Institution: University of Ulster

Unit of Assessment: 3B Allied Health Professions, Dentistry, Nursing and Pharmacy – Biomedical Sciences

Title of case study: Establishment, commercialisation, and impact of unique pancreatic derived clonal beta cells for end users in the global biopharmaceutical industry and international research community.

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

Fundamental to effective treatment of diabetes is the understanding of complex mechanisms regulating the function and demise of insulin-secreting pancreatic beta-cells. Inherent limitations relating to pancreatic beta-cell supply coupled with short functional life in culture prompted the challenge to establish model clonal human beta-cells. Ulster exploited an innovative approach to first establish clonal rodent beta-cells. Further development of our novel technology resulted in the generation, patent protection, and commercialisation of world-first electrofusion-derived functional human beta-cells. Our unique and valuable beta-cell lines have been licensed to multi-national pharmaceutical companies for diabetes drug development and further commercialised by sales through ECACC (now Public Health England) to directly impact on both bio-industry and the international research community by providing a limitless supply of high quality model beta-cells for translational research and diabetes drug development.

2. Underpinning research (indicative maximum 500 words)

The Diabetes Research Group (DRG), located within the SAAD Centre for Pharmacy & Diabetes at University of Ulster, is one of the pioneering and leading international centres of pancreatic beta-cell research. The key researchers responsible for this case study are Professor NH McClenaghan and Professor PR Flatt, who were employed full-time at Ulster when the research was carried out.

Studies using human islets to evaluate the function of beta-cells and the action of nutrients, hormones and drugs on insulin secretion are scarce, due to global scarcity of viable human donor pancreases. While much has been learned from the use of rodent islets, these are an imperfect proxy for human tissue and, like human islets, are notoriously difficult to isolate in large numbers in good functional state. These difficulties have prompted world-wide research efforts by various beta-cell researchers to generate model clonal insulin-secreting cells that can be grown in the laboratory to provide a limitless supply of tissue that has long-life and good functional stability. This approach also reduces usage of animals for research and the need to obtain donor human tissue. Of course, this is much easier said than done, and early research efforts by others established rodent insulin-secreting cells which, while useful, were relatively poor models of isolated rodent beta-cells.

In 1996, we published our innovative world-first application of electrofusion to generate clonal hybrid rodent pancreatic BRIN-BD11 beta-cells [1]. The impact of the provision of these bioengineered beta-cells is evident by the global adoption of BRIN cells by both eminent researchers in our field and major biopharmaceutical companies. Published outputs utilising BRIN cells cover a broad spectrum of research studies encompassing numerous aspects of pancreatic beta-cell function, demise, and destruction. As examples, BRIN-BD11 cells have been instrumental in discovery that plasma membrane Ca^{2+}-ATPase overexpression depletes Ca^{2+} stores and triggers beta-cell apoptosis (*Jiang et al. J Biol Chem* 285: 30634-30643, 2010); dysregulation of beta-cell Hnf1b gene expression occurs in response to cytotoxic fatty acid (*Johnstone et al. JOP*, 12, 6-10, 2011), structure-activity relationships influencing lipid-induced changes in eIF2alpha phosphorylation and beta-cell viability (*Dhayal et al. FEBS Lett* 585: 2243-2248, 2011), cytoprotective effects of citrus flavonoids on beta-cells (*Felipe et al. Nat Prod Res* 27: 925-928, 2013), effects of exposure to high glucose on the beta-cell metabolome (*Wallace et al. Biochim Biophys Acta* 1830: 2583-2590, 2013), and demonstration that whey protein hydrolysate promotes insulin secretion from beta-cells (*Gaudel et al. J Nutr* 143: 1109-1114, 2013). Importantly, BRIN-BD11 cells have also been used worldwide by industry, by ourselves and other members of the

international research community for evaluating potential beta-cell drug targets, together with the testing and screening of novel candidate drugs for diabetes [2].

Given that a comprehensive understanding of the beta-cell is a central driver of translational research into the aetiology and treatment of diabetes, the impact of provision of model clonal human beta-cells to both the global diabetes research field and bio-industry cannot be understated. This prompted us to further capitalise on our innovative expertise in beta-cell bioengineering with the goal of targeted establishment of world-first electrofusion-derived clonal human pancreatic beta-cells. Following provision of freshly isolated human pancreatic beta-cells we successfully utilised our electrofusion technology to generate and isolate functional model glucose-responsive human insulin-secreting beta-cell clones [3]. We filed our first patent on this technology on 02 August 2000. Subsequent to granting of patent and further research, we published our first paper in June 2011 in the *Journal of Biological Chemistry* [3]. We also made four clones (namely 1.1B4, 1.1E7, 1.4E7, 1.2B4) commercially available through ECACC (recently retitled Public Health England), in October 2010. We have recently published key data establishing these cells as excellent models for research on human beta-cell function and demise when cultured either as monolayers or aggregated with cell-to-cell contacts in the form of 'pseudoislets' [3-6].

Key Researchers: Professor PR Flatt (Professor and Head DRG; 1989 – present) and Dr/Professor NH McClenaghan (Senior Lecturer then Head of School then Professor; 1998 – present) (both 1.0 FTE University employees throughout the time of the research).

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

[1] McClenaghan, N.H., Barnett, C.R., Ah-Sing, E., Abdel-Wahab, Y.H., O'Harte, F.P., Yoon, T.W., Swanston-Flatt, S.K., Flatt, P.R. (1996). Characterization of a novel glucose-responsive insulin-secreting cell line, BRIN-BD11, produced by electrofusion. *Diabetes*, 45, 1132-1140.
Times Cited: 207 SJR: 3.810 SNIP: 2.093 Impact Factor: 7.895

[2] McClenaghan, N. H. (2007). Physiological regulation of the pancreatic beta-cell: functional insights for understanding and therapy of diabetes. *Experimental Physiology*, 292, 481-496.
Times Cited: 20 SJR: 1.041 SNIP: 1.178 Impact Factor: 2.790

[3] McCluskey, J. T., Hamid, M. H., Guo-Parke, H., McClenaghan, N. H., Gomis, R., Flatt, P. R. (2011). Development and functional characterisation of insulin-releasing human pancreatic beta cell lines produced by electrofusion. *Journal of Biological Chemistry*, 286, 21982-21992.
DOI: 10.1074/jbc.M111.226795.
Times Cited: 11 SJR: 2.723 SNIP: 1.234 Impact Factor: 4.651

[4] Guo-Parke, H., McCluskey, J. T., Kelly, C., Hamid, M., McClenaghan, N. H., Flatt, P. R. (2012). Configuration of electrofusion-derived human insulin-secreting cell line as pseudoislets enhances functionality and therapeutic utility. *Journal of Endocrinology*, 214, 257-265.
DOI: 10.1530/JOE-12-0188.
Times Cited: 4 SJR: 1.355 SNIP: 1.299 Impact Factor: 4.058

[5] Vasu, S., McClenaghan, N. H., McCluskey, J.T., Flatt, P.R. (2013). Effects of lipotoxicity on novel insulin secreting human pancreatic beta cell line, 1.1B4. *Biological Chemistry*, 394, 909-918.
DOI: 10.1515/hsz-2013-0115.
Times Cited: 0 SJR: 1.300 SNIP: 0.796 Impact Factor: 2.683

[6] Vasu S, McClenaghan NH, McCluskey JT & Flatt PR (2013). Mechanisms of toxicity by pro-inflammatory cytokines in a novel human pancreatic beta cell line, 1.1B4. Biochim Biophys Acta.
DOI:pii: S0304-4165(13)00366-8/j.bbagen.2013.08.022.
Times Cited: 0 SJR: 1.703 SNIP: 1.476 Impact Factor: 3.848

Selected grants awarded

Relating to rodent electrofusion-derived pancreatic beta-cells:

- Flatt PR, Lenzen S, and collaborators. Islet Research European Network. European Union, Concerted Action - Multi-Centre Study, 1997-2001, £250,000 (to Ulster).

- Flatt PR, Herchuelz A, and collaborators. Bioengineered cells for gene therapy of diabetes. European Union, Alfa Programme - Multi-Centre Study, 1999-2001, £100,000 (to Ulster).

- McClenaghan NH, Flatt PR, Newsholme P, Malthouse JPG. A NMR study of amino acid metabolism and its relationship to insulin secretion in pancreatic beta cells. Health Research Board North-South Cooperation Research Project Grant, 1999-2002, £70,500.

- Flatt PR, Shaw C, McClenaghan NH, Diabetes proteomics, pancreatic beta-cell targets and drug discovery, Northern Ireland Research & Development Office for the HPSS. Recognized Research Group in Endocrinology and Diabetes, 2001-2006, £185,000.

- Lenzen S, Bailey CJ, Flatt PR, Jones P, Herchuelz A, Soria B, Meda P, and collaborators. Bioengineering surrogate islets for gene therapy of diabetes. European Union 5th Framework, 2002-2005, £1,540,000.

- Flatt PR. Evaluation of novel GLP-1 antidiabetic agent. [text removed for publication], 2007-2010, £456,843.

- Flatt PR. Pharmacology feasibility study of incretin receptor antagonists. [text removed for publication] 2013, £98,598.

Relating to human electrofusion-derived pancreatic beta-cells:

- British Diabetic Association (Prof PR Flatt; 1998-1999): £32,819; Engineering immortal human insulin-secreting cells for studies of pancreatic beta cell function and potential gene therapy of IDDM. (Pilot work).

- McClenaghan NH, Flatt PR. Generation of novel insulin secreting cells. INI Proof of Concept Grant, 2004-2006, £152,582.

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

There are a range of indicators of the impact of the research and outcomes described in this Case Study. These include: (i) granting of patents on engineering human beta-cells by electrofusion with growth of an IP portfolio around our innovative technology; (ii) licensing of engineered beta-cells to the biopharmaceutical industry together with their deposit, commercialisation and sales through ECACC (recently retitled Public Health England); (iii) demonstration of the utility of our cell products by bio-industry and the international research community for drug discovery programmes and generation of high impact publications.

Filing and granting of initial patent on engineering human beta-cells by electrofusion:
Our first patent entitled “Human Insulin Producing Cell Line” that described our human clonal beta-cell products was filed in August 2000 and subsequently granted, inherently demonstrating the minimum legislative criteria of non-obviousness, novelty, and capability of being applied in trade/industry. This initial priority filing (GB0018808.6) led to growth of our related IP portfolio to include 2 granted/issued patents (covering 9 countries) on our innovative electrofusion technology for generation of clonal human beta-cells, which strategically allowed us freedom to operate in the field, and provided sufficient opportunity for commercialisation during the impact reporting period as described below.

Commercialisation and impact on non-academic end users:
In order to maximise the impact of our cells and allow other researchers and industry to benefit from their availability, we deposited three of our rat cells (BRIN-BD11, BRIN-BG5, BRIN-BG7), and four of our human cells (1.1B4, 1.1E7, 1.4E7, 1.2B4) in October 2010 with the ECACC. Testament to the impact of availability of these unique cells, ECACC has been impressed by the interest and

uptake of our cells by both research scientists and the bio-industry (see ECACC reference letter, Section 5 below). Our human cells and rat cells lodged with ECACC have generated 37 sales since January 2011 and derived total income of £23,356. As well as the direct impact on ECACC as an end user, arising from sales, the commercialisation of our 4 human and 3 rodent bioengineered insulin-secreting cell lines have proven utility as commercial and non-commercial research tools to study pancreatic beta cell function/dysfunction, including discovery and screening of new drugs/targets. As detailed in separate Case Study, Ulster has developed a strong portfolio of 12 granted patents since 2008 on peptide therapeutics for diabetes based around initial in vitro data evaluating the effects of innovative stable analogues of incretin gut hormones (GIP, GLP-1 and CCK-8) on insulin secretion using BRIN-BD11 cells. Since 2007 we have also conducted contract research on GLP-1 and other therapeutic gut hormones using BRIN-BD11 cells for [text removed for publication] (total income £555,441).

As further evidence of the direct impact of our cells on end users in industry, our human 1.1B4 cells have been licensed to [text removed for publication], Sanofi Aventis and Domain Therapeutics for commercial use (income £62,567), and evaluation licenses have been granted for 8 other pharmaceutical companies ([text removed for publication], Celther Polska, Eli Lilly, Nordic Bioscience, Novartis, [text removed for publication], and Tranzyme Pharma,). The impact of our human cells on industry is evidenced by the testimonial from Domain Therapeutics (see Section 5 below), a company who have used our human 1.1E7 cells to evaluate novel GLP-1 therapeutics for diabetes, clearly stating in their letter how our cells have impacted on their business.

Commercialisation and impact on academic end-users:
The impact of recent availability of our human cells and sales through ECACC on end users in the scientific research community is already being evidenced including, but not limited to, novel data presented in original papers reporting: discovery of molecular pathway by which nicotinamide-functionalised multiwalled carbon nanotubes can increase human beta-cell insulin production (*Ilie et al. Int J Nanomedicine*, 8, 3345-3353) using our human 1.4E7 cells; and a direct effect of hypoxia on human beta-cell proliferation and up-regulation of *Reg* and *HGF* genes (*Ota et al. Life Sci*ences, doi:pii: S0024-3205(13)00513-4), using our human 1.1B4 cells (see Section 5 below).

Other details of impact:
The impact that this research has had in the field of diabetes is also evident through recognition and esteem. Professor PR Flatt and Professor NH McClenaghan (together with Professor FPM O'Harte (employed at Ulster since 1993) were winners of the prestigious inaugural Academic Enterprise Awards (ACES) Europe for Life Sciences presented in Stockholm, 2008. This is testimony to outstanding translational research and associated commercialisation. Professor PR Flatt was elected Member Royal Irish Academy (2006) and awarded Dorothy Hodgkin Lecture of Diabetes UK (2007). Professor NH McClenaghan was awarded the Physiology Society Sharpey-Schafer Lecture (2005) for his research on insulin secretion and beta-cell engineering.

5. Sources to corroborate the impact (indicative maximum of 10 references)

Sources available from http://biomed.science.ulster.ac.uk/drgcellsimpactcasestudy/indexpage.htm

ECACC / PHE Culture Collections On-line Calatogue - availability of our rodent and human cells.
Impact of availability and sales of our rodent and human cells - end user ECACC.
Impact of availability and utilisation of our human cells - end user Domain Therapeutics.
Impact of availability and utilisation of our human cells - end users in scientific community.
Cells - end users in industry - agreements with third parties.
Cells - Ulster scientific publications.
Cells - Ulster patents. Key Researchers – Relevant publicity and press releases.