HUMAN ISLET RESEARCH NETWORK (HIRN):

YEAR 3 EXECUTIVE SUMMARY REPORT

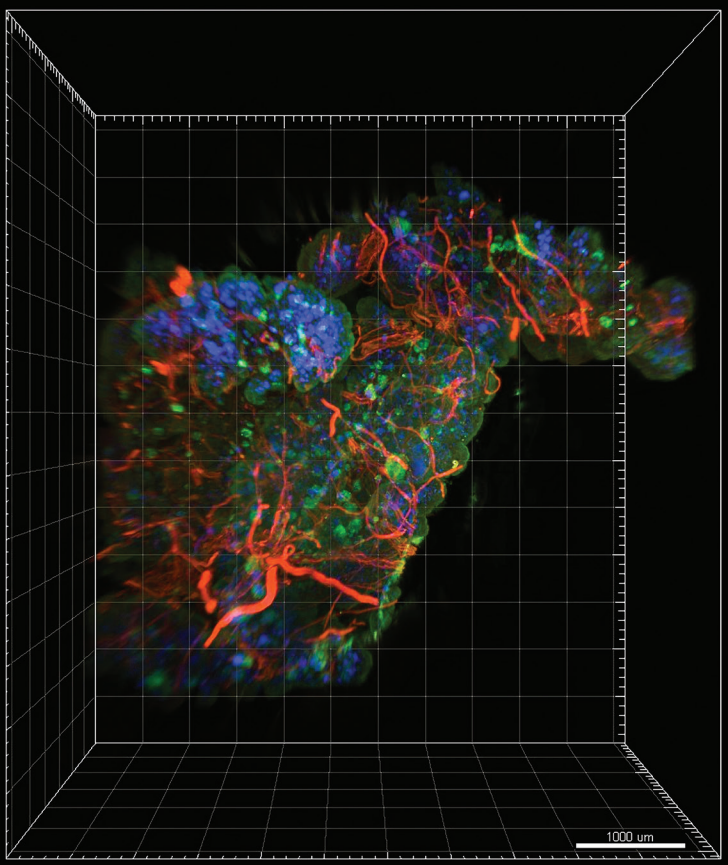

Photo Caption: 3-D projection of light-sheet analysis of a large block of perinatal human pancreas tissue, which shows the arrangement of TH (tyrosine hydoxylase)-positive neurons and their projections, glucagon-producing cells (green) and insulin-producing cells (blue). Tissue from Vanderbilt (Powers Lab) was hydrogel-embedded and immunolabeled at Vanderbilt (Wright Lab) and shipped to CalTech (Gradinaru Lab) for imaging on a custom-built light-sheet fluorescence microscope.

Photo Credit: *Min Jee Jang (Gradinaru Lab, Biology and Biological Engineering Division, Caltech), Jeff Duryea and Brenda Jarvis (Wright Lab, Dept. of Cell & Developmental Biology, Vanderbilt University)*

TABLE OF CONTENTS

FOREWORD

Type 1 diabetes (T1D) is an autoimmune disease caused by the destruction of insulin-producing beta cells in the pancreas. For reasons that are still being studied, in some individuals who are genetically at risk for T1D, the immune system fails to recognize the beta cells as "self" — that is, as part of the person's own body. Instead, in individuals with T1D, immune cells work to destroy beta cells in a manner similar to the way a bacterial or viral infection might be eliminated. Researchers believe that many cases of T1D-related autoimmunity may actually begin in the very first few years of life, at the same time that the cellular structure of the pancreas and the proper number of beta cells are being established. During this period, beta cells continue to duplicate themselves or to arise from progenitor (parent) cells and a tug of war ensues between the immune system and beta cell defenses. If too many beta cells are lost, the body is no longer provided with sufficient insulin, a hormone that is required for the body to use glucose (sugar) as fuel. Without insulin, the person cannot process the glucose taken in from food and T1D develops. A serious and lifelong disease, T1D often begins at a young age. Indeed, nearly 18,000 children and adolescents are newly diagnosed each year in the United States alone.

From the moment of diagnosis, individuals with T1D, or their caregivers, must undertake a complex management routine, replacing the person's own insulin either by multiple injections every day or by continuous infusion using an insulin pump. Their insulin needs must be balanced against multiple factors, including diet and physical activity. Since the discovery of insulin almost 100 years ago, researchers have made tremendous advances in understanding T1D and refining insulin therapy. Continuous glucose monitors, insulin pumps, better insulin formulations and now, automated insulin delivery systems (that is, an artificial pancreas) have all lessened the daily burden of T1D and dramatically improved the health of those living with the disease. Yet, current insulin therapy does not eliminate beta cell autoimmunity nor does it restore beta cells in the pancreas and there is always the possibility of life-threatening hypoglycemia when too much insulin is given.

In 2014, the National Institutes of Health (NIH) created the Human Islet Research Network (HIRN) with the goal of accelerating state-of-the-art research and discovery in T1D. The Network is charged with discovering how beta cells are lost to autoimmunity and finding innovative solutions for functional restoration or replacement of beta cells. The HIRN is comprised of five consortia and an Administrative Hub and employs a collaborative "team science" approach to T1D research. Frequent, open communication and sharing of data, resources and technologies are hallmarks of the HIRN that reflect this community's commitment to finding better treatments and, ultimately, a cure for T1D as rapidly as possible. Importantly, HIRN operates with a grassroots leadership paradigm, using investigator-driven committees to make decisions and set goals for the Network as a whole and within each consortium.

In Year 3, the HIRN consists of an international group of 110 investigators and co-investigators with broad expertise covering beta cell biology, immunology, bioengineering, advanced imaging, bioinformatics and other groundbreaking technologies. HIRN scientists generated new insights in many important areas in this year of the program: the identification of new biomarkers of beta cell injury and death, possibly yielding new ways to predict risk or identify the earliest stages of T1D before symptoms become manifest; the development of advanced microdevices that enable pioneering research on beta cell survival, growth and death; the engineering of *in vivo* models that can host human beta cells and human immune cells for vital research on autoimmunity in T1D; and innovative, new understanding of how beta cell proliferation declines during aging, pointing to additional strategies for beta cell restoration. The Human Pancreas Analysis Program, newly added to HIRN in Year 3, continues the HIRN focus on human T1D by performing multiplexed analysis of extremely rare samples of human pancreata from individuals with T1D or at high risk for T1D. HIRN consortia research projects collectively used more than 500 novel resources (including antibodies, datasets, protocols, technologies) that are shared freely across and beyond the Network for the advancement of T1D research. Finally, following through on the plan made at HIRN's inception, leadership changes within consortia and the HIRN Trans-

Network Committee in Year 3 are enriching the Network by providing new and unique perspective to the HIRN leadership as the Network grows and evolves.

On behalf of the HIRN research community, we are excited to release this *HIRN Year 3 Executive Summary Report*. We hope that all those with an interest in T1D share our enthusiasm for the progress made in the past year and for the very promising research avenues now being pursued. We look forward to a day when beta cell protection or replacement is a safe, effective and routine therapy to prevent or reverse T1D.

Sincerely,

The HIRN Trans-Network Committee (TNC)

***Mark Atkinson, Ph.D.**, Past TNC Chair and CBDS Representative*

***Alvin C. Powers, M.D.**, TNC Chair and CTAR Representative*

***Kristin Abraham, Ph.D.**, NIH Program Staff*

***Ashu Agarwal, Ph.D.**, CHIB Representative*

***Olivier Blondel, Ph.D.**, NIH Program Staff*

***Todd Brusko, Ph.D.**, CMAI Representative*

***Dale Greiner, Ph.D.**, Past CMAI Representative*

***John Kaddis, Ph.D.**, Bioinformatics Center Representative*

***Klaus Kaestner, Ph.D.**, HPAP Representative*

***Joyce Niland, Ph.D.**, Coordinating Center Representative*

***Layla Rouse, M.S.**, Project Manager*

***Sheryl Sato, Ph.D.**, NIH Program Staff*

***Lori Sussel, Ph.D.**, CBDS Representative*

THE HUMAN ISLET RESEARCH NETWORK:

HIRN: TEAM SCIENCE TO ADVANCE TYPE 1 DIABETES RESEARCH

The mission of the Human Islet Research Network (HIRN) is *to better understand how human beta cells are lost in type 1 diabetes (T1D) and to find innovative strategies to protect or replace functional beta cell mass in diabetic patients*. Since its establishment in 2014, the HIRN has vigorously pursued that mission through the pioneering research and resource development of multidisciplinary, multi-institutional consortia of outstanding T1D investigators. In Year 3, the Network included five consortia: the Consortium on Beta Cell Death and Survival (CBDS), the Consortium on Human Islet Biomimetics (CHIB), the Consortium on Modeling Autoimmune Interactions (CMAI), the Consortium on Targeting and Regeneration (CTAR) and the newly created Human Pancreas Analysis Program (HPAP). As of September 30, 2017, a total of 110 investigators and co-Investigators, representing 25 National Institutes of Health (NIH)-funded research projects and two administrative teams participated in the HIRN. Each consortia has continued to build on past successes to increase our knowledge of the human islet with the ultimate goals of better treating and curing T1D. Highlights of accomplishments are described in the "Summary of Significant Progress" chapter and additional information on scientific progress in HIRN's third year is presented in the individual chapters for each consortium.

The HIRN is administered by a Coordinating Center (CC) located at City of Hope in California. The HIRN CC manages the day-to-day operations of the entire Network with open, transparent processes to ensure that HIRN functions as a resource for the broader T1D research and patient communities. A primary goal of the HIRN CC is to foster opportunities and resources that promote scientific collaboration and communication among HIRN investigators who represent research institutions across the country and around the world. To accomplish this goal, the CC pursued multiple efforts in Year 3, including organizing an annual Network-wide investigator meeting and consortia-specific meetings as needed; coordinating online research resources in collaboration with the Bioinformatics

Center (BC; see below); implementing and facilitating the logistics for HIRN Working Groups (Appendix 1); and maintaining the website (hirnetwork.org) and social media accounts (Twitter, Facebook) with updates of scientific discoveries for the benefit of the wider research community and people affected by T1D. Additionally, the CC collates and displays over 200 HIRN publications which can be viewed at **https://hirnetwork.org/all-hirn-publications**

The CC works closely with the Trans-Network Committee (TNC), a network-wide leadership group comprised of representatives from each consortium, as well as from the CC, BC, and NIH program staff. The TNC facilitates communication across the Network and oversees Trans-Network activities and decision-making.

The HIRN BC, also located at City of Hope, provides bioinformatics capability and infrastructure to support the Network. This function is vital for HIRN investigators to be able to take advantage of the most up-to-date, high-throughput technologies that often generate massive amounts of data for analysis. The BC develops and/or hosts resources to assist investigators with long-term sharing, as well as maintenance and management of HIRN-developed resources, including datasets, technologies, documents and bioreagents.

PROMOTING COLLABORATION AND COMMUNICATION ACROSS THE NETWORK

Highlights of some of the HIRN CC and BC efforts to connect investigators across the Network and with the public, in Year 3, are described below.

HIRN COORDINATING CENTER

HIRN 2017 Annual Investigator Meeting

The HIRN Annual Investigator Meeting is an opportunity for HIRN investigators and their lab members from across the Network to meet for a robust exchange of ideas and information. The Year 3 event, planned by the Annual Meeting Planning Group (Appendix 1), was held in Bethesda, Maryland, in March 2017. A total of 213 HIRN investigators and trainees, NIH staff, HIRN CC and BC staff and invited guests from dkNET (the NIDDK Information Network), the Integrated Islet

Distribution Program, JDRF, the Network for Pancreatic Organ Donors with Diabetes and the T1D Exchange participated in the meeting, a 15 percent increase from 186 attendees in 2016.

Two days of scientific sessions included keynote presentations from members of the HIRN External Scientific Panel: Matthias von Herrath of the La Jolla Institute for Allergy and Immunology gave a talk entitled "Update on Human Pathology of Type 1 Diabetes and Treatment Considerations" and Chris Newgard of Duke University presented "Novel Pathways for Expansion of Functional Beta Cell Mass." Breakout sessions gave attendees a deep dive into key topics: biomarkers and diagnosis, disease modeling platform for beta cell immune interactions, human immunity to islets, *in vitro* modeling of autoimmune interactions between beta cells and the immune system, the alpha cell in T1D, and new approaches to therapies. In addition, 90 posters (increased from 65 posters in 2016) were displayed and discussed during the poster sessions. Ample time was set aside for investigators to meet within their own research teams and consortia, as well as with others across the HIRN, its advisors and guests, in order to foster new ideas for scientific collaboration to advance the HIRN mission.

The CC held a workshop on "Social Media for Scientists", 88 percent of attendees evaluated the workshop as "excellent/very good." The presentation slides from the workshop were shared with a HIRN investigator at the University of Florida to use as part of an ethics in research lecture series.

CHIB 2016 Investigator Meeting

The four research teams that participated in the CHIB in Year 3 are each working toward the goal of developing new technologies and devices for the study of human islets and beta cells in the laboratory setting. These teams communicate closely and regularly to exchange information about challenges and progress they've made in engineering these devices and developing new assays to probe islet function. In October 2016, the CC assisted in the planning, organization and travel logistics for a CHIB in-person meeting in Philadelphia, with guidance from the CHIB chair and NIH project scientist. The one-day meeting attracted 46 attendees, including representatives from each of the CHIB teams, the Helmsley Trust, the JDRF, the NIDDK and HIRN CC and BC staff. Members of another related NIH-funded research team at the University of Pennsylvania attended part of this meeting.

HIRN Resource Catalog

The HIRN CC and BC made significant progress toward ensuring that the scientific findings and resources generated by HIRN investigators are preserved, robust, and efficiently exchanged. Two iterations of the HIRN Resource Catalog were released in Year 3, with listings of antibodies, cell lines/cell systems, mouse strains and vectors and research protocols available to all investigators across the Network. This important effort is key to effective scientific collaboration and communication both within and across the HIRN consortia. A total of 506 resources (e.g., bioreagents, datasets, documents and technologies) have been documented by HIRN and can be viewed at **https://resourcebrowser.hirnetwork.org.**

HIRN BIOINFORMATICS CENTER

Information Technology Infrastructure and Services

The HIRN utilizes many different websites, services and technologies to store, share and exchange information. This includes the main HIRN website, as well as applications that facilitate data storage, sharing, analysis, visualization and integration. System administration includes security, domain name management, user registration and data backup and recovery services. There were 131 assets used and maintained by HIRN in Year 3, including servers, storage accounts, public IP addresses, web applications, NoSQL and SQL azure databases. These services are required for the day-to-day operational activities of HIRN.

Data Standards

Whenever information is generated, it has the potential to be re-used by others. Given the specialized nature of the data created and catalogued by HIRN, it is important that the information provided is structured in a way that adheres to emerging and established guidelines. This ensures that these resources are robust and reproducibly used. Using available metadata tools from authoritative aggregating communities, relevant standards or ontologies have been identified and were placed into use during Year 3. Models and frameworks were also established to address the FAIR (Findable, Accessible, Interoperable and Re-usable) and JDDCT (Joint Declaration of Data Citation Principles) guidelines. This activity aids in the preservation of relevant diabetes resources for use by the scientific community in the immediate and long-term future.

Resource Sharing and Data Analysis

Advanced scientific tools, technologies and specialized reagents are used by researchers in HIRN to examine blood, cells, or tissues that play a role in or might be affected by diabetes. Information describing each of these resources are deposited in public repositories and/or cataloged by HIRN (see the HIRN Resource Catalog section). In addition, assistance with data analysis and sharing tasks are offered to HIRN investigators in the form of existing tool support or via customized engineered products. HIRN also reaches out to collaborating organizations, such as the NIDDK's dkNET program to exchange and share resources. Sharing of resources enhances and enriches the science being conducted by HIRN researchers.

EXPANDING THE HIRN COMMUNITY TO ACCELERATE T1D RESEARCH

The HIRN was designed to be a flexible, transparent community of investigators that can evolve to accommodate new investigators, technologies, opportunities and priorities in T1D research. In Year 3, the HPAP consortium joined the Network to fill a critical research need — deep phenotyping of human pancreatic islets and related tissues and cells (e.g., immune cells, blood, lymph nodes) from individuals with T1D autoimmunity. These rare and valuable tissues have the potential to provide essential, new information on the state of islets/beta cells during the early stages of T1D. Data from the extensively characterized tissues and cells collected by HPAP investigators represent a unique resource that the entire HIRN community can use to address innovative questions in T1D research. In addition, a new research team brought a novel perspective to the CMAI in Year 3, focusing on the role of the thymus in the T1D autoimmune process.

Looking to the future, the HIRN welcomes 10 new research teams in Year 4 — five in the CTAR, three in the CBDS and one each in the CMAI and CHIB. For Year 5, the NIH expects to fund new CBDS research teams that have expertise on the discovery of early T1D disease markers in the pancreas, as well as new HPAP teams that can contribute state-of-the-art, high resolution technologies to study the physical and functional organization of human pancreas tissue.

PILOTING INNOVATIVE RESEARCH CONCEPTS IN TYPE 1 DIABETES: OPPORTUNITY POOL PROJECTS

Opportunity Pool Projects (OPPs) allow the HIRN to quickly respond to new T1D research ideas and emerging technologies that could advance the Network's mission. HIRN investigators are invited to propose short-term, innovative projects related to the causes, potential cures, or prevention of T1D. Projects may involve collaboration with HIRN investigators across consortia or with researchers outside of the Network. To date, 17 highly meritorious OPPs have been funded (Appendix 2); three multi-investigator OPPs were completed in Year 3. The HIRN CC manages the OPP process, including organization of application reviews, execution of OPP subawards to investigators' institutions and collection of final progress reports. More information on the HIRN Opportunity Pool Projects can be viewed at **https://hirnetwork.org/consortium/opportunity-pool**

SUMMARIES OF COMPLETED OPPORTUNITY POOL PROJECTS

Mass Spectrometry-based Proteome Maps for Human Islet Cells

Klaus Kaestner (CTAR, HPAP), University of Pennsylvania; Wei-Jun Qian (CBDS), Pacific Northwest National Laboratory

A proteome map is a catalog of proteins made in a particular tissue or cell or under different conditions (e.g., an islet in a person with diabetes versus in a nondiabetic person). The goal of this OPP was to create in-depth proteome maps for whole human islets, as well as for insulin-producing beta cells and glucagon-producing alpha cells. The investigators identified and measured the amount of more than 10,000 proteins in islets from six donors, including alpha and beta cells from three donors. They next compared the catalogs of all proteins found in islets versus other tissues in the body and discovered 530 proteins that were either unique to islets or made in much greater amounts in islets. Further analysis revealed distinctive protein patterns found in beta cells compared to alpha cells. These detailed proteome maps represent a foundational resource for HIRN and the diabetes research community. In the long term, the rich data developed by this OPP can help scientists better define functional beta cells, identify biomarkers of T1D risk, understand how beta cells react to autoimmune attack in T1D, develop strategies for beta cell regeneration or replacement and address other key questions in T1D research.

Characterization of In Silico Reconstruction of TCRs for Modeling Autoreactive T Cells in Type 1 Diabetes

Todd Brusko (CMAI), University of Florida; Sally Kent (CMAI), University of Massachusetts Medical School; Maki Nakayama (CMAI), University of Colorado, Denver

T cells of the immune system are key players in the autoimmune attack that destroys beta cells in people with T1D. Researchers have extensive knowledge about T cells circulating in the blood, but historically, it has been difficult, if not nearly impossible, to study T cells located in human pancreatic islets, at the forefront of the autoimmune process. This OPP focused on isolating and characterizing T cells in the islets of organ donors with T1D. The investigators were able to isolate hundreds of T cells and they found that some of those cells recognized beta cell proteins that are important biomarkers of the T1D disease process — GAD65 and insulin. These findings and ongoing work to identify other T cell targets in the islets will help researchers develop new T1D biomarkers and innovative strategies for the development of drugs to directly block T cell-mediated destruction within the islets. Another key goal of this OPP was to immortalize the islet-specific T cells in a way that would make them available for future research. The team has successfully created "avatars" (copies) of many of these rare, isolated T cells, which are now available for HIRN researchers to explore fundamental issues related to the causes, natural history and treatment of T1D autoimmunity.

Antibodies for Beta Cell Subtype Identification by Immunohistochemistry

Marcus Grompe (CTAR), Oregon Health and Science University; Philip Streeter (Collaborator), Oregon Health and Science University; Mark Atkinson (CBDS, HPAP), University of Florida

Research from HIRN investigators and others has shown that four subtypes of beta cells exist in human pancreatic islets. The objective of this OPP was to develop new tools (antibodies) to study two of these subtypes in more detail. The investigators took several approaches to making new antibodies or identifying existing antibodies that could selectively recognize either CD9 or ST8SIA1 — proteins that each mark one of the beta cell subtypes. They found one antibody made by a biotechnology company (Novus Biologicals) that recognized CD9 on stored human pancreas tissues. Efforts to find a suitable ST8SIA1

antibody are ongoing with other funding. With the CD9 antibody, the investigators can begin to examine key questions about the CD9-beta cell subtype — for example, is this subtype more or less prone to immune attack than other beta cells?

COORDINATING CENTER (CC) AND BIOINFORMATICS CENTER (BC) INVESTIGATORS, YEAR 3

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

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

TRANS-NETWORK COMMITTEE (TNC) MEMBERS, YEAR 3

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*

Klaus Kaestner, Ph.D., HPAP Representative, *University of Pennsylvania*

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*

* Year 4 TNC Chair, **Alvin C. Powers, M.D.,** *Vanderbilt University*

† Year 4 CBDS Representative, **Lori Sussel, Ph.D.,** *University of Colorado, Denver*

‡ Year 4 CMAI Representative, **Todd Brusko, Ph.D.,** *University of Florida*

SUMMARY OF SIGNIFICANT PROGRESS IN THE HUMAN ISLET RESEARCH NETWORK, YEAR 3

This chapter highlights significant scientific and organizational accomplishments of the Human Islet Research Network (HIRN) and its member consortia during the third year of the Network (first year for the Human Pancreas Analysis Program - HPAP). Selected publications are listed where applicable. Additional details and other significant progress in type 1 diabetes (T1D) research for each HIRN consortia are described in other chapters of this Executive Summary Report.

CONSORTIUM ON BETA CELL DEATH AND SURVIVAL (CBDS)

The CBDS made progress in three major areas. One revolved around the groups involved in optimizing beta cell death biomarker assays using circulating methylated genomic DNA, another was related to the development of new technologies to characterize the pathophysiology of human diabetic pancreatic tissues, and a third revealed novel mechanisms related to T1D pathogenesis.

Beta Cell Death Assays

The development of novel biomarkers to assess beta cell death in T1D has been a major focus of several CBDS groups and represents a promising advance that can find its way to the clinic in coming years.[1-4] Three CBDS teams are simultaneously developing assays to use DNA methylation status as a biomarker for beta cell death in T1D. All three groups have published successful use of this technology and it appears to be useful for monitoring the success or failure of islet transplantation. Moving forward, all three groups know that there is need to improve assay sensitivity and specificity in order to reliably apply this approach to the detection of early beta cell death in T1D. With this goal in mind, the CBDS groups are working together to compare efficacy and reproducibility of the different assays. This is a remarkable example of how HIRN has brought scientists together to accelerate discovery in T1D. Results from this working group will be reported in Year 4.

New Technologies

Many CBDS teams are sharing their respective expertise to develop and apply state-of-the-art technologies to facilitate the in-depth

exploration of human pancreatic tissues. Importantly, the HIRN has brought together experts in pancreatic islet biology with leaders in the area of novel technology development to facilitate the adaptation and application of the newest technologies to facilitate the in-depth characterization of islets or the islet microenvironment in pancreatic tissue. This community effort has led to acquisition of new knowledge regarding the etiology of human diabetes, which will significantly impact the way we treat diabetes in the years to come.[5-10]

New Understanding About the Pathogenesis of Type 1 Diabetes

Several CBDS teams made important new findings to clarify novel early immune mediators of beta cell dysfunction and death in T1D.[11-14] This is important not only in the search for novel therapies, but also to indicate possible differences in the biomarkers present early, during the innate immunity phase of the disease and late, during the full adaptive immunity attack on the islets when there is overt insulitis.

CONSORTIUM ON HUMAN BIOMIMETICS (CHIB)

One significant advancement from CHIB has been in the global development of useful platforms for interrogating human islets and pluripotent-cell derived beta cells, *in vitro*. In the future, these platforms will be used for advancing studies in human islet biology, the validation of T1D biomarkers, drug/toxicity testing of diabetes therapies and modeling aspects of human T1D, including beta cell/immune interactions.

Improved Survival and Function of Human Islets in the Laboratory

CHIB investigators generated new information on how to culture human cadaveric islets in their biomimetic devices, maintaining survival and function over the long term (weeks to months, rather than days). They also developed innovative dynamic systems to measure human islet function *ex vivo*. Advancements included continuous and repeated monitoring of glucose-stimulated insulin secretion; improved sensitivity of quantitative insulin assays; and methods to monitor electrophysiological responses to glucose stimulation.

More Efficient Differentiation of Islet Cells from Pluripotent Cells

Human pluripotent cells represent a potential source of replacement insulin-producing beta cells for treatment of T1D, if researchers can learn how to reliably turn on and maintain beta cell-specific programming

in these cells. Toward this goal, CHIB investigators generated new protocols for dispersing and reaggregating pluripotent cell-derived beta and alpha cells in order to increase long-term survival and function. New insulin and glucagon reporter lines were created. The teams also developed novel devices for long-term cell culture that improved the efficiency of differentiating pluripotent cells into beta cells.

Recreating the Islet Microenvironment

Important progress was made in CHIB on recreating the physiologic islet niche by engineering microenvironments that support survival and function of human cadaveric islets, as well as beta cells derived from pluripotent cells, in the laboratory setting. These microenvironments incorporate or mimic a number of diverse elements that support islets in the body, such as mesenchymal cells, vasculature (blood vessels), hydrogel platforms and extracellular matrix components and systems to model and monitor oxygen consumption.

CONSORTIUM ON MODELING AUTOIMMUNE INTERACTIONS (CMAI)

CMAI is developing innovative approaches to model basic aspects of human T1D immunobiology using novel *in vivo* and *in vitro* platforms. In Year 3 of the consortium, investigators in CMAI have published more than 40 manuscripts, including articles in such highly ranked journals as *Diabetes, Immunity, Journal of Clinical Investigation, PNAS* and *Nature Medicine*.

Enhanced Understanding of Human Beta Cell Autoreactive Specificities

The vast majority of experiments defining T1D T cell and B cell autoreactivity to date have derived from clinical studies conducted using peripheral blood from patients.[15-16] Efforts of CMAI investigators, in conjunction with work other investigators in HIRN (HPAP, CBDS) and the Network for Pancreatic Organ Donor with Diabetes consortium, are now providing an unprecedented view into the specificity and function of lymphocytes found in the islets of pancreata obtained from T1D donors. In a project facilitated by the HIRN-BC, data has now been combined into a web-based resource of antigen specificities that can be used to inform experiments devised by CMAI investigators where synthetic lentiviral TCR expression constructs are used to transfer T1D antigen reactivities into *in vivo* models. This CMAI-supported T1D "toolkit" that includes antigen receptor sequences and expression vectors will also be broadly

enabling for the community and for HIRN, as other HIRN consortia seek to: (a) identify new potential biomarkers of disease (CBDS), (b) test interventions aimed at combatting autoimmunity to protect beta cell mass in synthetic systems (CHIB) and (c) to analyze cellular approaches to enhance beta cell regeneration (CTAR).

Synthetic tools and bioinformatics resources — the islet infiltrating lymphocytes noted above have been catalogued in online data sharing repositories (e.g., clonesearch.jdrfnpod.org). Moreover, a growing number of receptors have been manufactured to create synthetic lentiviral TCR expression constructs available in HIRN Resource Catalogue.

Optimizing Human Pluripotent Cell Differentiation and Function in Vitro and in Vivo

In Year 3, CMAI investigators made major advances in enhancing the development of key human cell populations needed to model T1D from human pluripotent cells.[17-19] It is now possible to produce human hematopoietic pluripotent cells, human beta cells using nuclear transfer pluripotent cells in addition to human beta cells from other forms of pluripotent cells and human endothelium and human thymic epithelial cells, paving the way for the development of new "personalized" mouse models that incorporate these major cell populations in Year 4. Year 3 also saw the creation and validation of a suite of molecular tools that enable high fidelity gene targeting of human pluripotent cell lines. Moving forward, these tools are being applied to pluripotent cells obtained from a genotyped cell bank supported by CMAI, in order to create isogenic systems for analysis of T1D physiology and autoreactivity.

Advancing in Vitro and in Vivo Model Systems to Explore Human Autoreactivity in T1D

To date, a considerable amount of progress has been made in refining experimental mouse models for use in T1D research.[20-24] While existing strains are being used routinely to measure the functionality of human islets and of various pluripotent cells and their derivatives (including pluripotent cell-generated beta cells and some immune populations), in the last year, CMAI investigators focused on overcoming key remaining roadblocks in model development. In Year 3, CMAI investigators improved human NK, myeloid and B lymphocyte development in mouse models by incorporating additional human components into

the NSG background, setting the stage for *in vivo* investigation of both human adaptive and innate immune responses in immunity and in T1D in year 4. Moreover, *in vitro* systems to investigate the role of genetic susceptibility in T1D have been developed. Finally, in Year 3, the consortium saw publication of the first demonstration of overt islet-specific autoimmunity in an experimental mouse model and a demonstration that clonal deletion of autoreactive beta cells can occur in experimental mice, paving the way for studies to evaluate mechanisms of human autoreactivity and tolerance induction in Year 4.

CONSORTIUM ON TARGETING AND REGENERATION (CTAR)

All Beta Cells Are Not the Same

Transcriptional profiling of individual islet cells or isolated islet cell populations found that adult beta cells differ in their gene expression pattern and function; at least four beta cell types have been identified.[25-26] Discovery of this beta cell heterogeneity raises a number of interesting questions that CTAR and HIRN investigators are pursuing, such as whether the beta cell composition changes with age, under different physiologic states, or in diabetes.

Enhanced Understanding of Human Islet Cell Proliferation and Plasticity

Human islet cells have a very low proliferative rate and new approaches are needed to safely stimulate proliferation.[27-31] Complementary work by CTAR groups discovered that human beta cells proliferate in normal physiologic states and how beta cells proliferation might be stimulated.

CTAR investigators described age-dependent beta cell proliferation and identified the signaling pathways and mechanisms responsible for the greater human beta cell proliferative rate in young human beta cells compared to adult beta cells. Interestingly, young human beta cells, but not adult beta cells, could be stimulated to proliferate further by treatment with an agonist of the glucagon-like peptide 1 receptor, which is used clinically to treat type 2 diabetes. Responsible mechanisms include calcineurin/nuclear factor of activated T cells (NFAT) signaling, histone modifications and the expression of *SIX2* and *SIX3* transcription factors. This builds on prior work by CTAR investigators showing that agents such as harmine, which target dual-specificity tyrosine-regulated kinase-1a (DYRK1A) and the NFAT family of transcription factors, can induce adult human beta cell proliferation. CTAR Investigators discoverer how to convert alpha cells, which normally produce glucagon,

into insulin-producing cells by forcing the production of two beta-cell specific proteins. Hopefully, this information can be harnessed for therapeutic expansion of human beta cells.

A Genomic Recipe or Wiring Diagram for Human Islet Cell Proliferation Mechanisms

In order to elucidate novel pathways to therapeutic human beta cell proliferation, CTAR investigators collected a large series of very rare, benign human tumors called "insulinomas."[32] They reasoned that insulinomas must hold the genomic "recipe" or "wiring diagram" explaining how these tumors proliferate and continue to produce insulin, yet remain benign. Detailed DNA (30,000 genes) and RNA sequencing (17,000 RNAs) of 38 human insulinomas — the largest series ever described — revealed a large number of novel and unanticipated molecular pathways that control human beta cell proliferation. Several of these pathways were shown to be amenable to therapeutic induction of proliferation in normal human beta cells. The beta cell research world now has an encyclopedia or data mine that will lead to additional novel druggable pathways to human beta cell regeneration in both types 1 and 2 diabetes.

HUMAN PANCREAS ANALYSIS PROGRAM (HPAP)

HPAP established an efficient pipeline for the identification, selection, procurement, processing and analysis of pancreatic tissue, pancreatic islets and immune tissue from organ donors with T1D or autoantibodies indicating a high risk for T1D. Collaborative, interdisciplinary studies involving metabolic, genetic, immunologic approaches and emerging technologies on these tissues and cells are underway.

HIRN BIOINFORMATICS CENTER (BC)

In the past year, the BC made significant advancements toward ensuring that the scientific findings and resources generated by HIRN are preserved, efficiently exchanged, robust and unbiased. A data model and framework was described that employed controlled vocabularies, existing ontologies, emerging and established guidelines, metadata and reporting standards; this was used to complete revisions to the HIRN Resource Registry in accordance with FAIR (Findable, Accessible, Interoperable and Reusable) and JDDCP (Joint Declaration of Data Citation Principles) guidelines. An IT, computing and software development infrastructure was expanded to include maintenance

of different web-based applications for HIRN data storage, sharing, analysis, visualization and integration.

HIRN COORDINATING CENTER

Administrative Infrastructure

The CC has continued to provide optimal administrative infrastructure to the Network, including organization of the Annual Investigator Meeting, execution of Opportunity Pool Project subawards, development of HIRN literature (Executive Summary Report, pamphlets, etc.), maintenance of the HIRN website (including project content, publications, funding notifications and more), outreach to ensure the scientific and lay community are familiar with the advancements of the network and day-to-day related tasks for the entire Network.

Promoting the Sharing of Resources

Two iterations of the HIRN Resource Catalog were released this year, enabling HIRN Investigators to collect, curate and share resources, such as antibodies, cell lines/cell systems, mouse strains, vectors/viruses and protocols.

Footnotes

1 *Transplantation* 2018 Jan 11. DOI: 10.1097 PMID: 29329189

2 *J Diabetes Complications* 2017 Oct 31. pii: S1056-8727(17)31186-8. PMID: 29175121

3 *J Vis Exp* 2016 Dec 21;(118). DOI: 10.3791/54838. PMID: 28060259

4 *Am J Transplant* 2016 Sep 19. PMID: 27643615

5 *Nat Commun* 2018, in press

6 *Nucleic Acids Res* 2018 Jan 25;46(2):e7. PMID:29040675

7 *Cell Syst* 2017 Dec 26. pii: S2405-4712(17)30543-4. PMID: 29289569

8 *PLoS One* 2017 Sep 6;12(9):e0183908. DOI: 10.1371 PMID: 28877242

9 *J Chromatogr A* 2017 Jul 21. pii: S0021-9673(17) 31056-7. PMID: 28765000

10 *Mol Biosyst* 2017 Mar 30. DOI: 10.1039/c6mb00861e. PMID: 28357434

11 *Diabetologia* 2016 Nov;59(11):2448–2458. DOI: 10.1007/s00125-016-4067-4. Epub 2016 Aug 9. PMID: 27506584

12 *Diabetologia* 2017 Apr;60(4):656–667. DOI: 10.1007/s00125-016-4201-3. Epub 2017 Jan 6. PMID: 28062922

13 *PLoS One* 2017 Sep 6;12(9):e0183908. DOI: 10.1371 PMID: 28877242

14 *Diabetes* 2017 Dec;66(12):3061–3071. DOI: 10.2337/db17-0106. Epub 2017 Sep 6. PMID: 28877912

15 *Diabetes* 66:722–734, 2017 PMID:27920090

16 *Nat Med* 22:1482–1487, 2016 PMID: 27798614

17 *J Clin Invest* 127:2433–2437, 2017 PMID: 28481220

18 *Diabetes* 67:26–35, 2018 PMID: 28931519

19 *Front Endocrinol* (Lausanne) 8:276, 2017 PMID: 29093700

20 *Proc Natl Acad Sci U S A* 2017 PMID: 28874533

21 *J Clin Invest* 2017 PMID: 28920919

22 *Front Endocrinol* (Lausanne) 8:276, 2017 PMID: 29093700

23 *Blood Adv* 1:2007–2018, 2017 PMID:29296847

24 *Annu Rev Pathol* 12:187–215, 2017 PMID: 26588186

25 *Nat Commun* 2016 Jul 11;7:11756. DOI: 10.1038/ncomms11756

26 *Diabetes* 2016 Oct;65(10):3028–3038. DOI: 10.2337/db16-0405

27 *J Clin Invest* 2017 Oct 2;127(10):3835–3844. DOI: 10.1172/JCI91761

28 Cell *Metab* 2016 May 10;23(5):909–920. DOI: 10.1016/j.cmet.2016.04.002

29 *Cell Metab* 2016 Oct 11;24(4):616–626. DOI: 10.1016/j.cmet.2016.09.007

30 *Nat Commun* 2017 Oct 3;8(1):767. DOI: 10.1038/s41467-017-00992-9

31 *Nat Med* 2015 Apr;21(4):383–388. DOI: 10.1038/nm.3820

32 *Nat Commun* 2017 Oct 3;8(1):767; DOI.10.1038/s41467-017-00992-9, 2017

CONSORTIUM ON BETA CELL DEATH AND SURVIVAL (CBDS)

THE GOAL: DETECT AND HALT BETA CELL DESTRUCTION IN TYPE 1 DIABETES

Type 1 diabetes (T1D) develops when immune system cells selectively and silently attack and destroy insulin-producing pancreatic beta cells. The Consortium on Beta Cell Death and Survival (CBDS) was established to advance research and technology development that would lead to new strategies to detect and prevent beta cell death in the early stages of T1D development. In Year 3, the CBDS worked to identify and validate biological markers ("biomarkers") of beta cell injury or death that can be measured in blood from people with or at risk for developing T1D. Biomarkers have many potential uses in the future, including uncovering molecular pathways involved in T1D that may be targets for new drug development, diagnosing autoimmunity before the onset of T1D symptoms, and monitoring the effect of therapies to restore/replace beta cells or preserve remaining beta cells in those recently diagnosed with symptomatic disease. Some of the most promising biomarkers are differential methylation patterns of insulin DNA from beta cells. Methylation is a process that fine tunes control of gene expression by placing methyl groups (a small chemical) onto the DNA. In addition, the CBDS created valuable new resources and optimized cutting-edge technologies that enable analysis of human islets and beta cells at an unprecedented level of molecular detail. The high-resolution data generated by these technologies have revealed new pathways of early beta cell development, stress and death that may be targeted for the treatment and, ultimately, a cure for T1D.

IDENTIFYING BIOMARKERS TO DETECT BETA CELL DEATH

A multi-institution team based at the University of Florida and the Hebrew University of Jerusalem measured beta cell DNA circulating in the blood of individuals who had undergone an islet transplant. They discovered that the amount of beta cell methylated DNA 24 hours after the transplant procedure predicted how well the transplanted islets would be working three to six months later, suggesting that the assay is a good biomarker for beta cell death (Figure 1). The team refined their methods, so that they can now measure up to 24 different DNA

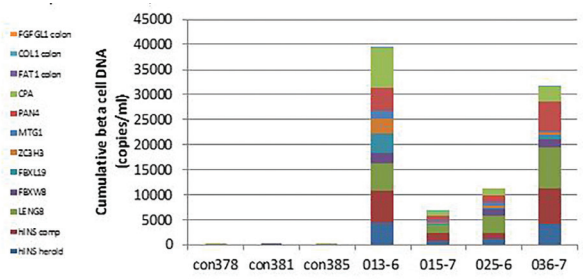

Figure 1. *Multiplex analysis of plasma. Healthy controls vs islet graft recipients were tested for markers of beta cells, exocrine pancreas and colon. Note presence of beta cell DNA, exocrine pancreas DNA, but not colon.* ***(CBDS: Schatz UC4, University of Florida)***

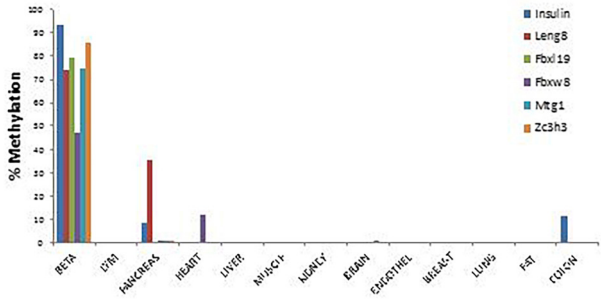

Figure 2. *Specificity of 6 of our beta cell markers. Note that insulin is unmethylated also in colon.* ***(CBDS: Schatz UC4, University of Florida)***

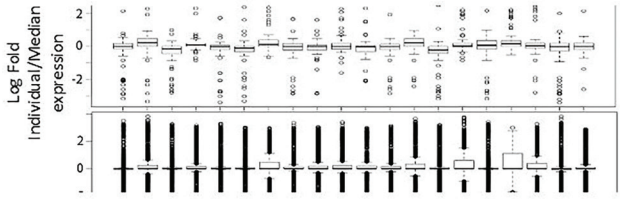

Figure 3. *Relative expression of 239 beta cell-enriched miRs/isomiRs in individual subjects.* ***(CBDS: Schatz UC4, University of Florida)***

methylation markers in a sensitive and specific multiplex assay. Research is ongoing to examine DNA and RNA markers in different cohorts, such as nondiabetic individuals of all ages, children at risk of T1D, people with recently diagnosed T1D, those with longstanding T1D and individuals with hyperinsulinism (excessive production of insulin) or T2D (Figure 2). These studies will help the team gather vital information about beta cell health and death at different stages in the development of T1D (Figure 3).

An international team of researchers coordinated by Indiana University is exploring several potential biomarkers. In Year 3, they showed that the ratio of proinsulin (an unprocessed form of insulin) to C-peptide (a segment of proinsulin that is removed to create active insulin) is a valid biomarker of high T1D risk in people with autoantibodies. In a companion study, the team identified 100 genes with the largest differences — either more or less methylation — in beta cells compared to other cell types. They are using these data to develop a new method for detecting beta cell death. One gene found in this screen, CHTOP, has unusually low levels of methylation in first degree

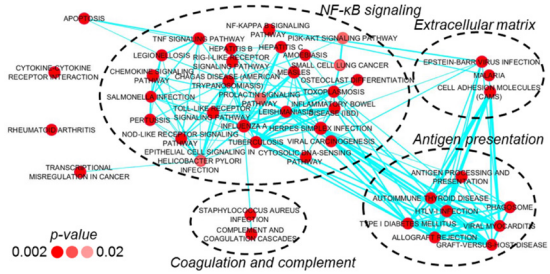

Figure 4. *Image of a human islet exposed to IFN-gamma and IL-1beta for 24 h, followed by staining for reactive oxygen species (CellROX) in red and nuclei (DAPI) in blue.* ***(CBDS: Mirmira UC4, Indiana University)***

relatives of individuals with T1D, indicating that under-methylated CHTOP DNA could also be a new biomarker of T1D risk. Having robust biomarkers for T1D risk helps researchers identify people who could most benefit from potential T1D prevention treatments and offer enrollment them in future clinical trials. Finally, the team discovered a network of proteins and other molecules whose amounts change when human islets are under stress, revealing new targets for biomarker and therapy development (Figures 4 and 5).

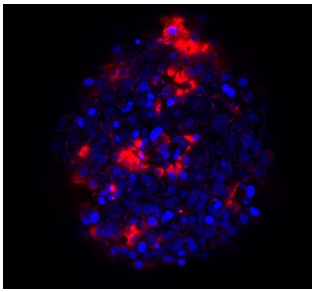

Figure 5. *Shown is the function-enrichment analysis of the differentially abundant proteins in human islets upon IFN-gamma and IL-1beta treatment. The intensity of red colors of the nodes represents how significant the enrichment is while the thicknesses of the edges represent the degree of similarity (shared proteins) between pathways.* ***(CBDS: Mirmira UC4, Indiana University)***

At Yale University and Columbia University, a CBDS team identified several changes in beta cells in response to the autoimmune attack that leads to T1D. They found that methylation of the insulin gene in beta cells correlates with decreased production of insulin as T1D develops. The changes to the insulin gene may fundamentally change how the beta cells are viewed by the immune system. In addition, they discovered that autoantibodies to the beta cell protein prolyl-4-hydroxylase (P4Hb) could be found in 43 percent of individuals with recently diagnosed T1D and 66 percent of those with established T1D. Intriguingly, the presence of autoantibodies for P4Hb could always be detected before the onset of autoantibodies for insulin, suggesting that P4Hb antibodies could be a biomarker of a very early stage of T1D in some individuals. In a third study, the team described a new population of immature beta cells that appears when immune cells begin to

infiltrate the pancreatic islets. These new beta cells seem to be in an immature state that is resistant to immune attack and death. Future research will focus on identifying the source of this cell population and exploring whether it can be used to generate new, fully functional beta cells (Figure 6).

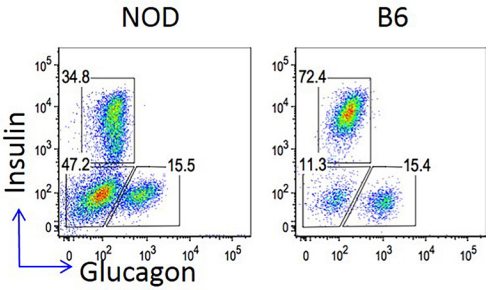

Figure 6. *Beta cells in normal B6 mice and in prediabetic NOD mice. Islets were harvested from B6 mice and NOD mice of 9 weeks of age. Islets were handpicked and dissociated. The islet cells were identified by staining with anti-insulin and anti-glucagon antibodies. Compared to B6 mice, beta cells from NOD mice have a reduced frequency of beta cells and the cells have reduced expression of insulin (reflected by lower insulin staining).* ***(CBDS: Herold UC4, Yale University)***

DEVELOPING AND APPLYING ADVANCED TECHNOLOGIES TO STUDY HUMAN PANCREAS, ISLETS AND BETA CELLS

At the Pacific Northwest National Laboratory and the University of Colorado Denver, a CBDS team is developing new technologies for detailed characterization of molecular and metabolic signatures related to beta cell stress, dysfunction and death. Standard technologies utilize large heterogeneous tissue fragments, which can mask subtle changes in individual islets/beta cells during T1D development. To address this issue, the team refined two powerful, advanced technology platforms — called combFISH and nanoDESI — that allow them to analyze single cells or small clusters of cells. In-depth characterization of gene activity, metabolite levels and lipid (fat) composition in individual islets or beta cells is generating key

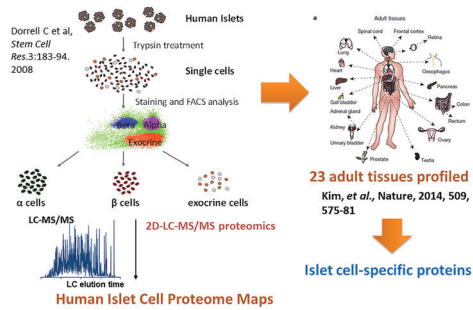

Figure 7. *Schematic workflow of establishing human islet proteome map. Human islets were procured from nondiabetic donors. Islets are subjected to cell sorting into alpha and beta cells. Each type of sample is then subjected to deep proteome profiling to generate islet cell proteome data. By comparative analysis with the 23 other human adult tissues, we identify proteins preferentially, or specifically expressed in the human islets, alpha cells or beta cells.* ***(CBDS: Qian UC4, Pacific Northwest National Laboratory)***

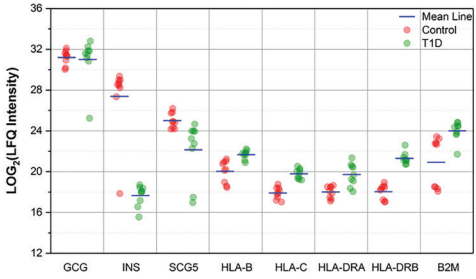

Figure 8. *Ultrasensitive proteomics analysis of single islets. Shown are the abundance levels of selected proteins from single islet sections (n=9) isolated by laser micro-dissection from a control donor and a T1D donor, respectively. The loss of insulin and SCG5 in T1D islets and the increased levels of HLA proteins are observed.* ***(CBDS: Qian UC4, Pacific Northwest National Laboratory)***

insights into T1D progression. In a collaborative study with another CBDS investigator, the team is using their novel technologies to analyze proteins in rare, live, replicating beta cells compared to nonreplicating beta cells. This exciting study may point to molecular pathways that could be targeted with new therapies to stimulate beta cell regeneration.

A proteome is an inventory of all proteins present in a specific cell type or tissue. A multi-institution team led by Pacific Northwest National Laboratory investigators focused on applying advanced technologies to map the proteomes of key T1D tissues (Figures 7 and 8). They created a transformative resource for the HIRN and diabetes research communities: a comprehensive proteome atlas of human islets, alpha cells and beta cells (see also the Opportunity Pool Projects discussion in the Human Islet Research Network chapter). In another study, they used a recently developed system, the Simplified Nanoproteomics Platform, coupled with a technique known as laser microdissection to map the proteomes of human islets and non-islet pancreas tissue from pre-T1D (autoantibody-positive) and nondiabetic organ donors. Approximately 280 proteins were significantly different between the pre-T1D and nondiabetic islets. A number of molecular pathways related to infections, stress responses and cell death were among the most highly elevated

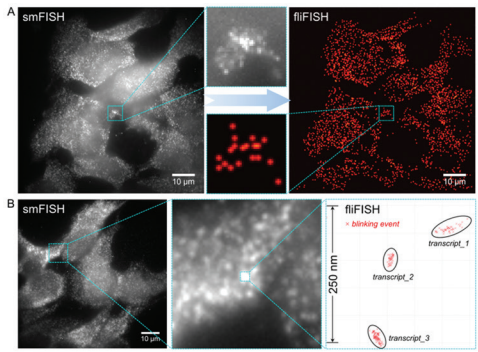

Figure 9. *fliFISH improves the resolution and reliability of counting RNA copies, especially when using a small number of probes. (a) Comparison between conventional smFISH (left image) and fliFISH (right image) using 8 probes to target Ins2 mRNA in pancreatic beta cells (MIN6) in culture. (b) fliFISH enables accurate localization of individual blinking events and the distinction between multiple transcripts within a diffraction-limited area.* ***(CBDS: Ansong UC4, Pacific Northwest National Laboratory)***

pathways in pre-T1D islets. These data provide a solid foundation for mechanistic research to understand the early molecular events that trigger beta cell dysfunction in T1D and identify new targets for early intervention or diagnosis (Figures 9 and 10).

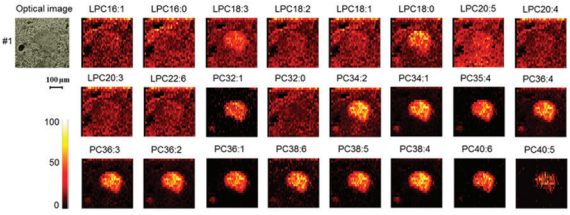

Figure 10. *Spatial distribution of selected LPC and PC species between mouse WT pancreatic islets and surrounding tissue.* ***(CBDS: Ansong UC4, Pacific Northwest National Laboratory)***

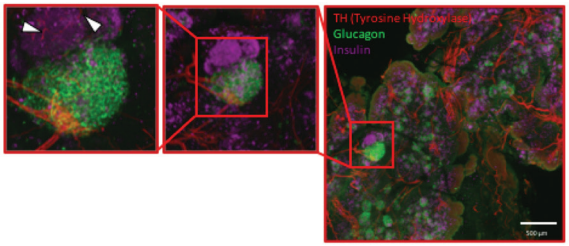

Figure 11. *3-D CLARITY "Google Earth"-type analysis, six week postnatal ("G42.1") human pancreas, here showing hormone–expressing cells and neurons. Many neural processes invest the proto–islets (arrows), and at this stage, human pancreas contains huge numbers of beta cells dispersed as single or small clusters of cells.* ***(CBDS: Wright UC4, Vanderbilt University)***

In a collaboration between Vanderbilt University and the California Institute of Technology, a multidisciplinary team of investigators is building a portfolio of advanced technologies that can be used to create a "Google Earth"-like view of pancreas architecture (Figure 11). They want to be able to move from a macro diagram of all islet-blood vessel-nerve connections across the entire pancreas to an extreme microscale understanding of the biology of individual cells and even detecting single molecules within cells under a variety of conditions. In one area of research, the team is optimizing their technologies to study juvenile islets (<5 years of age) when the pancreas architecture and beta cell mass are still being established, which also represents the time that autoimmunity begins in many individuals at risk for T1D (Figure 12). Using a technology known as "single-molecule HCR," the team measured the RNA

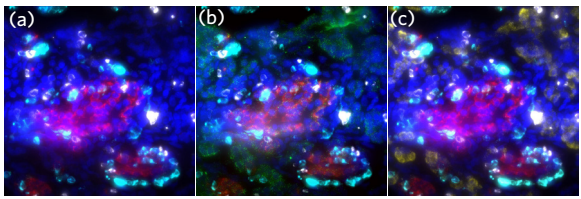

Figure 12. *Image of smHCR in juvenile human pancreas. Image represents a single region of interest (ROI) within the tissue sample. (a) RNA dots in the image are z-projected over 20×m. (a) Insulin (red), Glucagon (gray), SST(cyan) (b) Insulin (red), Glucagon (gray), SST(cyan), PDX1 (green) (c) -Insulin (red), Glucagon (gray), SST (cyan), GP2 (yellow).* ***(CBDS: Wright UC4, Vanderbilt University)***

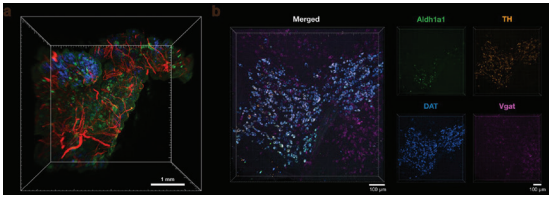

Figure 13. *(a) Light-sheet image, human pancreas (red: neurons; green: glucagon; blue: insulin). (b) In situ HCR analysis of four transcripts in cleared mouse brain (green: Aldh1a1; orange: TH; blue: DAT; magenta: Vgat).* ***(CBDS: Wright UC4, Vanderbilt University)***

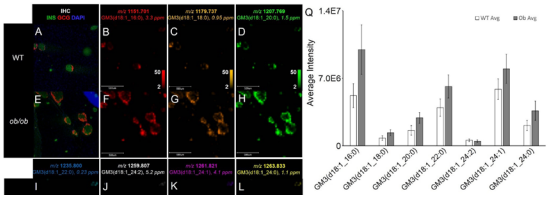

Figure 14. *Comparison of ganglioside distributions in a WT and ob/ob mouse.* ***(CBDS: Wright UC4, Vanderbilt University)***

molecules made by 55 genes in juvenile pancreas to gain insights into islet formation and cell identity in early development. The investigators also established a state-of-the-art "light-sheet" microscope system for fast, multicolor, three-dimensional imaging of human pancreas tissue (Figure 13). This technology enables them to collect high-quality, uniform data across the tissue sample in only two hours, rather than 12 hours using standard techniques. Data from these and other technologies have established that juvenile islets are substantially different than adult human islets. Drilling down to the molecular details of these differences will enable the team to better understand beta cell development and susceptibility to autoimmune destruction (Figures 14 and 15).

ADVANCING OUR UNDERSTANDING OF THE T1D DISEASE PROCESS

At Harvard University, a CBDS team is studying the earliest stages in the onset of T1D. They transplanted beta cells derived from human pluripotent cells into a mouse model in order to identify differences in healthy beta cells compared to beta cells that are under autoimmune attack. The group has significantly improved the function and differentiation of the pluripotent cell-derived beta cells after transplantation, particularly by identifying and correcting a defect in how the cells

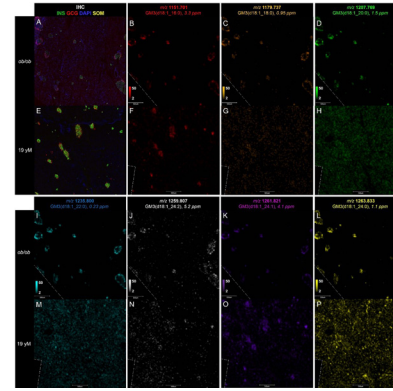

Figure 15. *Imaging mass spectroscopy for ganglioside distributions in ob/ob mouse pancreas and 19-year-old human pancreas.* ***(CBDS: Wright UC4, Vanderbilt University)***

sense and respond to glucose. The team can now expose the improved transplanted cells to diabetic conditions and autoimmunity to learn how and why the beta cells are lost early in diabetes. Identifying key pathways in this process, such as changes in proteins that are released from the islets, may reveal novel biomarkers that can identify early onset of T1D before patients lose the majority of their beta cells (Figure 16).

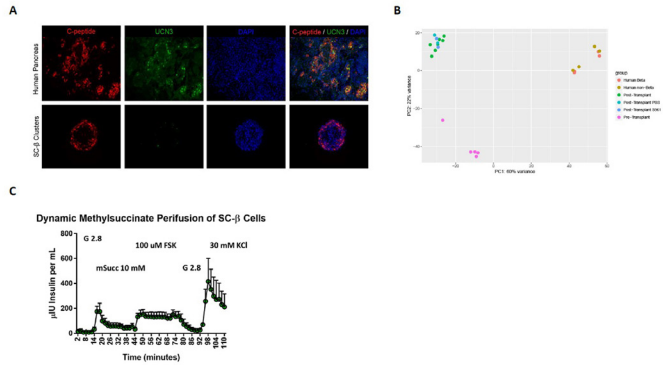

Figure 16. *Maturation state of derived beta cells. (a) Expression of UCN3 maturation marker; (b) Principle component analysis of in vitro vs in vivo SC derived beta cell; (c) Dynamic methylsuccinate perifusion analysis.* ***(CBDS: Melton UC4, Harvard University)***

Similar to a proteome, a transcriptome is a catalog of all genes that are active in a particular cell or tissue. A team of investigators at the University of Tennessee and University of Florida collected a transcriptome dataset from 120 individual islets of 11 T1D and 12 nondiabetic organ donors. An additional 140 islets, including many from nondiabetic donors with T1D autoantibodies, are also being analyzed. This unique and valuable dataset is a major step toward the goal of defining islet heterogeneity both between T1D and healthy islets and among islets within a single person. The team discovered that many different types of islets can exist

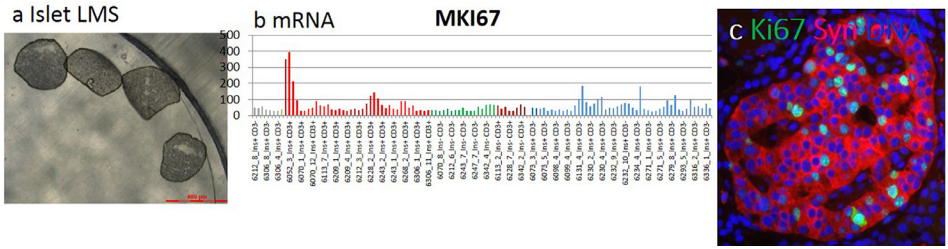

Figure 17. *High correlation between in situ single islet mRNA and protein levels for Ki67. Analysis of islets from a 12 year old male African American organ donor with type 1 diabetes for one year (nPOD 6052). Representative islet sections following laser microdissection are shown in the microfuge cap (a). Islet sections are processed for total RNA and analyzed by microarray as shown for MKi67 from 120 single islets (b). Multiple immunofluorescence image shows high numbers of Ki67+ endocrine cells (c) in this donor which correlates to the high Ki67 mRNA levels (green box in b).* ***(CBDS: Gerling UC4, University of Tennessee Health Sciences Center)***

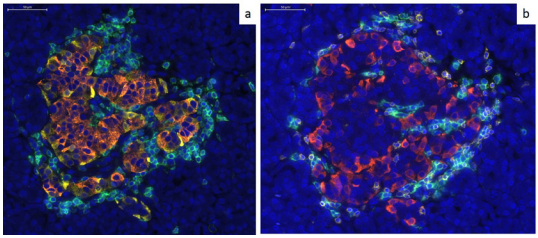

Figure 18. *Comparison of CD45 to CD3 immunotyping of insulitic islets. Serial stained sections of a single islet are shown from a 12-year-old patient with type 1 diabetes for one year (nPOD 6052). Sections were stained by multiple fluorescence followed by whole slide fluorescence scanning at 40x. Immunotyping of mononuclear cells by CD45 staining (green) with glucagon (yellow) and insulin (orange) detection is shown (a) in comparison to immunotyping for CD20 (green) and CD3 (yellow) lymphocytes cells staining with glucagon (red) (b). A linear correlation was obtained between total leucocytes and B and T cells. Scale bar = 50um.* ***(CBDS: Gerling UC4, University of Tennessee Health Sciences Center)***

within the same individual, particularly among people with T1D. Moreover, T1D islets that look "normal" under a microscope do not have identical gene activity patterns as healthy islets. Ongoing research to define and explore T1D-specific gene activity may reveal novel strategies for returning T1D islets to a healthy, insulin-producing state. Importantly, the data from this study are being shared collaboratively with other HIRN investigators who are collecting complementary data on islet biology from the same organ donors (Figures 17 and 18).

A multinational team centered at the University of Florida and the University of Zurich is optimizing and validating "highly multiplexed tissue imaging (HMI)" technologies to study the molecular and biochemical events leading to T1D at the single cell level. HMI technologies enable researchers to simultaneously analyze multiple proteins, protein modifications, genes, RNA, or other molecules in a cell or tissue sample. Using HMI and other state-of-the-art imaging technologies, the team has gained new insights into islet cell plasticity — the ability of non-beta cells to convert to insulin-producing beta cells — in human pancreata and mouse models (Figure 19). They demonstrated the presence of cells that contain proinsulin (the insulin precursor protein), but not insulin itself, in pancreas tissues from organ

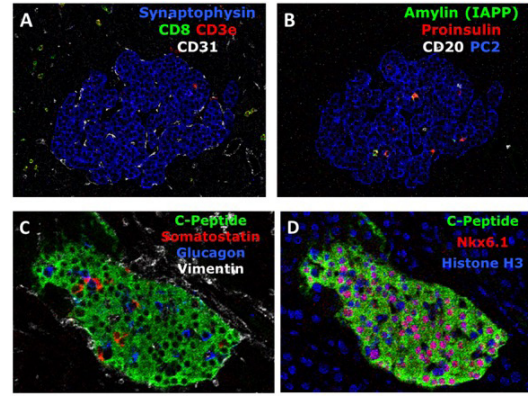

Figure 19. *Pancreas sections from a donor with T1D (A,B) and from mouse (C,D) imaged by IMC.* ***(CBDS: Atkinson UC4, University of Florida)***

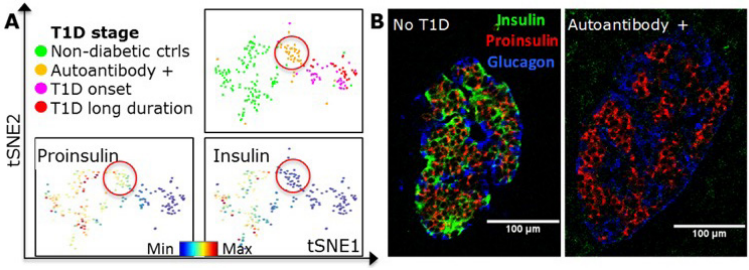

Figure 20. *(A) tSNE map of islets from six patients, each dot represents a single islet. Islets tend to cluster by disease stage (top). When the dots in A are colored to reflect marker expression, we oberve that in islets from the AAb+ donor (red circle) proinsulin, but not mature insulin is detected (bottom). (B) Images illustrating loss of mature insulin in the AAb+ donor.* ***(CBDS: Atkinson UC4, University of Florida)***

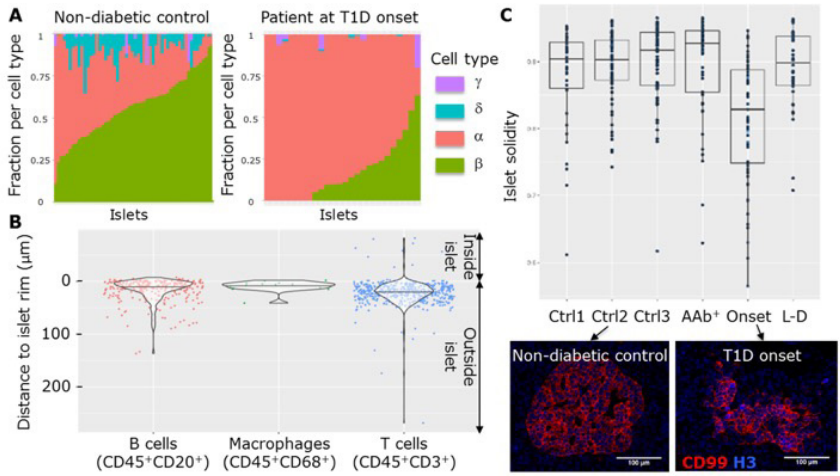

Figure 21. *(A) Islet cell composition in two donors. Columns represent individual islets. (B) Distance of immune cells to islet in a patient at T1D onset. Dots represent single cells. (C) Spatial measurements reflect differences in islet shape through T1D progression. In the T1D-onset donor, islet shape is altered due to immune cell infiltration.* ***(CBDS: Atkinson UC4, University of Florida)***

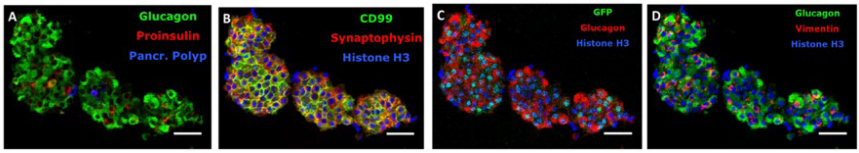

Figure 22. *Two monotypic pseudo-islets of human alpha cells which were transduced with adenoviruses expressing GFP and imaged by IMC.* ***(CBDS: Atkinson UC4, University of Florida)***

donors with T1D; this finding may corroborate previous studies showing the potential of alpha cells to reprogram to beta cells (Figure 20). The team has also discovered that a molecular pathway linked to DNA

repair and cell death is turned on in pancreas tissue from T1D donors (Figure 21). With continued research to understand these phenomena at the single cell level, the team hopes to develop innovative strategies to increase insulin production in individuals with T1D or possibly even prevent the loss of beta cell capacity before the onset of disease (Figure 22).

EXPANDING THE CONSORTIUM WITH NEW COLLABORATIVE RESEARCH TEAMS

In Year 4, the scope of research pursued by the CBDS will continue to evolve with the addition of three new research teams, representing seven T1D-focused investigators. Two teams will contribute new perspectives on the role of methylation in T1D: One will explore whether RNA methylation triggers beta cell death preceding T1D and the other will develop an innovative, sensitive assay that can detect beta cell death prior to and during progression of T1D by simultaneously measuring numerous markers of DNA methylation. A third team will validate new technologies for research on why DNA is damaged in beta cells and how that damage contributes to beta cell death and, ultimately, diabetes.

A total of 331 resources (e.g., bioreagents, datasets, documents and technologies) have been attributed to CBDS investigators, alone or in collaboration with other HIRN Consortia. All HIRN resources can be viewed at **https://resourcebrowser.hirnetwork.org** and publications can be viewed at **https://hirnetwork.org/all-hirn-publications**

CBDS INVESTIGATORS, YEAR 3

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

Raghavendra Mirmira, M.D., Ph.D., Investigator, *Indiana University*

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

Carmella Evans-Molina, M.D., Ph.D., Investigator, *Indiana University*

Thomas Metz, Ph.D., Investigator, *Pacific Northwest National Laboratory*

Margaret Morris, Ph.D., Co-investigator, *Eastern Virginia Medical School*

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*

Desmond Schatz, M.D., Investigator, *University of Florida*

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*

Mark Atkinson, Ph.D., Investigator, *University of Florida*

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*

Kevan Herold, M.D., Investigator, *Yale University*

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*

Mark Atkinson, Ph.D., Investigator, *University of Florida*

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, D.Phil., Investigator, *Vanderbilt University*

Marcela Brissova, Ph.D., Co-investigator, *Vanderbilt University*

Long Cai, Ph.D., Investigator, *California Institute of Technology*

Richard 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*

* Klaus Kaestner, Ph.D., Investigator, *University of Pennsylvania*

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

Ali Naji, M.D., Ph.D., Co-investigator, *University of Pennsylvania*

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

Chuan He, Ph.D., Investigator, *University of Chicago*

* Desmond Schatz, M.D., *University of Florida*

Carla Greenbaum, M.D., Co-investigator,
Benaroya Research Institute at Virginia Mason

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

* New teams to be added in Year 4.

CONSORTIUM ON HUMAN ISLET BIOMIMETICS (CHIB)

THE GOAL: CREATE *EX VIVO* MODELS OF HUMAN PANCREATIC ISLETS AND BETA CELLS

Pancreatic islets are complex micro-organs made up of multiple hormone-producing cells, including alpha cells (glucagon), beta cells (insulin), delta cells (somatostatin) and PP cells (pancreatic polypeptide). Clusters containing a mixture of these cells are embedded throughout the pancreas within a rich environment of blood vessels, nerves and an extracellular, gel-like matrix. A major challenge in type 1 diabetes (T1D) research has been the difficulty in studying islets outside of the human body (*ex vivo*) because they rapidly lose function and viability when removed from the body and grown in the laboratory. Individual beta cells in the lab — whether isolated from their islet community or made from pluripotent cells — are less able to release insulin in response to glucose compared to beta cells in their natural environment in the body. The Consortium on Human Islet Biomimetics (CHIB) has assembled world-class teams of investigators with diverse expertise on diabetes, islet biology, stem cell biology, bioengineering, fluid dynamics and other essential technologies to solve this challenge. In Year 3, CHIB investigators made exciting progress on the development of *ex vivo* models that can be used for advanced studies of human islet biology, validation of T1D biomarkers, drug development studies and research on the interaction of beta cells and the immune system in T1D.

FINDING CONDITIONS FOR LONG-TERM ISLET FUNCTION AND SURVIVAL

An investigator at the University of Pennsylvania led a multi-institution team that used a device they developed in prior years to search for conditions that support long-term function and survival of islets in the lab. The device allowed them to simultaneously and in real time test two different functions of islets — changes in the amount of calcium inside the cells and release of insulin from the cells — in response to higher levels of glucose in the culture liquid surrounding the islets. With these tests, the team could measure how well the *ex vivo* islets performed these functions in comparison to islets in the body. Next, they conducted these tests under varying conditions (e.g., media composition, physical features

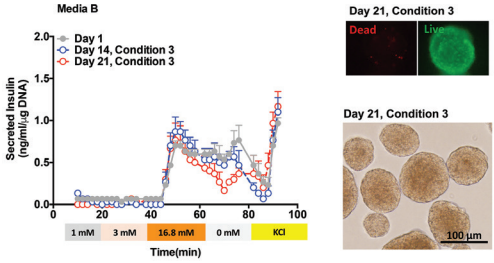

Figure 1. *Islets were obtained from a donor and cultured in specialized medium and matrix conditions for 14 or 21 days. Islets maintained their viability, morphology and function (as assessed by perifusion) throughout this period.* ***(CHIB: Stanger UC4, University of Pennsylvania)***

and matrix) and discovered a set of conditions in which the islets survived and functioned efficiently for more than a month — a marked improvement over the previous limit of a few days. This exciting advance has important applications in T1D research. Long-term culture of human islets will help drug discovery efforts and other islet biology research. Scaling up to larger numbers of islets could improve islet transplantation by allowing human islets to remain viable past the current 48-hour window between isolation and transplant into a person with T1D (Figure 1).

BUILDING DEVICES FOR *EX VIVO* RESEARCH ON ISLETS AND BETA CELLS

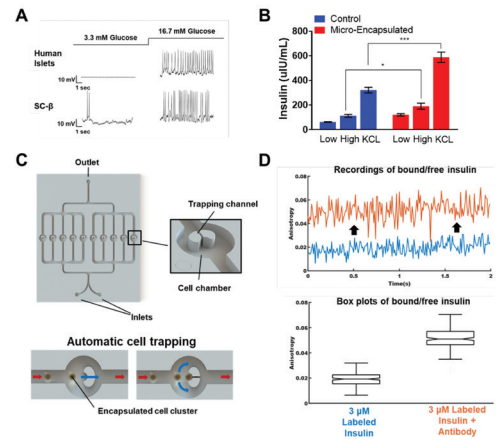

Figure 2. *A) Electrophysiological responses of human islets and SC-beta cells to low and high glucose. B) Glucose stimulated insulin response of non-encapsulated (control) and micro-encapsulated SC beta cell after five days in culture. C) Islet-on-a-chip design, highlighting automatic cell trapping mechanism. D) Anisotropy differences between fluorophore-tagged insulin versus fluorophore-tagged insulin with insulin antibody.* ***(CHIB: Melton UC4, Harvard University)***

At Harvard University, a research group continued to improve on the design of their "islet on a chip." The chip is a small device for holding human islets or clusters of islet cells made from pluripotent cells that allows the investigators to measure the long-term function and survival of the islets. The device could also be used to optimize the conditions for generating beta or other islet cells from pluripotent cells (Figure 2). The team's design changes make the device cheaper to manufacture and easier to use in the laboratory — thus, significantly improving the usability of the device by the broader

islet research community (Figure 3). In addition, the team is collaborating with an investigator at Florida State University, who will lead a new CHIB team in Year 4. Together, they are developing a real-time, sensitive test of insulin release from single islets. This test will speed research by reducing the time and cost of islet function tests compared to traditional methods.

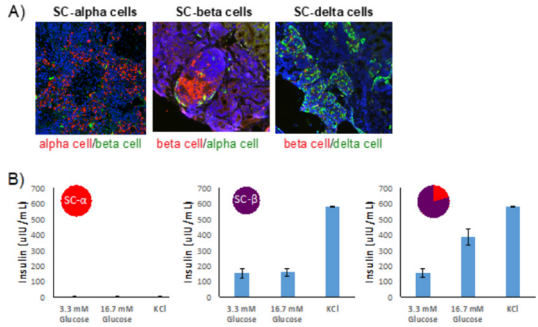

Figure 3. *Generation of organoids composed of multiple endocrine cell types. A) Organoids were transplanted under the kidney capsule in mice and grafts were analyzed for hormone expression. B) Glucose stimulated insulin secretion under low and high glucose conditions of organoids.* ***(CHIB: Melton UC4, Harvard University)***

At the University of Florida and University of Miami, another CHIB team refined their own microfluidic device with the goals of improving ease of use and adding new capabilities. The updated platform includes engineering changes that permit researchers to better image the islets within the device and to improve the delivery of oxygen to the cells. Other modifications make it easier to switch the liquid surrounding the islets — thus, helping the researchers to test different culture conditions (e.g., add or subtract nutrients in the liquid) and also to measure islet function (e.g., release of insulin from the islets in response to glucose levels in the liquid). The group shared detailed protocols, including PowerPoint and video instructions, for making and using the device with investigators across the HIRN so that the research community can apply this state-of-the-art technology to important questions in islet biology, islet transplantation and the discovery of new drugs for T1D (Figure 4).

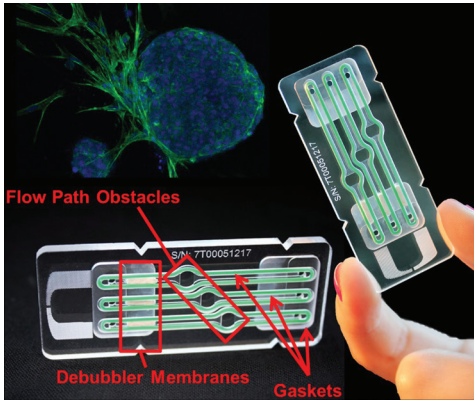

Figure 4. *Photography of microfluidic platform for dynamic culture of pancreatic islets. Microwells contain islets within a defined 3-D system, while perfusion provides dynamic interrogation. Top left image: Human islets cultured within a 3-D extracellular matrix exhibit a 3-D morphology, with sprouts that connect to neighboring islets.* ***(CHIB: Stabler UC4, University of Florida)***

RECREATING THE ISLET MICROENVIRONMENT IN THE LAB

A multi-institution team headed by an investigator from the University of California San Diego, is creating an optimal microenvironment for islets to thrive in the laboratory. Extracellular matrix (ECM) is a gel-like substance composed of proteins and other complex molecules that surround and nurture the islets in the body. The ECM is stripped away when islets are isolated from a pancreas and it is absent when islet cells are made from pluripotent cells. To test whether ECM can improve islet viability in the lab, the team carefully identified and measured all components of the ECM found around human islets. Using this information as a recipe, they began testing each component in their *ex vivo* system to determine which could most improve islet function and survival. The team also made progress toward creating a blood vessel network to deliver oxygen and nutrients to islets in culture. Together, these studies advance a key goal of making a stable, long-term, functional model of islet biology in the lab (Figure 5).

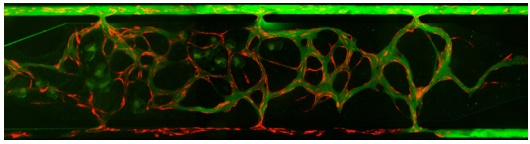

Figure 5. *hiPSC-derived pseudo-islets supported by a perfused vascular network (red) in microfluidic device.* ***(CHIB: Sander UC4, University of California, San Diego)***

EXPANDING THE CONSORTIUM

In Year 4, one new research team, representing three institutions, will add its expertise on engineering and analytical chemistry to the CHIB community. The new team develops novel technologies that can be connected to CHIB devices in a modular format to allow near real-time monitoring of insulin secretion from *ex vivo* islets.

In closing, a total of 41 resources (e.g., bioreagents, datasets, documents and technologies) have been attributed to CHIB investigators, alone or in collaboration with other HIRN Consortia. All HIRN resources can be viewed at **https://resourcebrowser.hirnetwork.org** and publications can be viewed at **https://hirnetwork.org/all-hirn-publications**

CHIB INVESTIGATORS, YEAR 3

Douglas Melton, Ph.D., Investigator, *Harvard University*
Jeff Karp, Ph.D., Co-investigator, *Brigham and Women's Hospital*
Kit Parker, Ph.D., Co-investigator, *Harvard University*

Maike Sander, M.D., Investigator, *University of California San Diego*
Karen Christman, Ph.D., Investigator, *University of California San Diego*
Steven George, M.D., Ph.D., Investigator, *Washington University*
Christopher 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*
Dan Huh, Ph.D., Investigator, *University of Pennsylvania*

* Michael Roper, Ph.D., Investigator, *Florida State University*
Christopher Hughes, Ph.D., Investigator, *University of California Irvine*
Ryan White, Ph.D., Investigator, *University of Cincinnati*

* New team to be added in Year 4.

CONSORTIUM ON MODELING AUTOIMMUNE INTERACTIONS (CMAI)

THE GOAL: UNDERSTAND THE INTERACTIONS BETWEEN BETA CELLS AND THE IMMUNE SYSTEM

Type 1 diabetes (T1D) is caused by an autoimmune attack that specifically destroys the insulin-producing beta cells of the pancreas. It is not clear why immune cells fail to recognize the beta cells as a part of a person's own body and, instead, attack them as they would an infection, such as might be caused by bacteria or viruses. Because the beta cells are nestled deep within the pancreas, researchers cannot directly study the interactions between beta cells and the immune system in a person living with T1D. To address this issue, the Consortium on Modeling Autoimmune Interactions (CMAI) was created to develop innovative tools that will advance our understanding of autoimmunity in T1D. In Year 3, CMAI teams made important progress on a primary objective of the consortium: the development of experimental mouse models that can measure key features of human T1D. The model strategy starts with induced pluripotent cells from bone marrow or skin cells of individuals with T1D. The investigators then develop methods to reliably differentiate these cells into three cell types that have critical roles in T1D: beta cells; pluripotent hematopoietic cells that can, in turn, be triggered to develop into any blood cell, including all types of immune system cells; and thymus cells that educate immune system cells on how to recognize the body's own tissues as "self." A final piece of the model is a mouse strain with a deficient immune system; these mice serve as a blank canvas upon which the human beta, blood and thymus cells can be placed to learn how they all interact with one another. By approaching this challenge from multiple angles, the CMAI collectively has created multiple tools and generated important new insights into T1D pathogenesis.

CREATING NEW MODELS OF HUMAN T1D PATHOGENESIS

A multi-institution team coordinated from the University of Massachusetts Medical School assembled a bank of induced pluripotent cells from both nondiabetic individuals and those with T1D, each of whom has a unique genetic background. They showed that when human beta cells made from the pluripotent cells were put into immunodeficient

mice, the beta cells responded to glucose and released insulin in a way that was identical to how beta cells from donated human pancreata behave. Significant progress was also made in creating thymus and hematopoietic cells that function correctly in the mouse system. When all three human cells types are successfully combined in the mouse model, the investigators will be able to learn more about how and why T1D develops in people (Figure 1).

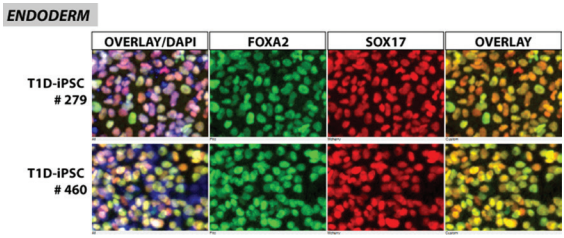

Figure 1. *Immunofluorescence analysis of definitive endoderm derived from type 1 diabetic patient-specific induced pluripotent stem cells. Anti-SOX17 and anti-FOXA2 immufluorescence is displayed individually, as well as overlap of the two stains alone or together with a nuclei stain.* ***(CMAI: Greiner UC4, University of Massachusetts Medical Center)***

A team at Columbia University is constructing immune systems *de novo* from blood and immune cell progenitor cells of T1D patients in immunodeficient mice. They have demonstrated that these immune systems recapitulate abnormalities that have been shown to exist in T1D patients. They are also using induced pluripotent cells as a starting point to generate genetically identical beta cells and thymus cells to study with these patient-specific immune systems. The team achieved a critical milestone in this project by creating a mouse line in which the immune system makes a specific type of T cell that has been shown to be capable of inducing diabetes in experimental mice and they are now investigating the fate of

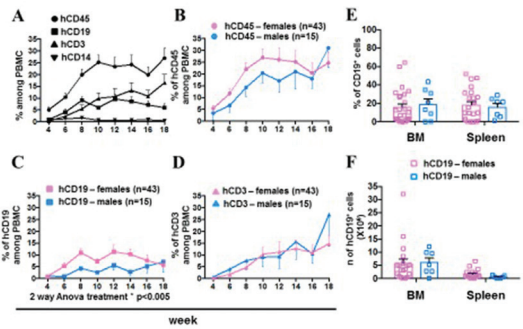

Figure 2. *Human B cell levels in peripheral blood are higher in female (pink lines and symbols) than male (blue lines and symbols) recipient mice injected with human CD34+ cells. Immunodeficient mice received adult human bone marrow CD34+ cells intravenously and were grafted under the kidney capsule with a partially HLA-matched human thymus. (A-D) Average levels of human immune reconstitution in the blood over time in 28 recipients. (E-F) Levels of human B cells in spleen and bone marrow 20-22 weeks after transplant. Each square represents a single mouse.* ***(CMAI: Sykes UC4, Columbia University)***

this T cell type in T1D-derived immune systems. This line represents a valuable tool for research to understand the development and progression of T1D (Figure 2). In addition, they demonstrated that thymus cells can be produced from pluripotent cells, paving the way for incorporating patient-specific thymus cells into this experimental model (Figure 3).

At the University of California San Francisco, a CMAI team focused on the development of thymus cells and beta cells from induced pluripotent cells for use in T1D models. The investigators tested different ways to graft their thymus cells into mice with compromised immune systems so that the thymus cells survive and function well (Figure 4). They also discovered a 27-day method for efficiently and reproducibly making functional beta cells from pluripotent cells. These beta cells can prevent diabetes when grafted into their experimental mouse model.

The CMAI teams' progress in developing groundbreaking models of human T1D results from extensive collaboration within and among these research groups, as well as with other HIRN and non-HIRN investigators. This line

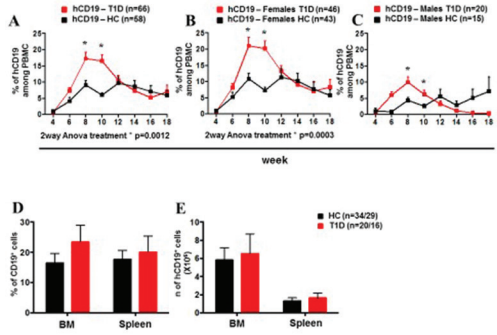

Figure 3. *Human B cell reconstitution is higher in mice receiving bone marrow cells from T1D compared to healthy control (HC) donors. Immunodeficient mice received adult bone marrow CD34+ cells and were grafted under the kidney capsule with a partially HLA-matched human thymus. (A-C) Mean plus SEM human peripheral B cell (CD19+) levels over time. (10 HC donors - n=58 mice, 9 T1D donors - n=66 mice). * indicates statistically significance calculated by Sidak's multiple comparison test. (D-E) Mean +SEM percentage (D) and number (E) of human B cells in bone marrow (BM) and spleen 14-18 weeks after transplantation were collected. Animals with <1% CD19+ cells were excluded from the analysis.* ***(CMAI: Sykes UC4, Columbia University)***

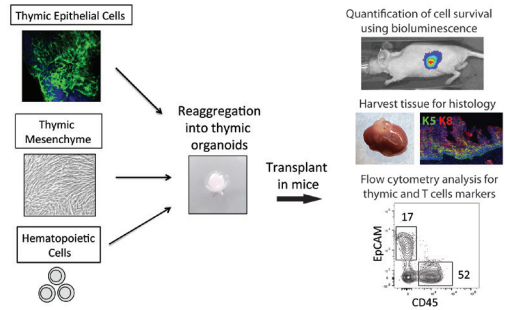

Figure 4. *Thymic epithelial cells produced in vitro are purified and reaggregated with thymic mesenchyme and hematopoietic cells to form thymic organoids. Cell survival upon transplantion in mice is monitored using bioluminescence quantification and function of thymic cells is assessed by histology and flow cytometric analysis of thymic and T cell maturation markers.* ***(CMAI: Anderson UC4, University of California, San Francisco)***

of research benefits the entire T1D research community, as these models have many important applications, including understanding the interaction of beta cells and the immune system; testing the ability of human pluripotent cell-derived beta cells to survive an immune system attack; and developing a renewable, abundant, safe and effective source of beta cells to treat T1D.

UNDERSTANDING HOW T CELLS CONTRIBUTE TO T1D

The CMAI team at the University of Colorado Denver is working to identify specific features of T cells that are involved in the immune attack on beta cells. To accomplish this, the investigators are characterizing proteins known as T cell receptors (TCRs) that impart T cells with the capacity to specifically target islet beta cells. Using valuable human pancreas tissue obtained through the Network for Pancreatic Donors with Diabetes (nPOD) program, the team identified several TCRs that respond to proteins in the pancreas, including parts of the insulin molecule itself (Figure 5). Importantly, they discovered that these TCRs were mainly found in the pancreatic islets, but not circulating in the blood. Moreover, a collaborative project with the Columbia University CMAI demonstrated that arming human T cells with such islet-reactive TCRs could result in overt disease using the

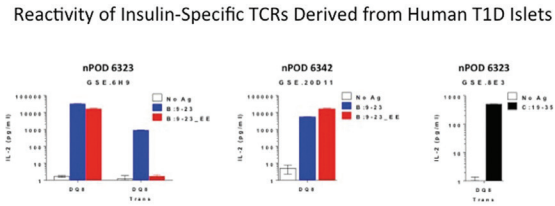

Figure 5. *DQ8-restricted, insulin peptide reactive TCRs derived from T1D islets. TCRs were identified using single-cell PCR from T cells associated with islets derived from T1D donors through the nPOD program. Such TCRs were used to transduce T cell hybridomas and then tested for reactivity to native (B:9-23) or modified (B:9-23_EE) insulin B chain peptides. Note that insulin-specific TCRs from nPOD donor 6323 responds to peptide presented by DQ8 trans.* ***(CMAI: Gill UC4, University of Colorado, Denver)***

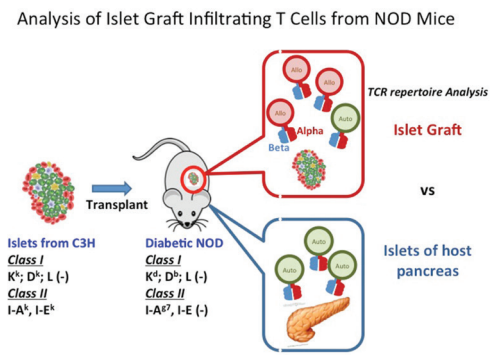

Figure 6. *Strategy for isolating and characterizing autoreactive and/or alloreactive TCRs from T cells infiltrating either islet transplants or endogenous host pancreatic islets in the NOD mouse model of T1D.* ***(CMAI: Gill UC4, University of Colorado, Denver)***

types of mouse models described above. This line of research provides key insights into how T cells recognize and attack the body's beta cells. Results from this study will inform future research on beta cell-T cell interactions in the mouse models of human T1D (Figure 6).

UNRAVELING GENETIC FACTORS IN T1D

A team centered at the University of Florida is studying *PTPN22*, a gene linked to high risk for the development of T1D. The group showed that the PTPN22 protein is involved in multiple molecular pathways in immune and non-immune cells, suggesting that the role of PTPN22 in T1D development is more complex than previously appreciated. In addition, they made human induced pluripotent cells from samples collected by the TrialNet Living Biobank and used these cells to make immune cells and endothelial cells (i.e., cells that line the inner surface of blood vessels) that carry either the protective or T1D-susceptible version of *PTPN22* (Figure 7). The team can now directly assess how and why immune cells with the susceptible *PTPN22* version more easily

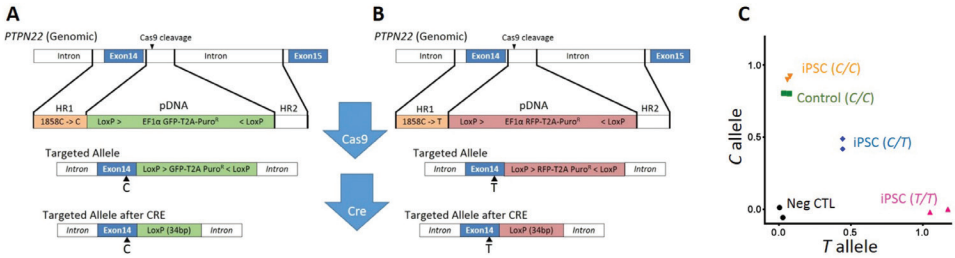

Figure 7. *1. CRIPSR/Cas9 modification of iPSC to generate isogenic cells with variation at rs2476601. To create iPSCs that carry either protective or risk variant of PTPN22 at the SNPrs2476601, a CRIPSR/Cas9 strategy was used. The nucleotide defined by rs2476601 is base 1858 where C encodes arginine and is protective and T encodes tryptophan and is risk. A CRIPSR guide RNA was designed to cleave with an intron proximal to the 3' end of Exon 14 that contains base 1858. A donor pDNA was delivered at the same time to drive template mediated repair of the CRISPR double strand break. (A) One pDNA carries a homologous repair cassette with 'C' at a position corresponding to 1858 and a GFP-Puro selection cassette. (B) A second pDNA carries a homologous repair cassette with 'T' at a position corresponding to 1858 and an RFP-Puro selection cassette. In the presence of Cas9, the two cassettes will drive repair with either C1858 (GFP) or T1858 (RFP). (C) When GFP+, GFP+RFP+ or RFP+ iPSC clones were screened by a TaqMan SNP assay for rs2476601, the genotypes were interpreted as C/C, C/T and T/T, respectively. Thus, the allele modification worked as designed. The large donor fragment that was deposited in an intron, is flanked by LoxP sites so that delivery of Cre recombinase will excise the extra DNA leaving only a small LoxP sequence in the intron but outside of the RNA splice site.* ***(CMAI: Mathews UC4, University of Florida)***

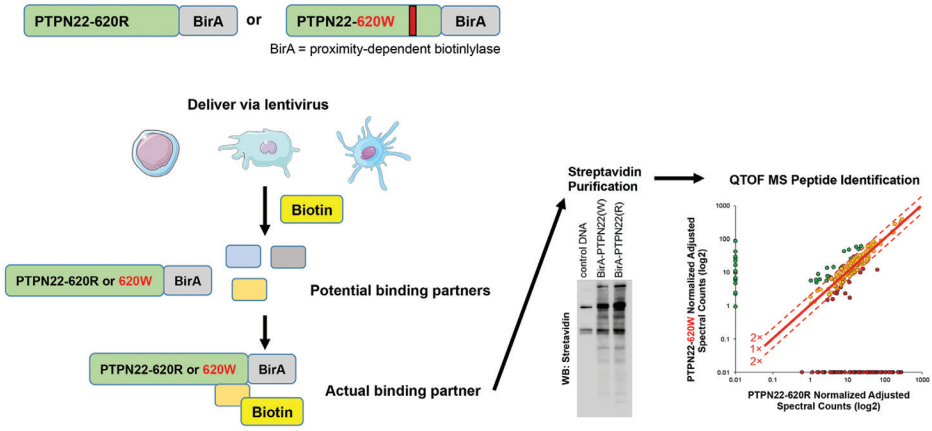

Figure 8. *Identification of PTPN22-620R and PTPN22-620W protein binding partners. T cells, macrophages (THP-1 or iPSC-derived macrophages) or DCs (iPSC-derived) are transduced with one of two unique lentiviral constructs. Each lentivirus encodes the gene for PTPN22 fused to a proximity-dependent biotinylase (BirA) — one version encodes the 620R residue (common variant) and the other encodes 620W (T1D risk variant). Cells expressing either PTPN22 variant are provided a biotin substrate and any proteins in close proximity to PTPN22-BirA become biotinylated as demonstrated by streptavidin-HRP Western blot. Total protein lysates are then enriched for biotinylated proteins on a streptavidin column. Biotinylated proteins (PTPN22 binding partners) are identified by tandem mass spectroscopy.* ***(CMAI: Mathews UC4, University of Florida)***

infiltrate the pancreatic islets and attack the body's own beta cells than immune cells with the protective *PTPN22* version (Figure 8).

EXPANDING THE CONSORTIUM

In Year 4, CMAI welcomes one new research team of four investigators based at the University of Massachusetts Medical School and Icahn School of Medicine at Mount Sinai. This team has previously collaborated with HIRN investigators as part of an Opportunity Pool Project, among other studies. After joining the CMAI, the team will comprehensively characterize autoimmune system T cells that have been isolated from the pancreatic islets of people with T1D.

To date, a total of 234 resources (e.g., bioreagents, datasets, documents and technologies) have been attributed to CMAI investigators, alone or in collaboration with other HIRN Consortia. All HIRN resources can be viewed at **https://resourcebrowser.hirnetwork.org** and CMAI publications can be viewed at **https://hirnetwork.org/all-hirn-publications.**

CMAI INVESTIGATORS, YEAR 3

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

Xiaojuan Chen, M.D., Ph.D., Co-investigator, *Columbia University*

Nichole Danzl, M.D., Co-investigator, *Columbia University*

Dieter Egli, Ph.D., Co-investigator, *Columbia University*

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

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

Yong-Guang Yang, 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 & 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 Brusko, 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

Naohiro Terada, M.D., Ph.D., Co-investigator, *University of Florida*

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

Dale Greiner, Ph.D., Investigator,
University of Massachusetts Medical School

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

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

George Daley, M.D., Ph.D., Investigator, *Harvard University*

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

* Sally Kent, Ph.D., Investigator,
University of Massachusetts Medical School

David Harlan, M.D., Investigator,
University of Massachusetts Medical School

Lawrence Stern, Ph.D., Investigator,
University of Massachusetts Medical School

Dirk Homann, M.D., Co-investigator,
Icahn School of Medicine at Mount Sinai

* New team to be added in Year 4.

CONSORTIUM ON TARGETING AND REGENERATION (CTAR)

THE GOAL: BETA CELL REGENERATION AND PROTECTION TO REVERSE TYPE 1 DIABETES

In people with type 1 diabetes (T1D), the immune system attacks and destroys most, if not all, insulin-producing beta cells in the pancreas. To cure the disease, researchers must figure out how to preserve or replace enough beta cells to meet the body's need for insulin and how to protect the beta cells from ongoing autoimmunity. In Year 3, the Consortium on Targeting and Regeneration (CTAR) made significant progress toward those goals. Stimulating proliferation (reproduction or expansion) of any beta cells that remain in the pancreas during or after the onset of T1D is one approach to replacing insulin production. CTAR investigators used two models — aging and insulinomas — to better understand the molecular pathways of beta cell proliferation in unprecedented detail. The rich datasets developed through this research have revealed previously unknown, high-priority targets for T1D drug development. Other exciting avenues of research included understanding the similarities and differences between alpha and beta cells in order to use alpha cells as a possible source of new beta cells in T1D patients and developing new drugs and delivery systems that selectively target beta cells to trigger proliferation or immune protection.

HARNESSING ISLET CELL PLASTICITY

A team at the University of Geneva is exploring ways to exploit islet cell plasticity — the ability of some cells to take on the characteristics of other cells within the islets — as a strategy to replace beta cells lost in T1D. The investigators successfully converted healthy human alpha cells, which normally

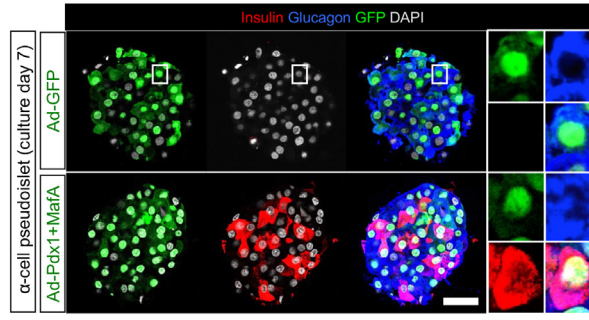

Figure 1. *Pseudoislets made of lineage-traced GFP-labeled human alpha-cells, after seven days of culture. Upper row, control pseudoislet. Lower row, pseudoislet of alpha-cells, expressing mouse Pdx1 and MafA: alpha-cells, start to produce insulin, while maintaining glucagon expression, and lead to diabetes remission if transplanted to diabetic mice.* ***(CTAR: Herrera UC4, University of Geneva)***

produce glucagon, into insulin-producing cells by forcing the production of two beta cell-specific proteins. The converted cells restored normal blood glucose levels and reversed diabetes when injected into diabetic mice. Interestingly, the team was able to repeat this outcome with alpha cells from individuals with type 2 diabetes (T2D), suggesting that alpha cells in diabetes patients retain the capacity to make insulin if given the proper stimuli (Figure 1).

NEW INSIGHTS ON BETA CELL PROLIFERATION IN AGING AND DISEASE

A multi-institution team at Vanderbilt University and Stanford University discovered why beta cells dramatically lose their ability to proliferate as people age. The Vanderbilt/Stanford teams found that Exendin-4 (Ex-4), a small protein similar to glucagon-like peptide-1 (GLP-1), a hormone used clinically to treat T2D, could stimulate proliferation of human juvenile (<10 years of age), but not adult, beta cells. By mapping differences in the proteins made in juvenile versus adult beta cells when exposed to Ex-4, they discovered an age-dependent molecular pathway for beta cell proliferation that is specific to young beta cells (Figure 2). In a parallel study at Stanford University, investigators identified several hundred genes whose amounts change with age, either increasing or decreasing, in human islets. Of particular interest for future research are two proteins, SIX2 and SIX3, that were not previously known to have important functions in the pancreas.

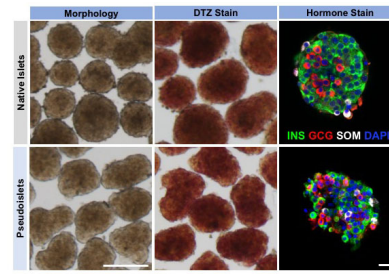

Figure 2. *Morphology, dithizone staining and hormone composition of human pseudoislets compared to native human islets.* ***(CTAR: Powers UC4, Vanderbilt University)***

A University of Pennsylvania team investigated how islet cells mature over the lifespan and how the process is perturbed by diabetes. They isolated individual alpha and beta cells from donors of varying ages and cataloged the types and amounts of RNAs found in each cell. These data allowed the team to create detailed molecular signatures of alpha and beta cells during aging and in T2D. They discovered that in T2D, islet cells lose some of their specialized characteristics and begin to regress to a more immature, progenitor-like state (Figures 3 and 4).

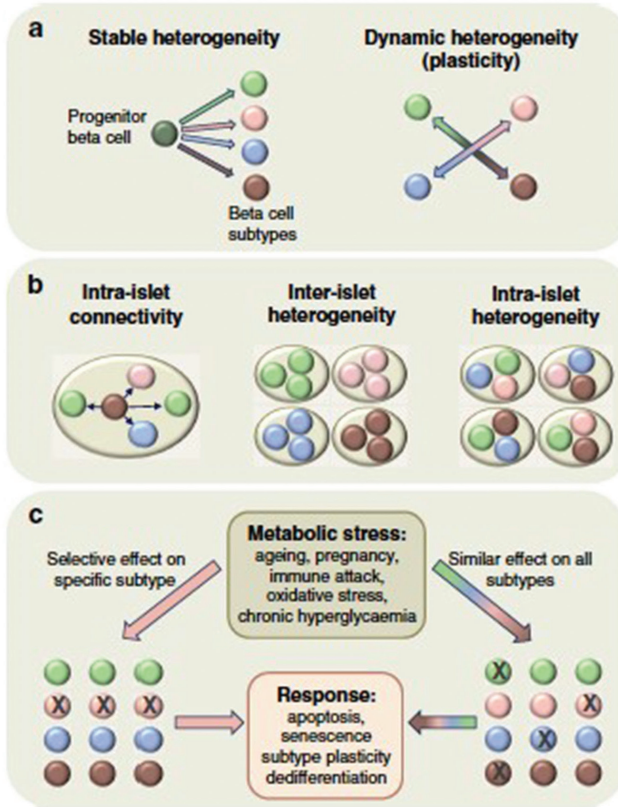

Figure 3. *Schematic representation of open questions related to the origin, status and stress-mediated fate of four hypothetical beta cell subtypes, each represented by a different color (a) The origin of beta cell heterogeneity. Are the different beta cell subtypes stable or are they able to switch from one phenotype to another? If so, what signals regulate such switches metabolic demand, pregnancy, age? (b) Distribution of beta cell subtypes. Do different beta cell subtypes interact within islets? Are they clustered in different islets (perhaps in different regions of the pancreas) or are they scattered randomly among the islets? (c) Effects of metabolic stress on beta cell heterogeneity. When stressed, how do different beta cell subtypes react? Do they undergo apoptosis, senescence, subtype plasticity of trans-differentiation to non-beta cells (the last is not shown)? Are some subtypes more sensitive or resistant to specific stress?* ***(CTAR: Kaestner UC4, University of Pennsylvania)***

A research group at the Icahn School of Medicine at Mt. Sinai studied a different model of beta cell proliferation that works in the opposite direction of aging — insulinomas, or benign pancreatic tumors caused by uncontrolled proliferation of insulin-producing beta cells. Investigators profiled the DNA (30,000 genes) and RNA (17,000 RNAs) from 38 human insulinomas and compared the results with those of beta cells from 25 normal pancreata. They identified multiple novel pathways and molecular mechanisms that contribute to beta cell proliferation in the insulinomas.

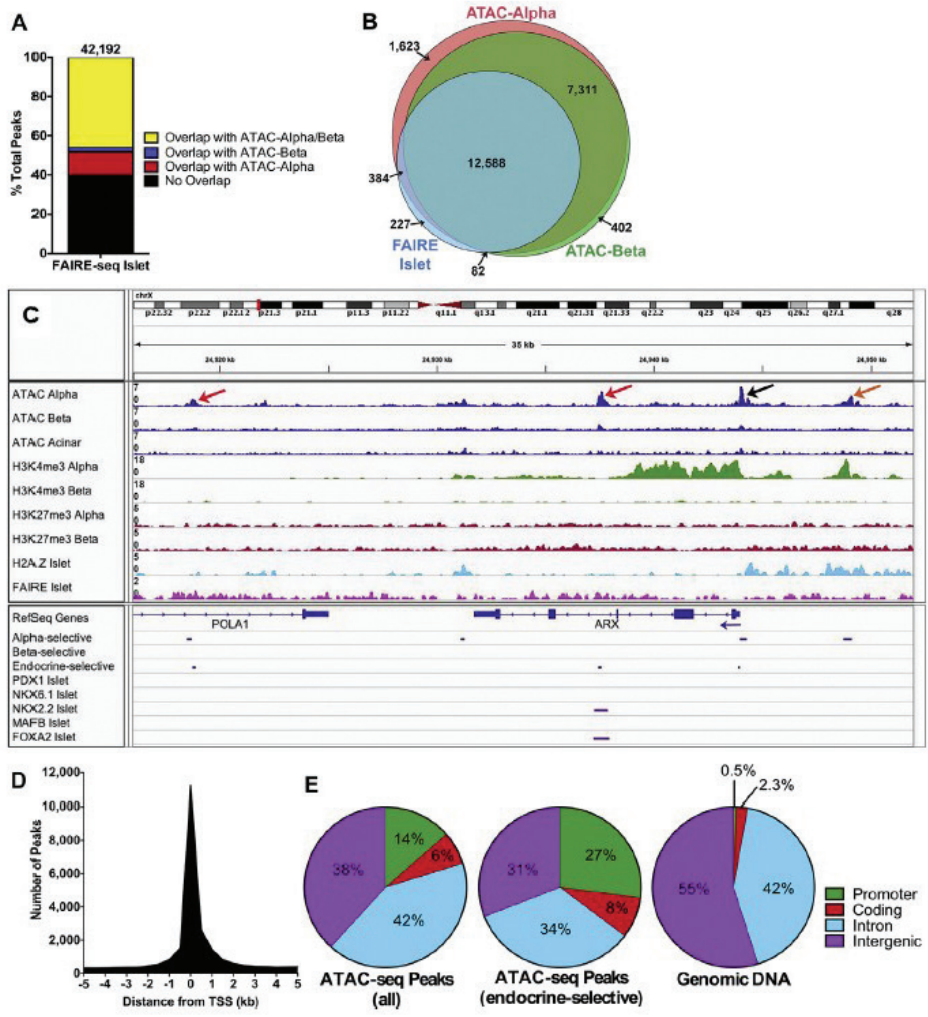

Figure 4. *Integration of ATAC-seq data with other genomics datasets. (A) Bar graph of % of overlapping open chromatic regions identified by FAIRE-seq [32] in whole islets versus by ATAC-seq in alpha- and beta-cells (including peaks also found in acinar cells). Total number of FAIRE-seq peaks in noted at top. (B) Venn diagram of distinct genes with open chromatin regions in alpha- and beta-cells identified by ATAC-seq (including peaks also found in acinar cells) versus in whole islets identified by FAIRE-seq. (C) Sequencing tracks for the ARX locus shows distinct alpha-cell-specific ATAC-seq peaks at the promoter (black arrow), at known intronic and distal enhancers (red arrows), and at a putative 5' enhancer (orange arrow), non of which were identified by FAIRE-seq. (D) Histogram of distance from the nearest transcriptional start site (TSS) for all ATAC-seq peaks within 5 kb of the nearest TSS that were identified in alpha- and beta-cells. Not shown are peaks 5 - 280 kb form the nearest TSS (E) Proportions of the ATAC-seq peak regions identified in alpha- and beta-cells that represent the various genome annotations, compared to the representation of a given sequence element in the human genome [47].* ***(CTAR: Kaestner UC4, University of Pennsylvania)***

These important new insights from aging beta cells and insulinomas together create a rich and unprecedented "wiring diagram" that has greatly improved our understanding of how human beta cells proliferate. The research community can begin to build on these findings and develop innovative strategies to safely trigger beta cell proliferation in people with T1D. Multiple laboratories from the CTAR, as well as from CBDS and CMAI, collaborated on these areas of research.

DEVELOPING NEW THERAPIES AND APPROACHES TO PREVENT BETA CELL LOSS

In previous years, a group at the University of Alabama at Birmingham showed that a protein called TXNIP promotes beta cell death and stops insulin production when islets are under immune attack. An FDA-approved blood pressure drug, verapamil, blocks these unwanted actions of TXNIP and reverses diabetes in mouse models. Although verapamil is safe for use in people and it is being tested in clinical trials for diabetes treatement, its effects are not very specific. In Year 3, this team screened a library of 300,000 chemicals to find and optimize molecules that work 100-fold better than verapamil to block TXNIP activity. These molecules can protect human islets in the lab and prevent the onset of diabetes in mouse models. These exciting findings pave the way for translational studies to develop a safe and effective TXNIP-blocking drug for beta cell protection that can be tested in people (Figure 5).

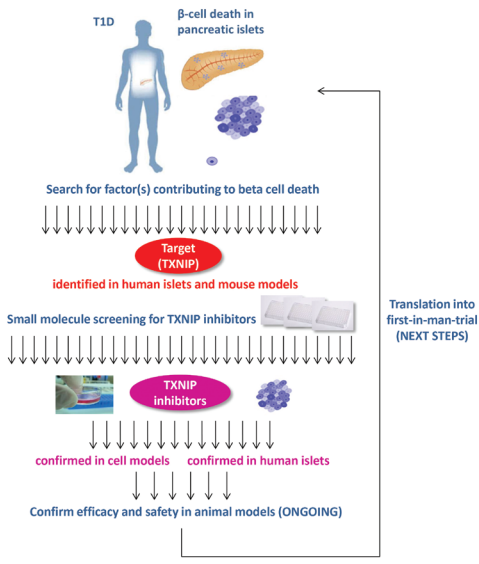

Figure 5. *Overview of the development of a novel small molecule approach to promote pancreatic beta cell survival in patients with T1D.* ***(CTAR: Shalev UC4, University of Alabama at Birmingham)***

A team at the Oregon Health & Science University made important progress on creating tools that may be useful for delivering molecules to improve beta cell function or number in people with diabetes. These investigators developed innovative virus vectors that can deliver new genes into human alpha and beta cells more efficiently and with less

toxicity than those used in early gene therapy studies (Figure 6). Importantly, these vectors can also be used to guide research on making replacement beta cells from pluripotent cells by enabling delivery of genes and molecules to beta cells. This project, which involved investigators from CMAI and CHIB, is another outstanding example of collaboration among teams across HIRN.

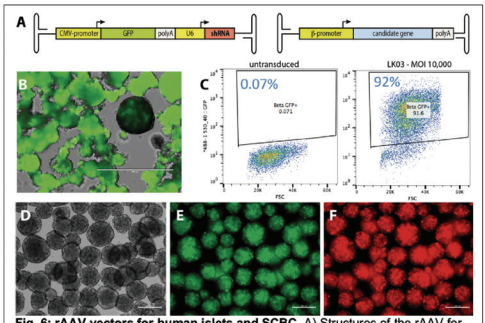

Fig. 6: rAAV vectors for human islets and SCBC A) Structures of the rAAV for overexpression and knockdown of candidate genes. Bb) Human cadaveric islets transduced with rAAVLK03 expressing GFP. C) FACS analysis showing 92% of β cells are GFP positive after 3 days. D-F: Transduction of SCBC with rAAVLK03 expressing tomoato red on day 4. D) Phase contrast; E) GFP = insulin reporter; F) Tomato red reporter. 100% of the insulin positive cells are rAAV transduced.

Figure 6. *The rAAV LK03 capsid enables high efficiency transduction of human beta cells or derived beta cells at low multiplicity of infection.* ***(CTAR: Grompe UC4, Oregon Health and Science University)***

EXPANDING CTAR WITH NEW INVESTIGATORS, HYPOTHESES AND TECHNOLOGIES

Looking forward, Year 4 brings a significant expansion of CTAR with the addition of five new research teams, representing 17 laboratories. These new teams will explore innovative strategies to shut down the immune attack on beta cells without turning off the entire immune system or to specifically stimulate beta cell proliferation. One group is developing islet-specific RNA aptamers, which are small, stable, therapeutic RNA molecules that could control selectively genes that regulate cell proliferation, apoptosis and resistance to autoimmunity. The development of systems that can deliver beta cell proliferation drugs directly to islets, thus potentially avoiding side effects of drugs that circulate throughout the entire body and engineering cells from the stomach lining to make insulin and serve as replacement beta cells are other approaches being tested. Other teams are modifying beneficial T cells ("Tregs") so that they directly home in on pancreatic islets, where they can suppress autoimmunity or deliver drugs to preserve beta cells.

A total of 280 resources (e.g., bioreagents, datasets, documents and technologies) have been attributed to CTAR investigators, alone or in collaboration with other HIRN Consortia. All HIRN resources can be viewed at **https://resourcebrowser.hirnetwork.org** and publications can be viewed at **https://hirnetwork.org/all-hirn-publications.**

CTAR INVESTIGATORS, YEAR 3

Anath Shalev, M.D., Investigator, *University of Alabama at Birmingham*

Markus Grompe, M.D., Investigator, *Oregon Health & Science University*
Mark Kay, M.D., Ph.D., Investigator, *Stanford University*
Hiroyuki Nakai, M.D., Ph.D., Investigator, *Oregon Health & Science University*

Pedro Herrera, Ph.D., Investigator, *University of Geneva*
Kenichiro Furuyama, M.D., Ph.D., Co-investigator, *University of Geneva*
Fabrizio Thorel, Ph.D., Co-investigator, *University of Geneva*

Klaus Kaestner, Ph.D., M.S., Investigator, *University of Pennsylvania*
Benjamin Glaser, M.D., Investigator, *Hadassah-Hebrew University*
Dana Avrahami-Tzfati, Ph.D., Co-investigator, *Hadassah-Hebrew University*

Alvin C. Powers, M.D., Investigator, *Vanderbilt University*
Andrew Stewart, M.D., Investigator, *Icahn School of Medicine at Mt. Sinai*
Seung Kim, M.D., Ph.D., Investigator, *Stanford University School of Medicine*
Rita Bottino, Ph.D., Co-investigator, *Children's Hospital of Pittsburgh*
Marcela Brissova, M.D., Co-investigator, *Vanderbilt University*
Chunhua Dai, M.D., Co-investigator, *Vanderbilt University*
Peng Wang, Ph.D., Co-investigator, *Icahn School of Medicine at Mt. Sinai*

* Paolo Serafini, Ph.D., Investigator, *University of Miami*
Midhat Abdulreda, Ph.D., Investigator, *University of Miami*
Peter Buchwald, Ph.D., Co-investigator, *University of Miami*
Camillo Ricord, M.D., Co-investigator, *University of Miami*
Natasa Strbo, M.D., Co-investigator, *University of Miami*

* Seung Kim, M.D., Ph.D., Investigator, *Stanford University*
Everett Meyer, Ph.D., Investigator, *Stanford University*

Philip Streeter, Ph.D., Co-investigator, *Oregon Health & Science University*

* **Amit Choudhary, Ph.D.,** Investigator, *Broad Institute*
Bridget Wagner, Ph.D., Investigator, *Broad Institute*
Rohit Kulkarni, M.D., Ph.D., Investigator, *Joslin Diabetes Institute*

* **Joe Zhou, Ph.D.,** Investigator, *Harvard University*
Stephan Kissler, Ph.D., Co-investigator, *Joslin Diabetes Institute*

* **Jeffrey Bluestone, Ph.D.,** Investigator, *University of California San Francisco*
Wendell Lim, Ph.D., Investigator, *University of California San Francisco*
Qizhi Tang, Ph.D., Co-investigator, *University of California San Francisco*
Kole Roybal, Ph.D., Co-investigator, *University of California San Francisco*

* New teams to be added in Year 4.

HUMAN PANCREAS ANALYSIS PROGRAM (HPAP)

THE GOAL: COLLECTION AND IN DEPTH STUDY OF TISSUES RELEVANT TO TYPE 1 DIABETES

One of the most daunting challenges in understanding type 1 diabetes (T1D) is that scientists cannot safely biopsy or effectively image the human pancreas and its islets. This limits their ability to investigate how the immune system attacks pancreatic islets and how the function of insulin-producing beta cells is lost as people transition from healthy to autoantibody-positive (pre-T1D) to diagnosed T1D. To tackle this issue, the HIRN created the Human Pancreas Analysis Program (HPAP) in Year 2. The HPAP works with U.S. organ procurement organizations (OPOs) to identify organ donors with recent-onset T1D or with autoantibodies that indicate high risk for T1D. The HPAP then procures and processes the pancreas, islets and immune tissues (e.g., blood, lymph nodes). The next step is analysis of the samples using multiple, interdisciplinary techniques with the ultimate goal of mapping out a detailed blueprint of the pancreas and the immune response in people with pre-T1D or T1D. Importantly, the HPAP maximizes the knowledge gained from the donated organs by making the data collected by the Program available to the broader T1D research community for further study. In its first year in the HIRN, the HPAP established a robust pipeline for procurement and processing of donated organs and began extensive and in-depth characterization of the collected tissues.

BUILDING A PIPELINE FOR COLLECTION OF HUMAN PANCREAS AND IMMUNE TISSUES

At the University of Florida, a team of investigators greatly expanded and enhanced an existing program for procurement of donated organs from people with newly diagnosed T1D or who were discovered to carry one or more T1D-related autoantibodies. The team increased their outreach to OPOs throughout the country and made more customized screening kits available, giving OPOs the resources they need to identify appropriate donors. Their efforts paid off significantly. The number of OPOs screening for T1D autoantibodies grew from 16 in 2016 to 27 in 2017, reaching 56 percent of the national organ donor pool in 2017, compared to 38 percent in 2016. Since 2017, the Florida team

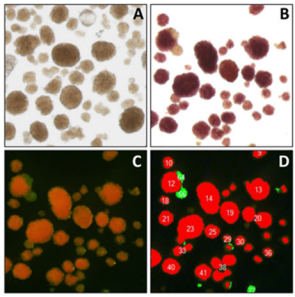

Figure 1. *HPAP analysis of islet number and purity. A - Islet morphology is first captured without stain. B,C - Islet preparation is then stained with DTZ to distinguish islets from exocrine tissue and imaged under the bright field (B) and dark field (C). D - Image is segmented with CellSens software to measure islet number, size and purity.* ***(HPAP: Naji UC4, University of Pennsylvania)***

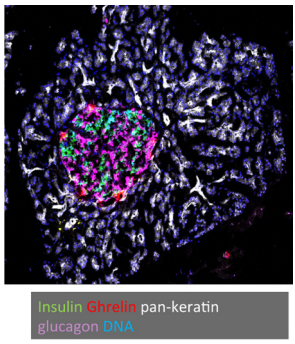

Figure 2. *Imaging mass cytometry (CyTOF) image of normal human pancreas. This section was stained simultaneously with 33 heavy metal conjugated antibodies. Shown here are Insulin (green), Ghrelin (red), pan-keratin (white), Glucagon (purple) and DNA (blue).* ***(HPAP: Naji UC4, University of Pennsylvania)***

identified tissues from 11 donors, including one donor with diabetic ketoacidosis (suspect T1D at onset), two with T1D and eight with potential autoantibodies (Figures 1 and 2). Collaboratively, a team at the University of Pennsylvania worked with a Philadelphia-based OPO to collect tissues from nine donors, including six as controls and three with type 2 diabetes (Figure 3).

ANALYZING AND SHARING HUMAN ISLETS AND DATA

The University of Pennsylvania team and a Vanderbilt University group both conducted extensive studies using state-of-the-art techniques to learn about the metabolic, genetic and immunologic processes underway in the donated tissues. For example, the teams routinely evaluated islet purity, number and viability. They also determined RNA content of single islet cells, measured hormone (insulin and glucagon)

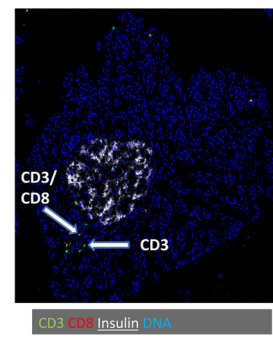

Figure 3. *Imaging mass cytometry (CyTOF) image of human pancreas from a single autoantibody positive donor. This section was stained simultaneously with 33 heavy metal conjugated antibodies. Visualized here are the T cell markers CD8 (red) and CD3 (green), insulin (white) and DNA (blue).* ***(HPAP: Powers UC4, Vanderbilt University)***

secretion and examined the composition of the islets with histology (studying cells and tissues at a microscopic level) and multispectral imaging technologies. The high-quality data collected at both sites is being pooled into a central database that will be made available to all HIRN investigators so that they can bring unique perspectives and questions to building the T1D-islet blueprint.

All HIRN Publications can be viewed at
https://hirnetwork.org/all-hirn-publications.

HPAP INVESTIGATORS, YEAR 1

Ali Naji, M.D., Ph.D., Investigator, *University of Pennsylvania*

Klaus Kaestner, Ph.D., Investigator, *University of Pennsylvania*

Michael Betts, Ph.D., Co-investigator, *University of Pennsylvania*

Michael Feldman, M.D., Ph.D., Co-Investigator, *University of Pennsylvania*

Jason Moore, Ph.D., Co-investigator, *University of Pennsylvania*

Doris Stoffers, M.D., Ph.D., Co-investigator, *University of Pennsylvania*

Al Powers, M.D., Investigator, *Vanderbilt University*

Mark Atkinson, Ph.D., Investigator, *University of Florida*

Marcela Brissova, Ph.D., Co-investigator, *Vanderbilt University*

Irina Kusmartseva, Ph.D., Co-investigator, *University of Florida*

Mingder Yang, Ph.D., Co-investigator, *University of Florida*

ACKNOWLEDGMENTS

Support for HIRN is provided by the National Institute of Diabetes and Digestive and Kidney Diseases and by the Type 1 Diabetes Special Statutory Funding Program. HIRN gratefully acknowledges funding provided by a grant from The Leona M. and Harry B. Helmsley Charitable Trust in support of the HIRN Annual Investigator Meeting. Additionally, a joint grant from the JDRF and the Leona M. and Harry B. Helmsley Charitable Trust supported the October 2016 CHIB in-person meeting.

APPENDIX 1
WORKING GROUP MEMBERSHIP, YEAR 3

2017 Annual Meeting Planning Committee

Raghu Mirmira, M.D., Ph.D., Co-Chair, CBDS Representative, *Indiana University*

Maike Sander, M.D., Co-Chair, CHIB Representative, *University of California, San Diego*

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

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

Nelly Berger, CC Representative, *City of Hope*

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

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

Clayton Mathews, Ph.D., CBDS Representative, *University of Florida*

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

Quinn Peterson, Ph.D., CHIB Representative, *Harvard University*

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

Layla Rouse, M.S., CC Representative, *City of Hope*

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

Anath Shalev, M.D., CTAR Representative, *University of Alabama at Birmingham*

Doris Stoffers, M.D., Ph.D., HPAP Representative, *University of Pennsylvania*

Megan Sykes, M.D., CMAI Representative, *Columbia University*

Fabrizio Thorel, Ph.D., CTAR Representative, *University of Geneva*

What Is a Beta Cell? Working Group

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

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

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

Yuval Dor, Ph.D., CBDS Representative, *Hebrew University of Jerusalem*

Ronald Gill, Ph.D., CMAI Representative, *University of Colorado, Denver*

Klaus Kaestner, Ph.D., CTAR Representative, *University of Pennsylvania*

Seung Kim, M.D., Ph.D., CTAR Representative, *Stanford University*

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

Cherie Stabler, Ph.D., CHIB Representative, *University of Florida*

Andrew Stewart, M.D., CTAR Representative, *Mt. Sinai Hospital*

Translational Working Group

Carmella Evans-Molina, M.D., Ph.D., Chair, CBDS Representative, *Indiana University*

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

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

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

Peter Gottlieb, M.D., CMAI Representative, *University of Colorado, Denver*

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

Mark Mamula, Ph.D., CBDS Representative, *Yale University*

Clayton Mathews, Ph.D., CBDS/CMAI Representative, *University of Florida*

Thomas Metz, Ph.D., CBDS Representative, *Pacific Northwest National Laboratory*

Raghavendra Mirmira, M.D., Ph.D., CBDS Representative, *Indiana University*

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

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

Christopher Rhodes, Ph.D., CBDS External Scientific Panel, *MedImmune*

Bart Roep, M.D., Ph.D., CBDS External Scientific Panel, *City of Hope*

Layla Rouse, M.S., CC Representative, *City of Hope*

Anath Shalev, M.D., CTAR Representative, *University of Alabama at Birmingham*

Cherie Stabler, Ph.D., CHIB Representative, *University of Florida*

Matthias von Herrath, M.D., CBDS External Scientific Panel, *La Jolla Institute for Allergy and Immunology*

Single Cell -Omics Working Group

Christopher Wright, DPhil, Chair, CBDS Representative, *Vanderbilt University*

Charles Ansong, Ph.D., CBDS Representative, *Pacific Northwest National Laboratory*

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

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

Bernd Bodenmiller, Ph.D., CBDS Representative, *University of Zurich*

Marcela Brissova, Ph.D., CBDS/CTAR Representative, *Vanderbilt University*

Kristin Burnum-Johnson, Ph.D., CBDS Representative, *Pacific Northwest National Laboratory*

Long Cai, Ph.D., CBDS Representative, *California Institute of Technology*

Viviana Gradinaru, Ph.D., CBDS Representative, *California Institute of Technology*

Pedro Herrera, Ph.D., CBDS/CTAR Representative, *University of Geneva*

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

Klaus Kaestner, Ph.D., CTAR Representative, *University of Pennsylvania*

Julia Laskin, Ph.D., CBDS Representative, *Pacific Northwest National Laboratory*

Thomas Metz, Ph.D., CBDS Representative, *Pacific Northwest National Laboratory*

Harry Nick, Ph.D., CBDS Representative, *University of Florida*

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

Jeremy Norris, Ph.D., CBDS Representative, *Vanderbilt University*

Galya Orr, Ph.D., CBDS Representative, *Pacific Northwest National Laboratory*

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

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

Layla Rouse, M.S., CC Representative, *City of Hope*

Lori Sussel, Ph.D., CBDS Representative, *University of Colorado, Denver*

Fabrizio Thorel, Ph.D., CBDS/CTAR Representative, *University of Geneva*

Islet Advisory Committee

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

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

Marcela Brissova, Ph.D., CBDS/CTAR Representative, *Vanderbilt University*

Rohit Kulkarni, M.D., Ph.D., CBDS Representative, *Joslin Diabetes Center*

Clayton Mathews, Ph.D., CBDS/CMAI Representative, *University of Florida*

Camillo Ricordi, M.D., CHIB Representative, *University of Miami*

Layla Rouse, M.S., CC Representative, *City of Hope*

iPSC Interest Group

Naohiro Terada, M.D., Ph.D., Chair, CMAI Representative, *University of Florida*

Clayton Mathews, Ph.D., CBDS/CMAI Representative, *University of Florida*

Website Advisory Group

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

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

Nelly Berger, CC Representative, *City of Hope*

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

Yi-Ju Chen, Ph.D., CHIB Representative, *University of Pennsylvania*

Klaus Kaestner, Ph.D., CTAR Representative, *University of Pennsylvania*

Layla Rouse, M.S., CC Representative, *City of Hope*

Sarah Tersey, Ph.D., CBDS Representative, *Indiana University*

Mark Wallet, Ph.D., CMAI Representative, *University of Florida*

APPENDIX 2
OPPORTUNITY POOL PROJECTS

	TITLE	PI	INSTITUTION
Completed projects — Year 2			
CHIB	December 2015 Investigator In Person Meeting	Cherie Stabler	University of Florida
Completed projects — Year 3			
CBDS CTAR	Mass spectrometry-based proteome maps for human islet cells	Wei-Jun Qian	Pacific Northwest National Laboratory
		Klaus Kaestner	University of Pennsylvania
CMAI	Characterization of in silico reconstruction of TCRs for modeling autoreactive T cells in type 1 diabetes	Todd Brusko	University of Florida
		Sally Kent	University of Massachusetts Medical School
		Maki Nakayama	University of Colorado
CTAR CBDS	Antibodies for beta-cell subtype identification by immunohistochemistry	Markus Grompe Philip Streeter	Oregon Health & Science University
		Mark Atkinson	University of Florida
CHIB	October 2016 Investigator In Person Meeting	Benjamin Stanger	University of Pennsylvania
Ongoing projects			
CHIB	Quantitative Mass Spectrometry Analysis of Human Islet and Pancreas ECM	Karen Christman	University of Massachusetts Medical School
		Kirk Hansen	University of Colorado
CBDS CHIB	Workshop for Continued Harmonization of Beta Cell Death Assays	Carmella Evans-Molina	Indiana University
		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 CTAR	Exploiting the power of CyTOF/ mass cytometry to elucidate the complex interactions of islet and immune cells in human type 1 diabetes pancreata	Clayton Mathews	University of Florida
		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
CHIB	Generation of functional endocrine cell receptor stem cell lines	Paul Gadue	Children's Hospital of Philadelphia

	TITLE	PI	INSTITUTION
Approved in Year 3			
CHIB	Functional Testing of Candidate HSC-Derived Islet Cells	Maike Sander	University of California San Diego
CMAI	Islet Reactive TCR Clones in Mice Generated with Type 1 diabetes Patient vs. Healthy Control Hematopoietic Stem Cells	Megan Sykes	Columbia University
		Todd Brusko	University of Florida
CTAR	A New Immunodeficient Mouse Model with Stable Hyperglycemia for the Study of Human Beta Cells	Klaus Kaestner	University of Pennsylvania
CTAR	The Role of Beta Cell Senescence in the Pathogenesis of Diabetes	Benjamin Glaser	Hadassah-Hebrew University
CBDS CTAR	Can Genome Mosaicism Explain the Lobular Nature of Type 1 Diabetes?	Klaus Kaestner	University of Pennsylvania

NOTES

NOTES