HUMAN ISLET RESEARCH NETWORK (HIRN): YEAR 2 EXECUTIVE SUMMARY REPORT
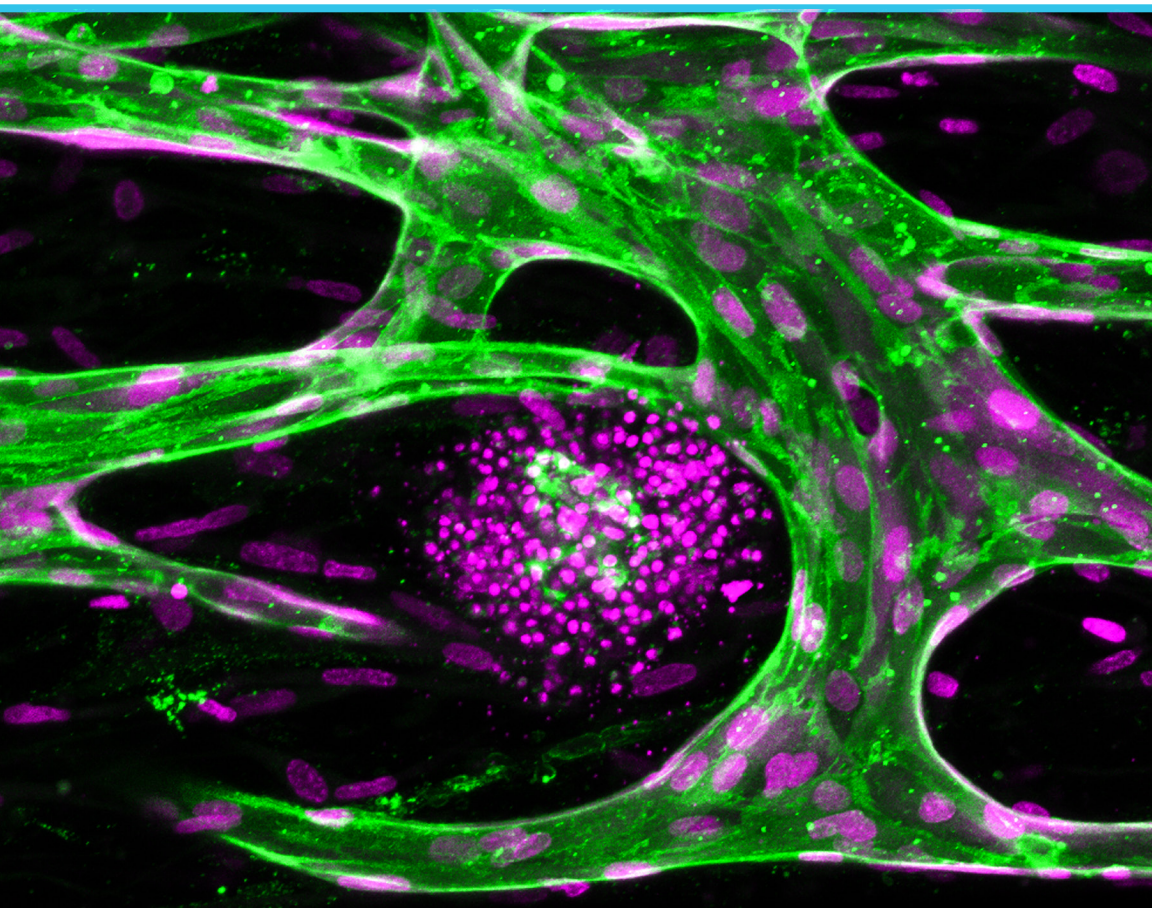
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WELCOME

Type 1 diabetes is a serious disease that usually begins in childhood or adolescence that has a lifelong impact on patients and their families. In persons with type 1 diabetes, the immune system — for reasons still unknown — destroys the insulin-producing beta cells located within the pancreas. Insulin is a hormone that helps the body change glucose (sugar) from food into energy. Without insulin, glucose levels in the blood increase, but the body's cells slowly starve because they cannot use the glucose for fuel. The destruction of the beta cells is considered a "silent" process because it can take months or even years with no signs or symptoms that anything is wrong until most beta cells have been lost and diabetes is established. After diagnosis, the daily burden of disease is heavy. Those with type 1 diabetes, or their caregivers, must replace their insulin either with multiple injections every day or continuous infusion by an insulin pump. Insulin replacement must be carefully balanced with the individual's diet and physical activity. Thus, while type 1 diabetes can be managed, it requires lifelong effort with no days off and no known cure at this time.

Fortunately, decades of research have led us to the most promising time ever in the type 1 diabetes field. Advanced technologies for genetic analysis, microdevice development, single cell analysis, and immune cell characterization — among many other methodologies — mean that scientists can collect data at a speed and level of detail that was unimaginable just a few years ago. Other scientific advances — such as the ability to derive stem cells from the skin or blood of individuals with or without type 1 diabetes — have also opened up new frontiers for understanding this disease in humans, which is not mirrored perfectly in standard animal or cell models. New insights into the immune process leading to type 1 diabetes (referred to as "autoimmunity" when a person's immune system destroys their own cells) paired with advances in beta cell biology have led to increased collaboration and communication among researchers in two traditionally separate fields — immunologists who are looking for ways to prevent or reverse autoimmunity and beta cell biologists who are exploring ways to protect beta cells from autoimmune destruction. Epidemiologic cohort

studies have increased our understanding of the natural history of type 1 diabetes in at-risk children and young adults, and clinical trials of potential immune therapies have revealed important new information about the autoimmune process. Finally, significant investments from funders over the past decade have attracted the energy and expertise of researchers from many other diverse fields — for example bioengineering, microscopy, clinical research, genomics, and animal model development — to create a truly multidisciplinary environment for type 1 diabetes research.

In this fertile environment, the National Institutes of Health (NIH) launched the Human Islet Research Network (HIRN) in 2014 with the mission of better understanding how human beta cells are lost in type 1 diabetes and finding innovative strategies to protect or replace functional beta cell mass in individuals with diabetes. The HIRN, built with the goal of promoting innovative "team science" in the type 1 diabetes field, encourages and supports collaboration, cooperation, and sharing of data and resources with a specific focus on understanding the interface between the immune system and beta cells in health and disease. In addition to working together within and across consortia, HIRN teams are actively collaborating and interacting with investigators and groups in the broader type 1 diabetes research community, including TrialNet for Type 1 Diabetes and the JDRF Network for Pancreatic Organ Donors with Diabetes (nPOD). In this way, the HIRN is accelerating scientific discovery toward understanding human physiology and pathophysiology in order to find better treatments and a cure that will improve the lives and health of people with type 1 diabetes.

The HIRN is excited by the new opportunities in type 1 diabetes research. Building on our successes in the first year, in Year 2 , HIRN investigators are working within and across HIRN consortia, taking a multidisciplinary approach to studying important research questions; applying innovative, state-of-the-art technologies to type 1 diabetes research; and most importantly, increasing our knowledge of how the immune system and beta cells function and interact in human type 1 diabetes. The HIRN added new research groups for Year 2, and we are looking forward to continued expansion in Year 3 — including the addition of a fifth consortium, the Human Pancreas Analysis Program.

On behalf of the HIRN community of researchers, we invite all people interested in type 1 diabetes — patients, caregivers, advocates, and scientists alike — to read about these and other advances and future directions in this *HIRN Year 2 Executive Summary Report*. We hope that you are as encouraged as we are about the progress made toward a day when safe and effective therapies for beta cell protection and regeneration are available to all those at risk for or living with type 1 diabetes.

Sincerely,

The HIRN Trans-Network Committee (TNC)

Mark Atkinson, Ph.D., TNC Chair and Consortium on Beta Cell Death and Survival (CBDS) Representative

Kristin Abraham, Ph.D., NIDDK Program Staff

Ashu Agarwal, Ph.D., Consortium on Human Islet Biomimetics (CHIB) Representative

Olivier Blondel, Ph.D., NIDDK Program Staff

Dale Greiner, Ph.D., Consortium on Modeling Autoimmune Interactions (CMAI) Representative

John Kaddis, Ph.D., Bioinformatics Center Representative

Klaus Kaestner, Ph.D., Consortium of the Human Pancreas Analysis Program (HPAP) Representative

Joyce Niland, Ph.D., Coordinating Center Representative

Alvin C. Powers, M.D., Consortium on Targeting and Regeneration (CTAR) Representative

Sheryl Sato, Ph.D., NIDDK Program Staff

THE HUMAN ISLET RESEARCH NETWORK:

HIRN: TEAM SCIENCE TO ADVANCE TYPE 1 DIABETES RESEARCH

The Human Islet Research Network (HIRN) was established in 2014 to support research that will allow us to better understand how human beta cells are lost in type 1 diabetes and to accelerate the development of innovative strategies to protect or replace functional beta cell mass in diabetic patients. As of September 30, 2016, the HIRN was composed of 94 investigators and co-investigators organized into four consortia — the Consortium on Beta Cell Death and Survival (CBDS), the Consortium on Human Islet Biomimetics (CHIB), the Consortium on Modeling Autoimmune Interactions (CMAI), and the Consortium on Targeting and Regeneration (CTAR). In addition, one new consortium is scheduled to join the network in the coming year: the Human Pancreas Analysis Program (HPAP; see Ongoing Expansion of the HIRN Community, below). In Year 2, HIRN investigators in each consortium reported exciting and significant progress. HIRN investigators continue to develop and apply state-of-the-art tools, reagents, and technologies to advance type 1 diabetes research. Scientific accomplishments of each team of HIRN investigators are highlighted in the summary reports for each of the four initial consortia.

The Human Islet Reserach Network (HIRN) is a large, complex organization with robust interactions among its members and between its investigators and other groups studying type 1 diabetes. In Year 2, the HIRN Coordinating Center (CC) continued to provide the necessary administrative framework to promote research synergy across the Network. The CC organized teleconferences and in-person meetings within and among HIRN consortia, the Trans-Network Committee (TNC) and various working groups, external scientific oversight panels, and external research partners or interested groups. Further, the CC maintained a frequently updated website to disseminate information and enhance sharing of data and resources and managed the application and review processes for Opportunity Pool Projects. The TNC, made up of members from each consortium, the CC, the Bioinformatics Center (BC), and NIH program staff, facilitated communication and collaboration across the Network and provided oversight and guidance for trans-network meetings and activities.

In Year 2, the trans-network Bioinformatics Center (BC) created a number of new tools and resources to promote data sharing and technology development among HIRN teams. The web-based BC labs portal (bclabs.hirnetwork.org) was launched as a site for making all experimental, pre-release, and supported applications available to HIRN investigators, the public, or both. New software tools for data analysis were made available, and webinars were developed to engage the community and train new users on the innovative resources and tools. In addition, the BC partnered with Dr. Todd Brusko, a CMAI investigator, the JDRF Network for Pancreatic Organ Donors With Diabetes (nPOD), and Adaptive Biotechnologies (a biotechnology company in Seattle, Washington) to build a T and B Cell Receptor Sequence Database that enables innovative analyses of immune cells associated with type 1 diabetes. Finally, the BC piloted a resource registry that catalogs biologic reagents, data sets, technologies, and protocols that are essential to HIRN research. The BC is interacting with other type 1 diabetes-related research networks to exchange information and further develop this registry as a valuable resource for the entire scientific community.

FOSTERING COMMUNICATION AND INTERACTION

HIRN 2016 Annual Investigator Meeting

Throughout Year 2, the Human Islet Research Network (HIRN) promoted scientific exchange within the network and interactions with other type 1 diabetes research groups in face-to-face meetings with the goal of promoting communication and stimulating new and novel research collaborations. The 2016 Annual Investigator Meeting was held in May 2016 in Bethesda, Maryland. The annual meeting was supported by the HIRN Coordinating Center (funded by NIDDK) with additional financial support provided by the Leona M. and Harry B. Helmsley Charitable Trust. The meeting, which spanned 4 days, was organized under the guidance of a HIRN trans-network *2016 Annual Meeting Planning Committee,* with the HIRN CC carrying out all logistics. This meeting attracted nearly 190 participants — a 70% increase in attendance compared to the inaugural meeting of the network held in 2015. Highlights of the meeting included a keynote address from Dr. Carla Greenbaum, Chair of the Type 1 Diabetes TrialNet steering committee. Dr. Greenbaum's presentation provided the basis for expanded discussions between HIRN and TrialNet investigators about new collaborations in areas of common interest. In addition, poster

sessions, breakout meetings and workshops, and consortium-specific meetings provided the environment needed to promote discussion, interaction, and "deep dives" into scientific progress and challenges encountered across the Network. Members of the HIRN External Scientific Panel attended the meeting and provided feedback to the Trans Network Committee on progress within each Consortium, as well as progress by the overall Network.

HIRN OUTREACH AT OTHER SCIENTIFIC MEETINGS

HIRN investigators Dr. Alvin C. Powers and Dr. Mark Atkinson, along with NIDDK program director Dr. Olivier Blondel, organized a meeting entitled "Emerging Technologies to Study the Human Pancreas and Islet: from the Whole Organ to a Single Cell" at the 8th Annual JDRF nPOD scientific meeting in Miami, Florida, in February 2016, facilitated by the HIRN CC. The satellite meeting highlighted innovative and emerging technologies that will be useful to study the human pancreas with an emphasis on innovative technologies with an emphasis on innovative technologies being used to study the human pancreas in HIRN-related projects. A total of 127 attendees registered for the meeting, investigators from HIRN made up nearly a third of the attendees. Funding was provided by the Leona M. and Harry B. Helmsley Charitable Trust.

The HIRN Coordinating Center (CC) set up informational tables at the nPOD scientific meeting and at the 9th Annual Midwest Islet Club Meeting at Indiana University in March 2016. These tables offered information on the goals and objectives of each consortium to promote awareness of the Network and its research agenda.

HIRN OUTREACH TO THE TYPE 1 DIABETES COMMUNITY VIA SOCIAL MEDIA

The HIRN website (hirnetwork.org) is another means of fostering communication between the Network and the type 1 diabetes research community, as well as with the public at large. The network **Website Advisory Group** provides input regarding the design, functionality, and content of the website, which serves as a portal for sharing HIRN goals, objectives, and progress. In Year 2, the website was enhanced with an interactive investigator diagram that visually represents the growth of the HIRN community, type 1 diabetes-related progress reports from HIRN and the NIH Special Program for Type 1 Diabetes, and a job posting section. In addition, HIRN utilizes a variety of social media outlets (Facebook, Twitter, Instagram, and LinkedIn) to communicate with the public regarding publications, meetings, and other relevant announcements.

ONGOING EXPANSION OF THE HIRN COMMUNITY

The Human Islet Research Network (HIRN) is a dynamic community with the flexibility to expand as new scientific and technological opportunities arise. In Year 2, the Consortium on Beta Cell Death and Survival (CBDS) added three new research teams with expertise in single cell analysis of human islets and in beta cell biology during the first years of life — a crucial time for type 1 diabetes development. Their research progress is detailed in the CBDS summary report.

Looking to the future, in Year 3, the CMAI is adding a new team of investigators to focus on understanding the role of the thymus in type 1 diabetes. Their expertise and methodologies contribute an important dimension to the CMAI's development of experimental, personalized mouse models of type 1 diabetes. Also in Year 3, HIRN is welcoming a fifth consortium — the Human Pancreas Analysis Program (HPAP) — with combined efforts from teams of investigators at the University of Pennsylvania, Vanderbilt University, and University of Florida. HPAP investigators will collect and characterize pancreatic tissue from organ donors with type 1 diabetes, pre-type 1 diabetes, or other forms of islet dysfunction. The HPAP will provide a vital resource for the entire diabetes research community by distributing extensive datasets obtained as a result of in depth phenotyping of pancreata using multiple experimental modalities, which will be available via an open-access resource database.

To support the growth and evolution of HIRN in Year 4, the NIH released two Requests for Applications (RFAs) for continued growth of HIRN in Year 4. These RFAs solicit applications for the formation of new research collaborations that will expand innovation in the HIRN overall and for research teams with a specific focus on targeting therapies to human islets that will join the CTAR. By continually expanding its science and technology base, HIRN and its community of investigators are able to quickly respond to new opportunities and explore groundbreaking strategies for treating type 1 diabetes.

COORDINATING CENTER (CC) AND BIOINFORMATICS CENTER (BC) INVESTIGATORS, YEAR 2

Joyce Niland, Ph.D., CC Principal Investigator, BC Co-Principal Investigator, *City of Hope*

John Kaddis, Ph.D., CC Co-Investigator, BC Principal Investigator, *City of Hope*

Mark Atkinson, Ph.D., TNC Chair and CBDS Representative, *University of Florida*

Kristin Abraham, Ph.D., Program Staff, *National Institute of Diabetes and Digestive and Kidney Diseases*

Ashu Agarwal, Ph.D., CHIB Representative, *University of Miami*

Olivier Blondel, Ph.D., Program Staff, *National Institute of Diabetes and Digestive and Kidney Diseases*

Dale Greiner, Ph.D., CMAI Representative, *University of Massachusetts Medical School*

John Kaddis, Ph.D., BC Representative, *City of Hope*

Joyce Niland, Ph.D., CC Representative, *City of Hope*

Alvin C. Powers, M.D., CTAR Representative, *Vanderbilt University*

Layla Rouse, M.S., Project Manager, *City of Hope*

Sheryl Sato, Ph.D., Program Staff, *National Institute of Diabetes and Digestive and Kidney Diseases*

TRANS-NETWORK COLLABORATION:

FACILITATING COLLABORATION AND OUTREACH ON SHARED SCIENTIFIC QUESTIONS

A guiding principle of the Human Islet Research Network (HIRN) is that collaboration is a key to rapidly advancing scientific knowledge about type 1 diabetes and its treatment. HIRN investigators are committed to working together within and across consortia and to reaching out to other investigators and groups with an interest in type 1 diabetes. Many examples of research collaborations are described in the summary reports for the individual consortia. In addition, trans-network working groups (Appendix 1) are addressing shared research questions and resource access and utilization issues. Opportunity Pool Projects support collaborative projects among HIRN investigators.

WORKING GROUPS ENHANCE RESEARCH RESOURCES FOR THE NETWORK AND THE BROADER RESEARCH COMMUNITY

The ***What is a Beta Cell? Working Group***, an interdisciplinary group with representation from each of the four consortia, is developing a position statement that will influence work within the HIRN and also serve as a guide for the field of islet biology. The statement may propose nomenclature and criteria for defining a beta cell and hopes to establishes standards for future research on creating or sustaining insulin-producing cells. The Working Group expects to publish its statement in 2017.

The ***Translational Working Group*** provides assistance to HIRN investigators who are ready to translate laboratory discoveries or candidate biomarkers (biological markers of a disease process) into assays that can help predict the onset of type 1 diabetes in clinical practice. One of the highest priorities for this Working Group is to establish a system by which high-quality clinical samples from individuals with and without type 1 diabetes, and at various stages of the disease, can be provided to investigators in the form and volume needed to advance biomarker discovery and validation. As part of its efforts, the Working Group serves as a bridge between HIRN and external clinical research groups that collect or maintain relevant samples, including TrialNet for Type 1 Diabetes, The Environmental Determinants of Type

1 Diabetes in the Young (TEDDY) cohort study, the Type 1 Diabetes Exchange, and the NIDDK Central Repository.

The **Single Cell -Omics Working Group** brings together HIRN investigators with an interest in genomics (the study of all genes in a given cell or organism), proteomics (the study of all proteins expressed in a cell or organism), or metabolomics (the study of all chemicals or small molecules, i.e., "metabolites," in a cell or organism). Technologies for "-omics" research often generate extremely large and complex data sets that require sophisticated tools for meaningful analysis. The Working Group is coordinating efforts for efficient, long-term data integration and collection among its investigators.

The **Islet Advisory Committee** focuses on issues, needs and processes to procure optimal islets in support of HIRN research, on behalf of HIRN investigators. For example, this Group provides valuable feedback and suggestions to the NIH-sponsored Integrated Islet Distribution Program (IIDP). At the request of the Committee, the IIDP has implemented a new mobile responsiveness screen allows investigators to access offers for available islets from any mobile device — a change that improves access for physician-investigators who may be in clinic without access to a computer when offers are broadcast. In addition, the IIDP increased the availability of islets from donors with type 2 diabetes and initiated negotiations with the United Network for Organ Sharing (UNOS) to make more data on organ donors available to investigators. Importantly, these changes to the IIDP system benefit not just HIRN investigators, but also every diabetes researcher who uses human islets from the IIDP for basic research.

The **iPSC Interest Group** facilitates iPSC (induced pluripotent stem cell) research for type 1 diabetes by promoting communication and resource sharing among HIRN investigators. iPSCs are created by turning back the developmental program of adult cells, such as skin or blood, to generate stem cells with the potential to form different types of specialized cells, such as beta cells or cells of immune system. The Group is developing a website to share information about iPSCs derived from individuals with type 1 diabetes or at high risk for the disease and has initiated a research collaboration with an investigator at the New York Stem Cell Foundation Research Institute.

OPPORTUNITY POOL PROJECTS ENCOURAGE COLLABORATION AND COMMUNICATION

HIRN investigators submitted short-term, innovative, collaborative concept proposals that advance the HIRN's missions to be considered for support by the HIRN Opportunity Pool Project fund (Appendix 2). In Year 2, nine projects were approved for funding, including at least five that involved collaboration with investigators outside of the Network. One project funded a workshop designed by the Translational Working Group to compare three measures of beta cell death developed by HIRN investigators. The workshop involved blinded testing of biologic samples obtained shortly after islet transplantation, in collaboration with islet transplant centers and the JDRF Core for Assay Validation, which accepted the samples and distributed them to participating investigators. Another project supported the participation of trainees and junior researchers in a meeting of CHIB investigators held in December 2015.

CONSORTIUM ON BETA CELL DEATH AND SURVIVAL (CBDS)

OVERVIEW: DETECTING AND HALTING BETA CELL DESTRUCTION IN TYPE 1 DIABETES

The autoimmune process that destroys pancreatic beta cells in the early stages of type 1 diabetes begins in a silent mode (that is, before presenting with notable symptoms of the disease). Tests to measure for autoantibodies — specific immune system components in the blood that identify people who are at high risk of developing type 1 diabetes — do not reveal whether a person's beta cells are working properly or whether destruction is occurring at the time of the measurements. This is clearly a key and practical gap in predicting the course of autoimmunity in individuals who are progressing toward diabetes, and who would likely benefit from future therapies to halt the destructive process. Blocking destruction while it is still in its initial stages would be optimal. To bridge this gap, the Consortium on Beta Cell Death and Survival (CBDS) is searching for new biological markers ("biomarkers") of beta cell health, stress, and death that can be easily measured in blood, urine, or saliva, and especially for ones that can be detected at the earliest onset of autoimmune attack. The Consortium is also developing state-of-the-art tools to study single beta cells or islets in the pancreas at all stages of the autoimmune process. This research should uncover how beta cells initially become stressed and ultimately die in response to the autoimmune reaction, lead to the discovery of improved blood tests for predicting type 1 diabetes, and point to new targets for drug development. Importantly, in Year 2 of the HIRN, the CBDS added three new teams that brought fresh perspectives, expertise, and technologies in pursuit of these important goals.

BETA CELL BIOMARKERS REVEAL TYPE 1 DIABETES RISK AND THE PROGRESSION OF AUTOIMMUNITY

Several CBDS teams focused on beta cell biomarkers, with each team taking different approaches to finding new measures of the health state of beta cells, assessing their level of function, and detecting their death using non-invasive methods. Opportunity Pool Projects supported close collaborations within the Consortium, including a major effort to compare the usefulness of novel DNA-based biomarkers, carried in the bloodstream, of beta cell death that have been developed by three different teams.

Indiana University-based CBDS investigators found three biomarkers that effectively identify individuals who are likely to develop type 1 diabetes. The first, the ratio of proinsulin to C-peptide (enzymatic processing of proinsulin [a precursor protein] within beta cells produces two smaller secreted proteins: insulin and C-peptide) in the blood, suggests new clues about the nature of beta cell stress during autoimmunity. The team showed that this biomarker is elevated in young children prior to the development of type 1 diabetes (Figure 1). The second marker, an innovative measure of differentially methylated DNA (DNA carrying a specific chemical tag) in urine and blood, was shown to be a reflection of beta cell death and of the inflammation process that injures beta cells in both type 1 and type 2 diabetes. This marker is also elevated in relatives of type 1 diabetic subjects, suggesting that underlying genetic risk might be detectable using this biomarker. Finally, the third marker, again present in blood, is a particular "messenger RNA" (RNA derived from the genome that encodes the production of a specific protein) that is elevated when beta cells are stressed; importantly, this sensitive marker can detect such stress in both diabetic and nondiabetic individuals. (For an additional figure related to this project see Figure 10).

IFNα induces MHC class I expression in human beta cells
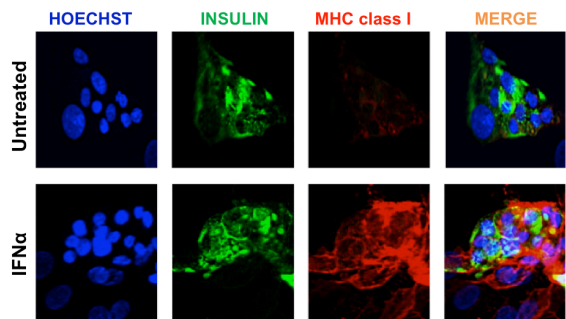
Figure 1. *IFNα increases MHC class I expression in human β-cells.* (CBDS: Mirmira UC4, Indiana University)

A CBDS team based at the University of Florida made exciting progress in developing a robust blood-borne DNA biomarker of beta cell death (Figure 2). They measured significant differences in the level of this biomarker under

Figure 2. *Illustration of method to detect beta cell death.* (CBDS: Schatz UC4, University of Florida)

various conditions, including newly diagnosed type 1 diabetes. Strikingly, when comparing their assay to other DNA "death biomarkers," the team discovered more beta cell death in young, healthy children than expected. This intriguing finding has consequences for the development of a biomarker-based blood test for autoimmunity that can be simply, cheaply, and easily used for at-risk individuals of all ages. The team also advanced the use of microRNAs (small pieces of RNA that do not encode specific proteins but often regulate the production of proteins from other messenger RNAs) in the blood as biomarkers of beta cell death and dysfunction. (For an additional figure related to this project see Figure 11).

A team at the Pacific Northwest National Laboratory worked closely with investigators from the University of Florida and the HIRN Consortium on Targeting and Regeneration (CTAR) to develop a catalog of all proteins in purified alpha cells and beta cells. In part as a result of this collaboration, the team identified up to 40 beta cell-specific proteins that are good candidates for biomarkers of type 1 diabetes progression; they are now developing assays to measure those proteins in blood (Figure 3). The investigators also worked with a team from the Consortium on Modeling Autoimmune Interactions (CMAI) to map a subset of proteins in T cells, leading to the discovery of new pathways that may be involved in beta cell autoimmunity. (For an additional figure related to this project see Figure 12).

Sex	Age	BMI
M	45	36.4
F	47	21.1
F*	47	36.5
M*	63	38.6
F*	42	23.1
F*	57	20.6

500–1,000 islets/donor

Human islets (n=6)

Tryptic peptides

Offline HpH fractionation
(24 fractions/sample)

Online nano RPLC-MS/MS

Human islet proteome

23 adult tissues proteome

Kim, *et al., Nature*, 2014

Islet-enhanced proteins
Islet-specific proteins
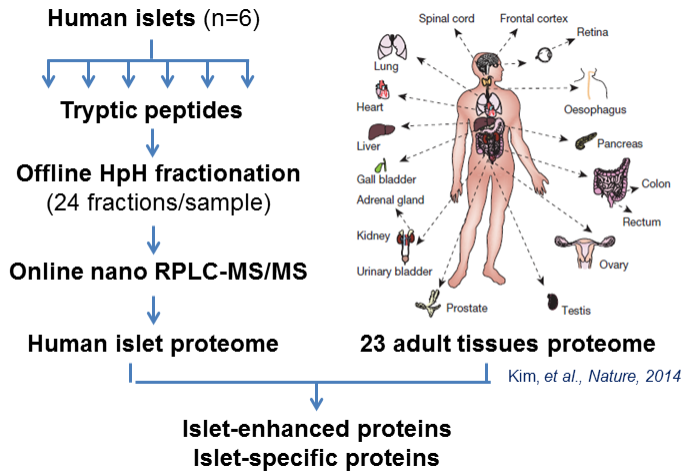
Figure 3. *Schematic workflow of establishing a human islet proteome map as a community resource for diabetes research. The comparison with data from 23 adult human tissues identifies islet-specific proteins.* (CBDS: Qian UC4, Pacific Northwest National Laboratory)

The CBDS team at Harvard University used beta cells derived from pluripotent stem cells to identify molecular changes in beta cells during diabetic stress(Figure 4). Several candidate biomarkers that distinguish mature, glucose-responsive, insulin-producing cells were identified. In addition, a large collection of serum samples from mice at various time points after the onset of diabetic stress was assembled. Together, these tools will help investigators generate a timeline for beta cell stress and will also provide a better beta cell model for many experimental systems across the HIRN, such as the development of islet-on-a-chip technology by the Consortium on Human Islet Biomimetics (CHIB) and new experimental type 1 diabetic mouse models for the consortium on modeling autoimmune interactions (CMAI).
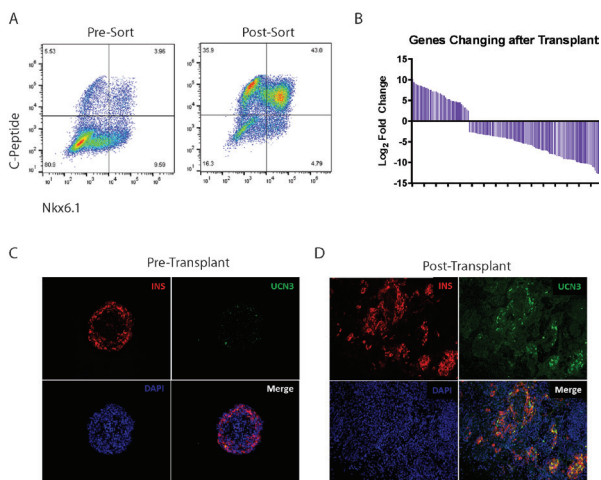
Figure 4. *A,B) Using the Zinc labeling dye TSQ, RNA Seq of SC-β cells after transplantation shows over 150 genes at least 2-fold up or down-regulated in all samples. C,D) SC-β cells show improved expression of maturation-associated genes post-transplant, including UCN3.* (CBDS: Melton UC4, Harvard University)

CHARTING A PATH TOWARD BETA CELL HEALTH AND REGENERATION ONE ISLET AT A TIME

Other CBDS teams actively collaborated with each other, as well as with CTAR investigators, using advanced technologies to study the properties of individual islets or beta cells, to begin collecting data on their degree of functional variation within one person's pancreas or between individuals. By sharing data and tissue samples from the same organ donors, the teams began to build a multidimensional picture of beta cell health and function during initial autoimmune attack and overt type 1 diabetes.

Another University of Florida-based team along with investigators from the University of Geneva and the University of Zurich used a technique known as "highly multiplexed tissue imaging (HMI)" to make an

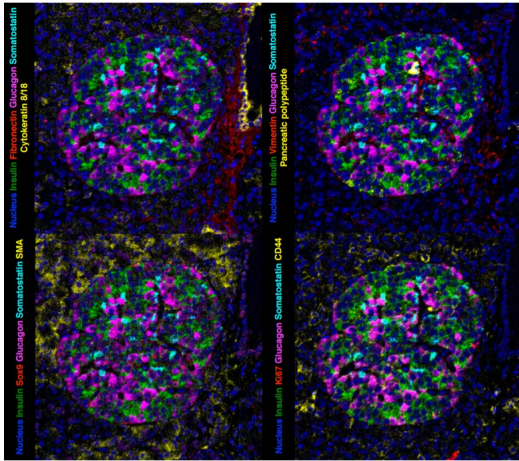exciting and unexpected discovery. (HMI allows the team to look for the presence or absence of more than 50 known molecules in a single tissue sample in about an hour — an experiment that previously would have required processing many samples over several days.) They detected proinsulin (the unprocessed form of insulin), but not insulin nor insulin mRNA, in most pancreata from donors with type 1 diabetes (Figure 5). This finding opens up new pathways for research on insulin processing during the development of the disease. The team also obtained preliminary evidence for islet "plasticity" — the potential for non-beta cells within islets (such as glucagon-producing alpha cells or somatostatin-producing delta cells) to be converted to beta cells — which may lead to new strategies for regenerating beta cells in individuals with type 1 diabetes. (For additional figures related to this project see Figures 13 and 14).

Figure 5. *Healthy pancreas analyzed by IMC. False color images show markers listed to the left hand of each quadrant.* (CBDS: Atkinson UC4, University of Florida)

A team of investigators at the University of Tennessee and the University of Florida mapped all RNAs derived from the genome in each of 120 islets from 11 donors with type 1 diabetes and 12 nondiabetic donors. They discovered that islets are not all alike (Figure 6). In fact, any individual, particularly someone with type 1 diabetes, can have many different types of islets in his or her pancreas. Remarkably, in several pancreas samples from type 1 diabetes patients,
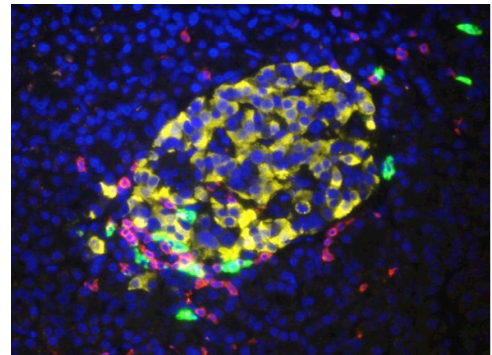
Figure 6. *Lymphocytic infiltration of an islet from a donor with type 1 diabetes (6052, 12 years old male African American with diabetes for 1 year). Shown is an islet with CD20+ (β cells) and CD3+ (T cells) surrounding alpha cells (glucagon).* (CBDS: Gerling UC4, University of Tennessee Health Sciences Center)

the team found insulin-expressing islets that were not yet invaded by immune cells (and were thus not yet in the later, more severe stage of autoimmune attack), yet all of these islets in these individuals were already detectably "abnormal" in the sense of expressing a large number of genes at much different levels compared to islets from nondiabetic pancreas tissue. This information represents a key step toward creating a molecular model of diabetes progression, especially the early stages.

A second team of researchers at the Pacific Northwest National Laboratory established two innovative technologies for single beta cell or islet analysis. With these tools, they can detect subtle changes in the levels of proteins, lipids, and other molecules in beta cells during the progression of autoimmunity (Figure 7). In collaboration with another CBDS team, they will use these powerful technologies to also detect differences between rare beta cells that retain the capacity to self-replicate and older beta cells that cannot reproduce. (For an addition figure related to this project see Figure 15).
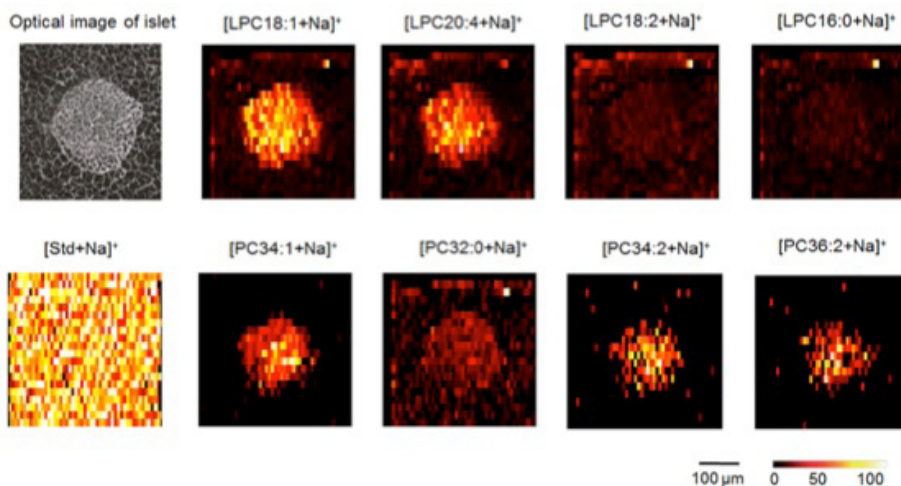
Figure 7. *Optical image of a 200 ×m in diameter pancreatic islet along with representative ion images of the standard added to the solvent, [Std+Na]+, and several endogenous phospholipids.* (CBDS: Ansong UC4, Pacific Northwest National Laboratory)

The Vanderbilt University-based team expanded their collection of juvenile pancreas tissue to 45 specimens. This rare tissue is critical for cataloging molecular differences between juvenile and adult islets — and for understanding what those differences can tell us about how to prevent autoimmune destruction of beta cells or regenerate beta

cells in type 1 diabetes. Team members are utilizing several state-of-the-art imaging technologies to investigate islet formation after birth, the maturation and plasticity of juvenile islets, networks of blood vessels and nerves in islets, and interactions between juvenile islets and the immune system (Figure 8). One critical hypothesis is that improper development of tissue architecture in the early juvenile period initiates a bad interaction with the immune system, thereby predisposing to earlier and more severe type 1 diabetes.
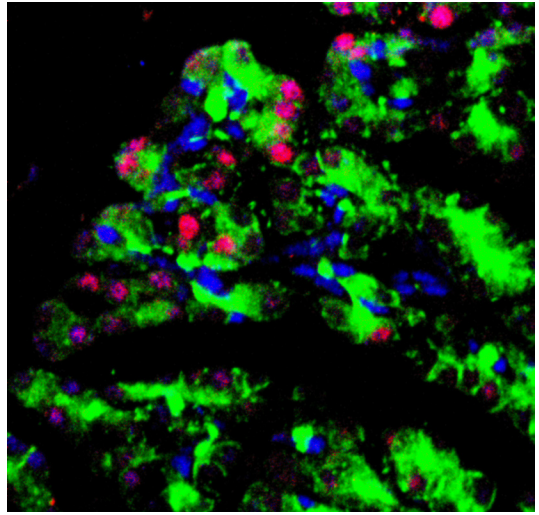
Figure 8. *Islet formation in human pancreas after birth.* (CBDS: Wright UC4, Vanderbilt University)

At Yale University, the CBDS team identified strategies that beta cells may use to survive the autoimmune process. They found that some beta cells lost certain immune features that appear to make them targets for autoimmune destruction, and at the same time, the cells acquired other features that made them more similar to replication-competent stem cells. This novel discovery could explain why some beta cells can still be detected in patients who have had type 1 diabetes for even decades. The team also analyzed all genes that were active in single beta cells to begin piecing together why some beta cells lose their specialized characteristics when stressed by the immune system (Figure 9).

Figure 9. *xpression of DNMT and INS genes in human beta cells. Human islets were cultured in medium without (white bars) or with TNF plus IFN-g plus IL-1beta (black bars) for 48 h and then gene transcription was analysed. Data are the means +/- SEM from 4 experiments, each with a single islet donor. ANOVA: **p<0.01, ****p<0.0001.* (CBDS: Herold UC4, Yale University)
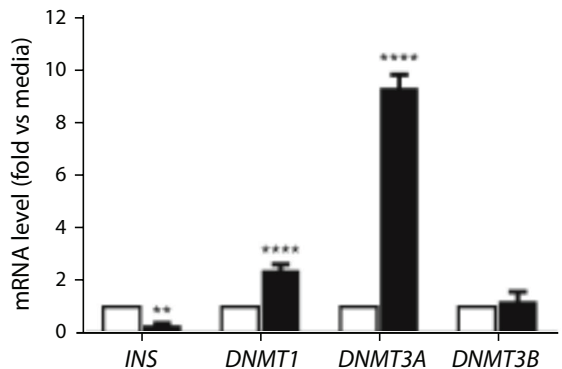
Douglas Melton, Ph.D., Investigator, *Harvard University*

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Decio Eizirik, M.D., Ph.D., Investigator, *University of Brussels*

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Jerry Nadler, M.D., Investigator, *Eastern Virginia Medical School*

Ernesto Nakayasu, Ph.D., Co-Investigator, *Pacific Northwest National Laboratory*

Julius Nyalwidhe, Ph.D., Co-Investigator, *Eastern Virginia Medical School*

Bobbie-Jo Webb-Robertson, Ph.D., Co-Investigator, *Pacific Northwest National Laboratory*

Wei-Jun Qian, Ph.D., Investigator, *Pacific Northwest National Laboratory*

Rohit Kulkarni, M.D., Ph.D., Investigator, *Joslin Diabetes Center*

Clayton Mathews, Ph.D., Investigator, *University of Florida*

Jason McDermott, Ph.D., Co-Investigator, *Pacific Northwest National Laboratory*

Vladislav Petyuk, Ph.D., Co-Investigator, *Pacific Northwest National Laboratory*

Tujin Shi, Ph.D.,, Co-Investigator, *Pacific Northwest National Laboratory*

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Yuval Dor, Ph.D., Co-Investigator, *Hebrew University of Jerusalem*

Jorge Ferrer, M.D., Ph.D., Co-Investigator, *Imperial College of London*

Ruth Shemer, Ph.D., Co-Investigator, *Hebrew University of Jerusalem*

Ivan Gerling, Ph.D., Investigator, *University of Tennessee*

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Martha Campbell-Thompson, D.V.M., Ph.D., Investigator, *University of Florida*

Hao Chen, Ph.D., Co-Investigator, *University of Tennessee*

Clayton Mathews, Ph.D., Co-Investigator, *University of Florida*

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Domenico Accilli, M.D., Co-Investigator, *Columbia University*
Mark Mamula, Ph.D., Co-Investigator, *Yale University*

*Charles Ansong, Ph.D., Investigator, *Pacific Northwest National Laboratory*
Lori Sussel, Ph.D., Investigator, *University of Colorado Denver*
Kristin Burnum-Johnson, Ph.D., Co-Investigator, *Pacific Northwest National Laboratory*
Julia Laskin, Ph.D., Co-Investigator, *Pacific Northwest National Laboratory*
Thomas Metz, Ph.D., Co-Investigator, *Pacific Northwest National Laboratory*
Gayla Orr, Ph.D., Co-Investigator, *Pacific Northwest National Laboratory*

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Bernd Bodenmiller, Ph.D., Investigator, *University of Zurich*
Pedro Herrera, Ph.D., Investigator, *University of Geneva*
Harry Nick, Ph.D., Co-Investigator, *University of Florida*
Fabrizio Thorel, Ph.D., Co-Investigator, *University of Geneva*

*Christopher Wright, DPhil, Investigator, *Vanderbilt University*
Marcela Brissova, Ph.D., Co-Investigator, *Vanderbilt University*
Long Cai, Ph.D., Investigator, *California Institute of Technology*
Jeremy Caprioli, Ph.D., Investigator, *Vanderbilt University*
Viviana Gradinaru, Ph.D., Investigator, *California Institute of Technology*
Jeremy Norris, Ph.D., Investigator, *Vanderbilt University*
Alvin Powers, M.D., Investigator, *Vanderbilt University*

*New CBDS teams added in Year 2

ADDITIONAL FIGURES ILLUSTRATING CBDS RESEARCH
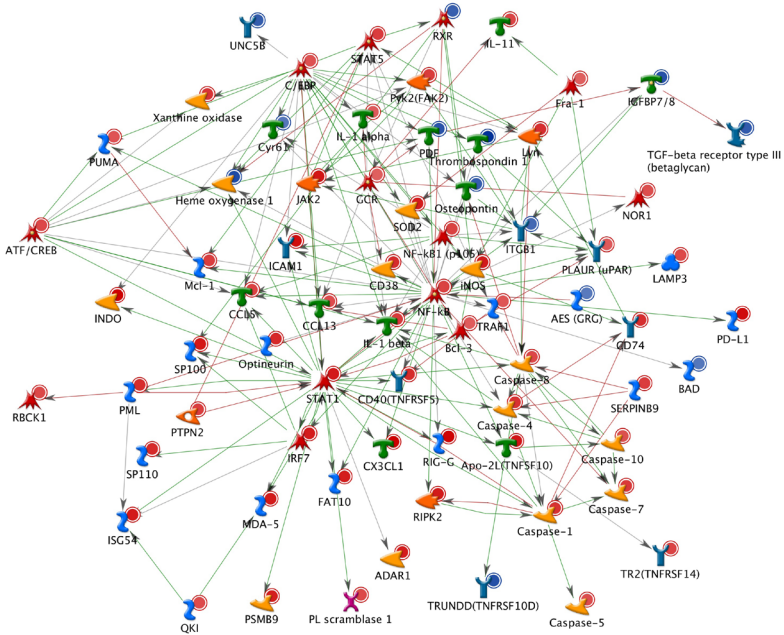
Figure 10. *This network diagram depicts the result of proteomics analysis of human islets exposed to proinflammatory cytokines, which shows enrichment in pathways related to the innate immune response (NOD-like receptor, Toll-like receptor, RIG-I-like receptor and cytokine signaling), antigen presentation and apoptosis.* **(CBDS: Mirmira UC4, Indiana University)**
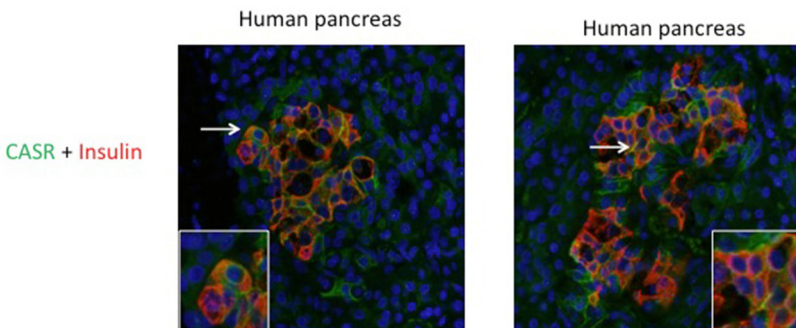

Figure 11. *Magnified images of the IHC for CASR in human pancreas. The co-staining with insulin indicates specificity to beta cells.* **(CBDS: Qian UC4, Pacific Northwest National Laboratory)**

Figure 12. *Antibodies tested for IMC on formalin fixed and paraffin embedded human pancreas. Three different antigen retrieval methods were tested (see heading of table). Red shows that no meaningful signal was detected and green means that the antibody showed the expected staining pattern.* (CBDS: Atkinson UC4, University of Florida)
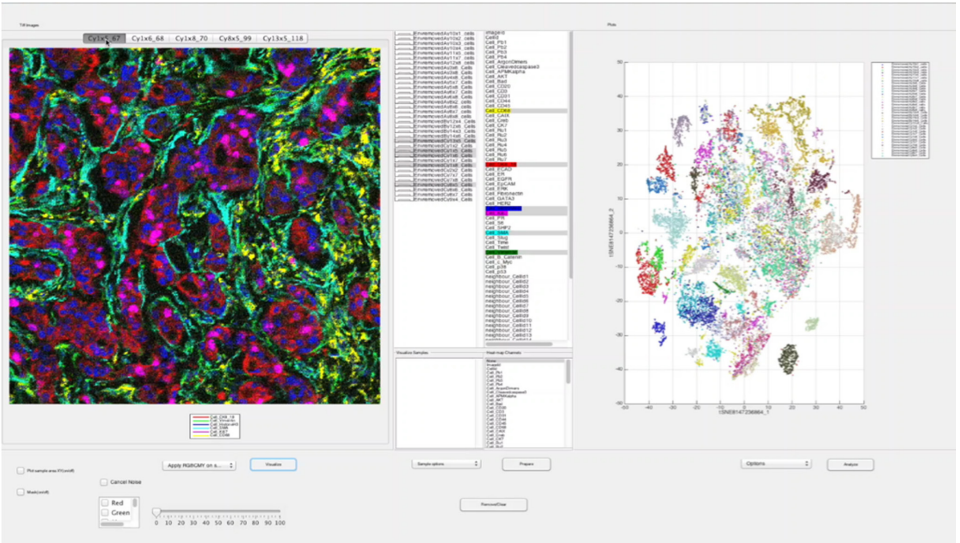

Figure 13. *Screenshot of miCAT. To the left the tissue visualization and interaction module is shown. To the right the cell type and cell microenvironment analysis module is shown. As an example an analyzed breast cancer tissue image is shown, to the right a corresponding cell type analysis by tSNE.* (CBDS: Atkinson UC4, University of Florida)

Figure 14. *Establish barcoded FISH using sub-probes with different colors, targeting the mRNA for elastase in the acinar cell line (266.6). A and C. STORM images of cells treated with sub-probes targeting elastase mRNA, tagged with either two (A) or three (C) different colors. Overlapping sub-probes are marked by the circles in the insets. B and D. The number of mRNA copies per cell (outlined by the dashed line) is then calculated by the number of overlapping sub-probes.* (CBDS: Ansong UC4, Pacific Northwest National Laboratory)
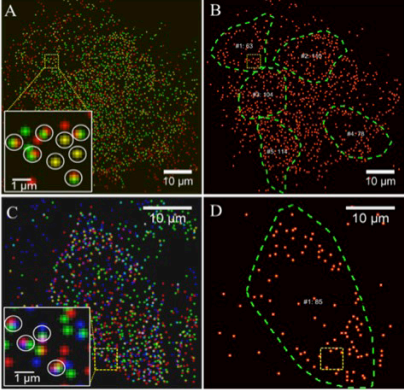
CONSORTIUM ON HUMAN ISLET BIOMIMETICS (CHIB)

OVERVIEW: MODELING HUMAN PANCREATIC ISLET STRUCTURE, FUNCTION AND ENVIRONMENT

In the human pancreas, insulin-producing beta cells live in islets — small communities of hormone-producing cells, including other cells such as alpha, delta, and PP cells, which all work together to control blood sugar levels. The islet is supported by a network of blood vessels, nerves, and extracellular matrix scaffolding. Recreating this complex islet community in the laboratory would offer several significant benefits. For example, it could improve the ability of beta cells derived from stem cells to work as well as beta cells do in the body. Also, mimicking the islet environment in the lab would help researchers better understand how beta cells are lost to autoimmunity, and it would provide a much-needed platform for testing potential new drugs to protect or restore beta cells in people with or at risk of diabetes. The Consortium on Human Islet Biomimetics (CHIB) combines advances in beta cell biology, stem cell biology, microengineering (building miniature machines or structures — some so small that they might not be visible to the human eye), and related technologies with the goal of developing state-of-the-art microdevices that support three-dimensional islet structure, function, and survival in the lab. In Year 2, CHIB investigators made excellent progress in creating such devices that will advance the goals of the entire diabetes research community.

BIOENGINEERED MICRODEVICES: A NEW DIMENSION IN TYPE 1 DIABETES RESEARCH

A CHIB team of investigators based at the University of California, San Diego, focused on two major upgrades to their strategy for maintaining healthy islets in a microfluidic device — a small gadget with tiny channels that scientists can use to change islets' environment, for example, by injecting nutrients or drugs into the liquid surrounding the islets (Figure 1). First, they identified specific proteins from the "extracellular matrix" that surrounds and physically supports islets in the human pancreas. By adding these proteins to the microdevice, they developed an environment that improved islet/beta cell function and survival. Second, the team developed new techniques for building
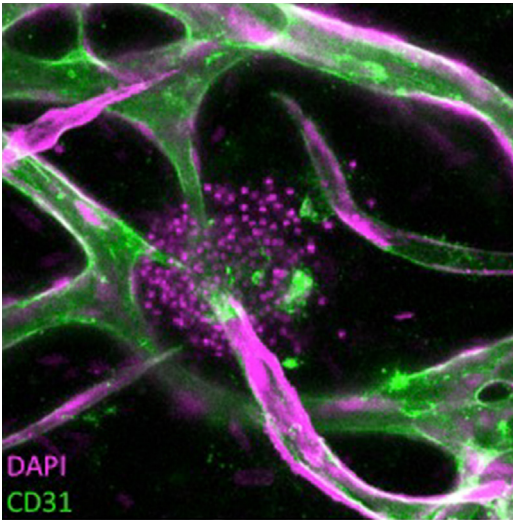
Figure 1. CHIB: *Incorporation of islets into the vascularized micro-organ platform: Conformal imaging of an islet surrounded by vasculature (CD31+, green). DAPI staining (purple) highlights the densely-packed cells in the islets.*
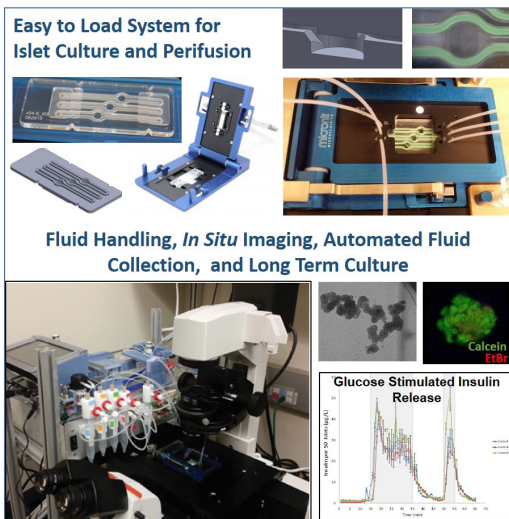(CHIB: Sander UC4, University of California, San Diego)

Figure 2. CHIB: *Outline of physiomimetic system developed.*
(CHIB: Stabler UC4, University of Florida)

a network of blood vessels around islets within the microdevice; these vessels deliver oxygen and other nutrients to further promote islet health. The team will begin working with a University of Florida investigator from the Consortium on Modeling Autoimmune Interactions to apply their optimized microdevice to research the effects of immune cells from type 1 diabetes patients on islets in the lab.

Another CHIB team, based at the University of Florida and University of Miami, developed new computational tools for designing islet culture devices and engineered a new prototype microdevice for three-dimensional islet or beta cell culture in the lab (Figure 2). Their new device reflected several advancements over older models, including ease of use and improved ability to take pictures of islets in the device. New measurements to assess the health and survival of islets were also developed and validated. In the HIRN tradition of collaboration, the team shared detailed information on their device

with other CHIB members to accelerate the development and characterization of islet-supporting microdevices, as well as with investigators across the HIRN who want to use this innovative device to address new questions in islet biology and autoimmunity.

At Harvard University, CHIB investigators refined their islet-on-a-chip microfluidic device that was developed in the first year (Figure 3). In one notable advance, the team used a nanofiber manufacturing technique to develop an extracellular matrix capsule to support

Figure 3. *Islet-on-a-Chip design and associated features. (a) Schematic and image of our chip holder and clamping platform, (b) image of SC-beta cell clusters micro-encapsulated within an alginate hydrogel, (c) image of SC-beta cell clusters housed on a nanofibrous scaffold.* (CHIB: Melton UC4, Harvard University)

islets in the device. These nanofibers — threads that are 50 to 100 times more narrow than an average human hair — more closely mimic the extracellular matrix scaffold that surrounds islets in the body compared to the gel-like matrices traditionally used in cell cultures. The nanofiber scaffold noticeably improved the viability and function of islets within the microdevice. The team also worked closely with an investigator at Florida State University to integrate a new measure of islet function directly into the microdevice. This innovative feature marks an exciting leap towards the team's vision of an automated technology that permits real-time, low-cost, simultaneous measurement of all hormones from a single islet — an accomplishment that would provide an invaluable tool for drug screening, disease modeling, and islet biology research (Figure 4).
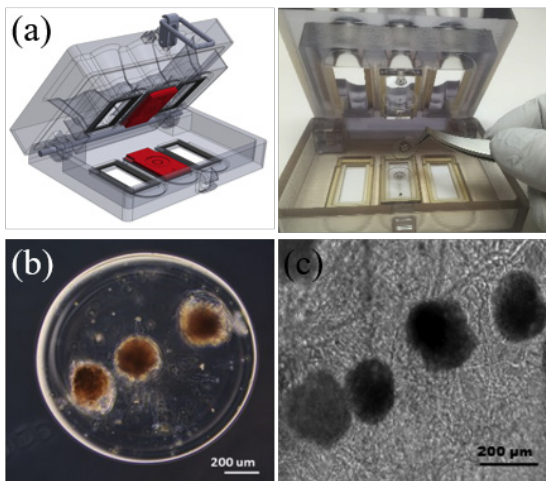

Figure 4. *Differentiation screening platform.* (CHIB: Melton UC4, Harvard University)

Identifying conditions by which

human islets can survive and function outside of the body for long periods of time is the overarching goal of the CHIB team of researchers at the University of Pennsylvania (Figure 5). In year 2, the team found a simple means of prolonging islet function beyond one week — an advance that can be incorporated into future designs to improve the performance of their islet microdevices. In addition, the team created a number of robust new tools for measuring islet function, including microdevices with flexible components that can be swapped and rearranged for different research purposes, such as imaging or simultaneously measuring the effects of multiple culture conditions on the islets.
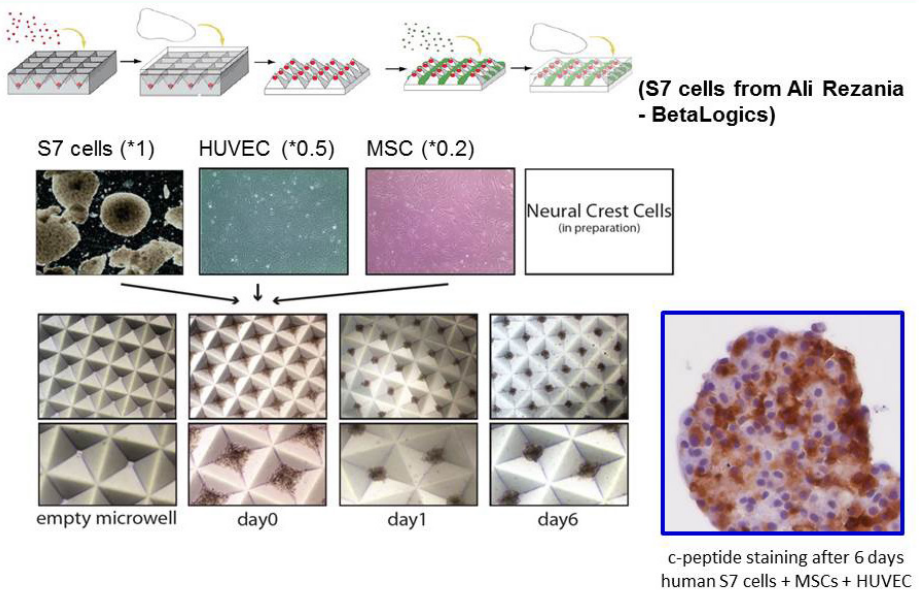
Figure 5. *Embryonic stem cell-derived pancreatic endocrine cells (S7 cells) are mixed with mesenchymal stem cells (MSCs) and/or endothelial cells (HUVECs) and placed into the inverted pyramids of the invert mold. After 6 days, the cells aggregate into islet-sized clusters containing a large number of C-peptide positive cells.* (CHIB: Stanger UC4, University of Pennsylvania)

OPEN COMMUNICATION AND COLLABORATION ACCELERATE PROGRESS

In December 2015, 33 CHIB Investigators and their lab members, as well as representatives from the NIH, JDRF, and the HIRN Coordinating Center, traveled to Miami, Florida, for a two-day, Consortium-wide meeting. At an opening-night poster session, postdoctoral fellows and students from each CHIB team presented their work-in-progress, showcasing their latest results and unpublished data. The following day provided ample time for each CHIB team to present their research and freely discuss their progress, challenges, opportunities, and new techniques. Collectively, the investigators discussed their grand vision for the Consortium and future directions that will have the greatest impact for type 1 diabetes. The meeting, which was sponsored in part by the Diabetes Research Institute and the JDRF, is a prime example of how the HIRN is fostering communication and collaboration among scientists across the country. By breaking through barriers that often separate research teams, the CHIB aims to move the field toward a new level of understanding in islet biology that can accelerate the development of new treatments to prevent or reverse type 1 diabetes.

CHIB INVESTIGATORS, YEAR 2

Douglas Melton, Ph.D., Investigator, *Harvard University*

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Kit Parker, Ph.D., Co-Investigator, *Harvard University*

Maike Sander, M.D., Investigator, *University of California, San Diego*

Karen Christman, Ph.D., FAHA, Investigator, *University of California, San Diego*

Steven George, M.D., Ph.D., Investigator, *Washington University*

Chris Hughes, Ph.D., Investigator, *University of California, Irvine*

Cherie Stabler, Ph.D., Investigator, *University of Florida*

Ashutosh Agarwal, Ph.D., Investigator, *University of Miami*

Peter Buchwald, Ph.D., Investigator, *University of Miami*

Camillo Ricordi, M.D., Investigator, *University of Miami*

Ben Stanger, M.D., Ph.D., Investigator, *University of Pennsylvania*

Chris Chen, M.D., Ph.D., Investigator, *Boston University*

Sangeeta Bhatia, M.D., Ph.D., Investigator, *Massachusetts Institute of Technology*

Paul Gadue, Ph.D., Investigator, *Children's Hospital of Philadelphia*

Kenneth Zaret, Ph.D., Investigator, *University of Pennsylvania*

CONSORTIUM ON MODELING AUTOIMMUNE INTERACTION (CMAI)

OVERVIEW: WHY AND HOW DOES THE IMMUNE SYSTEM TARGET BETA CELLS IN TYPE 1 DIABETES?

The autoimmune process that destroys insulin-producing beta cells, leading to type 1 diabetes, begins silently. Clinical symptoms of type 1 diabetes are only obvious after autoimmunity is well underway and the majority of beta cells have been lost. During the progression of autoimmunity, scientists can detect changes in the immune response in the blood of individuals known to have genetic (inherited) risk of type 1 diabetes, but it is much more difficult — if not impossible — to observe what's happening deep within the pancreas where the beta cells reside. For these reasons, it is challenging for researchers to track down the precise molecular and cellular mechanisms of beta cell injury in human type 1 diabetes — and to find ways to intervene in the process. The Consortium on Modeling Autoimmune Interactions (CMAI) is facing this challenge by incorporating human genes, beta cells, and immune cells into experimental mouse and cell models that more accurately mimic human type 1 diabetes than traditional models. In Year 2, CMAI investigators worked together within the Consortium and with investigators across the HIRN Network to refine these models and use them to gain key insights into type 1 diabetes.

BUILDING A FLEXIBLE, INNOVATIVE MODEL OF TYPE 1 DIABETES IN A LAB DISH

The CMAI team of researchers at the University of Florida collaborated with teams from the Consortium on Human Islet Biomimetics (CHIB) and the Consortium on Beta Cell Death and Survival (CBDS) that are also based at the University of Florida to create an innovative three-dimensional culture system that supports the survival and function of human beta cells and immune cells. They used modern 3-D printing technologies to add or remove cells and biological agents or small molecule drugs with pinpoint accuracy — even making gradients to form different microenvironments across the system. This state-of-the-art model is breaking new ground in the understanding of how immune cells and beta cells interact on a molecular level during the autoimmune process (Figure 1).
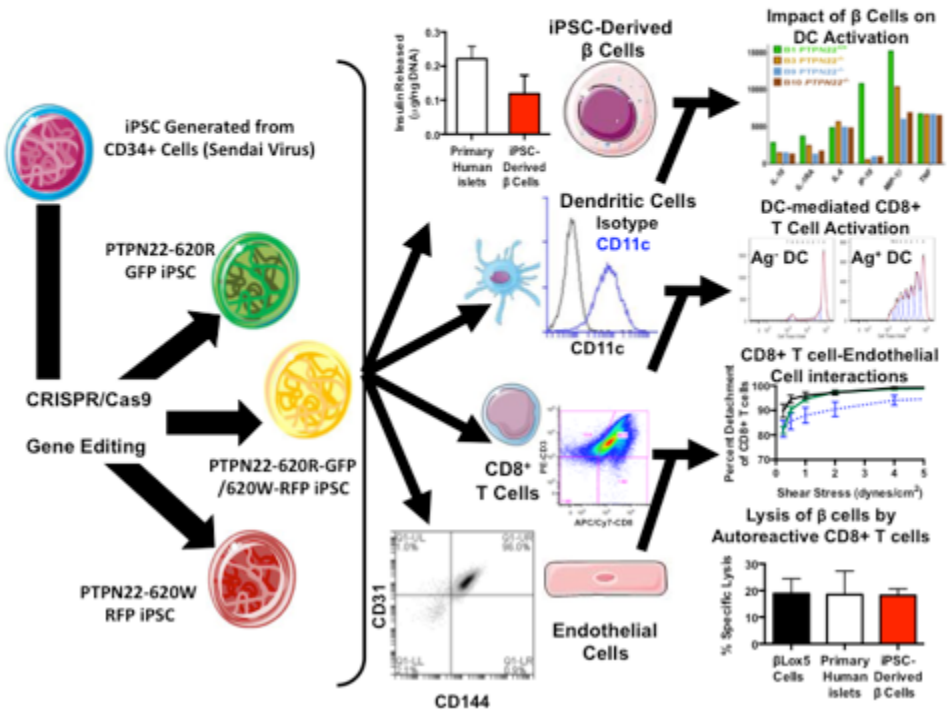
Figure 1. *Establishing and Employing a T1D Relevant Human Isogenic System to Elucidate the Genetics of Beta cell Destruction. (*CMAI: Mathews UC4, University of Florida)

PERSONALIZED, EXPERIMENTAL MOUSE MODELS OPEN NEW DOORS IN TYPE 1 DIABETES RESEARCH

At the University of Massachusetts Medical School, a CMAI team built a bank of human blood cell-derived stem cells (in a form known as "induced pluripotent stem cells") from individuals with type 1 diabetes who have different genetic backgrounds, as well as from individuals without diabetes (Figure 2). They transformed these stem cells into islet beta-like cells — a crucial component of a experimental mouse model of type 1 diabetes. In collaboration with a Consortium on Targeting and
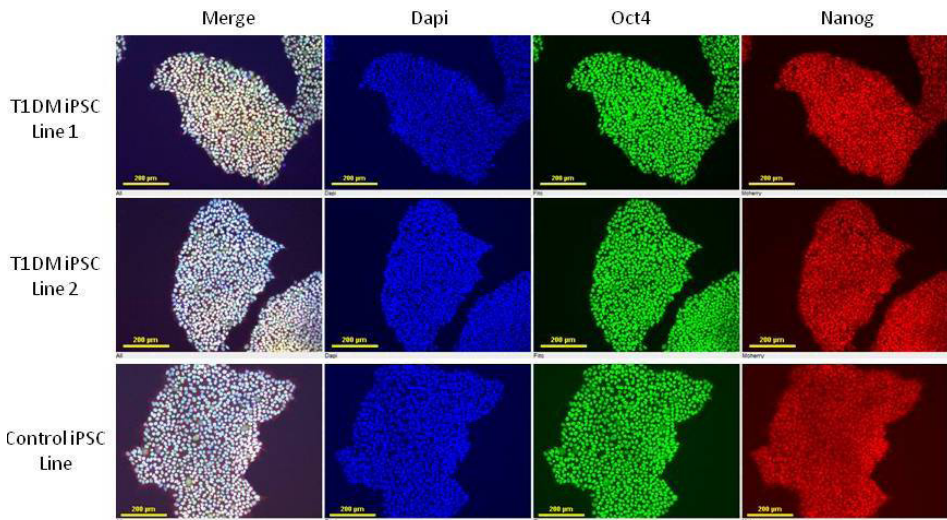
Figure 2. *Pluripotency Marker Expression in T1DM Patient Derived iPS Lines.* (CMAI: Greiner UC4, University of Massachusetts Medical School)

Regeneration (CTAR) team at Vanderbilt University, the group also characterized a new mouse strain for testing the function of implanted human beta cells. Human islets survived and functioned to control blood glucose levels when transplanted into these mice. When the mice were given a small amount of diphtheria toxin (a poison made by the bacteria that causes diphtheria), the mouse beta cells died, leaving the human beta cells as the only source of insulin for the animals. Thus, the team now has a robust model for studying how human beta cells work in a living system that can be used to study beta cells derived from human stem cells. (For additional figures related to this project see Figure 5).

A "personalized" experimental mouse model incorporates genetically identical immune cells and beta cells from a single type 1 diabetes patient. The CMAI team of investigators at Columbia University took several steps towards developing such an individualized model of type 1 diabetes biology (Figure 3). They
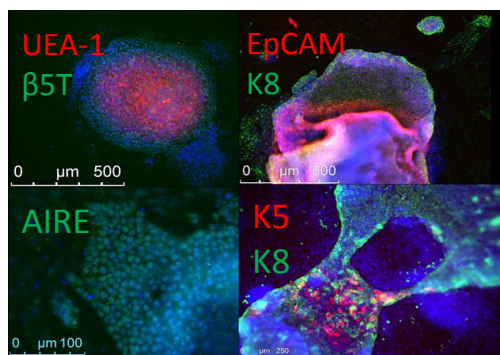

Figure 3. *Differentiation of thymic epithelial cells (TEC) from embryonic stem (ES) cells. TEC markers shown by immunofluorescence at day 30 of our TEC differentiation protocol from human ES cells. (*CMAI: Sykes UC4, Columbia University)

found new strategies for recreating a human immune system in mice using stem cells derived from bone marrow of individuals with type 1 diabetes. In addition, the team tested new ways to produce beta cells from human stem cells and to verify their insulin-producing capability by transplanting these human tissues into mice. The group established multiple partnerships with other CMAI teams, as well as those in CBDS and CTAR, in order to fully characterize these stem cells and stem cell-derived beta cells from multiple angles. Their thorough and collaborative approach to building a personalized model of type 1 diabetes benefits the entire research community interested in beta cell replacement therapies. (For additional figures related to this project see Figure 6 and 7).

The CMAI team at the University of Colorado Denver made significant progress identifying human T cell receptors found in islets of organ donors with type 1 diabetes in collaboration with other CMAI groups (Figure 4). These receptors can now be incorporated into experimental mouse models to understand their role in autoimmunity and beta cell death. Importantly, the team could find T cells that recognize insulin in the pancreatic islets but not in the bloodstream. This illustrated the benefits of investigating the actual pancreatic site where islet injury occurs as a means of discovering human T cells involved in the disease process. This key finding is one of the first identifications of bona fide insulin-specific T cells found in islets from multiple donors and opens up new avenues for exploration of how these immune cells actually target and destroy beta cells (For additional figures related to this project see Figure 8 and 9).

Functional Analysis of Cloned TCR Genes

Generate retroviral vectors encoding T cell receptor genes

Generate "artificial" T cells
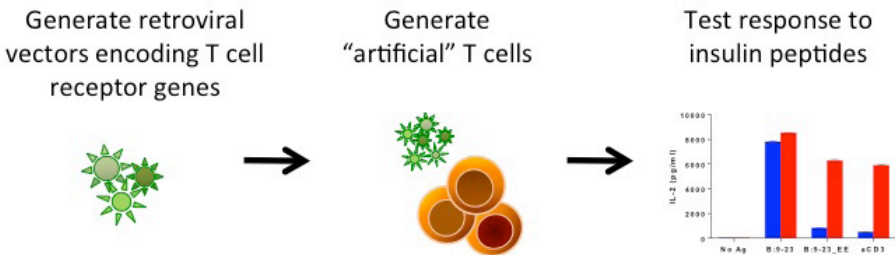
Test response to insulin peptides

Figure 4. *Schematic depicting the experimental approach for isolating, purifying, and single cell sequencing of human TCRs derived from isolated pancreatic islets from T1D cadaveric donors.* (CMAI: Gill UC4, University of Colorado Denver)

THE FUTURE: SEPARATING "SELF" FROM "NON-SELF" IN EXPERIMENTAL MODELS OF TYPE 1 DIABETES

Looking ahead to Year 3, the CMAI will build on these advances with the addition of a fifth research team, based at the University of California, San Francisco. This team of three investigators will contribute their innovative method for differentiating human stem cells into a type of cell that can mature into functional thymus tissue after transplantation into mice. (The thymus gland, located near the heart, is a central organ of the immune system where T cells learn to distinguish between "self" [a person's own cells] and "non-self" [abnormal cells, such as cancer or bacteria]. This learning process malfunctions in autoimmune diseases, including type 1 diabetes.) This unique tool represents a crucial step in creating fully experimental mouse models of autoimmune type 1 diabetes that can be used to understand the autoimmune process and find ways to intervene in it.

CMAI INVESTIGATORS, YEAR 2

Megan Sykes, M.D., Investigator, *Columbia University*

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Robin Goland, M.D., Co-Investigator, *Columbia University*

Hans Snoeck, M.D., Co-Investigator, *Columbia University*

Yong-Guang Tang, M.D., Ph.D., Co-Investigator, *Columbia University*

Ronald Gill, Ph.D., Investigator, *University of Colorado Denver*

Peter Gottlieb, M.D., Co-Investigator, *University of Colorado Denver*

John Kappler, Ph.D., Co-Investigator, *National Jewish Medical and Research Center*

Aaron Michels, M.D., Co-Investigator, *University of Colorado Denver*

Maki Nakayama, M.D., Ph.D., Co-Investigator, *University of Colorado Denver*

Clayton Mathews, Ph.D., Investigator, *University of Florida*

Todd Brisko, Ph.D., Co-Investigator, *University of Florida*

Jing Chen, Ph.D., Co-Investigator, *University of Florida*

Alexei Savinov, M.D., Co-Investigator, *Sanford Research/ University of South Dakota*

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Mark Wallet, Ph.D., Co-Investigator, *University of Florida*

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Rita Bortell, Ph.D., Co-Investigator, *University of Massachusetts Medical School*

Michael Brehm, Ph.D., Investigator, *University of Massachusetts Medical School*

David Harlan, Ph.D., Co-Investigator, *University of Massachusetts Medical School*

Rene Maehr, Ph.D., Co-Investigator, *University of Massachusetts Medical School*

Douglas Melton, Ph.D., Investigator, *Harvard University*

Leonard Shultz, Ph.D., Investigator, *The Jackson Laboratory*

Derrick Rossi, Ph.D., Investigator, *Children's Hospital Corporation*

*Mark Anderson, M.D., Ph.D., Investigator, *University of California, San Francisco*

Jeffrey Bluestone, Ph.D., Investigator, *University of California, San Francisco*

Matthias Hebrok, Ph.D., Investigator, *University of California, San Francisco*

*New team to be added in Year 3

ADDITIONAL FIGURES ILLUSTRATING CMAI RESEARCH
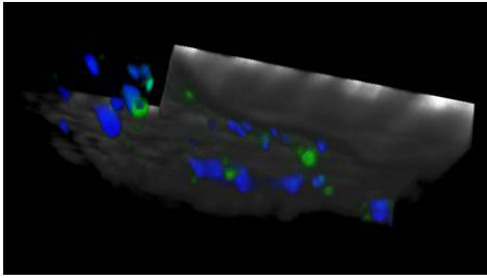
Blue: FUT6 modRNA transfected
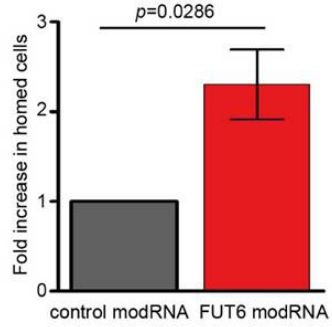Green: Control modRNA transfected
Gray: Bone

Figure 5. *Human HSCs modified using FUT6 modified-mRNA (blue) enhances homing and extravasation of transplanted hiPS-HSPCs into the calvarium of NSG mice (gray) compared to control human iPS-derived HSCs (green)*
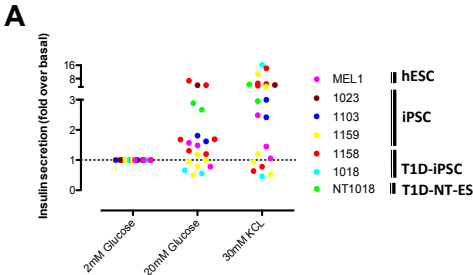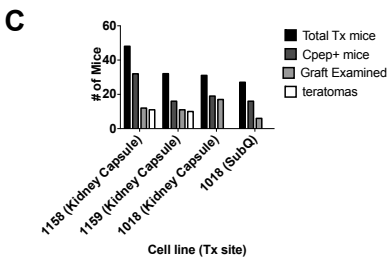(CMAI: Greiner UC4, University of Massachusetts Medical School)

Figure 6. *Function of beta cells from induced pluripotent stems (iPSC). a. Induced insulin secretion in vitro b. Human insulin secretion in vivo. c. Summary of graft function and teratoma formation in vivo.* (CMAI: Sykes UC4, Columbia University)
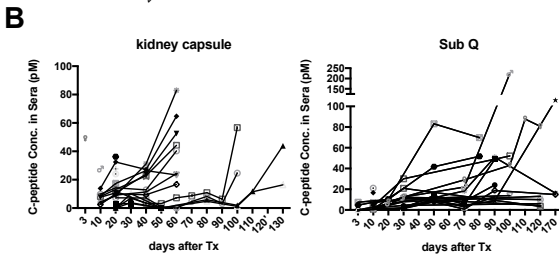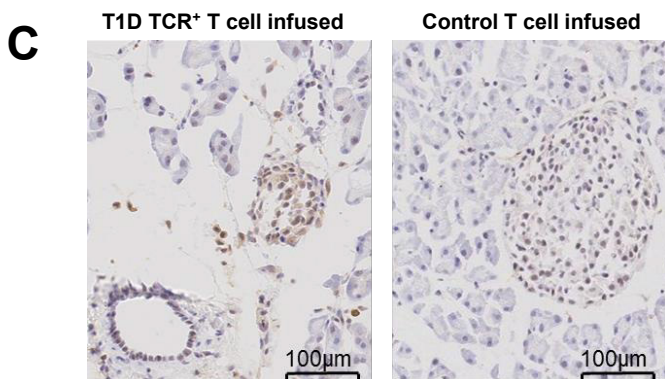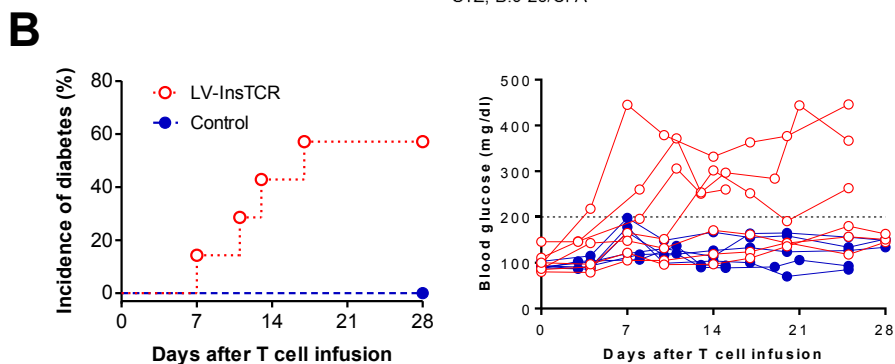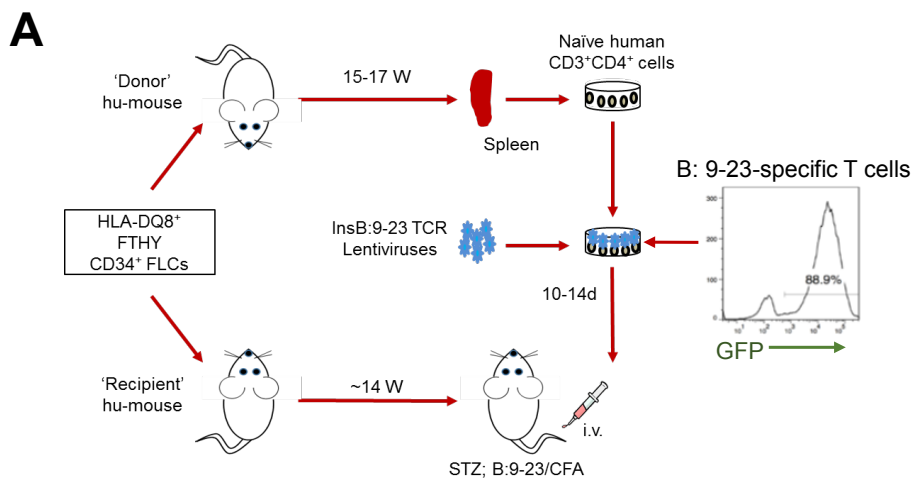
Figure 7. *Induction of diabetes by adoptive transfer of human T cells expressing an insulin-specific transgenic T cell receptor. a. Scheme for producing transgenic T cells from experimental mice and transferring them to mice with human immune systems made with the same stem cells. Transduction efficiency at day 14 is shown. b. Diabetes development in mice infused with transgenic (red) vs. control T cells (blue). c. Human T cell infiltrates in diabetic but not control pancreas.* (CMAI: Sykes UC4, Columbia University)

Single Cell PCR of Islet-Associated CD4 T Cells
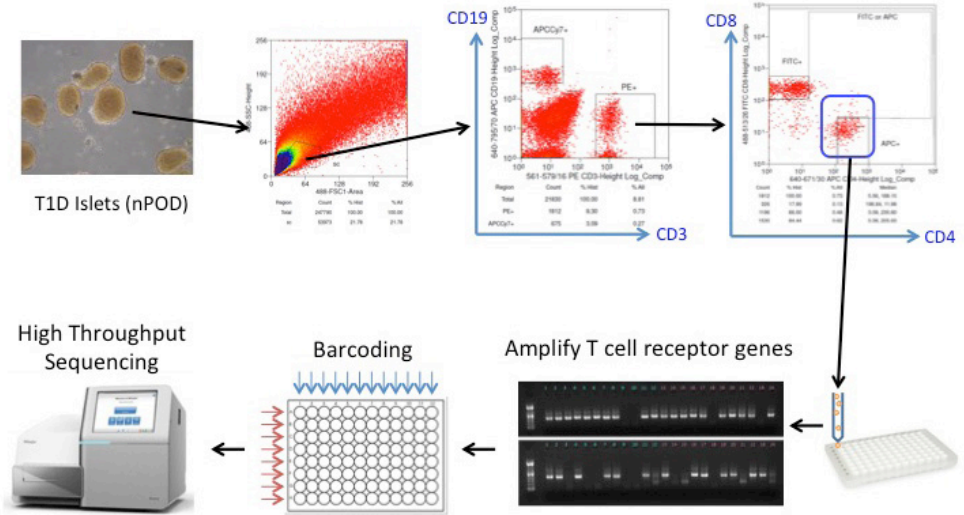
Figure 8. *Schematic depicting the experimental approach for isolating, purifying, and single cell sequencing of human TCRs derived from isolated pancreatic islets from T1D cadaveric donors.* (CMAI: Gill UC4, University of Colorado Denver)

Proinsulin-reactive T cells
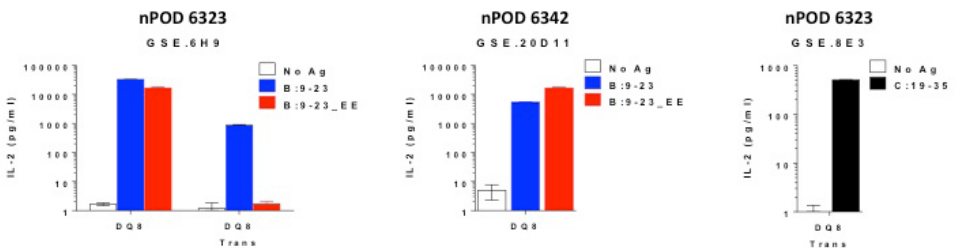
Figure 9. *HLA DQ8-restricted, insulin peptide reactive TCRs derived from T1D islets. Note that insulin-specific TCR from nPOD donor 6323 responds to peptide presented by DQ8 trans.* (CMAI: Gill UC4, University of Colorado Denver)

CONSORTIUM ON TARGETING AND REGENERATION (CTAR)

OVERVIEW: REGENERATING INSULIN-PRODUCING BETA CELLS IN PERSONS WITH TYPE 1 DIABETES

Developing strategies to replenish or protect the pancreatic beta cells that are lost or being lost in people with type 1 diabetes (or in those with insulin-dependent type 2 diabetes) is a high priority for diabetes research. Beta cells resulting from such regenerative strategies must be able to survive the autoimmune processes that has destroyed most or all original beta cells and also produce insulin in response to a person's diet and activity — exactly as beta cells work in those without diabetes. The Consortium on Targeting and Regeneration (CTAR) is tackling these problems head-on by learning how to change non-beta cells into insulin-producing cells, designing methods to selectively target molecules to the pancreas that can help replace or regrow beta cells, and developing new drugs to block beta cell death. In Year 2, CTAR investigators capitalized on the highly collaborative Network environment to make exciting new advances towards their goal of beta cell regeneration to prevent or reverse diabetes.

RESTORING INSULIN PRODUCTION AND PROTECTING ISLETS FROM IMMUNE DESTRUCTION

The CTAR team at University of Geneva (UG) worked with the Oregon Health & Science University (OHSU) CTAR team on a process for
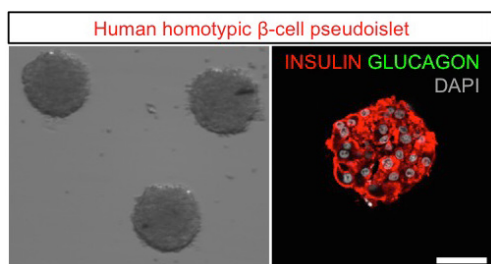
Figure 1. *Human homotypic (β-cell-only) pseudoislets Human β-cells can be sorted by flow cytometry with high purity (99%) and reaggregated in vitro. Such pseudoislets are stable and β-cells display normal glucose-dependent insulin secretion, comparable to native islets.* (CTAR: Herrera UC4, University of Geneva)

splitting up human pancreatic islets and sorting them into four sets containing exclusively alpha, beta, delta, or PP cells. The UG team then identified one approach for reprogramming normal alpha cells so that nearly 40 percent of those cells, which make the hormone glucagon, turned into cells that also produce insulin and become bihormonal (Figure 1). The Stanford University

and UG CTAR teams collaborated to identify genes that maintain islet alpha cell fate and function. They identified a second approach for reprogramming alpha cells into cells remarkably similar to beta cells in gene expression, electrophysiology, and regulated insulin secretion. Together, collaborative work by these teams has demonstrated the flexibility of alpha cell programming, establishing proof-of-principle for a possible future therapy to convert alpha cells into beta cells in persons with diabetes.

The OHSU team discovered the existence of four different kinds of beta cell subsets and collaborated with investigators in the Consortium on Modeling Autoimmune Interaction (CMAI) on a project utilizing pancreatic islets isolated from organ donors with type 1 diabetes (Figure 2). This project will clarify whether all beta cells are equally sensitive to autoimmune attack or whether there are more sensitive and hardy subtypes. This knowledge could be used to make beta cells more resistant to autoimmune attack.
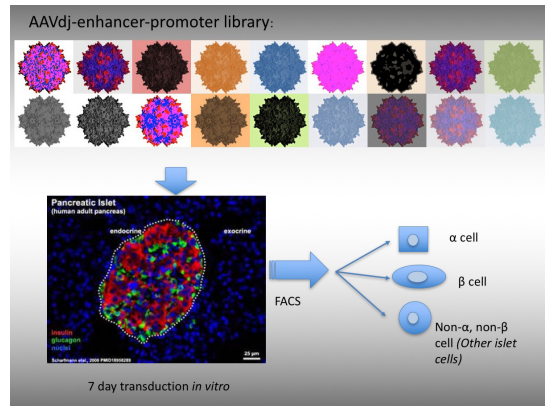
Figure 2. *Schematic of our alpha- and beta-cell promoter screen. A library of rAAV vectors, each containing a different candidate promoter and RNA bar-code is used to transduce human islets, followed by FACS isolation of the different endocrine subtypes. RNA-Seq is then used to identified the promoters with the highest activity and cell-type specificity.* (CTAR: Grompe UC4, Oregon Health and Science University)

CTAR investigators based at the University of Pennsylvania (Penn), Stanford University, and Vanderbilt University investigated changes in the state, function, and gene expression of islet cells during the human aging process and found important differences in adult and young islets. Juvenile human beta cells can replicate themselves, while adult beta cells have lost that ability. Investigators at the Icahn School of Medicine at Mt. Sinai catalogued differences in gene activity among normal adult beta cells and insulinomas (a type of pancreatic tumor caused by excessive, uncontrolled growth of beta cells) and also found that a group of small molecules known as "harmines" stimulate beta cell

replication. Thus, these studies might identify new harmine-based drugs that can drive human beta cell regeneration in individuals with type 1 diabetes (Figure 3).

Figure 3. *Expression of age-related transcription factor, SIX3, in human beta cells.* (CTAR: Powers UC4, Vanderbilt University)

One of the most striking outcomes from CTAR research in Year 2 is the growing appreciation that beta cells are not all alike — in fact, several subtypes of beta cells have been discovered. As discussed above, the OHSU investigators identified four distinct su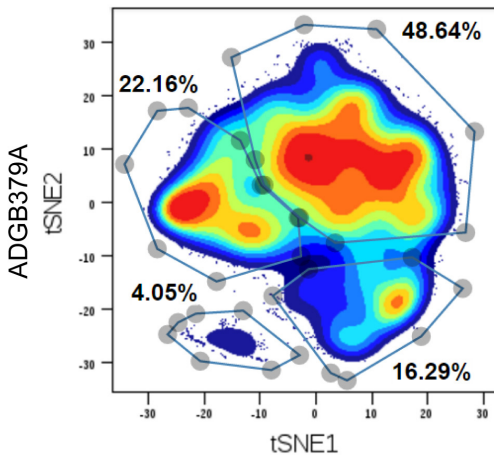btypes of beta cells in adult human islets. These four populations have unique gene expression patterns and differ in their ability to release insulin in response to glucose. At Penn, investigators used a technology that allowed them to individually examine each cell in an islet. By this analysis, three major types of beta cells could be distinguished (Figure 4). Combined with the studies mentioned above regarding changes in beta cell biology during the aging process, these studies reveal that human beta cells are more complex and dynamic than previously thought.

Figure 4. *Beta-cells contain multiple subtypes, with proliferating cells concentrated within one cluster.* (CTAR: Kaestner UC4, University of Pennsylvania)

The recognition that human beta cells exist in multiple subtypes suggests that some beta cells might be more or less susceptible to immune system destruction or more amenable to regeneration therapies. (For an additional figure related to this project see Figures 6 and 7).

At the University of Alabama Birmingham, a CTAR group discovered that a blood pressure control drug also reduces expression of a gene, called TXNIP, involved in beta cell death. This year, the team generated new molecules based on this drug that are 100-fold more effective at reducing TXNIP expression in beta cells (Figure 5). Remarkably, these
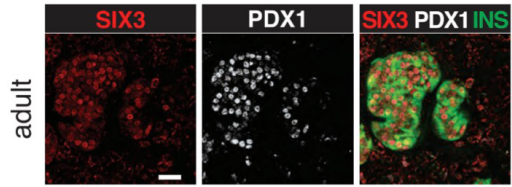
new molecules also protect beta cells from increases in TXNIP expression triggered by the autoimmune process in type 1 diabetes. (For an additional figure related to this project see Figure 8). In parallel, the team interacted closely with investigators from the Consortium on Beta Cell Death and Survival (CBDS) with the goal of discovering new ways to measure human beta cell numbers and beta cell death. Such measures will be

Figure 5. *Human TXNIP promoter activity in response to verapamil, TI-3 and new TI as assessed by dual luciferase assays.* (CTAR: Shalev UC4, University of Alabama Birmingham)

essential for future clinical trials of TXNIP-inhibiting drugs to prevent or reverse beta cell loss in people with type 1 diabetes.

COLLABORATION CREATES NEW RESOURCES FOR THE TYPE 1 DIABETES RESEARCH FIELD

Throughout Year 2, CTAR members actively collaborated on the development of new technologies, tools, and reagents for diabetes research. For example, several teams from CTAR and CMAI jointly created a consortium of researchers who use a new technology, known as "CyTOF (cytometry by time of flight)," to study individual cells within complex cell populations — such as those found in pancreatic islets or in the immune system. This important partnership will speed up the use of state-of-the-art CyTOF technology in beta cell and autoimmunity research. In addition, CTAR teams at OHSU and Penn collaborated on the development of viruses that can preferentially infect human alpha or beta cells — meaning that they could potentially deliver new genes for alpha cell reprogramming or beta cell regeneration to only those specific cells in an individual with diabetes. This technology opens up the possibility of innovative gene therapy approaches to beta cell replacement.

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Peng Wang, Ph.D., Co-Investigator, *Icahn School of Medicine at Mt. Sinai*

ADDITIONAL FIGURES ILLUSTRATING CTAR RESEARCH

Figure 6. *Single-cell RNA-seq of human islets identifies pancreatic cell types and gene signatures.* (CTAR; Kaestner UC4, University of Pennsylvania)

Figure 7. *Single-cell mass cytometry analysis of the human endocrine pancreas. Cover art: Cell metabolism.* (CTAR: Kaestner UC4, University of Pennsylvania)

Figure 8. *Effects of new TIs on T1D-associated inflammatory cytokine-induced TXNIP expression in human islets; n=3; *p<0.05.* (CTAR: Shalev UC4, University of Alabama Birmingham)

ACKNOWLEDGMENTS

APPENDIX 1
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APPENDIX 2

OPPORTUNITY POOL PROJECTS

	TITLE	PI	INSTITUTION
CHIB	Dec 2015 Investigator In Person Meeting	Cherie Stabler	University of Florida
CBDS	Mass spectrometry-based proteome maps for human islet cells	Wei-Jun Qian	Pacific Northwest National Laboratory
CTAR		Klaus Kaestner	University of Pennsylvania
CMAI	Characterization of in silico reconstruction of TCRs for modeling autoreactive T cells in T1D	Todd Brusko	University of Florida
		Sally Kent	University of Massachusetts Medical School
		Maki Nakayama	University of Colorado
CTAR	Antibodies for beta-cell subtype identification by immunohistochemistry*	Markus Grompe Philip Streeter	Oregon Health & Science University
CBDS		Mark Atkinson	University of Florida
CHIB	Quantitative mass spectrometry analysis of human islet and pancreas ECM*	Karen Christman	University of California, San Diego
		Kirk Hansen	University of Colorado
CBDS	Workshop for Continued Harmonization of Beta Cell Death Assays*	Carmella Evans-Molina	Indiana University
CHIB		Camillo Ricordi	University of Miami
CHIB	Generation of reporter stem cell lines to allow quantification of endocrine differentiation and functional analysis at the single cell level	Paul Gadue	Children's Hospital of Philadelphia
CMAI	Exploiting the power of CyTOF/ mass cytometry (MC) to elucidate the complex interactions of islet and immune cells in human type 1 diabetes pancreata*	Clayton Mathews	University of Florida
CTAR		Dirk Homann Andrew Stewart	Icahn School of Medicine at Mt. Sinai
CHIB	Real-time Detection of Insulin Surrogate Markers within Physiomimetic Islet Microsystems*	Ashu Agarwal Alejandro Caicedo	University of Miami

* Includes collaboration with investigators or groups outside of the HIRN.

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