Chapter 2. Islets

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Section A. Introduction

The pancreatic islet: a cell society

The endocrine pancreas consists of approximately one million pancreatic islets each composed of around 1000-2000 cells, of which ~60 percent of the endocrine cells are insulin-producing beta cells, and the rest are glucagon-producing alpha cells, somatostatin-producing delta cells, pancreatic polypeptide-producing PP cells and ghrelin-producing epsilon cells. The islets are densely innervated and are perfused with one of the highest blood flow rates in the body. The endocrine cells have direct contact with the bloodstream via fenestrated endothelium, enabling the cells to deliver their hormonal products directly to the circulation. In addition, the islets contain a few other non-endocrine cells, such as dendritic cells (immune cells). The architecture of the islets varies between species, with alpha and delta cells forming a demarcated margin around the beta cell core in rodents and efferent blood flow moving from beta to alpha to delta cells, whereas this distribution and perfusion sequence is less clear in human islets. The endocrine cells communicate via their hormonal products in a paracrine fashion and also directly via gap junctions. This intercellular communication is critical for normal endocrine function, and the importance of the bio-sociology of the islet cells is underlined by the fact that if dispersed islet cells are allowed to re-aggregate in culture they assume the same distribution as before dispersion. This re-aggregation is dependent on several adhesion molecules. Further, the extracellular matrix surrounding the islet cells and the islet micro-organs has important influence on islet function.

Causes of diabetes residing in the pancreatic islet

Diabetes mellitus arises when beta cells are unable to meet insulin demands in the body, due to failure and destruction of the beta cell mass or increased insulin needs due to insulin resistance or growth that are greater than insulin secretory capacity. With the exception of rare mutations in insulin signalling, insulin resistance is, however, neither *sufficient* nor *necessary* for the development of diabetes. Thus, most insulin resistant obese individuals are not diabetic, and patients with maturity onset diabetes of the young (MODY) and 15 percent of individuals with type 2 diabetes are not significantly insulin resistant. Either absolute or relative insulin deficiency is both *necessary* and *sufficient* to cause diabetes. Without impaired beta cell sensitivity to glucose or beta cell decompensation, insulin resistance does not cause type 2 diabetes. In type 1 diabetes, beta cell functional repression and autoimmune destruction and, in MODY, beta cell signalling defects alone are sufficient to cause diabetes. These observations highlight the key role played by the beta cell in diabetes. This in turn makes this cell a central target for pharmacological and therapeutic intervention.

It is much debated whether beta cell decompensation leading to type 2 diabetes occurs by impaired glucose sensing and/or secretory function, by reduced beta cell mass or both. The term ‘reduction in functional beta cell mass’ has been coined to indicate that reductions in function and mass are rarely independent in diabetes, at least as the disease progress. Beta cell function depends on the normal regulation of insulin synthesis and secretion and the maintenance of the functional beta cell mass, i.e. the balance between beta cell renewal by *de novo* formation or replication, and destruction. This delicate balance is controlled by both genetic and environmental factors and is subject to dysregulation by pathogenetic elements.

Genetics and epigenetics of diabetes with relevance to the pancreatic islet

In type 1 diabetes, genome-wide association studies have identified susceptibility genes mainly controlling the response of the immune system. In the case of type 2 diabetes, it is no coincidence that many of the novel type 2 diabetes genes identified by genome-wide scans appear to be expressed in beta cells and possibly involved in cell cycle control, suggesting that genetic susceptibility to type 2 diabetes is also linked to the ability of the beta cell to replicate in response to increased demands for insulin.

It is unlikely that structural mutations in key beta cell proteins directly cause common diabetes. This is supported by the fact that most patients who develop type 2 diabetes do so at an advanced age and have apparently had adequate beta cell function for 50-60 years prior to the onset of the disease. Mutations in key beta cell proteins have been described, but they result in MODY or neonatal diabetes. Accordingly, diabetes results when the capacity of the beta cell to secrete insulin is not sufficient for the body's requirement.

Genetics define the risk of developing diabetes, but even individuals who carry multiple risk alleles are not doomed to become diabetic. Identifying the roles of the novel type 1 diabetes and type 2 diabetes genes is an important Milestone, as is defining the genetic and epigenetic factors regulating their penetrance. Although some of the factors affecting beta cell function are non-modifiable (genetics, ageing, sex), most are potentially modifiable and therefore therapeutic targets. These factors are diverse and range from lifestyle (diet, exercise), environmental (infections), extrinsic (gut micro-flora, intrauterine environment), physiological (pregnancy, islet bio-sociology, blood flow and innervation, tissue cross-talk including signals from the gut) and pathophysiological (metabolic stress, inflammation and autoimmunity) conditions.

A delicate balance of beta cell 'dynamic instability'

Interestingly, inherent properties of the beta cell seem to predispose to pathology triggered by these factors. The evolutionary and physiological benefits of these inherent properties may be linked to the need for dynamic up- and down-regulation of beta cell function and mass during physiological states, such as involution of beta cell mass at birth, expansion during and reduction after pregnancy and increase in function and mass during growth or development of obesity. Further, since uncontrolled insulin secretion is potentially dangerous, the beta cell may be equipped with mechanisms for rapidly regulating not only function but also mass. Thus, the delicate balance of beta cell function and mass may be easily perturbed, and understanding factors maintaining as well as factors disturbing this balance is an important Milestone.

In type 1 diabetes and it is now believed also in type 2 diabetes, the beta cell is exposed to an inflammatory environment that leads to or accelerates its death. As beta cell mass and function are reduced below a critical threshold, the beta cell also becomes subject to metabolic stress as blood glucose and free fatty acids start to increase. While the initial trigger leading to islet inflammation is surely different in type 1 compared with type 2 diabetes, some downstream factors may be common to both. A dynamic state of instability may thus exist in the beta cell at several levels. For example, the beta cell may be prone to apoptosis in response to a number of stimuli related to its inherent properties. Because of these features that are the key to its differentiated function, even the healthiest beta cell is balanced on a knife's edge.

There is accumulating evidence that features of the highly specialised phenotype of the beta cell, one of which is energy metabolism tightly coupled to beta cell secretory function, may also make the beta cell susceptible to events leading to type 2 diabetes. The ratio between adenosine triphosphate (ATP) and diphosphate (ADP) is commonly used as a measure of a cell's metabolic state. An age- and/or lifestyle-dependent decrease in the ability of beta cell metabolism to increase the ATP/ADP ratio can therefore be expected to compromise insulin secretion. The metabolic disturbance will not be confined to the beta cell, but the functional consequences are particularly dramatic in this cell because its function is under direct metabolic control. However, most obese and/or older individuals do not become diabetic, and the genetic and epigenetic factors determining this dichotomy need to be identified.

Towards a cure focussed on the beta cell

What matters to people with diabetes, or with increased risk for developing the disease, is the capacity of the beta cells to secrete insulin, their ability to adapt to changing demand and/or inflammatory or metabolic stress. It follows that the major aim of this road map is to *restore and preserve beta cell function.* Ultimately it is hoped that this research could lead to a *cure for both type 1 and type 2 diabetes.* Improved understanding of the process leading to the destruction of beta cells may allow for *prevention of onset of type 1 diabetes and progression towards clinically manifest type 2 diabetes.*

Section B. Scientific advances and major challenges

There have been remarkable advances in our understanding of beta cell development, function, dysfunction, death, and defence over the past 10-20 years that set the stage for the studies proposed in the road maps and have in some cases already influenced therapeutic strategy.

Beta cell function

Recent advances in this field include those that have allowed for a detailed molecular understanding of many aspects of insulin production and secretion as well as the regulation of these processes. However, there is still much to be to be learned about the normal function of the beta cell and, most specifically, the molecular lesions that underlie disturbed function in diabetes.

Insulin gene transcription and its regulation

The advances in understanding the transcription factor network in beta cell ontogeny have added significantly also to our comprehension of how insulin gene transcription is regulated. One transcription factor in particular, pancreatic duodenal homeobox 1 (Pdx-1), is important in this respect, but what is less well understood is how dysregulation of this factor contributes to dysfunction and destruction of the beta cells.

Intragranular processing of proinsulin, ultrastructure of beta cell, granule traffic

There have been great advances in our understanding of the critical events that underlie the ability of the beta cell to produce, store and secrete insulin. Many of these appear to be perturbed in type 2 diabetes and may offer novel targets for development of drugs that improve beta cell function.

Substrate-site glucose recognition/glucokinase as glucose sensor

The glucose sensor in beta cell stimulus-secretion coupling is glucokinase. Dysfunction in this enzyme is the cause of a MODY subtype, and glucokinase has thereby attracted attention as a promising therapeutic target for developing novel insulin secretagogues.

Electrical activity/role of calcium in insulin secretion/potassium (K_{ATP}) channels

Important breakthroughs have been made in the understanding and treatment of rare neonatal diabetes forms arising due to mutations in the beta cell ATP-dependent potassium (K_{ATP}) channels. These advances have demonstrated clearly the importance of understanding basic cell biology to devise intelligent treatments, and there is hope that they can be generalised to the more common type 2 diabetes.

Biphasic insulin secretion/granule pools

It is remarkable that the timely delivery and thereby the kinetics of insulin secretion are more critical to normal glucose homeostasis than the total insulin output from the beta cells. Loss of first phase insulin secretion is a sign of beta cell failure and a hallmark of both developing type 1 and type 2 diabetes as well as pancreatic and islet graft failure. Thus, advances in this field have been crucial to focus attention on therapies where targets in the molecular processes controlling the first phase of insulin secretion are explored.

Triggering vs amplifying pathways in the regulation of insulin secretion

Significant advances have been made in understanding the fine-tuning of glucose-stimulated insulin production and release. Many hormonal, neuronal and inflammatory stimuli cross-talk with glucose stimulus-secretion coupling and this research has led, for example, to the development and use of glucagon-like peptide-1 (GLP-1) based therapies.

Specific features of beta cell intermediary metabolism/energy formation underlying function

Traditionally, glucose-oxidation has been regarded as central to metabolic control of beta cell function. However, advances in the understanding of the importance of the uptake, accumulation and/or metabolism of other nutrients, such as fatty acids, amino acids, and lipoproteins as well as trace metals, demonstrate that many other aspects of beta cell metabolism are potential therapeutic targets.

Type 2 diabetes is associated with reduced beta cell mass and beta cell dysfunction

An important major advance based on clinical studies has been the paradigm shift from considering type 2 diabetes only as the result of functional inability of the beta cell to compensate for increased insulin demand to the concept of this being a progressive disease of reduced beta cell function and mass. However, the relative importance of each remains unclear.

Regulation of insulin secretion by microRNAs

A revolutionary discovery has been the finding of small non-coding ribonucleic acids (RNAs) that regulate gene translation, so-called micro-(mi)RNAs. Islet research has contributed much to the field not only specific for the beta cell, but also

for a general understanding of miRNA biology. Inhibitors of miRNAs, so-called antagomirs, have shown efficacy *in vivo* and should pave the way for assessment of the clinical potential of this novel class of drug.

Beta cell mass

Beta cell size vs number
Although it is well established that cells can increase mass by either increasing size (cellular hypertrophy) or number (cellular hyperplasia), very few if any advances have been made in understanding the importance of the regulation of beta cell size in normal glucose homeostasis and in the diabetic state. This is clearly an area where technological breakthroughs are needed to assess beta cell size *in vivo*. There has been a remarkable increase in the understanding of the processes that determine rodent beta cell replication, but their relevance to humans remains to be established.

Pancreas developmental biology and delineation of the transcription factor hierarchy in pancreas development
The major milestones in this area encompass the unravelling of the endodermal origin of endocrine pancreas and the precise timing of the expression of key transcription factors along the beta cell linage. This has provided the essential key for *in vitro* derivation of beta cell-like cells from stem cells (see the following).

Adult pancreas regeneration
The development of two distinct models to study pancreatic regeneration, combined with sophisticated lineage tracing, has revealed that beta cell replication is the major form of beta cell regeneration in normal mice and mice subjected to partial pancreatectomy or non-immune beta cell ablation. Beta cell neogenesis from ductal precursor cells of adult mouse pancreas can be induced by duct ligation. The relevance of these findings to the situation in adult humans remains hotly debated. There have also been important studies, again in rodents, demonstrating trans-differentiation or re-programming of adult (i.e. exocrine pancreatic) cells into beta cells.

Regulation of beta cell mass
Preclinical studies have shed new light on factors promoting increased beta cell mass, including: the finding of growth factors causing rodent beta cell replication; the role of gastrointestinal peptides in regulating islet functional mass; the modulation of beta cell functional mass by extracellular matrix; involvement of ductal cells in islet regeneration. Again, the relevance of these findings to man remains to be established and will depend on a means of measuring changes in beta cell mass in living individuals. Despite this cautionary note, these findings have generated mounting enthusiasm for development of therapy for diabetes based on beta cell regeneration.

Replacement therapy

Recent advances in the field of islet transplantation
Although islet and beta cell grafts are gradually destroyed and graft function deteriorates much quicker in the islet compared to the segmental pancreatic graft, the advances in this field have been: the proof of principle that an islet graft can normalise glucose metabolism in patients with type 1 diabetes; the demonstration of preserved beta cell function and improved glycaemic control even in graft recipients who have relapse in insulin requirement; the technical and medical refinements and optimisation of pancreas transplantation; T cell biomarkers for islet graft survival in patients; the role of innate immunity in islet transplantation and replacement therapy; and the proof of principle that *in vitro* differentiated human embryonic stem (hES) cells can cure diabetes in experimental animals.

Directed differentiation of pluripotent stem cells into beta cells
An unlimited source of therapeutic-grade beta (or islet) cells is essential for future cell replacement therapy of diabetes. Recent general technical advances that set the stage for producing surrogate beta cells include the derivation of definitive endoderm from hES cells and the virus-free generation of induced pluripotent stem (iPS) cells from adult human cells. The ability to derive definitive endoderm opened the way to derivation of beta cell-like cells that could be further differentiated following implantation in mice. However, these cells may not yet be sufficiently well differentiated and, most importantly, development of teratomas (tumours derived from more than one germ layer) in several mice exemplifies the inherent dangers of using stem cells that must be overcome before clinical use can be envisaged.

Destruction

Apoptosis is a key mechanism of beta cell death in both type 1 and type 2 diabetes, with metabolic and immune/inflammatory pathways converging on common signals leading to beta cell apoptosis.
The advances in research into beta cell failure and destruction that have identified this important paradigm have been: refining isolation and culture processes for rodent and human islets;

mechanisms of beta cell pro-apoptotic action of inflammatory cytokines; understanding of oxidative damage and the role of mitochondria in beta cell demise; the beta cell as a producer of cytokines and chemokines; signalling networks, including the prototypic inflammatory and stress transcription factor nuclear factor (NF)-κB and stress kinases and downstream intracellular mechanisms regulating beta cell function, apoptosis/necrosis, defence and survival including oxidative and smooth endoplasmic reticulum (ER) stress; the mapping of mitochondrial events and the implication of the Fas pathway; and modulation of the sulphonylurea receptor (SUR).

Beta cell destruction with special relevance to islet autoimmunity in type 1 diabetes

In addition to most of the general recent advances summarised above, the following advances have particular relevance to type 1 diabetes: risk assessment by and standardisation of assays for circulating autoantibodies; cell-mediated autoimmunity to islets; genetic definition of pathogenesis by tissue type or major histocompatibility complex (MHC) antigens; identification of beta cell autoantigens and effector pathways in the type 1 diabetic non-obese (NOD) mouse; demonstration of major epitopes (including preproinsulin) recognised by peripheral T cells from patients; critical role of NKT cells in preventing development of spontaneous and virus-induced diabetes using various mouse models; the importance of innate immunity including inflammatory cytokines in diabetes pathogenesis; preservation of beta cell function by immunosuppression with cyclosporine; transient preservation of beta cell function by short-course anti-cluster of differentiation (CD) 3 antibody, anti-CD20 antibody or glutamic acid decarboxylase (GAD) 65 immunisation.

Beta cell destruction with special relevance to type 2 diabetes

With the understanding that type 2 diabetes is caused by decreased beta cell function and mass there has been increased focus on the mechanisms underlying this decrease and therapeutic strategies to reverse or prevent it. The following advances have particular relevance to type 2 diabetes: mechanisms of gluco- and lipotoxicity on the beta cell; islet inflammation in type 2 diabetes as a major cause of loss of insulin secretion and inappropriate glucagon production; first clinical studies suggesting that blocking interleukin-1β (IL-1β) action can improve glycaemic control through enhanced beta cell function; and the use of gastrin plus GLP-1 to preserve or prevent loss of beta cell mass.

Relationship between differentiated phenotype and defence systems

Advances in *in vitro* models have indicated that the beta cell loses defence mechanisms against metabolic and inflammatory attack as it differentiates. However, there is a general lack of knowledge as to if and how this translates into beta cell destruction *in vivo* and what defence pathways are suitable intervention targets. It is proposed that advances in understanding beta cell development and normal function on one side and insights into dysfunction and destruction on the other will synergise to clarify why the beta cell fails and dies in diabetes, and how protection and defence can be enhanced for therapeutic use.

Intercellular communication

Between islet cells

Pancreatic islets are complex micro-organs and contain many other endocrine cells in addition to the beta cells. Of these, glucagon-producing alpha cells (15-25 percent of the islet cells) and somatostatin-releasing delta cells (5-10 percent) are the most abundant. Diabetes research has so far largely focussed on the beta cells, but it has been recognised for several decades that diabetes is (at least) a bi-hormonal disorder and that impaired control of glucagon release exacerbates the metabolic consequences of impaired insulin secretion. Recent advances in understanding the cross-talk between islet cells and the mechanism underlying beta cell inhibition of glucagon secretion may help clarify the pathophysiology of islet dysfunction in both type 1 and type 2 diabetes.

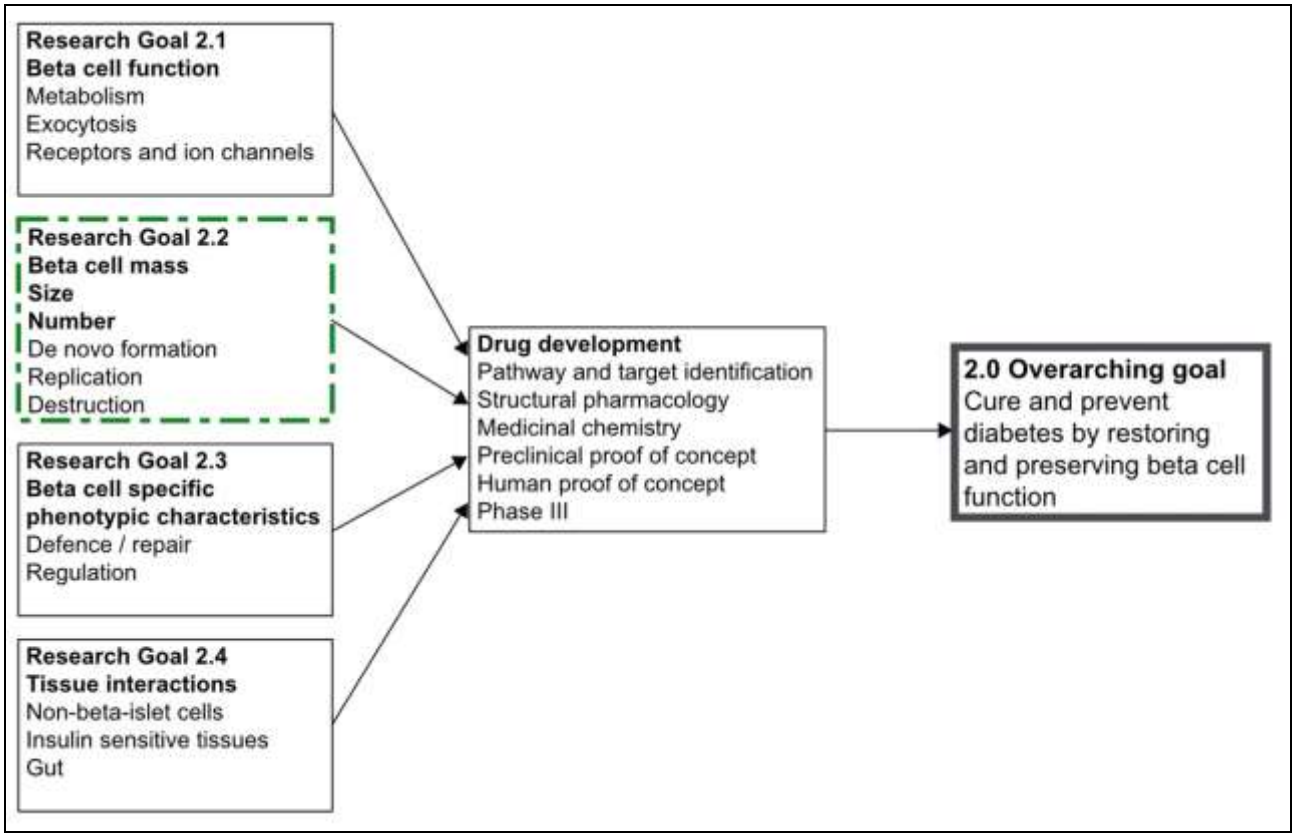
Between insulin-sensitive tissues and beta cells

The metabolic role of the major insulin-sensitive tissues (liver, fat and skeletal muscle) was initially assumed to be limited to the storage, mobilisation and uptake/metabolism of carbohydrates and fat. It is now clear, however, that these tissues play an important endocrine function and secrete a growing number of humoural signals (hepatokines, adipokines and myokines), e.g. leptin, adiponectin, tumour necrosis factor alpha (TNFα), interleukin (IL)-1β, IL-6, and IL-1 receptor antagonist (IL-1Ra). These factors affect not only insulin-sensitive tissues but also act on other cells, including the pancreatic beta cells. Insulin resistance alters profoundly the profile of these secreted products and may in turn change their biological action on beta cells.

Section C. Road maps in islet research towards cure and prevention of type 1 and type 2 diabetes

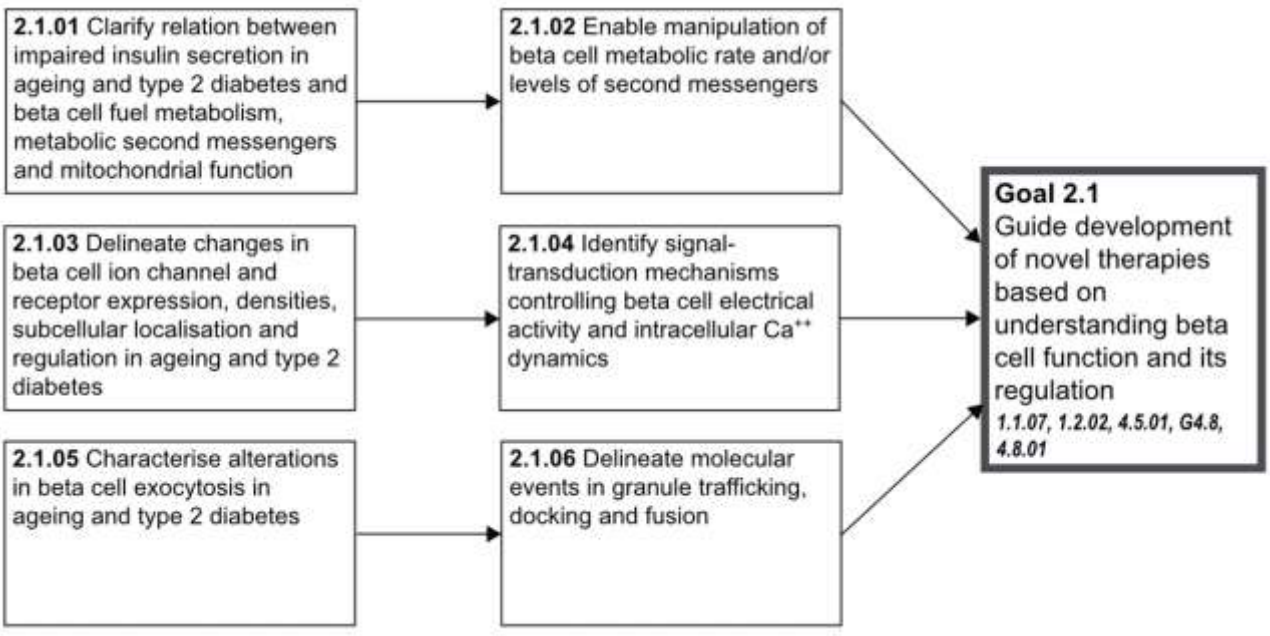
Links between Goals and Milestones in the DIAMAP report are noted within the text and also in the diagrams in *italic*s. Roadblocks are listed below the diagrams and described at the end of the chapter. Goals and Milestones considered a priority are indicated with a broken green line.

Overarching Goal 2.0: Cure and prevent diabetes by restoring and preserving beta cell function



The overarching Goal of the islet research road map is to cure and prevent type 1 and type 2 diabetes by restoring and preserving beta cell function. Four main road maps (2.1-2.4) lead to this overarching Goal. It is assumed that novel scientific discoveries in each of the four main thematic research areas 2.1-2.4 will provide the knowledge fundament to guide development of new therapies by employing translational methodologies of drug discovery and development.

Goal 2.1. Guide development of novel therapies based on understanding beta cell function and its regulation



Roadblock 1. Limited access to human beta cells/islets/pancreatic tissue

Introduction and background

The central role of the beta cell and insulin secretion in the metabolic regulation of the entire organism is well established. Although the normal regulation of insulin secretion is fairly well understood, researchers still only have fragmentary knowledge about the processes that culminate in impaired beta cell function and insufficient insulin secretion in type 2 diabetes. The research Milestones below should be illuminating in this respect but represent a phenomenal undertaking that can best be accomplished by an international/pan-European collaborative research network. It is expected that this will result in the discovery of molecular mechanisms that can be used as targets for novel anti-diabetic medicines superior to those currently available (*see also* Milestone 1.2.02, Goal 4.8).

Milestone 2.1.01. Clarify relation between impaired insulin secretion in ageing and type 2 diabetes and fuel metabolism, metabolic second messengers and mitochondrial function

In most people, there is sufficient spare capacity of beta cells to ensure adequate insulin supply throughout life. Although beta cell function at the age of 70 may be much reduced compared to that at the age of 20, it remains sufficient to keep plasma glucose within the 'healthy' range in most individuals. However, some individuals may be born with less beta cell capacity to spare and/or may not be able to compensate with suitably increased beta cell functional mass, and at one point in life the insulin demand may exceed what can be delivered by the beta cells (diabetes threshold). Nevertheless, a person with reduced beta cell mass is not doomed to become diabetic. If the individual puts on weight and becomes insulin resistant, that person will cross the line earlier than if lean and fit. Indeed, in the latter case it may not happen at all.

Once the balance between insulin delivery and demand has been perturbed, vicious cycles of events are initiated that collectively result in a progressive further decline in beta cell function that eventually culminate in overt type 2 diabetes. For example, a small decrease in insulin secretion may result in slight elevations of plasma glucose and free fatty acid levels that in turn result in a further reduction of insulin secretion. Thus, in individuals born with a slightly reduced beta cell mass (because of a genetic predisposition), the age-dependent reduction of mitochondrial function may be sufficient to tip the balance. More research is needed to improve knowledge about the interactions among ageing, mitochondrial function and metabolism, lifestyle factors and beta cell function.

Milestone 2.1.02. Enable manipulation of beta cell metabolic rate and/or levels of second messengers

Pancreatic beta cells couple changes in plasma glucose/nutrient levels to stimulation or inhibition of insulin secretion via electrical signals. The sulphonylurea drugs that have been used to treat type 2 diabetes for almost 60 years stimulate electrical activity in beta cells by blocking potassium channels regulated physiologically by metabolically induced changes in the intracellular ATP/ADP-ratio that reflect variations of the plasma glucose concentration. This has led to the proposal that the metabolic defects considered above culminate in impaired beta cell electrical activity and, in turn, reduced insulin secretion. However, to date there is no direct evidence that this is actually the case. There is a need for research to devise novel ways to manipulate beta cell metabolic rate and second messengers with the aim of improving insulin secretion in functionally impaired beta cells as well as in beta cells regenerating *in situ* or beta cell/islet grafts.

Milestone 2.1.03. Delineate changes in ion channel and receptor expression, densities, subcellular localisation and regulation in ageing and type 2 diabetes

Electrical signals generated in the beta cells play a critical role in the regulation of insulin secretion. These signals result from the activity of a group of membrane proteins specialised for a high rate of ion transport (ion channels). The human genome contains hundreds of different ion channels, and each cell type in the body expresses a distinct subset tailored to the functional needs and tasks of that particular cell. There is a fair understanding of which ion channels are important in rodent beta cells, but even this research area is incomplete. Further, recent data indicate that human beta cells express a different complement of ion channels than rodent beta cells. Thus, in order to understand how the beta cell responds to changes in plasma glucose or other fuels with stimulation or inhibition of electrical activity coupled to insulin secretion, a thorough description of the ion channels present in human beta cells is essential. This line of research will include determination of their number and regulation, and how expression and function are affected during ageing and type 2 diabetes.

Insulin secretion is controlled by hormones (released locally within the islets or reaching the beta cells via the blood supply) and neurotransmitters (released from intra-islet nerve endings). Some of these stimulate (like GLP-1), others inhibit (like somatostatin or adrenaline) insulin release. Precise information about the density and subtype of these receptors in islet beta and non-beta cells and whether they are affected in type 2 diabetes would be valuable because an altered balance between stimulatory and inhibitory pathways may contribute to the insulin and glucagon secretion defects that are a hallmark of type 2 diabetes.

Milestone 2.1.04. Identify signal transduction mechanisms controlling beta cell electrical activity and intracellular calcium (Ca^{++}) dynamics

When glucose is metabolised by the beta cell, the change in energy balance of the cell leads to closure of ATP-dependent potassium channels. This closure depolarises the cell membrane leading to opening of a subtype of voltage-gated channels. The influx of calcium into the beta cell eventually activates insulin release. However, many other cellular processes contribute to beta cell calcium handling, including mobilisation of calcium from intracellular calcium stores, such as the mitochondria or the endoplasmic reticulum. Mobilisation of calcium is regulated by many factors, including cellular stress signals, and inappropriate amplitude or frequency of alterations of the cytosolic calcium concentration is a potent signal for apoptosis. It is clear that better understanding of beta cell calcium handling that is key for the physiology of insulin secretion and that also integrates stress and death signals is extremely important for the development of new therapies for diabetes.

Milestone 2.1.05. Characterise alterations in beta cell exocytosis in ageing and type 2 diabetes

Insulin secretion involves the fusion of insulin-containing secretory granules with the surrounding plasma membrane. The release competence of the secretory granules is under metabolic, hormonal and neuronal control. The ability of glucose to evoke electrical activity in the beta cell is referred to as ‘the triggering pathway’. In addition, glucose exerts an amplifying effect. The amplifying action is quantitatively important and accounts for up to 80 percent of glucose-evoked insulin secretion. Certain hormones also exert amplifying effects. These include GLP-1, which is now exploited to treat type 2 diabetes. There is also evidence that the expression of the proteins that are involved in the fusion of the secretory granules is reduced in diabetes. Recent findings further suggest a direct link between a high-fat diet, circulating fats and obesity and impaired insulin secretion. It is thus conceivable that lowered insulin exocytotic capacity is a feature of type 2 diabetes, but this is an understudied area where more work is needed. There is a good case for such studies as the molecular machinery involved in the release of

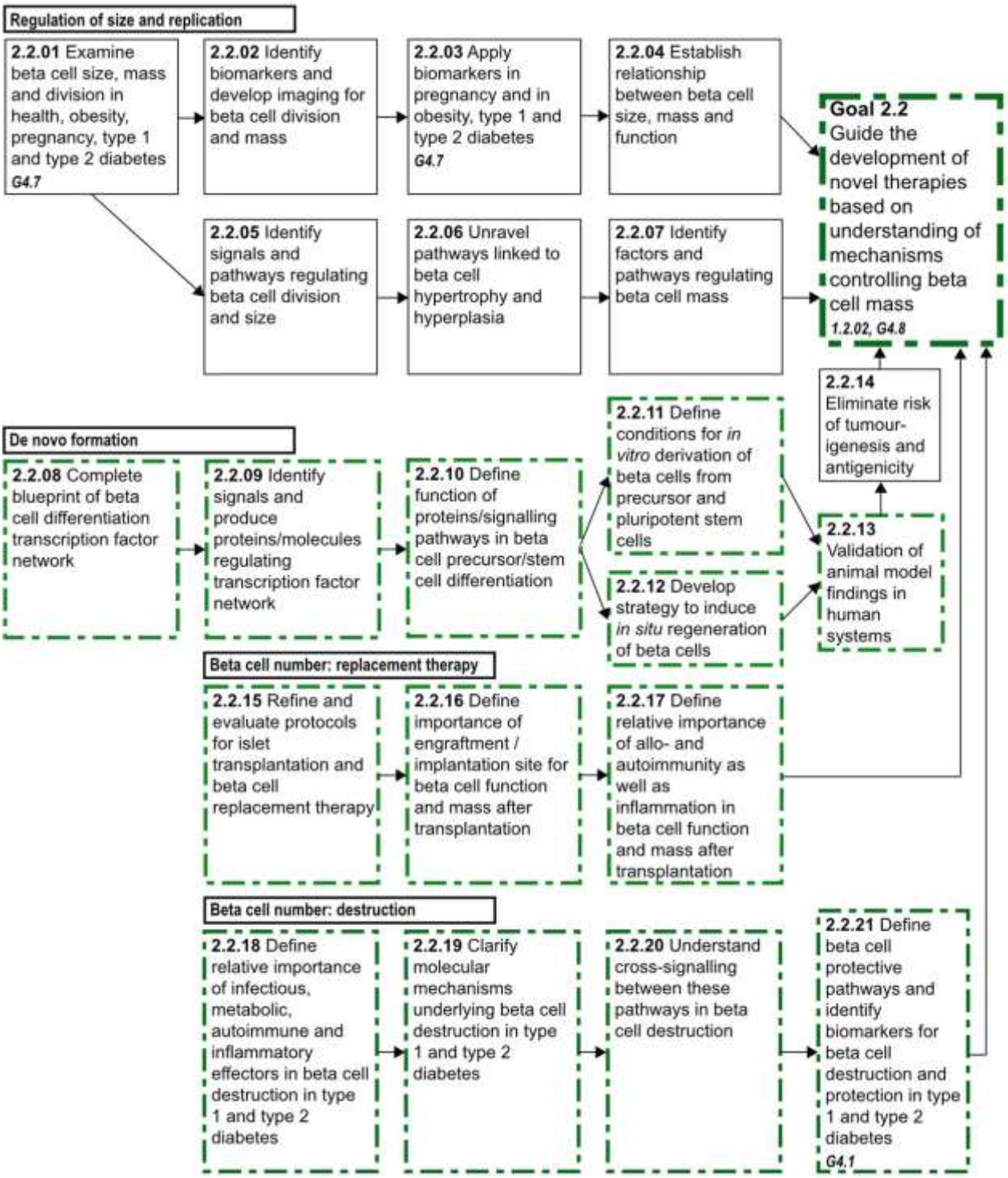
insulin is subject to strong metabolic, hormonal and pharmacological modulation and exhibits some unique features. Thus, it seems possible that studies of the defects of insulin secretion in type 2 diabetes may provide clues to a novel group of diabetes therapies that target the amplifying rather than the triggering action of glucose on the beta cell.

Milestone 2.1.06. Delineate molecular events in granule trafficking, docking, and fusion

Insulin is transported from the Golgi apparatus in secretory vesicles that are not just passive conveyors of the protein but are also responsible for insulin processing and stability. Further, these vesicles contain many other proteins, the function of which is incompletely understood. The physical movement of the vesicles along elements of the cytoskeleton and their docking at the cell membrane involve several proteins and are energy requiring processes, the regulation of which needs to be clarified in detail. After the docking process, the fusion of the vesicle membrane with the plasma membrane and the release of insulin to the bloodstream is a complex process, which is also only partly clarified. Therefore, much further research is needed in these areas to allow the development of therapies that target the export of insulin to the circulation. Also, recent reports indicate an intriguing feedback relationship between granule exocytosis and granulogenesis by release of peptides from granules that then accumulate in the nucleus. This requires further study since it may reveal a means to regulate beta cell granule stores by therapeutic intervention.

Since Milestones 2.1.01-06 are linked via a focus on insulin stimulus-secretion coupling, it is envisaged that completing any one Milestone will facilitate reaching the others.

Goal 2.2. Guide the development of novel therapies based on understanding of mechanisms controlling beta cell mass



Roadblock 1. Limited access to human beta cells/islets/pancreatic tissue
Roadblock 2. Lack of beta cell-specific surface ligands
Roadblock 3. Lack of a unified European Union stem cell strategy
Roadblock 4. Ethical and regulatory issues

Introduction and background
An adequate functional beta cell mass is essential to ensure normal blood glucose regulation, and an absolute or relative beta cell deficiency characterises diabetes phenotypes in both type 1 and type 2 diabetes. To normalise the functional beta cell mass as a treatment or cure for diabetes, it is not only important to understand how beta cells are born during fetal development and regenerate in adult life, but also how beta cell replication and death are controlled throughout life. Detailed knowledge from basic pancreatic and beta cell developmental biology is envisioned to translate into *ex vivo* production of therapeutic beta cells from stem cells. Optimal and safe conditions for beta cell replacement and regenerative therapy need to be developed.

The testing of novel therapies targeting restoration of the beta cell functional mass has been greatly hampered by the lack of understanding of the precise relation between beta cell mass and function. It is unclear if changes in secretory capacity are due to altered function of an existing mass, by altering the mass of beta cells without changing the function per beta cell or both. It will be critical to understand this relation, how it changes over time in physiological and pathophysiological situations, and how it is modulated by therapy.

Regulation of size and replication

Milestone 2.2.01. Examine beta cell size, mass and division in health, obesity, pregnancy, type 1 and type 2 diabetes
Access to cadaveric pancreatic tissue from patients with detailed clinical history and ideally laparoscopic or endoscopic pancreatic biopsies from living individuals after appropriate ethical issues have been overcome (*see also* Milestones 2.2.19-21) would allow studies on beta cell size, mass and division in health, obesity, pregnancy (*see also* Goal 4.7), type 1 and type 2 diabetes. Functional beta cell mass compensation may include beta cell hypertrophy. Very little is known about this phenomenon in diabetes, and whether an increase in beta cell size actually associates with an increase in functional mass remains a critical knowledge gap.

Milestone 2.2.02. Identify biomarkers and develop imaging for beta cell division and mass
Data from research in pursuit of Milestone 2.2.01 will allow the identification of biomarkers for beta cell mass and even beta cell division. The identification of such biomarkers should proceed alongside the development of imaging technologies, with the common goal of allowing non-invasive measurement of beta cell mass and mitotic activity in health and disease and their changes over time. As mentioned in the Introduction, beta cell number is a function of the balance between *de novo* formation of beta cells from stem cells or other precursor cells (neogenesis) and self-replication of existing beta cells on the one hand, and beta cell destruction on the other. Beta cell proliferative capacity can readily be activated to replenish rodent beta cell mass *in vitro* and *in vivo*, but it has been difficult to induce human beta cell proliferation *in vitro*. New studies are therefore needed to demonstrate that factors driving rodent beta cell proliferation can improve beta cell function in humans in a sustained fashion and to use new biomarkers or imaging techniques to determine whether this is due to increased beta cell function, mass or both.

Milestone 2.2.03. Apply biomarkers in pregnancy and in obesity, type 1 and type 2 diabetes
Descriptive studies are needed to profile beta cell mass, proliferation and apoptosis rates in tissues derived from healthy and diabetic individuals with states that exhibit the range of beta cell plasticity in health (puberty, pregnancy) and disease (obesity, type 1 and type 2 diabetes) (*see also* Goal 4.7).

Milestone 2.2.04. Establish relationship between beta cell size, mass and function
The morphological and histological characterisation of beta cell mass related to function in healthy and diabetic individuals as categorised under Milestone 2.2.03 would provide the gold standards for manifestations of beta cell plasticity and should be exploited for the validation of tools to define human beta cell mass, e.g. non-invasive imaging techniques and surrogate biochemical biomarkers. *In vivo* imaging is critical to fully establish the relationship between beta cell mass and function.

In parallel to descriptive studies aimed at developing biomarkers and imaging for the definition of the relation between beta cell mass and function, research is needed to identify signals and pathways involved in regulating human beta cell size and division (*see also* Milestones 2.2.05-07).

Milestone 2.2.05. Identify signals and pathways regulating beta cell division and size
Both unbiased systems biology technologies, such as subtraction microarray and transcriptome analysis using second generation (deep) sequencing, and hypothesis-driven candidate experimental approaches based on existing knowledge about cell replication and volume

homeostasis are needed. However, since human beta cells have not yet been shown to grow robustly in size or replicate *in vitro*, advances must be made to establish suitable *in vitro* models of human beta cell growth and replication to pave the way for the mentioned studies.

Milestone 2.2.06. Unravel pathways linked to beta cell hypertrophy and hyperplasia
Based on bioinformatic analysis of the vast amounts of data generated by systems biology tools and hypothesis-driven candidate studies, *in silico* bio-modelling approaches need to be developed to comprehend the complex non-linear and redundant cellular signalling pathways. This research will also be necessary due to the scarcity of human islets and inter-sample variability.

Milestone 2.2.07. Identify factors and pathways regulating beta cell mass
Information technologies to integrate genomic information on putative aetiological genetic variations with expression arrays, including splice variants, microRNA and other non-coding RNA profiling, post-translational modifications and the kinome, will be key to bringing forward new hypotheses and models for these processes, in order to direct validation in actual cellular studies.

Beta cell number: De novo formation

Milestone 2.2.08. Complete blueprint of beta cell differentiation transcription factor network
A hierarchy of transcription factor networks characterises pancreatic endocrine development at distinct stages and is highly conserved among vertebrates. The recent identification of endocrine progenitor cells in the adult mouse pancreas predicts that such transcription factor networks can be re-activated in the postnatal pancreas to result in beta cell neogenesis. A complete description/mapping of the transcription factor network blueprint in beta cell development in humans is fundamental for progression toward subsequent Milestones.

Milestone 2.2.09. Identify signals and produce proteins/molecules regulating transcription factor network
Extrinsic signals are responsible for activating key transcription factors in the signalling networks that operate in early pancreas progenitors and that guide the sequential development into mature beta cells. Research is needed to define these signals better in order to direct efforts to differentiate stem cells into functional beta cells *in vitro*.

Milestone 2.2.10. Define function of proteins/ signalling pathways in beta cell precursor/stem cell differentiation
Unravelling these processes constitutes a formidable task but is essential for the understanding of mammalian developmental biology in general and beta cell ontogenesis in particular. The goal is to provide the complete step-wise map/list of the growth factors and/or cell-cell interactions that instruct the pluripotent inner-cell-mass to differentiate into pancreatic progenitor cells and eventually glucose-responsive mature beta cells that can be used for therapeutic interventions.

Milestone 2.2.11. Define conditions for *in vitro* derivation of beta cells from precursor and pluripotent stem cells
Robust protocols for generating functional beta cells, based on precisely defined conditions, will be equally effective on different sources of pluripotent stem cells (hES and iPS cells). Access to 'patient-specific beta cell material' is contingent on the success of this research.

Milestone 2.2.12. Develop strategy to induce *in situ* regeneration of beta cells
In parallel to translating the understanding of the transcription factor network driving differentiation of beta cell progenitors into the generation of transplantable beta cell mass, research is needed to find safe and strictly controlled ways of regenerating the diminished and suffering beta cell mass *in situ*, either by growth signals that reactivate the proliferative potential or by factors that deactivate anti-proliferative signals.

Milestone 2.2.13. Validation of animal model findings in human systems
Much work in the beta cell biology field has been performed in mouse model systems. Although mouse research has led to important insights into the biology of beta cells in health and in diabetes, mouse beta cells are not identical to human beta cells. Research to systematically reproduce and validate important beta cell biology advances in human cells is needed in order to ensure that new therapies that are developed in mice can be translated for the benefit of human patients.

Milestone 2.2.14. Eliminate risk of tumourigenesis and antigenicity
Therapeutic beta cell preparations from pluripotent stem cell sources need to be well defined and of high purity. Contamination with undifferentiated (pluripotent) stem cells will cause teratomas. It will be an integral part of the protocol development to ensure that the end product will be 100 percent devoid of such contaminants. Long-term preclinical testing must ensure that this Milestone is achieved.

Even after establishment of Standard Operating Procedures (SOPs) for hES/iPS cell generation, culture, differentiation, preservation and production, several major hurdles remain before clinical trials can be initiated. These can be divided in two main categories: a) tumourigenicity, the risk of uncontrolled hES/iPS cell proliferation and formation of malignancies, and b) immunogenicity, the risk of inducing rejection and hES/iPS cell graft destruction. Within the next 10-year period it is unlikely that means will be obtained to control these problems at the cellular level. However, by transplantation of the generated insulin-producing cells in biochambers, both the problems of tumourigenicity and of immunogenicity may be solved, still allowing the transplanted cells to fulfil the biological functions to cure type 1 diabetes. Any strategy for *in situ* regeneration of beta cell mass must also avoid tumourigenicity and must be controlled to avoid overgrowth/hyperinsulinism.

Beta cell number: replacement therapy

Milestone 2.2.15. Refine and evaluate protocols for islet transplantation and beta cell replacement therapy
The indications for replacement therapy cannot be broadened until the inadequacies and side effects of current immuno-suppression have been eliminated or circumvented (*see also* Milestone 2.2.17), with a limitless supply of beta cells. Huge multidisciplinary efforts are needed to translate this into an established treatment for patients with type 1 and possibly type 2 diabetes. The importance of encompassing expertise covering all aspects of such a programme is exemplified by the fact that almost all available hES cell lines today are expected not to fulfil the regulatory requirements of good medical practice (GMP) and the relevant European and United States agencies (EMA and FDA).

Milestone 2.2.16. Define importance of engraftment/implantation site for beta cell function and mass after transplantation
Clinical islet transplantation today utilises infusion of the islet suspension into the portal vein of the liver, allowing the islets to settle in the portal vascular tree where they form micro-thrombi and blood clots causing downstream ischaemia, hepatic cell necrosis, inflammation, liver cell fatty involution (steatosis) and fibrosis. The coagulation process in the clot leads to activation of damaging pathways that acutely impair graft function and contribute to early graft failure. Animal experiments have pointed to several alternative transplantation sites superior to portal delivery, e.g. in the small omental pouch of the abdominal cavity. Much controlled clinical research is needed for validation of these promising findings in humans. Such multicentre efforts would be particularly suited within Europe due to existing research networks. The clinical use of beta cell replacement requires large investments and extensive studies that would benefit from public-private partnerships, and it is therefore important that guidelines are developed to facilitate and encourage industry involvement.

Milestone 2.2.17. Define relative importance of allo- and autoimmunity as well as inflammation in beta cell function and mass after transplantation
Irrespective of the source, grafted beta cells will have to be protected from the immune/inflammatory environment in the recipient (*see also* Milestones 2.2.18-21). Once understood, this environment could be modulated in the recipient (i.e. induction of tolerance in individuals with type 1 diabetes). Equally, the cells could be engineered to survive better after transplantation and/or bio-barriers developed to encapsulate the grafted beta cell mass. Further clinical trials of inhibitors of innate immunity alone and in combination with modulators of adaptive immunity/tolerance-inducing therapies are needed.

Beta cell number: destruction

Although type 1 and type 2 diabetes are two genetically distinct disorders, there is increasing evidence that metabolic factors in type 2 diabetes and islet autoimmunity in type 1 diabetes may converge on a common inflammatory pathogenesis involving overlapping destructive signalling pathways in beta cells. The rerouting of these signalling pathways from protective to deleterious signals depends in part upon inherent properties of the specialised pancreatic beta cell that render it susceptible to apoptosis as a consequence of the activation of these pathways. However, many other molecular mechanisms may be involved, and disentangling the relative importance of these pathogenetic factors is important in order to develop specific therapies.

The following sections highlight some of the aspects that may be restricted to either of the two major forms of diabetes, but which are covered by the overall flow in the road map Milestones:
2.2.18. Define relative importance of infectious, metabolic, autoimmune and inflammatory effectors in beta cell destruction in type 1 and type 2 diabetes
2.2.19. Clarify molecular mechanisms underlying beta cell destruction in type 1 and type 2 diabetes
2.2.20. Understand cross-signalling between these pathways in beta cell destruction

2.2.21. Define beta cell protective pathways and identify biomarkers for beta cell destruction and protection in type 1 and type 2 diabetes

Milestones 2.2.18-21. Type 1 diabetes
Population studies reveal marked regional differences in the incidence of type 1 diabetes, indicating an important contribution of the environment, including presumably infectious factors, in the aetiology of the disease (*see also* Chapter 1). At present, understanding of the aetiology of type 1 diabetes is limited and originates to a large extent from information gained in two animal models, the NOD mouse and the BB (bio-breeding) rat. In both models, hyperglycaemia develops due to a progressive immune-mediated destruction of the beta cells. Unfortunately, these small animal models of type 1 diabetes show little similarity with the human disease, and no suitable spontaneous large animal model for type 1 diabetes is available. At onset of type 1 diabetes, recent or manifest infections and auto-antibodies (IA-2 and GAD) are present in about 70 percent of cases. Human pancreatic specimens obtained at the onset of disease rarely display evidence of an ongoing *forceful* immune attack on the islets. Insulitis is discrete and heterogeneous in time and space in contrast to that observed in the rodent models. Immunosuppressive therapies (cyclosporine or anti-CD3/CD20 antibodies) or immuno-modulation (insulin, heat shock protein peptides or GAD) initiated at the time of diagnosis transiently preserve beta cell function but do not reverse the disease, and after cessation of immunosuppressive therapy a progressive decline in beta cell function is observed in most patients.

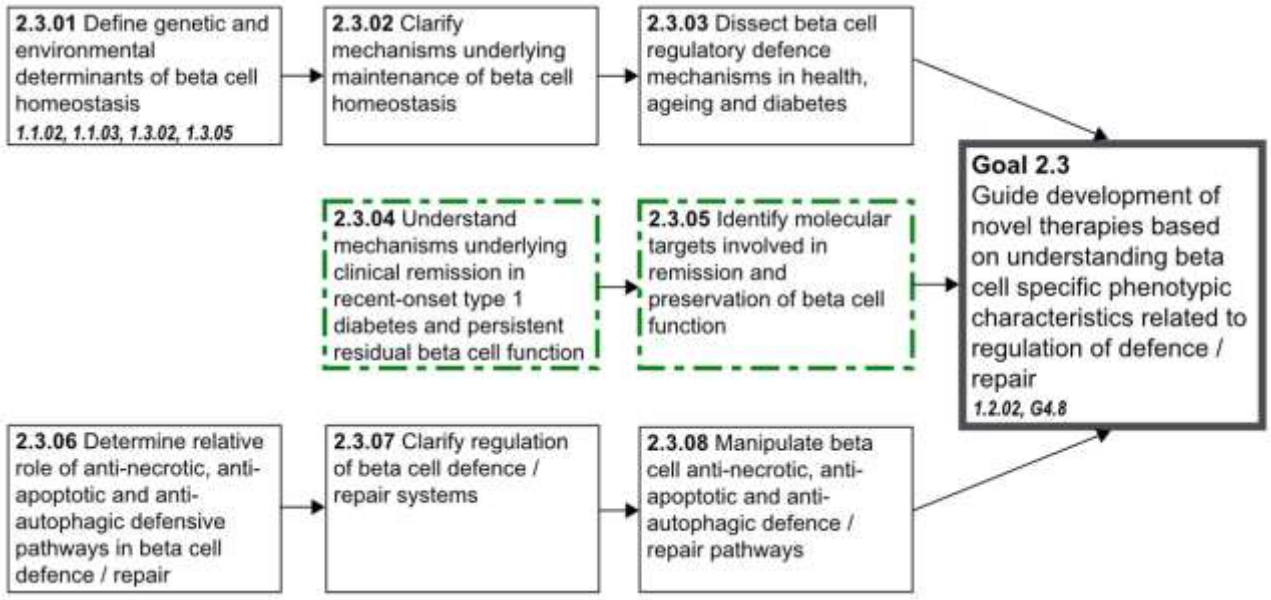
Therefore, emphasis should be redirected from studying the disease in rodent models to the study of human specimens obtained at different times during the development of the disease, as well as better informed and more sophisticated studies on peripheral blood cells and use of new biomarkers of autoimmune disease. It is critical that disease mechanisms disentangled *in vitro* or in animal models are validated in humans with respect to the relative contributions of inflammatory, immune, infectious and metabolic death effectors involved in autoimmune beta cell destruction, e.g. inflammatory cytokines, T cell effector molecules, role of NKT cells, viruses and their products, Toll-like receptors, fatty acids and glucose. Safe technologies are urgently required to obtain such specimens, and preliminary attempts to obtain laparoscopic or endoscopic pancreatic biopsies should be validated under carefully controlled and ethically acceptable conditions. The time-course for development of type 1 diabetes in humans, the aetiological factor(s) and the superimposed adverse effects on remaining beta cells induced by the metabolic derangements induced by impaired access to insulin deficiency, e.g. glucose- and lipotoxicity, need to be defined.

Extensive registries with associated biobanks of subjects at genetic risk for developing type 1 diabetes and of newly diagnosed type 1 diabetes patients would provide unique resources for the study of type 1 diabetes aetiology and pathogenesis as well as novel approaches to prevention and intervention therapies (*see also* Goal 4.1).

Milestones 2.2.18-21. Type 2 diabetes
Many factors responsible for beta cell destruction in type 2 diabetes have been suggested. These include (but are not limited to) high glucose, elevated plasma lipids, cytokines, leptin, autoimmune factors, amyloid deposition and the exhaustion of the beta cell. With respect to these causal factors, their mechanisms of action involve changes in the level and/or activity of the Fas pathway, the ATP-dependent potassium channel, insulin receptor substrate (IRS) 2, inflammation and oxidative stress, NFκB, endoplasmic reticulum stress, and mitochondrial dysfunction. Furthermore, islet-invading macrophages may contribute to this destructive process. Thus, beta cell failure and destruction in type 2 diabetes is a multi-factorial process that culminates in a reduced functional beta cell mass. The next steps will therefore be to define the precise role of each of these factors/systems to determine their relative importance (major/minor), their hierarchy (primary/secondary), at which stage they become significant (early/late stages) and how they interact with one another. This may also lead to sub-classification of type 2 diabetes, opening the way to individualised therapy.

Several of the mentioned inflammatory and destructive processes have also been implied in the pathogenesis of insulin resistance (*see also* Chapter 3) and endothelial damage in diabetic vasculopathy (Chapters 3 and 5). *See* Goal 4.8 for discussion of the implications of preserving beta cell functional mass for clinical outcomes and the prevention of acute and late diabetic complications.

Goal 2.3. Guide development of novel therapies based on understanding beta cell specific phenotypic characteristics related to regulation of defence/repair



Roadblock 1. Limited access to human beta cells/islets/pancreatic tissue

Introduction and background

Genome-wide association studies point at discrete genes predisposing to type 1 and 2 diabetes that seem to be responsible for regulating beta cell function, apoptosis and replication. Although many of these genes are expressed in beta cells, this does not mean that they are not also expressed in other cells. Indeed, there are examples that genes which lead to an increased diabetes risk also are associated with reduced risk of developing certain cancers and vice versa. This finding illustrates the need to fully understand the specific beta cell phenotypic characteristics in relation to other cell types (*see also* Milestone 1.2.02, Goal 4.8).

Milestone 2.3.01. Define genetic and environmental determinants of beta cell homeostasis

The precise physiological and pathophysiological role of genes identified by genome-wide association studies (or other genetic approaches) on beta cell function or regulation of mass needs characterisation. This research will require high-throughput functional assay platforms as well as novel methodological approaches, such as bio-modelling *in silico*.

Milestone 2.3.02. Clarify mechanisms underlying maintenance of beta cell homeostasis

In most individuals who do not develop diabetes, the endocrine pancreas has sufficient spare capacity to preserve beta cell function and ensure adequate insulin supply throughout life. Research on the mechanisms that support maintenance of beta cell homeostasis would help scientists understand why some people are more susceptible to beta cell loss and develop strategies to halt or reverse beta cell decline.

Milestone 2.3.03. Dissect beta cell regulatory defence mechanisms in health, ageing and diabetes

A genetically determined and age-related inadequate compensatory response will influence how the individual responds to immune, inflammatory and infectious insults and other stressors. For example, a slight decrease in insulin secretion caused by genetic variations insufficient to cause diabetes itself may interact with lifestyle factors, such as diet and exercise, to initiate multiple mechanisms by which hyperlipidaemia and hyperglycaemia can compromise beta cell function and survival by inducing apoptosis. This may activate both innate and adaptive immunity. It is therefore important to understand the interaction between epigenetic and genetic determinants in the maintenance of normal beta cell homeostasis and how this balance is perturbed in ageing and pathological states. Research should also focus on assessment of beta cell mass at different ages and in different genetic populations in lean and obese individuals with and without diabetes.

Milestone 2.3.04. Understand mechanisms underlying clinical remission in recent-onset type 1 diabetes and persistent residual beta cell function
Although the natural history of the remission or "honeymoon" period in recent onset type 1 diabetes has been described in many studies, very little is known about the mechanisms that underlie this transient period of improved beta cell function and reduced insulin requirements. Very likely this process is under both genetic and epigenetic control.

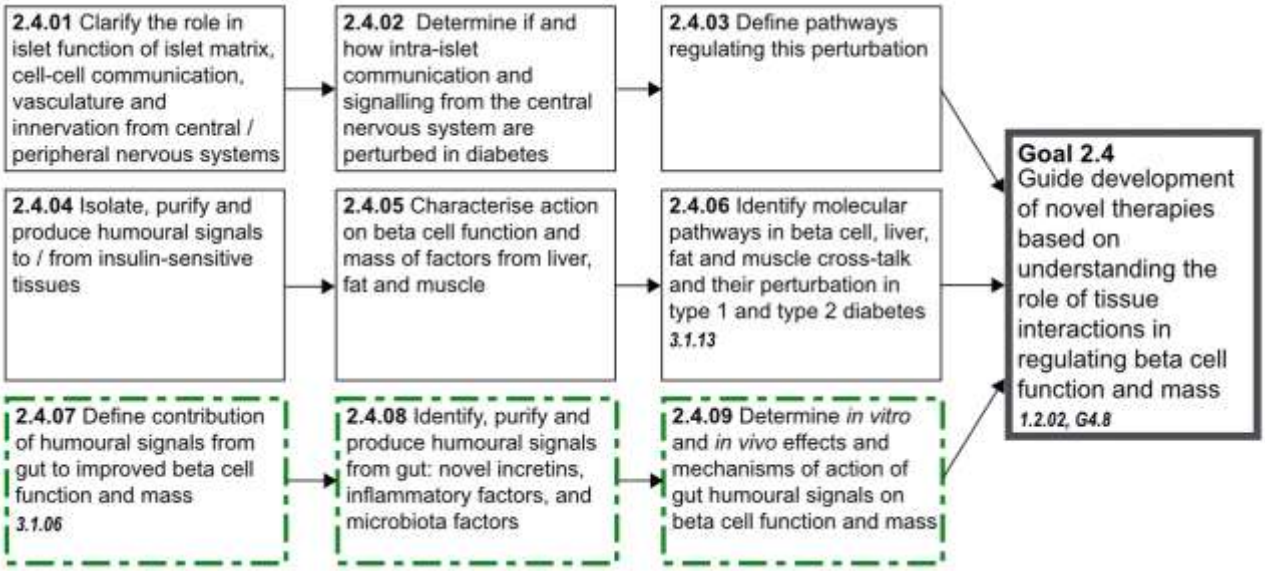
Milestone 2.3.05. Identify molecular targets involved in remission and preservation of beta cell function
The molecular mechanisms explaining why a significant number of individuals with type 1 diabetes, particularly with onset in adulthood, have notable residual beta cell function despite many years of disease duration are completely unclear. Unravelling this enigma will not only teach us important lessons about the causes of beta cell destruction, regeneration and survival but also improve the chances of developing interventions to preserve beta cell mass after the onset of overt diabetes.

Milestone 2.3.06. Determine relative role of anti-necrotic, anti-apoptotic and anti-autophagic defensive pathways in beta cell defence/repair
Little is known about the role of defensive pathways protecting human beta cells from destruction in response to cellular stressors. Although there is emerging evidence that apoptosis can be incriminated as one process causing beta cell destruction, the relative importance of necrosis, apoptosis, autophagy and other death pathways is unclear. Unravelling this will identify the defensive pathways that should be reinforced to prevent beta cell loss.

Milestone 2.3.07. Clarify regulation of beta cell defence/repair systems
It follows from Milestone 2.3.06 that it will be central to define the relative importance and modes of *regulation* of defence mechanisms against various death pathways.

Milestone 2.3.08. Manipulate beta cell anti-necrotic, anti-apoptotic and anti-autophagic defence/repair pathways
Complementary to inhibiting the death pathways that are dominant in beta cell destruction, systematic testing of pharmacological interventions that stimulate beta cell defence needs to be undertaken.

Goal 2.4. Guide development of novel therapies based on understanding the role of tissue interactions in regulating beta cell function and mass



Roadblock 5. Methods of maintaining myotubes, neuronal cells and intestinal tissue in culture

Introduction and background

Beta cells release insulin into the systemic circulation and affect the function of almost all tissues of the body. Conversely, they receive a rich input via the circulation (hormones and metabolites) and via nerve terminals (neurotransmitters), and are also influenced by the very specific physical micro-environment of the islet. This cross-talk between the beta cell, their immediate environment and the rest of the body is far more extensive than thought previously. It seems possible that disturbances in this cross-talk may contribute to impaired insulin secretion and diabetes.

A thorough understanding of the role and nature of the interactions between the beta cell and its environment in health and disease is required to achieve the overarching Goal of curing and/or preventing diabetes.

Milestone 2.4.01. Clarify the role in islet function of islet matrix, cell-cell communication, vasculature and innervation from central / peripheral nervous systems

The beta cells comprise approximately 60 percent of the endocrine cells within the pancreatic islets. The control of hormone release from the non-beta islet cells (in particular the alpha/glucagon, delta/somatostatin and epsilon/ghrelin cells) is much less well characterised than that of the beta cell, and this must be investigated using human islet cells. There are several hypotheses for the control of glucagon secretion that emphasise the role of paracrine signals. There is evidence to suggest that alpha cells also possess an intrinsic glucose sensor, but the processes involved remain unclear and should be clarified. The role of the delta cells in the physiology and pathophysiology of diabetes remains unclear, but somatostatin is a very powerful inhibitor of both glucagon and insulin secretion, raising the possibility that perturbed islet paracrine regulation may result in the defects of islet hormone secretion associated with human diabetes, including the loss of an adequate counter-regulatory glucagon secretion during hypoglycaemia.

Islets are richly vascularised. It is not known to what extent the vascular and thereby hormonal flow in human islets follows precisely that seen in rodents. However, paracrine signalling can also occur independently of the circulation via gap junctions and diffusion in the islet cell interstitium, influencing hormone release from cells in close proximity. Blueprints of these regulatory circuits are far from complete and will be essential for complete understanding of the coordinated regulation of islet hormone secretion.

Islet endothelial cells further contribute to the physical environment of the endocrine cells by deposition of extracellular matrix (ECM). In human but not rodent islets, the beta cell contributes towards creation of the bi-lamellar ECM surrounding them. While there has been some progress in understanding how ECM impacts on

beta cell function, replication and survival, more needs to be discovered, and any impact on non-beta cells remains to be studied. Finally, the layer of ECM or 'capsule' surrounding each islet has yet to be studied in detail, although it may be an important barrier in islet inflammation.

Milestone 2.4.02. Determine if and how intra-islet communication and signalling from the central nervous system are perturbed in diabetes
The islets are richly innervated with both vagal and splanchnic inputs. There is also evidence for intra-pancreatic ganglia that may serve to coordinate the secretion from many islets and in turn oscillatory secretion. Of note, most preparations used to study islet function involve the severing of the nerves entering the islets. Biopsies would be valuable to address how innervation is influenced by physiological and pathophysiological states.

Milestone 2.4.03. Define pathways regulating this perturbation
More research on human rather than rodent islets is needed. Indeed, paracrine signalling pathways may have different effects in rodent and human islets. It is likewise not known to what extent abnormal paracrine signalling contributes to the reduction of insulin secretion that is a hallmark of type 2 diabetes, or to beta cell defence and regeneration in type 2 diabetes. It would also be desirable to establish the receptor complements of the different islet cell types and to document how they are affected by obesity and diabetes and the potential pathophysiological significance of altered membrane receptor density. Such information also helps to ensure tissue specificity of any drugs targeting proteins/processes in the beta cell.

Tissue interactions: insulin-sensitive tissues

Milestone 2.4.04. Isolate, purify and produce humoural signals to/from insulin-sensitive tissues
It is now clear that fat and skeletal muscle play an important endocrine function and secrete many humoural factors, including leptin, adiponectin, TNFα, IL-1β, IL-6, and IL-1 receptor antagonist (IL-1Ra). These factors affect not only other major insulin-sensitive tissues but also act on other cells including the pancreatic beta cells. Research is needed to understand better how these factors transfer information among the different tissues and how this is modified by insulin resistance.

Milestone 2.4.05. Characterise action on beta cell function and mass of factors from liver, fat, and muscle
Research should identify muscle, fat and liver factors released under normal and pathological conditions with respect to their ability to regulate beta cell function and mass. Thus, insulin resistance may precipitate diabetes not only because of increased insulin requirement but also due to direct deleterious effect of the above-mentioned factors on islets. This is likely in type 2 diabetes but may also hold true in type 1 diabetes. Indeed, increased body weight is also a risk factor for development of type 1 diabetes.

Milestone 2.4.06. Identify molecular pathways in beta cell, liver, fat, and muscle cross-talk and their perturbation in type 1 and type 2 diabetes
The precise molecular mechanisms and pathways of cross-talk among these tissues should be identified. The involvement of these and other humoural factors in the interaction among non-beta cells is discussed in Milestone 3.1.14.

Tissue interactions: gut

Milestones 2.4.07-09. Incretins allow for communication between the gut and islet cells, and these peptide hormones are powerful modulators of postprandial insulin and glucagon secretion. Understanding this system allowed for the development of GLP-1-based therapy now in clinical use for type 2 diabetes patients (GLP-1 analogues/receptor agonists and DPP-4 inhibitors). It is however likely that other gut factors remain to be identified, including novel incretins and also possibly microbial factors derived from the gut flora. This topic is also covered in Goal 3.1, but here the major focus will be on exploring the mode of action of any novel gut factors on beta cell function as the platform for drug development.

Roadblocks Chapter 2

Roadblock 1. Limited access to human beta cells/islets/pancreatic tissue from diabetic and non-diabetic (and obese and pregnant) individuals
Human islet cells differ from their rodent counterparts. It is important, building on the firm knowledge base provided by studies in cell lines and rodent models, to characterise the human beta cell. The ability to interpret correctly the genetic association and expression data that are now emerging depends critically on accurate information about gene expression in pure fractions of human beta cells. Currently, human islets are obtained from organ donors. Given the major advances in modern endoscopic techniques, it is conceivable that safe and ethical bioptic methodologies for obtaining functional pancreatic tissue could be developed.

Roadblock 2. Lack of beta cell-specific surface ligands and safe labels of such ligands that have sufficient tissue signal penetration
Lack of beta cell-specific surface ligands that allow clinical imaging with sufficient resolution is an obstacle to prospective non-invasive imaging of islet and beta cell mass.

Roadblock 3. Lack of a unified European Union stem cell strategy
Many European Union Member States now allow experimental research with human embryonic stem (hES) cells, but there is still need for coordinated and harmonised ethical policies across Europe.

Roadblock 4. Ethical and regulatory issues
In order to translate novel therapeutic targets from multiple biochemical pathways into the clinic, which no doubt will require a combinatorial approach, there is an urgent need for facilitated processes to promote clinical trials testing combinations of therapy. This will pose demands on ethical and regulatory authorities and on funding agencies and industry.

Roadblock 5. Modern methods of maintaining myotubes, neuronal cells and intestinal tissue in culture are established, but scientific advances in this area will critically depend on the ability to study neuronal, hormonal and other humoural signals and responses in human specimens obtained from healthy and diabetic individuals, e.g. *in vitro* co-culture experiments and exposure of tissues to conditioned media from culture of other tissues. Organisationally, this could be linked to a European pancreas and islet procurement network. Further, identification of novel circulating humoural signals requires revival of the experimental techniques of classical clinical physiology and endocrinology. For example the demonstration of humoural signals as mediators of improved beta-cell function after bariatric surgery in experimental animals could be resolved by co-circulation shunts between operated and non-operated animals.