HIRN
HUMAN ISLET RESEARCH NETWORK

# HUMAN ISLET RESEARCH NETWORK (HIRN):

## YEAR 4 EXECUTIVE SUMMARY REPORT

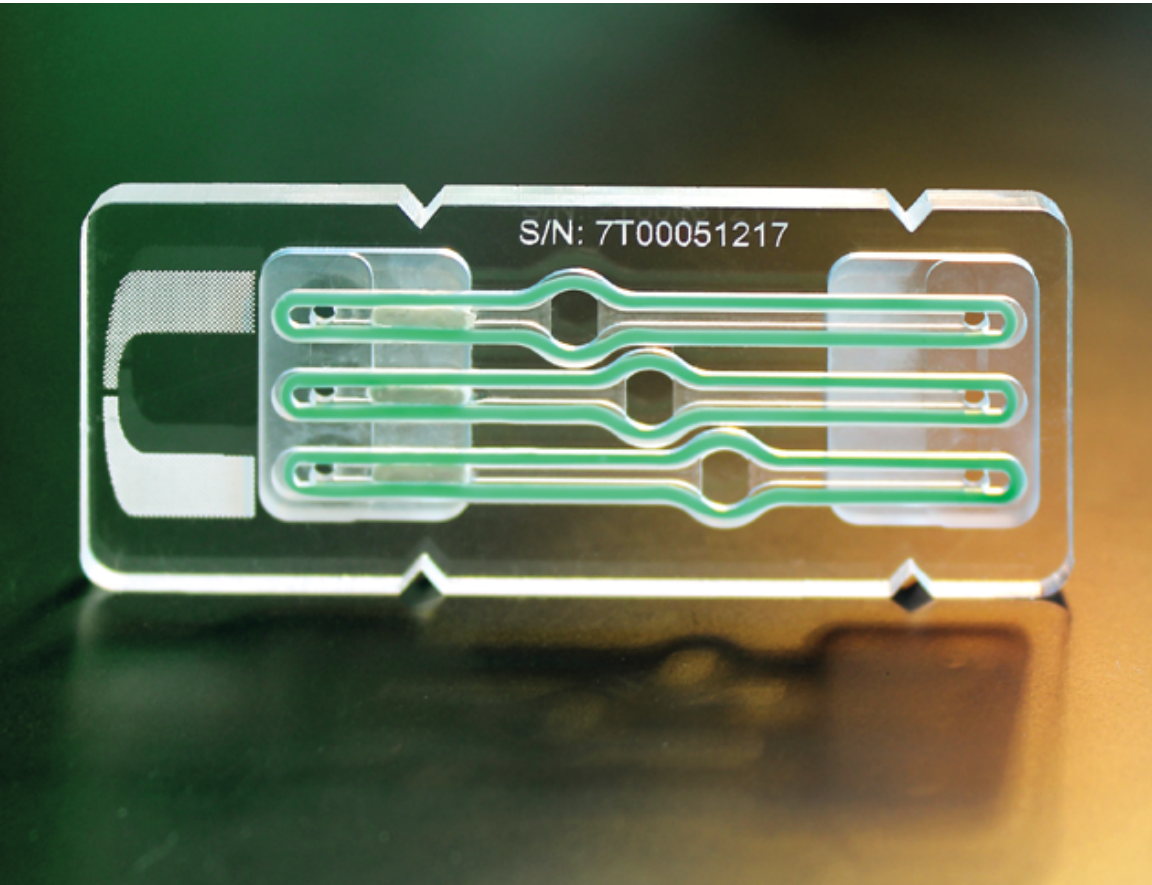

**Photo Caption:** Reversibly sealing plastic chip for perfusion, culture, and interrogation of pancreatic islets and beta cells derived from human pluripotent cells.

**Photo Credit:** *Agarwal Lab (Department of Biomedical Engineering, University of Miami)*

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*Report reflects activity as of September 30, 2018*

# FOREWORD

This year marks the 150th anniversary of the discovery of the pancreatic islets of Langerhans in 1869 by a German scientist, Paul Langerhans. He reported on small clusters of cells that looked distinctly different from the rest of the pancreas when viewed under a microscope. It would be more than 30 years before other scientists made the connection between the islets and diabetes, and another 20 years after that before insulin, which is produced in the islets, was successfully isolated and proven to treat diabetes. Over time, researchers learned that islets are complex organoids made up of multiple types of hormone-producing cells embedded in a network of blood vessels, neurons and other support cells and matrices. Notably, by the 1970s, the field knew that one particular islet cell type — the insulin-producing beta cell — can be targeted and destroyed by an autoimmune process, leading to type 1 diabetes.

Although the link between islet beta cells and type 1 diabetes is now firmly established, many questions remain unanswered. We do not fully understand why beta cells attract an autoimmune response in individuals who are genetically at risk for type 1 diabetes. Some part of the answer might lie in beta cell stress or dysfunction that precedes autoimmunity, but the exact cause or nature of that dysfunction is unclear. We are still unraveling how and why the normal safeguards that prevent the immune system from recognizing a person's own cells and tissues fail in type 1 diabetes. Nor do we know why some beta cells appear to escape autoimmune destruction, even in individuals who have had type 1 diabetes for many years. Tackling fundamental questions in human islet/beta cell biology is critically important if we are to devise ways to predict and prevent the development of autoimmunity, protect beta cells from destruction, regenerate or replace beta cells that are already lost and, ultimately, find a safe, effective and routine cure for the millions of people living with type 1 diabetes worldwide.

In 2014, the National Institutes of Health (NIH) established the Human Islet Research Network (HIRN) with the goal of advancing research on human islet biology in health and type 1 diabetes. NIH designed HIRN to be an evolving research community, with a fluid structure that is adaptable to new scientific concepts and opportunities as they arise. In the HIRN's fourth year, we were joined by 10 new research teams whose research

complemented and expanded the range of science being pursued across the network. All HIRN teams are committed to the principle of robust collaboration and communication within and across Consortia. This culture of open and ongoing interaction allows us to work together with colleagues across scientific disciplines, share ideas and results before publication, brainstorm about new directions and accelerate type 1 diabetes research in tangible ways.

In these pages, you will read about the technology revolution in human pancreas science that is being driven with HIRN support. For example, scientists in the Consortium on Beta Cell Death and Survival (CBDS) are developing and applying advanced technologies for analysis of single islet cells, uncovering an entirely new understanding of human islet structure, function and dysfunction in health and diabetes. In the Consortium on Human Islet Biomimetics (CHIB), several research teams are creating islet-on-a-chip microdevices that are revolutionizing the type of basic and translational research that can be conducted with human islets. A meticulously curated database derived from analyses performed on human pancreata from organ donors with type 1 diabetes or diabetes-related autoantibodies was released by the Human Pancreas Analysis Program (HPAP) in the past year. The broad diabetes research community can access and use this database to gain insights and formulate hypotheses, thus fostering progress across the field.

This report also describes how HIRN teams are making progress on previously understudied areas of human type 1 diabetes biology. For example, scientists from the Consortium on Modeling Autoimmune Interactions (CMAI) created an unprecedented library of immune cells isolated from organ donors with or without type 1 diabetes or diabetes-related autoantibodies. Studying these rare cells has already improved our knowledge of the autoimmune process in human islets. Teams in the Consortium on Targeting and Regeneration (CTAR) are developing multiple promising strategies that could, for the first time, deliver small molecules, drugs or cellular therapies directly to the islets without off-target effects on other cells or tissues.

On behalf of the HIRN community, we are pleased to present this *HIRN Year 4 Executive Summary Report*, which contains these and other highlights of compelling scientific discoveries and technology advances made by the research teams in the Network. We welcome all who have an interest in type 1 diabetes to learn about the exciting progress made in human islet biology over the past year and the promise it holds for a future when type 1 diabetes is routinely prevented or reversed.

Sincerely,

The HIRN Trans-Network Committee (TNC)

***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*
***John Kaddis, Ph.D.**, BC Representative*
***Klaus Kaestner, Ph.D.**, HPAP/HPAC Representative*
***Joyce Niland, Ph.D.**, CC Representative*
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***Sheryl Sato, Ph.D.**, NIH Program Staff*
***Lori Sussel, Ph.D.**, CBDS Representative*

# SUMMARY OF SIGNIFICANT PROGRESS IN THE HUMAN ISLET RESEARCH NETWORK, YEAR 4

The Human Islet Research Network (HIRN) and its member consortia made significant scientific and organizational progress during the fourth year of the Network (second year for the Human Pancreas Analysis Program). Highlights of this progress are described below, with selected publications listed where applicable. Additional major advances in type 1 diabetes research and technology development for each HIRN component are described in other chapters of this Executive Summary Report, along with selected image(s) from each research team's overall project. An up-to-date list of all publications by HIRN investigators is posted at: **hirnetwork.org/publications**.

## CONSORTIUM ON BETA CELL DEATH AND SURVIVAL (CBDS)

### *Technology Innovations Reveal Key Insights Into Pancreas Biology*

Understanding the cellular stresses and injuries that affect human pancreatic beta cells in a prediabetic environment and the contribution of these pathological events to the development of autoimmunity are priorities for the CBDS. This greater understanding can only come from using high-resolution technologies to study diseased human islets and their tissue environment. Four separate CBDS teams have made significant progress in developing or applying for the very first time a suite of state-of-the-art technologies for the exploration of the human pancreas[1-4]. The use of these new tools to examine pancreata from deceased organ donors is already yielding important information related to type 1 diabetes progression and heterogeneity that lays the foundation for hypothesis generation and follow-up experiments[1]. CBDS investigators are not restricting their exploration of the diseased pancreas to insulin-producing beta cells and have demonstrated that other cell types in and around pancreatic islets display severe abnormalities that may contribute to disease initiation, including glucagon-secreting alpha cells[5], as well as cells that constitute the islet vasculature[6].

### *Beta Cell Failure in Diverse Conditions May Hold Clues to New Strategies for Beta Cell Protection*

CBDS investigators are also studying beta cell failure in disease situations other than type 1 diabetes to better understand why pancreatic beta cells are so sensitive to a variety of stressors and are so prone to targeting by immune cells. Specifically, CBDS investigators have demonstrated that cystic fibrosis (CF)-related diabetes (CFRD), an increasingly common and devastating comorbidity of CF, is caused by beta cell loss and intraislet inflammation in the setting of a complex pleiotropic disease and not by intrinsic islet dysfunction from the CF mutation[7]. Another CBDS team reported for the first time that insulin-dependent diabetes often occurs in patients with cancers who are treated with a certain class of checkpoint inhibitors, and they showed that this syndrome has similarities and differences compared with classic type 1 diabetes[8]. Both reports have important implications for the therapeutic management of these conditions with associated diabetes.

## CONSORTIUM ON HUMAN ISLET BIOMIMETICS (CHIB)

### *Interdisciplinary Approaches Foster Technology Development for Islet Research*

Through harmonization of interdisciplinary research approaches between engineers and islet biologists, CHIB investigators have generated microphysiological (MPS) devices that support cells to closely mimic a functional human islet. MPS devices support long-term culture of multicellular organoids that recapitulate native microenvironments, optimize nutrient delivery and allow for longitudinal, multiparameter assessments. In addition, the MPS devices support quantitative assays for monitoring human islet function.

## CONSORTIUM ON MODELING AUTOIMMUNE INTERACTIONS (CMAI)

### *Researchers Build the Most Extensive Library of Rare Human Type 1 Diabetes T Cell Lines*

Central and peripheral immune tolerance mechanisms regulate autoimmunity. CMAI investigators have acquired the largest collection in the world of islet autoreactive T cell lines and clones obtained from the islets of individuals with type 1 diabetes. These cell lines and clones will

allow the study of human islet autoreactive T cells, their specificities and their effector functions that lead to beta cell destruction in type 1 diabetes. These include T cell receptors that recognize neoantigens, which are new antigens (molecules that provoke an immune reaction) that have not been previously recognized by the immune system. Neoantigens generated as a result of cell stress could unmask autoimmunity. CMAI investigators discovered that endoplasmic reticulum stress in beta cells induced the activity of enzymes that create post-translational modifications that can become neoantigens and provoke autoimmune T cells[9].

### *Previously Unknown Population of Cells Is Discovered in the Thymus*

A new population of thymus cells, termed "thymic tuft" cells, and their role in regulating development of lymphocyte populations in the thymus have been discovered in the thymus of mice[10]. These studies will improve our fundamental understanding of central tolerance mechanisms and shaping/seeding the peripheral immune repertoire. Extension of these studies to determine whether this cell population in humans has the same functions of thymic tuft cells in the mouse will be critical in our understanding of human type 1 diabetes.

### *The Genetics of Type 1 Diabetes in Underrepresented Populations Is a Critical Research Gap*

Genetics play a major role in autoimmunity. T1D genetic studies that have been performed have focused largely on Caucasian populations or populations with Northern European ancestry. In contrast, there has been little focus on type 1 diabetes genetics in underrepresented or health care disparity populations (URM; Black/African American or Hispanic/Latino populations). Investigators in CMAI demonstrated that the major genetic elements contributing to disease in these URM populations are similar, but not identical, to Caucasian groups[11]. These findings highlight the need for additional studies into the genetic mechanisms of type 1 diabetes in URM populations to inform the development of type 1 diabetes models that are representative of the diverse mechanisms that contribute to disease.

### *Robust Models for Human Type 1 Diabetes Accelerate Research and Discovery*

Advances have been made in the development of complex mouse models for the study of human type 1 diabetes, including genetically modified

mice that can be used to investigate the function of human autoreactivity *in vivo* in the absence of the confounding effects of graft-versus-host disease[12]. Significant progress in the development of human beta cells, hematopoietic progenitor cells and thymic epithelial cells (TECs) from pluripotent cells continues in the laboratories of CMAI investigators. The development of a roadmap charting the transcriptional profile of murine TECs during development has set the stage for making key advances toward directed differentiation of human TECs[13]. The generation of these three cell populations from human pluripotent cells is critical for reaching the ultimate goal of creating a complex mouse model with key human cell types that will be needed to recapitulate human type 1 diabetes.

## CONSORTIUM ON TARGETING AND REGENERATION (CTAR)

Scientists are pursuing two major therapeutic strategies to increase the number of insulin-producing beta cells in the pancreas of diabetic patients, in which this particular pool of cells is severely depleted: make the residual beta cells divide in a controlled and safe manner, or turn some of the pancreatic islet's non-beta cells into glucose-responsive, insulin-secreting cells. CTAR investigators have made significant progress in both of these areas.

### *Research on Beta Cell Replacement Points to Strategies for Diabetes Treatment*

First, building on several years of research in the mouse, they have demonstrated for the first time that human glucagon-producing alpha cells, which are very abundant in type 1 diabetes patients, can be triggered to secrete insulin in response to glucose and reverse diabetes once transplanted into autoimmune diabetic mice[14]. This pioneering work opens the door to therapeutic strategies to turn a type 1 diabetes patient's own alpha cells into insulin-producing cells that may escape the autoimmune environment that depleted the beta cell pool in the first place. Second, three CTAR teams have identified three separate regulatory pathways in beta cells that can be targeted to trigger efficient and controlled human beta cell replication[15–17]. In two of these cases, first-generation therapeutic molecules are being synthesized and tested[15,18].

### *First Report of a Beta Cell-Specific Cell Surface Marker Is a Game-Changer in Diabetes Research*

Since most therapeutic small molecules are unlikely to be specific for beta cells (and could therefore have significant associated off-target effects), a challenge in the field has always been to find molecular anchors on the surface of beta cells that could help make the delivery of a variety of therapeutic molecules more specific. CTAR investigators have described for the first time a beta cell-specific cell surface protein, called NTPDase3[19]. Antibodies against NTPDase3 can now be used to deliver therapeutic cargos to beta cells *in vivo* and to facilitate the isolation of pure beta cell preparations for research purposes.

## HUMAN PANCREAS ANALYSIS PROGRAM/CONSORTIUM (HPAP/HPAC)

Two years ago, HIRN launched the Human Pancreas Analysis Program (HPAP), a highly collaborative group of investigators dedicated to collecting and sharing an unprecedented amount of phenotypic data related to human pancreata obtained from rare organ donors with type 1 diabetes or prediabetic autoimmunity. The team has made tremendous progress in HPAP Year 2, with 30 organs processed and analyzed. The team applies multiple cutting-edge technologies to each pancreas, and the collected data are deposited in the public PancDB database.

### *Sharing Data from Rare Type 1 Diabetic and Prediabetic Pancreata Benefits the Entire Field*

In HPAP Year 2, University of Pennsylvania (UPenn) investigators developed and launched a state-of-the-art web portal that allows type 1 diabetes researchers from all HIRN consortia and the general research community to access, sort and download data and images from the HPAP human pancreas analysis collection. The team also made progress on developing an open-source, user-friendly artificial intelligence software package that will help users practice machine learning in an automated way, as well as a number of visualization tools that enhance the utility and accessibility of the unique, rich datasets available through the portal. Finally, HPAP is starting to demonstrate how the use of its most advanced technologies, such as imaging mass cytometry, can provide new insights on the pathophysiology of type 1 diabetes[20].

## HIRN BIOINFORMATICS CENTER (BC)

### *Disseminating HIRN Data and Resources Accelerates Type 1 Diabetes Research*

The HIRN BC focused on the curation of resources used or generated by HIRN, registration of metadata in available public repositories and the continued development and maintenance of tools that enable analysis, organization and sharing of data. Resources are grouped into four major categories, including bioreagents, datasets, documents and technologies. All resources are accessible to everyone through the HIRN website.

## HIRN COORDINATING CENTER (CC)

### *Efficient Administrative Infrastructure Supports HIRN Research and Community Outreach*

The HIRN CC continued to develop and execute policies and procedures that ensure the efficient operation of the Network and its constituent Consortia and research teams. The CC organized the largest HIRN Annual Investigator Meeting to date with 260 attendees, including HIRN investigators, their lab members, external advisors and representatives from other organizations with an interest in type 1 diabetes research. On the HIRN website, the CC continually updated and maintained current information about the Network, its research goals and progress, publications, resource development, funding opportunities and notifications and more for the benefit of HIRN scientists and the broader community of type 1 diabetes researchers, advocates and patients. Successful implementation of an online peer-reviewed competitive application process allowed the CC to manage the review and funding of multiple New Investigator Pilot Awards. These awards are a new funding mechanism that allows investigators who are at an early stage of their careers to pursue innovative human islet research projects within the Network structure.

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# HUMAN ISLET RESEARCH NETWORK:

## PROMOTING INNOVATION, COMMUNICATION AND RESOURCE DEVELOPMENT IN TYPE 1 DIABETES RESEARCH

The Human Islet Research Network (HIRN) mission is *to better understand how human beta cells are lost in type 1 diabetes and to find innovative strategies to protect or replace functional beta cell mass in diabetic patients*.

### HIRN: TEAM SCIENCE AT THE FOREFRONT OF TYPE 1 DIABETES RESEARCH

Since 2014, the National Institutes of Health (NIH)-funded Human Islet Research Network (HIRN) has fostered and supported collaborative research related to the loss of functional pancreatic beta cell mass in type 1 diabetes. The Network was originally made up of four consortia, composed of between 2 and 12 multidisciplinary, multi-institutional research teams. In Year 4, HIRN was re-organized into five consortia focused on specific scientific themes that included the: 1) Consortium on Beta Cell Death and Survival (CBDS); 2) Consortium on Human Islet Biomimetics (CHIB); 3) Consortium on Modeling Autoimmune Interactions (CMAI); 4) Consortium on Targeting and Regeneration (CTAR); and 5) Human Pancreas Analysis Program/Consortium (HPAP/HPAC). HIRN is supported by a Coordinating Center (HIRN CC) that manages all administrative aspects of the Network, including facilitating regular interactions within the Network and between HIRN and the broader type 1 diabetes community, and by the HIRN Bioinformatics Center (HIRN BC) that provides bioinformatics support for HIRN investigators and ensures that resources developed with Network support are shared with the field.

As of September 30, 2018, 160 diabetes investigators and co-investigators from research institutions across the United States and around the world participated in HIRN research teams. HIRN consortia continued to create new technologies, develop unique research resources and datasets, and to generate novel insights into human islet biology and type 1 diabetes pathogenesis. Especially noteworthy research findings from Year 4 are described in the "Summary of Significant Progress" chapter of this report. Additional examples of important scientific progress and resource

development that have advanced type 1 diabetes research in the past year can be found in the individual chapters for each consortium, along with selected image(s) from each research team's overall project. The next section describes how HIRN fosters innovation by soliciting new research teams, funding collaborative short-term Opportunity Pool Projects and supporting investigators who are beginning careers in islet research.

## HIRN FUNDING OPPORTUNITIES: RAPID RESPONSE TO INNOVATION

HIRN is purposely structured to be a dynamic, evolving community of diabetes researchers who develop and apply leading-edge technologies in pursuit of innovative hypotheses related to type 1 diabetes. The HIRN structure offers frequent opportunities for the development of new concepts and the addition of new projects. The length of HIRN grant awards varies from two to five years, and multiple initiatives are issued every year (when funds are available), enabling the creation of a fluid community that can respond to novel scientific concepts as they arise.

In Year 4, HIRN expanded with 10 new research teams — five in CTAR, three in CBDS and one each in CHIB and CMAI. Those teams are engaged in a range of pioneering research projects, including new models of type 1 diabetes pathogenesis based on DNA damage responses or RNA modification; development of a portable optical system for measuring insulin release from cultured beta cells; derivation and characterization of islet-infiltrating T cells; strategies to reprogram stomach cells into insulin-producing cells; and diverse approaches for targeting beta cells with therapeutic immune cells or small molecules. Looking ahead, in Year 5, CBDS welcomes a new investigator who will continue promising research on a target for type 1 diabetes diagnosis and treatment that was initiated in CTAR. And, the addition of four human islet-focused research teams to the ongoing resource development mission of the HPAP will reorganize that program into the Human Pancreas Analysis Consortium (HPAC). Also in Year 5, the NIH will review applications submitted in response to five Requests for Applications (RFAs) offering funding for new projects to join CHIB, CMAI, CTAR and HPAC.

In addition to supporting new consortium-based teams, HIRN has developed two short-term funding mechanisms to promote exploratory research by HIRN investigators and others from the broader research community — Opportunity Pool Projects and New Investigator Pilot Awards.

## OPPORTUNITY POOL PROJECTS

HIRN developed the Opportunity Pool Project initiative to foster new research ideas and to support the development of emerging technologies for type 1 diabetes research. HIRN investigators were eligible to propose short-term, innovative projects related to the cause, prevention or cure of type 1 diabetes. Projects could involve one or multiple investigators within or across consortia; researchers could also recruit collaborators from outside of the Network. In Years 1–4, HIRN funded 18 highly meritorious Opportunity Pool Projects (Appendix 1), including an award to provide support for three annual CHIB-wide meetings to encourage communication and coordination of technology development within the consortium. The HIRN CC manages all aspects of the Opportunity Pool initiative, including the peer-review of applications, execution of subawards to investigators' institutions and collection of final progress reports. More information on HIRN Opportunity Pool Projects can be found at hirnetwork.org/consortium/opportunity-pool.

In Year 4, two Opportunity Pool Projects completed work and submitted progress reports. Summaries of those projects are provided below.

### *Exploiting the Power of CyTOF/Mass Cytometry (MC) to Elucidate the Complex Interactions of Islet and Immune Cells in Human Type 1 Diabetes Pancreata*

*Dirk Homann, Icahn School of Medicine at Mount Sinai; Andrew Stewart, Icahn School of Medicine at Mount Sinai; Clayton Mathews, University of Florida*

Summary: Understanding the properties of pancreatic exocrine (digestive), endocrine (hormonal), ductal, immune and other cell types in health and disease is essential for the development of improved and novel treatments for type 1 diabetes. This collaboration among CMAI and CTAR investigators focused on development of an advanced technology platform that enables comprehensive, versatile and "sample-sparing" analysis of the pancreas proteome (a catalog of proteins) at the single-cell level. The team successfully selected, tested and validated multiple probes for detection of all human endocrine cell populations (alpha, beta, delta and PP cells), as well as endocrine-specific transcription factors and cell surface proteins. Preliminary results showed that pancreatic alpha, beta and delta cells exhibit heterogeneous characteristics and a broad spectrum of transcription factor expression patterns. In addition, the team developed strategies for analyzing immune cells that infiltrate nondiabetic and type 1

diabetic pancreata. This exciting line of research provides a robust, high-dimensional platform with broad applications in human islet cell biology research, such as studies of beta cell transdifferentiation from other islets cells, progenitor cell differentiation, profiling of infiltrating immune cell populations and refinement of drug development strategies.

### *Islet-Reactive TCR Clones in Experimental Mice Generated with Type 1 Diabetes Patient Vs. Healthy Control Hematopoietic Progenitor Cells*

*Megan Sykes, Columbia University; Todd Brusko, University of Florida*

Summary: T cells that recognize proteins on the body's own beta cells — called "autoreactive" T cells — are a driving force in type 1 diabetes autoimmunity. This collaboration between CMAI investigators explored two explanations for production of autoreactive T cells — a defect in the education of T cells in the thymus, where they normally learn to not attack self-molecules, or an underlying genetic defect that causes autoreactive T cells to multiply aberrantly after they leave the thymus. The team developed a personalized immune mouse model in which human hematopoietic progenitor cells were engrafted with genetically matched human thymus organoids. With this model, investigators can analyze T cells in the thymus and the periphery (e.g., spleen, lymph nodes) to determine where autoreactive T cells are most common and how they compare to autoreactive T cells that are known to attack beta cells. These studies provide proof-of-concept data that the earliest selection events in the thymus can be effectively modeled in experimental mouse models and provide a framework for future research to understand how genetic susceptibility shapes T cell populations in people at high risk for type 1 diabetes.

## NEW INVESTIGATOR PILOT AWARDS

In Year 4, HIRN utilized Opportunity Pool funds for a new initiative — the New Investigator Pilot Awards program. These awards support new investigators of exceptional creativity who propose to apply bold and highly innovative research approaches to biological problems under current investigation in HIRN. The Awards provide up to $150,000 in funding for up to 2 years for early career investigators to explore the feasibility of a novel concept that could support an eventual NIH R01 application. New Investigator Pilot Award applications underwent rigorous external peer-review for scientific and technical merit, and seven Awards were granted

near the end of Year 4 (see a list of Awards in Appendix 2). The scientific goals of the Awards are summarized in the CBDS, CMAI, CTAR and HPAC chapters of this annual report, and more information can be found at hirnetwork.org/news-awards/2018-new-investigator-recipients.

A unique feature of the New Investigator Pilot Awards is that successful recipients are assigned to a host consortium, and an investigator from that consortium is designated as a mentor to each recipient. The mentors serve as primary points of contact for the recipients, who are encouraged to interact broadly with the HIRN community during the Award tenure. Recipients participate in monthly consortium conference calls and attend the HIRN Annual Investigator Meeting. The mentors accelerate integration of the recipients within their assigned consortia and the Network as a whole. They also introduce the recipients to other scientists within the type 1 diabetes research field and the general scientific community. These senior investigators assist their mentees in data interpretation, review of pending journal manuscripts, development of R01 grant applications, and understanding of the NIH study section and grant review processes. By providing overall career guidance, HIRN mentors ensure that these creative, motivated new investigators have a meaningful opportunity to establish themselves in type 1 diabetes research careers and, thereby, contribute their talents to progress in T1D research.

## HIRN COORDINATING CENTER (HIRN CC)

The HIRN Coordinating Center (HIRN CC) located at City of Hope in Duarte, California, administers all Network operations and provides critical support of the HIRN mission. The primary objectives of the HIRN CC are to advance type 1 diabetes research by: establishing an optimal administrative infrastructure to facilitate consortia activities; fostering an environment of trust and collaboration; facilitating scientific communication among HIRN investigators; and promoting sharing of data, materials and resources within and across the HIRN consortia and with the broader scientific community. The HIRN CC assists with the coordination of centralized HIRN meetings, including conference calls for the consortia, working groups, External Scientific Panels and individual research teams, as well as the Annual Investigator Meeting and consortium-specific meetings. The HIRN CC serves as a bridge for communication between the HIRN and

other diabetes-relevant research communities, as well as with the type 1 diabetes patient community and the public at large. In collaboration with the HIRN Bioinformatics Center (HIRN BC), the HIRN CC coordinates and publicizes information about novel resources for diabetes research that are generated by the consortia.

The HIRN CC facilitates the operations of the investigator-led Trans-Network Committee (TNC), a Network-wide group with representation from each of the five consortia, the HIRN CC, HIRN BC and NIH program staff. The TNC oversees central activities, such as the Annual Investigator Meeting, and makes decisions that affect the Network as a whole. Monthly TNC meetings facilitate collaboration and communication among the consortia. To ensure broad investigator participation and input, a new TNC chair and new representatives for CBDS and CMAI were named in Year 4. Each consortium also convenes on a regular basis, often by conference calls, to discuss research progress, challenges and new opportunities.

## COMMUNICATING PROGRESS AND RESOURCE DEVELOPMENT FOR TYPE 1 DIABETES RESEARCH

A crucial part of the HIRN mission is to promote transparency and communication by sharing information, data and resources among HIRN investigators and with the scientific community and public at large. A primary venue for those communications is the HIRN website (**hirnetwork.org**), which was developed and is maintained by the HIRN CC in collaboration with the HIRN BC. The website provides important information and updates on the Network and each consortium, the NIH-supported UC4 grants, Opportunity Pool Projects (Appendix 1), New Investigator Pilot Awards (Appendix 2), HIRN working groups (Appendix 3), publications and research resources.

In Year 4, the HIRN CC expanded its outreach efforts within and outside of the Network. The HIRN CC and HIRN BC collaborated on an updated version of the HIRN Resource Browser. The HIRN CC worked proactively with HIRN research teams to collect, curate, load and display information about newly developed resources; this extraordinary collection of data and resources can be accessed on the HIRN website under the "Resources" tab. (Additional details on the Resource Browser are described in the HIRN BC section below.) The HIRN CC began distributing monthly newsletters to all HIRN investigators, trainees and staff. The newsletters are posted on the HIRN website for members of the public who are interested in learning more about

Network activities and progress. Also, the HIRN CC maintains an active social media presence to share information about HIRN or related topics of interest to the greater type 1 diabetes community; anyone can follow HIRN on Twitter (@HIRN_CC), Facebook (@HumanIsletResearchNetwork) and LinkedIn. In the future, the HIRN CC plans to create an informative YouTube channel to share type 1 diabetes-related video resources with the community.

## ORGANIZING INVESTIGATOR MEETINGS AND INTERACTIONS TO FOSTER COMMUNICATION

As part of its responsibility for facilitating communication across the Network, the HIRN CC organizes in-person meetings among the consortia and research teams, including the HIRN Annual Investigator Meeting. This Network-wide Meeting is an opportunity for HIRN investigators, their lab members and invited guests to further the HIRN goals of communication and collaboration to accelerate type 1 diabetes research. In May 2018, the HIRN convened in Washington, DC, for its annual meeting planned by the 2018 working group (Appendix 3) and organized by the HIRN CC, which managed the logistics. The meeting saw a record-high turnout of 260 attendees (up from 213 in 2017), including representatives from the NIH, The Leona M. and Harry B. Helmsley Charitable Trust, NIDDK Information Network (dkNET), Integrated Islet Distribution Program (IIDP), JDRF and the JDRF-funded Network for Pancreatic Organ Donors with Diabetes (nPOD).

The Annual Investigator Meeting agenda covered a range of cross-disciplinary topics of interest to investigators throughout the five consortia, including: innovative models and approaches to understanding diabetes; novel therapy and biomarkers; development and persistence of the autoimmune repertoire; understanding pancreas pathology through big data processing and analytics; regulatory and physiological-immunological insights in islet biology; advances in beta cell delivery and physiological manipulation; and new insights in beta cell proliferation and function. Investigators from each research team who joined the HIRN in Year 4 introduced themselves and presented their experimental plans to the Network. Seven break-out sessions allowed for deeper discussions on key scientific issues, and 114 poster presentations (up from 90 in 2017) showcased the breadth of innovative research being pursued by laboratories across the HIRN.

Importantly, blocks of time were allotted for open communication among all investigators and for in-person meetings of each consortium.

In Year 4, the HIRN CC also assisted in organizing the third annual in-person CHIB meeting, held in San Diego, California, in October 2017. A total of 51 participants attended, including representatives from each CHIB research team, the NIH, JDRF and HIRN CC and HIRN BC staff. The meeting featured a cross-consortium discussion between members of CHIB and CMAI to discuss mutual interests in modeling the immune component of type 1 diabetes using the islet microdevices.

## HIRN BIOINFORMATICS CENTER (HIRN BC)

The primary objectives of the HIRN BC, also located at City of Hope, are to provide shared bioinformatics capabilities and infrastructure for HIRN and to support the sharing of HIRN resources and findings with the scientific community and the public. In Year 4, the HIRN BC focused on the curation of resources used or generated by HIRN investigators, registration of metadata in available public repositories and continued development of tools that enable analysis, organization and sharing of data.

### HIRN RESOURCE BROWSER

In Year 4, the HIRN BC and CC released version 2 of the HIRN Resource Browser. This valuable tool was originally established in Year 2 to catalog the myriad resources used or generated by HIRN investigators, including bioreagents (antibodies, constructs, differentiated cells, model organisms, other cell lines, primary cells, progenitor cells), datasets (epigenomics, genomics, metabolomics, proteomics, transcriptomics), documents (protocols), and technologies (assays, code/pipeline, devices/equipment, software/databases). Revisions in the past year included expanded web searches for data acquisition; implementation of a content curation process that includes quality control procedures for data checking, standardization and processing; a new application programming interface for data sharing; an updated user interface; and other technical upgrades. All of these features were designed by the HIRN BC with the goal of increasing the use of HIRN-generated research resources to accelerate progress across the field.

All available datasets catalogued in the HIRN Resource Browser are deposited into the National Center for Biotechnology Information (NCBI) Gene Expression Omnibus (GEO), a public functional genomics data repository. All HIRN datasets in GEO were indexed with terms from six ontologies that can be used to search for studies related to HIRN datasets. The Resource Browser fulfills a major goal of the HIRN by facilitating rapid and open sharing of data and resources among HIRN investigators and, when appropriate, with the diabetes research community at large. The resource browser is available online and can be viewed at resourcebrowser.hirnetwork.org.

## COLLABORATION BETWEEN THE HIRN BC AND INVESTIGATORS PROMOTES USE OF NOVEL RESOURCES

The HIRN BC actively engages with all HIRN components to promote resource usage and respond to upcoming needs created by the Network's expansion and research progress. In Year 4, the HIRN BC worked with Dr. Mark Wallet (CMAI, University of Florida) to establish a version-controlled process for protocol curation and registration. A webinar was held in February 2018 to demonstrate the process, and the BC continues to follow up with additional investigators as needed. For CHIB investigators, the BC created an internal file share system to facilitate collaboration among investigators who are restricted from accessing commercial file sharing solutions. The HIRN BC also worked with NIDDK's dkNET program to mint stable unique identifiers for research resources offered through HIRN. This process is part of an ongoing effort to address the FAIR (Findable, Accessible, Interoperable and Re-useable) principles, established by the Force11 community, as well as NIH rigor and reproducibility requirements. Finally, the HIRN BC published the results of a study on the effect of hospitalization time prior to death on immune and replicating beta cells in the pancreas of organ donors. All of the code, calculations, quantitative histopathology data and macros were catalogued in the HIRN Resource Browser, as well an open access data repository, for use by all investigators.

## COORDINATING CENTER (CC) AND BIOINFORMATICS CENTER (BC) INVESTIGATORS, YEAR 4

**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 4

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

**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*

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

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

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

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

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

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

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

# CONSORTIUM ON BETA CELL DEATH AND SURVIVAL (CBDS)

## TIPPING THE SCALES TOWARD BETA CELL SURVIVAL

The autoimmune destruction of insulin-producing beta cells during the development of type 1 diabetes is a silent process that can take years. Up until the onset of clinical symptoms of diabetes, beta cells continue to release precise amounts of insulin to the rest of the body as glucose levels fluctuate in response to food, physical activity, illness or other events in daily life. Yet, deep within the pancreatic islets of people who are susceptible to type 1 diabetes, this basic beta cell function becomes dysregulated, the immune system is activated to attack the beta cells and, over time, the balance shifts from beta cell survival to death, triggering what may seem to be sudden onset of the disease. The Consortium on Beta Cell Death and Survival (CBDS) is searching for biological markers (biomarkers) of the disease process that can be used to monitor type 1 diabetes initiation and progression, reveal important information about beta cell health and function, and evaluate responses to potential treatments. In addition, the CBDS is developing cutting-edge technologies for probing beta cell biology that might reveal new targets for diabetes intervention. In Year 4, CBDS investigators advanced multiple strategies for beta cell biomarker discovery, generated unique data resources and high-resolution technologies to accelerate research across the diabetes community and pursued innovative hypotheses that may revolutionize the understanding of how beta cells either survive or succumb to autoimmunity.

## IDENTIFYING BIOMARKERS TO MONITOR AND UNDERSTAND BETA CELL HEALTH AND STRESS

Desmond Schatz, University of Florida, led two international teams of investigators who worked to develop noninvasive, quantitative measures of circulating cell-free (cf) DNA in the blood that could serve as biomarkers of cell death. The assay seeks to determine the existence of new biomarkers that identify several of the dysfunctional processes that occur during the progression of type 1 diabetes, including early loss of beta cells, changes in exocrine (i.e., digestive) pancreas cells, alterations in the immune system linked to autoimmunity and even the development of long-term diabetes complications. The team continued to optimize the specificity and sensitivity

of the cfDNA measurement system, bringing it closer to the clinical goal of monitoring beta cell death in individuals at risk for or diagnosed with type 1 diabetes. In addition, the team pursued a related assay measuring tiny RNA molecules released into the blood from dying beta cells. This cfRNA assay effectively detected the massive loss of beta cells observed after islet transplantation. Research is ongoing to discover more sensitive beta cell-specific cfRNA signatures that can detect extremely small levels of RNAs released when a few beta cells die at any point in time during type 1 diabetes development. It is likely that distinct cfDNA or cfRNA biomarkers of beta cell death, dysfunction or deficiency will be useful to monitor different stages of the disease (Figure 1).

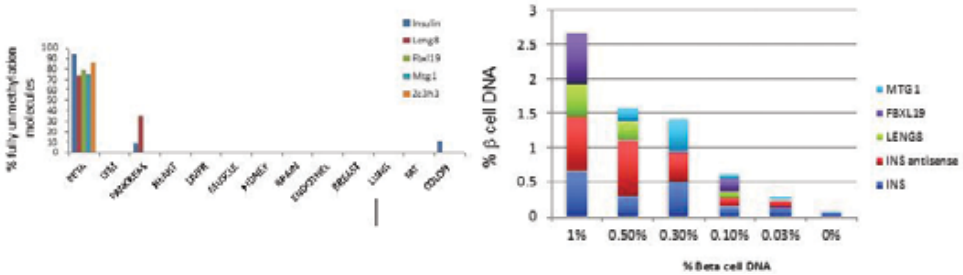



Figure 1. *Beta cell methylation markers. Left, specificity of markers reflected in fraction of unmethylated molecules from each locus in genomic DNA from the indicated tissues. Note that the classic marker of beta cells, unmethylated insulin, is in fact unmethylated also in a subset of molecules (cells) from the pancreas (beyond the 1% that can be explained by beta cells) and the colon. Right, sensitivity of assay tested by mixing beta cell DNA into leukocyte DNA in the indicated percentage. The assay is capable of robustly detecting beta cell DNA when comprising 1:3000 of the DNA in the mixture.* ***(CBDS: Schatz UC4, University of Florida)***

Wei-Jun Qian, Pacific Northwest National Laboratory, and a multi-institutional team of investigators generated the first comprehensive catalog of the proteins found in human islets, alpha cells or beta cells. The team characterized more than 10,000 proteins in islet cells, corresponding to about half of the human genome. Among these, they identified 1,000 islet-specific or islet-enriched proteins and mapped protein signatures that might serve as biomarkers for type 1 diabetes progression. The catalog addresses a major HIRN goal by creating a unique resource for the entire type 1 diabetes research community to generate novel hypotheses and gain new insights. In a major technological advance, the investigators developed ultrasensitive proteomics technologies to improve the analysis of proteins in single islets or small populations of isolated alpha or beta cells (Figure 2).

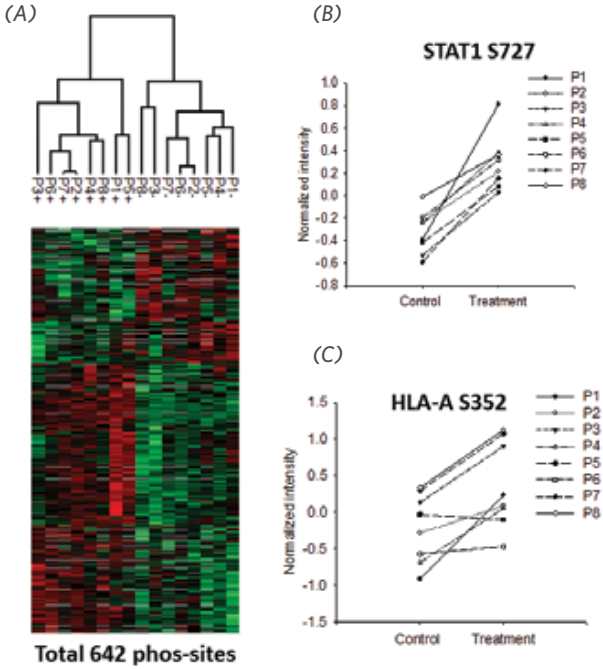



Figure 2. *Cytokine-induced phosphoproteome changes in human islets. Human islets from 8 individual donors were treated by IL1beta and IFN-gamma. (A) heatmap of phosphorylation levels for individual sites; (B) STAT1 S727 phosphorylation for 8 donors before and after treatment. (C) HLA-A S352 phosphorylation.* ***(CBDS: Qian UC4, Pacific Northwest National Laboratory)***

Raghavendra Mirmira, Indiana University, led an international, multi-institutional team that identified key biomarkers of type 1 diabetes and revealed fundamental mechanisms by which beta cell stress contributes to the disease (Figure 3). The team found that high levels of insulin gene DNA persist in the bloodstream of individuals with longstanding type 1 diabetes; circulating proinsulin (the precursor protein that is processed to form insulin and C-peptide) was also found in individuals with established diabetes. The presence of these biomarkers in the blood suggests that beta cell turnover continues in some people for many years after diagnosis and may point to opportunities to intervene in the cycle of beta cell growth or regeneration, stress and death.

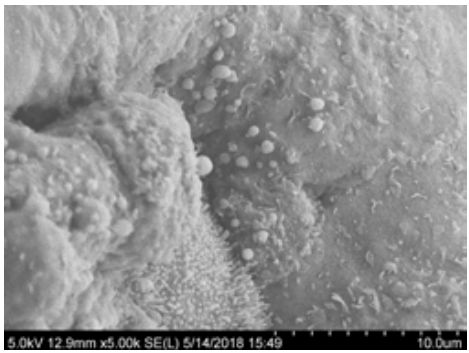



Figure 3. *The image shows a scanning electron microscopic image of extracellular vesicles (EVs) on the external plasma membrane of a human islet cell. EVs harbor proteins and nucleic acids derived from the cell interior that have the potential to serve as biomarkers of the state of islet cell health.* ***(CBDS: Mirmira UC4, Indiana University)***

Beta cells derived from human progenitor cells have a similar impairment in glucose-responsive insulin secretion as human islets from individuals with type 1 diabetes. Douglas Melton, Harvard University, identified a specific metabolic pathway in the beta cell mitochondria that appears to be responsible for defective glucose responsiveness. Further, Dr. Melton showed that the defect could be rescued by treating the beta cells with metabolites that are able to enter the cells. If the same process is confirmed in human diabetic islets, this line of research has several implications, including the possibility of harnessing the defective pathway as a biomarker of early beta cell stress, targeting the pathway to preserve beta cell/islet function early in the development of type 1 diabetes and improving methods to generate replacement insulin-producing beta cells from human progenitor cells (Figure 4).

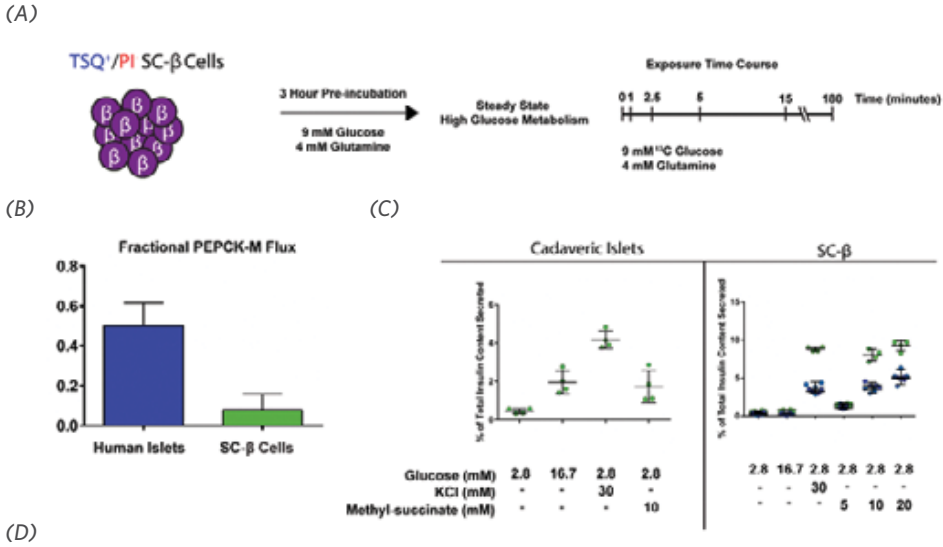



Figure 4. *Metabolic profiling of beta cells derived from human pluripotent cells (SC-beta cells). (A) A schematic of metabolic profiling of SC-beta cells using the MIMOSA technique with the Kibbey Laboratory. (B) Metabolic flux through the PEPCK-M reaction in SC-beta cells is much slower than in human islets. (C) Use of cell-permeable mono-methyl succinate results in significantly higher insulin secretion than glucose in SC-beta cells and similar in magnitude to human islets. (D) Dynamic perifusion of early (purple) and late (green) glycolysis metabolites results in different magnitudes of insulin secretion. (CBDS: Melton UC4, Harvard University)*

## DEVELOPING INNOVATIVE HIGH-RESOLUTION TECHNOLOGIES TO STUDY ISLET BIOLOGY

Ivan Gerling, University of Tennessee, led a group of investigators who defined the complete library of gene expression patterns (the "transcriptomes") of islets extracted directly from donated human pancreata. The team assembled a unique dataset comprising transcriptome data and associated information on beta cell-relevant proteins from 260 individual islets collected from donors with either (1) type 1 diabetes, (2) diabetes-associated autoantibodies, but no diabetes or (3) neither diabetes nor autoantibodies (Figure 5). This is the largest dataset of its kind and has already generated important biological insights. For example, genes associated with islet regeneration appear to be turned off in all islets from autoantibody-positive donors but are active in a small number of islets from diabetic or autoantibody-negative, nondiabetic donors. In addition, many islets from diabetic donors that appear "normal" under a microscope do not have normal gene expression patterns. The team openly shared this rich dataset with diabetes researchers in and outside of HIRN.

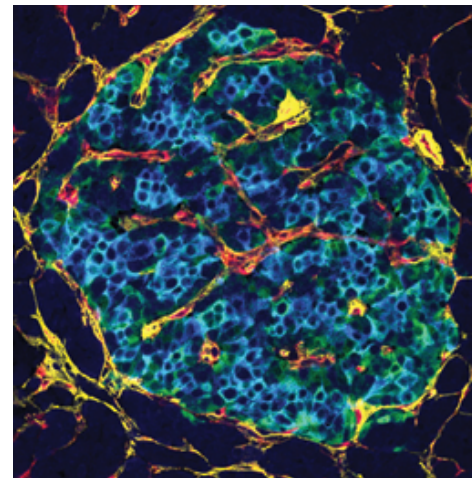


Figure 5. *Human islet from a 25 year old Caucasian male donor with type 1 diabetes at onset of diabetes. Endocrine cells are stained for insulin (blue) and secretogranin 3 (green, colocalization with insulin is dark blue) and the vessels are shown with smooth muscle actin (yellow) and CD34 (red). The islet microvascular was altered after islet loss of beta cells.* ***(CBDS: Gerling UC4, University of Tennessee Health Sciences Center)***

Mark Atkinson, University of Florida, and an international group of scientists made progress in mapping the molecular and biochemical events leading to type 1 diabetes at the single cell level utilizing highly multiplexed imaging (HMI) technologies. In one line of research, the team imaged 35 biomarkers at single cell resolution in pancreata from 12 human organ donors with recent-onset or longstanding type 1 diabetes or no diabetes diagnosis. Using a computational algorithm that predicts timing of events, the team reconstructed islet evolution over type 1 diabetes progression. They discovered that beta cell loss appears to be preceded by changes in beta cell function,

such as reduction in insulin production. In addition, they showed that data from islets and immune cells could be meaningfully connected to study how islet-immune cell interactions evolve during the development of type 1 diabetes (Figures 6-7). In other studies using HMI, the investigators found evidence that glucagon-producing alpha cells may have the ability to convert to insulin-producing beta cells when the original beta cells are destroyed.

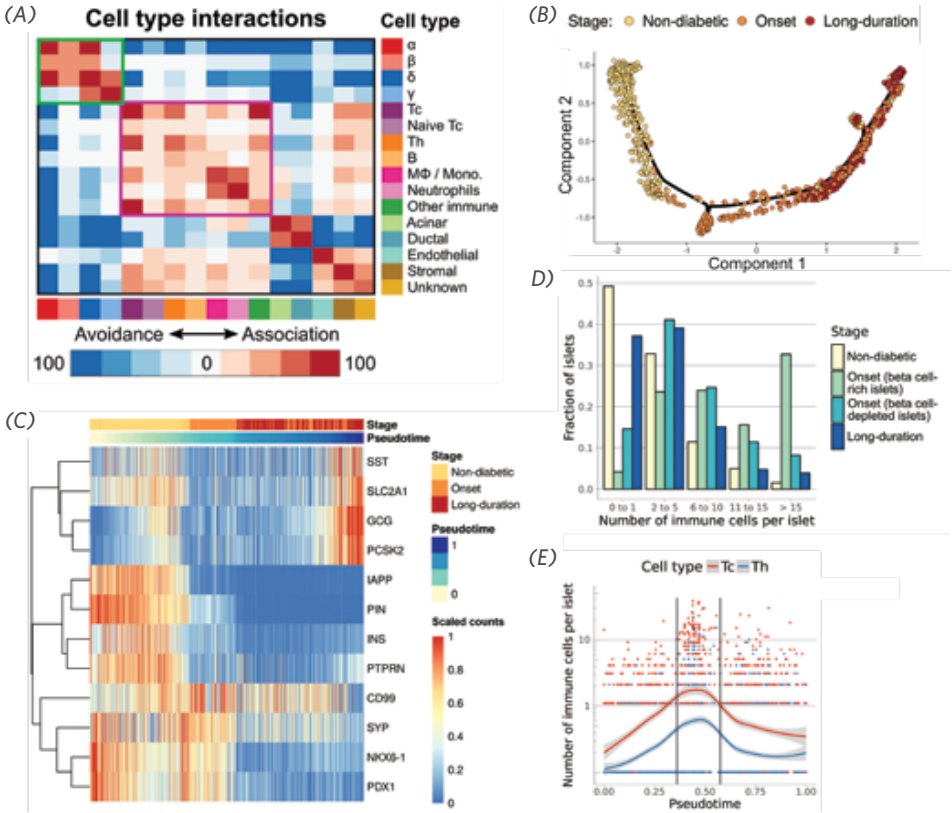



Figure 6. *(A) Frequency of cell-cell interactions in islets and in the 20 μm surrounding area. Colors indicate the percentage of donors that display significant association (red) or avoidance (blue) between two cell types. The green rectangle indicates interactions between islet cells and the magenta rectangle indicates interactions between immune cells. (B) Pseudotime analysis showing the evolution of islet expression profiles (represented by circles) from donors at different T1D stages. Islets from nondiabetic donors have profiles clearly distinct from those from donors with T1D. The black line represents the pseudotime trajectory. (C) Heatmap showing expression of islet markers with columns representing islets arranged according to pseudotime. The abundance of beta cell markers decreases with pseudotime progression. (D) Fraction of islets containing given numbers of associated immune cells (inside the islet or within 20 μm of the islet rim), in function of disease stage. (E) Number of Tc (red) and Th (blue) associated with individual islets in function of islet pseudotime. Curves represent smoothed conditional means.* ***(CBDS: Atkinson UC4, University of Florida)***

Figure 7. **PANCREATIC AND MISC MARKERS**

| Target Protein | Vendor | Reference |
|---|---|---|
| **Amylin (IAPP)** | Sigma | HPA053194 |
| **Carbonic anhydrase IX** | R&D Systems | AF2188 |
| **CD31** | Dako | M0823 |
| **CD99** | Biolegend | 318002 |
| **Cleaved caspase 3** | Becton Dickinson | 559565 |
| **Cleaved PARP** | Becton Dickinson | 558576 |
| **Cytokeratin 19** | CST | #13092 |
| **E-/P-cadherin** | Beckton Dickinson | 610182 |
| **Glucagon** | CST | #8233 |
| **Glucose transporter 1** | Abcam | ab115730 |
| **Histone H3** | CST | #4499 |
| **Insulin** | CST | #3014 |
| **Ki-67** | BD Biosciences | 556003 |
| **Nkx6.1** | CST | #54551 |
| **Pancreatic amylase** | Abcam | ab21156 |
| **Pancreatic polypeptide** | R&D Systems | MAB62971 |
| **Phospho-histone H3** | Biolegend | 641002 |
| **Phospho-retinoblastoma** | CST | #8516 |
| **Phospho-S6** | CST | #4858 |
| **Prohormone convertase 2** | Millipore | AB15610-I |
| **Pdx1** | R&D Systems | AF2419 |
| **Proinsulin** | Abcam | ab8301 |
| **PTPRN** | Sigma | HPA007179 |
| **Smooth muscle actin** | Abcam | ab76549 |
| **Somatostatin** | Dako | A0566 |
| **Synaptophysin** | Abcam | ab32127 |
| **Vimentin** | CST | #5741 |

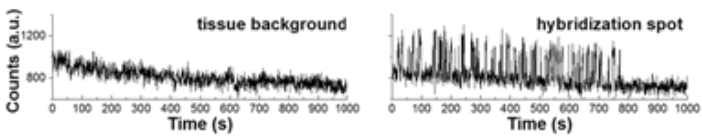
**IMMUNE MARKERS**

| Target Protein | Vendor | Reference |
|---|---|---|
| **CD3ε** | CST | 85061 |
| **CD4** | R&D Systems | AF379-NA |
| **CD8a** | eBioscience | 14-0085-82 |
| **CD20** | eBioscience | 14-0202-82 |
| **CD38** | Abcam | ab108403 |
| **CD44** | BD Biosciences | 553134 |
| **CD45** | eBioscience | 14-9457-82 |
| **CD45RA** | Biolegend | 304102 |
| **CD68** | eBioscience | 14-0688-82 |
| **Forkhead box P3** | Thermo (eBioscience) | 14-4777-82 |
| **Myeloperoxidase** | Dako | A0398 |

Figure 7. *Pancreatic and immune cell markers validated for Imaging Mass Cytometry (IMC) (CBDS: Atkinson UC4, University of Florida)*

Charles Ansong and investigators at Pacific Northwest National Laboratory and University of Colorado, Denver developed advanced technologies for high-resolution analysis of molecular events that occur in single islet cells in response to autoimmunity. Analysis of RNA, protein and lipid differences in mouse islet cells has already produced interesting findings. For example, the team identified differences in the lipid composition of islets from mice with autoimmune diabetes compared to those without diabetes, as well as differences in lipids among islets within a single pancreas. The investigators are adapting these novel technologies to human islets from organ donors with and without type 1 diabetes to search for lipid profiles that can distinguish islets that are more susceptible or resistant to autoimmunity. Other studies uncovered a novel enzyme that might have a role in regulating lipid composition to improve islet function or survival and could represent a new target for drug development (Figures 8-10).

*(A)*




*(B)*

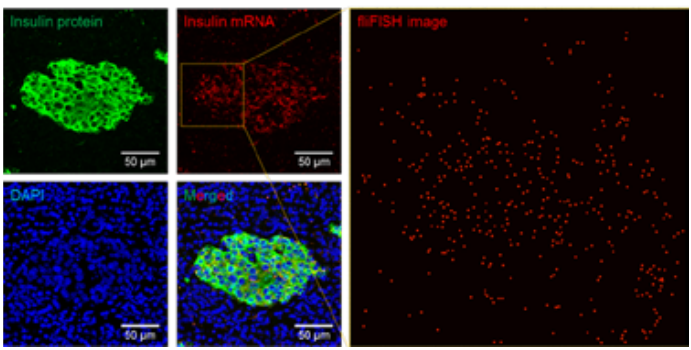
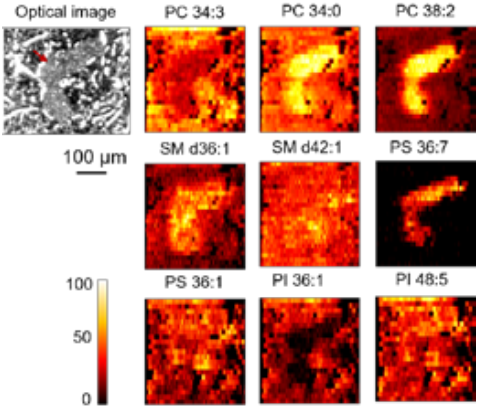
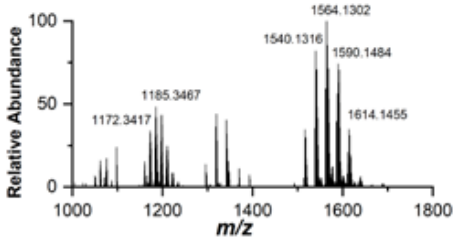



Figure 8. *Demonstration of fluctuation localization imaging-based fluorescence in situ hybridization (fliFISH) in formalin-fixed paraffin-embedded (FFPE) human tissues. (A) Photoblinking events can be clearly differentiated from the strong background in FFPE tissue sections, making fliFISH an applicable alternative for RNA quantification in complex biological samples. (B) The islet of Langerhans is located by immunofluorescence staining targeting insulin protein and the designed FISH probes show a high co-localization within insulin-positive cells. By fliFISH processing, the position and number of insulin mRNA can be precisely determined. (CBDS: Ansong UC4, Pacific Northwest National Laboratory)*

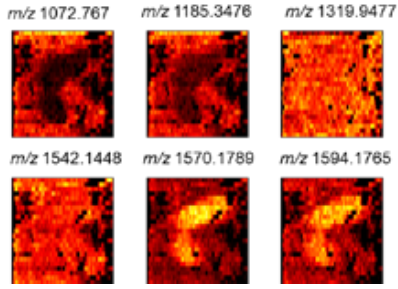



(A)

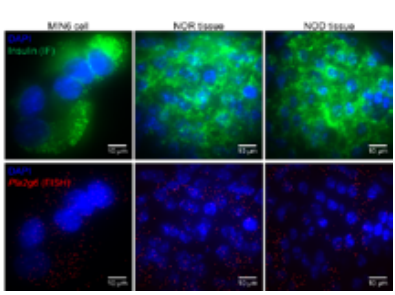
Figure 9. *Low-abundance phospholipid species observed in a pancreatic islet by coupling nano desorption electrospray ionisation mass spectrometry imaging (DESI-MSI) with a Q Exactive- HF-x mass spectrometer. The optical image of the analyzed region is shown for comparison. (A) Ion images of selected low-abundance PC, SM, PS, and PI species. PC and SM species were detected in positive mode; PS and PI species were observed in negative mode. (B) The positive mode mass spectrum in a range of m/z 1000 -1800 acquired from the islet. (C) Typical ion images of the molecules with a m/z of more than 1000.* ***(CBDS: Ansong UC4, Pacific Northwest National Laboratory)***




(B)




(C)



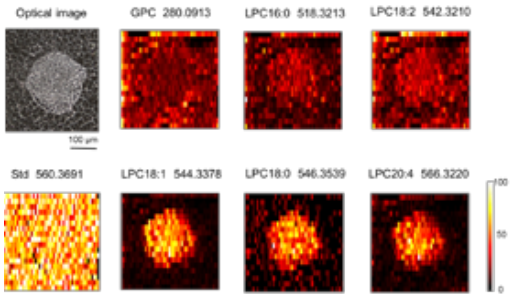



Figure 10. *Left panel. Integrated immunofluorescence (IF) and RNA fluorescence in situ hybridization (FISH) showing Pla2g6 enrichment in islets and beta cells. Right panel. NanoDESI imaging showing enrichment of specific lysophopholipid species in islets.* ***(CBDS: Ansong UC4, Pacific Northwest National Laboratory)***

A team at Vanderbilt University and the California Institute of Technology led by Chris Wright focused on the development of state-of-the-art methods for detection of single RNA molecules and for probing links between signaling lipids, proteins or metabolites and the autoimmune process. The team also developed a new method using the beta cell-specific protein ectonucleoside triphosphate diphosphohydrolase 3 (NTPDase 3) to sort adult beta cells from other types of islet cells (Figure 11). This technology will facilitate HIRN-wide research on isolated beta cells at various ages, including the critical first years of life, and at all stages of type 1 diabetes. The team's research positions technology and analysis in the pancreas at the leading-edge of innovative molecular analytical methods and high-resolution technologies, which HIRN and the diabetes research community can use to map the growth and maturation of the pancreas in unprecedented detail.

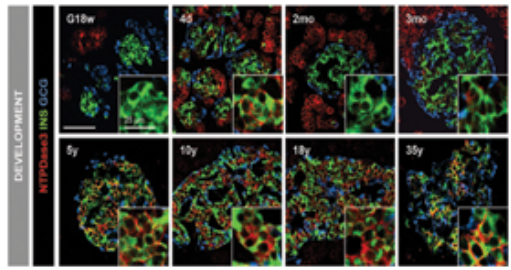



Figure 11. *Pattern of NTPDase expression within human pancreas during postnatal development. NTPDase3 expression (red), beta cells (INS, green); NTPDase3 has a different pattern of expression in the human pancreas at different stages of development; in top row, NTPDase3 is initially restricted to pancreatic epithelium and acinar cells, but by five years of age, acinar cell expression has ceased and only beta cells specifically express NTPDase3 (bottom row)* ***(CBDS: Wright UC4, Vanderbilt University)***

## EXPLORING NOVEL MODELS OF TYPE 1 DIABETES DEVELOPMENT

Klaus Kaestner and colleagues at the University of Pennsylvania and Hebrew University of Jerusalem investigated an intriguing new model for type 1 diabetes pathogenesis. They hypothesized that the stress on beta cells during the development of type 1 diabetes might damage DNA and increase the mutational load on beta cell genes. Those mutated genes may, in turn, produce abnormal proteins that trigger the aggressive autoimmune response against beta cells. The team showed that beta cells in mice and humans with type 1 diabetes have elevated levels of the DNA damage response to double strand breaks in the DNA. A key damage response protein was enriched at many genes that are active in beta cells and was also found at glucose-enriched genes when the cells were exposed to high levels of glucose. The team developed new technologies for mutation analysis and freely shared their methods and reagents (Figure 12).

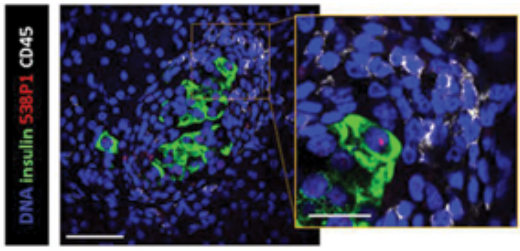



Figure 12. *Association of human beta cell DNA damage with islet immune infiltration. Costaining for beta cells DNA damage and immune infiltration in a patient with recently diagnosed T1D. A beta cell (stained for insulin) with a 53BP1 nuclear focus is surrounded by a mass of small cells with compact nuclei that stained positive for the pan-leukocyte marker CD45 (inset). Scale bar = 10 µm, scale bar for inset = 3 µm.* ***(CBDS: Kaestner UC4, University of Pennsylvania)***

Investigators at Yale University and Columbia University led by Kevan Herold reported that autoimmunity modifies proteins and causes DNA changes that may alter how beta cells are recognized by the immune system (Figure 13). They showed that inflammation triggers chemical modification of the beta cell enzyme prolyl-4-hydroxylase (P4Hb), which interferes with its role in insulin production and secretion. Moreover, 43 percent and 66 percent of patients with short- or long-term type 1 diabetes, respectively, had both anti-P4Hb and anti-insulin antibodies, with anti-P4Hb always preceding the appearance of anti-insulin. P4Hb may be a target for early intervention to prevent beta cell killing. The team also identified cells in diabetic mice that expressed both beta and alpha cell genes. Their research suggests that these "intermediate" cells might be newly forming beta cells that arise from non-beta cell progenitors.

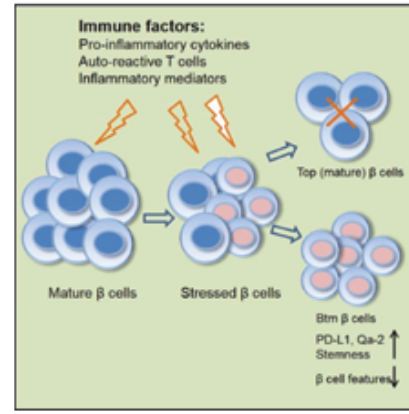



Figure 13. *Proposed changes in beta cells during progression of Type 1 diabetes. Data from NOD mouse experiments and studies with human islets exposed to inflammatory cytokines suggest that beta cells may undergo changes involving decreased expression of genes that define beta cells such as Nkx6.1, increased proliferation, and expression of immune modulatory molecules such as PD-L1 and IDO.* ***(CBDS: Herold UC4, Yale University)***

Rohit Kulkarni, Joslin Diabetes Center, and Chuan He, University of Chicago, proposed that chemical modification of protein-coding RNAs could function as novel regulators of gene expression in beta cells that may contribute to the control of beta cell function and survival. The investigators began developing a technology for assessing RNA modifications in small samples of beta cells or islets. In addition, the team discovered that several RNA-modifying enzymes were downregulated in islets from prediabetic mice, which could affect either the composition or level of proteins produced from islet RNAs. These findings open up a previously unexplored pathway for the development of therapies to restore healthy beta cell function and prevent or reverse type 1 diabetes (Figures 14-15).

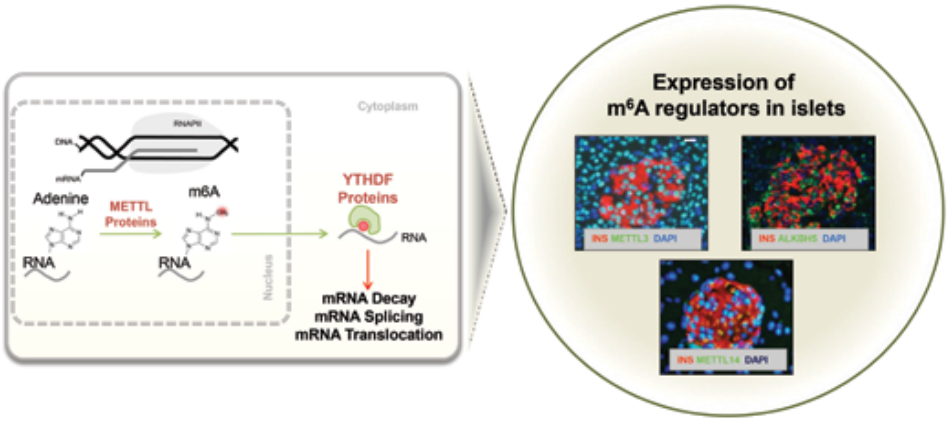



Figure 14. *Schematic of relevance of methyltransferase-like (METTL) proteins for methylation of mRNA and YTH domain family (YTHDF) proteins as readers important for mRNA decay, splicing and translocation. The right panel shows co-immunostaining of insulin (INS) and m6A modulators (METTL3, METTL14 and ALKBH5) in human pancreas sections. DAPI is the nuclear marker.* ***(CBDS: Kulkarni UC4, Joslin Diabetes Center)***

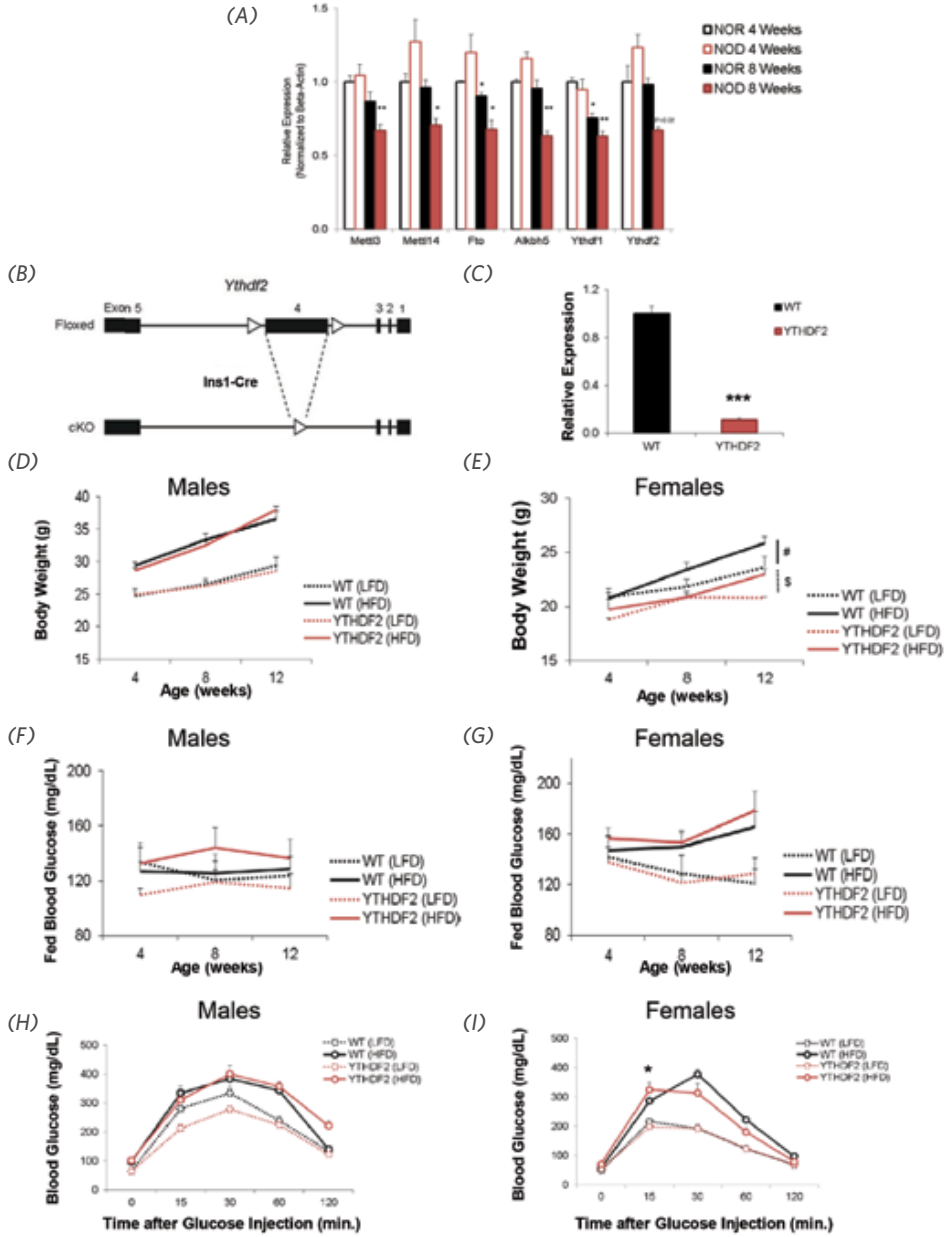



Figure 15. *N6-methyladenosine (m6A) modulators are impacted by prediabetes (A) RT-PCR analyses of m6A modulators in whole islets from diabetes-free NOR, nondiabetic NOD and pre-diabetic NOD mice (n=4/group). (B) Strategy for generation of beta cell-specific Ythdf2 KO mouse. (C) mRNA expression analyses of Ythdf2 in in whole islets. (D-E) Body weight trajectories in controls and ythdf2 KO mice in chow and high fat diet (HFD). (F-G) Random-fed blood glucose levels in controls (black) and Ythdf2 KO mouse (red). (H-I) Glucose tolerance tests in controls and Ythdf2 KO mice on chow or HFD for 12 weeks. (n=5-10). *p<0.05; ** p<0.01; ***p<0.001.* ***(CBDS: Kulkarni UC4, Joslin Diabetes Center)***

## NEW INVESTIGATORS BRING NOVEL RESEARCH DIRECTIONS TO THE CBDS

In Year 5, CBDS is joined by Anath Shalev, an Investigator at the University of Alabama at Birmingham. Dr. Shalev is continuing a promising line of research initiated in the Consortium on Targeting and Regeneration (CTAR) focused on thioredoxin-interacting protein (TXNIP) as a viable target for the early diagnosis and treatment of type 1 diabetes.

In addition, CBDS welcomes three New Investigator Pilot Award grantees who are pursuing innovative research ideas to advance the Consortium's mission. At the University of Colorado, Denver, Holger Russ is mapping all beta cell proteins and their variants produced under normal and stress conditions; these "translatome" maps may reveal diabetogenic proteins that attract the autoimmune response leading to beta cell death. Prashanth Vallabhajosyula, University of Pennsylvania, is studying exosomes — small, spherical vessels ("microvesicles") containing RNA and protein that are released by many cells, including beta cells, into the blood or other body fluids. Characterizing insulin-containing beta cell exosomes in the bloodstream might provide a useful, minimally invasive tool for diagnosing beta cell injury or death. Amelia Linnemann, Indiana University, is exploring the buildup of reactive oxygen species that contributes to beta cell death and the development of type 1 diabetes. New imaging methods will be used to assess how human beta cells handle reactive oxygen species and to test whether small molecules can rescue defects in this process.

## CBDS INVESTIGATORS, YEAR 4

**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*

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

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 Accili, M.D., Co-investigator, *Columbia University*
Mark Mamula, Ph.D., Co-investigator, *Yale University*

Charles Ansong, Ph.D., Investigator, *Pacific Northwest National Laboratory*
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*
Lori Sussel, Ph.D., Investigator, *University of Colorado, Denver*

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., Co-investigator, *Vanderbilt University*
Alvin C. 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., Investigator, *Joslin Diabetes Center*
Chuan He, Ph.D., Investigator, *University of Chicago*

Desmond Schatz, M.D., Investigator, *University of Florida*
Carla Greenbaum, M.D., Co-investigator, *Benaroya Research Institute*
Yuval Dor, Ph.D., Co-investigator, *Hebrew University of Jerusalem*

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

†Amelia Linnemann, Ph.D., Investigator, *Indiana University*

†Holger Russ, Ph.D., Investigator, *University of Colorado, Denver*

†Prashanth Vallabhajosyula, M.D., Investigator, *University of Pennsylvania*

*New team to be added in Year 5
†New Investigator Pilot Award, 2018

# CONSORTIUM ON HUMAN ISLET BIOMIMETICS (CHIB)

## ISLET BIOMIMETICS ADDRESS KEY CHALLENGES IN TYPE 1 DIABETES RESEARCH

Pancreatic islets are small, spherical clusters of hormone-producing cells embedded in a sea of exocrine tissue (the part of the pancreas involved in digestion). Islets make up around 1 to 2 percent of a human pancreas, and the insulin-producing beta cells that are damaged or destroyed in type 1 diabetes are only one of at least four islet cell types. Add in the fact that the pancreas is a relatively small organ located deep within the abdominal cavity — making it very difficult to image or biopsy in a living person — and the challenges of studying human islets or beta cells become clear. The Consortium on Human Islet Biomimetics (CHIB) is tackling these challenges with research designed to mimic islet structure and function in the lab. Using beta cells, alpha cells, blood vessels, neurons, extracellular support matrices and other components, CHIB investigators are building copies or "biomimetics" of the human islets and their environment in a dish. They are also engineering state-of-the-art microfluidic devices that can precisely measure activities, such as insulin release, from single human islets that are isolated from pancreata or from islets they build in the lab from progenitor cells. These microdevices offer new opportunities for innovative type 1 diabetes research that would be impossible to pursue in a living organism. In its fourth year, CHIB added one new research team and continued its longstanding tradition of collaboration with labs across the HIRN by sharing devices and protocols.

## NOVEL TECHNOLOGIES ADVANCE THE STATE OF THE SCIENCE IN HUMAN ISLET RESEARCH

In the past, research on human islets was limited because islets die or lose their ability to release insulin in response to glucose within days of being removed from the body. Islet isolation disrupts vital components of the microenvironment, including the islets' three-dimensional architecture and network of blood vessels. In Year 4, CHIB investigators have made remarkable progress in improving islet viability in the lab and pioneering new technological solutions for human islet research.

A team of investigators led by Maike Sander, University of California, San Diego, reconstructed islet structures in a culture system that combined hormone-producing cells with endothelial cells (that form blood vessels), fibroblasts (a type of support cell) and extracellular matrix (a complex mixture of proteins and other molecules that surround and support islets). The team identified components of the matrix around human islets and made a hydrogel that mimics that matrix for use in islet cultures. They then developed a strategy to reestablish islet cell-matrix interactions that are broken when islets are isolated from the pancreas. The team also improved the survival and function of islets in their microdevice, observing that the islets released insulin in a pattern that is similar to that found in the body — an important criterion for research to test new beta cell therapeutics (Figure 1).

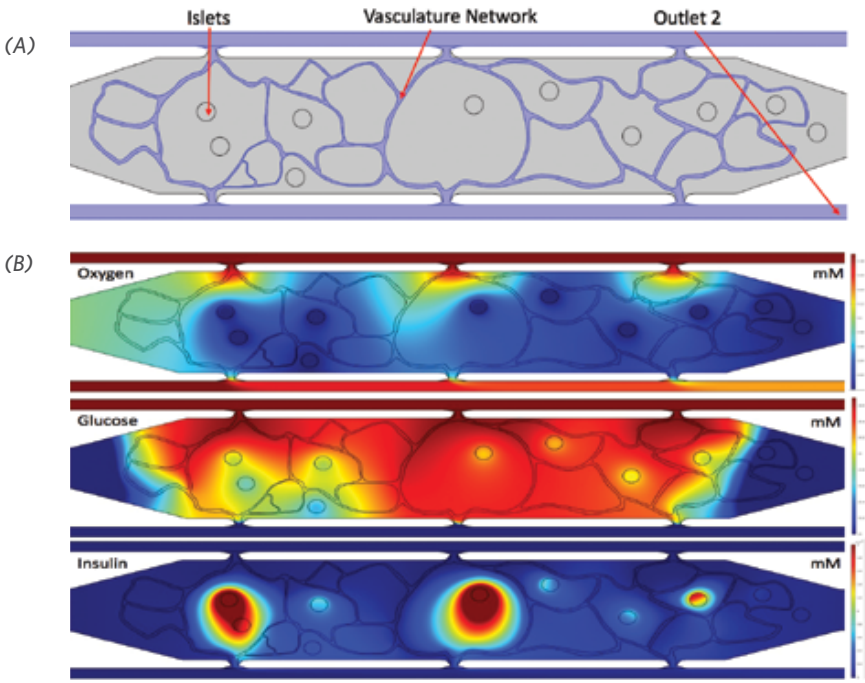



Figure 1. *COMSOL model of experimental device. (A) Schematic image of the model which consists of human pancreatic islets surrounded by a vascular network embedded within the central tissue chamber. The tissue chamber connects to adjacent microfluidic lines that provide nutrients (e.g., glucose) and effluent secreted products (e.g., insulin). (B) Concentration distribution of oxygen (top), glucose (mid) and insulin (bottom) at steady state high glucose input. Model highlights where in the device oxygen and glucose is being consumed, and where insulin is being accumulated at steady state conditions.* ***(CHIB: Sander UC4, University of California, San Diego)***

At the University of Florida and University of Miami, a team led by Cherie Stabler made progress toward their final deliverable by embedding islets in a pancreas-sourced matrix and culturing them in a dynamic microfluidic platform. They found that glucose-stimulated insulin secretion improved when the three-dimensional islets were cultured in a chip that maintains a continual flow of nutrients around the islets. A device that enhances long-term islet culture is critical for beta cell research, and it has the potential to extend the therapeutic window for islet transplantation by permitting more time for a suitable recipient to be identified after islet isolation. Their current system features a reduced footprint, sturdier materials so that the chip can easily be transferred to other labs and commercially available parts to further boost dissemination of the technology to collaborators. These design upgrades help the investigators address the HIRN mission of developing resources and technologies that benefit the entire beta cell research field (Figure 2).

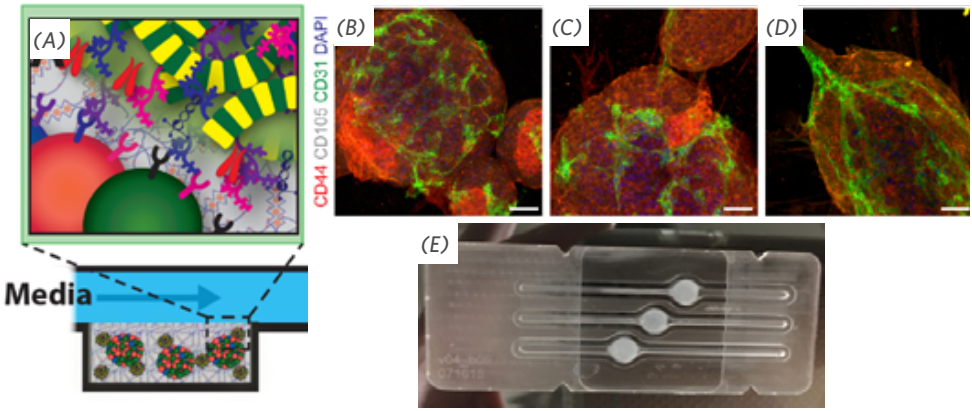



Figure 2. *(A) Schematic of islets within 3-D physiomimetic hydrogel. (B-D) Whole mount immunohistochemistry staining of human islets within extracellular matrix (ECM) hydrogels and cultured for up to 6 days with staining of the endothelial cell network on the surface of the islet. (E) ECM matrix within chip platform illustrating ease of loading islets within 3-D matrices into our chip system.* ***(CHIB: Stabler UC4, University of Florida)***

Ben Stanger led a group of investigators at the University of Pennsylvania, Children's Hospital of Philadelphia, and other institutions who developed a different microfluidic platform strategy. By testing various ratios of islets, blood vessels and fibroblasts, they created three-dimensional islet cultures that became vascularized (or interlaced with blood vessels) within a week after isolation. Their microdevice was engineered with multiple channels that allowed the investigators to measure insulin release in as many as five different islet cultures at a time. This innovation was key to rapidly evaluating a range of conditions that might affect islet survival and function in culture. The team was able to maintain islet viability in the lab for up to 83 days — a marked improvement that will support research on human islet health, stress, loss and treatment in type 1 diabetes (Figure 3).

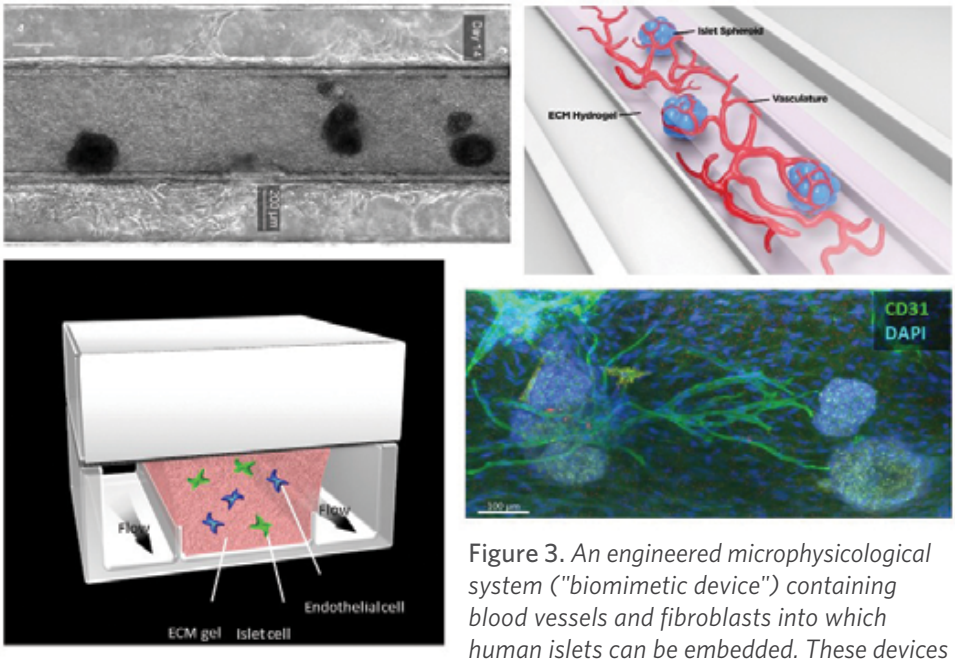



Figure 3. *An engineered microphysicological system ("biomimetic device") containing blood vessels and fibroblasts into which human islets can be embedded. These devices have flow channels on either side, permitting the introduction of different media. In these devices, islets are penetrated by blood vessels, which are in direct communication with the flanking channels. Islets in these conditions maintain their morphology and viability for weeks.* ***(CHIB: Stanger UC4, University of Pennsylvania)***

Two CHIB groups — one led by Michael Roper, Florida State University (FSU), and the other by Douglas Melton, Harvard University — collaborated on the development of a sensor for automated, continuous, real-time measurement of insulin release from islets cultured in their microdevice. This novel technology overcomes the limitations of conventional measurement protocols and greatly reduces the costs of insulin measurement, for the benefit of islet research across CHIB and HIRN. The FSU group, in its first year as a CHIB team, also began development of a portable optical system for measuring insulin that can be adapted to many of the microfluidic systems being created by Consortium teams (Figure 4).

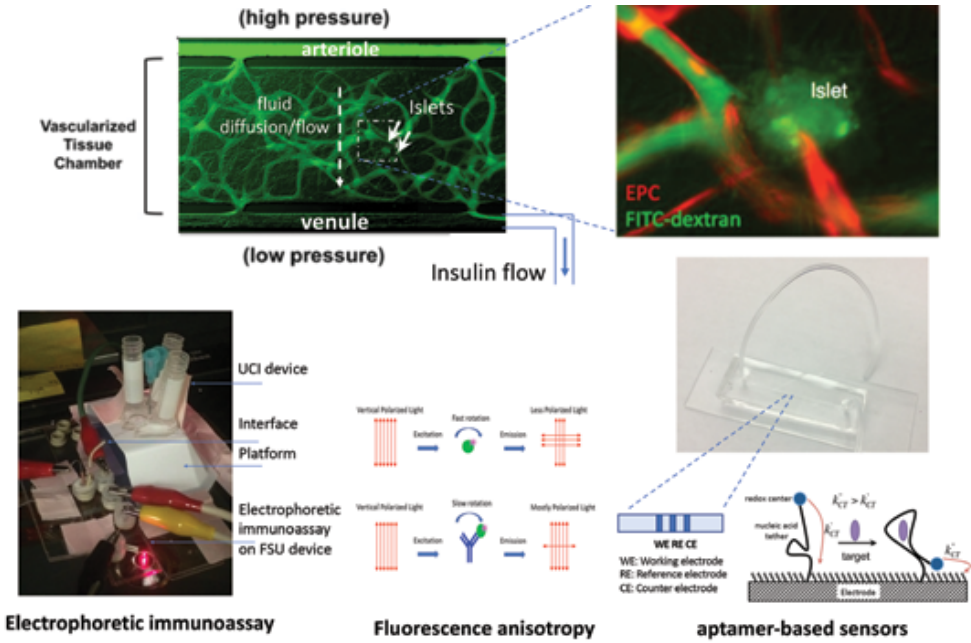



Figure 4. *The top of the figure shows a vascularized islet network. On the bottom half of the figure, three different methods to measure the output insulin flow from the vascularized network are being developed. On the left is an image of the University of California, Irvine device coupled into the FSU device for measurement of insulin via an electrophoretic immunoassay. The middle scheme on the bottom describes the use of fluorescence anisotropy for measurement of insulin. The bottom right figure shows the integration of an electrochemical aptasensor for insulin detection.* ***(CHIB: Roper UC4, Florida State University)***

In other work, the Harvard University team optimized protocols for deriving three different types of hormone-producing islet cells (alpha, beta and delta cells) from progenitor cells. They studied how mixing these progenitor-derived alpha and delta cells with beta cells into islet-like "organoids" affected insulin secretion, as measured in their microdevice. The technologies they developed may enable more precise studies of how islets function normally and how they fail in type 1 diabetes. The team also took an exciting step toward translating their advances to human diabetes by launching a small clinical study with individuals who have diabetes due to the surgical removal of the pancreas. Using blood cells from these individuals, the team induced the production of new progenitor cells that they could then differentiate into insulin-producing beta-like cells for potential transplantation back into the original blood cell donor. In the future, being able to grow viable, functional beta cells that are exactly matched to a specific patient's genetic makeup could revolutionize islet transplantation as an effective and more widely available treatment for type 1 diabetes (Figures 5-6).

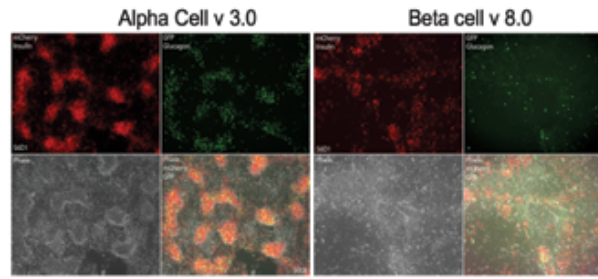



Figure 5. *Immunostaining analysis of cells for mCherry insulin and GFP glucagon of the iPS dual reporter cell line differentiated towards the alpha and beta cell lineages, respectively 100X.* ***(CHIB: Melton, Harvard University)***

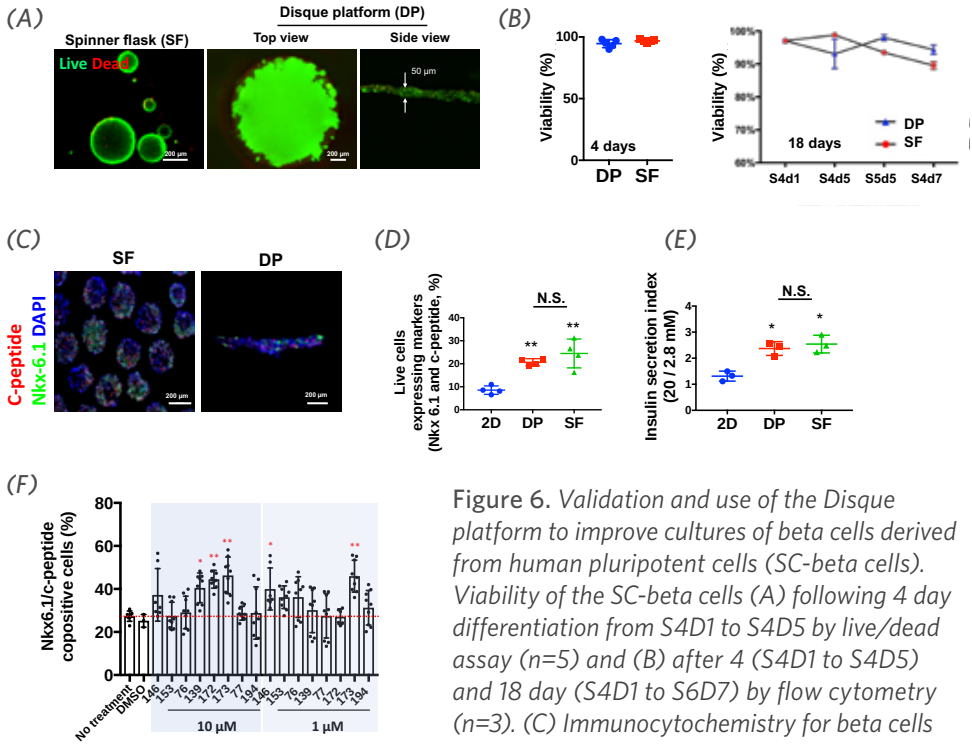



Figure 6. *Validation and use of the Disque platform to improve cultures of beta cells derived from human pluripotent cells (SC-beta cells). Viability of the SC-beta cells (A) following 4 day differentiation from S4D1 to S4D5 by live/dead assay (n=5) and (B) after 4 (S4D1 to S4D5) and 18 day (S4D1 to S6D7) by flow cytometry (n=3). (C) Immunocytochemistry for beta cells differentiation markers (c-peptide and Nkx6.1) in SC-beta cells. (D) Flow cytometry was conducted to examine the expression of Nkx6.1/c-peptide (n =3, **;P < 0.01 vs. 2D). (E) Glucose stimulated insulin secretion index (GSISI, 20 mM/2.8 mM glucose) of the cells cultured in 2D, Disque platform, and spinner flask (n =3, *;P < 0.05 vs. 2D). (F) Imaging quantification of c-peptide marker expressed cells (n=4~9, *;P < 0.05, **;P<0.01 vs. no treatment).* ***(CHIB: Melton UC4, Harvard University)***

## NEW RESOURCES CREATE OPPORTUNITIES FOR COLLABORATIVE RESEARCH

CHIB's work on engineering islet biomimetics and functional microdevices over the past four years has produced groundbreaking resources and technologies that are being applied to pressing questions in human type 1 diabetes research. In Year 4, CHIB teams held a one-day retreat with their counterparts from the Consortium on Modeling Autoimmune Interactions (CMAI) to discuss how the microdevices could be utilized to model immune cell-beta cell interactions that are at the heart of type 1 diabetes autoimmunity. This meeting stimulated new CHIB-CMAI collaborations that are being pursued in the upcoming year.

## CHIB INVESTIGATORS, YEAR 4

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, *University of California, Davis*
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., Co-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*

# CONSORTIUM ON MODELING AUTOIMMUNE INTERACTIONS (CMAI)

## FINDING CREATIVE SOLUTIONS TO CHALLENGES IN HUMAN TYPE 1 DIABETES RESEARCH

Cell types of multiple origins are central to type 1 diabetes pathogenesis: insulin-producing pancreatic beta cells that become targets of the immune system; immune cells that recognize beta cells as "non-self" and lead the autoimmune attack against them (including both innate and adaptive immune cells); and thymic epithelial cells (TECs) that educate immature T cells on the difference between "self" (the person's own cells) and "non-self" (infectious agents, such as bacteria or viruses). A major challenge in type 1 diabetes research has been that the biology of these cells is difficult to study in the human body. Beta cells, TECs and many innate immune cells are embedded in organs (the pancreas, thymus and lymph nodes) that cannot be routinely imaged or biopsied for research purposes. Adaptive immune cells (T cells and B cells), in contrast, are relatively abundant and accessible with a simple blood draw, but the cells found in blood might not be representative of the rare, diabetes-associated cells that infiltrate the pancreatic islets. Moreover, standard animal models do not accurately reflect the biology of these cells and tissues in humans. The Consortium on Modeling Autoimmune Interactions (CMAI) develops innovative models for the study of human type 1 diabetes that are designed to address these challenges. In Year 4, CMAI teams advanced research on mouse models of type 1 diabetes that reconstruct human immune system-beta cell interactions and took steps toward better understanding the cells that activate autoimmunity and lead to targeting of islet cells, causing injury and eventual beta cell death.

## BUILDING A COMPREHENSIVE MODEL OF TYPE 1 DIABETES

A Columbia University team led by Megan Sykes made significant progress on developing reproducible, personalized models of human type 1 diabetes biology that incorporate immune systems and beta cells from type 1 diabetes patients or nondiabetic donors. The team developed a protocol to create TECs from human pluripotent cells and showed that the differentiated cells could direct the development of mature T cells when transplanted into mice whose own thymuses had been removed. In related experiments,

they designed an *in vitro* method to educate human T cells to recognize "self" proteins using thymus tissue that will be extended to tissue from individuals with type 1 diabetes. This technology opens up novel avenues of research into how self-reactive T cells may escape thymic deletion in individuals who are susceptible to type 1 diabetes. Along with their successes in combining human beta cells and immune cells in a mouse model, these advances in human thymus biology bring the team much closer to realizing the vision of a fully integrated and personalized model of type 1 diabetes autoimmunity (Figures 1-3).

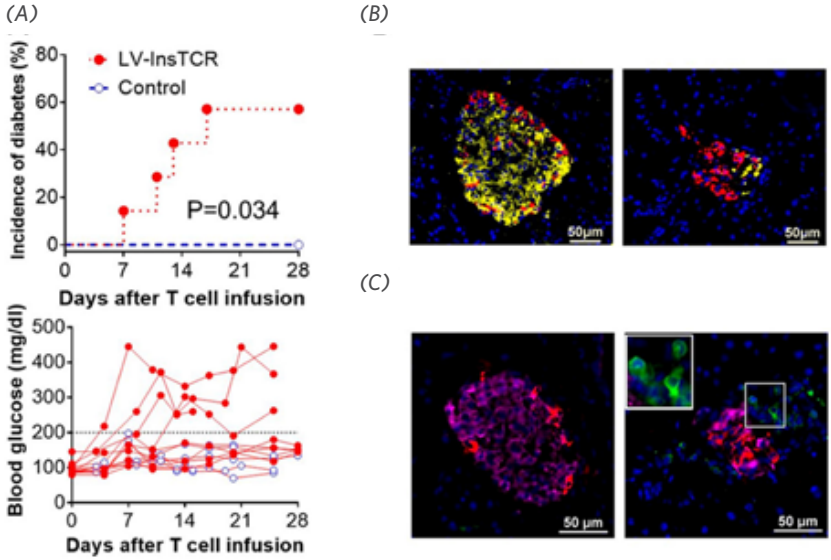



Figure 1. *Induction of diabetes in HLA-DQ8-Tg experimental mice by adoptive transfer of autologous diabetogenic human CD4+ T cells. HLA-DQ8-Tg mice were treated with low-dose streptozotocin and injected 1-2 d later with 5 × $10^6$ expanded LV-insTCR-transduced or control (i.e., the same mouse-derived human CD4+ T cells that were similarly expanded in vitro as the LV-insTCR-transduced CD4+ T cells) human CD4+ T cells, followed 1 d later by immunization with InsB:9-23 peptides. (A) Cumulative incidence of diabetes (Top) and levels of blood glucose (Bottom) in mice receiving LV-insTCR-transduced (solid symbol; n = 7) or control (opened symbol; n = 6) human T cells. Mice were defined as hyperglycemic if two consecutive blood glucose measurements were >200 mg/dL. (B and C) Immunofluorescent staining of pancreas samples prepared between 3 and 4 weeks after injection of CD4+ T cells from mice receiving control (Left) or LV-InsTCR-transduced (Right) human T cells (n = 3 per group). (B) Staining of mouse insulin (yellow) and glucagon (red). (C) Staining of human CD3+ cells (green), mouse insulin (pink) and glucagon (red). Nuclear is stained blue by DAPI.* ***(CMAI: Sykes UC4, Columbia University)***

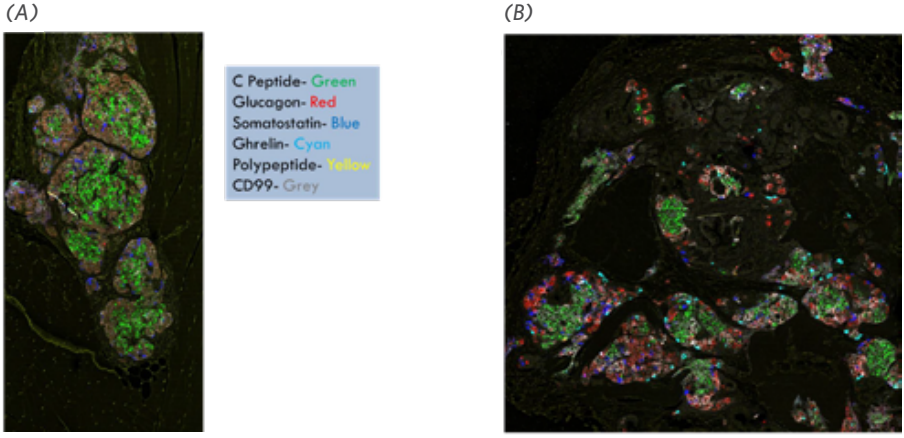



Figure 2. *Mass Cytometry Imaging of human pluripotent cell-derived beta cells (SC-beta cells) grafts five months post-transplant: (A) SC-beta cells engrafted in the intra-muscle site. (B) SC-beta cells engrafted in the sub-cutaneous tissue. The sc-beta cells have formed Islet-like structures which are polyhormonal and similar in configuration to mouse islets. Hormone positive cells are color coordinated per the accompanying legend.* ***(CMAI: Sykes UC4, Columbia University)***

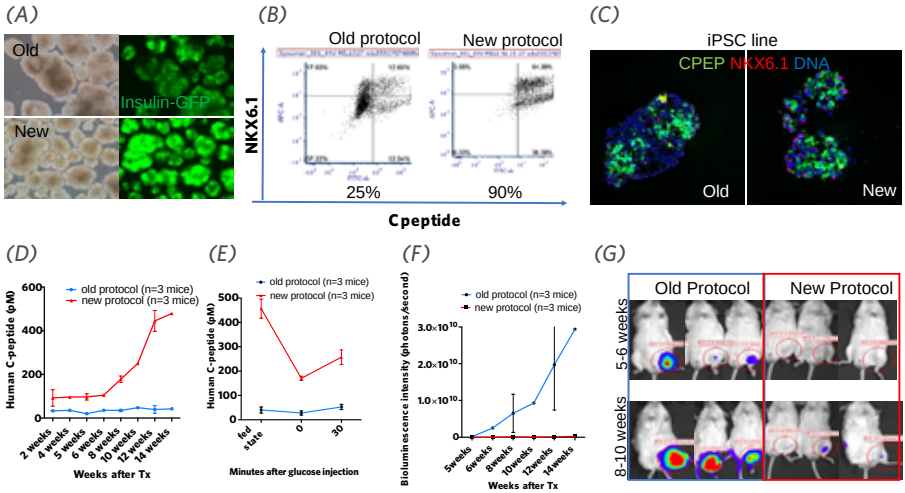



Figure 3. *(A) Example of human pluripotent cell derived beta cell clusters using old and new protocols. (B) Differentiation efficiency of pluripotent cells into C-peptide and NKX6.1 positive cells using indicated protocols. (C) Example of human induced pluripotent cell derived beta cell clusters using indicated protocols. (D) Human C-peptide levels were detected in mice transplanted with pluripotent cell derived beta cells with indicated protocols. (E) Human C-peptide levels when mice were at fed state, fasting state and 30 min after glucose injection. (F) Bioluminescence intensity of grafts after 5 to 14 weeks of transplantation. (G) In vivo imaging pictures were taken after 5-10 weeks of transplantation to monitor the growth of grafted cells.* ***(CMAI: Sykes UC4, Columbia University)***

Dale Greiner, University of Massachusetts Medical School, led a team that uses pluripotent cells from individuals with type 1 diabetes to develop renewable sources of beta cells, TECs and hematopoietic progenitor cells that can give rise to different types of immune cells (Figure 4). They are integrating these human cells into mice with deficient immune systems (OPTI-MICE) in order to create a robust model of human autoimmune type 1 diabetes.

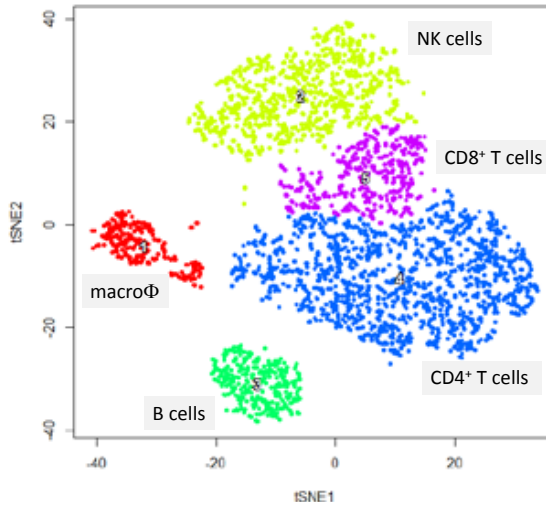



Figure 4. *t-SNE map of human peripheral blood mononuclear cells generated by single cell analysis of protein expression and transcriptome expression using CITE-seq.* ***(CMAI: Greiner UC4, University of Massachusetts Medical School)***

In Year 4, the team developed a bank of pluripotent cells from genetically diverse individuals with or without type 1 diabetes and differentiated them into human beta cells that function much like human islets when transplanted into mice. The investigators also made progress in generating functional TECs and hematopoietic progenitor cells. As each component of this system is successfully created, this research affords new opportunities for understanding autoimmunity and for developing and testing new drugs for type 1 diabetes and other autoimmune diseases.

A team of investigators at the University of California, San Francisco, led by Mark Anderson optimized a protocol for producing TECs from human pluripotent cells (Figure 5). In collaboration with the Sykes team, they used this platform to begin to generate a thymus for transplantation into experimental mouse models of type 1 diabetes. This line of research allows the investigators to explore the role of the thymus in the early stages of type 1 diabetes when the immune system's tolerance for "self" proteins breaks down. In parallel, the team developed a 27-day protocol to produce clusters of functional beta cells from human pluripotent cells. They showed that these cells worked as well as islets from human donors and did not form tumors when transplanted into the mouse model.

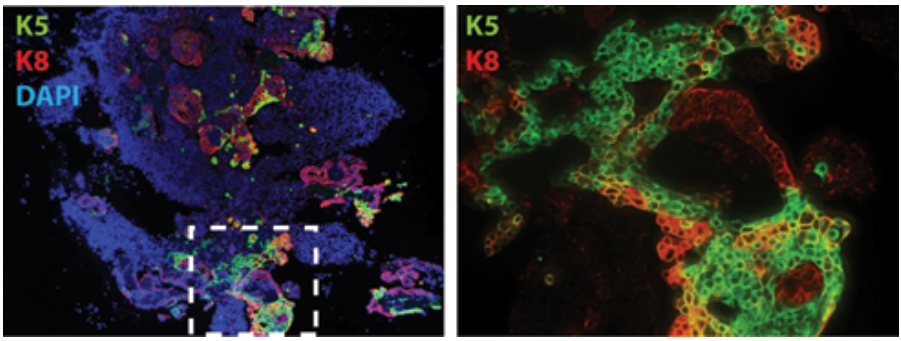



Figure 5. *Immunofluorescence analysis of thymic epithelial cells derived from human pluripotent cells. K8 (red) and K5 (green) staining identify cortical and medullary TECs, respectively, while K5+/K8+ double positivity (yellow) indicates progenitor cells. A higher magnification of dashed lines area is shown in the right panel.* ***(CMAI: Anderson UC4, University of California, San Francisco)***

Clayton Mathews, University of Florida, led a team that is building a disease-in-a-dish model of type 1 diabetes. The team used high efficiency gene editing technology to generate pluripotent cells carrying a variant of a specific gene, *PTPN22*, that is linked to high risk for type 1 diabetes. They have successfully derived several types of cells from the pluripotent cells, including immune cells and beta cells. In Year 4, macrophages made with the high-risk *PTPN22* variant were activated with T cells and found to inhibit beta cell function more strongly than macrophages with the typical (low-risk) version of *PTPN22* (Figure 6). This research shows how a specific gene mutation can promote inflammation and enhance the autoimmune response against beta cells and suggests a novel pathway that can be targeted to stop type 1 diabetes.

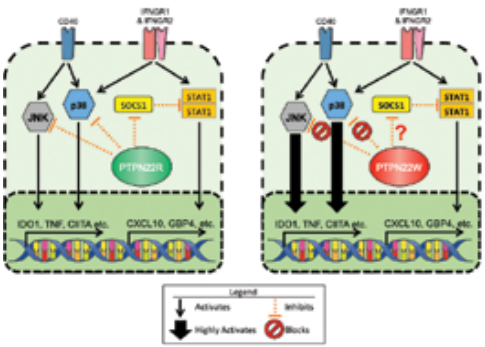



Figure 6. *PTPN22 dampens inflammation by inhibiting JNK and p38 MAPK signaling in macrophages. (Left) The common allele of PTPN22, PTPN22-620R, functions to reduce or inhibit signaling downstream of CD40 and the Interferon-gamma receptor. This dampened signaling reduces the impact of T cell help and limits the expression of chemokines, HLA Class II, and TNF. (Right) In cells that encode the T1D risk allele of PTPN22, PTPN22-620W, the signaling downstream of CD40 and Interferon-gamma receptor is not regulated, leading to enhanced expression of inflammatory mediators (i.e. CXCL10, TNF) and increased antigen presentation. The failure of PTPN22-620W to reduce inflammation is tightly linked to autoimmunity and the attack on beta cells.* ***(CMAI: Mathews UC4, University of Florida)***

## CHARACTERIZING IMMUNE CELLS THAT INFILTRATE PANCREATIC ISLETS IN TYPE 1 DIABETES

Pathogenic T cells recognize specific protein targets on beta/islet cells and direct the autoimmune process that eventually destroys the beta cells. A type of pathogenic T cell, "memory" T cells, have seen a particular target once and have been primed to mount an aggressive response when they encounter it again in the body. Regulatory T cells (Tregs) counteract autoimmune responses and help repair tissues; researchers are developing ways to harness healthy Tregs to treat disease. In Year 4, CMAI teams made progress in isolating rare T cells that infiltrate the pancreatic islets and identifying the beta cell/islet proteins that they recognize.

Using a mouse model of type 1 diabetes, a team led by Ronald Gill, University of Colorado Denver, isolated T cells from islets. They discovered that Tregs recognize very similar protein targets as pathogenic T cells (Figures 7-8). This finding lays the groundwork for parallel research in human T cells and may point to strategies for improving Treg therapy for type 1 diabetes. In another study, using a model of islet transplantation, the team found that islet-associated T cells were highly enriched with T cells that displayed an unusual property. These pathogenic T cells recognized two protein targets simultaneously — one specific to islets and another specific to the donor's immune system. This phenomenon reveals a previously unknown pathway of immune recognition of transplanted islets or beta cells from different donors.

Interrogation of Pathogenicity of Autoantigen-Specific T Cell Receptors in Retrogenic Mice

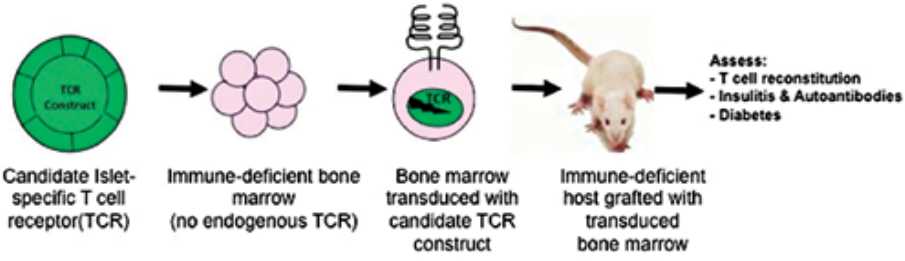



Figure 7. *Schematic of strategy for testing candidate autoreactive TCRs genes for expression and pathogenicity via retrogenic technology. (CMAI: Gill UC4, University of Colorado, Denver)*

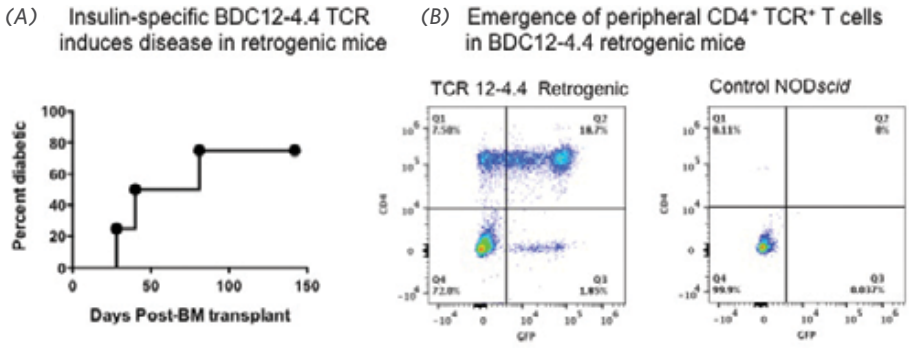



Figure 8. *Capacity of TCR gene constructs derived from insulin-specific CD4 T cells (BDC 12-4.4) to be expressed and initiate disease in retrogenic mice. (A) demonstrates that transfer of the BDC 12-4.4 TCR alpha/beta chain sequences can trigger overt disease in NOD mice. (B) illustrates robust detection of peripheral CD4 T cells (TCR+/CD4+/GFP+) in retrogenic mice.* ***(CMAI: Gill UC4, University of Colorado, Denver)***

Sally Kent, University of Massachusetts Medical School, led a team that derived 468 cell lines from T cells found in islets from 23 human organ donors — the largest bank of human islet-associated T cell lines in existence. The islets came from three types of donors: five donors positive for diabetes-related autoantibodies but no diabetes; six donors with recently diagnosed type 1 diabetes; and 12 donors with longstanding type 1 diabetes. Additional lines were derived from donors with no diabetes or related autoantibodies. Using this novel resource, the team has already made important discoveries about the nature of islet-associated T cells. For example, they found that most of the T cells are memory T cells. Also, the targets for those T cells came from multiple islet-associated proteins, some of which were modified from their usual forms. Further in-depth characterization of the T cells lines is ongoing in collaboration with other CMAI and HIRN investigators (Figure 9).

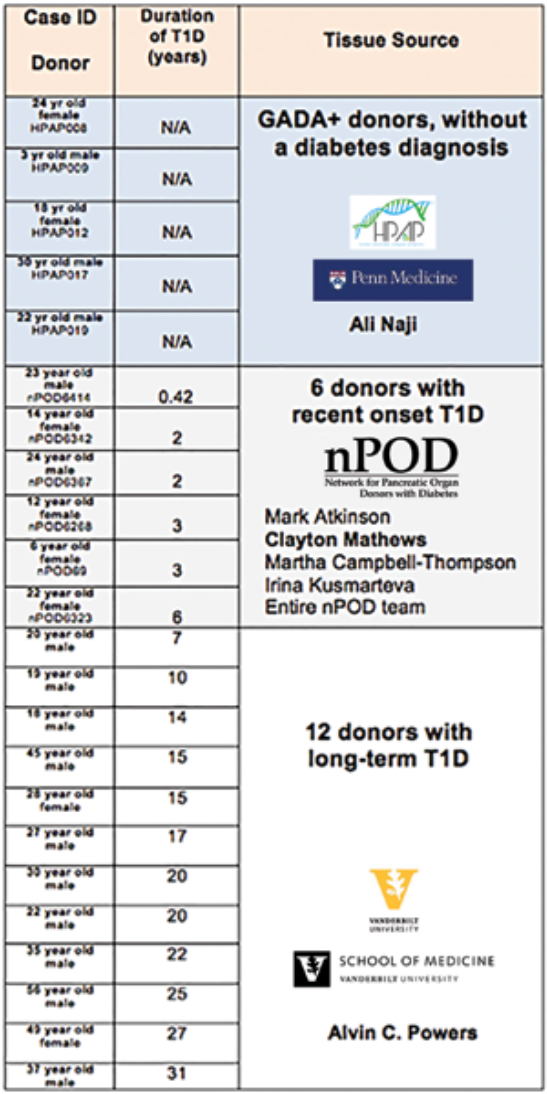



Figure 9. *Tissue donors for the isolation and expansion of islet-derived T cells. The tissue donors are from three sources and are of three broad types. Recently, we have received islets from 5 donors, positive for autoantibody to glutamic acid decarboxylase (GADA+), but without a diagnosis of T1D. From these donors, we have a total of 88 T cell lines and clones (both CD4 and CD8 represented). We received islets from 6 donors with recent onset T1D from the nPOD Pilot Islet Program. We have received islets from 12 donors with longer term T1D (7-31 years). In total, we have 468 T cell lines and clones derived directly from the islets of these donors to aid in the characterization of the islet-infiltrating T cells' targets, phenotype and function. (CMAI: Kent UC4, University of Massachusetts Medical School)*

## WELCOMING NEW RESEARCH TALENT IN TYPE 1 DIABETES-RELATED AUTOIMMUNITY

In Year 5, CMAI is joined by one New Investigator Pilot Awardee. Eddie James, Benaroya Research Institute, in collaboration with Sally Kent, is studying T cell lines isolated from islets of four nondiabetic organ donors who were positive for type 1 diabetes-related autoantibodies, with the goal of investigating their targets and functional attributes. Understanding T cells in the pre-type 1 diabetes stage may illuminate how the balance of the immune system tips from autoimmunity to clinical diabetes.

## CMAI INVESTIGATORS, YEAR 4

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

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

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

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*

*Eddie James, Ph.D., Investigator, *Benaroya Research Institute*

*New Investigator Pilot Award, 2018

# CONSORTIUM ON TARGETING AND REGENERATION (CTAR)

## TARGETING UNIQUE BETA CELL PROPERTIES TO PROTECT AND RESTORE INSULIN PRODUCTION

Insulin-producing beta cells are rare, unique cells in the human body. They are found in only one organ, the pancreas, where they constitute fewer than 2 percent of the cells. And, beta cells have a highly specialized mission to produce just the right amount of insulin in response to the level of glucose in a person's blood — a function that no other cell in the body can perform. To carry out their mission, beta cells turn on specific genes, display distinct proteins on their surface and operate dedicated molecular pathways that are not replicated in any other cells. This uniqueness is part of why the autoimmune process can selectively target and destroy only beta cells in individuals who are at risk for type 1 diabetes. But, those same unique properties also impart a significant advantage to researchers who are developing precisely targeted therapies to protect, restore or regenerate beta cell function without unwanted effects on other cells or tissues. In Year 4, the Consortium on Targeting and Regeneration (CTAR) advanced the science behind multiple beta cell-targeting strategies, uncovered previously unknown beta cell-specific pathways and molecules and validated new methods to reprogram non-beta cells with beta cell functions. Collectively, this body of research lights the way toward innovative therapies that restore beta cells and relieve the burden of daily insulin therapy for those with type 1 diabetes.

## EXPLORING DIVERSE STRATEGIES TO SELECTIVELY TARGET BETA CELLS

In Year 4, CTAR teams took several approaches to address the central question of how to target protective or regenerative therapies to beta cells with research spanning immune cells, small molecules and viruses as possible vehicles for beta cell-specific therapies.

The immune system is often portrayed as the enemy in type 1 diabetes — the disease is triggered when a person's own immune system destroys the beta cells in a process known as autoimmunity. But, a certain type of immune system cells, regulatory T cells or "Tregs," normally act as a brake on autoimmunity and promote tissue repair. Unfortunately, Tregs are

defective in many with type 1 diabetes. Jeffrey Bluestone and colleagues at the University of California, San Francisco, pioneered an approach to isolate Tregs from persons with type 1 diabetes, expand the cells *ex vivo* and safely inject them back into the patients, where the repaired cells appeared to stabilize insulin production. In the past year, the investigators began to engineer a second generation human Treg therapy that can reestablish immune balance and prevent ongoing autoimmunity in the islet environment. The team is modifying Tregs in ways that improve their ability to specifically target human islets and enhance their survival and function in the body. In animal models, some of these changes resulted in a 1,000-fold improvement in Treg function compared to first generation Treg therapy (Figure 1).

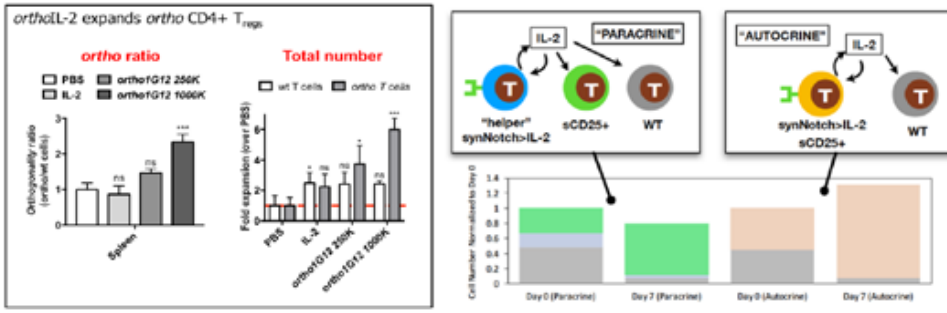



Figure 1. *IL-2 circuitry engineering for Treg enhancement. Left: Tregs expressing an orthogonal receptor for IL-2 were co-transferred into mice along with wt Tregs. The mice were divided into 4 treatment groups receiving PBS, standard IL-2, or two different doses of IL-2. The result shows that Tregs expressing the orthogonal receptor selectively responded to IL-2. Right: T cells expressing high-affinity CD25 (sCD25) preferentially responded to IL-2 induced by a ligand via a SynNotch receptor in the paracrine or autocrine fashion in vitro. (CTAR: Bluestone UC4, University of California, San Francisco)*

At Stanford University and Oregon Health & Science University, a team led by Seung Kim investigated how to target genetically modified CAR (chimeric antigen receptor)-Tregs to human islets in two ways. In the first set of experiments, a panel of antibodies was screened to find those that specifically bind to the surface of beta cells. In the second set, the team asked whether targeting proteins released from islet cells, such as insulin or C-peptide, might enable CAR-Tregs to localize to and activate in the pancreatic islet environment. They successfully demonstrated that CAR-Tregs modified to recognize SDF1α, a beta cell protein that is upregulated

by inflammation, migrate toward the protein *in vitro* and toward human islets engrafted in animal models *in vivo*. The investigators established multiple collaborations with other CTAR groups to identify additional beta cell proteins that can help target CAR-Tregs to beta cells (Figure 2).

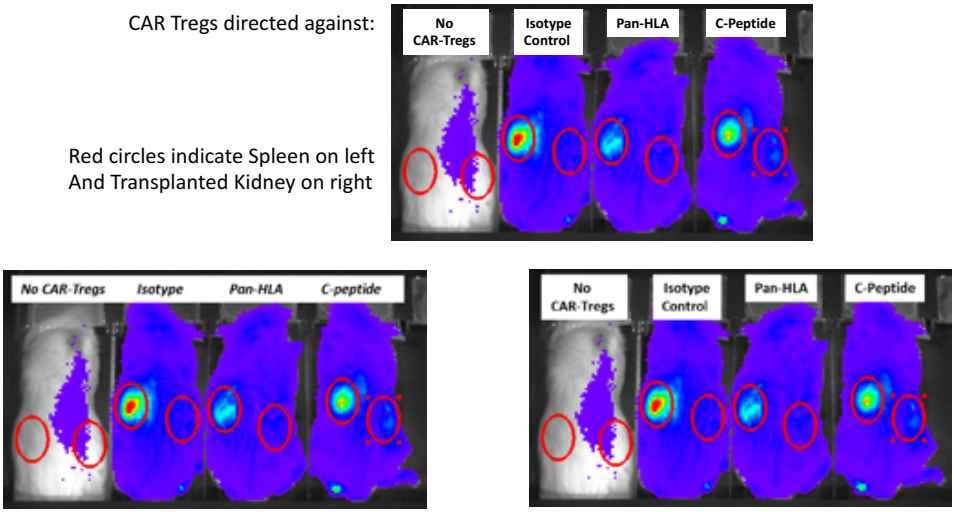



Figure 2. *Evidence for localization of CAR T-cells to islet grafts in vivo.* ***(CTAR: Kim UC4, Stanford University)***

Paolo Serafini and his team at the University of Miami designed small molecules called "aptamers" that can be linked to therapeutic molecules, such as RNA or a drug, and once injected into the body, specifically target and enter into the beta cells. Using an innovative aptamer-RNA system, the team effectively stimulated beta cell proliferation within human islets that had been transplanted into animal models. With an aptamer attached to a different RNA, they protected human beta cells from cell death during culture in the lab and *in vivo*.

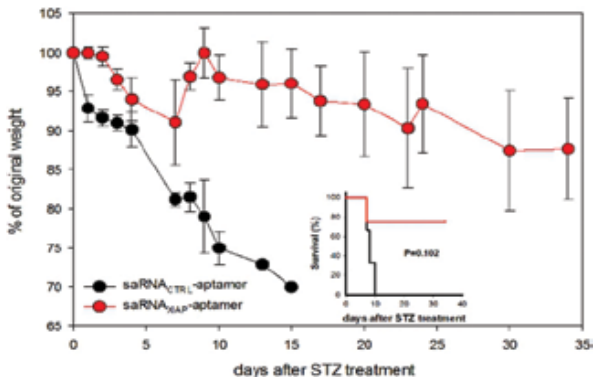



Figure 3. *saRNAXIAP-aptamer prevents early graft loss. NSG mice were transplanted with a suboptimal number of human islets treated with aptamer conjugated with saRNAXIAP or saRNACTRL, and with STZ to eliminate endogenous beta cells.* ***(CTAR: Serafini UC4, University of Miami)***

These and related experiments established proof of principle that the aptamer system can be used to modulate beta cell biology, while leaving other tissues unaltered. The investigators are collaborating with other labs in and outside of the HIRN to generate new aptamer-therapy combinations that will target additional beta cell properties (Figure 3).

Amit Choudhary and his colleagues at the Broad Institute and Joslin Diabetes Center made progress on developing a unique delivery system that can carry therapeutic molecules directly to human islets without affecting other tissues. In the past year, the team attached a fluorescent molecule — a visual marker — to their system to help them optimize its ability to precisely target islets. Their next step is attaching molecules that stimulate beta cell growth to prevent or reverse type 1 diabetes (Figure 4).

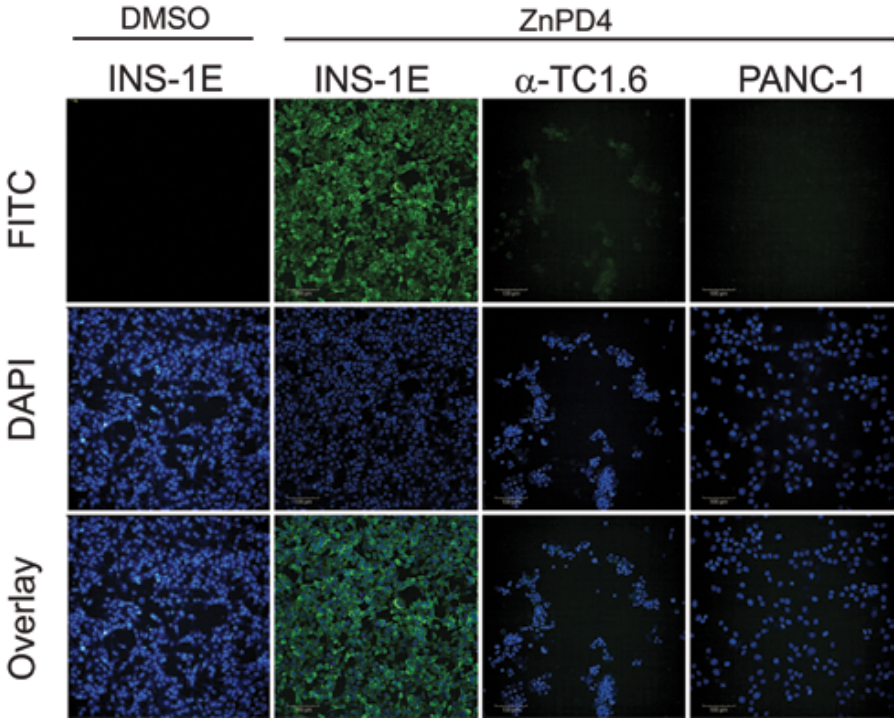



Figure 4. *Selective fluorophore delivery to INS-1E cells using our delivery system. (CTAR: Choudhary UC4, Broad Institute)*

A team led by Markus Grompe at Oregon Health & Science University and colleagues at Stanford University developed a series of viruses that target intact human islets with 95 percent efficiency at delivering a new gene to beta cells. Also, the team tested more than 1,000 DNA sequences that control the expression levels of genes and identified the top five that were most efficient at driving beta cell-specific gene expression.. These findings together mean that the investigators now have a way to selectively target and genetically reprogram beta cells for a variety of purposes, such as making the cells less vulnerable to autoimmune attack, more able to replicate new beta cells or more protected from cell death. To follow up on the significant clinical potential of this research, the team is planning a preclinical study to test the viruses in nonhuman primates (Figures 5-6).

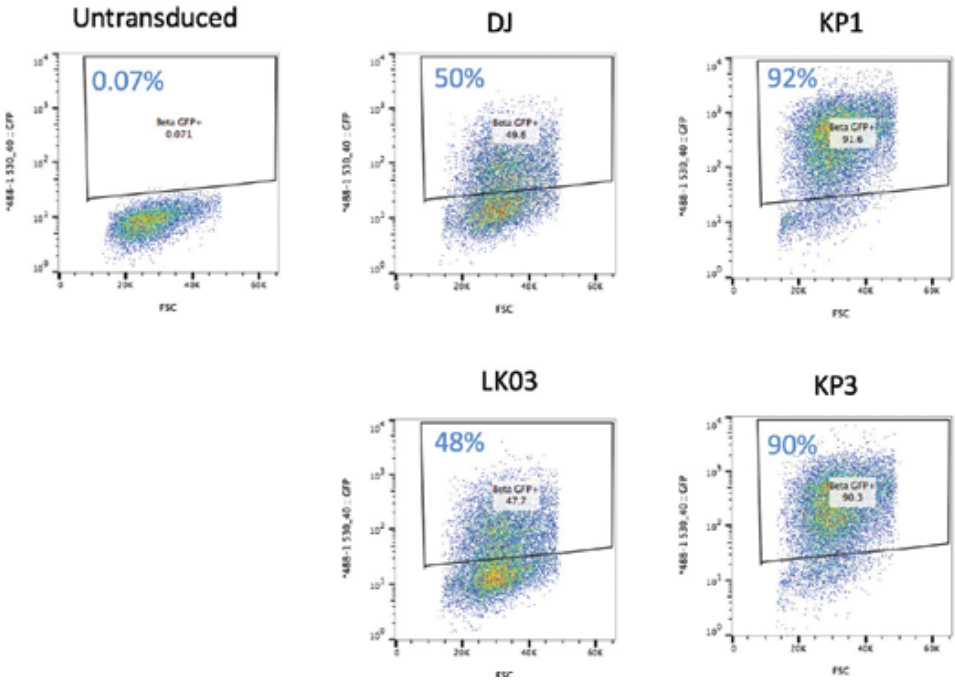



Figure 5. *rAAV transduction of human beta cells in intact islets. Cultured human islets were transduced with GFP-expressing rAAV of different serotypes. Three days later the islets were dispersed and beta cells were identified by FACS. Untransduced beta cells displayed no GFP expression. rAAV DJ, LK03, KP1 and KP3 produced GFP expression in 50, 48, 92 and 90% of beta cells respectively.* ***(CTAR: Grompe UC4, Oregon Health & Science University)***

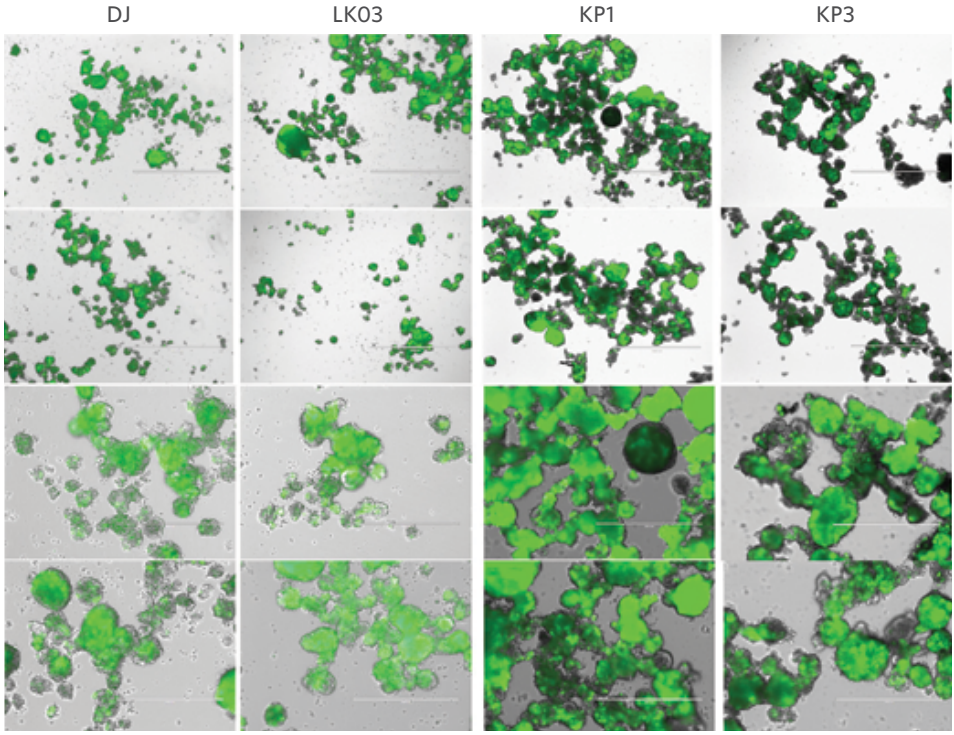



Figure 6. *Green fluorescent protein (GFP) expression in intact human islets transduced with different rAAV serotypes (names listed at top). GFP is expressed in the majority of beta cells using the new KP1 and KP3 serotypes.* ***(CTAR: Grompe UC4, Oregon Health & Science University)***

## REGENERATING BETA CELL FUNCTION TO REVERSE DIABETES

Four years ago, before the start of the HIRN, human beta cell regeneration was widely considered to be an impossibility. In Year 4, CTAR teams continue to turn this belief on its head with their groundbreaking research revealing several potential pathways for restoring beta cell mass and insulin production.

Al Powers, Vanderbilt University, led a multi-institution team focused on mapping molecular pathways of human beta cell function, proliferation and regeneration. In one example of their progress, Andrew Stewart's lab at the Icahn School of Medicine at Mount Sinai generated a beta cell proliferation "roadmap" by comparing how genes are turned on or off in normal human beta cells compared to cells from insulinomas (rare beta cell tumors). This roadmap represents an important data resource that has already revealed numerous previously unappreciated targets for drug

discovery in human beta cell replication and regeneration. Dr. Stewart and other investigators are working to develop potential new drugs based on insights gained from the roadmap (Figures 7-9). In another significant advance, Dr. Powers' lab identified a protein, called NTPDase3, that is the first to be found on the surface of all adult human beta cells, but no other islet cells. Thus, NTPDase3 offers a novel, more precise reagent for beta cell isolation, imaging and therapeutic targeting, and multiple teams across CTAR and the HIRN are collaborating on cutting-edge research that takes advantage of this important discovery.

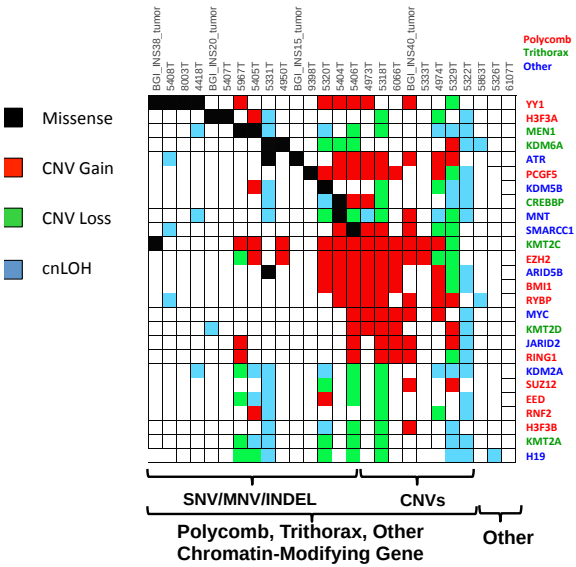



Figure 7. *A summary of mutations (black) and copy number variants (CNVs, red, green, blue) in human insulinomas. The key points are that among the 26 insulinomas (denoted along the top y-axis) with whole exome seq, most had different mutations, but they all converge on trithorax, polycomb or other epigenetic regulatory genes.* ***(CTAR: Powers UC4, Vanderbilt University)***

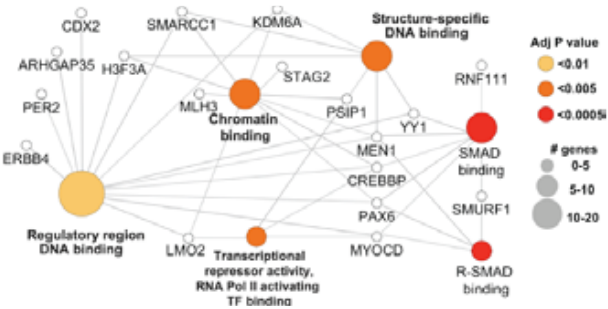



Figure 8. *Gene ontology pathway analysis of the 278 mutations and CNVs in the 26 insulinomas. The most statistically important pathways are illustrated. Note that KDM6A, SMADs, R-SMADs, YY1 are predicted to be of therapeutic interest in beta cell regeneration, and each has been confirmed experimentally. This illustrates the importance of further insulinoma data-mining for human beta cell regenerative research.* ***(CTAR: Powers UC4, Vanderbilt University)***

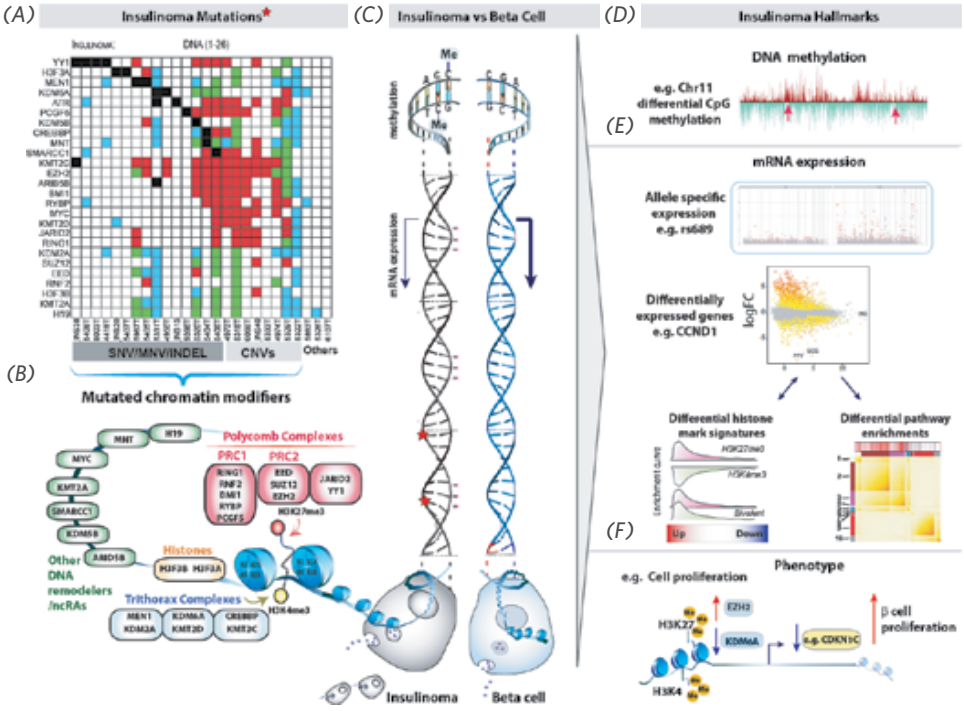



Figure 9. *The complete insulinoma "wiring diagram" or "Roadmap 1.0". This view includes the whole exome seq findings in Figs 7 and 8, and adds insulinoma vs. beta cell transcriptome analysis and biological confirmation of genomic and transcriptomic predictions. We now have an Insulinoma Roadmap 1.0 and are refining it further with increasing numbers of insulinomas and additional therapeutic drug data-mining and validation.* ***(CTAR: Powers UC4, Vanderbilt University)***

A team led by Klaus Kaestner at the University of Pennsylvania and Ben Glaser and Dana Avrahami at Hadassah-Hebrew University in Jerusalem developed a novel technology that allows for targeting the epigenome of human beta cells at specific, defined loci, increasing cellular proliferation without altering the DNA sequence of the modified cells. Specifically, by targeting an "epimutation" to the imprinting control region at the *CDKN1C/p57* locus, they were able to reduce p57 protein levels in human beta cells, causing them to divide in a xenotransplant setting. This type of targeting approach — coupled with the novel delivery systems developed by colleagues in the consortium — holds the promise that we will soon be able to alter the epigenetic state of human beta cells *in vivo* in a targeted

fashion (Figures 10-11). In the course of their research, the team developed several new technologies and novel reagents for the analysis of mouse and human islets and freely shared those with teams across the HIRN.

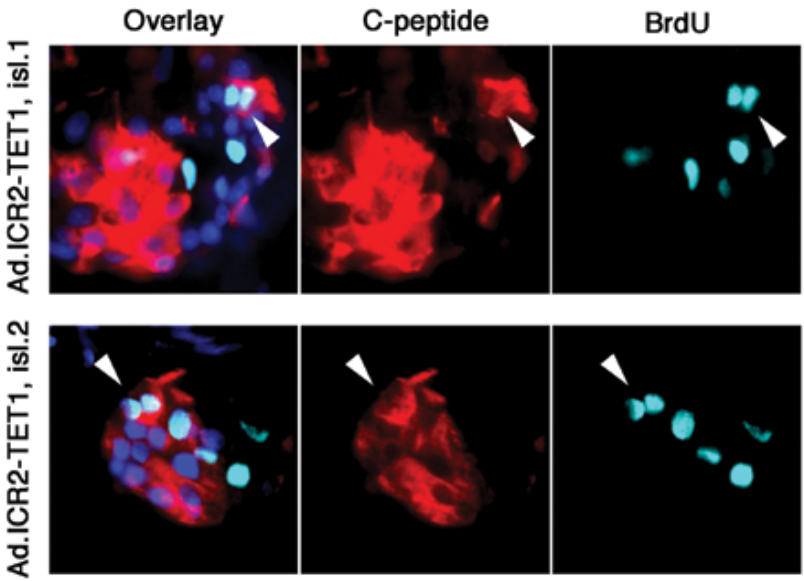



Figure 10. *Targeted epimutation of the imprinting control region at the CDKN1C/p57 locus increases proliferation of human beta cells in the xenograft model. BrdU/C-peptide double-positive doublets, indicated by the white arrows, in Ad.ICR2-TET1 transduced xenografts indicate successful completion of the cell cycle.* ***(CTAR: Kaestner UC4, University of Pennsylvania)***

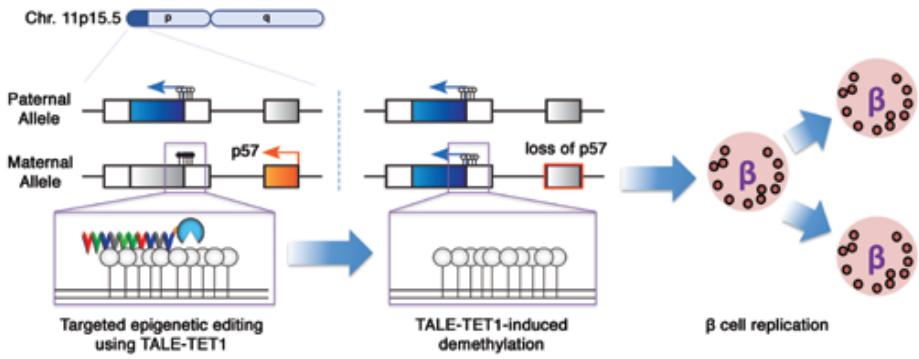



Figure 11. *Targeted epimutation of the imprinting control region at the CDKN1C/p57 locus increases proliferation of human beta cells in the xenograft model. A TALE-TET construct was designed to demethylate specifically the imprinting control region 2 at the CDKN1C/p57 locus to increase replication of human beta cells.* ***(CTAR: Kaestner UC4, University of Pennsylvania)***

Pedro Herrera and co-Investigators at the University of Geneva explored the hypothesis that non-beta islet cells could be reprogrammed to become insulin-producing beta cells. They showed that a small fraction of glucagon-producing alpha cells and somatostatin-producing delta cells spontaneously express insulin and reverse diabetes in a mouse model after induction of beta cell ablation. This discovery led the team to explore whether human islets display the same capacity. The scientists provided the first direct evidence of the plasticity of human non-beta islet cells, with an approach based on the production of "pseudoislets," or small clusters of cells that mimic how islet cells fit together in the body. Type 1 diabetes autoimmunity only targets the beta

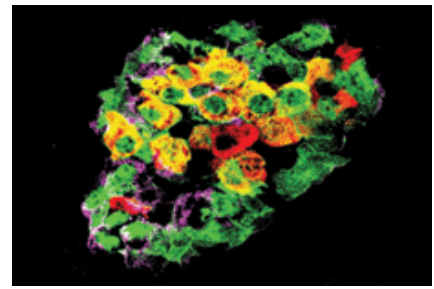


Figure 12. *Beta cell-ablated mouse pancreatic islet in which the glucagon-producing alpha-cells have been genetically (i.e. irreversibly) labeled with a fluorescent tag (green). Some of them have started to produce insulin (red), and appear as yellow (green-and-red merge).* ***(CTAR: Herrera UC4, University of Geneva)***

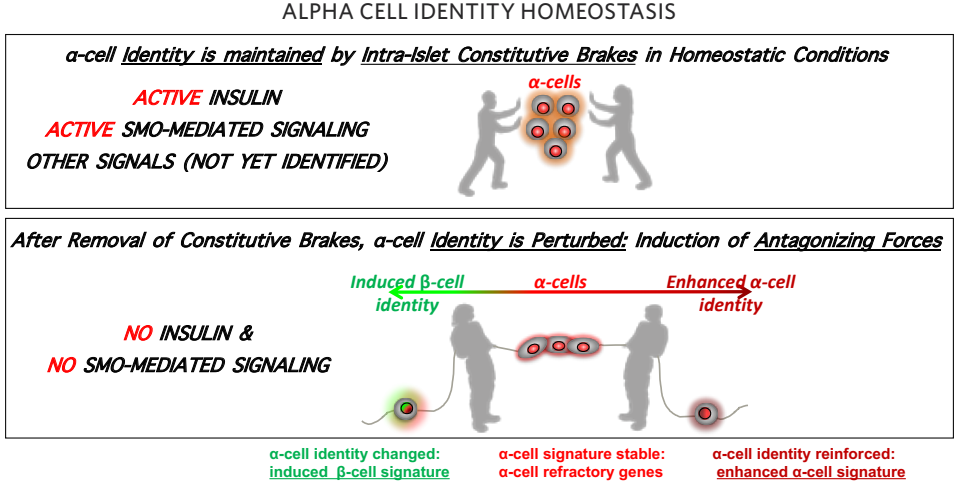



Figure 13. *Islet-autonomous signals control alpha cell identity. In homeostatic conditions, alpha cell identity is maintained through constitutive insulin and smoothened-mediated signaling, among others. Removal of the insulin signaling brake induces molecular changes resulting in the induction of antagonizing forces in alpha cells: one opposes change of identity and the other redirects alpha cells toward a beta-like cell phenotype. This dual response results in the reprogramming of few alpha cells into insulin producers whose number is enhanced when insulin deprivation is combined with blockade of smoothened-mediated signaling. Re-establishment of optimal insulin levels reverses this response, and leads to blockade of insulin production in alpha cells: the alpha cell identity change is thus, at least to some extent, reversible.* ***(CTAR: Herrera UC4, University of Geneva)***

cells within islets, leaving behind the alpha, delta and other cells, so these new findings point the way to a potential source for regenerated beta cells in those with the disease (Figures 12-13).

Joe Zhou, Harvard University, and his co-Investigator at the Joslin Diabetes Center worked on reprogramming cells from the stomach into insulin-producing beta-like cells. The potential advantage of this innovative approach is that the reprogrammed stomach cells may be less likely to attract attention, and eventual destruction, from the immune system, while still being able to release insulin in response to blood glucose levels. In the past year, the investigators worked on optimizing methods for robust and reliable generation of insulin-producing cells from stomach organoids derived from human progenitor cells and testing their function in animal models. This team established collaborations with other CTAR and HIRN investigators to fully understand the properties of the reprogrammed cells and study how they interact with the immune system (Figure 14).

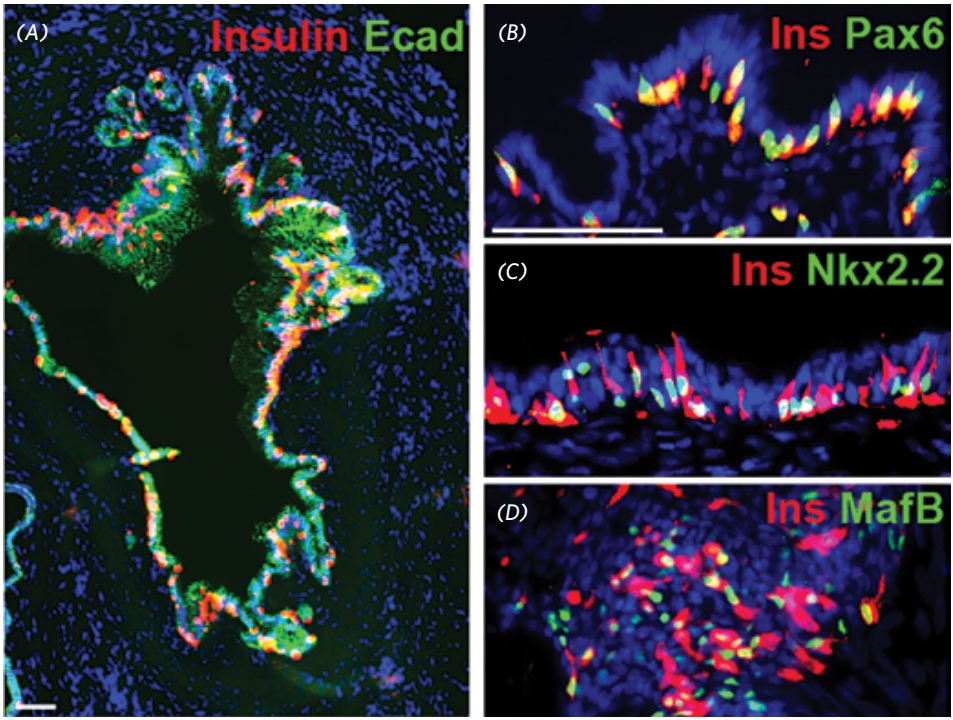



Figure 14. *The image shows immunohistochemistry analysis of a transplanted stomach organoid derived from human pluripotent cells. The organoid has been engineered to express NPM (Ngn3, Pdx1, Mafa) factors, and after induction of NPM, many insulin+ cells appeared in the organoid (A). The induced gastric insulin+ cells express endocrine and beta cell markers Pax6, Nkx2.2, and MafB (B-D).* ***(CTAR: Zhou UC4, Harvard University)***

Anath Shalev, University of Alabama at Birmingham, completed a randomized, double blind, placebo controlled clinical trial of the drug verapamil in adults with recently diagnosed type 1 diabetes. Verapamil inhibits a protein called TXNIP that promotes beta cell death and reduces insulin production. The trial showed that addition of verapamil to standard insulin therapy led to improved glucose control and reduced insulin requirements compared to the placebo. Dr. Shalev also developed next generation TXNIP-specific inhibitors that protect beta cell lines, mouse models of diabetes and human islets from inflammation associated with type 1 diabetes. The positive findings of the verapamil trial suggest that further development of these new inhibitors may lead to novel therapies to improve glucose control in type 1 diabetes by protecting and regenerating a patient's own beta cells (Figure 15).

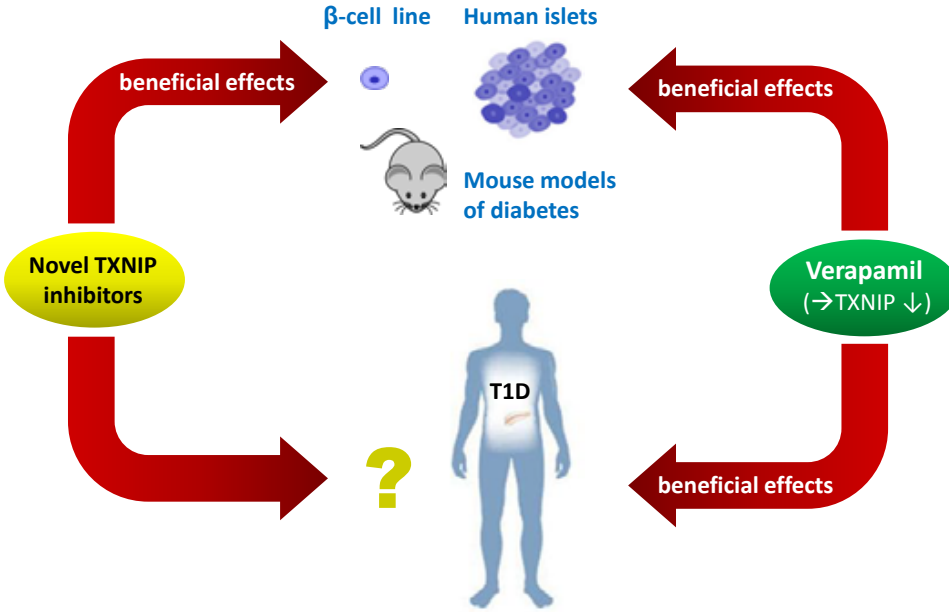



Figure 15. *Inhibiting TXNIP has protective effects on beta cell lines, mouse models of diabetes and isolated human islets and some of these benefits are translatable to human subjects with T1D.* ***(CTAR: Shalev, University of Alabama, at Birmingham)***

## FOSTERING NEW TALENT IN BETA CELL REGENERATION

In Year 5, CTAR is joined by two New Investigator Pilot Award recipients. Sangeeta Dhawan, City of Hope, is investigating how a type of chemical modification of genes, known as hydromethylation, affects beta cell function. This line of research has implications for understanding type 1 diabetes pathogenesis, as well as for improving strategies to regenerate beta cell function. Abdelfattah El Ouaamari, Rutgers University, is unraveling the molecular blueprint of pancreas-specific projecting neurons. This information will be leveraged to develop a neuromodulation therapy aimed at restoring beta cell function by sending signals through neurons that can selectively target the beta cells.

## CTAR INVESTIGATORS, YEAR 4

**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*

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

**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.,** 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 Mount Sinai*
**Seung Kim, M.D., Ph.D.,** Investigator, *Stanford University School of Medicine*
**Rita Bottino, Ph.D.,** Co-investigator, *Allegheny Health Network*
**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 Mount 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 Ricordi, 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*

***Sangeeta Dhawan, Ph.D.,** Investigator, City of Hope

***Abdelfattah El Ouaamari, Ph.D.,** Investigator, Rutgers University

†Current Affiliation: Weill Cornell Medical College

*New Investigator Pilot Award, 2018

# HUMAN PANCREAS ANALYSIS PROGRAM/CONSORTIUM (HPAP/HPAC)

## HUMAN PANCREAS RESEARCH IS VITAL FOR TYPE 1 DIABETES

A fundamental challenge in type 1 diabetes research is that the pancreas — the home of the insulin-producing beta cells that are destroyed by autoimmunity — cannot be safely biopsied or effectively imaged in humans. Moreover, the process of identifying, procuring and analyzing extremely rare organ donors with type 1 diabetes or prediabetic autoimmunity is logistically complex and costly — beyond the capability of individual laboratories. Yet, studying the human pancreas offers enormous potential for gaining new understandings of the causes and possible cures for type 1 diabetes that simply cannot be achieved in other ways. For this reason, the HIRN launched the Human Pancreas Analysis Program (HPAP), a highly collaborative group of investigators that is making large amounts of information about the composition and function of the human pancreas accessible to the entire type 1 diabetes community. Over the past year, the HPAP continued to refine its protocols for screening the U.S. organ donor pool and procuring pancreata and other tissues from those with recent-onset type 1 diabetes or signs of islet autoimmunity for analysis. After processing, preserving and analyzing the tissues, HPAP investigators deposited meticulously curated data into a public database that is an unparalleled resource for research on the human pancreas. Already, HPAP's efforts are paying off with novel and important findings about how type 1 diabetes affects human islet biology.

## COOPERATION AND COMMUNICATION TO TRANSFORM HUMAN PANCREAS RESEARCH

The three HPAP institutions — University of Florida (UF), University of Pennsylvania (UPenn), and Vanderbilt University (VU) — exemplify the collaborative spirit at the heart of HIRN. In their second year working together, the institutions further streamlined communication among eight core laboratories. Together, these labs have created a seamless process for (1) identifying and procuring rare human pancreata and other tissues from organ donors with type 1 diabetes or related autoantibodies (UF), (2) processing those tissues and isolating islets (UPenn) and (3) assessing islet number, purity, viability and function (VU). To date, this coordinated strategy has made it possible for the HPAP to obtain and analyze more than 32 human pancreata for transformative type 1 diabetes research.

In HPAP Year 2, the HPAP Executive Committee convened bimonthly conference calls to share data and to address progress and challenges across the program. In addition, the HPAP instituted semiannual in-person meetings to discuss scientific and logistic issues; investigators from the three institutions met in Philadelphia in May and in Nashville in October. These regular opportunities for cross-institutional communication ensure that the HPAP's efforts to advance human pancreas research are efficient and productive.

At UF, investigators expanded their outreach to organ procurement organizations (OPOs) emphasizing the importance of studying human organs in type 1 diabetes research (Figure 1). As a result, the number of OPOs participating in the UF-directed pancreas screening for type 1 diabetes-related autoantibodies increased from 27 to 32 by the end of 2018, capturing 59 percent of the U.S. organ donor pool (up from 56 percent in the HPAP's first year). That expansion enabled UF to identify 13 new donors with type 1 diabetes and eight new autoantibody-positive donors through the third quarter of 2018, despite lowering the upper age limit for donation from 50 to 30 years.

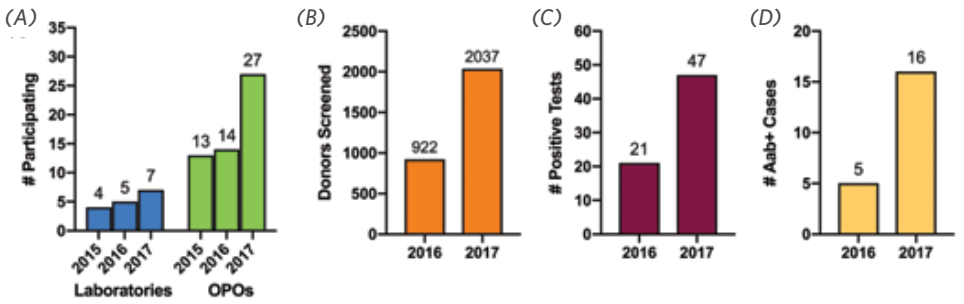
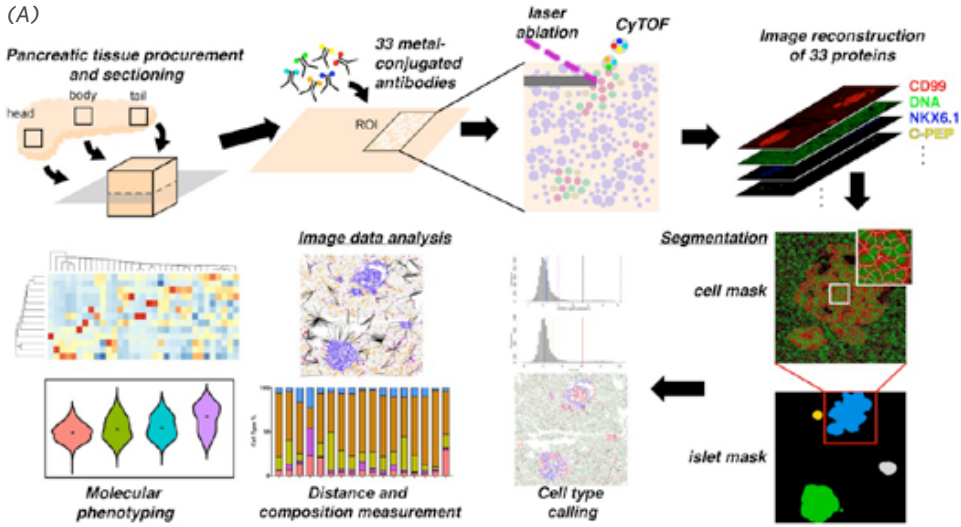



Figure 1. *HPAP efforts led to an increased number of participating laboratories and organ procurement organizations (Figure 1A), and increased screening efforts led to an increase in the number of organs for research (Figure 1B, C, D). (HPAP: Powers UC4, Vanderbilt University)*

## BUILDING AN UNPARALLELED DATA RESOURCE FOR THE TYPE 1 DIABETES RESEARCH COMMUNITY

Pancreata and other tissues of interest (e.g., lymph nodes) collected through the UF screening program are shipped to UPenn for processing, islet isolation and cryopreservation. In addition to preserving tissue samples for future analyses, a primary goal of the UPenn team is to collect and curate standard datasets on each donor pancreas. The team applies multiple cutting-edge technologies to each pancreas (Figure 2), and the collected data undergo a rigorous quality assurance protocol before being deposited in the public PancDB database. In HPAP Year 2, UPenn investigators developed and launched a state-of-the-art web portal (**hpap.pmacs.upenn.edu**) that allows type 1 diabetes researchers from all HIRN consortia and the general research community to access, sort and download data and images from the HPAP human pancreas collection. The team also made progress on developing an open-source, user-friendly artificial intelligence software package that will help users practice machine learning in an automated way, as well as a number of visualization tools, which enhance the utility and accessibility of the unique, rich datasets available through the portal.



*(B)*

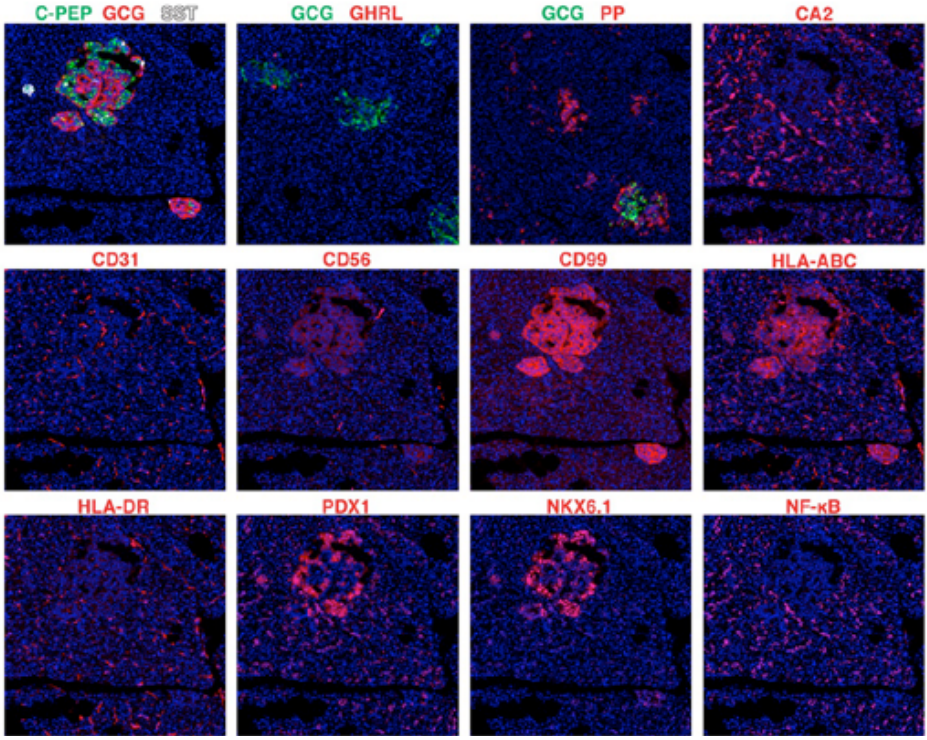



Figure 2. *Workflow and antibody validation for imaging mass cytometry study of the human pancreas. (A) Schema of the experimental platform. 4-8 μm FFPE sections from human pancreatic tissues are labelled simultaneously with 33 metal conjugated antibodies. 1000 μm x 1000 μm regions of interest (ROIs) around islets are selected for laser ablation. Plumes of particles are carried over to CyTOF for signal quantification. Antibody labeling patterns are reconstructed and output as 32-bit images. Islet-level and cell-level segmentation are performed to enable downstream image analyses. (B) Staining patterns of the 13 newly developed metal-conjugated antibodies confirm their specificity. Displayed channels from top to bottom, left to right are: C-PEP (green)/GCG (red)/SST(white), GCG (green)/GHRL (red), GCG (green)/PP (red), CA2 (red), CD31 (red), CD56 (red), CD99 (red), HLA-ABC (red), HLA-DR (red), PDX1 (red), NKX6.1 (red), and NF-κB (red). Iridium-DNA staining is shown in blue in all panels. All stainings are from the same ROI except GCG/GHRL and GCG/PP panels.* ***(HPAP/HPAC: Naji UC4, University of Pennsylvania)***

## NOVEL INSIGHTS INTO HUMAN PANCREAS BIOLOGY

Human islets isolated at UPenn are shipped to VU for additional analyses, such as computer-guided islet counting and protocols to assess islet purity that were established in HPAP Year 2. The team also developed a technique for simultaneous measurement of insulin and glucagon secretion. Using that approach, investigators found that islets from autoantibody-positive donors have similar or better insulin secretion compared to islets from autoantibody-negative donors (Figure 3). In other experiments, the team discovered a new explanation and a molecular mechanism for abnormal glucagon secretion in type 1 diabetes. This is a common complication in individuals with type 1 diabetes that interferes with the body's normal response to low blood sugar (hypoglycemia). Investigators found changes in how multiple genes were turned on in pancreatic alpha cells from a type 1 diabetes donor, suggesting that an intrinsic alpha cell defect could be responsible for impaired glucagon secretion. This finding will help researchers develop therapies to better regulate glucagon secretion and treat hypoglycemia in people with type 1 diabetes.

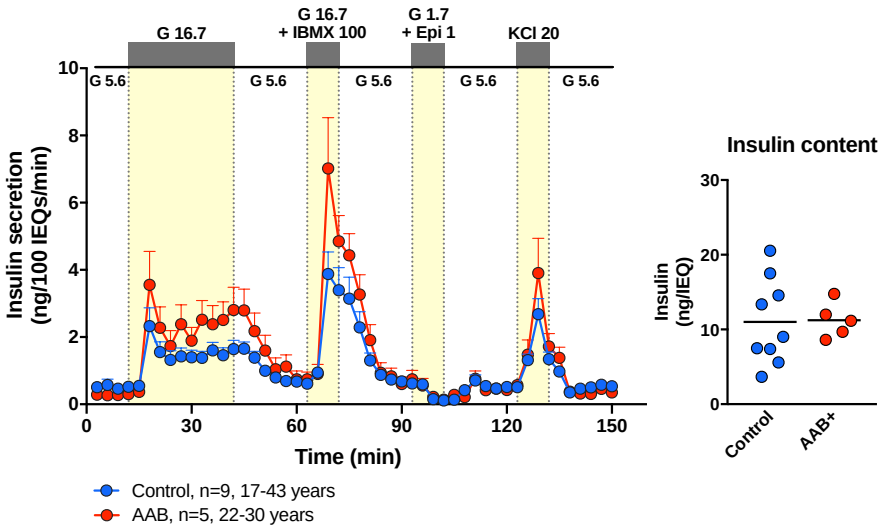



Figure 3. *Analysis of islet insulin secretion at Vanderbilt. Islets are challenged with 16.7 mM glucose for 30 minutes to resolve biphasic insulin secretory response, followed by 15-minute stimulations with 16.7 mM glucose + 50 mM IBMX, 1.7 mM glucose + 1 mM epinephrine, and 20 mM KCl. Insulin secretory response to these stimuli is shown on the left and islet insulin content on the right.* ***(HPAP: Powers UC4, Vanderbilt University)***

## LOOKING TO THE FUTURE: NEW APPROACHES AND INNOVATIVE TECHNOLOGIES

As its third year gets underway, the HPAP is joined by four multi-investigator research teams, as well as the recipient of a New Investigator Pilot Award, to expand studies in human pancreas biology. With this growth in new talent and technologies, the program will become the "Human Pancreas Analysis Consortium (HPAC)." Patrick MacDonald, University of Alberta, is leading an international, multi-institution team to understand how islet cell identity and function is influenced by the local pancreas microenvironment. Another international team, led by Alejandro Caicedo, University of Miami, is mapping islet development in early life — a critical time when beta cell autoimmunity is strongly associated with the development of overt type 1 diabetes. Juan Dominguez-Bendala and a team of investigators at the University of Miami focus on identifying and characterizing islet progenitor cells that could pave the way for beta cell regeneration therapies. At the University of California, San Diego, Maike Sander is leading investigators applying single cell RNA analysis technology to identify cells, molecular pathways and genes that may have causal roles in type 1 diabetes. Joana Almaca, University of Miami, is studying how the network of blood vessels surrounding pancreatic islets changes in type 1 diabetes and how those changes affect beta cell survival and function.

## HPAP/HPAC INVESTIGATORS, YEAR 2

**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*

**Alvin C. 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*

*Alejandro Caicedo, Ph.D., Investigator, *University of Miami*
Stephen Speier, Ph.D., Investigator, *Paul Langerhans Institute Dresden*
Marcela Brissova, Ph.D., Investigator, *Vanderbilt University*

*Juan Dominguez-Bendala, Ph.D., Investigator, *University of Miami*
Ricardo Pastori, Ph.D., Investigator, *University of Miami*
Alejandro Caicedo, Ph.D., Co-investigator, *University of Miami*
Camillo Ricordi, M.D., Co-investigator, *University of Miami*

*Patrick MacDonald, Ph.D., Investigator, *University of Alberta*
Martin W. Hetzer, Ph.D., Investigator, *Salk Institute for Biological Sciences*
Emma Lundberg, Ph.D., Investigator, *KTH Royal Institute of Technology*
Stephen R. Quake, D.Phil., Investigator, *Stanford University*
Seung Kim, M.D., Ph.D., Co-investigator, *Stanford University*

*Maike Sander, M.D., Investigator, *University of California, San Diego*
Kyle Jeffrie Gaulton, Ph.D., Investigator, *University of California, San Diego*
Sebastian Preissl, Ph.D., Co-investigator, *University of California, San Diego*
David Gorkin, Ph.D., Co-investigator, *University of California, San Diego*
Alvin C. Powers, M.D., Co-investigator, *Vanderbilt University*

†Joana Almaca, Ph.D., Investigator, *University of Miami*

*New teams to be added in Year 5
†New Investigator Pilot Award, 2018

# 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 grant from the JDRF supported the October 2017 CHIB in-person meeting.

# APPENDIX 1
## OPPORTUNITY POOL PROJECTS

| | PROJECT TITLE | PRINCIPAL INVESTIGATOR | INSTITUTION |
|---|---|---|---|
| **Completed projects — Year 2** | | | |
| CHIB | December 2015 Investigator In Person Meeting | Cherie Stabler | University of Florida |
| **Completed projects — Year 3** | | | |
| CBDS<br>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<br>CBDS | Antibodies for beta cell subtype identification by immunohistochemistry | Markus Grompe<br>Philip Streeter | Oregon Health & Science University |
| | | Mark Atkinson | University of Florida |
| CHIB | October 2016 Investigator In Person Meeting | Benjamin Stanger | University of Pennsylvania |
| **Completed projects — Year 4** | | | |
| CMAI<br>CTAR | Exploiting the Power of CyTOF/ Mass Cytometry (MC) to Elucidate the Complex Interactions of Islet and Immune Cells in Human Type 1 Diabetes Pancreata | Clayton Mathews | University of Florida |
| | | Dirk Homann<br>Andrew Stewart | Icahn School of Medicine at Mount Sinai |
| CMAI | Islet Reactive TCR Clones in Experimental Mice Generated With Type 1 Diabetes Patient Vs. Healthy Control Hematopoietic Progenitor Cells | Megan Sykes | Columbia University |
| | | Todd Brusko | University of Florida |
| CHIB | October 2017 Investigator In Person Meeting | Maike Sander | University of California, San Diego |

| | PROJECT TITLE | PRINCIPAL INVESTIGATOR | INSTITUTION |
|---|---|---|---|
| **Ongoing projects** | | | |
| CHIB | Quantitative Mass Spectrometry Analysis of Human Islet and Pancreas ECM | Karen Christman | University of California, San Diego |
| | | Kirk Hansen | University of Colorado |
| CBDS<br>CHIB | Workshop for Continued Harmonization of Beta Cell Death Assays | Carmella Evans-Molina | Indiana University |
| | | Camillo Ricordi | University of Miami |
| CHIB | Real-time Detection of Insulin Surrogate Markers Within Physiomimetic Islet Microsystems | Ashu Agarwal<br>Alejandro Caicedo | University of Miami |
| CHIB | Generation of reporter progenitor cell lines to allow quantification of endocrine differentiation and functional analysis at the single cell level | Paul Gadue | Children's Hospital of Philadelphia |
| CHIB | Functional Testing of Candidate HSC-derived Islet Cells | Maike Sander | University of California, San Diego |
| 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<br>CTAR | Can Genome Mosaicism Explain the Lobular Nature of Type 1 Diabetes? | Klaus Kaestner | University of Pennsylvania |
| CBDS | The Proteome of Replicating Cells | Charles Ansong | Pacific Northwest National Laboratory |
| | | Yuval Dor | Hebrew University of Jerusalem |
| CHIB | October 2018 Investigator In Person Meeting | Kevin Parker | Harvard University |

# APPENDIX 2
## NEW INVESTIGATOR PILOT AWARDS

| | PROJECT TITLE | RECIPIENT | INSTITUTION |
|---|---|---|---|
| **Awarded Year 4** | | | |
| CBDS | Real-time In Vivo Analysis of Islet Redox Dynamics* | Amelia Linnemann | Indiana University |
| CBDS | Elucidating the Human Beta Cell Translatome in Heath and Disease* | Holger Russ | University of Colorado, Denver |
| CBDS | Discovery and Investigation of Noninvasive Diagnostic Potential of Circulating Pancreatic Islet Beta Cell Exosomes as Biomarkers of Beta Cell Injury* | Prashanth Vallabhajosyula | University of Pennsylvania |
| CMAI | HLA Multimer Based Characterization of Islet Resident CD4+ T Cells That Target Beta Cell Epitopes and Non-Epitopes† | Eddie James | Benaroya Research Institute |
| CTAR | Targeting DNA Hydroxymethylation to Promote Human Beta Cell Function* | Sangeeta Dhawan | City of Hope |
| CTAR | Neuromodulation for Type 1 Diabetes: Harnessing Sensory Innervation to Promote Regeneration and Function of Insulin-Producing Cells* | Abdelfattah El Ouaamari | Rutgers University |
| HPAC | Changes in Human Islet Micro-vascularization During Type 1 Diabetes* | Joana Almaca | University of Miami |

*2-year project
†1-year project

# APPENDIX 3
# WORKING GROUP MEMBERSHIP, YEAR 4

## 2018 ANNUAL MEETING PLANNING COMMITTEE

Maria Golson, Ph.D., Co-chair, HPAP Representative, *University of Pennsylvania*

Clayton Mathews, Ph.D., Co-chair, CMAI Representative, *University of Florida*

Christopher Wright, D.Phil., Co-chair, CBDS Representative, *Vanderbilt University*

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

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

Nelly Berger, CC Representative, *City of Hope*

Michael Betts, Ph.D., HPAP Representative, *University of Pennsylvania*

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

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

Markus Grompe, M.D., CTAR Representative, *Oregon Health & Science University*

Rene Maehr, Ph.D., CMAI Representative, *University of Massachusetts Medical School*

Margaret Morris, Ph.D., CBDS Representative, *Eastern Virginia Medical School*

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

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

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

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

## WHAT IS A BETA CELL? WORKING GROUP

Alvin C. Powers, M.D., Chair, TNC and 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, *Mount 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., 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, 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, D.Phil., Chair, CBDS Representative, *Vanderbilt University*

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

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## NOTES

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