BETA CELL REPLACEMENT THERAPIES IN T1D
JDRF-NIH WORKSHOP

May 18–19, 2022

MEETING REPORT

Contents

Summary

- The FDA guidelines for allogeneic islet transplantation were released in 2009. The field has made significant progress since then in several areas, including the development of stem cell-derived cell products, porcine islets and beta cells for xenotransplantation, use of implantation sites other than the portal vein, innovative devices, and large-scale manufacturing of islets or beta cells.

- FDA representatives emphasized the need for developers of beta cell replacement products to communicate with the agency early in the development process and throughout development and clinical testing. Each product will be reviewed based on its own characteristics and supporting data, and advice specific to each product will be provided.

- Since 2009, significant clinical advances have been realized in artificial pancreas technology and continuous glucose monitoring (CGM). The target population of type 1 diabetes patients who experience severe hypoglycemic events (SHE) has decreased due to these advances. Coupled with advances to reduce or eliminate immunosuppression, there may be an opportunity to expand the transplant-eligible patient population. Meeting participants discussed the potential for the FDA to accept CGM data for patient selection and for outcome measures in transplantation trials.

- Innovative platform trial designs could be harnessed to accelerate testing and approval of multiple advances across several domains relevant to islet transplantation (e.g., cell source, immune protection methods, devices).

- People with type 1 diabetes are urgently seeking solutions to alleviate the significant daily burden of the disease and its management. Many patients are willing to accept more risk in return for the potential of better glucose control without intensive daily management and real-life risks of hypoglycemia and other complications.

- Presenters described current challenges with identifying optimal strategies and sources for transplantable islets and beta cells. Progress in the stem cell field has resulted in multiple protocols for differentiation into beta cells or mixed islet cell populations. Researchers are working to define reproducible outcomes of stem cell differentiation and develop ways to automate and scale the process.

- Gene editing strategies may be combined with stem cell approaches to create beta cells that are protected from immune destruction after transplantation. Gene editing is being explored as a means of developing a renewable source of porcine islets for safe, viable, and functional beta cell replacement.

- Participants discussed the benefits and challenges with different animal models for preclinical testing of islet replacement. Different models may be applied to address specific questions in the field.

- Multiple strategies are in development to facilitate islet transplantation in immune protected sites with or without encapsulation, often using devices that are scalable, removable, or replaceable, and/or reloadable with minimally invasive procedures. Some devices promote vascularization in or around the transplanted islets or devices.

- As safe and efficacious cell replacement strategies are developed, the field must address CMC and manufacturing issues to ensure that successful therapies are available to the broad type 1 diabetes population.

Welcome Remarks and Introduction

William Cefalu, MD, National Institute of Diabetes and Digestive and Kidney Diseases (NIDDK)

The Special Statutory Funding Program for Type 1 Diabetes (or Special Diabetes Program [SDP]) supports resources for islet transplantation research. Several SDP-supported programs, including the Clinical Islet Transplantation Consortium (CIT), Integrated Islet Distribution Program (IIDP), Beta Cell Biology Consortium (BCBC), and Human Islet Research Network (HIRN), have facilitated significant advances in the field over the past several years. Dr. Cefalu welcomed all participants to this workshop. Multiple stakeholders will gauge the current state of the field and chart a path for continued progress. The outcome of this effort will help inform research strategies at the NIDDK and create opportunities for partnership among the NIDDK, NIAID, JDRF, FDA, and others.

Sanjoy Dutta, PhD, JDRF

Dr. Dutta reminded participants that the last workshop on beta cell replacement therapy for type 1 diabetes was held in 2009. That meeting largely focused on defining how healthy beta cells need to be for successful transplantation. Now, many different beta cell sources are being tested in clinical trials. But, barriers and questions remain to be addressed before beta cell replacement therapy is widely available to those with type 1 diabetes. Achieving the goal of routine, accessible beta cell replacement requires a multidisciplinary approach to reduce redundancies and increase efficiency.

JDRF has a roadmap for cell replacement therapy research, but Dr. Dutta asked workshop participants to think beyond that roadmap for innovative ways to make beta cell replacement and islet transplantation a clinical reality. With 80 percent of people with type 1 diabetes not meeting glycemic goals and the ongoing burden of long-term complications, such therapies would fulfill a critical need.

Elizabeth Hart, MD, U.S. Food and Drug Administration (FDA)

Dr. Hart stated that the patient perspective is important throughout the life cycle of product development, including during the FDA regulatory process. They can provide insights on what aspects of the disease are most troubling and what risks are acceptable for a specific potential benefit.

The FDA is committed to the efficient development of new cell therapies for type 1 diabetes, with regulators working together with patients, physicians, researchers, and industry. Dr. Hart recommended that those seeking to develop a new IND speak with FDA regulators early in the process of product development to receive advice and guidance tailored to each product.

Session One: Clinical Trial Considerations for Beta Cell Therapies

Moderator: Michael Rickels, MD, MS, University of Pennsylvania

Patricia Beaston, MD, PhD, FDA

Regulatory Considerations for Beta Cell Replacement Therapies

Products that are relevant to cell replacement therapies for type 1 diabetes include pancreatic islets from deceased donors, human islet/beta-like cells from stem cells or genetically transformed cells, xeno islets obtained from animals, immunomodulatory drugs or biologics, and devices. Limited data are available on how closely the physiologic function of induced, modified, or xeno cells approximates that of the human islet. In addition to known risks of cadaveric islet transplantation, safety concerns from newer islet sources include off-target hormone production, hormone dysregulation, clonal expansion from off-target gene insertion, and risks specific to xeno islets, such as zoonosis. The development of islet transplantation strategies that eliminate immunosuppression would be a significant advance; however, it would also remove a safety measure in that islets, particularly those from animal sources, could not be eliminated by withdrawing immunosuppression. This would require new strategies to

manage hormonal dysregulation or clonal expansion, if they arise from non-cadaveric cells. Each route of islet administration (e.g., portal, omental, subcutaneous, etc.) has its own potential risks that must be considered in a safety assessment and clinical monitoring plan.

The FDA's 2009 guidance limited islet transplantation to people with established type 1 diabetes and impaired hypoglycemia awareness who were unable to achieve glycemic goals due to severe hypoglycemic events (SHE). The potential benefit of improving recurrent SHE was balanced against the risks of allogeneic islet administration and immunosuppression. The guidance does not allow SHE identified using CGM or glucose meters for the purpose of clinical trials (inclusion or efficacy). The FDA also has guidance available on xenotransplantation and gene therapy.

Dr. Beaston encouraged product developers to engage with the FDA early and often to understand regulatory requirements and avoid unnecessary delays. She also encouraged application of a risk:benefit approach, identifying the risks and describing how those risks can be mitigated at each step in a program. Several opportunities are available for discussions before an IND application. One informal INTERACT (pre-pre-IND) meeting can be held prior to completion of preclinical studies. A pre-IND meeting and discussions at the end of each clinical phase are also possible.

Tom Eggerman, MD, PhD, NIDDK

Clinical Trial Considerations for Beta Cell Therapies: Perspectives From Clinical Islet Transplantation

As of 2022, data from approximately 1,400 allogeneic and 1,300 autologous islet transplants have been added to the CITR. Significant current outcomes include: 5-year insulin independence rate of >50%, >90% absence of SHE, and improvements in hypoglycemia awareness and HRQOL. Safety concerns remain, including implantation procedure adverse events, infection from contaminated islets, immunosuppression risks, and potential development of anti-donor antibodies.

FDA guidance allows for open-label, single arm trials because the potential improvements are significantly better than standard of care, so a control arm is unnecessary. However, this approach may need to be reconsidered in the future given advances in artificial pancreas technology.

The FDA guidance issued in 2009 addresses manufacturing considerations, preclinical studies, and trial design. In 2014, the FDA issued guidance on manufacturing islets used in preparation of the CIT Master Batch Record publication. Phase III trial design approaches used for the CITC were established after pre-phase III meetings with the FDA. Although each future beta cell product will be individually reviewed, these historical guidance documents provide a framework for FDA oversight of new beta cell therapies.

Several downsides to allogeneic islet transplantation are spurring the development of new therapies (e.g., insufficient donors, inefficient islet isolation, immunosuppression, development challenges for academic investigators, etc.). Experience from clinical islet transplantation provides a framework with respect to trial design, regulatory oversight, and manufacturing. The promise and hope of stem cell-based therapies come from their scalability and unlimited supply, off-the-shelf availability, elimination or minimization of immunosuppression, and broad use by patients, if shown to be safe and effective.

Jason Gaglia, MD, Joslin Diabetes Center

Clinical Trial Considerations in Investigational Islet Replacement Programs: Participant Characteristics and Outcome Measures

Dr. Gaglia proposed that the focus on patients with SHE is most important in the setting of immunosuppression; as the field moves away from the need for chronic immunosuppression, it might make more sense to focus on other characteristics for enrollment. SHE is now rare with current treatment modalities, i.e., when patients are on a closed-loop system.

Stimulated C-peptide is a good surrogate marker, and different levels may indicate clinically meaningful benefit, including potential reduction of hypoglycemia (200 pmol/L), elimination of SHEs (450 pmol/L), and insulin independence (750 pmol/L). In islet transplant recipients, CGM metrics improve as C-peptide levels increase, with or without insulin independence.

HbA1c provides a surrogate marker for long-term diabetes complications. Most diabetes care guidelines set an HbA1c target of 7%, but benefits increase when patients can safely achieve lower levels. Dr. Gaglia argued that islet replacement trials should enroll patients with lower A1c and target lower levels for treatment. Patients with lower A1c levels would have a more conducive (i.e., less hyperglycemic) environment for islet products to survive and function. Data from transplantation trials show that insulin processing is better in recipients with lower A1c and that insulin independence rates improve with lower baseline A1c. Thus, people with good A1c levels may benefit the most from these therapies, have lower risk for surgical complications and provide the best environment to test the efficacy of an islet product.

Dr. Gaglia advocated that islet replacement trials should avoid enrollment of patients with high A1c levels and that all potential participants should be put on a closed loop system to improve glycemic control before any study.

Using CGM provides more information than A1c, such as time in range (TIR), which correlates with lower risk of microvascular complications. Dr. Gaglia proposed targeting TIR in future trials.

To define insulin independence, Dr. Gaglia would target an A1c <6.5% or even <6% to promote normal beta cell function, among other criteria. Further, he suggested that the community discuss and decide criteria for remission of type 1 diabetes that could be targeted in future trials, similar to the criteria for remission of type 2 diabetes after bariatric surgery that are in development.

Manasi Sinha Jaiman, MD, MPH, ViaCyte

How to Detect Success in Early Clinical Development in Stem Cell Therapy

ViaCyte's goal is to offer replacement endogenous hormone production by achieving homeostatic glucose control. C-peptide and histology are early markers of success. A1c is another key marker, but it does not reflect daily glycemic variability. Evolving CGM technology can complement A1c as it is more widely available, is more accurate, and provides more data, including on variability, excursions, and TIR.

An international consensus recommendation stated that TIR as a metric of glycemic control provides more actionable information than A1c alone. Thus, CGM TIR data can complement A1c in clinical trials and clinical decision making. Increased uptake of CGM and closed loop technology will reduce the patient pool for clinical trials that enroll patients with SHEs. The field must consider what other metrics are important inclusion/exclusion factors and how to monitor and measure success in clinical trials.

The past decade has seen an exponential increase in CGM accuracy to the point that some now mimic SMBG accuracy. The 2009 FDA guidance on allogeneic pancreatic islet cell products was issued at a time before CGM metrics could provide accurate information on daily glycemic variability. The 2012 FDA guidance on applications for artificial pancreas device systems (APDS) included endpoints related to patient outcomes and surrogate markers for meaningful clinical outcomes. The use of CGM was indicated as being appropriate and acceptable for these endpoints. The guidance recognized that data on tight control of glycemic variation can only come from real-time monitoring.

ViaCyte is developing an implantable medical device containing fully encapsulated cells that secrete insulin and glucagon. In their PEC-Direct clinical trial, CGM metrics were used to demonstrate tightening of glycemic control and increased TIR as exogenous insulin dosing decreased.

Dr. Jaiman concluded that CGM technology provides robust, clinically meaningful endpoints beyond A1c. Having consensus on CGM endpoints is critical for developing and advancing cell therapy products.

Roger J. Lewis, MD, PhD, Berry Consultants

Rationale for an Adaptive Platform Clinical Trial for Accelerating Development of Beta Cell Replacement Therapies

Adaptive clinical trials are designed to take advantage of accumulating information by allowing modification of key trial elements (e.g., randomization, sampling rules) according to predefined criteria. This strategy mitigates risks of failure that are specific to a particular clinical development program. A platform trial evaluates multiple treatments for a group of diseases (or subcategories of a disease) and is intended to continue beyond the evaluation of any individual treatment. Adaptive trials rely on criteria for stopping arms for harm, futility, or demonstrated efficacy and rules for revising randomization ratios among the remaining arms to continue data collection. All approaches being tested are not necessarily available at all times or in all centers.

A challenge to beta cell replacement trials is that a stepwise approach will be slow and expensive. A potential solution is a seamless platform trial with the capability to change enrolled populations (e.g., with/without organ transplant, with/without SHEs) and available treatment arms over time, including the capability to draw separate conclusions about risk:benefit by subpopulation.

Once an effective therapy becomes available, subsequent progress may require controlled trials. A solution to this problem is to begin a platform trial with only one factor in the beta cell domain (i.e., a single arm strategy against an objective criterion), but allow the seamless addition of new factors or approaches within the domain once another option becomes worthy of evaluation, with a traditional randomized comparison.

There are multiple type 1 diabetes populations with potentially different optimal treatment strategies. An approach that begins with the neediest or easiest to treat population (i.e., those who already require immunosuppression) makes sense, but a design that has the built-in capability to expand the trial to additional population subtypes as the risk:benefit changes would decrease development time.

Testing many different immunosuppressive regiments, different cell sources or preparation methods, and protection/encapsulation methods is not feasible with a limited sample size. Using response-adaptive randomization to shift available patient resources to the combination of factors that appear most promising would be more efficient.

Using longitudinal modeling can leverage early information from proximate outcomes (e.g., time since last SHE, changes in A1c) to predict long-term outcomes and improve interim decision-making or speed trial completion.

Dr. Lewis discussed a potential platform design to increase the efficiency of beta cell replacement programs, testing various options for cell source, immune suppression regimen, or patient population.

Discussion

Q (C. Ricordi): Previously, the FDA stated that insulin independence is not a sufficient endpoint for trials. And, children with type 1 diabetes potentially lose decades of life, which could indicate an unmet need for type 1 diabetes—that is, insulin therapy is not enough. Why is SHE still required for islet transplant trials? Why is insulin independence required?

M. Rickels: Insulin independence is clearly attributed to the cell product, whereas better glycemic control could be attributed to better management, rather than clearly to the intervention.

P. Beaston: Every product will have an individual benefit:risk assessment. To date, for islet cell products that require immunosuppression, the patient population that would have a reasonable benefit:risk is those who are unable to achieve glycemic control because of recurrent hypoglycemia. Insulin independence is a very clear-cut endpoint and raises the expectation that a patient would not have SHEs. A sponsor could propose another reasonable endpoint. If patients could use only basal insulin with no more hypoglycemic events, that could be compelling. Currently, there is no clear understanding of a clinically meaningful reduction in insulin dose, because so many factors impact insulin dose.

Q (Jose Oberholzer): What does the field need to do for the FDA to change their stance on CGM criteria for enrollment and as an endpoint in clinical trials?

P. Beaston: CGMs have advanced significantly but they remain inadequate for regulatory decision making. While average error has improved, the accuracy of CGM devices and blood glucose meters is very poor in the hypoglycemic range. CGM data could be supportive, but more information is needed, depending on the type of CGM. A group of sponsors could approach the FDA with their data to discuss their approaches and the potential for using CGM for enrollment. The decision comes down to product, risk, and population.

Q: A1c as an enrollment criterion—the recommendation is that it be low for enrollment, but it is not a criterion for pancreas transplantation. The potential complications are real, but there is interest in increasing the A1c threshold for enrollment because the current value excludes many patients who could benefit.

J. Gaglia: Many trials use a lower A1c limit of 7%, because that is the ADA goal for diabetes therapy. He was arguing that below 7%, there is much room for patients to benefit from islet transplantation, perhaps even more than those at 7% or above. With regard to an upper limit, the comparison to pancreas transplantation is difficult because that procedure works immediately to control glucose. He suggests that for a therapy that does not work immediately, glucose control should be as good as possible to improve the likelihood of success and reduce surgical risk, but he does not advocate for an upper A1c limit.

T. Eggerman: At the peri-transplant and immediate post-transplant periods, the beta cells are under significant stress. Having good glucose control would likely result in better transplantation efficiency and longer duration of grafts or devices.

M. Jaiman: For ViaCyte, A1c <10% is required to optimize success from a surgical standpoint, but there is no lower limit. The type of cell delivery system will determine the optimal range.

Q: What in addition to C-peptide is sufficient to attribute efficacy to the cell product (e.g., change in A1c, improvement in glycemic control)? Are there recommendations for what supportive metrics in addition to C-peptide are required?

P. Beaston: C-peptide is not an efficacy endpoint for outcomes; it demonstrates that islets are able to provide endogenous insulin, but there are no outcome studies for C-peptide values. Trials need to demonstrate a clinically meaningful change that is attributable to the product. Many endpoints, such as insulin dose and A1c, are heavily influenced by subject selection, subject behavior, treatment guidance, etc. The FDA has been proposing an adaptive approach to many sponsors. They would like sponsors to start with patients for whom it is easy to make a benefit:risk assessment and then expand the population as information is gained on safety and potential efficacy and as risks are better understood. They do not want people in trials with severe hyperglycemia, because glucose toxicity would not be favorable for transplanted islets, or those are already on their glycemic target, who are not the best first

candidates. The initial patient population should have room to improve and have something to gain, such as reduction in SHE.

Q: Are there any insights in how commercial entities can successfully participate in platform trials?

R. Lewis: An example is the REMAP-CAP platform trial for hospitalized patients with COVID-19, which had hundreds of sites around the world and multiple sponsors. Commercial entities come to this trial with an asset to test in order to decrease their time to first patient in or gain access to sites that they might otherwise have difficulty accessing. They put in a proposal and work with sites of interest to develop a clinical trial and data management/data sharing agreement. Regulatory, consent, and procedural issues are worked out much quicker in the context of an ongoing trial than when starting from scratch.

Complicated issues regarding data access may arise when the commercial entity wants to submit data for regulatory review. For example, control patients will include patients who received randomized treatments in other domains that are not relevant to that entity's product. Those unblinded treatment assignments are not released to commercial sponsors, so firewall and honest broker issues must be resolved so that a complete regulatory package can be delivered without compromising the integrity of other comparisons being investigated in the trial. Some SOPs and questions are available to help commercial entities decide whether the trial is a good/viable path for them.

Session Two: Incorporating the Patient Perspective

Moderator: Marjana Marinac, Pharm D, JDRF

Anna Casu, MD, AdventHealth

Clinical Perspective on Unmet Needs for Type 1 Diabetes

Dr. Casu is an endocrinologist and has had type 1 diabetes since 1978. She conducts research with preclinical models of islet transplantation and previously led a islet transplantation program in Sicily. Today's insulin therapy is not perfect—even when HbA1c is <7%, mortality is still twice as high among people with type 1 diabetes and is worse in females than males. Data from the T1D Exchange Registry show that glycemic goals are not being met despite the increased use of new technologies. Hypoglycemia and hypoglycemia unawareness remain relevant and are especially difficult to control in older people. Diabetes treatment is a 24/7 burden, and it is not easy to stay on top of it and accomplish good control. Patients often over- or underestimate their insulin boluses based on the nutrient content of meals; however, glucose excursions are comparable between patients who estimated correctly versus those who did not.

Anne Lacey, patient panelist

Patient Perspective on Transplantation

Ms. Lacey was diagnosed with type 1 diabetes in 1968 and has a child with type 1 diabetes. She underwent islet transplantation in 2001, briefly achieving insulin independence, and she had a whole pancreas transplant in 2008. Prior to the islet transplant, Ms. Lacey had severe hypoglycemia unawareness and retinopathy and was beginning to exhibit microalbuminuria. These increasing complications led to a car accident, and she frequently experienced confusion between her own diabetes treatment and that of her young son, putting both of them at risk. For her, the potential benefits of enrolling in a clinical trial for islet transplantation far outweighed the risks of doing nothing and continuing her trajectory with type 1 diabetes.

Kyle Rose, patient panelist

Patient Perspective on the Future of Transplantation

Mr. Rose is a medical device consultant who has had type 1 diabetes for 26 years. He is on the patient advisory committee for the INNODIA consortium. Mr. Rose described how mentally and physically exhausting daily diabetes management can be. Life is not a controlled environment, and hypoglycemia is a constant concern for him, especially when caring for his young child. He has found type 1 diabetes to be very invasive for many aspects of everyday life.

Discussion

Q: How do type 1 diabetes patients weigh the risks and benefits of being in a clinical trial?

A. Lacey: Living with type 1 diabetes ruled every moment of every day. Comparing this reality with the risks of immunosuppression made her decision easy. Since her pancreas transplant, she does not get sick, does not get infections, and her HbA1c has never been >5.4%. The importance of these therapies for people living with the considerable daily risks of type 1 diabetes was indisputable.

Q: How can the risks and benefits best be communicated to patients considering future trials?

A. Casu: Recently diagnosed patients are interested but are not currently eligible for islet transplantation. Support is needed from behaviorist and psychologists who are trained in transplantation care; these professionals are critical to helping patients and the medical community understand the risks of immunosuppression. Long conversations with patients are needed to discuss their burden, the risks of diabetes versus the risks of therapies, and potential outcomes from participating in trials. Physicians need to identify and monitor measures, e.g., HbA1c level or TIR, that would have a significant impact on patient quality of life.

Q: What information or benefits would patients look for in a potential beta cell replacement trial? What risks would patients want to know about and understand?

K. Rose: He experiences substantial risk every day with insulin dosing. Given that, he is open to more risk from participating in a clinical trial. He is looking for more options, especially for hypoglycemia prevention and reduction of the mental health burden of glucose excursions. He is open to exploring new options through clinical trials because he is not happy with the current state of diabetes treatment.

Q: What are the gaps in patient-reported outcomes (PRO) instruments?

K. Rose: Sleep is not asked about enough. The toll on mental health should be better documented, especially as related to productivity issues and daily activities.

Q (Ron Gill): How patients view risk:benefit tolerance is entirely different than the professional community. How can this perspective be brought to the field in a meaningful way?

A. Casu: Behavioral components are not quantitated sufficiently. What level of mental health burden is functional versus nonfunctional for patients? For the regulatory agencies, the transplantation field needs to develop measurable elements to aid in balancing physical and mental risks.

Audience comment (Camillo Ricordi): The field needs to rethink outdated rules for inclusion in islet transplantation trials. Thousands of patients must be screened for SHE, but a great medical need also exists for patients who have HbA1c <7% and glycemic variability.

Q (Jose Oberholzer): Are enough whole pancreas transplants being performed?

A. Lacey: A source of sufficient organs is a problem. Looking at cell sources will move the field forward.

A. Casu: Many potential patients are not referred for pancreas transplantation, so organs are not used. She would advocate for cell/islet transplantation, which has fewer risks than pancreas transplantation.

Q (online): A survey of 400 Dutch patients showed a surprising level of risk they were willing to take. How can more information be gathered on risks that patients are willing to take on?

K. Rose: His experiences with chronic diabetes management compel him to take on risks if the potential benefits could ease that burden even for a short time. He expects that many patients would be eager to enroll in such trials.

Session Three: Identifying Optimal Cell Source, Composition, and Testing

Moderators: Jon Odorico, MD, University of Wisconsin; Gopika Nair, PhD, EndoCrin Bio

M. Cristina Nostro, PhD, McEwan Stem Cell Institute, University of Toronto

Leveraging Vascularization Strategies to Improve Beta Cell Replacement Therapies

Islet transplantation has been limited by poor engraftment, rejection, inconsistency in islet preparation, requirement for immunosuppression, and declining insulin independence with time. Potential solutions include providing a fast connection with the host vasculature, manufacturing surrogate islets from human pluripotent stem cells to develop a consistent product, and developing hypoimmunogenic islets.

Dr. Nostro used a protocol to transplant pancreatic progenitors into immunocompromised mice to generate all multiple endocrine and exocrine cell types. She next isolated microvessel fragments from adipose tissue for transplantation with the pancreatic progenitors or human islets. The microvessels created functional vascular connections within one week post-transplant and reduced apoptosis in the graft. Vascularized grafts lowered glycemia faster than a control group of pancreatic progenitors transplanted without the microvessels (7 weeks versus 4–5 months). The microvessels improved engraftment and accelerated beta cell function, and they reduced the number of human islets needed to normalize hyperglycemia in mice when transplanted in the subcutaneous space.

Jeffrey Millman, PhD, Washington University

Stem Cell-Derived Islets: Status and Challenges

Stem cells offer a potentially renewable source of well-characterized, high-quality replacement cells for diabetes. Human embryonic stem cells can be differentiated by a month-long, 6-stage process into cells that fulfill in vitro characteristics of beta cells. These stem cell-derived islet cells (SC-islets) normalize blood glucose after transplantation into immunodeficient mice.

A challenge with SC-islets is cell line variability in differentiation that affects both composition and function. Each cell line requires some adjustment to an early stage of the differentiation protocol. The protocol also results in variability in the production of non-beta-like cells in the final product. Dr. Millman is working with ARMI (Advanced Regenerative Manufacturing Institute) to automate the differentiation process and minimize batch variation due to human intervention. Cell line variability depends on which protocol is used, i.e., differentiation of some cell lines is more or less effective with different protocols. Some cell lines are difficult to grow in suspension. A final issue is that SC-islets are immature and are not as functional in vitro as primary isolated cadaveric islets. Functional and transcriptional maturation occurs after transplantation, suggesting that maturation could be stimulated in vitro if SC-islets are given the correct molecular cues.

Addressing these challenges with SC-islets will improve manufacturability, decrease costs, and increase speed to the clinic.

Maike Sander, MD, University of California San Diego

Improving Stem Cell-Derived Islets Using Regulatory Maps From Single-Cell Genomics

Dr. Sander's stem cell (SC) differentiation protocol results in mostly endocrine pancreatic-type cells in proportions that approximate those in primary islets. Some endocrine-progenitor cells remain at the end of the differentiation process.

After differentiation, enterochromaffin (EC)-like cells are found that resemble intestinal endocrine cells. What are these cells? And, do they need to be removed or depleted for transplantation protocols or can they be further developed into beta cells? The SC-EC population decreases significantly after transplantation into mice. Dr. Sander proposed that purification protocols to isolate SC-beta cells would add another layer of difficulty to development of a cell replacement product. Current mixed populations of SC-derived cells may be a more functional cell product than a pure population of beta cells, due to paracrine actions.

SC-beta cells differ from primary beta cells in that they fail to activate beta cell maturation signals. In vitro, the SC-beta cells are not exposed to immune signals or nutrient signals as they would be in vivo; these pathways could account in some way for the failure to mature before transplantation. In addition, SC-beta cells hypersecrete insulin in response to pyruvate, which could increase risk of hypoglycemia.

Dr. Sander has a detailed roadmap of transcription factors that are needed to derive SC-beta cells. It may be possible to use RNA programming to program a specific lineage and influence cell production to be more homogeneous.

Holger Russ, PhD, University of Colorado

Senescent Pancreatic Beta Cells Marked by CD9 Increase Upon Transplantation and Display-Enriched Immunogenicity

Extended in vitro culture results in functional maturation of SC-beta cells, which present as heterogeneous cell subpopulations with distinct expression patterns.

A subpopulation of CD9+ SC-beta cells was identified that exhibits a senescence/senescence-associated secretory phenotype and is less glucose responsive. Senescent beta cells in vivo may contribute to progression of type 1 and type 2 diabetes. CD9+ SC-beta cells exhibit increased immunogenicity due to increased HLA class I expression with activation of CD8+ T cells and stimulation of multiple T cell receptors. Transplantation leads to increased levels of CD9+ SC-beta cells.

Questions that remain include: What are the mechanisms resulting in differential immunogenicity of in SC-beta cells? Which SC-beta cell subpopulation is ideal for cell therapy, i.e., immature cells that can better withstand stress or mature cells that functions sooner? Can we improve on generation of SC-beta cells or subtypes? How plastic are SC-beta cells? What mechanisms trigger phenotypic changes and are those changes permanent? How can the SC-beta cell phenotype be preserved after transplantation?

Discussion

Q (Gopika Nair): Why do SC cell lines behave so differently?

J. Millman: One explanation could be different levels of endogenous signaling among cell lines. Also, characteristics of how the lines grow as stem cells (e.g., proliferation rates, compaction, etc.) affects how receptive they are to differentiation signals.

H. Russ: Key morphogens are differentially expressed at the pluripotent stage across lines. Early stages are key for setting up success for differentiation. Cells that do not differentiate still secrete factors into the culture media that could compete with factors added for the protocol and influence differentiation of other cells in the population.

Q: Source of microvessels: Islets in the liver are protected because they have been revascularized. Are the microvessels recipient-derived?

C. Nostro: Autologous microvessels can be obtained through liposuction, frozen, and thawed. Thus, microvessels could be collected in advance and transplanted when islets become available.

Q (Felicia Pagliuca): Are there differences in senescence among cadaveric islet donors? Is this unique to beta cells?

H. Russ: Senescence seems to be upregulated in experimental models compared to cadaveric islets. He has not looked at senescence in non-beta cell types.

Q (Klearchos Papas): How important is glucagon release by alpha cells in vitro and in vivo?

M. Sander: Stem cell studies have not looked at glucagon secretion after transplantation in mice, so this has not been functionally characterized.

J. Millman: He does not know the answer to this, but having some alpha cells may be important. Unpublished data show some glucose-stimulated glucagon secretion in SC-derived mixed populations. SC-alpha cells persist after transplantation, but a good ELISA to distinguish mouse from human glucagon is not available.

Q (Ron Gill): Donor versus host-derived endothelium could be critical if host cells recapitulate autoimmunity. There is a need to test both.

C. Nostro: In the future, she wants to use hypoimmunogenic SC-beta cells.

Q (Lonnie Shea): For clinical safety and efficacy, how important is it to characterize other cells in a stem cell-derived mix? Will those other cells preclude use in humans?

J. Millman: As long as the preparation meets purity standards for endocrine cells, he has not seen anything that would raise a safety issue. Other cells in the preparation do not seem inherently problematic for safety. It will be impossible to characterize every cell in the mixed populations, and for the first generation products, perfect cell compositions may not be necessary.

Q: Microvessels may have pericytes. Do they recruit mouse cells? This may be critical for scaling up the technology.

C. Nostro: At 14 weeks post-transplant, rat microvessel cells persist in the graft along with recruited mouse cells; those data are being evaluated further.

Q (Joe Zhou): Is there a path forward for an iPSC approach to patient-specific transplantation?

J. Millman: In type 1 diabetes, autoimmunity is an issue. From a manufacturability perspective, it would be difficult to do patient-specific transplantation on a large scale for the entire type 1 diabetes population, although it could be done on a one-by-one basis. The field would need a universal (or small number of) differentiation protocol(s). Costs would be high for one cell line per person, and quality and other practical challenges would need to be addressed.

Q (Esther Latres): Has senescence linked to immunogenicity been described in other diseases or cells?

H. Russ: In NOD mice, ablation of senescent cells blocks development of type 1 diabetes. Senescence occurs in cadaveric islet transplantation, which could be an interesting model to study the basis for autoimmunity.

Q (Vincenzino Cirulli): A common theme is squeezing the differentiation process into a few weeks when it normally takes 40+ weeks during human development. Is anyone comparing scRNAseq data between distinct stages of human fetal pancreatic development and SC-derived islet cells?

M. Sander: SC-islet function improves over time, but it is not clear how to speed it up. SC-islet/beta cells have not been carefully compared to fetal cells.

Q: Is there an assay to predict performance or variation in the differentiation efficiency of cell lines?

J. Millman: Not currently. Lines must go through the entire process in order to assess efficiency.

Q: Given studies showing that nutrient signals can affect beta cell maturation, can that be mimicked to induce maturation of SC-beta cells?

M. Sander: In animals, beta cell maturation occurs postnatally, driven by diet (fasting/feeding) and circadian cues. This is difficult to do in a research setting, and it may be an issue of duration.

H. Russ: We need to know: What is the ideal starting material for cell therapy? Do more immature cells better withstand the stress of transplantation?

Audience Comment (Camillo Ricordi): The field needs to be extremely careful about safety. Lessons were learned from beta cell composition in cadaveric islet transplantation about what variations in islet composition can be tolerated. The science may be good enough now to learn what happens with transplantation of stem cell-derived islets. Islet cells have much plasticity, but there are challenges with uniformity in manufacturing.

J. Millman: He agrees that every detail of cell composition does not need to be known, but there are theoretical risks to the artificial tissue that is being created. A definition of a fully differentiated cell has not been universally agreed. Scalability amplifies these issues—the current scale of manufacturing works well for a phase 1/2 trial, but it may not be sufficient for the entire type 1 diabetes population.

Session Four: Gene Editing of Cell Sources

Moderator: Ron Gill, PhD, University of Colorado

Stephan Kissler, PhD, Joslin Diabetes Center

Hiding in Plain Sight: A Survival Guide for Beta Cells

The optimal gene editing strategy for beta cell replacement will depend on the cell source—autologous, allogeneic, or xenogeneic. Dr. Kissler advocated for the long-term goal of autologous cell replacement, as the other two pathways introduce a new problem of transplant rejection on top of autoimmunity.

MHC I deletion can prevent islet alloimmune rejection but not autoimmunity. CD8 T cells are not required for beta cell killing. Beta cells can be killed by CD4 T cells, and beta cells do not have to express MHC II to be killed. Thus, neither MHC I nor II is needed on the beta cell for autoimmune destruction. Hypoimmune strategies that rely on knocking out MHC I/II are probably not sufficient.

The mechanisms of beta cell killing during autoimmunity are not clear, so we do not know what pathway(s) beta cells need to be protected against. It is also not clear why beta cells are targeted by the immune system in the first place. Evidence suggests that the beta cells, through dysfunction or stress, might shape the autoimmune response, which could point to an opportunity for intervention by changing the beta cells to avoid immune killing.

Dr. Kissler used an unbiased approach to identify protective gene edits. One result that stood out is the renalase (RNLS) gene, which is associated with type 1 diabetes risk. Renalase has no known immune modulatory role, but its deletion seems to protect beta cells from immune destruction. Further exploratory, creative approaches are needed to find solutions for diminishing beta cell immunogenicity.

Sonja Schrepfer, MD, PhD, Sana Biotechnology

Protecting Transplanted iPSC-Derived Islets From Immune Rejection Is the Key to Bringing Cell-Based Therapies to Patients

Dr. Schrepfer uses a gene editing approach to create a hypoimmune cell product and protect cells from alloimmune responses. This would allow a single "off-the-shelf" cell product that could be transplanted into any patient. The strategy is to disrupt MHC I and II expression and overexpress CD47. Hypoimmune iPS cells survive and proliferate in allogeneic sensitized NHPs without immunosuppression. Hypoimmune iPSC-derived islet cells survive and function in diabetic humanized mice. Those cells do not trigger T or B cell immunogenicity or innate immune responses.

Cellular transplantation without immunosuppression appears to be achievable using hypoimmune cells.

Audrey Parent, PhD, University of California San Francisco

Selective Deletion of Human Leukocyte Antigens as a Strategy to Protect Stem Cell-Derived Beta Cells From Immune Rejection

Immunologic barriers to cell replacement therapy include: mismatches of HLAs, NK-mediated rejection (missing self), and beta cell-specific immune rejection. Dr. Parent explores a strategy of selective deletion of HLAs, specifically those that are more variable/more difficult to match between donor and recipient. More common alleles are retained to facilitate matching with the host.

Functional iPSC-beta cells can be derived efficiently from immunoengineered clones. Dr. Parent showed that selective deletion of HLA alleles can significantly reduce NK and allogeneic T cell-mediated rejection of SC-beta cells, while maintaining the cells' capability for immune surveillance through the retained HLA allele. The downside of the strategy is that the cells cannot be used universally; multiple cell lines with different retained HLA alleles would be needed for compatibility with more people. In addition, the cells are more immunogenic than those with complete HLA deletion because they can still present some beta cell antigens due to the retained allele. New preclinical models are needed to assess the impact of these genetic modifications on autoimmune responses.

Michele Youd, PhD, eGenesis Bio

Gene Edited Porcine Islets for Beta Cell Replacement

Dr. Youd uses CRISPR for gene editing of porcine cells that are used to create porcine donors for solid organ and tissue transplantation. Issues being addressed with editing strategies (either deletion of genes or insertion of human transgenes) include retroviral risk, hyperacute rejection, vascular injury (e.g., coagulation, complement system), and chronic rejection. eGenesis has created a reproducible and consistent neonatal porcine islet (NPI) product that is viable and functional in vitro and in vivo; on-target effects of gene editing have been verified by comparison to wildtype neonatal porcine islets.

Challenges with NPI transplantation include: determining the optimal dose for human trials given the need for *in vivo* maturation; minimizing loss and improving engraftment; evaluating the durability of transgene expression in NPIs in the liver post-transplant; defining the best immunosuppression regimen; optimizing genetic edits to protect the NPIs in vivo; determining whether NPI retrievability will be a focus for regulators if NPIs are delivered without encapsulation; evaluating susceptibility to autoimmune attack; and scaling pig production and NPI generation.

Discussion

Q (Ron Gill): There is a need to prevent pathways that are not well defined. Different immune responses are being jumbled together. How well can you assess whether products are protected from these pathways?

S. Kissler: The field's biggest challenge is to develop better humanized models to test these issues.

M. Youd: The xeno field relies on in vitro assays as much as possible, but better assays are needed to address these questions.

A. Parent: We need to simplify the questions before getting all of the players together.

S. Schrepfer: Each model has limitations. Studying full allo- and autoimmune issues is difficult with the current options and new models are needed.

Q: Is immune surveillance a problem in the hypoimmunogenic setting (e.g., neoplasia)?

A. Parent: This is definitely a concern for hypoimmune populations if cells overproliferate over time. Strategies to address this issue may include a suicide gene that could be turned on.

S. Schrepfer: Many strategies are possible using the immune system to clear the cells, e.g., block with a CD47 antibody to restore innate immune responses. It is important in islet transplantation to be able to control the cells.

Audience Comment: The field can create many models, but they may never fully reflect human physiology. We need to think about when to start clinical trials to learn about immune responses to these products in humans.

Q: *How are CD47 and renalase regulating immunity*?

S. Schrepfer: CD47 is expressed on all subpopulations of primary NK cells and can be upregulated when the cells are stimulated. NK cell lines do not express CD47.

Q (Yue Wang): What about pathogens with porcine trophism?

M. Youd: pCMV may have affected the recent porcine heart transplant; monitoring plans are in place to generate pCMV-deficient animals.

Q: In iPS cells, genomic imprinting on oncogenes is erased, and follow-on effects are complex.

A. Parent: That is an interesting point—can that problem be solved? Can you stimulate the immune system to eliminate the proliferating cells?

Q: We need a human model system for oncogenesis studies on iPS cells.

R. Gill: The potential neoplasia issue as not been well addressed; it is undefined.

S. Schrepfer: The field needs to focus on methods to control the cells (e.g., on/off switches, etc.). More work will be coming out in the next few years.

A. Parent: It may be possible to uncouple some signals and put them on a scaffold that could be physically removed if necessary.

Q: Can the combination of immune approaches with the anti-CD3 model cause exhaustion of T cells?

M. Youd: anti-CD3 has been used in many patients, so there is much clinical data. Targeting T cells is interesting for xenotransplantation, but more data and model systems are needed to convince the company that the pathway is worth pursuing.

R. Gill: Combination therapies will be important. Disarming direct MHC targeting of the beta cell surface may not block all pathways to killing beta cells, but it could be combined with other approaches. Gene therapy to target beta cell survival (e.g., ER stress, free radical processing, etc.) could be combined with complementary strategies.

S. Kissler: We do not have to do everything on the beta cell side, and combination of approaches could be useful. The drawback is that it may complicate regulatory issues and clinical trials.

Session Five: Preclinical Considerations for Translating Beta Cell Replacement Approaches to Clinical Testing

Moderators: Julia Greenstein, PhD, Life Science Advisors; Klearchos Papas, PhD, University of Arizona

Jose Oberholzer, MD, University of Virginia

Preclinical Considerations for Translating Beta Cell Replacement Approaches to Clinical Testing

Dr. Oberholzer discussed preclinical studies testing islet products in NHPs. This model is closest to humans, but it is by definition a xenogeneic model. NHPs do not have islet autoimmunity. Clinical islet transplantation protocols allow prevention of rejection of human islets or SC-islets for 30 days, which limits safety studies. NHPs have a significantly higher ratio of insulin per kg bodyweight needs than humans, which limits the feasibility of efficacy studies. Human islet transplantation rarely achieves insulin independence in NHPs. Similar issues are relevant to testing biomaterials, e.g., for encapsulation, in NHP models. Other animal models are needed to cover all efficacy and safety testing.

Possible tactics to move the field forward include: first generation islet transplantation using cadaveric islets with immunosuppression; second generation islet transplantation using encapsulated cadaveric islets without immunosuppression or a renewable cell source with immunosuppression; and third generation islet transplantations with an encapsulated renewable cell source.

Norma Kenyon, PhD, University of Miami

Dr. Kenyon addressed translational considerations related to: the animal model (e.g., mouse, pig, NHP); what is being tested (e.g., cell product, gene therapy, immune intervention, device, implant site); capsules/coating/scaffolds/devices (e.g., biocompatibility, fibrosis, vascularization, nutrient and oxygen delivery, insulin delivery); type of insulin-producing cell product (e.g., allo/xeno/stem cell-derived, adult or neonatal, reproducibility and potency, impact of implant site); type of immune intervention (local or systemic, effect on IPC function, risk of infection); immune monitoring; and scalability.

Results from mouse models enable selection of promising approaches. NHP models offer additional advantages, such as heterologous immunity, ability to test new immune intervention drugs or biologics and biocompatibility of materials and devices intended for human use, and the time course to rejection, changes in metabolic control, and immune-mediated events are similar to humans. Disadvantages are that NHPs are expensive and do not model islet autoimmunity. In addition, adverse events seen in clinical trials might not always manifest in NHP.

David Scharp, MD, Prodo Lab

Delivering Large-Scale Human and Rodent Islets to Global Academic and Corporate Investigators for Diabetes Research

Prodo Labs has a nine step human islet processing procedure for isolating islets for global researchers, including detailed criteria for acceptance of human organ donors. Cause of death and donor race do not significantly impact islet recovery. Increasing cold ischemia time to ≥11 hours reduces islet yields. Increased BMI leads to lower islet recovery. Post-culture islet recovery increases with donor age.

The lab is developing a polymer, light-based crosslinking formulation that can be placed uniformly around human islets using a light box that permits continuous mixing of human islets during the encapsulation process. This results in minimal encapsulating polymer volumes. Variables, such as the size and number of islets within the capsules, can be controlled in this process. Islets encapsulated by this method can correct glucose control when transplanted into immune-deficient mice.

Kate Dabirsiaghi, VMD, FDA

A common theme in CBER's preclinical review of INDs and other submissions is that no one size fits all. They have a flexible approach to reviewing all products. There is considerable diversity among beta cell replacement products, and CBER review depends on product-specific factors, such as cell source, cell composition, route of administration, proposed patient population, encapsulation status, etc.

Discussion

Q (Julia Greenstein): How can we build a set of models that would convince us that a product is worth testing in humans? *Is it possible that something that fails in a mouse model might still be promising for humans*?

N. Kenyon: Some products have failed in mice but work in humans. The issue is that it will be difficult to test every novel immune intervention agent in a large animal model without at least trying it in a mouse.

J. Oberholzer: It would be difficult to get any novel agent through an animal care committee without proof-of-concept in a mouse model or very strong in vitro data.

N. Kenyon: An exception could be certain devices that cannot be feasibly tested in mice.

J. Oberholzer: The question of building a set of models is critical. He would volunteer for a panel to talk to the FDA on guidance to industry that would facilitate a regulatory pathway in the field.

Q (Michael Rickels): In the NHP model for efficacy testing, IEQ/kg can be much higher, but if an unlimited number of cells are available to test, is it possible to assess proof-of-concept of efficacy for cell-derived beta cell sources?

J. Oberholzer: The goal of preclinical testing is to obtain IND approval; the risk of testing unlimited numbers of cells is that it may compromise the safety profile. He would do efficacy testing in immunocompromised rodent models.

N. Kenyon: It would be possible to do higher dosing for efficacy. The animals might not achieve insulin independence, but there could be effects on C-peptide production or long-term survival of islets.

J. Oberholzer: That's the difference between proof-of-concept experiments and preclinical IND data.

Audience Comment (Ron Gill): It is important to have clarity on what models can be applied for which questions. Different models are critical for addressing specific questions. For example, mice are needed to see how systems work in the autoimmune setting but are not useful for device development. Whereas large animal models are important for addressing biomaterial compatibility, scaling, etc.

Audience Comment (Camillo Ricordi): NHP models are critically important for proof-of-concept studies, but there are reservations about their usefulness for preclinical, IND-enabling data.

J. Oberholzer: In an ideal world, the field would not use NHPs, but in some situations, it is not feasible to go straight into humans for safety considerations.

K. Dabirsiaghi: The FDA's recommendations are flexible and made on a case-by-case basis; there is no default need for NHP or for models from two species. Safety and efficacy issues should be based on appropriate models with scientific justification. She encouraged sponsors and investigators to set up an INTERACT meeting to obtain informal, nonbinding feedback on new products. In addition, pre-IND meetings provide formal, nonbinding guidance on supporting data that would be needed for an IND for a new product. Those are the best opportunities to understand the FDA's expectations and concerns about specific products and discuss the design of pivotal IND-enabling studies.

Q (Klearchos Papas): Are there any thoughts about examining encapsulated products prior to transplantation? What can be done pre-transplant to optimize in vivo testing of these products?

D. Scharp: He advised learning as much as possible about encapsulation capacity in vitro before moving into animal models.

K. Papas: Investigators also need to think about potency and viability of the encapsulated cells before animal model testing.

Q (Cherie Stabler): Animal models are important tools, but in vitro assays (e.g., for islet potency or immunologic responses) have been used in place of in vivo testing and found to be predictive. *Is this valuable for investigators or from the FDA's perspective*?

J. Oberholzer: He agreed that in vitro assays are critical and should be used more often.

K. Dabirshiaghi: The FDA encourages looking at in vitro models in place of in vivo testing and/or using lower species over higher species when possible. In vitro assays must be qualified and validated and have appropriate scientific justification/rationale, especially for novel assays.

Session Six: Transplant Site Considerations and Strategies to Protect the Cells

Moderator: Cherie Stabler, PhD, University of Florida

Lonnie Shea, PhD, University of Michigan

Tunable Environments for Beta Cell Transplantation

Dr. Shea addressed the transplantation environment and the opportunity to influence beta cells after transplantation using biomaterials. A potential advantage of an open (i.e., non-encapsulated) system for beta cell transplantation is vascularization, which allows direct integration with the host. Challenges include inflammation and the adaptive immune response. SC-beta cells have the additional challenges related to the need to mature after implantation.

Dr. Shea is developing a microporous scaffold for extrahepatic transplantation, which provides an opportunity to regulate the microenvironment around transplanted islets and support engraftment and host integration. The extracellular matrix coating can provide signals to modulate inflammation and to replace the matrix that was lost during islet isolation. The platform supports the function of transplanted human islets and SC-beta cells. Localized delivery of proteins, such as exendin-4 or local presentation of Fas ligand, from the scaffold can reprogram the environment to support beta cell survival and function. Using the scaffold to cotransplant islets and Tregs results in long-term graft survival/function without immunosuppression. This scaffold technology would be a good candidate for testing in a flexible platform trial.

Alice Tomei, PhD, University of Miami

Conformal Coating of Insulin-Secreting Cells for Beta Cell Replacement Without Chronic Systemic Immunosuppression in Type 1 Diabetes

Dr. Tomei described the development of a conformal coating (Polyethylene glycol (PEG) hydrogel) that: conforms to islet diameter; minimizes delays in GSIS; allows transplantation in confined, well-vascularized sites, such as the omentum and is applicable to stem cell derived beta cells. Larger capsule technologies results in decreased islet diameter after transplantation and changes in islet composition, with an increase in alpha cells; conformal coating avoids these issues. The coating is permeable to glucose and insulin but not immunoglobulin. The mechanical properties of the coating are stable long-term. A limitation of the coating is biocompatibility in rat omentum, which elicits a strong foreign body response, unlike in mice which do not display this reaction.

Comparison of conformal coating with microencapsulation reveals several advantages of the coating. Nanomaterial-mediated targeted delivery of immunomodulatory drugs to the conformal coated graft was tested. The nanomaterials were taken up by immune cells, and drug release from the nanomaterials

was sustained and prolonged. The nanomaterials remained localized at the site of the graft or homed to the graft site when introduced intravenously.

Philip Toleikis, PhD, MSc, Sernova

Platform Approach: Early Functional Cure for Chronic Diseases

Dr. Toleikis described a platform that brings together three technologies. The cell pouch is an implantable medical device that promotes vascularization; it is made of a series of chambers, much like a scaffold. Second generation immune protection using conformal coating shields transplanted cells from immune attack to reduce or eliminate the need for immunosuppression. And the therapeutic cells reflect the transition from human donor cells to stem cell-generated insulin-producing cells.

The cell pouch has a series of plugs around which highly vascularized tissue will grow and create an organ-like environment; removing those plugs after 3 weeks leaves void spaces for inserting therapeutic cells. This platform exhibits no fibrosis, is scalable, accepts human donor cells or tissues or SC-islets, and is biocompatible with the natural cell environment.

A phase I/II study for safety and dosing was conducted at the University of Chicago with human islets. Sentinel devices removed after 90 days demonstrated cell survival and function. A 10-plug cell pouch device has been developed to increase the islet capacity of the system, while decreasing density, as a means to achieve insulin independence.

Sernova is partnering with Evotec for iPSC-beta cell technology to determine the ability of the cell pouch platform to deliver long-term insulin independence in mouse models and eventually in humans.

Omid Veiseh, PhD, Rice University

Developing Immunomodulatory and Vascularizing Microdevices

Dr. Veiseh is developing new materials that enable long-term viability and survival of grafted cells and a vascularized macrodevice that can minimized transplant volume for improved GSIS kinetics and nutrient delivery. Chemical modified alginates have been identified through HTS that eliminate fibrosis around the encapsulation materials. Cellular barcoding enables high-throughput materials screening in NHP model of the foreign body response, and lead analogs have been identified that enable survival of encapsulated cells. This advance allows encapsulation with higher islet density than in previous approaches.

He has created a device with degradable parts that allow growth of a vasculature within the device; 3D printing is being used to create a perfusable design with novel polymers and soft gels. Those gels can secrete factors that promote angiogenesis. Vascularization begins within 2 weeks, with vessel maturation within 8 weeks.

Melanie Graham, PhD, MPH, University of Minnesota

A Bioengineered Artificial Interstitium for Islet Cell Transplant Supports Long-Term Insulin Expression in Nonhuman Primates Without Immunosuppression

Dr. Graham's work addresses challenges with intraportal transplantation, using a dynamic approach to protecting islet grafts. A subcutaneous device allows accumulation of interstitial fluid and pumps that fluid through a cellhouse to create a noninvasive, retrievable, reloadable transplantation site. The potential for coagulation within the device is an important safety issue for devices that have perfusion with intravascular diffusion, which is not the case for this device (ultrafiltrate that lacks clotting factors).

In NHPs, the device supports long-term insulin expression without immunosuppression. Islet loss in some animals was not attributed to immune response but to response to hydrogels and shear stress

from the fluid environment. The ultrafiltrate accumulation disk attracts a foreign body response, which is beneficial for this device. This platform is biocompatible and safe, prevents hypoxia and has favorable mass transfer, is immunoprotective, supports unlimited islet sources, and can be easily reloaded, biopsied, or removed in a patient-friendly, out-patient procedure.

Discussion

Q (to O. Veiseh): Can your device, which seems to rely on diffusion through the nonbiodegradable layer, be scaled to human size? What site would be used for implantation?

O. Veiseh: The intraperitoneal space is under consideration for implantation. The device is modular, and different configurations are being explored. Eventually, the platform needs to fit 0.5 billion cells.

Q: What are the mechanisms to protect the cell pouch from the immune system?

P. Toleikis: The cell pouch is designed to protect beta cells, but it is not immune protected. The current clinical trial uses standard immunomodulatory drugs, but they are moving toward local immune protection within the chambers of the device. The goal is to have the cells sitting in a tissue matrix, which is a more natural environment.

Audience Comment: Islet and acinar endothelial cells are different. In all presentations, omental or adipose endothelial cells were used, but responses to Tregs, inflammation, etc. are completely different. Think about how to recapitulate a true islet endothelium.

O. Veiseh: The platform approach is agnostic to the type of endothelial cells that are used, so different cells could be tested within it.

C. Stabler: Islet vasculature and the potential lack of lymphatic drainage could be contributing to the autoimmune attack, so using different (non-islet) endothelial cells may be beneficial.

Q (Klearchos Papas): Regarding approaches to prevascularization and large-scale application of this technology, what are the considerations for having surgeons at different clinical sites deliver the device and, subsequently, the therapeutic cells?

P. Toleikis: They are developing a toolkit for surgeons to standardize how their device is utilized and how cells are implanted appropriately in terms of numbers and distribution across the device. The platform will be set up to be used identically any place in the world.

M. Graham: There is a 21-day wait time after implantation of the device she is developing. She is thinking about the process for standardizing the implantation and loading processes.

Q (Klearchos Papas): How will the consistency of loading islets or of viability and potency be evaluated?

C. Stabler: There is an issue of cell loss in the syringe.

A. Tomei: They are looking at the syringe to ensure that the product is delivered appropriately and using histology of cells in the device at different time points to be sure they know where the cells are

Q (Ron Gill to P. Toleikis): Have you considered using the already immunosuppressed IAK population? This is a large population that would benefit from this device and could provide useful proof of concept.

P. Toleikis: He is very interested in that population.

Q (Ron Gill): Reproducibility and longevity—how long can beta cells be expected to survive in an avascularized state?

A. Tomei: Once the immune isolation strategy is determined, they can ask this question for various products. Primary islets and SC-islets may have different needs. Can encapsulated islets still get the signals they need from soluble mediators.

R. Gill: This is an ideal application for genetic engineering of xeno or stem cells islets with a focus on pathways for islet survival and function (e.g., hypoxia, etc.).

L. Shea: Persistent function over the long term could be solved with an integrated approach with open and closed devices. With his approach, they seem to have good function, but how long can we protect islets/beta cells from the immune system.

Q (Simi Ahmed for A. Tomei): Have you compared the survival of nonencapsulated cell grafts plus targeted immune modulation versus the approach of immune isolation plus antifibrotic drugs? Which fairs better?

A. Tomei: She wants to look at this in the future.

O. Veiseh: Ultimately, combination approaches may be necessary to get the longest term islet function. He is excited about how some of the material properties can synergize to achieve different goals related to lack of fibrosis, immunoisolation, vascularization, and immunomodulation. These concepts may be able to come together in a device that is still simple enough for manufacturing and for regulatory purposes. That is the sweet spot for technology that has a clinical impact.

Q (Guillermo Arreaza-Rubin): What about innervation?

O. Veiseh: Some patients transplanted with the Edmonton protocol have been insulin independent out to 15 years. It is unclear whether the nervous system is playing a role.

Q (Paraish Misra): With alginate encapsulation and conformal coating, what happens when the islets vascularize? Do the blood vessels eventually cross through the barrier to communicate directly with the islets? If so, would that limit their immunoprotective properties?

O. Veiseh: In his device design, the materials are nondegradable, so the membrane will remain intact for the duration of the implant. They are building vessels around smaller, immune-isolating regions, so there is no contact between the islets and blood vessels.

A. Tomei: Blood vessels grow to the capsule surface but do not penetrate into the capsule.

Q (Cherie Stabler): Do endothelial cells left over from islet isolation become encapsulated? What happens with respect to vascularization if that occurs?

A. Tomei: They tried that experiment, but there were not many viable endothelial cells after transplantation.

Q (Wanxing Cui): What are the starting materials for the devices? Are islets cells or organs? And, vascularization and revascularization are different processes.

L. Shea: His material starts with a simple polymer, which are part of what sutures are commonly made of and the scaffold is a degradable platform that provides a temporary residence for the cells. The pore structure allows for cell in-growth; functional vessels are connecting through the transplanted islets. Several materials seem to work in different platforms, so the materials themselves do not seem to be directing the process.

A. Tomei: Islets would be considered mini-organs because different cell types have to work in a coordinated fashion, and changing the cell composition results in reduced metabolic control. Different stem cell products with various cellular compositions may have different functionality.

Audience member: The field needs to clarify the definitions of islets, stem cell-derived insulin-producing cells, and iPSC-derived islet-like cells, because these are all different and may have different needs and outcomes in the various platforms.

Q (Esther Latres): As human data are obtained regarding biomaterials, how reliable or predictive have data from rodents and NHPs been?

P. Toleikis: The device was designed to be highly scalable from mice to humans. The important biological parameters, such as pore size, the diameter of the device and plug, are identical across the devices for different species. The polymers used in the device are as simple as possible among those known to be biocompatible in humans. The pig model has been predictive of human responses, but the islet dose in pigs is relevant to humans in the subcutaneous site.

M. Graham: There is a track record in humans for silicone-based materials. The question differs based on whether materials are biodegradable or not, as well as the nature of the transplanted cells (e.g., allo versus auto versus xenograft).

A. Tomei: Brain implants last longer in humans than in rats or NHP. But, immune systems are heterogeneous in humans. Using multiple models and strategies to ask different questions will increase the success in humans.

Q: Regarding scalability, is one large device or multiple smaller devices better?

A. Tomei: What is best for the surgeons who will implant the devices? Multiple device allows for the possibility of retrieving some during a clinical trial for a better assessment of safety, among other issues.

P. Toleikis: Multiple devices implanted deep under the skin allow the flexibility to see how things are going with respect to efficacy and to add more modules, if necessary. Also, the platform has multiple chambers with plugs in each chamber, allowing for the addition of more cells, as needed. The field is in a learning process and needs the potential to try different strategies. Having a modular device allows for stepwise testing of added components, such as the conformal coating and iPSC technology.

Session Seven: Manufacturing and CMC Considerations

Moderator: Richard McFarland, MD, PhD, ARMI

Sukhanya Jayachandra, PhD, FDA

FDA Perspective: Chemistry, Manufacturing, and Controls (CMC) Considerations for Beta Cell Therapy Products

The FDA reviews investigational study and marking applications. Allogeneic islets and beta cell replacement products are regulated in several ways. Relevant regulations include human cells, tissues and cellular and tissue-based products, biologics, drugs, devices, xenotransplantation products, and combination products. Some CMC challenges in this field include: a multitude of products, increasing complexity of products, innovative manufacturing methods, and novel assay methods and characterization. Multiple safety issues must be considered. The manufacturing process should be maintained throughout the clinical trial process to facilitate FDA approval for progression. Donor testing and screening for infectious agents are concerns; regulations were released in 2005. Reagents must be qualified and meet FDA requirements. Lot release specifications must be set related to product safety and quality.

There are multiple opportunities for FDA engagement with investigators and sponsors. Dr. Jayachandra advised requesting a meeting as early in the development process as possible.

Jens Christian Wortmann, MS, Novo Nordisk

Delivering a Successful Beta Cell Therapy: Manufacturing and CMC Considerations and Challenges

CMC challenges include: the scale, reproducibility, and quality control of the stem cell differentiation process; the safety, virus, and sterility of the raw materials; and delivery of a route of administration,

formulation, and device. In addressing those challenges, it is critical to consider the clinical point of use of the cell product. Dose preparation and delivery technologies must be proven to provide cells that survive through the entire process up to the patient. Scaling up the cell production process from stem cells must be shown to maintain the cell differentiation result. Multiple raw materials and reagents (~20–30) must be qualified for stem cell differentiation compared to 1–3 new raw materials for other types of drugs. A good, smart, scalable delivery system is critical for getting cell therapy to patients.

Tom Bollenbach, PhD, ARMI

Building the Industrial Base for Human Cell, Tissue, and Organ Manufacturing

Dr. Bollenbach administers the BioFab USA program on half of the Department of Defense, a network of more than 175 organizations who can be drawn on for technology development. BioFab is a neutral enabler of scalable, consistent, and cost-effective manufacturing for cells, tissues, and organs. The current challenge is that most tissue engineering manufacturing practices in are not scalable. The processes rely largely on T flasks with a yield of ~10 million cells, which is not nearly enough to meet the clinical need. There is a great difference between culturing cells as medicine versus culturing cells to make medicines.

Scalable, modular, automated, closed (SMAC) manufacturing is a robotic, automated process that removes the risk of human intervention and variability. It does not remove variability that is inherent in the process being conducted (e.g., cell differentiation). They have developed an autonomous system to provide JDRF-funded investigators with a consistent SC-islet product. Their system results in a cell product that is comparable to that produced by small-scale beta cell production.

There is a data analytics challenge in ensuring the quality of the final product. It is important to know the attributes of the cells that correlate with clinical endpoints, i.e., critical quality attributes (potency, identity, purity). Addressing this challenge requires untargeted discovery of product quality attributes using modern analytics approaches (multi-omics, cell surface markers, media chemistry, deep characterization of the final product, etc.). Further, in-process quality control specifications for intermediate stages of the derivation process must be identified to determine whether a process will be success by the end.

Greg Korbutt, PhD, University of Alberta

Porcine Islet Manufacturing and the Path to the Clinic

Human islet transplantation is limited by immunosuppression risks, poor quality of islets, issues of reproducibility with the isolation process, and proinflammatory processes. Investigators are studying pig islets from the fetal through adult stages as a possible source of translatable islets. Islets only begin to form in the juvenile stage. In fetal and neonatal pigs, islets are not isolated; tissue culture is required to form cellular aggregates. This process is simpler than islet isolation from adult pigs or multi-step stem cell differentiation methods. Fetal and neonatal cells can correct diabetes on a delayed timescale and are robust; adult cells are functionally mature but fragile. Approximately 28 piglets would be needed to generate sufficient beta cells to achieve insulin independence in one human recipient.

Current limitations for porcine beta cell transplantation include: the donor source must be pathogen free; grafts must be retrievable; and life-long immunosuppression is required. Dr. Korbutt uses an allogeneic porcine model with a subcutaneous implantation site for a catheter that causes a foreign body response and vascularization. After 4 weeks, neonatal porcine islets are implanted combined with cyclosporine A and dexamethasone micelles to help prevent rejection. This could be a useful preclinical model.

Qizhi Tang, PhD, University of California San Francisco

Ancillary Immune Therapy to Beta Cell Replacement

Dr. Tang discussed Treg cell therapy as an approach to creating a tolerogenic environment to protect islet grafts. Several mechanisms impact islet rejection including both recurrent autoimmunity and alloimmune rejection. Current approaches to promoting endogenous Tregs lack specificity, whereas Treg cell therapy allows precise control of cell type that is manufactured, as well as antigen specificity. Polyclonal Treg infusion monotherapy has limited efficacy in islet transplantation. Deletion of alloreactive T cells combined with Treg therapy results in long-term protection and operational tolerance to an islet graft, but this does not provide systemic protection to other grafts (e.g., a skin graft from the same donor). Islet-specific Treg therapy could be combined with hypoimmune islets.

Discussion

Moderator Comment (Richard McFarland): FDA guidance for allogeneic islet transplantation was published in 2009. Dr. McFarland advocated for more intense community engagement with the FDA for new guidance as the field moves closer to commercialization.

Audience Comment (Camillo Ricordi): The field is moving toward an islet product that is a more complex, heterogeneous organ with different combinations of cells, as well as scaled-up manufacturing. The FDA is regulating islets as a drug, but the field needs to keep in mind that they are no longer islets, but advanced cell therapy products. The rest of the world is regulating islets as micro-organs rather than drugs.

S. Jayachandra: Each product will be reviewed and regulated on its own characteristics and the data that are provided. The field is trying to move away from unmodified cadaveric donor islets, so new methods and products will be reviewed differently.

Q (Wanxing Cui): How will the FDA regulate in terms of potency and purity?

S. Jayachandra: There are concerns—sponsors cannot just look at DZT staining for purity; additional, more robust markers for purity would be needed and other cells types would need to be looked at in addition to beta cells. Regarding potency, the FDA envisions matrix approaches that go beyond GSIS.

Q (Wanxing Cui): From the FDA perspective, is donor source material highly controlled by UNOS?

S. Jayachandra: She cannot comment.

Audience Comment (Wanxing Cui): The need to revascularize islets is strong evidence that they are organs, rather than cells. He appealed to the FDA to reconsider classification of islets under the human cell and tissue products category.

Q (Mrignayani Kotechato to T. Bollenbach): Is the automated foundry for cells only or also for biomaterials? Is anything in development to manufacture 3D tissues that involve biomaterials at BioFab USA?

T. Bollenbach: There have been a number of processes for scaffolds (e.g., retinal progenitor epithelial cells, skeletal muscle on porcine bladder matrix), but the challenge is to see them as they develop in a closed system. Costs are dropping—through automation, batch failure is lower, which decreases costs, and labor costs are also lower. The use of closed systems eliminates the need for expensive clean rooms.

Q (Jon Odorico to Greg Korbutt): Currently, 28 piglets are needed for one patient; reducing that number would lower costs. Is any work in progress to improve the functionality and maturation of islets in vitro

before transplantation? There may be potential for cross-fertilization and synergy between SC-islets and NPI, which are both immature prior to transplant, in terms of learning how to better induce maturation.

G. Korbutt: He has procedures to differentiate the cells and enrich for beta cells. His concern is that maturing the cells in vitro would increase costs for the differentiation and require more handling of the product, which can cause problems. He has shown that neonatal islets can induce insulin independence in 2 weeks when transplanted into juvenile pigs and 3 weeks in NHPs, so maturation may depend on the species. But, the neonatal cells do behave similarly to SC-beta cells and differentiation protocols may be similar. The costs of the pigs are minimal.

Q: If clinical quality attributes do not correlate with clinical outcomes, can a BLA be approved?

S. Jayachandra: The attributes are intended to ensure that the product is produced and works consistently over time in the clinic. The main issue with the attributes is product consistency.

Q (Richard McFarland): How often do people measure the same marker in the same way? *Can the community work together so that companies do not have to validate each assay themselves*?

J. Wortmann: It would be hugely beneficial to have standard assays, but that would take an agreement on the assays together in the community and with regulatory agencies.

T. Bollenbach: He proposed adopting standards in processes and automation whenever possible (e.g., cell counting methods, batch control, etc.). Using and demonstrating standards extensively should smooth the path for approval of BLAs and INDs.

G. Korbutt: Every lab has its own assay to measure GSIS. In Edmonton, they tried to look at islet measures in comparison to patient outcomes, but islet preparations and patients are both highly heterogeneous. A standardized product will have different outcomes in each patient.

Q. Tang: For Treg manufacturing centers, it would be beneficial, but assays are not harmonized yet.

Audience member: After 20 years of islet transplantation, the field still does not have critical quality attributes that correlate with clinical outcomes; new tools are needed to measure such attributes.

Q: Islets and recipients are heterogeneous. How can the field define an in vitro assay to use as a release criterion and predict in vivo activity? What assays should be included in a matrix as mentioned by the FDA?

T. Bollenbach: We do not have an answer for that. This is why he is taking an unbiased approach to find correlates. If variability in a factor of function does not correlate with clinical outcomes, then it does not matter.

Concluding Remarks

Esther Latres, PhD, JDRF

Dr. Latres thanked all participants of the workshop, who represented academia, industry, regulators, and the patient voice. Progress since the last workshop in 2009 was clearly reflected in all sessions. The presentations and discussion will help funding organizations identify gaps that can be strategically targeted with funding and collaboration. Dr. Latres thanked the patient representatives for their important insights on risk tolerance and psychosocial aspects of type 1 diabetes and its treatments.

Albert Hwa, PhD, NIDDK

Dr. Hwa recapped several themes that emerged from the meeting:

→ We were reminded of the experience of CIT trials and the exciting development of new stem cell derived products now in clinical testing. We now have better tools to evaluate and stratify patients,

for qualifying inclusion, and to have better outcome measures, as well as innovative clinical trial designs. With the continuing improvement in diabetes care, we need to consider carefully how to design trials as more new products for beta cell replacement enter clinical testing.

→ The patient panel discussion was informative regarding the reasons we are moving toward a goal of widely applicable beta cell therapy.

→ FDA personnel provided information on the regulatory framework for novel products.

→ As the complexity increases, there were productive discussions on useful preclinical models to inform the assessment of biocompatibility, safety, integration with the host, and cell function. But, challenges remain for models to predict human host responses; clearly, ongoing work is needed to evaluate such new research approaches for translation.

→ A multitude of approaches are being tested to improve engraftment efficiency at extra-hepatic sites and to minimize the uses of immunosuppression, including developments in genetic engineering of stem cells or pigs.

→ Increasingly, combinations of cells are being tested within devices.

→ Wound healing is being harnessed to establish prevascularized sites for implantation.

→ Improved technologies are providing more precise engineering of materials and devices.

→ Attention is needed on scaling up and CMC of cell production, as well as ways to characterize the heterogeneous cell populations.

In summary, Dr. Hwa noted that this is an exciting time for translation of beta cell replacement research from bench to bedside. The field has made great progress since the last JDRF-NIH workshop in 2009. He thanked all participants for their collaborative spirit and looked forward to continued progress in the field and opportunities to move beta cell replacement therapy closer to clinical reality.