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

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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 underly the more prevalent Type 2 diabetes. The establishment of robust drug development programmes aimed 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. In the present review, we describe the progress towards this goal achieved by the Innovative Medicines Initiative in Diabetes, a collaborative public–private consortium supported by the European Commission and by dedicated resources of pharmaceutical companies. We compare several of the available imaging methods and molecular targets and provide suggestions as to the likelihood 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.

Keywords: beta cell, GLP-1 analogue, islets, sulphonylureas, type 1 diabetes, VMAT2

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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. Faced with the urgent need to develop new sensitive tools that can be applied to humans, β-cell imaging appears to be 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 5 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]. The Innovative Medicine Initiative in Diabetes (IMIDIA) [12] is one of several programmes within IMI and was launched in 2009. Comprising 12 academic institutes, eight 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.

Imaging of β-cells has recently been reviewed in a number of excellent publications [13–17]. In the present review, 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 the development of knowledge on 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 (Figure 1A, left), and ending with a probe suitable for measuring β-cell mass (Figure 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. For in vivo imaging, however, other imaging methods 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 humans. Within IMIDIA, β-cell imaging is organized in six approaches, which together span all the steps considered to be important for the development of a tracer capable of measuring β-cell mass in humans (Figure 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

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 methods. 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 preclinical or clinical studies.
ablated [18,19] and the insulin antagonist S961 that was recently shown to increase β-cell mass [6].

Imaging Methods

Among available methods, MRI provides excellent anatomical resolution but suffers at the molecular scale because of 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, CT and ultrasonography 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 models). In addition, CT and MRI contrast agents are typically dosed at concentrations in the millimolar 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 [International Conference on Harmonisation (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 Zn2⁺ and Mn2⁺ ions, as well as sulphonylurea receptor-1 (SUR-1) and glucagon-like peptide-1 receptors (GLP-1Rs) 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 Kd of such a ligand needs to be less than 1nM [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 pharmacokinetics 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

‘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 T1DM [29,30] or T2DM [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 Virostko et al. [27,30,32,33], who pioneered the study of β-cell mass by bioluminescence imaging, we have studied a transgenic model which provides 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, before and after injection of a cytotoxic dose of diphtheria 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 people with T1DM. We further longitudinally monitored the mice, 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 (L. Vinet et al., unpublished data). At the end of the experiment, the increased bioluminescence imaging signal correlated with a partial recovery of the insulin content. The data show 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 humans, it serves a purpose in preclinical settings to evaluate potential candidate drugs before the trials in large animals and humans.

Transmembrane Protein 27 (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 on 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 [89Zr]-8/9-mAb, which revealed a high signal-to-background contrast in subcutaneous tumours 1 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.

Polysialylated Neural Cell Adhesion Molecule (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 modelling [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 cells, suggesting that this molecule may also reflect functional β-cell mass (Figure 2).

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 that 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 thus remains 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 labelled 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.

Glucagon-Like Peptide-1 Receptor (Ligand). Peptides targeting the GLP-1R are promising candidates for use in β-cell imaging because (i) 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 (ii) they are safe and already used in the clinic for treatment of T2DM. 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 and nuclear imaging and MRI of endogenous β cells [44,51,53,59–63], transplanted islets [64,65] and insulinomas [63,66–68], including in humans [45]. 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 in quantifying the low signals emanating from native islets [61,69]. Such limitations may prevent the detection of submaximal changes of β-cell mass; for example, 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 [61,69], raising serious safety concerns for use in longitudinal studies where repeated imaging is necessary.
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) analogue. 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 sulphonylurea receptor 1 (SUR-1) 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 exendin-4 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 enhance the contrast of magnetic resonance images by shortening the T1 relaxation time.

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, was internalized into the β cells while the antagonist (Ex4_9-39) remained at the plasma membrane. Furthermore, three-dimensional imaging (optical projection tomography) showed 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 [70]. In parallel, 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, ultrasmall superparamagnetic iron oxides, tagged with Alexa 647 fluorochrome [70]. 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].

Sulphonylurea Receptors (Small Molecule). Sulphonylureas are antidiabetes drugs used to mitigate T2DM symptoms by increasing insulin secretion. The drugs bind to specific sulphonylurea receptors (SUR-1) located in the plasma membrane of pancreatic islet cells [71,72] but not in the exocrine acinar cells [73]. SUR-1s are expressed at high density in β cells [71,72,74] but also other endocrine pancreatic cells [73]. Despite this, the established clinical use of sulphonylureas, such as glibenclamide [22,73–75], 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 [22,71,73,74,76]. We have thus tested modified versions of the original molecules carrying moieties detectable by different imaging methods, specifically in vivo fluorescence, which do not excessively disturb the binding affinity to SUR-1. 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 SUR-1. 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 the non-toxic polyamidoamine dendrimers [77,78] useful to increase hydrophilicity (thereby decreasing binding to plasma proteins). Spherical probes containing three to seven glib-B moieties (Ø ∼6 nm diameter) displayed the best compromise between
Vesicular Monoamine Transporter Type 2 (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 [79], and may have a possible role in insulin secretion [80]. PET-based quantitation of VMAT2 was made possible by the development of radiolabelled dihydrorhodamine (DTBZ) analogues, 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 [81–84], especially as 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 [46]. However, the specificity of molecules targeting VMAT2 was brought into question when Goland et al. [81] reported significant [13C]DTBZ uptake in long-standing T1DM, in line with Tsao et al. [85] who showed low non-specific binding of [18F]AV-133 to non-β-cells in rats. It recently appeared that non-specific binding as estimated by the negative enantiomer [18F]FP-(–)-DTBZ in T1D pancreata, normal human and non-human primate pancreas is most likely <15% of the total signal [86,87]. 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 cells [80,81] remains to be clarified. Yet, several studies have suggested that adult β cells might originate from duct or duct-associated cells [86,87] and VMAT2 was also shown to regulate differentiation in β-cell precursors [88]. Meanwhile, it appeared that rodents may not be optimal for DTBZ imaging of β cells [33,89], 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 [18F]FP-DTBZ could be visualized from in vivo PET images in the pancreas of normoglycaemic pigs and quantified by measuring the corresponding standardized uptake value. Individual results appeared to be associated with ~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 [18F]FP-DTBZ could be visualized in response to STZ treatment (Raphael Boisgard, 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.

‘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. ~30 mM) [90], of which a small but significant proportion (1–100 μM) is unbound [91], Zn2+ is also present in α-cell granules, albeit at lower levels [92]. Given the relative scarcity of zinc ions throughout the rest of the pancreas [93], 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 MRI agents for quantification of β-cell mass that focus on zinc binding. One example is GdDOTA-diBPEN, an MRI agent sensitive for Zn(II), as reported by Esqueda et al. [94] and Lubag et al. [95]. This compound shows enhancement in the magnetic resonance 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 pancreases were enhanced only after glucose was elevated to a level that stimulates insulin secretion, whilst the agent did not enhance magnetic resonance images of the pancreas in euglycaemic mice or in mice pretreated with STZ. Serial magnetic resonance 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 [96]. This is based around the ‘gold standard’ DOTA motif [97] featuring a Gd(III) centre (MRI) alongside a fluorophore, which acts as both the fluorescence probe and zinc-binding motif. The probe has a Ki of 22 μM, and undergoes a ratiometric shift in fluorescence emission from 410 to 500 nm, with a relaxivity of 4.2 mM/s. This value increases upon addition of half an equivalent of Zn(II) to 6.6 mM/s, decreasing back to 4.9 mM/s (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 T).
**Manganese.** Manganese ions (Mn$^{2+}$) significantly enhance the contrast of MRI by shortening the T1 relaxation time and enhances the contrast of the pancreas [98] by entering stimulated β-cells through calcium channels[99]. 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 [100], and progressively declined with the loss of β cell mass in a model of T1DM [101].

**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 (14.1 T) MRI machine to assess whether Mn$^{2+}$ could confer a contrast sufficient to visualize native, individual islets [99,100,102–104]. In normoglycaemic conditions as well as after glucose infusion, MEMRI distinguished different tissues within the pancreas, including individual islets with a diameter >50 μm, and correctly quantified their volume density [102]. MEMRI also detected a significant decrease in the numerical and volume density of islets in a model of T1DM [102].

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 normoglycaemic participants or people with T2DM [105]. Enhancement was significantly lower in the pancreas of diabetic than of normoglycaemic participants [105].

**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 programme. Consequently this meant that a number of agents known to target β cells were not analysed as part of the IMIDIA programme and are therefore not discussed in the present 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 [106].
- Single-chain antibodies (SCA), such as SCA-B1 [107]. The target of SCA-B1 is not known but was shown specific for β cells and suitable for MRI, 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 [108]; 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 [108] and have been able to differentiate people with T1DM from healthy people [86,109] Somatostatin receptors were also proposed as a target for imaging probes to monitor β-cell mass. Thus, Sako et al. [87] have synthesized a PET probe, $^{68}$Ga-DOTA-octreotide, and shown that its accumulation in the pancreas was reduced in STZ-treated rats.

### 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.

| Target          | Probe                              | Fluorescence | MRI                  | PET/SPECT          | Main challenges                                                                 | Reference |
|-----------------|------------------------------------|--------------|----------------------|--------------------|---------------------------------------------------------------------------------|-----------|
| TMEM27          | antiTMEM27                         | Successfully | Not attempted        | Successfully       | (1) Only recognizes human TMEM27                                               | [37]      |
| PSA-NCAM        | antiPSA                            | Not attempted | Not attempted        | Not attempted      | (2) Reformattting antibody to tracer (1) Specificity                            | [41,42]  |
| GLP-1R          | Ex4                                | Successfully | Successfully         | Successfully       | (2) Reformattting antibody to tracer (1) GLP-1R expression in disease models     | [44,45,70]|
| SUR-1           | Multimerized glibenclamide          | Successfully | Difficult            | Not attempted      | (1) Exposure time in vivo. (2) Retention in blood vessels                       | [22,71,73,74,76]|
| VMAT2           | FP-DTBZ                            | Difficult    | Successfully         | Successfully       | (1) VMAT2 expression in polypeptide cells (2) Validity of rodent models unclear | [46]      |
| Zinc            | Chelators                          | Successfully | Successfully         | Not attempted      | (1) Toxicity (2) Sensitivity                                                    | [94–96]  |
| Ca$^{2+}$ channel | Manganese                        | Not compatible | Successfully         | Not compatible      | (1) Non specific (2) Safety concern                                              | [98,100,102,105]|
| Insulin gene    | Luciferase                         | Successfully | Not compatible        | Not compatible      | (1) Limited to small transgenic animals                                          | [27,30]  |

FP-DTBZ, Fluoropropoxy-dihydrotetrabenazine; GLP-1R, glucagon-like peptide-1 receptor; PET, positron emission tomography; PSA-NCAM, polysialylated modification of neural cell adhesion molecule; SPECT, single-photon emission computed tomography; SUR-1, sulphoxylurea receptor 1; TMEM27, transmembrane protein 27; VMAT2, vesicular monoamine transporter 2.

**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 showing 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 (table 1), the VMAT2 and exendin-based PET tracer presently represent the most advanced approaches to assess β-cell mass in humans [45,46]. 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 to detect subtle changes over time. GLP1-R agonists may also show significant promise for an MRI application in mice and may already be worth considering for use in combination with Zn2+ or Mn2+ probes to assess β-cell function in preclinical studies.

An important conclusion from this IMIDIA programme 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. Because none of the probes investigated are likely to fulfill all criteria for a ‘perfect’ β-cell imaging agent, these models will provide a benchmark for the changes in β-cell mass 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. As these biomarkers should be more convenient and inexpensive compared with 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 in the present review.

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Conflict of Interest

D.L. is employed by Novartis. M.D., G.F., A.K. are employed by Servier, H.W., S. S. are employed by Roche, H-P.J., H.G., W.K. are employed by Sanofi, J.H-S. is employed by Novo Nordisk.

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