IMI1 Final Project Report
Public Summary

Project Acronym: IMIDIA
Project Title: Improving beta-cell function and identification of diagnostic biomarkers for treatment monitoring in diabetes

Grant Agreement: 115005
Project Duration: 01/02/2010 - 30/09/2015
1. Executive summary

1.1. Project rationale and overall objectives of the project

A complete or relative decrease in insulin secretion by pancreatic beta-cells underlies the development of type 1 and type 2 Diabetes, respectively. These diseases impose a huge burden to welfare systems, both in Europe and in other developed and developing countries. So far there are limited therapeutic options to treat both types of diabetes and none to cure or prevent the disease. This is due, in large part, to our limited knowledge of beta-cell biology in health and disease. Although a large body of knowledge has been gained on the function of beta-cells from animal models, knowledge on human beta-cell function, survival, and of the pathophysiological mechanisms that lead to their demise is still scarce.

The IMIDIA consortium, which consists of 14 leading European academic experts in the biology, physiology, and genetics of islet cells and in bioinformatics applied to systems biology, together with 8 major pharmaceutical industries and 1 SME, is an ambitious project to generate novel tools, biomarkers, and fundamental knowledge on islet-cell organization to accelerate the path to improved diabetes management.

1.2. Overall deliverables of the project

The scientific program aims at delivering:

1-Novel tools for the study of human beta-cell development, function and survival; their modulation by potential therapeutic compounds; and for in vivo beta-cell imaging.

2-Biomarkers for the diagnosis and prognosis of beta-cell failure and for monitoring diabetes progression and treatment.

3-Knowledge on novel pathways and sites that control beta-cell proliferation, differentiation and apoptosis, and on the role of known nutrient regulated pathways and sites in controlling beta-cell mass and function.
1.3 Summary of progress versus plan since last period

WP1:

Following the first description of a human beta-cell line (BetaH1), new generation of human beta-cell lines have been generated. The most recent one (BetaH3) was derived from BetaH2 to express an tamoxifen-inducible form of the CRE recombinase to allow conditional removal of the proliferation gene. The use of human beta-cell lines and the functional assessment has been validated by pharma partners. A new in vitro protocol has been established to discover conditions to study pancreatic progenitor cell proliferation and differentiation and factors have been characterized that induce in vivo functional beta cell differentiation from pancreatic progenitors. Several monoclonal antibodies raised against the human cell line have been subjected to antigen identification using proteomics approach (mass spectrometry analysis) – and a manuscript is in preparation.

WP2A:

Following successful completion of the bioinformatics analysis aimed at identifying modules of beta-cell-expressed genes that underlie the adaptation of beta-cells to metabolic stress, work has progressed in the study of hub genes that may regulate specific pathways required for beta-cell function. These studies involved partners of WP2A, 2B and 3. These genes are potentially associated with: i) lipid metabolism, ii) Fe” and Cu” beta-cell utilization, iii) regulated endocytosis, iv) energy production by the mitochondria, v) beta-cell transcription factors controlling glucose-stimulated insulin secretion. Specific genes under investigation are: Elovl2, CPT1A (involved in lipogenesis and ceramide pathway), shank3 (involved in exocytosis), Kcnj5 or GIRK4 (potassium inwardly-rectifying channel involved in exocytosis), Prokineticin 2 (involved in exocytosis), NDUF8 involved in oxidative phosphorylation, Klf6 involved in transcriptional regulations. Plasma lipid modules have also been identified that correlate with insulin secretion and insulin action. These modules have been validated as biomarkers predictive of diabetes susceptibility in a prospective cohort (DESIR); validation is being confirmed in a second prospective cohort (CoLaus), as part of ENSO (WP2). Physiological studies have been performed in mice with alpha cell ablation or with ablation of ~50 percent of the beta-cells. They show an unexpected increase in beta-cells in alpha cell-ablated mice. RNASeq analysis of islets from these mice has been performed.

WP2B:

WP2B team has continued to assemble the world largest biorepository of human islets, which includes islets from non diabetic individuals and from individuals with T2D, impaired glucose tolerance, and diabetes associated with pancreatic disorder (T3D). Islets have been collected form organ donors and from partial pancreatectomy tissues. Genetic, transcriptomic, lipidomic, microscopic, and functional analyses of the collected islets have been performed. Bioinformatic analyses of the sets has been refined over the last year and led to the identification of a restricted set of differentially expressed genes between T2D and NDs islets. A small set of genes similarly differentially expressed in organ donor and partial pancreatectomy samples has been selected for experimental validation. eQTL analysis of the RNASeq data together with genotyping information
from the same islets has been performed. Manuscripts describing these major achievements are now being finalized.

WP2 ENSO

In the previous year, we had identified in the preclinical studies of WP2A a lipid module, which correlates with insulin secretion and insulin tolerance. This lipid module has been tested in the plasma of subjects from the DESIR cohort, a prospective cohort of 6000 individuals followed for a period of 9 years for the development of diabetes. We found that this lipid module was elevated in the plasma of the individuals who would develop T2D, even after 9 years of follow-up. This module was significantly lower in the individuals who would never develop T2D. We have now replicated this study in a separate prospective cohort (CoLaus) analyzing this lipid module in 150 control individuals and a similar number of individuals who had progressed over time to T2DA. Data have been generated and are being analyzed.

WP3:

Almost all lines of investigation by WP3 partners have now led to publications on the role of selected signaling pathways that convey information of whole body metabolic status on beta-cell function. These include publications on the connectivity between beta-cells as regulated by gluco-incretins; genes that are up-regulated upon LKB1 knockout in beta-cells, a condition that increases these cell proliferation, and which includes glutamatergic signaling; a new regulator of glucokinase activity, Midnolin; the regulation and role of autocrine IGF2 biosynthesis and secretion on beta-cell mass and function; the identification and functional characterization of a new pro-apoptotic gene expressed by beta-cells (Clic4); the role of FXYD3 in controlling beta-cell glucose competence; new SOFIA reporter mice that allow the precise study of insulin granule dynamics and aging. Finally, mice that were generated in previous years to express lentiviral (TVA) receptors on beta cells and beta-cell precursors have been used to test cell-type specific delivery of gene constructs to modify gene expression. Although in vivo infection of these cells remains a challenge, it was demonstrated that using these mouse models, mature beta-cells as well as beta-cell precursors could be efficiently infected by the viruses in vitro.

WP4:

Progress has been made in 1) validating PSA-NCAM as a marker of beta-cells in human pancreas and isolated human islets; 2) the development of Zn++ probes coupled to Gadolinium and used for in vivo MRI experiments to detect beta-cells; 3) the development of ultra small iron oxide particles coupled exendin-4 to visualize GLP-1 receptor expressing beta-cells. This probe has been used in MRI studies in mice and shown to faithfully report on the decline in beta-cell mass using the DTR system that allows reduction of beta-cell mass by 50% or 100%; 4) validating the use of treatment of mice with
the insulin receptor antagonist S961 to increase beta-cell mass to further validate the exendin-4 MRI probe in vivo, 5) further studies of the VMAT2 marker DTBZ to assess beta-cell mass in pigs have been conducted.

WP5:

This work package continues to provide outstanding support and resources for the storage of data and their retrieval for bioinformatics analysis. The data from all WPs are stored in structured ways and are now transformed into RDF and CDISC formats in provision of future opening of the database to the public at large. Bioinformatic analysis of IMIDIA data have generated hypothesis for plasma biomarker candidates, pathways and gene modules involved in the pathogenesis of diabetes and the identification of novel biomarkers of beta-cell mass and function related to the studies of WP2A, B and WP2ENSO. One additional aspect of this WP is the training and workshop activities designed to trained IMIDIA young scientists for the independent use of bioinformatic tools to mine the IMIDIA database. This bioinformatics resources has continued to be of vital need for conserving and exploiting the data gathered during IMIDIA. A sustainability plan is being finalized to ensure that the IMIDIA data can still be used over the next years with a precise plan for preserving access to IMIDIA partners and providing access to new interested parties.

1.4 Significant achievements since last report

- Generation of a third generation human beta-cell lines with conditional growth-arrest control.
- Characterization of novel human beta-cell surface monoclonal antibodies.
- Identification of culture conditions and in vivo factor that control beta-cell progenitor differentiation.
- Replication study completed to further validate, in a prospective human cohort, a plasma lipid biomarker for diabetes susceptibility.
- Extensive characterization of the role in beta-cell biology of several genes identified in the Systems Biology investigation of beta-cell susceptibility to metabolic stress.
- Identification of novel human genes differentially expressed in islets from diabetes patients and identification of their role in human beta-cell function.
- Development of new imaging tools for beta-cell mass and demonstration of their validity as beta-cell markers in vitro and as imaging agents in vivo using various animal models and MRI approaches.
- Continued database structuration and adaptation of data format to CDISC, and integrated bioinformatics analysis of mouse and human beta-cell gene modules and plasma lipidomic data to develop new predictive biomarkers of beta-cell function and diabetes susceptibility.
1.5 Scientific and technical results/foregrounds of the project

Scientific results:

New knowledge was gained along the following axis:

- Definition of novel conditions for isolation and in vitro culture of beta-cell precursors.
- Identification of factors required for in vivo differentiation of beta-cell precursors.
- Identification of genes modules underlying beta-cell adaptation or resistance to metabolic stress. Characterization of the association of these gene modules with specific phenotypes or dysfunctions of glucose homeostasis.
- Initial characterization of the role of alpha cells in beta-cell mass regulation in different metabolic conditions.
- Characterization of genes that are differentially expressed in islets from impaired fasting glucose (pre-diabetes) patients and from T2D patients. Association of these genes with specific aspects of beta-cell biology.
- Characterization of novel pathways controlling beta-cell functions:
  - role of nutrient regulated kinases (AMP-K, LKB, PASK)
  - role of Zinc in beta-cell and alpha cell hormone secretion
  - role of different regulators of glucokinase regulators (midnolin)
  - role of autophagy in mouse and human beta-cell stress control
  - role of different genes in gluco-incretin signaling in beta-cells (IGF2, FXYD3, Clic4)
  - mechanisms underlying differential aging of insulin granules and capacity to be secreted

Technical Results:

New technical development have generated:

- Developed new monoclonal antibodies directed to the cell surface of human beta-cells.
- Commercialized new human beta-cell lines ready to be utilized for the elucidation of human beta-cell physiology and pathophysiology and drug discovery programs for drug candidates improving beta-cell function. 2 out of 3 beta-cell lines are in the process of implementation for screening and functional testing of compound libraries.
- Significant progress in the validation of novel and non-invasive beta-cell probes and imaging techniques outlining options and limitations of non-invasive beta-cell imaging
- Candidate biomarker (lipid signature in plasma) being predictive to develop T2D was identified in preclinical studies and verified independently in two different human cohorts.
- Identified genes modules underlying beta-cell adaptation or resistance to metabolic stress. Characterized the association of these gene modules with specific phenotypes or dysfunctions of glucose homeostasis.
- Extensive and complete tissue and knowledge platform for human beta-cells from healthy individuals and type 2 diabetes patients including corresponding samples, data and results from 6 different mice strains to bridge human and animal physiology established. This includes all the data generated during IMIDIA, annotated in a manner that ensures integrated analysis of all datasets as well as further integration of this database in a federated database to serve as a basis for further EU or worldwide collaboration on diabetes research and development projects. A sustainability model to enable further use of the integrated IMIDIA beta-cell platform in forthcoming consortia developed and in progress to be formally established.

- The Knowledge platform is used for validation of targets, biomarkers and selection of in vivo models.
1.6 Potential impact and main dissemination activities and exploitation of results

Many data generated by IMIDIA will significantly contribute to increase our knowledge on the pathophysiology of type 2 diabetes and to provide new tools for the research of treatments. It is strongly expected that this will contribute to a better management of the disease.

In our opinion, IMIDIA is a unique example of close collaboration between pharmaceutical companies and academic institutions which resulted in many important achievements (for details please see Section 2.4 of this report) paving the way of new tracks for the research in diabetes taking advantage from basic and industrial research. The attractiveness of the project was stressed by the interest showed by a very reputable American association, the Juvenile Diabetes Research Association (JDRF) which offered grants to support additional aspects1 in the context of IMIDIA research and participated in our Plenum and scientific meetings. Thanks to that JDRF is now a full partner of the IMI 2 project INNODIA.

About 70 scientific articles related to the data generated through IMIDIA have been published more than 20 are in the process of being published. These inform the scientific and industrial community on the most recent progress in the knowledge of the pathophysiology of type 2 diabetes and in the treatment of the disease. Moreover, the impact of the discovery and further improvement of the human β-cell line is a major step forward in Diabetes-research, since it allows for the 1st time intensive research on the physiology, pathophysiology and biochemistry of the HUMAN β-cell thereby delivering results and knowledge relevant for the human disease, not just nice publications about genes in mice with only a limited importance for human health. In conjunction with this overcomes the principal unlimited availability of human β-cell line the very limited availability of human material ex vivo necessary for extensive scientific elucidation. This, and the robustness of the cell line for the 1st time allows straightforward drug industrial drug discovery programs with a realistic chance to detect molecules of value to improve/restore β-cell function in DIABETIC PATIENTS.

One of the other main achievements of IMIDIA is the discovery of plasma biomarkers. When these biomarkers will be fully validated, they will provide an invaluable tool for type 2 diabetes taxonomy and the evaluation of treatments.

Within IMIDIA the most extensive and complete tissue and knowledge platform for human β-cells from healthy individuals and type 2 diabetes patients has been built up. This unique β-cell knowledge platform is the focus of the IMIDIA Sustainability plans and will enable its further use in new consortia and collaborations in the course of IMI-2 as well other collaborations.

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1.7 Lessons learned and further opportunities for research

Collaboration is key for the success of public private partnerships and this collaborative spirit needs to be implemented and constantly fostered at all levels of the project. These includes on the one hand to achieve sustainable win win situations for all participants on the other hand collaborative decision making at all levels of the project coupled with tight project and alliance management.

Within IMIDIA a collaborative leadership team was established from the beginning consisting of the Coordinator, Co-coordinator and the Managing Entity complemented by the IMIDIA project office. This specifically focused to remove cultural and all other (admin) barriers (especially between academic institutions and pharmaceutical companies).

This setup enabled empowered workpackage leader teams - consisting of an academic and a pharma work package leader. The work package leader teams were the operational driver of this collaboration. To monitor the progress of the project a simple traffic light system based on deliverables were deployed. Monthly updated and made available to all participants via the IMIDIA internal e-room, it served as very useful tool for open and transparent communication focusing on the key problems within the project. These were addressed by quarterly face to face Managing Board meeting (all Work package leaders + Triumvirate), where solutions were found and the across work package activities coordinated. To have these meetings face to face was vital to find quick solutions and develop the mutual trust and confidence. We also introduced annual Scientific meetings – these 2-day events provided all the scientific details and progress for all IMIDIA participants and were attended by 100 + people. This exchange also proved to be very important input for the Plenum meetings held right after the science meeting, where future directions (incl. sustainability plans) were presented, discussed and formally approved.

In summary it can be concluded that this PPP provides critical scientific mass to achieve unparalleled results of extremely high scientific quality coupled with a strong support team dealing with day-to-day management, the identification of problems and gaps and contributing to their solution have been key to archive all these goals. Also a key learning is that it is of outmost importance that key potential problems linked to management are recognized and solved at the beginning of the project.

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