3.1 Publishable summary

A summary description of project context and objectives

The scientific objective of HumEn is to understand the balance between differentiation and proliferation in embryonic progenitor cells of the human endoderm lineage with the aim of achieving robust generation of functional beta cells from hPSCs (hESCs)/hiPSCs).

Ultimately, these results are expected to be essential for up-scaled beta cell generation for cell-replacement therapy.
Despite progress in producing beta cells from hESCs, full differentiation has not been obtained in vitro. Currently, fully functional beta cells are generated from hESCs by grafting uncommitted beta cell progenitors into mice for long and "uncontrolled" maturation (>3 months) in vivo.

HumEn's starting point is to understand the cues that promote and halt differentiation at different points in the progression of hPSCs to beta cells as a means to develop "real" beta cells from hPSCs in vitro. The scientific objective is to exploit these cues as a means to identify and expand endodermal progenitors as a consistent and renewable source of cells for pancreatic beta cell differentiation.

HumEn focuses on precursors from two stages of pancreatic differentiation; definitive endoderm (DE) and pancreatic endoderm (PE) progenitors. Thus, HumEn will provide mechanistic insight into the signaling pathways and downstream targets that control the expansion of DE and PE and their differentiation into functional beta cells. Rigorous in vitro (regulated insulin-release) and in vivo (protection against experimentally induced Diabetes in mice) testing of insulin-producing cells will ensure a functional end product.

Main results during the 2nd period

New valuable research tools for isolating ADE were generated, including the HHEX-Cherry/FoxA2 double hESC reporter. The use of the new ADE reporters made it possible to develop conditions to expand ADE/VFG without affecting its ability to give rise to PDX1+ pancreatic progenitors. This provides a potential strategy for scaling up the generation of INS+ beta cells. We have also identified new cell surface markers that segregate ADE into a liver- and pancreas-generating ADE sub-populations.
The new cell surface marker for PDX1+ pancreatic progenitors has been confirmed to work independent of which differentiation protocol or hESC line is used. Thus, we have strengthened the evidence for the usefulness of the new cell surface marker in isolating PDX1+/NKX6-1+ progenitors capable of differentiating into glucose responsive INS+ cells. Preliminary data suggests that the new cell surface marker can not only be used for FACS, but also for magnetic cell sorting (MACS). Identification of additional cell surface markers have extended this work to permit isolation of endocrine progenitors and their hormone-producing derivatives from the human fetal pancreas.
Exciting new results have identified several polymers which increase the proliferation of hESC-derived PDX1+ pancreatic progenitors, suggesting new avenues for expanding pancreatic endoderm in vitro. In addition, using candidate and unbiased (Zebrafish chemical screen) approaches we have identified signaling pathways capable of increasing the number of Pdx1-expressing cells in vitro (hESCs) and in vivo (Zebrafish).

In our efforts to test how cell confinement (spreading) affect the fate of PDX1+ progenitors, we have discovered that changes in cell confinement at the single cell level affects conversion of PDX1+ progenitors into the endocrine lineage. Furthermore, in an unbiased screen in Zebrafish several compounds were identified as potential regulators of beta cell maturation.
Altogether, the project has made significant progress towards its main objective to identify hESC-derived expandable endodermal progenitors capable of generating INS+ beta cells and ways to regulate their expansion in vitro.

The expected final results and their potential impact and use (including the socio-economic impact and the wider societal implications of the project)

HumEn will contribute to the strategic objective of developing the knowledge, tools, and resources needed to exploit the full potential of pluripotent stem cells and apply it to human health while at the same time stimulating industrial and economic activity. It will fulfil EU policies targeted at strengthening the competitiveness of the European economy, generating a knowledge-based economy, and solving major societal questions. Through its contribution to the development of new regenerative cell therapies, HumEn will - on the level of the individual citizen - improve the future quality of life for people diagnosed with Type 1 and Type 2 Diabetes.

In relation to the overall objectives of the Health Theme, the approach taken in HumEn is focused on improving the health of European citizens, increasing the competitiveness and boosting the European innovative capacity of Europe. HumEn will specifically contribute significantly to the translation of basic discoveries into clinical applications related to new cell-based therapies. The SME-specific objectives are to emphasize the importance and integration of SMEs in health research projects. Supporting the advanced research and technology development of the two SMEs involved in HumEn, the proposal will improve the capacity and quality of the SME technologies while reducing costs, thereby significantly strengthening their competitiveness and opening up for new business opportunities. In particular, the stem cell market for cell therapy and drug discovery applications shows huge potential in the years to come for the SMEs involved in this proposal and HumEn will allow them to demonstrate the application of their innovative technologies in this field.

HumEn will deliver a reliable and scalable protocol for directed differentiation of hPSCs and expandable intermediates into bona fide beta cells. Human islet transplantation provides proof-of concept that cell-replacement therapy can restore a functional beta cell mass in Type 1 Diabetes patients. However, the lack of donor pancreases limits the number of patients who can benefit from this therapy.

Therefore, one of the most urgent goals towards treatment of Type 1 Diabetes is to identify an alternative source of unlimited supply of beta cells.

The facts that hESCs can self-renew forever and differentiate into functional beta cells in vivo but very inefficiently in vitro, indicate that one of the most urgent priorities at this point is to understand why it is so difficult to generate glucose-responsive beta cells in vitro. Moreover, while it is not practical to differentiate hPSCs directly to beta cells when needed, the creation of frozen stocks of endodermal progenitor cells (derived from hPSCs) that could readily be transformed to functional beta cells could potential meet the clinical need of TID patients.

HumEn seeks to provide several routes to this goal, with the promise of cost efficient beta cell generation from a number of potential hPSC derived sources. Creation of new knowledge: HumEn will create new knowledge, which will significantly contribute to solving key bottlenecks towards generating functional beta cells for future cell therapy in diabetes, as well as creating more relevant beta cell models for in vitro drug discovery and target validation.

In addition HumEn goes one step further; it attempts to solve the problem of efficient beta cell generation, from accessible and storable endodermal intermediates. The differentiation of these cell lines to beta cells should be less complex (avoid unnecessary early steps), less expensive and more efficient.

Specifically, HumEn will deliver:

- Knowledge, tools, and resources for exploiting the full potential of Pluripotent Stem Cells.
- Contribution to development of new regenerative cell therapies for Diabetes.
- A new, in depths understanding of the balance between differentiation and proliferation in embryonic progenitor cells of the human endodermal lineage.
- Mechanistic insight into the signaling pathways and downstream targets that control expansion of ADE and PE, and their differentiation into functional beta cells.
- Robust generation of functional beta cells from hESCs/hiPSCs.

Our studies on utilizing key check points regulating the balance between renewing cell divisions and differentiation will also have application to hPSC models in general, including organ systems from all three germ layers. HumEn contains both world leaders in endoderm and pancreas developmental biology as well as world leaders traditionally outside of the endoderm field including material science and epigenetics enabling effective cross-fertilization.

Development of new techniques: By combining basic mechanistic research with various small molecule screens and bioengineering, HumEn will provide new innovative technologies, including functionalised 3D and micropattern culture systems for expansion of human anterior definitive endoderm and pancreatic endoderm and differentiation of pancreatic endoderm into beta cells. Potentially this will include the generation of new and widely applicable endodermal stem/progenitor cell lines. In addition, detailed protocols for generating hESC reporter cell lines using gene targeting by homologous recombination will be provided. An in vitro model for human beta cells would also represent an important system to decipher disease mechanisms involved in Type 1 and Type 2 Diabetes.

Translation of the research results to drug discovery: In addition, these hES/iPS derived human beta cells, potentially polarized on micropatterned substrates, could yield a more relevant phenotype in vitro and represent a major progress towards a physiologically relevant in vitro cell based assay to decipher disease mechanisms involved in Type 1 and Type 2 Diabetes, carry out target validation as well as drug screening against Diabetes.

Translation of research to the clinic:

Almost 35 million people worldwide are diagnosed with Type 1 Diabetes and WHO foresees that Diabetes deaths will double between 2005 and 2030. Cell replacement therapies for Diabetes have been implemented in the clinic and have become increasingly efficient since the development of the Edmonton

protocol, leading to a better control of blood glucose over time. As the beta cell supply from deceased donors is limited, the generation of beta cells from hESCs will thus be important for diabetic patients. Bringing us closer to the generation of glucose responsive insulin producing pancreatic beta cells from different stem cell systems will significantly improve treatment of Diabetes, heightening quality of life for diabetics and lowering the costs for treatment of Diabetes related complications.

HumEn will contribute to the first three steps in the overall road-map towards stem cellreplacement therapy of Diabetes, specifically, by addressing at a mechanistic level how hPSCs efficiently can be coaxed into transplantable beta cells, i.e. glucose-responsive beta cells, via their endodermal progenitors, STEP 1 will be accomplished. These new differentiation protocols will then be ready to be plugged in for production of GMP-grade beta cells.

HumEn's thorough in vitro and in vivo functional validation of insulin-producing cells provides a state-of-the-art platform for STEP2, i.e. preclinical functional validation of clinical relevant cells. Lastly, by performing careful testing of the ability of clinically relevant beta cell populations to form tumours in mice part of STEP3 will be achieved.

The address of the project website:
Public website: http://www.hum-en.eu/
Members' area (project intranet): http://www.hum-en.eu/member-area[1]

[1] For requesting an access to the project intranet, please contact: naomi.dayan@sund.ku.dk