Institution: University of Kent

Unit of Assessment: 5, Biological Sciences

Title of case study: Biopharmaceutical characterisation, production and development. [Short name: *ICS3-BPH*]

1. Summary of the impact

Research by Smales has led to IP that protects novel technologies for mammalian recombinant cell line development. Based upon mass spectrometry and *in silico* modelling approaches, the technology has permitted the development of highly efficient cell lines for monoclonal antibody production in the commercial environment at Lonza Biologics. This IP has three important benefits to the pharmaceutical and biotechnology industries:
(a) It allows key biopharmaceuticals to be made using substantially less resource and with an overall higher efficiency.
(b) It reduces the time from transfection to production of cell banks.
(c) It accelerates bioreactor evaluation and the ability to predict cell line performance at the bioreactor scale early in cell line construction.

2. Underpinning research

Following his appointment at Kent in 2003, the research in the Smales' laboratory has largely focused on improving our understanding of the biology that underpins bioprocessing and recombinant protein (rP) production from eukaryotic cell expression systems via systems biology and/or more traditional targeted approaches. By developing close links with major biopharmaceutical companies (in particular Lonza Biologics) Smales has ensured rapid translation of the tools and technologies emerging from his research to the industrial sector. The key technological advance has been the development of a combined *in silico* and experimental approach to predict the performance of a given cell line, specifically the ability to produce high-levels of recombinant biotherapeutic monoclonal antibodies. The technology replaces a simple, but time-consuming, productivity-based approach that does not necessarily allow the effective identification of high-producing cell lines. The new technology meets a need in the sector to develop faster screening methods that can successfully predict the performance of a recombinant cell line at manufacturing scale using data obtained early on in the cell line development process at small scale.

The approach taken by Smales was to develop a screening system that would be able to select a small number of cell lines based upon the analysis of data generated in multi-well plates. Importantly, they showed that these could then be taken straight to a lab-scale bioreactor-evaluation stage with a high probability that the selected cell lines were highly productive in a bioreactor culture. Intact cell MALDI-ToF mass spectroscopy (MS) was used to create 'fingerprints' of cell lines in multi-well plates [*3.1, 3.3*] and this information was used to predict the behaviour of the individual lines in lab-scale (10 L) bioreactors. This also required the development of analytical and predictive software tools based upon development of a Partial Least Squares Discriminant Analysis (PLS-DA) model for use in early stage cell line development for the identification of patterns in MS spectra associated with different productivity levels. This model can be routinely used as a rapid screen to classify cell lines into high/low producers based on their MALDI-ToF profile and current work is aimed at making the method more widely applicable, for example for the classification of cell lines with different cellular phenotypes.

Since 2008 Smales has attracted in excess of £4 million competitive funding for his research in this area from both Research Councils (BBSRC, EPSRC) and industry, particularly with Lonza Biologics but also Pfizer, GlaxoSmithKline, UCB-Celltech, Medimmune, Fujifilm Diosyth Biotechnologies and Pall Europe (see Section 3). He has also been awarded 14 PhD studentships and in 2013 took up a Royal Society Industrial Fellowship that will ensure his research continues to be effectively translated to the end-user and focuses on areas of strategic importance to the biopharmaceutical sector.

Several lines of Smales' research, partly in collaboration with von der Haar and Mead (Kent; e.g.

3.3) and colleagues in academia (Hoare, UCL, e.g. *3.5*; Martin, Newcastle) and industry (Humphreys, UCB-Celltech, *3.4*)**,** have underpinned development of the technologies for the very early prediction of cell lines' performance in cGMP manufacturing-scale bioreactors and the foundation for methods and models for predicting other mammalian cell phenotype. These include:

(a) Elucidation of the molecular responses in mammalian cells to lowering temperature of culture incubation [*3.2*],
(b) The identification and modelling of the post-transcriptional limitations upon rP production in mammalian cells and establishing the role of mRNA translation and its control in determining rP yields at physiological and sub-physiological temperatures [*3.3, 3.5*; a collaboration with UCL & Newcastle University),
(c) Defining the CHO host cell proteome and how this changes with bioprocessing [*3.1*; a collaboration with UCL); and
(d) Generating novel antibodies using protein engineering [*3.4*; a collaboration with UCB-Celltech).

3. References to the research [NB: Kent based authors in **bold**]

3.1. Hogwood, C,E., Tait, A.S., **Koloteva-Levine, N.,** Bracewell, D.G. and **Smales, C.M.** (2013) The dynamics of the CHO host cell protein profile during clarification and protein A capture in a platform antibody purification process. *Biotechnology and Bioengineering* 110:240-251.

3.2. Masterton, R.J., Roobol, A., Al-Fageeh, M.B., Carden, M.J., Smales, C.M. (2010) Post-translational events of a model reporter protein proceed with higher fidelity and accuracy upon mild hypothermic culturing of Chinese hamster ovary cells *Biotechnology and Bioengineering* 105: 215-220.

3.3. Mead, E.J. Chiverton, L.M., Spurgeon, S.K., Martin, E.B., Montague, G.A., **Smales, C.M.,** von der Haar,T. (2012) Experimental and *in silico* modelling analyses of the gene expression pathway for recombinant antibody and by-product production in NS0 cell lines *PLoS ONE* 7:e47422.

3.4. Peters, S.J., **Smales, C.M.,** Henry, A.J., Stephens, P.E., West, S. and Humphreys, D.P. (2012) Engineering an improved IgG4 molecule with reduced disulfide bond heterogeneity and increased Fab domain thermal stability. *Journal of Biological Chemistry* 287:24525-33.

3.5. Reid, C.Q., Tait, A., Baldascini, H., Mohindra, A., Racher, A., Bilsborough, S., **Smales, C.M.,** Hoare, M. (2010) Rapid whole monoclonal antibody analysis by mass spectrometry: An ultra scale-down study of the effect of harvesting by centrifugation on the post-translational modification profile. *Biotechnology and Bioengineering* 107:85-95.

3.6. Patent: *Rapid method for targeted cell (line) selection*: WO2012055554 (A1); EP2447717 (B1) Filed Oct 2010 with co-inventors from the University of Kent (including **Smales**) and Lonza Biologics plc. In addition a second patent based on research carried out by the **Smales'** group (*Means and methods for the generation of mammalian producer cells for the production of recombinant proteins*) was filed August 2013 by Lonza Biologics plc with co-inventors at the University of Kent including **Smales**.

Major grants awarded to Smales, 2008-13

By industry: *Lonza Biologics plc,* 2008-2012. Development of predictive tools for the isolation of highly productive recombinant cell lines, £971k; *Lonza Biologics plc,* 2009-2012. Investigation of improved mRNA translation and growth characteristics in CHO cells for enhanced mAb production, £416k; *Pfizer Ltd.,* 2010-2013. Engineering antibody expression vectors with respect to exploiting miRNA expression during culture of CHO cells for enhanced IgG production, £164k; *Medimmune,* 2010-2013. Development of predictive tools and formulations for improved stability and delivery of recombinant protein formulations for bio-therapeutic use, £64k.

By BBSRC: 2008-2011: Enhancing global- and mRNA-specific translation for improved recombinant protein expression in *in vitro* cultured mammalian cells, £370k. Integrating upstream host cell line selection and development with improved downstream bioprocessing, £324k. 2011-2014: Defining novel mechanisms of mRNA translational control upon cold-shock in mammalian

cells, £375k. 2012-2015: Investigation and manipulation of mTOR cellular signalling to generate novel CHO host cells with high growth and productivity characteristics, £324k. 2013-2016: Tailor-made expression hosts depleted in protease activity for recombinant protein production, £342k. 2013-2016: Unravelling and engineering the role of metals on recombinant therapeutic protein synthesis and heterogeneity from Chinese hamster ovary cells, £399k.

4. Details of the impact

The impact of the technologies developed by Smales is primarily in the area of enhanced protein synthesis especially in relation to bioprocessing and the manufacture of biopharmaceuticals. The technology has allowed the rapid generation of highly productive industrial recombinant monoclonal antibody producing cell lines. Bioprocessing and biotherapeutics are a major growth area in the UK and are priority areas for funding bodies such as the Technology Strategy Board (TSB) and the Biotechnology and Biological Sciences Research Council (BBSRC) [*see 5.1*]. The global significance of protein-based therapeutics is highlighted by the fact that they represented 11% of total global pharma sales in 2010. Indeed, of the 21 new drugs approved by FDA in that year, six were proteins and there are now around 200 biopharmaceutical products on the market with about 15 having sales in the USA of greater than $1 billion. Out of the top ten pharmaceuticals, six are recombinant proteins and this is predicted to increase to eight out of ten by 2014, with monoclonal antibodies continuing to be the dominant class of protein. The importance of research and new technologies to underpin such processes was recognised in the BIGT (Bioscience 2015) report to the UK Government which, as one of its six recommendations, called for a need to "*build a strong bioprocessing sector*" [*see 5.2*].

The production of recombinant proteins in mammalian cells is very expensive and therefore even modest increases in the yield can represent a significant cost saving. Smales' research directly relates to the goal of improved recombinant protein production by providing technology that leads to the generation and rapid identification of advanced cell lines that can be used in production processes based on large-scale (anywhere from 100 to 20,000 L) bioreactors. By providing technology that reduces the time for engineering and identifying highly productive animal cell lines, end-users are now achieving significant savings in the production of the next generation of drugs used to treat a wide range of diseases. In particular, the technology has led to a 30% reduction in the time taken from transfection to having secured research/production cell banks and completed bioreactor evaluation from approximately 22 weeks to 15 weeks by reducing the number of rounds of screening [*see 5.3*].

The research outlined by Smales has five associated elements to the overall impact as follows:

1. Commercial production of high value recombinant proteins. By working with Lonza Biologics, a company involved in the custom manufacturing of biopharmaceuticals, the technology and developments in cell line construction and screening has not only been patented [*see 5.4*], but fed into the methods for the generation of recombinant cell lines, helping to reduce the overall time and cost of product development [*see 5.3*]. This has helped maintained Lonza's position as a world leader in process development for mammalian cell expression systems including cell line production.

2. Generation of new intellectual property. By continuing to work with Lonza Biologics on technologies for enhanced recombinant protein production, the Smales group have generated further intellectual property resulting in the filing of a second patent on the generation of mammalian producer cells [*see 5.5*].

3. Development of a strong bioprocessing community in the UK. Smales' success in developing practical solutions to some of the challenges the bioprocessing community faces has led to his inclusion in the Bioprocessing Research Industry Club (BRiC) panel. The BRiC initiative involves the BBSRC, EPSRC and 15 companies. Together with Dickson (University of Manchester) Smales is leading a 2013 BBSRC-Network in Industrial Biotechnology and Bioenergy (NIBBs) initiative called Bioprocessing Network: BioProNET [*see 5.6*].

4. Sustaining and developing industry-HE links. In the review period Smales trained 14 industrially-affiliated PhD students. Four of his PhD students graduating during this period have taken up posts in industry (Lonza Biologics x2, Isogenica and Pfizer) while two others were

employees of a company. By working closely with the biopharmaceutical sector, Smales has been able to train a new generation of young PhD level researchers uniquely skilled to work in the industrial bioprocessing/biopharmaceutical sector. This in turn fosters a closer working relationship between academia and industry.

5. Furthering the impact of the research in the field of bioprocessing. As a recognised leader in field of bioprocessing [*see 5.6, 5.7, 5.8*], Smales has been awarded a Royal Society Industrial Fellowship from October 2013 [*see 5.9*]. This involves part secondment of Smales to work with Lonza Biologics plc to continue his successful interaction with the company and application of the technology to enhanced therapeutic protein production.

4. Sources to corroborate the impact

5.1: Extending Capacity and Reach in Bioprocessing, a report prepared by the BBSRC indicating the priority given to research in bioprocessing.
See: www.bbsrc.ac.uk/web/FILES/Publications/bioprocessing-leaflt.pdf

5.2: BigT Report (Biosciences 2015: Improving National Health, Increasing National Wealth), 2003. Prepared by a government working group, Bioscience Innovation and Growth Team (BIGT)
See: www.bioindustry.org/document-library/bioscience-2015/

5.3: Letter from the Head of Process Development Sciences at Lonza Biologics plc confirming the current benefits of the technology to the company and its expected future applications.

5.4: Patent *Rapid method for targeted cell (line) selection*: EP2447717B1 (filed Oct 2010, published Sept 2013) jointly by Lonza Biologics plc and the University of Kent)

5.5: Acknowledgement of receipt by the European Patent Office of a patent application entitled *Means and methods for the generation of mammalian producer cells for the production of recombinant proteins.* Application number EP13179342, filed July 2013 jointly by Lonza Biologics plc and the University of Kent.

5.6: Letter from the co-Director of the Health Tech & Medicines division of the Knowledge Transfer Network, confirming the lead role played by Smales in national bioprocessing networks including BBSRC-BRiC and the NIBBS (Network in Industrial Biotechnology and Bioenergy) in the bioprocessing area (Bioprocessing Network: BioProNET)

5.7: Appointment of Smales to the Scientific Advisory Board at University of Manchester Centre of Excellence in Biopharmaceuticals (COEBP). See www.coebp.ls.manchester.ac.uk/workwithus/sab/

5.8: Feature presentations at leading industrial conferences to describe new innovations in the area: Example: Keynote Speaker at 'The Bioprocessing Summit', Boston, USA, Aug 2012 - see www.ibclifesciences.com/variants/overview.xml

5.9: Announcement of the award in 2013 of a Royal Society Industry Fellowship to Smales.