Institution: University of Cambridge		
Unit of Assessment: 5		
Title of case study: Optimised human cell products to improve therapy development		
Period when the underpinning research was undertaken: 2004 - present		
Details of staff conducting the underpinning research from the submitting unit:		
Name(s): Ludovic Vallier	**Role(s) (e.g. job title):** Professor of Regenerative Medicine	**Period(s) employed by submitting HEI:** 2002 - present
Period when the claimed impact occurred: 2013 - 2020		
Is this case study continued from a case study submitted in 2014? N		

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

Professor Ludovic Vallier's research group at the University of Cambridge has made major contributions to stem cell science by uncovering the molecular interplays directing the differentiation of human pluripotent stem cells. This research has led to 13 patents. Spin-out DefiniGEN was founded on Prof. Vallier's underpinning research and was the first company to use human pluripotent stem cells (hPSC) to produce customised predictive liver models for the pharmaceutical sector. They now provide hepatocyte, pancreas and gut cells to customers in Europe, the US and Japan. Since 2013, DefiniGEN has raised GBP9,000,000 in investment and currently employs 17 people. Prof. Vallier's research has also contributed to the development of three other companies that produce cell types of clinical interest for disease models and advances in regenerative therapy, including bit.bio, which employs 90 people and has generated USD50 million in investment.

2. Underpinning research (indicative maximum 500 words)

Research into hPSCs is being used to advance knowledge about how organisms develop and how diseases can affect healthy cells, offering hope for novel treatments for many serious illnesses including diabetes, heart failure, cystic fibrosis, and neurodegenerative disorders such as Parkinson's disease. However, the area has faced challenges due to a lack of understanding of how pluripotent stem cells function and, more importantly, poorly defined methods to induce their differentiation.

In 2004, Professor Ludovic Vallier and colleagues at the University of Cambridge discovered that Nodal signalling controls the pluripotency of hESCs and influences early cell fate decisions by inhibiting progression along the default neuroectoderm pathway whilst promoting cell differentiation into mesoderm and endoderm lineages [R1]. This discovery later led to a landmark manuscript describing the derivation of epiblast stem cells that demonstrated, for the first time, the existence of different pluripotent states during development [R2]. In the same year they demonstrated that small interfering RNA (siRNA) could knock down the expression of endogenous genes, a method that could be used to model human disease *in vitro* and to reveal the developmental role of specific human genes [R3].

Prof. Vallier and colleagues went on to correct a faulty gene in hPSCs derived from skin cells of people with an inherited liver disease. They were able to convert the stem cells into liver cells and correct the mutation using targeted genome editing [R4]. The resulting corrected cells could then be differentiated into healthy hepatocytes with the capacity to be grafted into the liver of a mouse model of acute hepatic failure. These experiments provided a first proof of concept that

human induced pluripotent stem cells (iPSCs) could be used for personalised cell-based therapy in the context of genetic diseases.

In 2010, Prof. Vallier and colleagues published a multistep protocol for producing hPSC-derived hepatocytes by mimicking the developmental changes occurring during natural development of the liver. These can be used for producing functional hepatocyte-like cells for modelling embryonic development, disease studies and toxicological investigations [R5,R6].

In 2017, they investigated genetic and phenotypic variability in iPSCs as a blueprint for their use as models for complex human traits and cancer. By analysing 711 iPS cell lines from 301 healthy individuals, they found that 5 – 46% of the variation in different iPSC phenotypes arises from differences between individuals. They produced a map of recurrent genetic abnormalities in human iPSCs and identified candidate targets for selection. This was the first time the majority of these recurrent loci were identified because previous studies had used much smaller sample sizes [R7].

Prof. Vallier has published 13 patents related to this work since 2006, including the induction of cell differentiation and methods for obtaining and generating progenitor cells [R6]. The licensing of these patents into commercial endeavours forms the basis of impact that has been achieved from 2013 to now.

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

R1. Vallier L, Reynolds D, Pedersen RA. Nodal inhibits differentiation of human embryonic stem cells along the neuroectodermal default pathway. Dev Biol. 2004;275(2):403-421. doi:10.1016/j.ydbio.2004.08.031*

R2. Brons IG, Smithers LE, Trotter MW, Rugg-Gunn P, Sun B, Chuva de Sousa Lopes SM, Howlett SK, Clarkson A, Ahrlund-Richter L, Pedersen RA, Vallier L. Derivation of pluripotent epiblast stem cells from mammalian embryos. Nature. 2007; 7150, 191-5. doi: 10.1038/nature05950*

R3. Vallier L, Rugg-Gunn PJ, Bouhon IA, Andersson FK, Sadler AJ, Pedersen RA. Enhancing and diminishing gene function in human embryonic stem cells. Stem Cells. 2004;22(1):2-11. doi:10.1634/stemcells.22-1-2 *

R4. Yusa, K. *et al.* Targeted gene correction of alpha1-antitrypsin deficiency in induced pluripotent stem cells. *Nature* 2011, **478**, 391-394, doi:10.1038/nature10424*

R5. Rashid ST, Corbineau S, Hannan N, Marciniak SJ, Miranda E, Alexander G, Huang-Doran I, Griffin J, Ahrlund-Richter L, Skepper J, Semple R, Weber A, Lomas DA, Vallier, L. Modelling inherited metabolic disorders of the liver using human induced pluripotent stem cells, Journal of Clinical Investigation, 2010, 120(9):3127-3136, doi:10.1172/JCI43122*

R6. Patents: US2010034785A1/US8323971B2 (15 May 2008); US2012238014A1/US8497125B2 (15 May 2008); US2013031645A1 (03 Jun 2011); WO2012025725A1 (01 Mar 2012); US2014093963A1/US9963681B2 (04 Oct 2012); US2015225698A1/US9790470B2 (27 Mar 2014); WO2014174047A1 (30 Oct 2014); WO2015052143A1 (16 Apr 2015); US2018187160A1 (29 Dec 2016); WO2018096343A1 (31 May 2018); WO2018234323A1 (27 Dec 2018); WO2020030821A1 (10 Aug 2018); WO2020030822A1 (10 Aug 2018)

R7. Kilpinen, H. et al. Common genetic variation drives molecular heterogeneity in human iPSCs. Nature 2017, 546, 370-375, doi:10.1038/nature22403*

*All research outputs have been published in peer-review journals.

Competitive funding received

2005-2008 MRC Career Development Fellowship, Molecular mechanisms controlling cell fate specification of human embryonic stem cells toward the endoderm germ layer, GBP130,000

2008-2010 Wellcome Trust, Programming embryonic stem cell derived endodermal cells towards a Beta cell phenotype (co-investigator), GBP110,000

2008-2015 European Union Fp7, LivES: Development of culture conditions for the differentiation of human Embryonic Stem Cells into hepatocytes, GBP420,000; InnovaLIV: Innovative strategies to generate human hepatocytes for treatment of metabolic liver diseases: tolls for personalized cell therapy, GBP450,000; TissueGEN: The production of a 3D human tissue disease platform to enable regenerative medicine therapy development, GBP570,000

2008-2013 MRC Senior non-clinical fellowship, Molecular mechanisms controlling differentiation of pluripotent cells into endoderm, GBP1,450,000

2009-2012 Technology Strategy Board, Manufacturing solutions for high value induced pluripotent stem cell products (co-investigator), GBP656,986

2011-2013 Evelyn Trust, *In vitro* modelling of lung diseases using human Induced Pluripotent Stem Cells, GBP145,366

2012-2017: ERC consolidator grant, Relive-IMDs: Reprogramming cell identity to develop new therapies against Inherited Metabolic Disorders of the liver, GBP1,373,000

2012-2017: Wellcome Trust / MRC Strategic award. UK human IPS consortium: genotype to phenotype (co-investigator); GBP12,400,000

2013-2018: MRC UK Regenerative Medicine Platform, The Pluripotent Stem Cells Platform. (co-investigator), GBP4,262,863

2013-2017: EU/IMI, European Bank for induced pluripotent Stem Cells (co-investigator), GBP35,000,000

2015-2017: MRC/UKRMPII: The development of 3-dimensional implantable liver organoids. (co-investigator), GBP1,999,858

2016-2018 CF Foundation, Personalized cell-based therapy for Cystic Fibrosis-related lung disease, GBP250 000

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

Prof. Vallier's research has generated economic, commercial and social impact through the production of cells used for research and clinical purposes, company and job creation, revenue generation, and patient interaction.

DefiniGEN

DefiniGEN was founded in 2012 by Prof. Vallier, based on patented technology for the differentiation of pluripotent cells into primary germ layer progenitors and the method for hepatic differentiation of definitive endoderm cells [R6] developed in Prof. Vallier's laboratory [E1]. The

company's mission is to develop, produce and commercialize highly-predictive human cell disease models to improve safety and efficacy during the drug testing process, improve attrition and ultimately reduce costs [E1]. DefiniGEN were the first company to use human iPSC to produce customised predictive liver models for the pharma sector [E1], based on Prof. Vallier's lab being the first to develop a methodology for doing so. DefiniGEN remains one of few companies developing hepatocytes from iPSCs. DefiniGEN produces *in vitro* disease models for drug development using the OptiDIFF technology derived from Prof. Vallier's research. OptiDIFF is a protocol for the differentiation of hPSCs and is a world-leading production platform designed to generate mature, primary-like human endoderm cell types from iPSC [E1]. The company's portfolio now includes iPSC derived human hepatocytes, intestinal organoids and pancreatic beta cells, providing mature human cells for these tissues [E1]. These cells are sold internationally and have been used to improve the safety and efficacy of the drug testing pipeline, which helps to accelerate research during the candidate drug selection process [E1].

Impact on economy and employment

DefiniGEN has raised GBP9,000,000 in investments within the REF period, indicating the uniqueness of their technology and commercial offering [E1, E2]. DefiniGEN employs 17 people in roles such as business development, research and development [E1], with world-leading expertise in iPSC production and metabolic disease modelling. Application of these technologies in drug discovery provides pharmaceutical companies with more predictive *in vitro* cell products enabling the development of safer and more effective treatments [E1]. As Chief Scientific Officer (CSO) at DefiniGEN, Prof. Vallier provides ongoing scientific guidance, directing the development of OptiDIFF and facilitating development of the company's market-leading offerings [E1]. His position ensures that the findings of his ongoing research are fed into the company's portfolio.

Enabling downstream innovation

DefiniGEN takes understanding from basic biology and translates it *in vitro*, providing commercial impact and benefitting downstream production. DefiniGEN's cell products serve the pharmaceutical and biotech industry as well as academic groups in Europe, the US and Japan [E1]. DefiniGEN has had a chain of collaborations during the current REF period that have helped to advance their work as a company, and support partners in meeting commercial goals. In 2017, DefiniGEN formed a collaboration with GenoMembrane, a world leader in drug transporter production. The CEO of GenoMembrane said that "collaboration with DefiniGEN will create innovative *in vitro* tools useful for drug development in pharmaceuticals as iPS cells can provide stable lots of differentiated human intestinal cells with same characteristics" [E3]. The technology developed by DefiniGEN, based on Prof Vallier's work, can be further expanded in reach by using genome editing tools. In November 2018, a licence agreement was announced between DefiniGEN and ERS Genomics Limited, giving DefiniGEN access to ERS Genomic's genome editing technology patent. The CEO of DefiniGEN said, "These tools will enable our customers to optimise the preclinical development of therapeutics for multiple rare liver diseases, Type 2 diabetes and non-alcoholic fatty liver disease" [E4]. In November 2019, DefiniGEN started a collaboration with InSphero AG with a GBP 750,000 grant from Eurostars (a programme co-funded by the European Commission to support international innovative projects within SMEs). DefiniGEN will provide iPSC-derived pancreatic endocrine cells that InSphero will develop for human *in vitro* model systems for diabetes drug discovery and safety testing [E5].

Contribution to emerging commercial biotech activity

Prof. Vallier's work has led to the development of three additional biotechnology companies:

1) bit.bio Limited was founded by Dr Mark Kotter at the University of Cambridge in 2016 based on a collaboration with Prof. Vallier's group that led to the development and patent of their OpiOX system (R6, patent WO2018096343A1) [E6]. Currently, bit.bio has 90 employees of which over 60 are involved in research and discovery activities. They have launched two products based on the technology: Human-Induced Glutamatergic Neurons are used as a high quality human model for the study of neurological activity and disease, and Human-Induced Skeletal Myocytes for research, disease modelling and high throughput screening across areas

such as muscle, neuromuscular, and associated metabolic disorders) Since its establishment, bit.bio has secured investment of USD50 million. The company's Chief Financial Officer/Chief Operating Officer states that their unique market position is "built upon the excellent research which came from academic research at the University of Cambridge, including that of Professor Ludovic Vallier" [E7].

2) Hepatotarget Therapeutics was founded in 2019 from Prof. Vallier's research (R6, patent WO2020030822A1) into correcting faulty genes in hPSCs derived from skin cells of people with an inherited liver disease. The company commercialises new cell-based approaches against acute liver failure [E8].

3) Bilitech was founded in 2017 based on Prof. Vallier's research (R6, patent WO2020030821A1) and has obtained Innovate seed funding worth GBP250,000 [E9]. Bilitech uses cholangiocyte (epithelial bile duct cell) organoids, for regenerative medicine application focusing on biliary disorders; it currently employs two people [E10].

Impact on learning via patient interaction and engagement

Prof. Vallier has been working with the Cystic Fibrosis Trust, the pre-eminent UK-based charity for this condition (charity no. 1079049), for eight years to support patients' understanding of their condition. The Director of Strategic Innovation at the Cystic Fibrosis Trust said that Prof. Vallier "has a remarkable ability to explain in non-technical language [about] the work in this area and is repeatedly asked to speak [to] the CF community. Importantly, he provides a level of realism in terms of the hurdles and timelines to find the cure. He is in demand from the community who are keen to hear directly from an expert especially when there is so much "hype" in the…press" [E11]. Prof. Vallier also participates in the first UK-based Cystic Fibrosis Trust Innovation Hub, which opened in Cambridge in 2017.

Prof. Vallier's landmark research into hPSCs has generated wide reaching impact, benefiting the economy through four new companies, allowing international pharmaceutical and biotech companies to test new drugs safely via novel model systems, and supporting patients to understand their conditions and treatments available through direct engagement.

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

E1 DefiniGEN CEO testimonial
E2. DefiniGEN Companies House record
E3. GenoMembrane press release
E4. ERS Genomics press release
E5. DDN news article
E6. bit.bio Companies House record and website combined
E7. Testimonial from CFO of bit.bio
E8. Hepatotarget Therapeutics certificate of incorporation
E9. Bilitech Innovate and UKRI funding combined (pages 18 and 32)
E10. Bilitech Companies House record (page 3)
E11. Testimonial from the Director of Strategic Innovation at Cystic Fibrosis Trust