Institution: University of Leeds

Unit of Assessment: Chemistry (UoA 8)

Title of case study 3:
Establishment of Photopharmica Ltd. to exploit novel photosensitising dyes as photoactivatable antibacterial therapeutics

1. Summary of the impact

Leeds researchers discovered a novel class of tissue penetrating, light-activated dyes that were selectively and rapidly taken up by bacteria. Based on the dyes' promising antimicrobial activity, the University of Leeds span-out Photopharmica Ltd. Further research at Leeds has progressed the development of a targeted antimicrobial for chronic wound infections. Photopharmica has raised £11.5M in external investment, around £6.0M of which has been deployed since 2009 to support a 57 patient phase IIb clinical trial. The results, which showed substantially reduced loads of all bacterial species, led to a further £250K investment in 2012 to support Photopharmica's strategy to bring an antimicrobial drug to the market.

2. Underpinning research

A new class of bioactive photosensitising dyes

In the mid-1990s, an interdisciplinary research collaboration at the University of Leeds between the groups of Professor John **Griffiths** (Department of Colour Chemistry then School of Chemistry) and Professor Stan Brown (Department of Biochemistry at the time) led to the discovery of novel photosensitising dyes that were capable of generating singlet oxygen efficiently with (red) light of a wavelength needed for good tissue penetration.

Recognising the potential of these dyes in photodynamic therapy (PDT), the researchers received substantial grants from Yorkshire Cancer Research from 1998 (£3.5M, including a programme grant). PDT requires the combination of light and a photosensitiser drug to achieve a therapeutic effect; the mechanism involves absorption of light by the photosensitiser, activation of molecular oxygen by the excited photosensitiser, and damage by the resulting highly reactive singlet oxygen leading to cell death. The need for a light source ensures that the process can be targeted precisely to the site of disease. This funding supported research by Griffiths and his colleagues to develop synthetic methods for the systematic preparation of water-soluble photosensitising dyes including phenothiaziniums(1) and phthalocyanines (2,3) In parallel with these synthetic studies, between 1996 and 2001, Brown's research group carried out experiments to evaluate the cellular uptake and *in vitro* photodynamic activity of these compounds for potential applications as anticancer or antimicrobial agents.

The collaborative team demonstrated the value of the novel photosensitisers as photoactivatable antimicrobial agents, and optimised their activity. Bacteria can be classified into two broad groups – Gram positive and Gram negative bacteria – and dangerous pathogens are found in both classes. Although it was well known that some photosensitising dyes were active against Gram positive bacteria, none had previously been found active against Gram negative bacteria. The novel photosensitisers are active against Gram negative bacteria, Gram positive bacteria (including antibiotic resistant bacteria such as methicillin-resistant *Staphylococcus Aureus*, MRSA) and fungi. Research conducted at Leeds showed that both Gram positive and Gram negative bacteria could be killed provided that the photosensitiser was cationic (4). Through a process of systematic structural modification, with parallel *in vitro* and *in vivo* screening, detailed structure-activity relationships were established. Specific phenothiaziniums, with far superior antibacterial activities compared to other known photosensitisers, were discovered, including the subsequent candidate agent PPA904 (5). A crucial advantage of these compounds, which made them suitable for treating infected tissue, was their highly selective uptake by bacteria compared to mammalian cells.

This intellectual property was transferred to the University spin-out company Photopharmica Limited and followed up by a range of national and regional applications. Photopharmica was established in 2001 to continue the clinical development of the candidate agent PPA904. The class of photosensitising dyes were patent protected worldwide through a patent cooperation treaty (PCT) application (5), with Brown and O'Grady as named inventors.

Phase IIa clinical trial

The first clinical trial, funded by Photopharmica, focused on the treatment of chronic infected leg and foot ulcers; it took place in 2004 under the 'DDX' ('Doctors and Dentists Exemption') rules which allowed clinicians to use the experimental therapy on a named patient basis. This DDX study involved nine patients, and led to regulatory approval of a Phase IIa clinical trial. The Phase IIa clinical trial, involving 32 patients from three centres (16 with chronic leg ulcers and 16 with diabetic foot ulcers), was led by Professor Lesley Rhodes (University of Manchester) and was undertaken in 2005-2007. Clinicians from Dundee, Manchester and Leeds carried out the clinical work with support from both Griffiths and Brown. This trial demonstrated a significant reduction of bacterial load in patients (6).

Key personnel

Department of Colour Chemistry/School of Chemistry
Professor John Griffiths (Lecturer 1969-81, Senior Lecturer 1981-99, Professor 1999-2004, Research Professor (part time) 2004-12)
Stephen Gorman (2001-8), Russell Cox (1998-2000), Jack Schofield (1992-2004) (Research Fellows)

Department of Biochemistry/Faculty of Biological Sciences
Professor Stan Brown (Professor of Biochemistry 1971-2009)
David Vernon (1995-2010), Simon Wood (1991-1996), Kirste Mellish (1998-2003), Denise Ball (1998-1999) (Research Fellows), Andrea Bell (Technician 1998-2002, Research Fellow 2002-10)
Andrew Minnock (1991-1995), Cassandra O'Grady (2000-3) (PhD students)

Key research grants and funding

i. "Programme grant", Yorkshire Cancer Research, PI: Prof. S. Brown, 1998-2003, £2.3M.
ii. "Photodynamic therapy", Yorkshire Cancer Research, PI: Prof. S. Brown, 1998-2005, £500k.
iii. "Lasers for photodynamic therapy", Yorkshire Cancer Research, PI: Prof. S. Brown, 1998-2003, £150k.
iv. Yorkshire Cancer Research, PI: Prof. S. Brown, 1998-2005, £500k.
v. Yorkshire Cancer Research, PI: Prof. S. Brown, 2002-2006, £230k.

Grants from Yorkshire Cancer Research are awarded following extensive peer review on a competitive basis, provided the proposed research meets stringent quality criteria.

3. References to the research

1. S.A. Gorman, A. L. Bell, J. Griffiths, D. Roberts, S.B. Brown, The synthesis and properties of unsymmetrical 3,7-diaminophenothiazin-5-ium iodide salts: Potential photosensitisers for photodynamic therapy, *Dyes and Pigments* 2006, **71**, 153-160. (10 citations; Source: Scopus, 24/10/13) http://dx.doi.org/10.1016/j.dyepig.2005.06.011
2. D J Ball, S Mayhew, S R Wood, J Griffiths, D I Vernon and S B Brown, A comparative study of the cellular uptake and photodynamic efficacy of three novel zinc phthalocyanines of differing charge, *Photochemistry and Photobiology,* 1999, **69**, 390-396. (51 citations; Source: Scopus, 24/10/13) http://dx.doi.org/10.1111/j.1751-1097.1999.tb03303.x
3. K J Mellish, R D Cox, D I Vernon, J Griffiths and S B Brown, *In Vitro* Photodynamic activity of series of Methylene Blue analogues, *Photochemistry and Photobiology,* 2002, **75**, 392-397. (79 citations; Source: Scopus, 24/10/13) http://dx.doi.org/10.1562/0031-8655(2002)0750392IVPAOA2.0.CO2
4. A. Minnock, D.I. Vernon, J. Schofield, J. Griffiths, J. H. Parish and S. B. Brown., Photoinactivation of bacteria. Use of a cationic water-soluble zinc phthalocyanine to photoinactivate both Gram-negative and Gram-positive bacteria, *J. Photochem. Photobiol. (B)* 1996, **32**,159-164. (206 citations; Source: Scopus, 24/10/13) http://dx.doi.org/10.1016/1011-1344(95)07148-2
5. S. B. Brown and C. C. O'Grady, Biologically active methylene blue derivatives, International PCT application WO02096896. This application established a filing date in all contracting states and was followed up by the filing and granting of national and regional patents (see corroborative evidence A).
6. S. Morley, J. Griffiths, G. Philips, H. Moseley, C. O'Grady, K. Mellish, C. L. Lankester, B. Faris, R. J. Young, S. B. Brown and L. E. Rhodes, Phase IIa randomized, placebo-controlled study of

antimicrobial photodynamic therapy in bacterially colonized, chronic leg ulcers and diabetic foot ulcers: a new approach to antimicrobial therapy, *Brit. J. Dermatol.* 2013, DOI: 10.1111/bjd.12098. (0 citations; Source: Scopus, 24/10/13)
http://dx.doi.org/10.1111/bjd.12098

All papers are in internationally-leading peer-reviewed journals and are hence ≥2*, but references 2-4 are particularly highlighted by the UoA to demonstrate the quality of the underpinning research.

4. Details of the impact

Context – a large market

Chronic wounds such as infected leg ulcers represent a significant burden to patients and to healthcare systems throughout the world. A study in *Nursing Times* (*Nursing Times* 2008, **104**, 44-45) describes the impact of chronic wounds on a representative healthcare system: approximately 200,000 patients in the UK have a chronic wound and the cost of caring for these patients is conservatively estimated at £2.3-3.1 billion per year i.e. around 3% of total NHS expenditure. The cost of treating those patients with venous ulceration, mostly in primary care and through community nursing services, is over £168-198 million per year. Pain and odour can affect quality of life, and these common symptoms are frequently associated with poor sleep, loss of mobility and social isolation.

As described in Section 2, the candidate photodynamic therapeutic PPA904 stems from the discovery and initial evaluations of the new class of photosensitising dye by Brown, Griffiths and colleagues. Subsequent studies by the Leeds team revealed that these dyes have properties which made them suitable for photodynamic therapy (PDT). The candidate agent PPA904 was identified as part of targeted chemical modifications led by Griffiths and laboratory antimicrobial screening studies led by Brown (5). The intellectual property generated by the two research groups was transferred to the spin-out Photopharmica for further development in 2001. **Patents have been granted** in all major territories in the world, all of which are current and are owned by Photopharmica, covering the application of specific phenothiazinium photosensitisers in photodynamic treatment (A,B,C,D)

Commercial impact – job creation

Photopharmica successfully raised £11.5M of external investment from IP Group PLC and others to support its research and development programme, £6.25M million of which has been deployed since 2008 (E,F) (see below). Some of these funds have supported further research and development of PPA904 and other candidate photosensitisers by researchers at the University of Leeds (outlined in Section 2).

The establishment of Photopharmica as a **viable spin-out company** has also **created jobs**. Since 2008, Photopharmica has employed on average 8 employees according to the needs of its R&D cycle, and additional personnel have been employed at contract research organisations providing services to support its clinical trials (F).

Health benefits – reducing infectious bacteria

Between 2009 and 2011, Photopharmica deployed around £6.0M to fund a Phase IIb trial of antimicrobial photodynamic therapy involving PPA904 in the treatment of chronic leg ulcers. Thus, a **new drug was trialled with patients**. This trial involved 11 leading UK institutes and recruited 57 patients at seven sites across the UK (G).

The trial showed that subjects receiving weekly PPA904 treatment (with light activation) showed a statistically greater reduction in the total load of bacteria in the wound compared to placebo and light. Crucially, significantly fewer PPA904-treated patients (compared to placebo) experienced post-treatment bacterial load levels above the recognised clinical threshold for prevention of wound healing. Significantly fewer PPA904-treated patients experienced very high post-treatment bacterial load levels, suggesting that PPA904 may reduce the risk of acquiring an infection. All bacterial species, including MRSA, were substantially and significantly reduced (H).

The trial reported that the treatment was well tolerated and no safety concerns were raised by the independent Data Safety Monitoring Board. Analysis of wound area and quality of life scores showed that the treatment had no adverse effect on either (H).

Commenting on this trial, the Head of Wound Healing at Cardiff University Medical School, Chief Investigator on the trial and Editor-in-Chief of International Wound Journal commented: *"In 30 years in the wound healing field, I have never seen a topical treatment with efficacy anywhere near that which Photopharmica has demonstrated. Very few significant advances have been made in this field over the last decade; I believe the Photopharmica technology is the disruptive change needed."* (I).

Following these promising Phase IIb clinical trial results, in December 2012, Photopharmica raised a further £250K in external investment to source an acquisition and/or co-development partner to help bring a novel photodynamic antimicrobial to market (E,F).

5. Sources to corroborate the impact

A. The international PCT application WO02096896 (S. B. Brown, C. C. O'Grady, J. Griffiths, K. J. Mellish, R. G. Tunstall, D. J. H. Roberts and D. I. Vernon, "Biologically Active Methylene Blue Derivatives") established a filing date (30.5.2002) in all contracting states. It was followed up by a range of regional and national applications, including the following granted patents: (a) AU2002256784 (Austrailia; filed 30.5.2002, granted 12.7.2007); (b) AU2007221946 (Australia; filed 11.10.2007, granted 1.4.2010; (c) CA2448303 (Canada; filed 25.11.2003, granted 13.7.2010); (d) CN100491362 (China; filed: 30.5.2002, granted 27.5.2009); (e) EP1392666 (Europe; filed 30.5.2002, granted 28.2.2007); (f) HK1063788 (Hong Kong; filed 1.9.2004, granted 29.6.2007); (g) JP4554198 (Japan; filed 30.5.2002, granted 29.9.2010); (h) KR100957260 (Korea; filed 15.1.2009, granted 12.5.2010); (i) NO324321 (Norway; filed 28.11.2003, granted 24.9.2007); (j) NZ529682 (New Zealand; filed 24.11.2003, granted 13.7.2006); (k) US7915254 (US; filed 4.4.2008, granted 29.3.2011); (l) US7855197 (US; filed 28.6.2008, granted 21.12.2010); (m) US8188074 (US; filed 12.5.2008, granted 29.5.2012); (n) US7732439 (US; filed 10.7.2008, granted 10.5.2010); (o) US7371744 (US; filed 26.11.2003, granted 13.5.2006); (p) ZA200309215 (South Africa; filed 26.11.2003, granted 25.5.2005).

B. The international PCT application WO2006032848 (J. Griffiths, S. A. Gorman and A. L. Bell, "Photosensitisers and Their Uses") established a filing date (14.9.2005) in all contracting states. It was followed up by a range of regional and national applications, including the granted patent US7407948 (US; filed 20.3.2007, granted 5.8.2005), which are currently being maintained.

C. The international PCT application WO0224226 (S. B. Brown, A. L. Bell, J. Griffiths and J. Schofield, "Photosensitisers") established a filing date (21.9.2001) in all contracting states. It was followed up by a range of regional and national applications, including the following granted patents: (a) AU2001287915 (Australia; filed 21.9.2001, granted 24.11.2005); (b) CA2423252 (Canada; filed 20.3.2003, granted 20.1.2009); (c) EP1320383 (Europe; filed 21.9.2001, granted 13.6.2007); (d) US7276494 (US; filed 21.3.2003, granted 2.10.2007).

D. The international PCT application WO2006032847 (S. B. Brown and C. C. O'Grady, "Wound Healing") established a filing date (14.9.2005) in all contracting states. It was followed up by a range of regional and national applications, including the following granted patents: (a) EP1797053 (Europe; filed 15.9.2005, granted 3.11.2010); (b) JP5118967 (Japan; filed 14.9.2005, granted 16.1.2013); (c) US7407953 (US; filed 20.3.2007, granted 5.8.2005).

E. Statement, Business Development Manager, IP Group PLC, 1st February 2013.

F. Statement, Senior Programme Manager, Photopharmica Ltd, 1st February 2013.

G. "Clinical Trial to Investigate Treatment With Photodynamic Therapy to Reduce Levels of Bacteria in Leg Ulcers", ClinicalTrials.gov identifier: NCT825760.

H. Statement, Chief Investigator, Phase IIb clinical trial; Director, Institute for Translation, Innovation, Methodology and Engagement, University of Cardiff; and Head, Wound Healing Research Unit, Dept. of Dermatology and Wound Healing, Univ of Cardiff, 29th January 2013).

I. Press releases: (a) http://www.photopharmica.com/latest_news.htm (accessed 4.12.12); and (b) http://www.ipgroupplc.com/media-centre/ip-group-news/2011/2011-10-05 (accessed 25.9.13).