Modelling Neonatal and MODY Diabetes *in Vitro* Using IPS Cell-derived human Pancreatic Beta Cells

Filipa Soares [1], Alex Armesilla-Diaz [2], Kalpesh Jhaveri [1], Rodrigo Santos [2], Tina McRunnel [1], Christine Schofield [2], Ludovic Vallier [1,3], Chris Lowe [2], Yeo, Marcus[1]

[1]DefiniGEN Ltd, Babraham Research Campus, Cambridge , UK. [2] Horizon Discovery Group Plc, Cambridge Research Park, Waterbeach, UK. [3] Cambridge Stem Cell Institute, University of Cambridge, UK

Introduction

DefiniGEN is a spin-out company from the University of Cambridge which utilises its directed differentiation platform OptiDIFF to provide high-functionality human cells to the drug discovery sector to enable the development of preclinical screens with improved predictivity of human health and efficacy. This platform has been combined with the CRISPR gene-editing platform by leading Cambridge life science company Horizon Discovery Ltd. The platform is generating monogenic IPS disease models for A1ATD, Familial Hypercholesterolemia, Neonatal and MODY diabetes which will be validated at both the genotypic and phenotypic level using an array of biochemical methodologies. Future work will focus on combined directed differentiation and genome-editing approaches to generate disease models for complex diseases such as diabetes type 2.
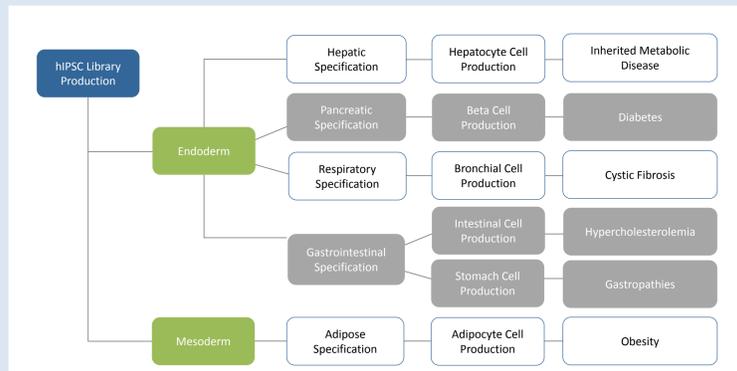

Figure 1. OptiDiff Platform. DefiniGEN is focussed on the production and disease modelling of related cell types including liver, pancreas, lung, intestinal and stomach cells.

Advantages of combining IPSC direct differentiation with CRISPR technology

Human IPSC circumvent many of the ethical and commercial barriers associated with embryonic stem cells as they are derived from adult cells that have been reprogrammed to the stem cell state. Stem cells have two major properties:

1) they can form any cell type of the body.
2) they can self-renew indefinitely, producing a limitless supply of cells.

Genome editing and CRISPR/CAS9 technology are an efficient system to introduce mutations to healthy donor lines whereby isogenic control can be used to distinguish disease-relevant changes. Healthy human IPSC lines can be edited using CRISPR/CAS9 technology to create KI/KO mutants that will help study gene function and develop therapeutic approaches.

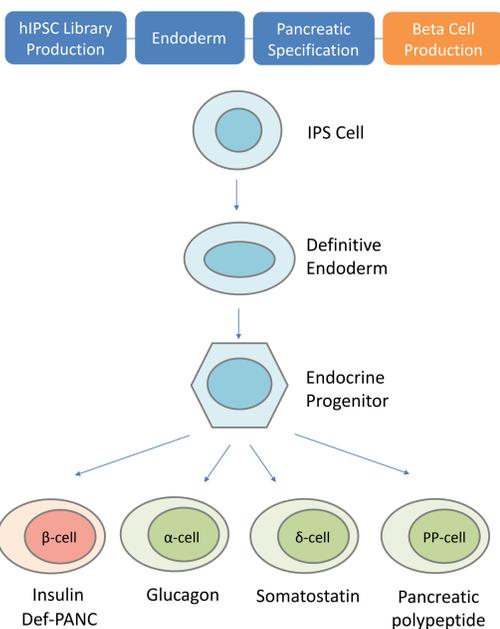## IPSC-derived Pancreatic Beta Cell Production

Figure 2. Def-PANC Pancreatic Beta Cell Production. Overviews the production of Def-PANC human pancreatic beta cell-like cells from human IPSC, using the OptiDIFF technology platform.

	Stage 1	Stage 2	Stage 3	Stage 4	Stage 5
E8	CDM-PVA	ADV-BSA	ADV-BSA	ADV-BSA	ADV-BSA
A/F	A/F/B/Ly/CHIR	RA/NOG/F10/SB	RA/NOG/F10/CYCP	B27/DAPT	B27
Day 0	Day 1-3	Day 4-6	Day 7-9	Day 13-15	Day 16-24

hIPSC → DE → Foregut → Pancreatic Progenitors → Endocrine Cells

Figure 3. Protocol to generate endocrine progenitors from hIPSCs. The culture conditions used to direct the differentiation of human IPSC to pancreatic endoderm. A: Activin, F: FGF, B: BMP, Ly: Ly294002, CYCP: Cyclopamine, NOG: Noggin, DAPT, SB and RA: Retinoic acid.
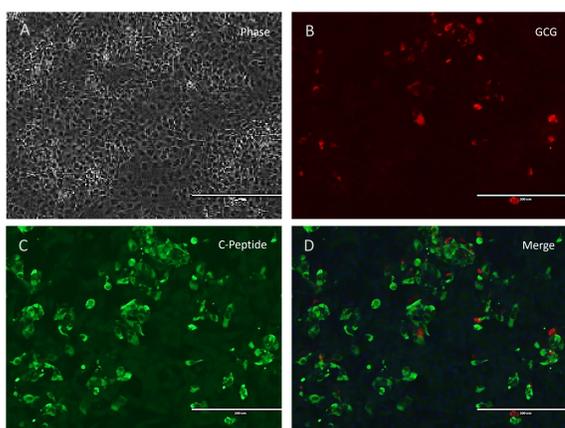

Figure 4. Immunostaining analysis of live Def-PANC pancreatic beta-like cells. A) Phase contrast image of pancreatic beta cell-like at the end of the differentiation process. Immunostaining confirms expression of glucagon (red) (B) and C-peptide (green) (C). Image (D) shows co-staining for glucagon (red), C-peptide (green) and DAPI (blue).
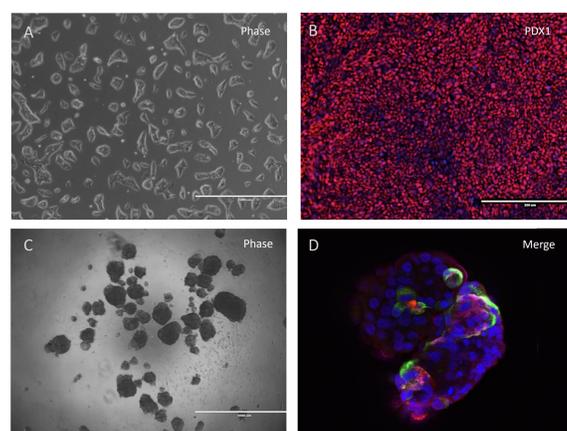
Cryopreserved IPSC-Derived Beta-Like Cells

Figure 5. Immunostaining analysis of Def-PANC pancreatic beta-like cells. A) Phase contrast image of hIPSC at the start of the differentiation protocol. B) Immunostaining confirms expression of PDX1 (red) and DAPI (blue) at the pancreatic progenitors stage. C) Phase contrast image of Def-PANC islet-like structures at the end of the differentiation protocol. D) Confocal image of a Def-PANC islet-like structure showing co-staining for glucagon (red), C-peptide (green), somatostatin (magenta) and DAPI (blue).
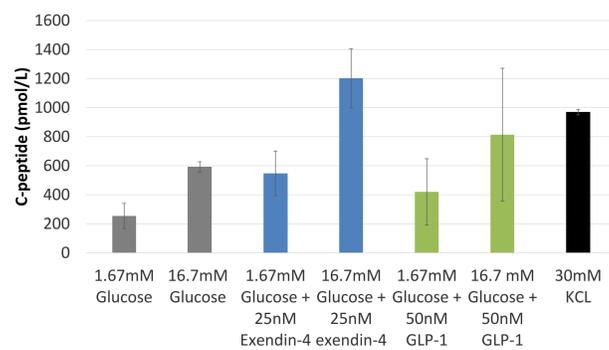

Figure 6. Glucose stimulated insulin secretion (GSIS) assay for cryopreserved Def-PANC using 96wp low adherent plates. Low and high glucose medium contains 1.67mM and 16.7mM of glucose, respectively. Compounds Exendin-4 and GLP-a are added at a concertation of 25nM and 50nM, respectively. KCL is added to the cells at 30mM. The graph shows the average of three different wells.
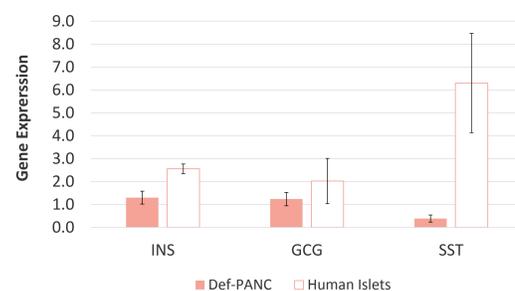

Figure 7. Relative expression of key pancreatic markers for cryopreserved Def-PANC using 96wp low adherent plates in comparison with Human islets. Relative expression was calculated for each gene of interest by comparison with the endogenous control human Glyceraldehyde-3-phosphate dehydrogenase (GAPDH) and the $2^{-\Delta CT}$. The graph shows the average of three different batches of Def-PANC. INS: Insulin, GCG: Glucagon and SST: somatostatin.

IPSC Gene Editing Using CRISPR

Neonatal Diabetes: KCNJ11

Neonatal diabetes mellitus is a monogenic form of diabetes that is diagnosed within the first 6 months of life. About 50% of the cases lead to permanent condition. The most common cause is the mutation in KCNJ11 gene which plays a major role in ATP-sensitive potassium channel (K_{ATP}) thus impairing insulin secretion.
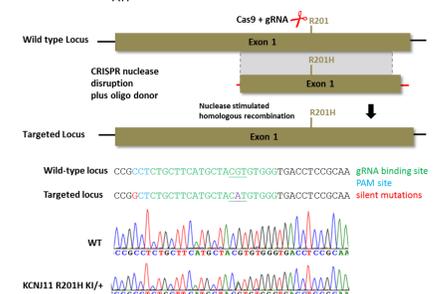

Figure 8. Schematic representation shows introduction of the most common KCNJ11 KI mutation (R201H) using CRISPR/CAS9 technology. Sanger sequencing data indicates the correct incorporation of the mutation CGT>CAT at codon 201.

MODY: HNF1a

MODY (Maturity onset diabetes of the young): is a monogenic form of diabetes which causes dysfunction of beta cell. Most common cause of MODY is the mutation in hepatocyte nuclear factor 1a (HNF1a) which accounts for about 50-70% of MODY cases. Patients with MODY tend to have hyperglycaemia which increases over time which results in progressive reduction in insulin secretion.
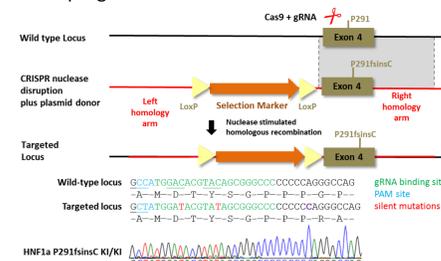

Figure 9. Schematic representation shows introduction of the most common HNF1a KI mutation (P291fsinsC) using CRISPR/CAS9 technology. Sanger sequencing data indicates the correct insertion of C at codon 872 leading to frameshift mutation.

Summary

Five step process for the generation of pancreatic diabetes disease models powered by Horizon CRISPR gene-editing technology:

1. CRISPR Design and Validation - vector design, synthesis and validation
2. CRISPR-modified IPS Cell Generation - screening, verification, expansion, and banking
3. Differentiation - using OptiDIFF platform to generate a range of cell types
4. Cryopreservation and banking of pancreatic beta cell products
5. Product Validation - genotype, phenotype and cell marker analysis