Institution: Royal Veterinary College (RVC)

Unit of Assessment: A 6 Agriculture, Veterinary and Food Science

Title of case study: Discovery, development and commercialisation of a novel nanotechnology for research, bioprocessing and antimicrobial therapy in human and animal health.

Period when the underpinning research was undertaken: 2010-2020

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:
Liam Good	Senior Lecturer -> Professor of Microbiology and Biotechnology	03/09/2007 - present

Period when the claimed impact occurred: 01/08/2013 - 31/12/2020

Is this case study continued from a case study submitted in 2014? N

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

Novel mechanisms of action for the antimicrobial polymer polyhexanide biguanide (PHMB) have been identified through RVC research demonstrating its penetration into cells, formation of nanoparticles with a range of compounds and mediation of functional delivery into cells. Following patent filings and company creation to commercialise the technology, impacts include investment from private individuals and venture capital; sales of research kits and services; influence on regulatory agencies' decisions and guidance, and out-licensing for both human and veterinary product applications. In addition to 3 (2 completed) clinical trials in human health, the technology platform has been developed via a multi-million dollar (USD) license deal to a global animal health business for both production and companion animal applications.

2. Underpinning research (indicative maximum 500 words)

Professor Liam Good was recruited to the RVC in 2007 from Karolinska Institute to add a new dimension to RVC's research addressing the global issue of antimicrobial resistance. Good's expertise in antisense approaches to inhibit essential genes in bacteria provided several new possibilities for development. In particular, the work led-on to the development of peptide nucleic acid fluorescence *in situ* hybridisation (PNA-FISH) probes for bacteria detection and his idea of overcoming cell delivery barriers by linking oligomers to cell wall active peptides has enabled drug target validation through RNA silencing (patented in 2001); the method is now widely used in early-stage drug development. However, as a possible therapy, antibacterial antisense agents and the delivery strategies face cost, stability and toxicity problems that limited developments. Nevertheless, the work showed that relatively large molecules can enter microbes and cationic peptides can even act as carriers to deliver reagents and drugs.

On arrival at the RVC, a major research goal was to devise ways to improve the delivery of antisense oligonucleotides into bacteria, since Good had shown they could be used to provide remarkable selectivity of antibacterial activity, something lacking from conventional antimicrobial drugs. To this end, he examined polymers that offered similar structural properties to the carrier peptides, but which could provide affordable, stable and safe solutions. The group was attracted to the polymer, polyhexanide biguanide (PHMB), also known as polyhexanide, which is widely used in industrial applications for its antimicrobial properties (e.g. in wound care and as contact lens disinfectant). Such cationic polymers are generally believed to provide antimicrobial effects through preferential targeting of the negatively charged lipids within bacterial cells; however, the team's experience with peptides suggested that the mechanism(s) may be more nuanced and involve cell entry. The first direct insight came from constructing a fluoro-tagged version of the polymer and tracking the localisation in pathogens and host cells. The results were surprising

and striking, showing that PHMB efficiently enters all microbial and mammalian cells types examined [1]. This raised new questions about its mechanism(s) of antimicrobial action and safety profile and raised possibilities for diverse practical applications that could potentially address otherwise intractable delivery challenges. To further investigate mechanisms, the team studied the uptake pathways, tracked localisation over time and measured the effects on cell membrane integrity. The results revealed that the polymer enters bacterial cells with little damage to cell membranes but then condenses chromosomes [1]. In mammalian cells, the polymer enters via dynamin-dependent endocytosis and is largely retained in endosomes and excluded from the nucleus, explaining its selective toxicity against microbes [2]. This supported the idea that the polymer can be used as a delivery technology and greatly broadened the scope for applications. In follow-on studies, the team assessed whether the polymer could be used to form complexes (nanoparticles) with reagents and drugs and mediate delivery into bacteria, fungi, parasites and mammalian cells [2-5]. In addition, the team demonstrated that the polymer is directly antimicrobial against hard to inhibit intracellular pathogens [2-4].

With these observations in place, multiple academic and industrial research collaborations have ensued to further define the antimicrobial mechanisms and understand opportunities for practical applications of the polymer-mediated delivery approach. The work has also influenced regulatory agencies' decisions and guidance. Thus, what commenced as research into efficient carriers to enhance cell penetration into bacteria led to the discovery of PHMB's novel mechanisms of action involving cell uptake and this knowledge has underpinned a range of impacts detailed below. Promising work with long-term potential for impact continues at RVC, to pursue the original aim of reagent and drug delivery into bacteria, specifically to develop alternatives to traditional antibiotic-based strategies for infection control.

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

1. Chindera K, Mahato M, Sharma AK, Horsley H, Kloc-Muniak K, McFarlane A, Stach J, Bentin T, **Good L** (2016) The antimicrobial polymer PHMB enters cells and selectively condenses bacterial chromosomes. *Scientific Reports* 6, 23121. https://doi.org/10.1038/srep23121
2. Firdessa R, **Good L,** Amstalden MC, Chindera K, Kamaruzzaman NF, Schultheis M, Röger B, Hecht N, Oelschlaeger TA, Meinel I, Lühmann T, Moll H (2015) Pathogen- and Host-Directed Antileishmanial Effects Mediated by Polyhexanide (PHMB). *PLoS Negl Trop Dis,* 9(10), e0004041. https://doi.org/10.1371/journal.pntd.0004041
3. Kamaruzzaman NF, Firdessa R, **Good L** (2016) Bactericidal effects of Polyhexamethylene Biguanide against intracellular Staphylococcus aureus EMRSA-15 and USA 300, *Journal of Antimicrobial Chemotherapy,* 71(5), 1252–1259. https://doi.org/10.1093/jac/dkv474
4. Kamaruzzaman NF, Chong SQY, Edmondson-Brown KM, Ntow-Boahene W, Bardiau M, **Good L** (2017) Bactericidal and Anti-biofilm Effects of Polyhexamethylene Biguanide in Models of Intracellular and Biofilm of Staphylococcus aureus Isolated from Bovine Mastitis. *Frontiers in microbiology,* 8, 1518. https://doi.org/10.3389/fmicb.2017.01518
5. Kamaruzzaman NF, de Fatima Pina M, Chivu A, **Good L** (2018) Polyhexamethylene Biguanide and Nadifloxacin Self-Assembled Nanoparticles: Antimicrobial Effects against Intracellular Methicillin-Resistant *Staphylococcus aureus*. *Polymers,* 10(5), 521. https://doi.org/10.3390/polym10050521

Other Quality Indicators

Professor Good also holds the following external positions:

1) Member of the Scientific Advisory Board, Blueberry Therapeutics Limited
[Text removed for publication]

Reference 1 was in the top 5% of papers for its field (multidisciplinary sciences) based on field weighted citation indices, and reference 3 was in the top 10% for Pharmacology and Pharmacy. The quality of the research cited and Prof Good's capacity to translate research into products and commercialisation has facilitated successful competitive follow-on grant applications to multiple agencies including Biotechnology and Biological Sciences Research Council

(BB/R022569/1), EU Horizon 2020 (EU H2020 AVANT Reference 862829), EU FP7-People (EU 612338) and Research England.

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

When the research team recognised the ability to improve cell and tissue delivery using a polymer with an excellent safety profile, RVC protected the relevant intellectual property through patent filing (WO2013054123) in all major territories and licensed the technology to a spin-out company, Tecrea Ltd, formed in 2012, as a vehicle for commercialisation and out-licensing of the platform technology in several sectors. Tecrea's progress in animal health has led to formation of a progeny spin-out: Tecrea Animal Health Ltd. Activities have delivered multiple impacts in the form of company formation, jobs created, inward investments, product sales, services sales, influence on court decisions, royalties to RVC, and progress in the development of new therapies for human (reaching clinical trial stage IIb) and animal health, as illustrated in Figure 1.

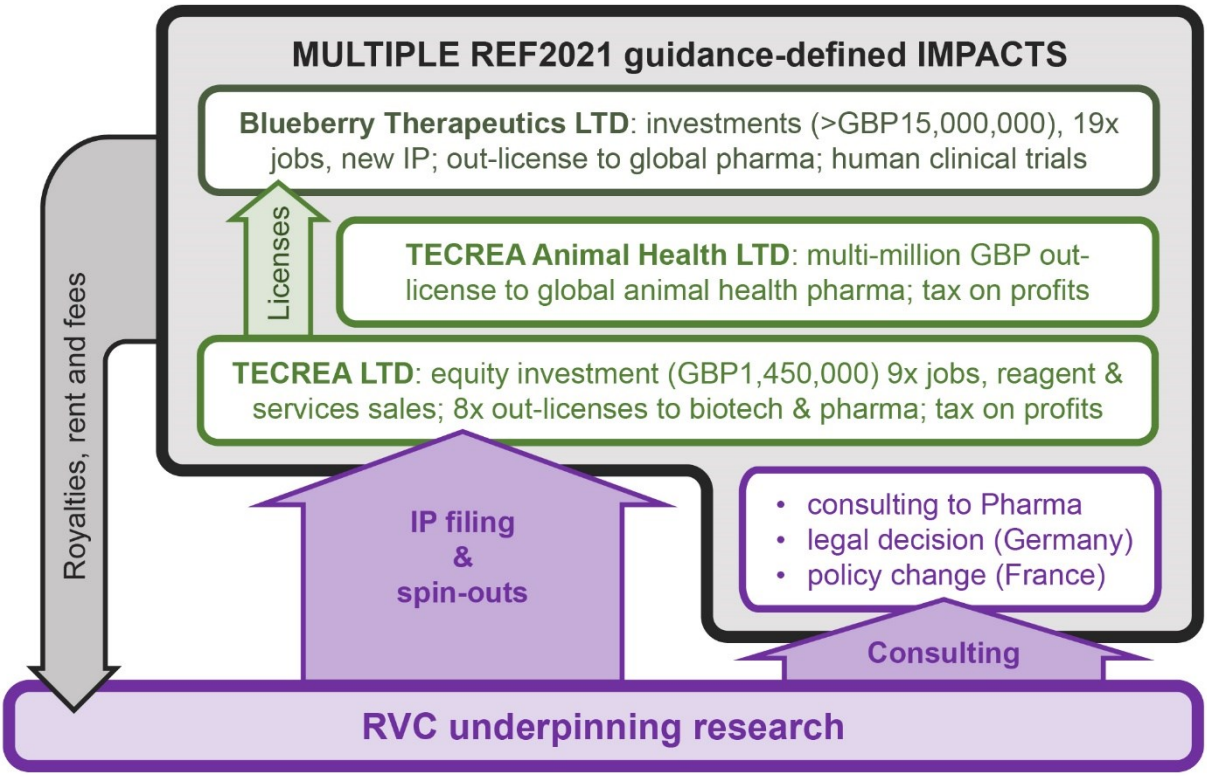

Figure 1: Illustration of RVC, Tecrea and partner relationships and impacts.

Tecrea's direct sales of research kits are principally intended to facilitate research and build long-term development partnerships with its customer base, but additionally generate revenue. Research kits are currently exported to 40 institutions in >20 countries, including major economies in the EU, North America and Asia. Between founding and October 2020, payments to HMRC/PAYE amount to GBP617,342, with additional yearly corporation tax and VAT payments [a]. Royalty payments to RVC amount to GBP137,350 [a]. Consequently, only limited equity investment has been sought, with GBP1,450,000 raised to provide security for its sales/marketing operation in Alderley Park and staff growth to 9 employees (headcount: 9; FTEs: 9) [a]. The current product range includes: Nanocin-plasmid: for transfection of plasmid DNA; Nanocin-RNA: for transfection of RNA; Nanocin-SM: for cell delivery of poorly cell permeable small molecules; and Nanocin-PRO: for cell delivery of proteins and peptides [a]. Drug formulation and cell screening fees for services to Biotech and Pharma since August 2013 have generated GBP588,000. IP Pragmatics Ltd estimated that the net value of Tecrea at the end of 2020 was between GBP12,400,000 and GBP19,100,000 [b].

Blueberry Therapeutics Ltd was set-up to in-license and exploit Tecrea's technology together with technology licensed in from AstraZeneca, in pursuit of certain human clinical applications and has attracted GBP15,000,000 of investment [c]. Building on Tecrea's technology, Blueberry has filed 11 patents and their pipeline has expanded to 5 drugs, having USD50,000,000,000 in commercial potential [c]. During the REF period, job numbers in Blueberry increased from 1 to 20 (FTE:15) [c]. The Nanocin technology is a key element of a formulation, which has completed 2 phase I/II clinical trials in humans relating to large market human dermatological conditions, meeting all primary end points [c, d, e]. Fungal nail bed infections (onchomycoses) are the primary disease target which has 10-23% prevalence in the major markets globally [c, d]. Building on this progress, a multi-site international Phase IIb trial initiated during 2019 was funded and has received FDA regulatory approval to proceed [c, d]. The test formulation reduces antifungal drug terbinafine usage by 10,000-fold, thus reducing liver and kidney exposure to damage caused by systemic terbinafine administration. Recruitment to this trial was initiated in early 2020, but was paused in March 2020 due to the COVID pandemic, before finally restarting in the last quarter of 2020 [c, d]. As an indication of the overall success of this programme, in a single agreement, Blueberry received USD12,000,000 (08-2018) funding and an out-licensing deal with an Asian-based global pharma [f].

Building on the strategy established with Blueberry, Tecrea's out-licencing has proved highly successful, with 8 active out-license agreements in place with human and animal health companies in UK, USA, Asia and Sweden – spanning SMEs and multi-nationals including a top 10 global animal health pharma [a, g, h]. Out-licensing revenue exceeded GBP1,000,000 during 2019/2020 tax year [a]. In 2020, Tecrea agreed a further out-license with the Asia-based global pharma, with a fee achieved; manufacturing for sales is expected in 2021 [a]. In Europe/USA, one of Tecrea's licensing partners (a Confidential Animal Health Corporation) has sponsored several animal trials and the results have led to an out-license being signed with Tecrea Animal Health Ltd. involving 7-figure (USD) development milestones with funds received early 2020 [a]. Multiple production animal and companion animal health applications using the Nanocin platform are being pursued in parallel, each with large market potential.

In addition to out-licensing for therapeutic product development, Tecrea Ltd has partnered with companies including AstraZeneca; Cobra Biologics; Nanoptima Ltd; [Text removed for publication] and Blueberry Therapeutics Ltd [a]. This has involved matched counter-funding from commercial partners within Innovate UK and EU supported projects, including a recent grant awarded for SARS-CoV-2 related product development. Project costs total GBP1,500,000 with commercial matched funding total of GBP394,000, with the majority spent on understanding and developing Tecrea's technology [a]. Areas of investigation that have attracted commercial partner investments in the technology include oncology, inflammation, bioprocessing and several areas of infection, including CoV-2 inactivation.

Improved fundamental understanding of PHMB's antimicrobial properties has underpinned a decision by the German Federal Institute for Drugs and Medical Devices (BfARM) leading to a change in classification of a PHMB containing product from a medical device to a pharmaceutical [i]. Professor Good was consulted through contract and questioned at the Cologne court case leading to the decision [i]. Additionally, in France, the Agency for Food, Environmental and Occupational Health & Safety (ANSES) has released a notice entitled "Évaluation de la résistance aux biocides antimicrobiens" which describes our new understanding of the mechanism of action of PHMB [j]. These government actions both cite the RVC research and the impacts are in diverse areas, including drug registration and infection control recommendations.

5. Sources to corroborate the impact (indicative maximum of 10 references)
All corroborating evidence has been uploaded into the submission system.

a. Letter from Tecrea corroborating sales, royalties, investments, taxes and new jobs, plus Tecrea's website corroborating products https://www.tecrea.com/overview/

b. Letter and valuation report from IP Pragmatics corroborating estimated value of Tecrea

c. Letter from Blueberry Ltd corroborating investment, patents, drugs, jobs and commercial potential

d. Clinical trials register NCT04188574 corroborating completion of phase I/II clinical trials and initiation of phase IIb trial during 2019 having received FDA regulatory approval plus delay due to COVID-19 https://clinicaltrials.gov/ct2/show/NCT04188574

e. Press release Blueberry Therapeutics Limited Announce Positive Results from a Phase I/II Clinical Trial: https://www.prnewswire.com/news-releases/blueberry-therapeutics-limited-announce-positive-results-from-a-phase-i-ii-clinical-trial-847837328.html

f. Press release corroborating new partners in Asia: https://endpts.com/dermatology-biotech-blueberry-therapeutics-banks-12m-series-b-and-new-partners-in-asia/

g. [Text removed for publication]

h. News release announcing deal with Confidential Animal Health Corporation. https://www.tecrea.com/tecrea-announce-exclusive-global-animal-healthcare-partnership/

i. Court records associated with a case involving a confidential German pharmaceutical company which corroborates a change in medical device/pharmaceutical classification of PHMB containing product https://openjur.de/u/2154698.html [in German].

j. French Agency for Food, Environmental and Occupational Health & Safety (ANSES) notice 'Évaluation de la résistance aux biocides antimicrobiens' citing RVC research in describing the new understanding of the mechanism of action of PHMB [in French]. https://www.anses.fr/fr/system/files/BIOC2016SA0238Ra.pdf