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Report of the Key Opinion Leaders Meeting on Stem Cell-derived Beta Cells

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Abstract

Beta cell replacement has the potential to restore euglycemia in patients with insulin-dependent diabetes. Although great progress has been made in establishing allogeneic islet transplantation from deceased donors as the standard of care for those with the most labile diabetes, it is also clear that the deceased donor organ supply cannot possibly treat all those who could benefit from restoration of a normal beta cell mass, especially if immunosuppression were not required. Against this background, the International Pancreas and Islet Transplant Association in collaboration with the Harvard Stem Cell Institute, the Juvenile Diabetes Research Foundation (JDRF), and the Helmsley Foundation held a 2-day Key Opinion Leaders Meeting in Boston in 2016 to bring together experts in generating and transplanting beta cells derived from stem cells. The following summary highlights current technology, recent significant breakthroughs, unmet needs and roadblocks to stem cell–derived beta cell therapies, with the aim of spurring future preclinical collaborative investigations and progress toward the clinical application of stem cell–derived beta cells.

IMPROVED DIFFERENTIATION AND CHARACTERIZATION OF STEM CELL–DERIVED CELLS

A variety of in vitro differentiation protocols have been published,[1–7] based on the original work of Rezania et al,[1] which can successfully differentiate human embryonic stem cells (ESCs) and induced pluripotent stem cells (iPSCs) into monohormonal insulin-expressing cells that phenotypically and functionally resemble adult beta cells. An important feature of these protocols is the efficient generation of PDX1 and NKX6.1 coexpressing pancreatic progenitors, through which improved yields of insulin-expressing beta cells can be obtained. The current focus is on improving the quality and functionality of these populations by identifying unique selectable markers and key signaling mechanisms that control the process.

Using glycocapture-based proteomics to reveal potential cell surface markers, Cristina Nostro[7] has identified a novel cell surface marker, glycoprotein-2, which distinguishes human pancreatic progenitors from pancreatic polyhormonal cells and can be used to phenotypically characterize and sort pancreatic progenitors, leading to enriched beta cell preparations in vitro. Differentiating stem cells into beta-like cells with functional characteristics of primary adult beta cells is critical to improving the technology.[4–6] Although beta cell preparations reverse diabetes after transplantation in rodent models and demonstrate glucose responsiveness on glucose-stimulated insulin secretion (GSIS) assays in vitro, on perifusion assays the insulin secretion kinetics and mitochondrial respiration are

functionally immature. Ali Rezania[4] has compared RNA sequencing on stem cell–derived beta cells at various stages of differentiation to human islets to identify upstream signaling pathways that could be modulated to improve maturation. Using this information, Rezania modified his recipe and now is producing stage-7 beta cells with 2-phase insulin secretion and mitochondrial oxygen consumption rates that approach adult islets, “although,” he cautions, “the insulin secretory responses remain subpar compared to adult human islets.”

Pancreatic islets are not comprised entirely of insulin-secreting beta cells, but include other hormone-secreting cells, such as alpha and delta cells. The islet-like clusters currently being produced in laboratories worldwide contain fewer functional beta cells than adult human islets. In addition, the long-term goal is to produce islet-like clusters that contain most or all of the islet endocrine cell populations with the aim of gaining better physiological control in vitro than has currently been achieved and more immediate reversal of diabetes in animals. Using inDrops (1CellBiO Inc., Cambridge, MA), a microfluidic-based platform for high throughput single cell RNA sequencing, Douglas Melton’s group has recently published a transcriptomic atlas of human and mouse pancreas that reveals the intercellular and intracellular population structure of islets, including 400 novel previously unknown, potentially secreted proteins.[8]

Jose Oberholzer[9] is studying the functionality and heterogeneity of stem cell–derived beta cells using biochip-based microfluidic and nanofluidic, multiparametric perifusion assays designed to study beta cell physiology and to phenotype islet surrogates from various sources. The islet biochips integrate islet micro and nanoperifusion with multiparametric imaging technology and measure not only insulin secretion kinetics but also insulin secretion coupling which is determined by measuring calcium influx and mitochondrial potentials. Integrated high throughput islet arrays and multiplexing have significantly increased the analytical power of these biochips, providing better understanding of the heterogeneity of stem cell–derived islet surrogates. Oberholzer’s beta cell testing facility is funded by the Juvenile Diabetes Research Foundation (JDRF) and is available to test various beta cell or islet surrogate populations. However, the correlation of test results with in vivo function is still under study.

Determining the functionality of stem cell–derived beta cells is a challenge Daniel Pipeleers[10] has studied by comparing the synthetic, storage, and secretory capabilities of insulin in stem cell–derived beta cells with those of human pancreatic beta cells, which can correct hyperglycemia following clinical intraportal transplantation in mice. Their analysis was conducted on implants that were generated by Viacyte stem cell–derived progenitor cells in a subcutaneous (SQ) Encaptra device; implants retrieved from recipient mice and newly formed beta cells were compared with those in human pancreatic islet cell isolates.[11] Beta cell number and functional maturation, the components responsible for homeostatic control of insulin in a functional beta cell mass, were followed over 50 weeks. Other endocrine and nonendocrine cells that affect outcomes in rodents were also quantified.[11] Emphasizing the utility of developing standardized markers and models, Pipeleers stressed that comparisons are not possible with data reported in the existing literature, because differing methods and reference values have been used and often insufficient information is provided on beta cell number and functional maturation.

GENOME EDITING—FUTURE IMPACT ON THE FIELD OF BETA CELL REPLACEMENT

In the 1990s, efforts to develop xenotransplantation as a renewable source of organs and tissues were thwarted by immunological problems and the risk of human infection by porcine endogenous retroviruses (PERVs), which reside in the pig genome. Benign to pigs, PERVs are potentially injurious to humans. Luhan Yang reported her experience with the CRISPR-Cas9 RNA-guided genome editing technology, which has allowed up to 65 simultaneous edits, where previously the maximum was 5, permitting the removal of all copies of the PERV *pol* gene from the pig genome.[12,13] CRISPR-Cas9 has provided a powerful tool to genetically modify porcine somatic cells, rendering them more compatible with the human immune system. Yang acknowledged the task of producing multiple edits to substantially reduce immunogenicity takes time, but noted the accuracy and multiplexability of the CRISPR-Cas system has injected new energy into the field that many believe will accelerate progress.

CRISPR-Cas9 provides an unprecedented opportunity to create stem cell-based disease-in-a-dish models. Chad Cowan detailed his discovery of SNP rs12740374, a mutation located in the noncoding regulatory region of *Sortilin*, a gene whose expression suppresses circulating LDL cholesterol. This mutation creates a transcription factor binding site that leads to increased expression of *Sortilin*. Challenged to prove this anomaly is present in humans, Cowan used CRISPR-Cas9 to create isogenic pairs of human stem cells, one with the mutation and one without, then differentiated them and made phenotypic comparisons. He showed that rs12740374 SNP in the *Sortilin* noncoding regulatory region exhibited a similar biological effect in human cells as he saw in the stem cell lines. Cowan also is using CRISPR-Cas9 to alter stem cells to reduce immunogenicity by eliminating the expression of HLA antigens. He further proposes to reduce HLA Class I expression by mutating beta2 microglobulin and HLA Class II expression by mutating the *CIITA* gene. Additionally, he plans to knock-in HLA-G to protect against NK cells. The long-term aim is to produce an off-the-shelf universal donor cell line for replacement therapies.

Danwei Huangfu[14–16] used the CRISPR-Cas9 system to develop inducible Cas9 H1 and HUES8 cell lines. Using these lines to knock out genes important in murine pancreas development, she then studied their role in human pancreas development. She is developing genetic models relevant to diabetes and pancreas, including both forward and reverse genetic approaches in hESCs and hiPSCs. For example, *Neurogenin-3 (NGN3)* mutations in patients with neonatal diabetes have greatly reduced numbers of beta cells. Huangfu introduced frameshift mutations into *NGN3* to create a variety of different lines, including a mutant line in which almost the entire *NGN3* gene is deleted. She also made disease-mimicking lines to recapitulate what might happen in patients. The phenotypes were similar across all lines. One of the phenotypes was specific to the last stage of beta cell differentiation; during the early stages everything was normal but in late stages beta cell formation was severely affected. Immunostaining of C-peptide+ cells that coexpress NKX6.1 was observed in wild type cells, whereas it was greatly reduced in the mutant lines.

Dieter Egli pioneered an alternative approach to reprogramming human pluripotent stem cells (hPSCs) termed somatic cell nuclear transfer. The nucleus is removed from a somatic cell, inserted into an enucleated ovum, and then the somatic cell nucleus is reprogrammed by the host cell. Using this approach, in combination with elements of differentiation protocols published by Rezania,[1,4] Melton,[8] and Nostro,[7] he routinely produces 60% C-peptide positive cells. Egli does report differences in differentiation efficiencies between isogenic iPS cells and nuclear transfer ES cells, necessitating cell line quality control. Quality controls based on functional assays are most informative, as isogenic cell lines have very similar gene expression and DNA methylation patterns, but can still have profound functional differences. The greater developmental competence of nuclear transfer ESCs compared with iPSCs is consistent with findings by others who generate mice from somatic cells. Repeated reprogramming of somatic cells to ESCs by nuclear transfer does not alter the efficiency of the cloning, but developmental competence decreases with repeated iPSC generation from somatic cells.[17,18] In his hands, the nuclear transfer ESCs more efficiently differentiate into beta cells and can protect mouse models of diabetes. Finally, the use of reprogrammed autologous cells would be expected to have some advantages since it would reduce or obviate the need for immune protection.

Avoiding the tumorigenicity of stem cell–derived beta cells after transplantation is a critical step in preclinical testing. The 3 principles of cell culture that can prevent teratoma formation and development of genetically aberrant cells are: (1) minimizing transfer of undifferentiated cells, (2) minimizing culture stress, and (3) routinely monitoring the genomic stability of cells in culture for early signs of aberration. Even with the best differentiation protocols, a few undifferentiated cells often remain among the differentiated progeny. Uri Ben-David[19–22] has identified multiple small molecule strategies to remove these undifferentiated cells and eliminate tumorigenic potential. One of these molecules, PluriSIn1, specifically inhibits Steroyl CoA Desaturase 1, the key enzyme involved in synthesizing the monounsaturated fatty acid oleate. hESCs and hiPSCs depend on this enzymatic activity. Inhibition by PluriSIn1 leads to ER stress, protein synthesis attenuation, and apoptosis.[19] Importantly, pretreatment with PluriSIn 1 completely prevents teratoma formation.[19,21,23]

IMMUNOGENICITY

Shane Grey presented a broad framework for understanding the barriers imposed by immune mechanisms when stem cell–derived beta cells are implanted into patients. Grey provided concrete examples of alloimmunity and autoimmunity operating in mouse islet models. Of note, he strongly encouraged the stem cell community to start testing their beta-cell preparations using in vivo models of human immunity, such as humanized mice,[24–26] to hasten the discovery of hidden immunological problems and to develop ways to subvert immune mechanisms.

Qizhi Tang reviewed the classic autoimmune and alloimmune mechanisms associated with islet graft rejection in T1D, followed by a detailed presentation of strategies that promote immune tolerance by altering the balance between effector autoimmune and alloimmune T cells and regulatory T (Treg) cells. She highlighted the Immune Tolerance Network study of

Alefacept to selectively deplete memory T cells[27] and the University of California San Francisco phase I study of Treg cell therapy[28] in T1D subjects. She pointed to a lesson learned from the University of California San Francisco experience with clinical islet transplantation, namely, potent immune modulating therapies are vital to long-term insulin independence.[29] Tang postulates that immunosuppression-free survival of stem cell–derived beta cell transplants can be achieved using a 3-pronged approach: (1) controlling inflammation immediately after transplant, (2) minimizing immunogenicity by either encapsulation or immunoengineering, and (3) promoting immune regulation.

Bart Roep reported the vulnerability of human embryonic stem cell–derived beta cells to innate and adaptive autoimmune and alloimmune responses in T1D.[30–32] Although embryonic stem cell–derived progenitors are hypoimmunogenic, in vivo differentiated endocrine cells are vulnerable to adaptive immune responses, particularly in the inflammatory milieu of diabetes. The ideal encapsulation device should be able to prevent cellular, antibody, and complement-mediated attack. Progenitor cells used in the ViaCyte trial showed blunted donor-specific immune responses compared with matured endocrine cells. This finding suggests that cells put into capsules and implanted in patients do not activate or cause changes, at least that can be measured with current tools.

ALTERNATIVE TRANSPLANT SITES AND PLATFORMS

Transplanting islets into the portal venous circulation stimulates an instantaneous blood-mediated inflammatory reaction and exposes the islets to high levels of toxic immunosuppressive drugs. Many are searching for alternative minimally invasive, well-vascularized, and retrievable sites. Camillo Ricordi views the omentum as a potentially advantageous site for implanting stem cell–derived beta cells. He presented a novel technique for layering islets, coagulated autologous plasma, and thrombin onto the omentum via a laparoscopic approach in a patient with T1D. The initial success of this strategy has inspired further study of the omentum as a potential site for transplantation of both free and microencapsulated insulin-producing cells, including stem cell–derived islet-like clusters. Furthermore, this “bio-degradable” scaffold technology platform may allow the inclusion of strategies to eliminate systemic immunosuppression, such as conformal encapsulation and/or local immunomodulation with cotransplantation of immunomodulatory cells, including mesenchymal stem cells, Treg cells, and so forth. Additional studies are under way to further test and validate this novel implantation approach, which may provide a safe alternative to intrahepatic islet transplantation.[33–35]

Ali Naji[35] underscored the need for alternative sites for transplanting insulin-producing cells. Summarizing the first University of Pennsylvania experience with the Collaborative Islet Transplant Consortium, he recounted the success of the Collaborative Islet Transplant protocol in which islets were transplanted intrahepatically, despite the ultimate loss of islet mass leading to suboptimal insulin independence rates. Loss of islet mass after intrahepatic transplantation is thought to be multifactorial, including instantaneous blood-mediated inflammatory reaction, amyloid deposition, and nonimmunologic consequences of the intraportal site.[36] He went on to propose SQ implantation of islets as a suitable alternative.[37] Although prior studies have shown the SQ site to be poor for islet engraftment, most likely

the result of poor vascularity, Naji presented contervailing data suggesting that SQ implantation could prove viable. He provided experimental evidence of a novel proprietary factor, which, when added to an islet graft, significantly enhanced the function of a minimal mass of islets in rodent allogeneic and syngeneic islet transplant models. Further studies are in progress to elucidate the mechanisms underlying these potentially important observations.

Giuseppe Orlando.[38,39] is a leading proponent of using decellularized organs as a scaffold for bioengineering new organs for transplantation. The organs first are perfusion-decellularized, leaving a scaffold of extracellular matrix, which is used as a template for regenerating the cellular compartment, ideally from the patient's own derived stem cells Experimental attempts to reseed nonhuman decellularized pancreatic grafts with islets, ductal cells, and endothelial cells have been reported.[40] Orlando[41] shared his experience with decellularized human pancreatic grafts reseeded with endothelial and endocrine cells. Whether such grafts can remain viable, free of thrombosis, and capable of sustaining functional islet tissue remains to be determined. A significant challenge to the clinical application of this approach is the need for an inexhaustible source of extracellular matrix.[42] An ideal platform might involve harvesting extracellular matrix scaffolds from pig organs, which then could be seeded with specialized cells, such as islets and endothelial cells derived from patient-derived iPSCs.[43] Interestingly, porcine pancreatic extracellular matrix-based hydrogels may also be incorporated within microcapsules to enhance islet viability, function, and lifespan, and ultimately improve islet encapsulated technology.[44]

ENCAPSULATION TECHNOLOGIES

The JDRF, in conjunction with The Helmsley Charitable Trust and other foundations and agencies, funds research to support preventive, restorative, and replacement therapies for T1D. The current emphasis on transplanting insulin-producing tissues into patients with T1D while avoiding immunosuppression has motivated the development of encapsulation systems for immunologically protected stem cell–derived beta cells. Immunologist, Julia Greenstein, summarized JDRF's leadership in the area of encapsulation which has brought together a 52-member Encapsulation Consortium comprised of principal investigators from leading universities worldwide and 5 companies all involved in novel biomaterials development, micro and macro device design, tools standardization, and preclinical and clinical translational studies.[45] The purpose of the consortium is to identify research priorities, share data and protocols, including cross team comparisons of successful technologies, and accelerate progress by fostering multidisciplinary collaboration among academia and industry.

The vulnerability of allogeneic and xenogeneic islet grafts to host immune and autoimmune attack is a primary challenge in islet transplantation and is being addressed through the development of immunoisolating microencapsulation and macroencapsulation systems. A decade ago Daniel Anderson's team designed a microcapsule that could support islet immune isolation. Recognizing that fibrotic responses contribute significantly to loss of function of microencapsulated islets over time, they sought to identify biomaterials and a capsule architecture that resists fibrosis. First, they discovered that capsules above 1.5 mm demonstrate significantly greater resistance to fibrosis in rodents and primates, causing them

to limit capsule size.[46] Because alginate, used as a base polymer material for decades, supports islet function in immune-deficient animal models, but fails in animals with strong immune systems, including primates and humans, they decided to modify the chemistry of alginate to resist fibrosis. Using a combinatorial approach, they created a library consisting of hundreds of chemically modified materials from which they identified a number of modifications that could significantly reduce fibrosis.[47] With these capsules, they have been able to provide long-term islet survival in mice using both rat islets and stem cell–derived beta cells.[48]

Mark Poznansky uses chemorepellents, including chemokines, such as CXCL12, to improve microencapsulation systems to reduce or eliminate the need for immunosuppression. Islets are placed in CXCL12-releasing alginate microcapsules, creating an elution gradient that prevents immune destruction. Poznansky showed that CXCL12-impregnated microbeads can repel cytotoxic T cells, along with other immune cells, and attract and retain Treg cells and M2 macrophages around the encapsulation site. CXCL12 also functions as a signaling molecule, exerting prosurvival effects on the islets themselves. In preclinical research in collaboration with other JDRF-supported scientists, including James Markmann and Ji Lei, Poznansky demonstrated that CXCL12-impregnated alginate microcapsules protect and prolong the survival of transplanted allogeneic islets in diabetic mice without systemic immunosuppression.[49,50] Preclinical studies in nonhuman primates (NHPs) have progressed from transplanting blank (CXCL12 negative) microencapsulated autologous and allogeneic islets in healthy animals, to transplanting CXCL12-positive microencapsulated allogeneic and xenogeneic islets in diabetic animals, without significant adverse events related to microbead implantation.

Remarkable progress was reported by Barbara Ludwig with a device for macroencapsulation that has undergone extensive testing in rats, NHPs, and humans in both allogeneic and xenogeneic settings. The BetaO_2 device for macroencapsulation is comprised of 3 compartments layered in a disc-shaped capsule. The outer 2 compartments house the islets, which are immobilized in alginate and then integrated into the capsule. A middle compartment houses an oxygen reservoir that can be intermittently replenished via an SQ gas access port using an external O_2 delivery device. Adequate oxygen delivery to the islets, in addition to well developed vascularization of the surrounding tissues, is seen as key to the successful application of this system. A preclinical safety and efficacy trial in NHPs was conducted without any detectable transmission of pathogens to the recipient animals and with excellent readouts including blood glucose levels and insulin secretory responses.[51] The lack of fibrosis demonstrated in this model upon explantation is striking, and probably the result of the robust health of the islets. A pilot clinical trial has been conducted in an allogeneic setting in humans, where the device was transplanted preperitoneally with a marginal mass of 2000 islets per kg body weight. The patient did not receive any immunosuppression, yet maintained excellent glycemic parameters for 10 months.[52] No change in islet autoantibody or donor alloantibody status has been observed. A follow-up pilot clinical trial with human islets is under way.[53]

Alice Tomei is developing conformal coating, a new microencapsulation platform technology. The technique wraps differently sized islets with uniformly thin (15 μm)

hydrogels. Conformal coating reduces the size and volume of the encapsulated islet to a few microns, comparable to a naked islet, thus reducing the volume of transplanted material and minimizing diffusion barriers to insulin, oxygen, and nutrients.[54] Instead of placing large free-floating capsules in the peritoneal cavity, the goal is to put very thin capsules into alternative confined and well-vascularized sites. Preclinical studies revealed physiological GSIS and oxygen consumption rates comparable to naked islets. Most importantly, despite their minimal thickness, conformal coatings confer immunoprotection in fully MHC mismatched allografts. Tomei plans to move the technology into clinical trials after altering the composition of the hydrogel to make it cGMP-compliant, xenoprotein-free, and nontoxic.

EFFICIENT PRE-IND STUDY DESIGN OF FIRST-IN-MAN COMBINED BIOLOGIC/DEVICE CLINICAL PRODUCT

Kevin D'Amour shared Viacyte's successful experience in steering a combined pluripotent stem cell and encapsulation technology through preclinical studies and Phase I clinical trial. ViaCyte, Inc., is developing a stem cell-based islet replacement therapy for treating patients with diabetes. The therapy is a combination biologic/device product, called VC-01, comprised of pancreatic endoderm cells (PEC) encapsulated within a retrievable delivery device known as the Encaptra Cell Delivery System. After implantation in immune-deficient rodent models, the encapsulated progenitor cells differentiate into glucose-responsive insulin-secreting cells. The renewable starting material for cell product manufacturing is human embryonic stem cells that are directed to differentiate to the PEC product using scalable processes.[55] The biostable delivery device is designed to fully contain cells and protect them from immune attack, with the goal of eliminating the need for immunosuppressant drugs.[56] Nonclinical evaluation of the efficacy of the product was conducted in immunocompromised rodent models and included therapeutic proof-of-concept studies in diabetic mice. Efficacy studies evaluated the differentiation of PEC-01 cells into glucose-responsive insulin-producing cells by measuring GSIS response and the capacity of grafts to regulate blood glucose in the host. Safety assessment included 3 GLP studies which characterized the toxicity, tumorigenicity, and tolerability associated with the VC-01. We anticipate additional details regarding islet survival and physiologic function upon completion of follow-up of the subjects participating in this trial.

CLINICALTARGETS

Unmet Needs in Diabetes

Defining the study population is a central aspect of clinical trial design. Bernhard Hering advocated the importance of the risk-benefit ratio and a sufficiently powered study to ensure the scientific objectives. *Stem cell–derived beta cell replacement technologies are evolving rapidly*. The individual characteristics of each investigational cell therapy product and the technologies used to prevent rejection will determine the patient group in whom product activity, whether beneficial or adverse, can be best detected and for whom participation in an early-phase stem cell–derived beta cell trial is the best option. Because early phase I pilot trials are likely to be small, single arm and open label studies, with outcomes compared with

baseline status, the anticipated metabolic and clinical effects must be sufficiently robust to be detected in the selected study population if meaningful information is to be obtained. *Medical treatments for diabetes are also evolving rapidly*. Improved educational programs involving behavioral therapies, insulin analogs, sensor-augmented insulin pumps, and other diabetes technologies will help greater numbers of insulin-treated diabetic patients meet treatment goals. For diabetes complicated by impaired awareness of hypoglycemia and recurrent severe hypoglycemia, extreme glycemic lability, and progressive microvascular lesions, *transplant interventions should be considered*.

Allogeneic and Autologous Modes of Pluripotent Stem Cell Transplantation in Beta-Cell Replacement. Which Way to Go?

Lorenzo Piemonti addressed issues related to the use of an allogeneic or autologous approach to designing stem cell–derived beta-cell replacement therapies for T1D, T2D, or pancreatogenic diabetes, a decision complicated by many factors, including the molecular, genetic and biologic diversity of these populations[57] and the regulatory and economic impact of selecting a particular path.[58] The choice has important implications for the manufacturing process, the associated infrastructure, and could even affect the design of preclinical studies. Lack of effective methods to induce immune tolerance to maintain graft survival is a major roadblock for cell-based therapies, and poor stem cell survival and engraftment after delivery is partly due to immune responses triggered by host immunity.[59] The great advantage of patient-derived autologous cells is avoidance of the host versus graft immunological reaction. However, this advantage may be limited by the autoimmune response in the context of T1D.[60] Those bothered by the possibility of choosing the incorrect path should be comforted by the fact that their innovation is certain to find utility in some patient subgroup.

Other faculty reviewed advanced humanized mouse models (Greiner)[24,61,62]; best practices in nonhuman transplantation models (Graham)[63–65]; regulatory considerations in the United States (Carpenter) and EU (Migliaccio), respectively; as well as the need for new global standards for stem cell research and clinical transplantation (Vallier).[66–68]

FUTURE PERSPECTIVES

Recent advances toward the successful translation of stem cell–derived beta cells for diabetic patients have been impressive, yet there is still much work to be done (Table 1). The consistent generation of pure populations of fully functional beta cells remains elusive and the question persists as to whether pure beta cells or a mix of beta cells with other islet endocrine cells will yield better performance. Perhaps just as problematic is the need to develop an immunoisolation device that provides a hospitable environment while preventing immune-mediated damage and to define an optimal site for its implantation. Despite these remaining hurdles, progress has been broad-based and tangible, with many groups working toward early phase trials in the near term. Moreover, the genome-editing revolution is likely to impact this field in a positive way in the near future by creating better disease models and producing safer and more effective therapeutic cell populations. It seems inevitable that stem

cell–derived beta cells will play an important role in the care of diabetic patients within the next decade.

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TABLE 1.

Unmet needs and future directions in stem cell-derived beta cell technology

• Stem cell-derived beta cells do not have robust physiologic function.
• Stem cell-derived islet-like clusters do not contain the full complement of endocrine cells.
• More efficient low-cost differentiation methods are needed.
• An effective delivery device that can support physiologic function and prevent immune attack has yet to be defined.
• The optimal site for implantation has not been established.
• Better T1D autoimmunity experimental models are needed for testing human cells.
• Understanding which genetic modifications and therapies could allow cells to evade immune destruction, suppress immune responses, enhance functionality or better sustain the viability of cells after transplantation is vital to the future development of this technology.