Institution: University of Sunderland

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

Title of case study: Improving clinical outcomes in the treatment of the 'superbug' bacterial pathogen – *Pseudomonas aeruginosa*

1. Summary of the impact

Research co-led by Prof Roz Anderson, in collaboration with a multi-disciplinary team, resulted in a new chromogenic substrate for the rapid detection and specific identification of the bacterial pathogen, *Pseudomonas aeruginosa*, a 'super-bug' that threatens many thousands of hospital patients annually, leading to poor clinical outcome and increased risk of mortality.

bioMérieux adopted the technology for a new product, ChromID® P. aeruginosa, for commercial realisation as a clinical microbiology test; it was launched in the EU, USA and Australia, supporting the company's commercial position as leaders in this field. This test has enhanced the care of patients, through more rapid detection of *P. aeruginosa* and earlier informed clinical decision-making.

2. Underpinning research

The opportunistic bacterium *Pseudomonas aeruginosa* is the source of problematic, multi-drug resistant, life-threatening infections, particularly in patients with a compromised immune system or those with cystic fibrosis. In the clinical setting, samples must be tested quickly to identify the infecting agent, so that the correct antibacterial treatment can be implemented as soon as possible; delayed or incorrect treatment leads to high mortality rates.

To facilitate its use across a wide range of clinical laboratories, which may not have access to expensive technology, we developed a simple, reliable, relatively quick and inexpensive, visual test for the presence of *Pseudomonas aeruginosa* in clinical samples, suitable for translation into a commercial product. [1,2]

Under the supervision of Professors Roz Anderson and Paul Groundwater (until 2009, now at University of Sydney, Australia) and Dr Mark Gray (since 2009), doctoral students have developed new synthetic compounds that decrease the time taken to detect and identify *Pseudomonas aeruginosa*, exploiting the unusual presence of β-alanylaminopeptidase activity observed in this 'super-bug.' [2,3,4] The synthetic compounds, with low intrinsic colour, are designed to be hydrolysed by specific target bacteria to release an intense colour for rapid visualisation of the results. In collaboration with Professor John Perry (clinical microbiologist at the Freeman Hospital, Newcastle upon Tyne), Professor Arthur James (consultant to bioMérieux), and Sylvain Orenga and team at bioMérieux, 7-*N*-(β-alanyl)amino-1-pentylphenoxazin-3-one was identified as the best agent for *P. aeruginosa* detection and was chosen for translation into product. This successful collaborative project was chosen as the cover story for the February 2008 issue of Organic and Biomolecular Chemistry (pubs.rsc.org/en/content/articlepdf/2008/OB/B716978G). [2]

The science underlying the impact relies upon the muted colour of certain heterocyclic molecules when covalently attached to an enzyme targeting group, prohibiting electron delocalisation throughout the chromogen. [2,3,4] The targeting group directs the molecule to a specific bacterial enzyme, which acts upon the linkage between the targeting group and the chromogen, breaking the covalent bond and releasing the coloured chromogen. Other substrates incorporate a nitro functional group, which inhibits resonance; upon reduction by bacterial enzymes, the strong electron-releasing effect of the resultant amino group enables delocalisation and the appearance of colour. [5] If a specific bacterial enzyme is present, then the substrate is cleaved and the delocalisation of electrons in the π-system of the chromogen is enabled, resulting in visualisation of colour due to the chromogen. A desirable substrate has low colour while attached to the enzyme targeting group and an intense defining colour that adheres to bacterial colonies for their

observation after cleavage by the specific bacterial enzyme.

Doctoral students engaged on the collaborative bacterial detection project:
Dr Yongxue Huang (2001 – 2004), Dr Andrey Zaytsev (2002 – 2005), Dr Alexandre Bedernjak (2005 – 2009), Dr Alice Rowan (2007 – 2010), Dr Linda Varadi (2009 – 2013), Keng Tiong Ng (2012 – current).

3. References to the research

This industrially-funded collaboration is based on confidential research, with significant commercial interest and potential due to the competition in developing commercial products for particular markets. Of necessity, there is some delay in converting research results into patents and publications. Those results already in the public domain are listed below; they describe the results of a range of PhD student programmes contributing to this project.

1. Nouveaux Substrats Enzymatiques Dérivés de Phénoxazinone et leur Utilisation comme Révélateur dans la Détection de Micro-organismes à Activité Peptidase. **R.J. Anderson**, P.W. Groundwater, A.L. James, D. Monget, A.V. Zaytsev. Patent No. *PCT/FR05/02249 (WO 2006/030119), 23/03/2006*; granted 11th Jan 2012 (EP 1786828 B1). *This patent claims priority on the art of detection and identification of various pathogenic bacteria in clinical, food and environmental samples through the use of derivatised 7-aminophenoxazin-3-ones designed as substrates for specific bacterial enzymes. In particular, β-alanylaminopeptidase-targeting substrates are named for the identification of* Pseudomonas aeruginosa*, of which 7-N-(β-alanyl)amino-1-pentylphenoxazin-3-one was most suited for this purpose. Prof Anderson is the first named author of this invention and made a considerable contribution to its development.*

2. Synthesis and Testing of Chromogenic Phenoxazinone Substrates for β-Alanyl Aminopeptidase, A.V. Zaytsev, **R.J. Anderson**, A. Bedernjak, P.W. Groundwater, Y. Huang, J.D. Perry, S. Orenga, C. Roger-Dalbert, and A. James, *Org. Biol. Chem.*, 2008, **6**, 682-692. *In this paper, we described the synthesis and evaluation of phenoxazinone-based substrates for the rapid and reliable identification of* Pseudomonas aeruginosa*. The synthetic methods were developed specifically for this project and provided a significant improvement on previous methods, giving reasonable yields of several related phenoxazinones in good purity. The citations from Europe and Asia provide evidence of its international significance. Prof Anderson was the joint PI on this project and made a considerable contribution to the manuscript.*

3. Synthesis and Evaluation of Novel Chromogenic Peptidase Substrates based on 9-(4′-Aminophenyl)-10-methylacridinium Salts as Diagnostic Tools in Clinical Bacteriology, **R.J. Anderson**, P.W. Groundwater, Y. Huang, A.L. James, S. Orenga, A. Rigby, C. Roger-Dalbert, J.D. Perry, *Bioorg. Med. Chem. Lett*., 2008, **18**, 832-835. *This paper describes the initial research with a chromogenic substrate for the selective detection of a range of bacteria in clinical samples, which relies on the hydrolysis of a particular substrate by specific bacterial enzymes, including β-alanylaminopeptidase activity for the identification of* Pseudomonas aeruginosa*. Professor Anderson was the PI on this project and the corresponding author of the manuscript.*

4. Synthesis and evaluation of fluorogenic 2-amino-1,8-naphthyridine derivatives for the detection of bacteria, L. Váradi, M. Gray, P.W. Groundwater, A.J. Hall, A.L. James, S. Orenga, J.D. Perry, and **R.J. Anderson**, *Org. Biomol. Chem*., 2012, **10**, 2578-2589. *The chromogenic substrates work was further extended by the use of fluorogenic substrates, which provide a signal more quickly due to the sensitivity of fluorescence spectroscopy. This work highlighted a substrate with the potential to identify Gram negative bacterial strains that could be resistant to colistin, which is of great interest in clinical samples, and has been cited internationally. Prof Anderson was the PI on this project and the corresponding author of the manuscript.*

5. Synthesis and evaluation of halogenated nitrophenoxazinones as nitroreductase substrates for the detection of pathogenic bacteria, A.F. Bedernjak, P.W. Groundwater, M. Gray, A.L.

James, S. Orenga, J.D. Perry and **R.J. Anderson**, *Tetrahedron*, 2013, **69**, 8456-8462. *The success of the aminophenoxazinones [ref 3] was followed up with the evaluation of the corresponding nitrophenoxazinones, some of which were substrates for bacterial arylnitroreductase enzymes and provided evidence that selective arylnitroreductase substrates were possible for the differentiation of selected bacterial pathogens. Prof Anderson was the PI on this project and the corresponding author of the manuscript.*

The research leading to the impact outlined here has been funded by a variety of sources and receives continued funding for future impact aims; in each case, Prof Anderson was/is the PI.

- bioMérieux (2004 – present): 7 projects £320,554
- University of Sunderland matched research funding (2005 – 2010): 2 projects £67,000
- Royal Pharmaceutical Society (2004 – 2007): £38,250
- Freeman Hospital (2004 – 2007): £9,000

4. Details of the impact

Pseudomonas aeruginosa is known to be a challenging, clinically relevant, infective agent in a wide range of patients, particularly in hospitals. Of the 300,000 hospital-acquired infections in England each year, costing the NHS more than £1 billion annually, one bacterium alone is responsible for about 10%: *P. aeruginosa*. In the UK it is the most common cause of pneumonia in intensive care units and the 2nd most common cause in hospitals generally. It causes high morbidity and mortality in neonatal patients, particularly pre-term babies, and the elderly, causes sepsis in burn wounds, is the most serious pathogen in ventilator-associated pneumonia (VAP), colonizes the respiratory system of up to 80% of patients with cystic fibrosis, and is widely found in immunocompromised patients, such as those with AIDS, neutropenia and organ transplant recipients.

Once established, *P. aeruginosa* infections are difficult to treat effectively due to the intrinsic resistance afforded by a thick mucus secretion and broad spectrum efflux pumps and, increasingly, acquired resistance of this bacterium to many antibacterial agents, including β-lactams, aminoglycosides and fluoroquinolones, through the transmission of genes encoding antibiotic-disabling enzymes or altered outer membrane pores that restrict the uptake of these agents. Delays in correctly directed treatment allow the bacteria to colonise a wound or the respiratory system and become established, resulting in poor clinical outcome and increased patient deaths, along with additional cost burden on the NHS. The production of necrosis-causing toxins in approx. 65% of hospital-acquired *P. aeruginosa* strains places an additional urgency on the rapid detection and appropriate treatment of this opportunistic pathogen.

The impact from this work is embodied in the production and adoption of a new product, ChromID® P. aeruginosa, launched commercially in April 2008, for the rapid diagnosis of this infecting agent in clinical specimens. It can also be used for the confirmation of phenotypic results. Impact demonstrated by this project includes:

- commercial adoption of a new diagnostic technology by bioMérieux for the EU, US and Australian markets;
- improved clinical outcomes for patients, with decreased delay in specific treatment and increased treatment success and reduced risk of transmission to other patients;
- markers of health have been enhanced with changes to care practice through the implementation of the technology in routine testing in hospital microbiology laboratories;
- sales of a new product, resulting from demonstrable collaboration with a multidisciplinary team, including industry, academia and hospital;
- development of new expertise, e.g. in in synthetic organic chemistry and the design of chromogenic and fluorogenic substrates, for the doctoral graduates associated with this project. Doctoral graduates have moved to employment outside the EU, translating their specialist skills and knowledge into new arenas for the benefit of international health and commerce. Other doctoral graduates are using their skills and knowledge for the benefit of education and industry in Europe.

To date, the purchase and use of ChromID® P. aeruginosa is largely limited to the few hospitals

with a specialist cystic fibrosis care unit in the target markets, where it has proved valuable for the more rapid and successful treatment of *Pseudomonas aeruginosa* infections in these patients, preventing unnecessary loss of life and transmission to other patients by decreasing the response time to implementation of treatment. This diagnostic tool remains a current part of the bioMérieux portfolio for bacterial detection and identification.

The success of this multidisciplinary collaboration has encouraged its continuation into new areas, generating new intellectual property and leading to further patents and publications; one patent and three manuscripts are currently in draft. Where the potential is not considered sufficiently commercial, the new knowledge generated is identified as making a significant contribution to knowledge in the field through publication in good quality peer-reviewed journals.

5. Sources to corroborate the impact

1. Commercial interest and success of the new technology can be corroborated by our industrial collaborators, bioMérieux, and from the website. Product information on ChromID® P. aeruginosa: www.biomerieux-diagnostics.com/servlet/srt/bio/clinical-diagnostics/dynPage?open=CNL_CLN_PRD&doc=CNL_PRD_CPL_G_PRD_CLN_21&pubparams.sform=9&lang=en [accessed 23rd Sept 2013].
   The continued agreement between bioMérieux and University of Sunderland was highlighted in the bioMérieux annual financial report in 2008. bioMérieux stated '*The detection and identification of bacteria is a key part of bioMérieux's business and Sunderland University's research has helped to strengthen bioMérieux's activities in the diagnostics field*.'

2. Clinical success showing improvement in outcome for patients, and enhancement of markers for health and care practice changes, can be corroborated through various professionals, for example, the Clinical Scientist at the Freeman Hospital, Newcastle upon Tyne and the Consultant Microbiologist at Nottingham University Hospitals. Supporting statements include '*The results of a clinical trial with this medium showed that ChromID® P. aeruginosa was at least as good as the best comparators for recovery of* P. aeruginosa *but also allowed simultaneous identification with high predictive value, thus improving time-to-detection and obviating the need for laborious identification tests*' and '*The PAID [*P. aeruginosa *identification] plate is useful in conjunction with other phenotypic tests to confirm the identification of PA [P. aeruginosa]. This is especially useful in certain clinical situations, such as cystic fibrosis, where accurate identification of Gram-negative pathogens is of critical importance.'*

3. The launch of this specific *Pseudomonas aeruginosa* test for clinical samples was reported by a number of web-based news sites:
   - Reuters: uk.reuters.com/article/2008/04/03/idUS53680+03-Apr-2008+BW20080403 [released 3rd April 2008; accessed 25th September 2013]
   - Life Sciences Europe: www.life-sciences-europe.com/news/press-release-university-sunderland-biomerieux-euronext-bim-merieux-2001-85226.html [released 3rd April 2008; accessed 25th September 2013]
   - Medical News Today: www.medicalnewstoday.com/releases/103793.php [released 13th April 2008; accessed 25th September 2013]

4. The successful development of new experts in synthetic heterocyclic and amino acid chemistry can be illustrated by their employment post-PhD graduation. Two examples, who have provided their details for corroboration, are impacting on the Worldwide economy through their employment in international industrial research environments:
   - Research Scientist, NewChem Technologies Ltd, based at University of Newcastle upon Tyne, UK;
   - Vice-President, AstaTech (Chengdu) Pharmaceutical Company Ltd.