Institution: University of Cambridge		
Unit of Assessment: 5: Biological Sciences		
Title of case study: Next generation growth factors for regenerative medicine		
Period when the underpinning research was undertaken: 2005 – present		
Details of staff conducting the underpinning research from the submitting unit:		
Name(s):	**Role(s) (e.g. job title):**	**Period(s) employed by submitting HEI:**
Marko Hyvönen	Reader in Protein Biochemistry	2001 - present
Period when the claimed impact occurred: 2016 - present		
Is this case study continued from a case study submitted in 2014? N		

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

Stem cell research is providing new avenues for both therapy and diagnosis but has been limited by the low availability of high-quality, affordable and consistent growth factors, which are required to maintain stem cells in culture. Using his expertise in basic research on growth factors, Dr Hyvönen at the University of Cambridge has developed a platform that represents a step-change in the production of growth factors through research undertaken between 2006 and 2019. His methodology for producing large amounts of high-quality growth factors led to the establishment of his spin-out company Qkine in 2016. This has since grown to encompass a portfolio of 16 products, employing 10 staff and exporting growth factors to Europe, Asia and North America. Qkine has generated [text removed for publication] in total sales and has generated over GBP 2,000,000 in venture capital investment.

2. Underpinning research (indicative maximum 500 words)

Activins are growth factors that bind specific cell receptors to control many cellular behaviors, including tissue growth and function. Activins are essential for maintaining stem cell pluripotency, making them of considerable importance in stem cell research and its clinical outputs. Progress in the field of stem cell research has laid the foundation for cell-based therapies of disease that cannot be cured by conventional medicines (Mahla, Int. J Cell Biol., 2016) but in order to meet this potential, research and clinical development need stable cell systems with which to work.

Activin signalling is regulated by the activin-binding protein follistatin, an important antagonist in the extracellular matrix, which neutralises activin's actions. However, activin also induces follistatin expression, generating a negative feedback loop that limits the extent and duration of activin signalling, restricting its use. Upregulation of Activin A (made of two alpha subunits; activin B is two beta subunits; activin AB is one of each), drives pluripotent stem cells into a mesoendodermal fate, i.e. early developmental and able to form all cell lineages, and thus provides a useful tool for stem cell differentiation and organoid formation.

Dr Marko Hyvönen at the University of Cambridge has dedicated research towards understanding activin A signalling at the molecular level. This required the production of significant quantities of activin A, but relied on methods that yield very little active protein. This led to Dr Hyvönen developing his own novel method capable of generating large amounts of high quality activin A. By 2003 Dr Hyvönen had developed and refined this core technology, enabling him to determine some of the first structures of activin A in complex with follistatin [R1]. He was able to do this entirely animal-free, the first time this had been

achieved. This new production method, which does not rely on the use of animal-derived products, means the growth factors can be used for clinical grade human stem cell cultures. Dr Hyvönen and colleagues made the unexpected finding that mutations at certain positions in the amino acid sequence of activin A dramatically increase the resistance of activin to follistatin inhibition without affecting its biological signalling activity, providing increased signalling potency in cell culture. Dr Hyvönen patented his methodology for producing a modified activin in 2018 [R2].

Dr Hyvönen saw the opportunity to use this method to produce other growth factors and used the technology to synthesise proteins which are widely used in stem cell research. This includes bone morphogenic proteins (BMPs), which were being increasingly used in bone regeneration treatments but without an identified optimal delivery system. Dr Hyvönen and colleagues synthesised BMPs and combined them with a novel nanocomposite (α-TCP/PLGA). They discovered the material produced osteo-induction and had potential application as an osteo-inductive agent in regenerative medicine [R3]. He also researched the interaction between BMPs and Gremlin-1, which regulates BMP signalling. Little was known about the molecular mechanism by which Gremlin-1 inhibits BMP signalling, but Dr Hyvönen and colleagues found that Gremlin-1 inhibits BMP by a distinct mechanism compared to other known inhibitors which led to them proposing a novel model of interaction for BMP and Gremlin-1. They found that Gremlin-1 can attach to more than one BMP molecule at a time, so BMP and Gremlin-1 form large complexes that assemble in alternating fashion [R4].

In 2019, Dr. Hyvönen showed how this technology could be applied in organoid media, which is used to study how cells interact together, the effect of diseases and to test the effectiveness of drugs. His technology of expression in bacteria [R5] was able to produce highly pure recombinant Gremlin-1 and R-spondin-1 growth factors, critical components in organoid media that were not previously widely available. In the course of this work it was clearly demonstrated that the new method was superior to anything else available in terms of yield and purity, and also could be more widely applied to other proteins.

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

R1. Harrington, A.E., Morris- Triggs, S.A., Ruotolo, B.T., Robinson, C.V., Ohnuma, S., **Hyvönen, M**., Structural basis for the inhibition of activin signalling by follistatin, EMBO Journal, 2006 25:1035-1045, DOI: 10.1038/sj.emboj.7601000*

R2. FOLLISTATIN-RESISTANT ACTIVIN. UK patent application number 1709636.3, Filed 16 June 2018, published 20 December 2018

R3. Sharma, A., Meyer, F., **Hyvönen**, **M**., Best, S.M., Ruth E. Cameron, R.E., Neil Rushton, N. Osteoinduction by combining bone morphogenetic protein (BMP)-2 with a bioactive novel nanocomposite, Bone Joint Res. 2012 1:145-51, DOI: 10.1302/2046-3758.17.2000082*

R4. Kišonaitė, M., Wang, X., and **Hyvönen, M.,** Structure of Gremlin-1 and analysis of its interaction with BMP-2. Biochemical Journal 2016 473:1593-1604 DOI:10.1042/BCJ20160254*

R5. Urbischek, M., Rannikmae, H., Foets, T., Ravn, K., **Hyvönen, M.,** and de la Roche, M. Organoid culture media formulated with growth factors of defined cellular activity. Scientific Reports 9: 6193. 2019 DOI: 10.1038/s41598-019-42604-0*

*Research outputs have been published in peer-review journals.

Competitive funding received
2008 – 2008 Production of recombinant activins and other TGFB growth factors for stem cell applications, BBSRC, PHZF/120, GBP 87,829 [RG 51204]

2017 – 2019 BBSRC Super Follow-on Fund BBSRC, PHZJ/555, GBP 412,877 [RG 87768]

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

Dr Hyvönen's basic research at the University of Cambridge on growth factors enabled him to develop a new way of producing exceptionally high purity growth factors and cytokines necessary for stem cell research. In the current REF period, the commercialisation of this technology has led to the establishment of a new company in 2016 with the creation of 10 new jobs, new investment of over GBP2,000,000 and a catalogue of 15 specialised products generating income of [text removed for publication] in total sales. Continued success of licensing agreements generated revenues in the reporting period of [text removed for publication].

Economic impact and job creation

In 2016 Dr Hyvönen established Qkine, a spin-out company, to commercialise this new technology for high quality activin A proteins which fills a major gap in the market [E1]. The manufacture of these proteins was transferred to Qkine in 2017, which generated early revenue and was an important factor in attracting seed investment [E1]. Qkine has grown strongly and steadily, highlighted as one of the "10 Cambridge-based start-ups to look out for in 2019 and beyond" [E2]. Between December 2017 and April 2019, Qkine was embedded in the Department of Biochemistry at the University of Cambridge. After successful completion of an Innovate UK funded project to develop growth factors for organoid culture [E1], Qkine received GBP 550,000 investment from Cambridge Enterprise and five angel investors which allowed it to open dedicated laboratories on the Cambridge Science Park in 2019 [E3]. Since 2016, Qkine has generated [text removed for publication] in total sales [E1]. Qkine now employs 10 staff including five scientists, marketing and scientific operations managers, and a senior management team of three [E1]. Dr Hyvönen continues to provide scientific guidance and practical insight to Qkine's scientists as the company's Chief Scientific Officer (0.1 FTE) and has supported the development of other high-quality products [E1], described below.

Products on the market

Qkine's product range now comprises 15 products, four of which were licensed from Dr Hyvönen's lab [E1]. These include growth factors such as Gremlin-1, a BMP inhibitor for several embryonic and organoid culture systems, and GDF15 (human growth differentiation factor 15), an exciting new cellular stress biomarker. Their latest offering, TGF (transforming growth factor) beta 1 is the first entirely animal-free recombinant human version of this protein, which has been extensively tested for maintenance of iPSC pluripotency by the specialist stem cell biotechnology company, Stemnovate; this product represents a unique offering in the market by Qkine [E4].

There are few primary manufacturers of the complex bioactive proteins made by Qkine, so the proprietary protein engineering and refolding technology developed from Dr Hyvönen's research strengthens the supply chain for stem cell research, biotechnology and drug discovery assays, with a focus on quality in terms of specific bioactivity. When Qkine secured its latest investments (details below), Chief Investment Officer of Parkwalk, a leading investor in university spin-out companies, commented: "There is a clear market need for high-quality protein reagents, especially for applications in stem cell biology and organoid research. Qkine has…a compelling technology to meet this need…" highlighting the unique position held by the company in the market. [E5]

Customer satisfaction

Many of Qkine's customers are based in the UK, however Qkine also serves a growing base of customers in mainland Europe and Nordic countries, Asia, the US and Canada [E1]. Customers are satisfied with the quality of products received from Qkine. The CEO of a customer, a biotech company specialising in the production of highly-predictive human cell disease models based on stem cell technology, said that growth factors purchased from

Qkine "have proved to be effective and reliable for the production of [their] cell products". They use activin A as a central factor in their industrial process and find the cost "attractive compared to alternative suppliers". They go on to say that "the consistency of supply, scale of supply and crucially the quality of the Qkine reagents do…confer a competitive advantage to Qkine in the stem cell reagent space. Procurement of quality reagents from Qkine has been an important aspect of generating products for [our] customer base, and we will continue to use them as a trusted supplier of high-quality reagents." as part of the production of their disease models for global pharma, biotech companies in Europe, the US and Japan, and academic groups. [E6]

Investment

The ability to supply high quality activin A protein commercially was important for generating early revenue and helped attract seed investment [E1]. In 2017, the company was awarded an Innovate UK grant worth GBP 68,500 for its next generation growth factors for organoid-driven precision medicine [E7]. In 2018, Qkine won a place on Accelerate@Babraham, a new initiative designed to support ambitious early-stage life science ventures, providing GBP 20,000 of non-dilutive funding, initial laboratory and office space, mentoring and business support [E7]. Since 2016, Qkine has secured investment worth more than GBP 2,000,000, including GBP 1,500,000 in June 2020 [E3, E8]. Their international customer base will expand due to this recent investment, which will allow them to roll out full commercial operations in East Asia, the US and Europe [E9].

Licensing agreements and direct protein sales

Technology derived from Dr Hyvönen's research has yielded continuing royalties through several licensing agreements. Production of mature, bioactive activin A from *E.coli* was licensed to [text removed for publication] in 2009; technology for producing an engineered form of activin A was also licensed to [text removed for publication] in 2011; in 2005 Dr Hyvönen provided purified activin B to a senior professor at [text removed for publication]. The total royalties received for licences to Dr Hyvönen's inventions in the reporting period 2013-2020 is [text removed for publication]. [E9]

Translation of research

The provision of purified activin B to [text removed for publication] in 2005, enabled the development of a highly sensitive diagnostic assay for inhibin B, a key test for fertility clinics linked to the involvement of activin and inhibin in regulation of the menstrual cycle and, specifically, follicle maturation. The assay exclusively licensed to [text removed for publication], a US corporation, which manufactures, markets and distributes the assay in many territories worldwide [E11. Since the second-half of 2013, [text removed for publication] have had annual sales of the assay totalling [text removed for publication] [E11].

Dr Hyvönen has taken his expertise in fundamental knowledge of cell biology, and research conducted at the University of Cambridge, to develop technology that represents a step-change in the production of growth factors, meeting a demand from those working in areas such as stem cell research, regenerative medicine, and diagnostics. Through commercial endeavours his work has led to provision of necessary reagents, with downstream benefits, as well as direct economic outcomes such as job creation.

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

E1. Qkine testimonial
E2. EU start up article highlighting Qkine as one of Cambridge start-ups to watch 2019
E3. Cambridge News article highlighting investment and Qkine move to dedicated premises
E4. Screenshot of Qkine online catalogue
E5. Screenshot of UKTN news article, Jun 2020
E6. Customer testimonial
E7. Innovate UK funded projects 2017, page 22
E8. Accelerate@Babraham funding

E9. Business cloud article highlighting GBP 1,500,000 investment to Qkine
E10. Licencing revenues
E11. Sales of diagnostic assay