y, Spinks, D**, **Stojanovski, L**, **Thomas, J**, **Thompson, S**, **Viayna Gaza**, **E**, **Wall, R, Zuccotto, F**, **Horn, D**, **Ferguson, M**, **Fairlamb, A**, Fiandor, JM, Martín, J, **Gray, D**, Miles, TJ, **Gilbert, I**, **Read, K**, Marco, M & **Wyatt, PG** (2019) 'Preclinical candidate for the treatment of visceral leishmaniasis that acts through proteasome inhibition', *Proceedings of the National Academy of Sciences*, vol. 116, no. 19, 201820175, pp. 9318-9323. DOI: 10.1073/pnas.1820175116

Key research grants relevant to this case study:

1. **Fairlamb, AH**. Characterization and validation of drug targets in the Kinetoplastida. Wellcome Principal Research Fellowship (2006-2016). Wellcome. Value: GBP 5,419,846.
2. **Wyatt, PG.** A pipeline of drugs for leishmaniasis and Chagas disease (2017-2022) Wellcome. Value: GBP 7,454,810.
3. **Wyatt, PG** Centre for Neglected Tropical Diseases Drug Discovery (2017-2022). Wellcome. Value: GBP 13,611,794.
4. **Wyatt, PG** Discovery and Development of Drug Candidates for Neglected Diseases. Strategic Award (2011-2017). Wellcome. Value: GBP 10,174,124.
5. **Gilbert, IH** Chemical biology: Leveraging phenotypic hits against kinetoplastids. (2015-2019). Wellcome. Value: GBP 2,301,742.
6. **Gray DW** Axenic assay development and screening of external compounds. Drugs for Neglected Diseases *initiative* (2012-2014). Value: GBP 179,000.
7. **Wyatt, PG** Identification of Drug Leads to Treat Leishmaniasis. Drugs for Neglected Diseases initiative (2009-2014). Value: GBP 1,800,000.
8. **Gilbert, IH** A Translational Engine for Biomedical Discoveries. Strategic Award (2013-2015). Wellcome. Value: GBP 800,000.

4. Details of the impact

Phase II clinical trial of fexinidazole for treatment of human VL, and application in dogs

The discovery by Fairlamb, Read and Wyllie that oral fexinidazole had anti-leishmania activity in mouse models prompted a re-evaluation by DND*i* that ultimately resulted in a Phase II clinical trial to determine the efficacy and safety of fexinidazole to treat VL patients **[E1]**. All patients benefited from clinical improvement during treatment and the trial delivered conclusive knowledge to DND*i* that fexinidazole reduces parasite burden. However, not all patients remained cured due to insufficient exposure to fexinidazole (requiring higher or longer dosing) and the trial terminated **[E1]**. Since anti-leishmanial drugs are subject to resistance, DND*i*'s goal is for two safe, orally administered drugs to be used in combination. Fexinidazole is one of only two orally-available therapies and it's superior safety profile compared to the other oral drug, miltefosine, means it could be a partner candidate for combination treatments with new oral drugs in development.

Leishmania infantum is the causative agent of infantile VL in the Mediterranean region and Latin America, with expanding geographical incidence. Dogs are the primary reservoir for infection of humans and up to 10% of dogs in endemic areas develop disease. Existing treatments do not

produce a reliable cure and have significant side effects. In 2014, Merial (now Boehringer Ingelheim Animal Health) filed a patent on fexinidazole as a treatment and cure for leishmaniasis in dogs, evidenced by trials they had undertaken **[E2]**. Their trials and patent are a clear direct consequence of the earlier published DDU research **[R1]** which they cite.

Delivery and clinical trial of new oral candidates for VL treatment in collaboration with GSK

There is an urgent need for new oral treatments for VL that work through novel modes of action. This enables combination therapies that will improve efficacy and reduce the risk of resistance. In April 2017, DND*i* and GlaxoSmithKline, in collaboration with the DDU, entered into a formal agreement for the preclinical development of two such compounds **[R4, R5]** discovered in Dundee for treatment of leishmaniasis **[E3]**. The most advanced compound was declared as a preclinical candidate by GlaxoSmithKline in July 2018 **[E4]** and human Phase 1 clinical trials began in May 2019 **[E5]**. The second compound was approved for Phase 1 clinical trials in June 2020 **[E6]** and the first subjects were dosed in October that year.

These clinical candidates represent step-changes in the development of treatments for VL since both are orally bioavailable in humans. Globally, only three other oral VL candidates are currently being clinically assessed **[E7]**. Dundee has therefore contributed 40% of the global new compounds in the pipeline for combination clinical testing. Addressing the impact of these new drug treatments, the Discovery Director at DND*i* said:

> "*To have a new compound with a novel mode of action against VL is a huge advance for the field as it enables testing and development of multiple new combination therapies with other new and existing drugs. For the DDU to identify not one, but two such new drug candidates in just a few years, is an outstanding achievement for an academic drug discovery unit*" **[E8]**.

Adoption of gold-standard screening assays for leishmaniasis drug development

An important impact of Dundee's research has been adoption by industry of a suite of screening and assay platforms developed by the DDU for VL drug discovery. These were transferred to GlaxoSmithKline (following training of GSK staff in Dundee) and are now widely used by industry and Product Development Partnerships **[E9]**. In total, DNDi and GlaxoSmithKline have screened >500,000 and 1.8 million compounds respectively using DDU assays **[E9]**, a feat previously impossible with earlier, laborious assays. DDU Mode of Action screening cascades also provided DND*i,* GlaxoSmithKline and others with knowledge that saved resources by enabling decisions not to proceed with compounds that might have negative outcomes **[E10]**. Overall, the DDU's drug discovery expertise has enhanced the productivity of the global VL drug discovery portfolio. DND*i* comment on DDU's gold standard screening assays thus:

> *"…continued development of high capacity, physiologically relevant assays using Leishmania by the Dundee DDU has been transformational for visceral leishmaniasis (VL) drug discovery. The high throughput* [parasite-killing] *assay and high content imaging platform for intracellular assays have revolutionized the capacity and efficiency of screening, enabling many millions of compounds to be screened for new chemical starting points…The Dundee L.donvani screening platform allowed rapid identification of hit series of molecules for both Dundee and other groups around the world, including major Pharma companies."* **[E8]**.

5. Sources to corroborate the impact

[E1] (i) Powerpoint presentation from DNDi presenting data from R1.
(ii) Phase II Clinical Trial: (NCT01980199). 8th November 2013.

[E2] Patent for fexinidazole as treatment and cure of canine leishmaniasis citing Dundee underpinning research R1: PCT/US2014/014134 (WO2014121064A1).

[E3] DND*i* R&D Portfolio updates about the two new oral candidates for VL, GSK3186899/DDD853651 and GSK3494245/DDD1305143.

[E4] Press release citing R1 on declaration of preclinical drug candidate to treat VL, GSK3186899/DDD853651.

[E5] Phase I Clinical Trial (NCT03874234) 14th March 2019.

[E6] Phase I Clinical Trial (NCT04504435) 7th August 2020.

[E7] DND*i* 2020 portfolio update validating that Dundee's GSK3186899/DDD853651 & GSK3494245/DDD1305143 are two of only five candidate drugs in Phase 1 for treatment of VL.

[E8] Corroboratory testimonial from Discovery Director, DND*i*.

[E9] DND*i* Visceral leishmaniasis R&D portfolio screening collaboration with Dundee to screen 500,000 compounds plus GlaxoSmithKline publication also using DDU assay to screen 1.8million compounds.

(i) DND*i* R&D Portfolio 2020, 'Screening leishmaniasis' *DNDi* 29th Feb 2020.

(ii) Publication by GSK: Peña, I, Pilar Manzano, M, Cantizani, J, Kessler, A, Alonso-Padilla, J, Bardera, AI, Alvarez, E, Colmenarejo, G, Cotillo, I, Roquero, I, de Dios-Anton, F, Barroso, V, Rodriguez, A, Gray, DW, Navarro, M, Kumar, V, Sherstnev, A, Drewry, DH, Brown, JR, Fiandor, JM & Julio Martin, J 2015, 'New compound sets identified from high throughput phenotypic screening against three kinetoplastid parasites: an open resource', *Scientific Reports*, vol. 5, 8771. https://doi.org/10.1038/srep08771

[E10] Press release announcing the GlaxoSmithKline Scientific Termination of Projects (STOP) Award 2017 to the University of Dundee Mode of Action group for producing data that enabled de-prioritisation of inappropriate compound series aimed at VL.