MECHANISMS IN ENDOCRINOLOGY

Towards the clinical translation of stem cell therapy for type 1 diabetes

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Abstract

Insulin-producing cells derived from human embryonic stem cells (hESCs) or induced pluripotent stem cells (iPSCs) have for long been a promising, but elusive treatment far from clinical translation into type 1 diabetes therapy. However, the field is now on the verge of moving such insulin-producing cells into clinical trials. Although stem cell therapies provide great opportunities, there are also potential risks such as teratoma formation associated with the treatment. Many considerations are needed on how to proceed with clinical translation, including whether to use hESCs or iPSCs, and whether encapsulation of tissue will be needed. This review aims to give an overview of the current knowledge of stem cell therapy outcomes in animal models of type 1 diabetes and a proposed road map towards the clinical setting with special focus on the potential risks and hurdles which needs to be considered. From a clinical point of view, transplantation of insulin-producing cells derived from stem cells must be performed without immune suppression in order to be an attractive treatment option. Although costly and highly labour intensive, patient-derived iPSCs would be the only solution, if not clinically successful encapsulation or tolerance induction protocols are introduced.

Introduction

Type 1 diabetes (T1D), resulting from the progressive loss of the insulin-producing cells following an immune attack, is one of the most common chronic diseases among children and adolescents with an estimated 500,000 children below the age of 15 years living with the disease (1). There is also evidence of an increase in incidence of approximately 3% per year (1). When insulin was discovered in 1921, the disease suddenly became treatable and during the initial years after its introduction, insulin was viewed upon as a cure for diabetes. Although the insulin therapy has been refined and technical aids have substantially improved over the years, it is still impossible to fully mimic the physiological fine tuning of blood glucose levels. In modern diabetes treatment, great efforts are made to monitor and prevent the long-term complications, but these can, in most cases, not be

Invited author’s profile

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completely avoided. As a consequence, the life expectancy for patients with T1D is reduced by more than 10 years despite modern treatment and clinical care (2).

In the 1960s, a technique for isolating islets of Langerhans from rodents with collagenase was described (3), and this has over the years been used in many studies concerning beta-cell physiology. In addition, the idea to transplant islets as a cure for diabetes rapidly evolved as a new field of research. However, the clinical results for islet transplantation in type 1 diabetes were poor (for a recent review of the development of the islet transplantation field, see (4)) until a modified immunosuppressive regime was introduced in 2000, the so-called Edmonton protocol (5). The long-term results have further improved since the introduction of the Edmonton protocol, and currently around 50% of the patients remain insulin independent even five years post transplantation (6, 7). A phase 3 clinical trial has recently been conducted (8), and has in many countries lead to a translation of the islet transplant procedure from experimental therapy to standard care for selected T1D patients. As opposed to whole pancreas transplantation, islet transplantation is a minimal invasive procedure performed in local anaesthesia during which the islets are infused into the portal vein and distributed in the vascular tree of the liver. However, both procedures require life-long immunosuppressive treatment, which is afflicted with a number of adverse side effects such as infections and increased risk of malignancies. There is also a need for tight follow-up of metabolic outcome and immunological parameters after the transplantation of pancreatic islets. In most cases more than one pancreas from diseased organ donors are required to give cure to one patient by allogeneic islet transplantation, whereas in patients autologously transplanted with pancreatic islets due to exocrine pancreatic disease these much smaller numbers of islets from one individual are often sufficient to maintain normoglycaemia. The reasons for this difference in efficacy are not certain, but are likely explained by differences in islet procurement, isolation and transplantation, and the need for use of immunosuppressive drugs in the allograft transplantation case (9). Our experimental studies also indicate that an improved vascular engraftment of autotransplanted islets may be the result of the pancreatectomy procedure (10).

Stem cell therapies have, for decades, been suggested as a future treatment option for a number of diseases including T1D. Stem cells can be defined either as totipotent cells that can form all cell types in a body, as well as the extraembryonic or placental cells, pluripotent cells that can give rise to all cell types in a body, or multipotent cells that can become more than one cell type e.g. mesenchymal stem cells that can differentiate into osteoblasts, adipocytes and chondroblasts. A fertilised egg has totipotency for approximately four days, thereafter the cells become pluripotent. Stem cells in adult mammals are generally multipotent. Mesenchymal stem (stromal) cells has, due to their modulatory effects on immunity, inflammation and tissue repair, been suggested to be used to either halt beta-cell loss during T1D development or be used to protect and support pancreatic islets when transplanted. For the discussion of the use of mesenchymal stem cells in T1D, we refer to a recent review (11). This review will instead focus on the use of pluripotent stem cells (embryonic or induced) to generate new beta-like cells, give an overview of the current knowledge of such stem cell therapy outcomes in animal models of T1D and a proposed road map towards the clinical setting with special focus on the potential risks and hurdles which need to be considered. Over the last five to ten years, there has been a number of advances on how to stimulate human embryonic stem cells (hESCs) and induced pluripotent stem cells (iPSCs) to differentiate into insulin-producing cells (for reviews see e.g. (12, 13)). In fact, the field is now on the verge of bringing stem cell-derived insulin-producing cells into clinical trials. Although stem cell therapies come with great potential, there are also great risks associated with the treatment. To clinically translate this field, people from many different disciplines will be needed to address the below identified hurdles.

Insulin-producing cells derived from stem cells

Already in 1997, an initial report was published in which pluripotent mouse pancreatic-derived stem cells were differentiated into cells expressing insulin and glucagon. When transplanted to diabetic mice, a reduction in blood glucose was observed. However, normoglycaemia could not be maintained (14). In the beginning of the new millennium, the first reports on the use of ESCs as a source for insulin-producing cells were published. By using undifferentiated ESCs from mice, Soria et al. could induce insulin-producing cells responsive to different secretagogues. Clusters of these cells had the capacity to normalise blood glucose when transplanted to diabetic mice (15). Shortly thereafter, another research group demonstrated that multiple pancreatic endocrine cells can be derived from ESCs, and that these cells self-assembled
into islet-like clusters that were rapidly vascularised and maintained their architecture after transplantation (16). Another report showed that insulin-producing cells derived from hESCs could secrete insulin in vitro (17). Following papers demonstrated that insulin-producing cells derived from hESCs in contrast to endogenous beta-cells are bi-hormonal (also glucagon producing), but similar to cells derived from mice form islet-like clusters (18). In 2007, a report was published in which hESCs were used to derive insulin-producing cells, also bi-hormonal, which expressed a number of genes important for beta-cell function and had the capacity to cure 30% of diabetic mice when transplanted (19).

In many publications, transplantation of the cells has been used as a part of the differentiation protocol, i.e. immature cells have been transplanted in order for them to further differentiate in vivo. For instance, immature cells have been co-transplanted with embryonic pancreas from mice which stimulated them to differentiate into insulin-producing cells (20). In vivo differentiation have also been successful without co-transplanting embryonic tissue by differentiating hESCs in vitro into pancreatic progenitor cells which then further differentiated into functional insulin-producing cells in vivo after transplantation beneath the kidney capsule (21). After differentiation and engraftment in vivo, these cells secreted human C-peptide and could cure pre-existing diabetes (21). The final differentiation in vivo has also been shown successful when progenitor cells derived from hESCs were macroencapsulated and transplanted in mice (22).

In later years, in vitro protocols for the differentiation of insulin-producing cells from both hESCs and human iPSCs have been published (23). Refined in vitro protocols for differentiating hESCs and human iPSCs into insulin-producing cells have also been described, and these cells have been proven functional both in vitro and in vivo with the capacity to cure diabetic mice (24, 25). Although the cells are not fully equal to adult human beta-cells with regard to their gene expression, they respond to nutrients with similar magnitude of insulin secretion, and do not express glucagon (24, 25). In addition, the protocols allow for large-scale production of insulin-producing cells, which opens up for the possibility for studies in large animal models and clinical trials.

**Transplantation of insulin-producing cells derived from stem cells**

The transplantation procedure has served as a part of the differentiation protocol in a number of publications, and therefore direct functional comparisons of cells prior to transplantation are not sufficient. Instead, in order to be able to compare the efficacy of cells developed in the different protocols, head-to-head comparisons in vivo several months post transplantation would be needed. A number of groups have reported that they can cure or prevent diabetes in mice by transplanting insulin-producing cells derived from stem cells in cell numbers between 1.25 and 7×10^6 cells (21, 24, 25, 26). It should be noted that when assessing functionality of cells in rodents, there are several pitfalls. One is that the glucose disposal after a glucose challenge to a large extent is independent of insulin release kinetics in mice (27), which means that C-peptide or insulin is needed to be evaluated in parallel with blood glucose values during the test. This also bears specific implications for encapsulated insulin-producing cells, since the encapsulation per se can affect insulin release kinetics (cf. below). Another is that animals transplanted with a marginal mass of insulin-producing cells may have fully normal blood glucose values only during daytime (28).

There have, so far, been no reports on transplantation of insulin-producing cells derived from stem cells in large animal models. There is, since 2014, an ongoing clinical study sponsored by the company ViaCyte Inc. in which macroencapsulated pancreatic progenitors derived from stem cells are transplanted subcutaneously to patients with T1D, but safety or efficacy data have so far not been reported (Clinicaltrials.gov identifier NCT02239354).

When evaluating cells developed in different protocols, clearly more than functional aspects need to be considered in vivo. The potential of hESCs and human iPSCs to form tumours/teratomas is a real threat, and there have, in fact, been reports on the formation of teratomas in several of the preclinical studies of stem cell-derived insulin-producing cells. When pancreatic endoderm cells derived from hESCs were macroencapsulated and transplanted to mice, seven out of totally 46 (15%) evaluated grafts contained teratomas (26). In the follow-up study from the same group, they found that when using un-enriched pancreatic endoderm cells as origin, the frequency of teratomas was even higher (46%) (29). However, if the cells were enriched based on the cellular marker CD142 prior to transplantation, no teratomas were observed in surviving grafts. Noteworthy, however, only seven grafts containing CD142 enriched cells were evaluated (29). In the study by Rezania et al. with transplantation of pancreatic progenitors, formation of cartilage and bone was found in 50% of the transplanted animals when the cells were transplanted under the kidney capsule.
in mice (21). It is possible that transplantation of more fully differentiated insulin-producing cells diminishes the risk of tumour and teratoma formation in grafts, since such was not reported or discussed in these more recent studies (24, 25). Nevertheless, in order to take the next step using stem cell-derived insulin-producing cells, i.e. into clinical trials, this is a crucial concern that needs to be addressed. There are also a number of ethical and legal perspectives regarding the use of stem cells to consider, since most of the cell lines used are derived from hESCs. The International Society for Stem Cell Research (ISSCR) recently published an update of their guidelines regarding the clinical translation of stem cell research (30). The guidelines cover scientific and clinical considerations as well as ethical. Our proposed ‘road map to the clinic’ for insulin-producing cells derived from stem cells is in line with these guidelines. In addition, the jurisdiction and political decisions will have a major impact on the use of all types of cells derived from stem cells in the clinical setting. Although of major importance, the political and legal perspective regarding the use of stem cells lies beyond the scope of this review.

The formation of tumours and teratomas can, as described, be reduced by enriching cells based on specific cellular markers, and probably by the use of more fully differentiated cells for transplantation. However, it must be kept in mind that these cells have not yet been evaluated in large animal models or in humans. In fact, it has been shown that there are species differences even between mice and rats regarding the differentiation and engraftment of insulin-producing cells derived from hESCs (31). From a safety point of view, the cells therefore initially need to be transplanted into a site where they can be radically retrieved if needed. Preferentially, the differentiation of the cells should be monitored longitudinally and without risk for the patients’ health. A safe route for transplantation of stem cell-derived insulin-producing cells in the clinical setting would therefore be to encapsulate them prior to transplantation. This has already been evaluated in the preclinical setting using macroencapsulation (22, 32) and microencapsulation (33).

An encapsulation approach adds a new dimension of demands when it comes to the biomechanical properties of the capsule, since it must be able to meet the high physiological demands of insulin-producing cells while also be able to prevent excessive cellular/tumour growth. In microencapsulation techniques, only one or a few islets or clusters of insulin-producing cells are encapsulated e.g. in alginate. Transplantation of many such capsules is therefore needed to transfer a substantial endocrine mass. Few sites can harbour such numbers, and experimentally the encapsulated cells are therefore infused into the peritoneal cavity of the recipient. In a clinical setting using a relevant number of cell clusters, the number of capsules would exceed 0.5 million for a normal weight recipient. Therefore, it is close to impossible to assess the integrity of each individual capsule and if needed to retrieve all capsules. Microencapsulation also has functional limitations which are well known from preclinical studies of islet transplantation, including size-dependent impaired kinetics of hormonal secretion (34), limitations of oxygen supply (35) and exaggerated islet amyloid formation which impairs both the survival and function (36).

When macroencapsulating cells, the integrity and function of the device can be carefully examined prior to transplantation. In addition, the device could easily be removed and the cells are retrieved for in depth in vitro studies of their differentiation and potential tumour development. However, since all cells are compiled in a small space without vascularisation, at least islet cell survival and function will be severely limited by the oxygen diffusion distance (37, 38). This has been shown to be overcome by using devices that are oxygenised e.g. by external refilling of oxygen or internal oxygen production through photosynthesis (39, 40, 41). Based on a pilot study (42), we are currently evaluating an oxygenised macrocapsule (Beta-Air) approved for clinical use in an investigator-driven clinical trial with human islets (Clinicaltrials.gov NCT02064309). When using a similar oxygenised macrocapsule in small animal models, it has been shown that pancreatic progenitor cells do survive and secrete detectable, although low, levels of human C-peptide, whereas undifferentiated hESCs do not survive (32). However, in a non-oxygenated macroencapsulation device implanted to mice, pancreatic progenitors survive and function similar to free (non-encapsulated) human islet grafts (43, 44). The discrepancy to findings with macroencapsulated islet cells may reflect that pancreatic progenitors are less vulnerable to hypoxia. However, hypoxia is also known to negatively affect the differentiation of immature cells into insulin-producing cells (45, 46). It should be noted that different macroencapsulation devices and cells were used in the studies. It is possible that more fully differentiated insulin-producing cells derived from stem cells may behave more like islet cells during macroencapsulation conditions. Moreover, the scaling of macroencapsulation from a small animal model into humans will severely affect oxygen
diffusion gradients and supply of oxygen to the tissue (38). Simultaneously, a scaling will likely affect kinetics of glucose sensing and insulin release into blood, and result in a delayed and blunted insulin response, as reported even when scaling up the size of microcapsules from 350 to 650µm (34). By using the macroencapsulation approach, it will however be possible to gain valuable knowledge about how the cells respond to the in vivo environment in humans without great risks regarding the health and safety of the recipient. Due to the size of macroencapsulation devices, few implantation sites are eligible, and the devices are commonly placed in subcutaneous tissue (37, 38, 39, 40, 41). Also, the peritoneal space has been used (42), whereas intraperitoneal placement is difficult in the clinical perspective.

Due to the functional limitations of encapsulation, and the ongoing successful development of more fully matured insulin-producing cell products, we believe that for efficacy non-encapsulated transplantations will be needed. However, such studies should not be initiated until clinical safety studies have been performed using encapsulated cells. For transplantation of cells without encapsulation, we carefully need to consider the optimal implantation site. Based on the concerns regarding potential tumour development, the liver, currently used clinically for islet transplantation, must clearly be considered as questionable. Since the islets/cells become widespread throughout the liver after infusion through the portal vein, they cannot be retrieved. Due to the distribution of cells throughout the parenchyma, and the size of the liver, it would not even be possible to harvest representative biopsies. At this stage, only the clinically not possible renal subcapsular site has been investigated for suitability of implantation of non-encapsulated stem cell-derived cells.

In clinical islet transplantation, an instant blood-mediated inflammatory reaction (IBMIR) is evoked when exposing the islet tissue to portal blood (47), but this reaction seems to be limited at extrahepatic sites (48). We have thoroughly investigated and compared the engraftment and function of rodent and human islets transplanted into several implantation sites (kidney, spleen, liver, striated muscle, omentum, pancreas, intra-peritoneal space and subcutaneous fat), and observed marked implantation site-dependent differences with regard to islet survival, revascularisation and function (49, 50, 51, 52). We have also investigated encapsulation-induced changes in islet function (53). Importantly, even adult beta-cells were observed to substantially change their function and gene expression depending on implantation organ, which could potentially explain the disappointing long-term outcome of clinical islet transplantation to the liver (52). In those studies, transplantation into the pancreas, omentum and striated muscle best supported maintained differentiation of the beta-cells (52, 54). In Table 1, we have summarised the most crucial physiological parameters of importance for islet engraftment and how they are met in different anatomical implantation sites in the preclinical setting. There are of course also other considerations specific for the different implantation sites, e.g. the risk of pancreatitis when injecting cells into the pancreatic parenchyma (55). Muscle is easily accessible, and there is vast experience from transplantation of parathyroid glands to the forearm muscle (56). However, large clusters of cells in an organ limit the tissue survival due to insufficient oxygenation of cells, and spreading of cells is highly important to limit cellular death and fibrosis in such grafts (57, 58). The possible impact of mechanical stress on insulin-producing cells in muscle also remains to be determined. The greater omentum has

<table>
<thead>
<tr>
<th>Anatomical site</th>
<th>Cell survival</th>
<th>Blood vessel density</th>
<th>Proliferation</th>
<th>Differentiation</th>
<th>Number of islets</th>
<th>Species</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Liver</td>
<td>++</td>
<td>+</td>
<td>++</td>
<td>+</td>
<td>150–350</td>
<td>Mouse</td>
<td>52, 57, 70, 71, 73</td>
</tr>
<tr>
<td>Kidney capsule</td>
<td>++</td>
<td>+</td>
<td>+++</td>
<td>++</td>
<td>250; 150–350</td>
<td>Rat</td>
<td>58</td>
</tr>
<tr>
<td>Spleen</td>
<td>++</td>
<td>+</td>
<td>+</td>
<td>?</td>
<td>150–350</td>
<td>Mouse</td>
<td>70, 71</td>
</tr>
<tr>
<td>Anterior chamber of the eye</td>
<td>+++</td>
<td>+++</td>
<td>?</td>
<td>?</td>
<td>30–300</td>
<td>Mouse</td>
<td>72</td>
</tr>
<tr>
<td>Pancreas</td>
<td>++</td>
<td>+++</td>
<td>?</td>
<td>++</td>
<td>200</td>
<td>Mouse</td>
<td>51, 52</td>
</tr>
<tr>
<td>Muscle</td>
<td>++</td>
<td>+++</td>
<td>?</td>
<td>+++</td>
<td>200–300; 250</td>
<td>Mouse</td>
<td>50, 54, 57, 58</td>
</tr>
<tr>
<td>Omentum</td>
<td>++</td>
<td>+++</td>
<td>?</td>
<td>+++</td>
<td>200–300</td>
<td>Mouse</td>
<td>49, 54</td>
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</table>

Sites are based on an arbitrary scale ranging from + to ++. Parameters that have not been evaluated for that given site are indicated with a question mark (?). The table is based on the following references (49, 50, 51, 52, 54, 57, 58, 70, 71, 72, 73).
the benefit of a portal drainage and implantation to this site is also a safe procedure, although not possible in local anaesthesia. There is an ongoing phase 1/2a clinical trial with allogeneic islet transplantation into the omentum (clinicaltrials.gov:NCT02213003).

Since the phenotype of cells clearly may change depending on implantation site, it will be most important to perform not only efficacy studies but also safety studies of implanted insulin-producing cells from stem cells i.e. several months of follow-up in experimental animals. The tendency for tumour and teratoma formation may differ between sites. It will also be important to evaluate conditions of relevance e.g. to include not only male but also female recipients in the experimental studies, which has to our knowledge not been done so far, as well as to investigate the impact of pregnancy on cell fate.

Ideally, the differentiation, survival and function of the transplanted cells should be possible to follow longitudinally by non-invasive or minimal invasive techniques. For some implantation sites such as striated muscle, magnetic resonance imaging and biopsies may easily be used (57), while implants in other organs may be more difficult to monitor. As we previously have shown for pancreatic islets, positron emission tomography (PET) could also serve as a valuable tool for in vivo imaging and endocrine cell quantification (59, 60, 61). However, it will be difficult to monitor implants in the liver or adjacent organs, since many PET tracers are metabolized in the liver which leads to a high background signal.

**Autologous vs allogeneic stem cells**

Another aspect regarding stem cell-derived insulin-producing cells is the source of stem cells. From a commercial point of view, there are great interests in establishing a generic stem cell line from embryonic cells or iPSCs. Although this would theoretically provide indefinite amount of insulin-producing cells and thereby diminish the need for organ donors, it would probably not solve the problem with immune suppression. A recent publication using cells originating from hESCs showed that although pancreatic progenitor cells are hypoimmunogenic, the cells that have differentiated in vivo to endocrine cells do mature and react with cytotoxic T-cells (62). There might be possibilities of downregulating human leukocyte antigen (HLA) class I molecules, a procedure which does not seem to affect the ability of these cells to differentiate into specific lineages (63), but induction of tolerance in clinical islet transplantation has proven difficult. Nevertheless, there are several promising strategies for tolerance induction, including costimulatory blockade, regulatory T-cell induction and dendritic cell targeting (for reviews on this field, see e.g. (64, 65)). However, since there are protocols developed using iPSCs, it could, at least theoretically, be possible to perform autologous transplantations. In a recent publication, it was in fact shown that iPSCs from patients with T1D can be differentiated into insulin-producing cells with functional capacity equal to that of cells derived from non-diabetic individuals (66). From a patient’s point of view, it would be a great benefit to receive autologous cells, since this would diminish the need for immune suppression or development of tolerance induction protocols with use of non-encapsulated transplantation. It has been argued that iPSCs are prone to have genetic defects that compromise their use in cell therapy. However, the karyotype analysis of more than 1700 human ESC and iPSC cultures from 97 investigators in 29 laboratories showed an overall incidence of karyotype abnormalities to be similar in hESCs (12.9%) and iPSCs (12.5%) (67). Nevertheless, iPSCs from older donors retain an epigenetic signature of age, but which can be reduced through passaging (68). Since type 1 diabetes is a disease with onset mainly in childhood or adolescence, future treatment of disease will, in many cases, be current in young people. An obstacle is presently the variability of differentiation efficiency between iPSC lines, which could make personalized cell therapies costly and highly labour intensive. There is also a theoretical...
possibility of recurrence of disease, i.e. an autoimmune attack to the implanted cells.

From a clinical point of view, transplantation of insulin-producing cells derived from stem cells must be performed without immune suppression in order to be an attractive treatment option. If not, islet transplantation must be considered a better treatment option for selected patients suffering from life-threatening hypoglycaemic events, since the outcome have dramatically improved over the last years and the islets do not possess a risk for tumour development. For islet transplantation today, the organ shortage is not an overwhelming problem, since the number of patients who actually fulfil the criteria and would benefit from islet transplantation only constitute less than 5% of the total patient population. If insulin-producing stem cells were to be transplanted with immune suppression, the only relevant indication would be for patients fulfilling the transplantation criteria where a matching donor cannot be found, in other words, a very limited number.

**Conclusion**

There are currently successful in vitro protocols for deriving insulin-producing cells from stem cells. These cells are functional in vitro and have the capacity to secrete insulin in response to glucose challenges in vivo when transplanted to mice. However, there is, especially with pancreatic progenitors, also alarming data on the formation of tumours and teratomas after transplantation to mice. Signals such as cytokines, growth factors and even oxygen levels are of major importance for the development and differentiation of these cells. Such factors cannot be controlled in vivo, and there will be large inter-individual differences as well as individual fluctuations over time. Since there are species differences regarding in vivo differentiation even between mice and rats, we can assume that there might be differences also between mice and men.

Based on this, we propose that the best strategy for clinical trials would be to first transplant these cells in a macrochamber, which meet the basic needs for beta-cells regarding oxygen and nutritional needs. This would be of major importance for gaining knowledge on how these cells behave in vivo in humans regarding differentiation and potential tumour development. However, the macroencapsulation field has yet to deliver a system functional even for clinical islet transplantation, and it is therefore unlikely that this approach will be fully sufficient to provide cure to T1D patients. If safety for the cells can be demonstrated in macroencapsulation studies, we therefore propose to move towards non-encapsulated transplantation. The cells for use should preferably be autologous insulin-producing cells, i.e. cells derived from iPSCs of the patient. When changing the implantation site there is always a risk that the differentiation of the cells is affected. Long-term follow-up animal experiments of safety and efficacy therefore needs to be tested for the considered implantation sites. For this consideration, we can learn from studies of transplanted islets, but also remember the aspect of possibilities for invasive or non-invasive monitoring the grafts. One site that will be clearly worthy of consideration is forearm muscle. There is a number of reports of clinical islet transplantation (57, 69), where preclinical studies have shown excellent engraftment of islets at this site (50, 57). The procedure for implantation at this site is minor surgery, biopsies could easily be harvested, and there are currently available imaging protocols that could be used in order to monitor the transplanted cells. And, most importantly, the transplanted cells could be surgically removed if needed. We have summarised our proposed ‘road map to the clinic’ in Fig. 1.

**Declaration of interest**

The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research reported.

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