Institution: University of Oxford

Unit of Assessment: UOA5

Title of case study:

Prolysis Ltd: novel methods for antibiotic discovery

1. Summary of the impact

From 1993 to 2005, Professor Errington and his colleagues at the University of Oxford addressed the increasingly serious global emergency of treating antibiotic-resistant bacteria. Their research led to the establishment in 1998 of the university spin-out company Prolysis Ltd and the discovery and development of two innovative series of antibiotics. The success of Prolysis Ltd was confirmed in 2009 when it was acquired by Biota Europe for £6.4 million, and gained an additional investment of £14.9 million. The subsequently formed Biota Pharmaceuticals Inc. continues to support the development of innovative broad-spectrum antibiotics essential to combat antibiotic-resistant bacteria.

2. Underpinning research

Antibiotic-resistant bacteria represent an increasing worldwide threat, and new antibiotics are urgently required to meet this emergency. An important area of innovative antibiotic development lies in the investigation of bacterial cell division. Methods of targeting FtsZ (one of a group of proteins known to be absent from humans, yet highly conserved throughout the bacterial kingdom) by using cell division inhibitors (CDIs), represents an extremely valuable approach in developing a new generation of effective broad-spectrum antibiotics.

From 1993 to 2005, Professor Errington and his group at the University of Oxford performed research into several key aspects of bacterial cell function, particularly RNA synthesis, chromosome replication and segregation, and cell division. They also pioneered the application of digital fluorescence imaging of protein and DNA in bacteria. One crucial finding was the identification of the SpoIIIE protein (a homologue of FtsZ) as a major player in homeostatic mechanisms designed to maintain chromosome integrity in bacteria[1,2]. The dependence of chromosome segregation on SpoIIIE also provided a highly sensitive and specific assay to identify inhibitors of SpoIIIE function or, indeed, inhibitors of other chromosome segregation factors[3]. Another pivotal result revealed that during the early stages of spore formation in *Bacillus subtilis*, key changes in gene expression were required for the cell division machinery to function. These gene expression changes were brought about by activity of the transcription factor sigma F, which is highly conserved in the bacterial sub-kingdom. This information provided a novel means of identifying CDIs, by screening for inhibitors of sigma F synthesis[4].

In 1998, patents protecting Errington's research, together with £2.5 million funding from Oxford Molecular (a company selling pharmaceutical software), were used to establish Prolysis Ltd (originally Microgenics Ltd) as a University spin-out company. From 2000 to 2008, research at Prolysis Ltd was further supported by funding totalling more than £12 million from a variety of sources, including equity investment led by the East Hill Management Company of Boston, USA (£3.25 million), Evotec OAI AG, the Wellcome Trust Seeding Drug Discovery programme and a LINK grant in Applied Genomics from the Biotechnology and Biological Sciences Research Council, the Medical Research Council, and the former Department of Trade and Industry[5]. In 2002, Prolysis Ltd was able to gain access to world-class chemical and drug discovery services through its collaboration with Evotec OAI AG. The early stage drug development carried out by Prolysis Ltd resulted in novel cell-based screening methodology for identifying antibacterial agents and represented a major advance that was essential for the rapid identification of bacteria-specific CDIs[6]. Prolysis Ltd expanded rapidly from 7 full-time employees in 2000 to a research team of up to 50 international scientists in 2009. This expansion enabled Prolysis Ltd to continue its

successful primary research programme to investigate more potent CDIs, and in addition to develop a second, highly innovative series of antibiotics that inhibit DNA supercoiling enzymes, specifically gyrases, that are essential for chromosome segregation.

3. References to the research

1. Wu LJ, Errington J. (1994) *Bacillus subtilis* SpoIIIE protein required for DNA segregation during asymmetric cell division. Science 264: 572-575. doi: 10.1126/science.8160014 ***Paper identifying the SpoIIIE protein as a crucial effector of chromosome segregation during sporulation in* Bacillus subtilis*.***

2. Sharpe ME, Errington J. (1995) Postseptational chromosome partitioning in bacteria. Proc Natl Acad Sci USA 92: 8630-8634. doi: 10.1073/pnas.92.19.8630 ***Paper demonstrating a general role for SpoIIIE protein in bacterial chromosome segregation.***

3. Sharpe ME, Errington J. (1996) The *Bacillus subtilis soj–spo0J* locus is required for a centromere-like function involved in prespore chromosome partitioning. Mol Microbiol. 21: 501–509. doi: 10.1111/j.1365-2958.1996.tb02559.x ***Paper identifying the Spo0J protein as a novel chromosome segregation factor.***

4. Feucht A, Daniel RA, Errington J. (1999) Characterisation of a morphological checkpoint coupling cell-specific transcription to septation in *Bacillus subtilis*. Mol Microbiol. 33: 1015–1026. doi: 10.1046/j.1365-2958.1999.01543.x ***Key paper demonstrating the dependence of sigma F activation on functioning of the cell division machinery.***

5. http://www.life-sciences-europe.com/index-term/prolysis-east-hill-investment-ltd-university-oxford-2005-2001-37517.html
http://www.thepinksheetdaily.com/deals/200530275 ***Example of websites confirming funding to Prolysis Ltd for their research.***

6. Stokes NR, Sievers J, Barker S, Bennett JM, Brown DR, Collins I, Errington VM, Foulger D, Hall M, Halsey R, Johnson H, Rose V, Thomaides HB, Haydon DJ, Czaplewski LG, Errington J. (2005) Novel inhibitors of bacterial cytokinesis identified by a cell-based antibiotic screening assay. J Biol Chem. 280: 39709–39715. doi: 10.1074/jbc.M506741200 ***Paper with mainly Prolysis Ltd personnel describing the application of the cell division inhibitor assay and its use in identifying novel inhibitors of the cell division protein FtsZ.***

Funding for research: Between 1993 and 2006, Professor Errington's research in Oxford was funded by £2.3M in grants from the BBSRC, EU and Human Frontiers Science Programme.

4. Details of the impact

The emergence of antibiotic-resistant bacteria represents a constant threat to world health. A headline in the *Independent* last year stated "Antibiotics crisis will mean routine infections are lethal" and Margaret Chan, head of the World Health Organization, warned of a global crisis in antibiotics[7]. This problem is particularly relevant for people living in close communities or who have health-associated problems, for example HIV sufferers. Examples of antibiotic-resistant bacteria include a multidrug-resistant tuberculosis that has been responsible for at least 150,000 deaths[7], and methicillin-resistant or multidrug-resistant *Staphylococcus aureus* (MRSA) that has shown resistance to vancomycin[8], a compound considered to be the antibiotic of last resort. Each year in the USA alone, MRSA infections are predicted to cost more than $3.2–$4.2 billion[8]. The necessity to develop more effective antibiotics is therefore of paramount importance. Professor Errington's research, identifying and targeting cellular processes essential for bacterial growth, represents a timely approach to a rising emergency.

Clinical Value
The expertise generated within the University of Oxford and Prolysis Ltd is at the vanguard of the

development of new, effective antibiotics. Using novel methodology, Professor Errington and colleagues identified novel compounds that were effective at targeting and killing a wide range of bacteria, including MRSA[9, 10]. These new classes of broad-spectrum antibacterials, by their very action of hitting pivotal cell division mechanisms, circumvent future problems arising from antibiotic resistance and thus represent real solutions to the emerging problem of multidrug-resistant bacteria. The achievements of Prolysis Ltd were reflected in 2009 when the company was acquired by Biota Europe[11]. This step facilitated further preclinical development of the key research programmes of Prolysis Ltd[12] and accelerated their entry into the clinic, providing new hope for the effective treatment of antibiotic-resistant pathogens.

Commercialisation

The importance and commercial attractiveness of the research and technology generated in Oxford was recognised with Errington/Prolysis Ltd as runner-up in the BBSRC Innovator of the Year Competition in 2009[13], and with the acquisition of Prolysis Ltd for £6.4M (A$10.8M) by the Australian ASX-listed biotechnology company Biota Holdings Ltd[11], a company based in Melbourne and Oxford (Biota Europe Ltd). Biota Holdings Ltd announced plans to invest £14.9M (A$25M) between 2009 and 2012 to develop the key CDI and DNA gyrase (GYR) programmes initiated in Oxford[11]. The CDI programme targeted staphylococcal infections, including MRSA, while the GYR programme, focused on DNA supercoiling inhibitors, to target the bacterial enzymes DNA gyrase and DNA topoisomerase, both of which are essential for bacterial survival. The acquisition therefore enabled the early stage development of antibacterial agents performed by Prolysis Ltd, to undergo further preclinical development and for the compounds to enter clinical trials. For example, an important asset of Prolysis Ltd was its *Clostridium difficile* programme; Biota Holdings Ltd. obtained £1.9M ($2.9M) from the National Institute of Health (ROI A1094456) for the continued preclinical development of a new candidate for treating virulent strains of *C. difficile*[14]. Since hospital costs associated with this infection are estimated at more than £2.1 billion ($3.2 billion) per annum in the USA, with treatment usually consisting of vancomycin or metronidazole[14], a successful antibiotic would have enormous benefit to the patient and reduce the cost of health care. Another important outcome of the acquisition by Biota Holdings Ltd was the continued full-time employment of all of the Prolysis Ltd staff and the maintenance of research activities in Oxford. Prolysis Ltd also retained the rights to 15% of shares in all milestones achieved, and in royalties earned on commercialisation. Professor Errington was elected to the Board of Biota Holdings Ltd on 1 February 2010.

Biota Holdings Ltd subsequently merged with Nabi Biopharmaceuticals in November 2012 to become the NASDAQ-listed group Biota Pharmaceuticals Inc. (worth £165.4M or $258M) with headquarters in the USA[15] and operations based in Melbourne and Oxford. Examples of Biota's commercialised products include Relenza and Inavir (antivirals for influenza). Biota Pharmaceuticals Inc. are continuing their broad-spectrum antibiotic programmes[15]. Indeed the GYR programme is considered to be one of the key components of Biota's strategy. Professor Errington continues to act as a Non-Executive Director at Biota Pharmaceuticals Inc.

Effective broad-spectrum antibacterial agents that target even antibiotic-resistant strains have resulted from underpinning research originating at the University of Oxford. The estimated sales potential of these products is £200m – £1 billion per year.

5. Sources to corroborate the impact

7. 'Alarming' rise in antibiotic resistance. NHS Choices. 2012. Available from: http://www.nhs.uk/news/2012/11November/Pages/Alarming-rise-in-antibiotic-resistance.aspx ***NHS Choices article on the rise in antibiotic resistance.***

8. New research estimates MRSA infections cost U.S. hospitals $3.2 billion to $4.2 billion annually. Infection Control Today. 2005. Available from: http://www.infectioncontroltoday.com/news/2005/05/new-research-estimates-mrsa-infections-cost-u-s-h.aspx ***Details of cost of MRSA in USA healthcare.***

9. Haydon DJ, Stokes NR, Ure R, Galbraith G, Bennett JM, Brown DR, Baker PJ, Barynin VV, Rice DW, Sedelnikova SE, Heal JR, Sheridan JM, Aiwale ST, Chauhan PK, Srivastava A, Taneja A, Collins I, Errington J, Czaplewski LG. (2008) An inhibitor of FtsZ with potent and selective anti-staphylococcal activity. Science 321: 1673–1675. doi: 10.1126/science.1159961 ***Paper with mainly Prolysis Ltd personnel describing pharmaceutical efficacy of a CDI.***

10. Czaplewski LG, Collins I, Boyd EA, Brown D, East SP, Gardiner M, Fletcher R, Haydon DJ, Henstock V, Ingram P, Jones C, Noula C, Kennison L, Rockley C, Rose V, Thomaides-Brears HB, Ure R, Whittaker M, Stokes NR. (2009) Antibacterial alkoxybenzamide inhibitors of the essential bacterial cell division protein FtsZ. Bioorg Med Chem Lett 19: 524–527. doi: 10.1016/j.bmcl.2008.11.021 ***Paper by Prolysis Ltd describing the development of potent anti-staphylococcal inhibitors.***

11. Biota announcement of acquisition of Prolysis assets, Nov 2009 and includes details of the deal, details of the Prolysis antibiotic programmes and their foundation in Prof Errington's basic science. http://www.biotapharma.com/uploaded/154/1021586_49biotaacquirestheantibac.pdf

12. Haydon DJ, Bennett JM, Brown D, Collins I, Galbraith G, Lancett P, MacDonald R, Stokes NR, Chauhan PK, Sutariya JK, Nayal N, Srivastava A, Beanland J, Hall R, Henstock V, Noula C, Rockley C, Czaplewski L. (2010) Creating an antibacterial with in vivo efficacy: synthesis and characterization of potent inhibitors of the bacterial cell division protein FtsZ with improved pharmaceutical properties. J Med Chem 53: 3927–3936. doi: 10.1021/jm9016366 ***Paper from Biota Europe Ltd describing the further validation of FtsZ as an antibacterial target and suitable for optimisation into new anti-staphylococcal therapy.***

13. BBSRC Press Release http://www.bbsrc.ac.uk/news/archive/2009/090325-pr-innovator-of-the-year.aspx ***Errington and Prolysis runners up as Inaugural BBSRC Innovators of the Year 2009.***

14. Biota obtains US$2.9m NIH grant for the development of novel antibacterial. Business Wire. 2011. Available from: http://www.businesswire.com/news/home/20110610005120/en/Biota-Obtains-US2.9m-NIH-Grant-Development-Antibacterial ***Website mentioning that in 2011 Biota Holdings Ltd was awarded an NIH grant for $2.9 million for the development of antibacterial agent for treating* C. difficile.**

15. Biota and Nabi merge to form Biota Pharmaceuticals. CenterWatch News Online. 2012. Available from: http://www.centerwatch.com/news-online/article/3247/biota-and-nabi-merge-to-form-biota-pharmaceuticals
Website describing the merger of Biota Holdings Ltd with Nabi Biopharmaceuticals and the continuation of the broad-spectrum antibiotic-targeting gyrase (GYR) programme.