Pancreatic β-cell imaging in man: fiction or option?

Didier Laurent[1], Laurent Vinet[2], Smaragda Lamprianou[2], Marie Daval[3], Gaelle Filhoulaud[3], Alain Ktorza[3], Haiyan Wang[4], Sabine Sewing[4], Hans-Paul Juretschke[5], Heiner Glombik[5], Paolo Meda[6], Raphael Boisgard[7], Duc Loc Nguyen[7], Graeme J. Stasiuk[8], Nicholas J. Long[8], Xavier Montet[9], Peter Hecht[10], Werner Kramer[11], Guy A. Rutter[12*] and Jacob Hecksher-Sørensen[13*]

This article has been accepted for publication and undergone full peer review but has not been through the copyediting, typesetting, pagination and proofreading process, which may lead to differences between this version and the Version of Record. Please cite this article as doi: 10.1002/dom.12544

1) Biomarker Department – Clinical Imaging, Novartis Institutes for Biomedical Research, CH-4056 Basel, Switzerland
2) Department of Genetic Medicine and Development, University of Geneva, Geneva, Switzerland
3) Servier Research Institute, Metabolic Diseases Department, Suresnes, France
4) Roche Pharma Research and Early Development, Innovation Center Basel, CH-4070 Basel, Switzerland
5) Sanofi-Aventis Deutschland GmbH, R&D DSAR/BBB D-65926 Frankfurt am Main, Deutschland
6) Department of Cell Physiology and Metabolism, University of Geneva, Geneva, Switzerland
7) Commissariat à l'Energie Atomique (CEA), Equipe d'Imagerie Moléculaire Expérimentale, Orsay, France
8) Department of Chemistry, Imperial College London, South Kensington Campus, London SW7 2AZ, UK.
9) Department of Radiology, Geneva University Hospital, Geneva, Switzerland
10) IMIDIA Project Office, Schirmleitenstrasse 95; 8046 Graz Austria
11) Scientific Consultant for Sanofi Deutschland GmbH, D-65926 Frankfurt am Main, Germany
12) Section of Cell Biology, Department of Medicine, Imperial Centre for Translational and Experimental Medicine, Imperial College London, Hammersmith Hospital, du Cane Road, London W12 0NN, UK.
13) Department of Histology and Imaging, Novo Nordisk Park, DK-2760 Måløv, Denmark.

*To whom correspondence should be addressed: Professor Guy A. Rutter (g.rutter@imperal.ac.uk) or Dr Jacob Hecksher-Sørensen (jhes@novonordisk.com) or Dr Didier Laurent (didier.laurent@novartis.com)

Main Text (excl abstract, refs, figure legends, acknows) = 5083 words

This is the peer reviewed version of the following article: Laurent, D., Vinet, L., Lamprianou, S., Daval, M., Filhoulaud, G., Ktorza, A., Wang, H., Sewing, S., Juretschke, H.-P., Glombik, H., Meda, P., Boisgard, R., Nguyen, D. L., Stasiuk, G. J., Long, N. J., Montet, X., Hecht, P., Kramer, W., Rutter, G. A. and Hecksher-Sørensen, J. (2016), Pancreatic β-cell imaging in humans: fiction or option?. Diabetes Obes Metab, 18: 6–15,which has been published in final form at http://dx.doi.org/10.1111/dom.12544. This article may be used for non-commercial purposes in accordance With Wiley Terms and Conditions for self-archiving.

Abbreviations,; IMIDIA, Innovative Medicines Initiative in DIAbetes; MRI, magnetic resonance imaging; OPT, optical projection tomography; PET, positron emission tomography; SPECT, single photon emission computed tomography; VMAT, vesicular monoamine transporter; GLP1, glucagon-like peptide-1; PSA-NCAM, polysialylated neural cell adhesion molecule; RIP-DTr, rat insulin promoter diphtheria toxin; SUR 1, sulfonylurea receptor 1; STZ, streptozotocin; T1DM type 1 diabetes; T2DM, type 2 diabetes; TMEM27, transmembrane protein 27; USPIO, ultrasmall superparamagnetic iron oxide

Abstract

Diabetes mellitus is a growing worldwide epidemic disease, currently affecting 1 in 12 adults. Treatment of disease complications typically consumes ~10% of healthcare budgets in developed societies. Whilst immune-mediated destruction of insulin-secreting pancreatic β-cells is responsible for Type 1 diabetes, both the loss and dysfunction of these cells underlies the more prevalent Type 2 diabetes. The establishment of robust drug development programmes aiming at β-cell restoration is still hampered by the absence of means to measure β-cell mass prospectively *in vivo*, an approach which would provide new opportunities for understanding disease mechanisms and ultimately assigning personalized treatments. Here, we describe progress towards this goal achieved by the Innovative Medicines Initiative in DIAbetes (IMIDIA), a collaborative public-private consortium supported by the European Commission and dedicated resources of pharmaceutical companies. We compare several of the available imaging modalities and molecular targets and provide suggestions as to the likeliest to lead to tractable approaches. Furthermore, we discuss the simultaneous development of animal models that can be used to measure subtle changes in β-cell mass, a prerequisite for validating the clinical potential of the different imaging tracers.

Introduction

Changes in pancreatic β-cell mass contribute to the development of both type 1 (T1DM) and Type 2 (T2DM) diabetes, diseases which currently affect >340 million individuals worldwide [1] and whose incidence in adults is projected to increase by ~20 % by 2030. Whereas immune cell-mediated mechanisms lead to near-complete (>90%) elimination of β-cells in T1DM, both the extent of the loss [estimates range from ~ 25 % [2] to 60 % [3]] and the underlying defect are less well established for T2DM [4]. Nonetheless, these changes are likely to involve the interplay of genetic and environmental factors.

β-cells comprise only 0.3-2.0 % of the total pancreatic mass [2]. Distinguishing such a rare population of cells from others in the pancreas is extremely difficult and, whilst several approaches provide information on *functional* β-cell mass, those quantifying the "*absolute*" β-cell number or volume are less well developed. Moreover, and as already indicated above, there is substantial variation in β-cell mass even between healthy subjects [2]. These challenges make it unlikely that β-cell mass imaging will be used as a predictive tool for diagnosing diabetes in humans in the near future. It is more likely that the value of β-cell mass imaging can be exploited in longitudinal studies or clinical trials where two or more time points will indicate increases or decreases in β-cell mass in the same individual.

In recent years, there has been significant progress in our understanding of how β-cell mass is regulated [5-8] and today drug development is geared towards compounds that can increase β-cell mass or prevent β-cell death. While the efficacy of such compounds is relatively easy to determine in mice and rats using post mortem histology, it is very challenging to translate these results to humans. At present, only measurements of circulating C-peptide and insulin levels may allow β-cell mass assessments, and these approaches are indirect, and lack sensitivity and reproducibility. Facing the urgent need of developing new sensitive tools that can be applied to human, β-cell imaging appears as an obvious candidate. However it should be noted that, given the high variability in β-cell mass, which can exceed 100% as typically found in healthy individuals [2], β-cell imaging may in fact be limited to the longitudinal monitoring of β-cell mass rather than being used as a pure diagnostic tool. Yet, the main challenge resides in the detection of subtle changes as

opposed to the almost complete loss of β-cell mass as found in people affected by T1DM, and long-term T2DM, respectively.

For the past four years, the pharmaceutical companies Novartis, Novo Nordisk, Roche, Sanofi and Servier have been working together in close collaboration with academia to develop new tools for β-cell imaging. Thus, in 2008, the European commission launched the Innovative Medicines Initiative (IMI) with the goal of 'significantly improving the efficiency and effectiveness of the drug development process with the long-term aim that the pharmaceutical sector produces more effective and safer innovative medicines' [9-11]. IMIDIA (The Innovative Medicine Initiative in Diabetes; [12]) is one of several programmes within IMI and was launched in 2009. Comprising 12 academic institutes, 8 pharmaceutical companies and one Biotechnology Company, the overall goal of the consortium is to generate new tools, biomarkers and knowledge on β-cell biology and this includes imaging pancreatic β-cell mass. Facing the urgent need of developing new sensitive tools that can be applied to human, β-cell imaging appears as an obvious candidate. However it should be noted that, given the high variability in β-cell mass, which can exceed 100% as typically found in healthy individuals [2], β-cell imaging may in fact be limited to the longitudinal monitoring of β-cell mass rather than being used as a pure diagnostic tool. Yet, the main challenge resides in the detection of subtle changes as opposed to the almost complete loss of BCβ-cell mass as found in T1DM and long-term T2DM, respectively

Imaging of β-cells has recently been reviewed in a number of excellent publications [13-17]. However, here we further discuss how we envisage that β-cell imaging will enable the development of new therapeutics for β-cell regeneration and transplantation, as well as contribute to knowledge development around personalized medicine, disease progression, biomarker validation, translational applications and drug design. Finally, we provide our vision on β-cell imaging in the future, and how we believe this is likely to advance drug discovery.

Development of Imaging Probes.

The process of developing imaging agents for the measurement of β-cell mass in humans can be structured into a number of consecutive stages, starting with the identification of a β-cell specific target and agent (Fig. 1A, left), and ending with a probe suitable for measuring β-cell mass (Fig. 1A, right). However, for each step the biological complexity and safety concerns increase and, as a consequence, the number of promising agents decreases. In the early stages, there is a need to establish at least some β-cell specificity and the cellular resolution offered by a fluorescent tracer is attractive. However, for *in vivo* imaging, other imaging modalities such as magnetic resonance imaging (MRI) and positron emission tomography/single photon emission computed tomography (PET/SPECT) are likely to be the only realistic options for studies in large animals and in man. Within IMIDIA, β-cell imaging is organised in six approaches, which together span all the steps considered to be important for the development of a tracer capable of measuring β-cells mass in humans (Fig. 1B). Furthermore, keeping in mind that the overall purpose of this consortium is to study drug-induced changes in β-cell mass over time, we are employing various models for β-cell regeneration. These include the rat insulin promoter diphtheria toxin (RIP-DTr) mice where 100% or 50% of the β-cell mass can be ablated [18, 19] and the insulin antagonist S961 that was recently shown to increase β-cell mass [6].

Imaging modalities

Among available modalities, MRI provides excellent anatomical resolution but suffers at the molecular scale due to its intrinsic low sensitivity: targets are therefore likely to be abundant, and their visualization may require high concentrations of tracer. PET imaging, on the other hand, provides higher sensitivity but brings challenges of low resolution, high costs and limited convenience. At present, computer tomography (CT) and ultrasound provide little specificity. As for fluorescence imaging, effective light penetration in the tissue being limited to a few cm, new near-infrared fluorescent β-cell imaging agents may only be used for studies in small rodents (*i.e.* mouse). In addition, CT and MRI contrast agents are typically dosed at concentrations in the mM range, which can carry a significant toxicological risk. This makes the development process of such tracers nearly as long and expensive as for a new therapeutic drug[20]. By contrast, PET radiotracers are typically administered in microdoses, which by definition should not elicit a pharmacological effect. Thus, under the new guidance from health authorities (ICH guidelines [21]), PET tracers may be in a better position to offer a first solution for clinical trials, until another generation of tracers, including those developed for use in clinical MRI scanners, become available.

Specific criteria for β-cell imaging

For MRI-based studies, the chief challenge is in identifying target molecules which are both abundant and, at least to some extent, selectively accumulated by the β-cell. As described in the next section, metal ions such as Zn^{2+} and Mn^{2+} ions, as well as sulphonylurea (SUR-1) and glucagon-like peptide-1 (GLP-1) receptors provide examples, which might be targeted in useful approaches to functional and total β-cell mass assessment, respectively.

For PET studies, the main challenge resides in the selection of a radiolabelled ligand with a reasonably high affinity for a β-cell specific target as well as high specific activity. Using a realistic number of 100,000 binding sites/cell and assuming a need for a contrast between specific and unspecific labelling of at least 5:1, one can estimate that the dissociation constant K_d of such a ligand needs to be less than 1 nM [22, 23]. The sensitivity may be greater if the selected tracer uses a specific mechanism to accumulate in β-cells. A slow dissociation from the target, fast elimination of the free extracellular tracer and low non-specific binding could reduce this requirement for high affinity by a factor of ~10.

Other practical aspects include high signal-to-noise ratio, favourable pharmaco-kinetics and absence of toxicity. Furthermore, the low spatial resolution of positron cameras prevents distinction of islets dispersed within the exocrine pancreas. The resulting partial volume effect can affect to some degree quantification of the pharmacological response, unless the β-cell ligand proves to be highly specific. Similarly to other tracer developments, acceptance criteria must be met for biological qualification (using e.g. tissue autoradiography) prior to costly imaging sessions in animal models and/or humans. Kinetic modelling may also be considered for accurate quantification. Stability in the expression of the target (i.e. number of binding sites/β-cell) should preferably be independent of disease status. At the stage of research and preclinical development, multimodal probes (e.g. combining fluorescent, MRI or even PET-active moieties within the same molecule) may be useful to facilitate preclinical studies in model systems including rodents.

Tools and targets for imaging of β-cell mass

(1) "Absolute" β-cell mass. The following examples are of targets whose expression is likely to reflect the total number (or volume) of β-cells, rather than secretory function.

Bioluminescence

Bioluminescence is the emission of light resulting from the catalysis of luciferin by a luciferase enzyme [24, 25]. Using highly sensitive cameras, the few photons emitted by a tissue source can be detected at the surface of an animal, making the method adaptable *in vivo* to many different systems [24, 26]. The only requirement is the prior provision of the enzyme substrate, usually by injection, to the organism under study. However, a serious limitation is that luciferase needs first to be expressed by transgenesis or transduction, possibly in selected cell types, using a cell-specific promoter [25]. Using part of the insulin promoter to target luciferase to pancreatic β–cells [27], bioluminescence imaging has been documented to provide a signal linearly related to the mass of rodent β-cells [27]. Thus, β-cell mass can be rapidly and repeatedly monitored over time in the very same animal, e.g. as loss of β–cells is chemically induced [28] or spontaneously develops in models of type 1 [29, 30] or type 2 diabetes [31]. In vivo bioluminescence imaging can also monitor the fate of islets after transplantation [32, 33], and the regeneration of β-cells exposed to candidate drugs [34]. It also significantly contributes to the evaluation of alternative imaging approaches [33].

Findings from IMIDIA

In collaboration with Powers and colleagues, who pioneered the study of beta cell mass by bioluminescence imaging [27, 30, 32, 33], we have studied a transgenic model which provides for a differential loss of β-cells in male (> 95 %) and female mice (~ 50%) of the RIP-DTr strain [18]. Two independent mouse strains expressing both the luciferase and the RIP-DTr transgenes in the β–cells were generated [33]. In both strains, the bioluminescence monitoring of the very same mice, prior and after injection of a cytotoxic dose of diphteria toxin, showed that the rapid, and almost complete loss of β–cells observed in male mice, correlated with a parallel depletion in the insulin content of the pancreas [33]. In female mice, the same experiment also showed that the loss of about half the β–cells, which correlated with a more modest loss of the insulin content and of the bioluminescence imaging signal [33]. The data show that bioluminescence imaging can detect a submaximal change of the β-cell mass, with an amplitude similar to that expected in people with T2DM, and differentiates this moderate change from the much larger change, which mimics the situation prevailing in most type 1 diabetics. We further longitudinally monitored the animals, for up to 9 months, and observed that both the insulin content and bioluminescence imaging signal of male mice remained at the low levels. In contrast, about 30% of the females showed a progressive

and regular increase of the bioluminescence imaging signal (Vinet *et al*., unpublished). At the end of the experiment, the increased bioluminescence imaging signal correlated with a partial recovery of the insulin content. The data demonstrate the lack of toxicity of the bioluminescence imaging procedure, and show that the approach has the sensitivity to prospectively monitor, in individual animals, subtle changes of the β-cell mass.

The findings summarized above stress the versatility of the bioluminescence approach, and its relevance in islet and diabetes research. Although the method does not translate to human, it serves a purpose in preclinical settings to evaluate potential candidate drugs before the trials on large animals and humans.

TMEM27 (antibody)

Although likely to be challenging, selective targeting and imaging of β–cells using an antibody-mediated approach is feasible *in vivo*[35], and may be useful for both preclinical and clinical applications. The transmembrane protein 27 (TMEM27) is selectively expressed on the β-cell surface and in kidney collecting ducts[36]. A monoclonal antibody, 8/9-mAb is specific to human TMEM27 (hTMEM27)[37], and upon suitable labelling, could be used for specific imaging of β-cells *in vivo* by fluorescence or PET imaging. The specificity of fluorescently-labelled 8/9-mAb was evaluated in human tissue sample microarrays and pancreatic sections from transgenic mice expressing hTMEM27 under the control of rat insulin promoter (RIP-hTMEM27-tg). The biodistribution of the antibody and its binding to hTMEM27 *in vivo* were assessed in a nude mouse subcutaneous insulinoma model expressing high levels of hTMEM27 using [^{89}Zr]-8/9-mAb, which revealed a high signal-to-background contrast in subcutaneous tumours one day after antibody injection. In addition, target-specific *in vivo* imaging was used to assess β-cells in RIP-hTMEM27-tg mice with fluorescent labelled AF 680–8/9-mAb and similarly high label retention in the pancreatic islets of the RIP-hTMEM27-tg mice was observed[37].

Findings from IMIDIA

The human TMEM27-specificity of 8/9-mAb limits its use for β-cell *in vivo* imaging in preclinical models of diabetes. Therefore changes in β-cell mass during disease progression or therapeutic intervention cannot be assessed with 8/9-mAb in rodent models. Cross species selective antibodies

against β–cell specific surface proteins can overcome this hurdle. Further studies need to be conducted to monitor changes in β-cell mass *in vivo* and assess the safety of such approaches.

PSA-NCAM (antibody)

The polysialylated Neural Cell Adhesion Molecule (PSA-NCAM) is a post-translationally modified form of NCAM (CD56) [38]. High expression of PSA-NCAM is found during development, specifically in the central nervous system, where it is involved in tissue modeling [39], but after birth, PSA-NCAM is mainly restricted to certain brain regions and pancreatic β-cells. The specific expression of PSA-NCAM in rodent pancreatic β-cells was shown in 1994 [40], and later confirmed [41, 42]. A positive correlation was also demonstrated between PSA-NCAM expression level and altered β-cell mass in animal models. However, PSA-NCAM expression at the cell membrane correlates with the secretory capacity of β-cell, suggesting that this molecule may also reflect functional β-cell mass.

Findings from IMIDIA

Consistent with its possible use as a marker for β-cell imaging, unpublished data obtained on human pancreatic sections indicated that PSA-NCAM is mainly expressed in the endocrine pancreas consistent with earlier findings [43]. However, in this study, PSA-NCAM staining performed on dispersed pancreatic islet cells was also detected in other (non-β) endocrine cells. Nonetheless, magnetic sorting of dispersed cells using PSA-NCAM antibody resulted in an enriched endocrine cell population, of which ~95% were β-cells [43]. Specific expression of PSA-NCAM in human β-cells remains thus to be confirmed.

The potential of PSA-NCAM as a marker for non-invasive imaging of β-cells can be assessed in rodents using a labeled PSA NCAM antibody. To determine whether an antibody-based approach to target PSA NCAM can be developed for β-cell imaging, biodistribution and toxicity studies will be essential. Preliminary data using whole islet isolated from rats indicate that antibody binding to PSA NCAM sites on β-cells does not affect glucose-stimulated insulin secretion, demonstrating the absence of functional toxicity. However, extended toxicity studies will be needed in both *in vitro* and *in vivo* models to validate this approach.

GLP-1R (ligand)

Peptides targeting the glucagon-like peptide-1 receptor (GLP-1R) are promising candidates for use in β-cell imaging because 1) they should target the β-cells with high specificity [44-55], given the abundant expression of these receptors on native islet β-cells [56] and 2) they are safe and already used in the clinic for treatment of type 2 diabetes. Among these, Exendin-4, found in the saliva of the Gila monster [57], binds to the extracellular domain of GLP-1R with pM affinity [58]. Exendin derivatives have, therefore, been developed for fluorescence, nuclear and MR imaging of endogenous β-cells [47, 53, 59-65], transplanted islets [66, 67] and insulinomas [65, 68-70], including in humans [51]. The recent PET and SPECT studies open up the possibility of testing the exendin-4 derivatives in a clinical setting. These studies, however, have also revealed the difficulty to quantify the low signals emanating from native islets [63, 71]. Such limitations may prevent the detection of submaximal changes of β-cell mass, *e.g.* in the monitoring of early onset T1DM or T2DM or potentially their use in regenerative β-cell-targeted therapies. Furthermore, the above studies have confirmed the high kidney uptake of the positron-emitting exendin probes [63, 71], raising serious safety concerns for use in longitudinal studies where repeated imaging is necessary.

Findings from IMIDIA

Following intravenous injection, the fluorescently-labelled probes targeted the β-cells in the pancreas to a very high extent. At the cellular level, it was possible to determine that the GLP-1R agonist, exendin-4 (Ex4), was internalised into the β-cells while the antagonist (Ex4_9-39) remained at the plasma membrane. Furthermore, 3D imaging (OPT) revealed that, in the mouse pancreas, the probes were highly specific for the β-cell. The signal in the β-cells was completely lost in mice lacking the GLP-1R confirming that the accumulation is mediated by this receptor. Efforts to characterize the specificity of the probe in various mouse models of diabetes are currently ongoing [72]. In parallel, in order to evaluate if MRI can be used to measure β-cell mass, we developed a different, dual modality nanoparticle probe, which targets GLP-1R, and is suitable for both fluorescence and MRI. The probe (Np647-Ex) consists of multiple copies of a modified exendin-4 peptide linked to USPIOs, tagged with Alexa 647 fluorochrome [72]. The probe selectively targets insulin-containing cells and allows longitudinal monitoring of individual animals, whilst differentiating between partial (~50 %) and almost complete loss of β-cells [18].

Sulfonylurea receptors (Small molecule)

Sulfonylureas are antidiabetes drugs used to mitigate T2DM symptoms by increasing insulin secretion. The drugs bind to specific sulfonylurea receptors (SUR 1) located in the plasma membrane of pancreatic islet cells [73, 74] but not in the exocrine acinar cells [75]. SUR1s are expressed at high density in β-cells [73, 74, 76] but also other endocrine pancreatic cells [75]. Despite this, the established clinical use of sulfonylureas, such as glibenclamide [22, 75-77] makes these drugs attractive candidates for β-cell mass imaging.

Findings from IMIDIA

Previous studies have revealed complications in the use of glibenclamide including binding to plasma proteins, rapid clearance and insufficient accumulation in the islets [22, 74-76, 78]. We have thus tested modified versions of the original molecules carrying moieties detectable by different imaging modalities, specifically *in vivo* fluorescence, which do not excessively disturb the binding affinity to SUR1. *In vitro* studies on living cells showed that several configurations, where multiple rhodamine molecules were directly attached to native glibenclamide resulted in probes without affinity for SUR1. We therefore decided to use a backbone permitting the attachment of both glibenclamide-derivatives (*e.g.*, glib-B) and the rhodamine fluorochromes.

Testing such backbones, we found useful the non-toxic PolyAMidoAMide (PAMAM) dendrimers [79, 80] to increase hydrophilicity (thereby decreasing binding to plasma proteins). Spherical probes containing 3 to 7 glib-B moieties (Ø~6 nm diameter) displayed the best compromise between specificity and affinity and specifically bound to SUR1 of insulin-containing cells in mouse and human islets (Babič, Lamprianou *et al*. in preparation). However, intravenous injection of selected probes in control mice resulted in intense staining of vessels in the endocrine pancreas, as well as in the glomeruli of the kidney. Moreover, hypoglycaemia was not observed, implying that the probes do not bind (or bind only weakly) *in vivo* to the SUR1 of the insulin-producing cells.

VMAT2 (Small molecule)

Vesicular monoamine transporter (VMAT) is a transmembrane protein that translocates monoamines from the cytoplasm into secretory vesicles. The type 2 protein (VMAT2) is highly expressed in pancreatic β-cells [81], and may have a possible role in insulin secretion [82]. PET-based quantitation of VMAT2 was made possible by the development of radiolabelled dihydrotetrabenazine (DTBZ) analogs, which specifically bind to VMAT2. Of the compounds

tested within IMIDIA, [18F]FP-(+)-DTBZ (or [18F]AV-133) appears to be the most promising candidate tracer, both in rodents and humans [83-86], especially since it was shown in a cross-sectional study to be able to discriminate people with T1DM from healthy volunteers, despite some residual binding (~30% of healthy subjects) in the pancreas [50]. However, the specificity of molecules targeting VMAT2 was brought into question when Goland et al. [83] reported significant [^{11}C]DTBZ uptake in long-standing type 1 diabetes, in line with Tsao et al. [87] showed low non-specific binding of [^{18}F]AV-133 to non-β-cells in rats. It recently appeared that non-specific binding as estimated by the negative enantiomer [^{18}F]FP-(-)-DTBZ in T1D pancreata, normal human and non-human primate pancreas is most likely less than 15% of the total signal [109,110]. This leaves the other portion of binding to be off-target (i.e. non β-cells) binding to VMAT2. To which extent such residual VMAT2-positive signal also emanates from insulin negative cells, including β-cell precursors and polypeptide (PP) cells [82,83] remains to be clarified. Yet, several studies have suggested that adult β-cells might originate from duct or duct-associated cells [109,110] and VMAT2 was also shown to regulate differentiation in β-cell precursors [111]. Meanwhile, it appeared that rodents may not be optimal for DTBZ imaging of β-cells [33, 88], while primates or pigs may be better suited to the study of the human disease.

Findings from IMIDIA

Our efforts were geared towards further validation of the VMAT2 target in a large animal species, as well as assessing the usefulness of this target to detect a longitudinal change in β-cell mass *in vivo*. To this end, minipigs were treated with streptozotocin (STZ) to induce β-cell loss. Clear uptake of [^{18}F]FP-DTBZ could be visualized from *in vivo* PET images in the pancreas of normoglycemic pigs and quantified by measuring the corresponding standardized uptake value (SUV). Individual results appeared to be associated with a ~10% variability as shown from test-retest measurements, whereas inter-individual variations exceeded 200%, in line with human data. A moderate decrease in the pancreatic uptake of [^{18}F]-FP-DTBZ could be visualized in response to STZ treatment (unpublished). Whether such a decrease correlates well with β-cell mass as measured *post-mortem* by histology, both with insulin and VMAT2 staining, still needs to be investigated.

(2) "Functional" β-cell mass. The following are examples of targets, which might be used to measure absolute or functional β-cell mass.

Zinc

High concentrations of Zn(II) ions exist in insulin granules (ca. around 30 mM) [89] of which a small but significant proportion (1-100 μM) is unbound[90]. Zn^{2+} is also present in alpha cell granules, albeit at lower levels [91]. Given the relative scarcity of zinc ions throughout the rest of the pancreas [92], and the widespread use of the colorimetric zinc probe dithizone to stain islets, imaging agents capable of binding Zn(II) may be useful as a means to quantify β-cell mass *in vivo*.

There are very few reported MR imaging agents for quantification of β-cell mass that focus on zinc binding. One example is GdDOTA-diBPEN, an MR agent sensitive for Zn(II), as reported by Lubag and colleagues [93, 94]. This compound shows enhancement in the MR images of mice in regions corresponding to the pancreas, and appears to measure zinc co-secreted with insulin into the bloodstream. Thus, images of the pancreas were enhanced only after glucose was elevated to a level that stimulates insulin secretion whilst the agent did not enhance MR images of the pancreas in euglycemic mice or in mice pretreated with STZ. Serial MR images of mice collected during a prolonged period of high-fat (60%) feeding showed a dramatic increase in contrast enhancement throughout the abdomen, consistent with expansion of the pancreas and a concomitant overall increase in β-cell function.

Findings from IMIDIA

We have recently developed a novel dual-modal MRI/optical probe for zinc [95]. This is based around the 'gold standard' DOTA motif[96] featuring a Gd(III) centre (MRI) alongside a fluorophore which acts as both the fluorescence probe and zinc binding motif. The probe has a Kd of 22 μM, and undergoes a ratiometric shift in fluorescence emission from 410 nm to 500 nm, with a relaxivity of 4.2 $mM^{-1}.s^{-1}$. This value increases upon addition of half an equivalent of Zn(II) to 6.6 $mM^{-1}.s^{-1}$, decreasing back to 4.9 $mM^{-1}.s^{-1}$ (at 400 MHz). Cellular imaging data show that this probe localises to the β-cell granule, consistent with binding to free zinc in this compartment. *In vivo* studies show limited uptake into the pancreas, consistent with selective uptake into the endocrine (β-cell) compartment. The probe is efficiently accumulated into secretory granules in β-cell-derived lines and isolated islets, but more poorly by non-endocrine cells, and leads to a reduction in T1 relaxation in human islets. Biodistribution studies demonstrated clear but low uptake of the agent into the pancreas, and suggest that this probe can potentially report either β-cell mass (total zinc-

positive volume) or function (average intensity per islet) if used in combination with other measures of β-cell mass. However, it should be noted that single islet resolution is presently unlikely to be achieved with existing clinical scanners (<7 Tesla).

Manganese

Manganese ions (Mn^{2+}) significantly enhance the contrast of MR images by shortening the T1 relaxation time and enhances the contrast of the pancreas [97] by entering stimulated β-cells through calcium channels[98]. Mn^{2+} has thus emerged as a potential tool for the *in vivo* evaluation of (*functional*) β-cell mass. Correspondingly, Mn^{2+}- enhanced MRI (MEMRI) of the pancreas was significantly decreased after an almost complete loss of β-cells in a mouse model of chemical ablation [99], and progressively declined with the loss of β-cell mass in a model of type 1 diabetes [100].

Findings from IMIDIA

To further evaluate the usefulness of Mn^{2+} as an MRI contrast agent to monitor the β-cell mass/function, we first used a high field (14.1T) MRI machine to assess whether Mn^{2+} could confer a contrast sufficient to visualize native, individual islets [98, 99, 101-103]. In normoglycemic conditions as well as after glucose infusion, MEMRI distinguished different tissues within the pancreas, including individual islets with a diameter larger than 50 μm, and correctly quantified their volume density [103]. MEMRI also detected a significant decrease in the numerical and volume density of islets in a model of T1DM [103].

To investigate the possibility that a clinically available MEMRI could be adequate to monitor the change in β-cell mass/function in people with T2DM, we retrospectively evaluated a series of whole-body MEMRI for normoglycemic participants or people with T2DM [104]. Enhancement was significantly lower in the pancreas of diabetic than of normoglycemic participants [104].

Other β-cell imaging agents not explored in IMIDIA

The scope of IMIDIA was designed according to the probes the different partners included in the program. Consequently this meant that a number of agents known to target β-cells were not analysed as part of IMIDIA program and therefore not discussed in this review. Below are listed the most promising of these agents and their targets.

- IC2, a rat monoclonal IgM Ab, which targets sphingomyelin on the β-cell surface [105].

- Single-chain antibodies (SCA), such as SCA-B1 [106]. The target of SCA-B1 is not known but was shown specific for β-cells and suitable for MR imaging, after coupling it with highly magnetic cobalt nanoparticles [34].

- Less specifically, hydroxy-tryptophan targets the serotonergic innervation, which is present in all the endocrine cells of the pancreas [107]. However, in spite of their wide expression, this probe appeared to show a correlation between β-cell mass and pancreatic signal in STZ treated rats [107] and have been able to differentiate people with T1DM from healthy people [108, 109] Somatostatin receptors were also proposed as a target for imaging probes to monitor β-cell mass. Thus, Sako et al. [110] have synthesized a PET probe, ^{68}Ga-DOTA-octreotide, and shown that its accumulation in the pancreas was reduced in STZ treated rats.

Concluding remarks: clinical and industrial perspective

The development of a non-invasive read-out of β-cell mass, combined with measurement of β-cell function, is expected to provide improvements in both disease management and clinical trial assessments. By demonstrating the mode of action of a candidate drug (*e.g.* a β-cell preserving or regenerating agent), a successful β-cell mass marker will help to decrease risk by allowing critical decisions earlier in the drug development process.

Of the agents studied in IMIDIA, the VMAT2 and Exendin based PET tracer presently represents the most advanced approaches to assess β-cell mass in humans [50, 51]. However, while both tracers may be able to detect differences between people suffering from T1DM and healthy controls further studies are required to determine if the sensitivity and specificity is good enough detect subtle changes over time. GLP1R agonists may also show significant promise for an MRI application in mice and may already be worth considering for use in combination with Zn^{2+} or Mn^{2+} probes to assess β-cell function in preclinical studies.

An important conclusion from this IMIDIA program is that the development of the tracers needs to go hand in hand with development of animal models where subtle changes in β-cell mass can be induced and subsequently measured in longitudinal studies. Since none of the probes investigated are likely to fulfil all criteria for a "perfect" β-cell imaging agent, these models will provide a benchmark for the changes in BCM which are actually possible to measure. Furthermore, it is likely that imaging of β-cell mass needs to be developed in combination with other biomarkers. If robust methods for imaging of β-cell mass can be developed, then they may serve as references for circulating biomarkers reflecting changes in β-cell mass. Since these biomarkers should be more convenient and inexpensive compared to imaging, they may become key elements of personalized medicine. However until then, it is clear that further hurdles need to be overcome in the development of a gold-standard imaging method, as well as appropriate animal models before β-cell imaging becomes available in the clinic. New imaging tools from which people with diabetes will undoubtedly greatly benefit should emerge from the combined efforts of academic and industrial organizations, as described here.

Target	**Probe**	**FL**	**MRI**	**PET/ SPECT**	**Main Challenges**	**Refs**
TMEM27	antiTMEM27	Successfully Attempted	Not Attempted	Successfully Attempted	1) Only recognizes human TMEM27 2) Reformatting antibody to tracer	[37]
PSA-NCAM	antiPSA	Not Attempted	Not Attempted	Not Attempted	1) Specificity 2) Reformatting antibody to tracer	[41, 42]
GLP1R	Ex4	Successfully Attempted	Successfully Attempted	Successfully Attempted	1) GLP-1R expression in disease models	[47, 51, 72]
SUR1	Multimerized Glibenclamide	Successfully Attempted	Difficult	Not Attempted	2) Exposure time in vivo. 3) Retention in blood vessels	[22, 74-76, 78]
VMAT2	FP-DTBZ	Difficult	Difficult	Successfully Attempted	1) VMAT2 expression in PP cells 2) Validity of rodent models unclear	[50]
Zn	Chelators	Successfully Attempted	Successfully Attempted	Not Attempted	1) Toxicity 2) Sensitivity	[93-95]
Ca^{2+} Channel	Manganese	Not Compatible	Successfully Attempted	Not Compatible	1) Non specific 2) Safety concern	[97, 99, 103, 104]
Insulín gene	Luciferase	Successfully Attempted	Not Compatible	Not Compatible	1) Limited to small transgenic animals	[27, 30]

Table 1: Summary of the different tracers used in IMIDIA and which tracers they have been used with to date. Also listed is the main challenge associated with a particular marker.

Acknowledgements

The work leading to this publication has received support from the Innovative Medicines Initiative Joint Undertaking under grant agreement n° 155005 (IMIDIA), resources of which are composed of a financial contribution from the European Union's Seventh Framework Programme (FP7/2007-2013) and EFPIA companies' in kind contribution. G.A.R. thanks the MRC (UK) for Programme grant MR/J0003042/1, the BBSRC (UK) for a Project grant (BB/J015873/1), the Royal Society for a Wolfson Research Merit Award and the Wellcome Trust for a Senior Investigator Award (WT098424AIA). G.A.R. and N.L. received an Imperial College/Wellcome Trust Institutional Strategic Support Funds (ISSF) grant.

Figure Legends

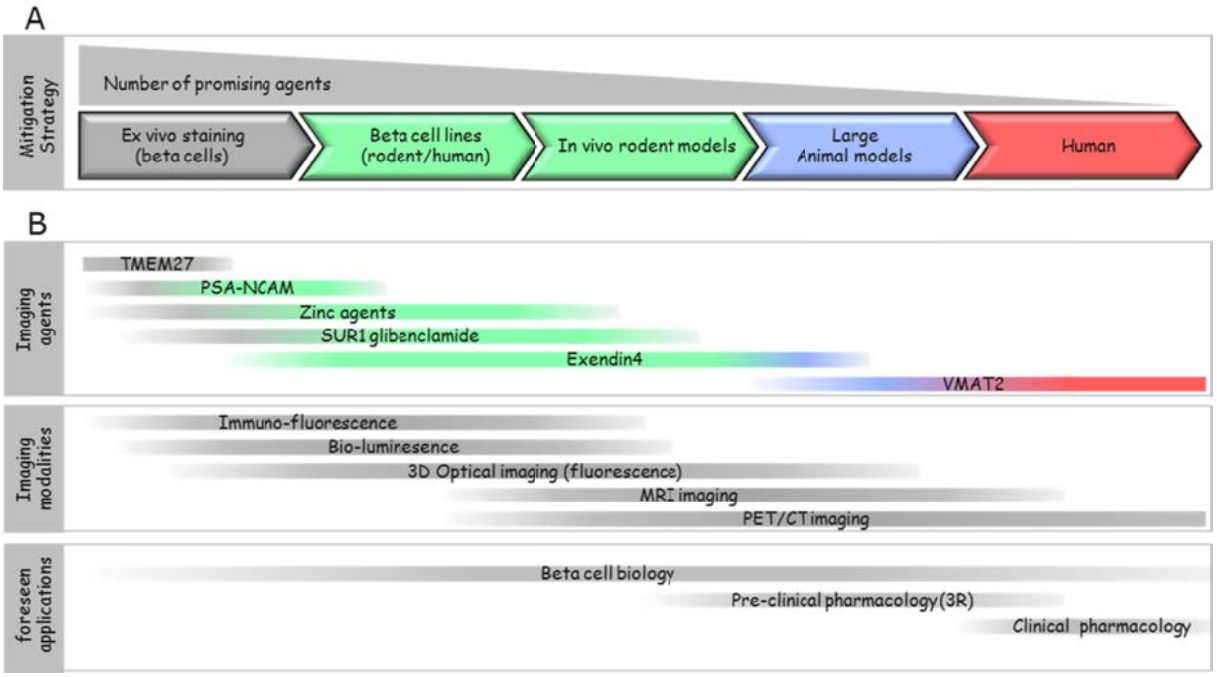

Figure 1: Illustration of the various stages in developing a tracer for β-cell imaging.

A) The logical process from discovering a new target up to its use for imaging in man. B) Mapping the progress of different tracers used in IMIDIA together with the various imaging modalities. The foreseen applications associated with different markers can also be correlated to the overall progress in this map. The early stage tracers can be applied to address questions in β-cell biology, while more developed tracers can be used in pre-clinical or clinical studies.

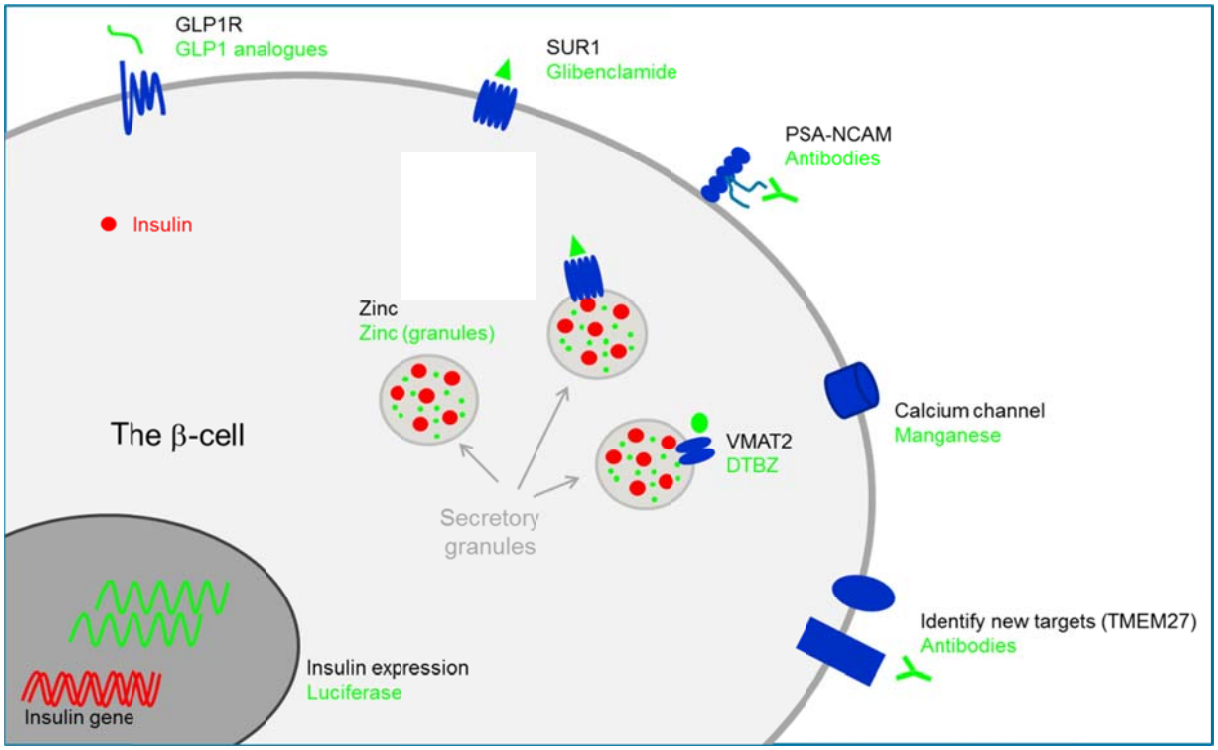

Figure 2: Schematic outline of the imaging tracers used to target the β-cell in IMIDIA. In transgenic mice luciferase can be used as a surrogate readout of β-cell mass when expressed under influence of the insulin promoter. The vesicular monoamine transporter 2 (VMAT2) is expressed on the secretory vesicles in the β-cell and can be targeted using a dihydrotetrabenazine (DTBZ) analog. The polysialylated modification of Neural Cell Adhesion Molecule (PSA-NCAM) is uniquely present in the β-cell and can be recognized with antibodies that only distinguish the modified molecule. Identification of novel antibodies will recognize epitopes that are highly specific for the β-cell. The sulfonyl urea receptor 1 (SUR1) is part of the inward-rectifier potassium ion channel and can be targeted with glibenclamide. The glucagon-like peptide-1 receptor (GLP-1R) is a G-

protein coupled receptor located on the surface of the β-cell and can be targeted with exendin4 and other GLP-1 analogues. Zinc is highly abundant in the secretory granules of the β-cell, and hence represents another potential target for both absolute and functional measures of β-cell mass. Manganese enters the β-cell via the calcium channels and enhances the contrast of MR images by shortening the T1 relaxation time.

References

1. Scully, T., *Diabetes in numbers.* Nature, 2012. **485**(7398): p. S2-3.
2. Rahier, J., et al., *Pancreatic beta-cell mass in European subjects with type 2 diabetes.* Diabetes Obes Metab, 2008. **10 Suppl 4**: p. 32-42.
3. Butler, A.E., et al., *Increased -Cell Apoptosis Prevents Adaptive Increase in -Cell Mass in Mouse Model of Type 2 Diabetes: Evidence for Role of Islet Amyloid Formation Rather Than Direct Action of Amyloid.* Diabetes, 2003. **52**(9): p. 2304-2314.
4. Rutter, G. and L. Parton, *The beta-cell in type 2 diabetes and in obesity.* OBESITY AND METABOLISM, 2008. **36**: p. 118-34.
5. Kulkarni, R.N., et al., *Human beta-cell proliferation and intracellular signaling: driving in the dark without a road map.* Diabetes, 2012. **61**(9): p. 2205-13.
6. Yi, P., J.S. Park, and D.A. Melton, *Betatrophin: a hormone that controls pancreatic beta cell proliferation.* Cell, 2013. **153**(4): p. 747-58.
7. Bouwens, L. and I. Rooman, *Regulation of pancreatic beta-cell mass.* Physiol Rev, 2005. **85**(4): p. 1255-70.
8. Ackermann, A.M. and M. Gannon, *Molecular regulation of pancreatic beta-cell mass development, maintenance, and expansion.* J Mol Endocrinol, 2007. **38**(1-2): p. 193-206.
9. Goldman, M., *Reflections on the Innovative Medicines Initiative.* Nat Rev Drug Discov, 2011. **10**(5): p. 321-2.
10. Goldman, M., N. Seigneuret, and H.G. Eichler, *The Innovative Medicines Initiative: an engine for regulatory science.* Nat Rev Drug Discov, 2014.
11. IMI. *http://www.imi.europa.eu*. 2015; Last date accessed 2/7 2015
12. IMIDIA, *http://www.imidia.org.* 2015. Last date accessed 2/7 2015
13. Yang, L., et al., *Imaging beta-cell mass and function in situ and in vivo.* J Mol Med (Berl), 2013. **91**(8): p. 929-38.
14. Blomberg, B.A., et al., *Beta-cell imaging: call for evidence-based and scientific approach.* Mol Imaging Biol, 2013. **15**(2): p. 123-30.
15. Di Gialleonardo, V., et al., *Imaging of beta-cell mass and insulitis in insulin-dependent (Type 1) diabetes mellitus.* Endocr Rev, 2012. **33**(6): p. 892-919.
16. Malaisse, W.J. and K. Maedler, *Imaging of the beta-cells of the islets of Langerhans.* Diabetes Res Clin Pract, 2012. **98**(1): p. 11-8.
17. Andralojc, K., et al., *Obstacles on the way to the clinical visualisation of beta cells: looking for the Aeneas of molecular imaging to navigate between Scylla and Charybdis.* Diabetologia, 2012. **55**(5): p. 1247-57.
18. Thorel, F., et al., *Conversion of adult pancreatic alpha-cells to beta-cells after extreme beta-cell loss.* Nature, 2010. **464**(7292): p. 1149-54.
19. Chera, S., et al., *Diabetes recovery by age-dependent conversion of pancreatic delta-cells into insulin producers.* Nature, 2014.
20. Agdeppa, E.D. and M.E. Spilker, *A review of imaging agent development.* AAPS J, 2009. **11**(2): p. 286-99.

21. *ICH Guidelines. http://www.ich.org/fileadmin/Public_Web_Site/ICH_Products/Guidelines/Multidisciplinary/M3_R2/Step4/M3_R2__Guideline.pdf*
22. Sweet, I.R., et al., *Non-invasive imaging of beta cell mass: a quantitative analysis.* Diabetes Technol Ther., 2004. **6**(5): p. 652-9.
23. Sweet, I.R., et al., *Systematic screening of potential beta-cell imaging agents.* Biochem Biophys Res Commun., 2004. **314**(4): p. 976-83.
24. Greer, L.F., 3rd and A.A. Szalay, *Imaging of light emission from the expression of luciferases in living cells and organisms: a review.* Luminescence, 2002. **17**(1): p. 43-74.
25. Sato, A., B. Klaunberg, and R. Tolwani, *In vivo bioluminescence imaging.* Comp Med, 2004. **54**(6): p. 631-4.
26. Contag, C.H., et al., *Visualizing gene expression in living mammals using a bioluminescent reporter.* Photochem Photobiol, 1997. **66**(4): p. 523-31.
27. Virostko, J., et al., *Bioluminescence imaging in mouse models quantifies beta cell mass in the pancreas and after islet transplantation.* Mol Imaging Biol, 2010. **12**(1): p. 42-53.
28. Smith, S.J., et al., *In vivo monitoring of pancreatic beta-cells in a transgenic mouse model.* Mol Imaging, 2006. **5**(2): p. 65-75.
29. Sever, D., et al., *Evaluation of impaired beta-cell function in nonobese-diabetic (NOD) mouse model using bioluminescence imaging.* FASEB J, 2011. **25**(2): p. 676-84.
30. Virostko, J., et al., *Bioluminescence imaging reveals dynamics of beta cell loss in the non-obese diabetic (NOD) mouse model.* PLoS One, 2013. **8**(3): p. e57784.
31. Patel, M., et al., *Non-invasive bioluminescence imaging of beta-cell function in obese-hyperglycemic [ob/ob] mice.* PLoS One, 2014. **9**(9): p. e106693.
32. Virostko, J., et al., *Factors influencing quantification of in vivo bioluminescence imaging: application to assessment of pancreatic islet transplants.* Mol Imaging, 2004. **3**(4): p. 333-42.
33. Virostko, J., et al., *Multimodal image coregistration and inducible selective cell ablation to evaluate imaging ligands.* Proc Natl Acad Sci U S A, 2011. **108**(51): p. 20719-24.
34. Balla, D.Z., et al., *In vivo visualization of single native pancreatic islets in the mouse.* Contrast Media Mol Imaging, 2013. **8**(6): p. 495-504.
35. Wu, A.M., *Antibodies and antimatter: the resurgence of immuno-PET.* J Nucl Med, 2009. **50**(1): p. 2-5.
36. Altirriba, J., et al., *The role of transmembrane protein 27 (TMEM27) in islet physiology and its potential use as a beta cell mass biomarker.* Diabetologia, 2010. **53**(7): p. 1406-14.
37. Vats, D., et al., *Multimodal imaging of pancreatic beta cells in vivo by targeting transmembrane protein 27 (TMEM27).* Diabetologia, 2012. **55**(9): p. 2407-16.

38. Finne, J., et al., *Occurrence of alpha 2-8 linked polysialosyl units in a neural cell adhesion molecule.* Biochem.Biophys.Res.Commun., 1983. **112**(2): p. 482-487.
39. Bonfanti, L., *PSA-NCAM in mammalian structural plasticity and neurogenesis.* Prog.Neurobiol., 2006. **80**(3): p. 129-164.
40. Kiss, J.Z., et al., *Activity-dependent mobilization of the adhesion molecule polysialic NCAM to the cell surface of neurons and endocrine cells.* EMBO J., 1994. **13**(22): p. 5284-5292.
41. Bernard-Kargar, C., et al., *Sialylated form of the neural cell adhesion molecule (NCAM): a new tool for the identification and sorting of beta-cell subpopulations with different functional activity.* Diabetes, 2001. **50 Suppl 1**: p. S125-S130.
42. Karaca, M., et al., *Exploring functional beta-cell heterogeneity in vivo using PSA-NCAM as a specific marker.* PLoS.One., 2009. **4**(5): p. e5555.
43. Banerjee, M. and T. Otonkoski, *A simple two-step protocol for the purification of human pancreatic beta cells.* Diabetologia, 2009. **52**(4): p. 621-625.
44. Roed, S.N., et al., *Real-time trafficking and signaling of the glucagon-like peptide-1 receptor.* Mol Cell Endocrinol, 2014. **382**(2): p. 938-49.
45. Ahnfelt-Ronne, J., et al., *A new view of the beta cell.* Diabetologia, 2012. **55**(9): p. 2316-8.
46. Secher, A., et al., *The arcuate nucleus mediates GLP-1 receptor agonist liraglutide-dependent weight loss.* J Clin Invest, 2014. **124**(10): p. 4473-88.
47. Reiner, T., et al., *Accurate measurement of pancreatic islet beta-cell mass using a second-generation fluorescent exendin-4 analog.* Proc Natl Acad Sci U S A, 2011. **108**(31): p. 12815-20.
48. Keliher, E.J., et al., *Efficient F-Labeling of Synthetic Exendin-4 Analogues for Imaging Beta Cells.* ChemistryOpen, 2012. **1**(4): p. 177-183.
49. Clardy, S.M., et al., *Fluorescent exendin-4 derivatives for pancreatic beta-cell analysis.* Bioconjug Chem, 2014. **25**(1): p. 171-7.
50. Normandin, M.D., et al., *In vivo imaging of endogenous pancreatic beta-cell mass in healthy and type 1 diabetic subjects using 18F-fluoropropyl-dihydrotetrabenazine and PET.* J Nucl Med, 2012. **53**(6): p. 908-16.
51. Brom, M., et al., *Non-invasive quantification of the beta cell mass by SPECT with (111)In-labelled exendin.* Diabetologia, 2014. **57**(5): p. 950-9.
52. Wicki, A., et al., *[Lys40(Ahx-DTPA-111In)NH2]-Exendin-4 is a highly efficient radiotherapeutic for glucagon-like peptide-1 receptor-targeted therapy for insulinoma.* Clin Cancer Res, 2007. **13**(12): p. 3696-705.
53. Brom, M., et al., *111In-exendin uptake in the pancreas correlates with the beta cell mass and not with the alpha cell mass.* Diabetes, 2014.
54. Boerman, O.C. and M. Gotthardt, *18F-Labelled exendin to image GLP-1 receptor-expressing tissues: from niche to blockbuster?* Eur J Nucl Med Mol Imaging, 2012. **39**(3): p. 461-2.

55. Mukai, E., et al., *GLP-1 receptor antagonist as a potential probe for pancreatic beta-cell imaging.* Biochem Biophys Res Commun, 2009. **389**(3): p. 523-6.
56. Richards, P., et al., *Identification and characterization of GLP-1 receptor-expressing cells using a new transgenic mouse model.* Diabetes, 2014. **63**(4): p. 1224-33.
57. Eng, J., et al., *Isolation and characterization of exendin-4, an exendin-3 analogue, from Heloderma suspectum venom. Further evidence for an exendin receptor on dispersed acini from guinea pig pancreas.* J Biol Chem, 1992. **267**(11): p. 7402-5.
58. Runge, S., et al., *Crystal structure of the ligand-bound glucagon-like peptide-1 receptor extracellular domain.* J Biol Chem, 2008. **283**(17): p. 11340-7.
59. Berclaz, C., et al., *Combined Optical Coherence and Fluorescence Microscopy to assess dynamics and specificity of pancreatic beta-cell tracers.* Sci Rep, 2015. **5**: p. 10385.
60. Clardy, S.M., et al., *Fluorescent Exendin-4 Derivatives for Pancreatic beta-Cell Analysis.* Bioconjug Chem, 2013.
61. Kirsi, M., et al., *Cu- and Ga-Labelled [Nle ,Lys (Ahx-NODAGA)NH]-Exendin-4 for Pancreatic Beta Cell Imaging in Rats.* Mol Imaging Biol, 2013.
62. Mikkola, K., et al., *64Cu- and 68Ga-labelled [Nle(14),Lys(40)(Ahx-NODAGA)NH2]-exendin-4 for pancreatic beta cell imaging in rats.* Mol Imaging Biol, 2014. **16**(2): p. 255-63.
63. Selvaraju, R.K., et al., *In Vivo Imaging of the Glucagonlike Peptide 1 Receptor in the Pancreas with 68Ga-Labeled DO3A-Exendin-4.* J Nucl Med, 2013.
64. Wang, P., et al., *GLP-1R-targeting magnetic nanoparticles for pancreatic islet imaging.* Diabetes, 2014. **63**(5): p. 1465-74.
65. Wild, D., et al., *[Lys40(Ahx-DTPA-111In)NH2]exendin-4, a very promising ligand for glucagon-like peptide-1 (GLP-1) receptor targeting.* J Nucl Med, 2006. **47**(12): p. 2025-33.
66. Pattou, F., J. Kerr-Conte, and D. Wild, *GLP-1-receptor scanning for imaging of human beta cells transplanted in muscle.* N Engl J Med, 2010. **363**(13): p. 1289-90.
67. Wu, Z., et al., *Development and evaluation of 18F-TTCO-Cys40-Exendin-4: a PET probe for imaging transplanted islets.* J Nucl Med, 2013. **54**(2): p. 244-51.
68. Antwi, K., et al., *Localization of hidden Insulinomas with 68Ga-DOTA-exendin-4 PET/CT: A Pilot Study.* J Nucl Med, 2015.
69. Brom, M., et al., *68Ga-labelled exendin-3, a new agent for the detection of insulinomas with PET.* Eur J Nucl Med Mol Imaging, 2010. **37**(7): p. 1345-55.
70. Kiesewetter, D.O., et al., *18F-radiolabeled analogs of exendin-4 for PET imaging of GLP-1 in insulinoma.* Eur J Nucl Med Mol Imaging, 2012. **39**(3): p. 463-73.

71. Mikkola, K., et al., *Erratum to: Cu- and Ga-Labelled [Nle ,Lys (Ahx-NODAGA)NH]-Exendin-4 for Pancreatic Beta Cell Imaging in Rats.* Mol Imaging Biol, 2013.
72. Vinet, L., et al., *Targeting GLP-1 receptors for repeated magnetic resonance imaging differentiates graded losses of pancreatic beta cells in mice.* Diabetologia, 2014.
73. Kaubisch, N., et al., *Specific receptors for sulfonylureas in brain and in a beta-cell tumor of the rat.* Biochem Pharmacol., 1982. **31**(6): p. 1171-4.
74. Ladriere, L., F. Malaisse-Lagae, and W.J. Malaisse, *Uptake of tritiated glibenclamide by endocrine and exocrine pancreas.* Endocrine, 2000. **12**(3): p. 329-32.
75. Guiot, Y., et al., *Morphological localisation of sulfonylurea receptor 1 in endocrine cells of human, mouse and rat pancreas.* Diabetologia, 2007. **50**(9): p. 1889-99.
76. Schneider, S., *Efforts to develop methods for in vivo evaluation of the native beta-cell mass.* Diabetes Obes Metab, 2008. **10 Suppl 4**: p. 109-18.
77. Carpentier, J.L., et al., *Internalization of 3H-glibenclamide in pancreatic islet cells.* Diabetologia., 1986. **29**(4): p. 259-61.
78. Schneider, S., et al., *Synthesis and evaluation of a glibenclamide glucose-conjugate: a potential new lead compound for substituted glibenclamide derivatives as islet imaging agents.* Regul Pept, 2007. **139**(1-3): p. 122-7.
79. Sadekar, S. and H. Ghandehari, *Transepithelial transport and toxicity of PAMAM dendrimers: implications for oral drug delivery.* Adv Drug Deliv Rev, 2012. **64**(6): p. 571-88.
80. Stiriba, S.E., H. Frey, and R. Haag, *Dendritic polymers in biomedical applications: from potential to clinical use in diagnostics and therapy.* Angew Chem Int Ed Engl, 2002. **41**(8): p. 1329-34.
81. Anlauf, M., et al., *Expression of the two isoforms of the vesicular monoamine transporter (VMAT1 and VMAT2) in the endocrine pancreas and pancreatic endocrine tumors.* J Histochem Cytochem, 2003. **51**(8): p. 1027-40.
82. Raffo, A., et al., *Role of vesicular monoamine transporter type 2 in rodent insulin secretion and glucose metabolism revealed by its specific antagonist tetrabenazine.* J Endocrinol, 2008. **198**(1): p. 41-9.
83. Goland, R., et al., *11C-dihydrotetrabenazine PET of the pancreas in subjects with long-standing type 1 diabetes and in healthy controls.* J Nucl Med, 2009. **50**(3): p. 382-9.
84. Kung, M.P., et al., *In vivo imaging of beta-cell mass in rats using 18F-FP-(+)-DTBZ: a potential PET ligand for studying diabetes mellitus.* J Nucl Med, 2008. **49**(7): p. 1171-6.
85. Simpson, N.R., et al., *Visualizing pancreatic beta-cell mass with [11C]DTBZ.* Nucl Med Biol, 2006. **33**(7): p. 855-64.

86. Singhal, T., et al., *Pancreatic beta cell mass PET imaging and quantification with [11C]DTBZ and [18F]FP-(+)-DTBZ in rodent models of diabetes.* Mol Imaging Biol, 2011. **13**(5): p. 973-84.
87. Tsao, H.H., et al., *Binding characteristics of 9-fluoropropyl-(+)-dihydrotetrabenzazine (AV-133) to the vesicular monoamine transporter type 2 in rats.* Nucl Med Biol, 2010. **37**(4): p. 413-9.
88. Fagerholm, V., et al., *Assessment of islet specificity of dihydrotetrabenazine radiotracer binding in rat pancreas and human pancreas.* J Nucl Med, 2010. **51**(9): p. 1439-46.
89. Hutton, J.C., E.J. Penn, and M. Peshavaria, *Low-molecular-weight constituents of isolated insulin-secretory granules. Bivalent cations, adenine nucleotides and inorganic phosphate.* Biochem J, 1983. **210**(2): p. 297-305.
90. Vinkenborg, J.L., et al., *Genetically encoded FRET sensors to monitor intracellular Zn2+ homeostasis.* Nat Methods, 2009. **6**(10): p. 737-40.
91. Kristiansen, L.H., et al., *Autometallography allows ultrastructural monitoring of zinc in the endocrine pancreas.* Histochem Cell Biol, 2001. **115**(2): p. 125-9.
92. Sondergaard, L.G., et al., *Zinc ions in the endocrine and exocrine pancreas of zinc deficient rats.* Histol Histopathol, 2006. **21**(6): p. 619-25.
93. Esqueda, A.C., et al., *A new gadolinium-based MRI zinc sensor.* J Am Chem Soc, 2009. **131**(32): p. 11387-91.
94. Lubag, A.J., et al., *Noninvasive MRI of beta-cell function using a Zn2+-responsive contrast agent.* Proc Natl Acad Sci U S A, 2011. **108**(45): p. 18400-5.
95. Stasiuk, G.J., et al., *Dual modal MR/fluorescent ratiometric zinc sensors towards pancreatic beta cell imaging.* Chem EU J, 2014.
96. Stasiuk, G.J. and N.J. Long, *The ubiquitous DOTA and its derivatives: the impact of 1,4,7,10-tetraazacyclododecane-1,4,7,10-tetraacetic acid on biomedical imaging.* Chem Commun (Camb), 2013. **49**(27): p. 2732-46.
97. Mayo-Smith, W.W., et al., *Pancreatic enhancement and pulse sequence analysis using low-dose mangafodipir trisodium.* AJR Am J Roentgenol., 1998. **170**(3): p. 649-52.
98. Gimi, B., et al., *Functional MR microimaging of pancreatic beta-cell activation.* Cell Transplant, 2006. **15**: p. 195-203.
99. Antkowiak, P.F., et al., *Noninvasive assessment of pancreatic beta-cell function in vivo with manganese-enhanced magnetic resonance imaging.* Am J Physiol Endocrinol Metab, 2009. **296**: p. E573-578.
100. Antkowiak, P.F., et al., *Manganese-enhanced magnetic resonance imaging detects declining pancreatic beta-cell mass in a cyclophosphamide-accelerated mouse model of type 1 diabetes.* Diabetes, 2013. **62**(1): p. 44-8.
101. Denis, M.C., et al., *Imaging inflammation of the pancreatic islets in type 1 diabetes.* Proc Natl Acad Sci U S A, 2004. **101**(34): p. 12634-9.

102. Evgenov, N.V., et al., *In vivo imaging of islet transplantation.* Nat Med, 2006. **12**(1): p. 144-8.
103. Lamprianou, S., et al., *High-resolution magnetic resonance imaging quantitatively detects individual pancreatic islets.* Diabetes, 2011. **60**(11): p. 2853-60.
104. Botsikas, D., et al., *Pancreatic magnetic resonance imaging after manganese injection distinguishes type 2 diabetic and normoglycemic patients.* Islets, 2012. **4**(3): p. 243-8.
105. Kavishwar, A., Z. Medarova, and A. Moore, *Unique sphingomyelin patches are targets of a beta-cell-specific antibody.* J Lipid Res, 2011. **52**(9): p. 1660-71.
106. Ueberberg, S., et al., *Generation of novel single-chain antibodies by phage-display technology to direct imaging agents highly selective to pancreatic beta- or alpha-cells in vivo.* Diabetes, 2009. **58**(10): p. 2324-34.
107. Eriksson, O., et al., *Quantitative imaging of serotonergic biosynthesis and degradation in the endocrine pancreas.* J Nucl Med, 2014. **55**(3): p. 460-5.
108. Eriksson, O., et al., *Positron Emission Tomography Ligand [11C]5-Hydroxy-Tryptophan Can Be Used as a Surrogate Marker for the Human Endocrine Pancreas.* Diabetes, 2014. **63**(10): p. 3428-37.
109. Ludvigsen, E., *Somatostatin receptor expression and biological functions in endocrine pancreatic cells: review based on a doctoral thesis.* Ups J Med Sci, 2007. **112**(1): p. 1-20.
110. Sako, T., et al., *Positron emission tomography study on pancreatic somatostatin receptors in normal and diabetic rats with 68Ga-DOTA-octreotide: a potential PET tracer for beta cell mass measurement.* Biochem Biophys Res Commun, 2013. **442**(1-2): p. 79-84.