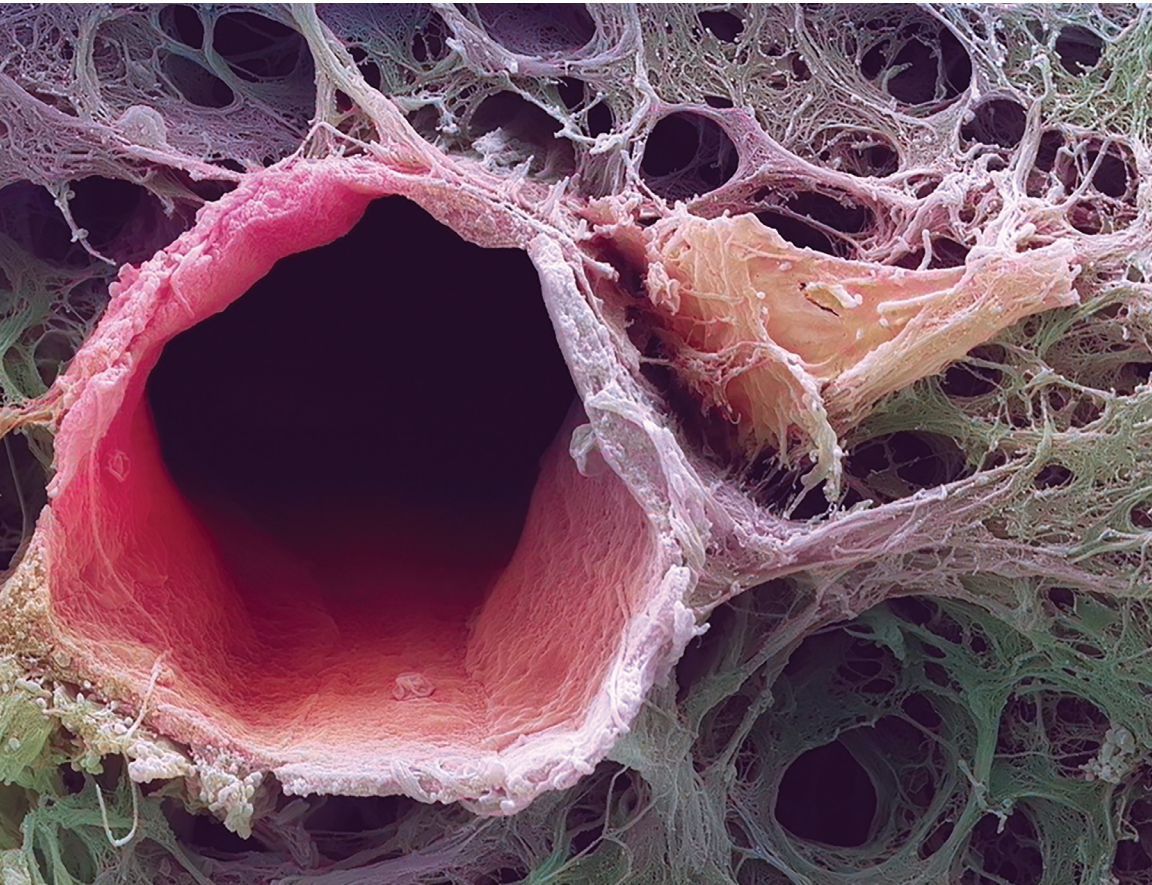
HIRN
HUMAN ISLET RESEARCH NETWORK

HUMAN ISLET RESEARCH NETWORK (HIRN):

YEARS 5 & 6
EXECUTIVE SUMMARY REPORT



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Cover photo: Cross-sectional view of a vascularized three-dimensional biomimetic device visualized by scanning electron microscopy. A micro-capillary is seen in cross-section along with a functional pericyte (upper right) and surrounding matrix.

Photo Credit: *Dan Huh, Ph.D., University of Pennsylvania, on behalf of the Stanger UG3.*

Report reflects activity from October 2018 through September 2020.

FOREWORD

January 2022 is the 100th anniversary of one of the most revolutionary advances in medical research. A team of scientists working at the University of Toronto — Frederick Banting, Charles Best, John Macleod and James Collip — achieved the first demonstration of insulin injection as a viable therapy for type 1 diabetes (T1D). The transformative power of their research is undeniable — to this day, insulin remains a lifesaving treatment for people with T1D. Nonetheless, there is more to do. Insulin therapy does not keep insulin-producing pancreatic beta cells safe from the immune process that causes T1D, nor does it replace beta cells that have already been lost to that process.

The Human Islet Research Network (HIRN) pursues innovative science and technology development with the ultimate goal of going beyond insulin therapy for T1D to the protection or regeneration of beta cell function. In an era of increasingly specialized scientific expertise and technologies, the HIRN is committed to fostering an environment of teamwork and multidisciplinary collaboration within and across its five research consortia. Moreover, the HIRN actively participates in the wider diabetes research community, extensively sharing datasets, new technologies and research resources for the benefit of people living with T1D.

This biannual report highlights major examples of research progress and new program developments made by the HIRN in Years 5 and 6 of the network (October 2018 through September 2020). Like everyone throughout the world, HIRN scientists were affected by the novel coronavirus pandemic that sprang up in early 2020 (the middle of HIRN's Year 6). Many universities and laboratories were shut down for weeks or months, and travel restrictions slowed collaborations among far-flung research groups. Organ procurement challenges led to a general shortage of human islets for biomedical research. Despite these temporary setbacks, the HIRN adapted to the times, and we remained focused on our core research mission.

Here are just a few of the HIRN's accomplishments during the period covered in this report:

- The launch of new career development programs was one of the most exciting changes in Years 5 and 6. These initiatives support up-and-coming scientists who have exceptional credentials and are committed

to building careers in islet/beta cell research. Bringing new investigators and emerging postdoctoral leaders into the HIRN helps us maintain a vibrant atmosphere of innovation and fresh perspectives.

- A key programmatic change was the addition of the Human Pancreas Analysis Program-Type 2 Diabetes (HPAP-T2D) in Year 6. HPAP-T2D synergizes with existing HPAP-T1D infrastructure for procurement and in-depth analysis of pancreata and related tissues from organ donors. HPAP-T2D expands the program's reach to donors with T2D and prediabetes.
- Three HIRN consortia took complementary approaches to modeling human islets and interactions between beta cells and the immune system that happen in early T1D. Scientists of the Consortium on Human Islet Biomimetics (CHIB) engineered microdevices that maintain the function and viability of human islets in the context of blood vessel networks and extracellular matrix. In the Consortium on Modeling Autoimmune Interactions (CMAI), teams worked to develop complex mouse models populated with human immune cells, beta cells and thymus cells, providing a living system for T1D research. Human Pancreas Analysis Consortium (HPAC) investigators refined and applied pancreas slice technology for high-resolution experiments on human islets that remain embedded in their native pancreatic milieu.

  All of these technologies, as detailed in this report, represent significant, groundbreaking advances from previous experimental systems that limited research to isolated islets or beta cells out of context from their physiologic environment.
- An HPAP-T1D team developed and released a new public, online resource called "Pancreatlas™." Funded in part by the HIRN, Pancreatlas is a game-changer for exploring data-rich images of pancreas tissue from nondiabetic and T1D organ donors.
- Researchers in the Consortium on Targeting and Regeneration (CTAR) pursued numerous strategies toward the goal of selectively targeting beta cells to deliver therapies that enhance beta cell protection, function or survival in T1D. CTAR teams made considerable progress toward harnessing small molecules, non-disease-causing viruses, RNA molecules and regulatory T cells (Tregs) as vehicles for beta cell targeting.

- In the Consortium on Beta Cell Death and Survival (CBDS) and the HPAC, research teams pioneered the use of sophisticated, multiplexed imaging technologies to visualize the composition of islet cells in extraordinary detail. Many of these high-resolution images were deposited in public databases, such as Pancreatlas or PANC-DB, and are available for study by the entire research community.

As we write this, the HIRN consortia continue to build on this progress toward our mission of understanding T1D pathogenesis and finding the keys to prevent or reverse it. Additional researchers have joined the consortia, stimulating new ideas and novel scientific approaches. And, as part of its ongoing communication with T1D stakeholders, the HIRN is publishing a series of review articles in *Molecular Metabolism*, featuring the network's efforts to solve fundamental issues in T1D research.

We are pleased to present this *HIRN Executive Summary Report, Years 5 and 6,* on behalf of the entire HIRN community of investigators, and we hope that all T1D stakeholders are encouraged by the scientific and technological achievements of each HIRN research team. Together, we look forward to a time when effectively preserving and replacing beta cell function is as routine as insulin therapy is today for people with T1D.

Sincerely,

The HIRN Trans-Network Committee (TNC) [1]
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[1] This list includes all investigators who served as TNC members during HIRN Years 5 and 6. Each consortium had one representative on the TNC per year.

THE HUMAN ISLET RESEARCH NETWORK

The mission of the Human Islet Research Network (HIRN) 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.*

THE DISCOVERY AND PURIFICATION OF INSULIN REVOLUTIONIZED DIABETES MEDICINE

One hundred years ago, researchers at the University of Toronto conducted experiments that produced one of the most important medical breakthroughs of the twentieth century. Scientists already knew that certain cells in the pancreas produce a substance, dubbed insulin, that helps keep blood glucose at healthy levels. They even knew that those insulin-producing "B" cells (now known as beta cells) are lost in people with type 1 diabetes (T1D). But no one had been able to isolate insulin in a pure form or show whether it could be used as a treatment for people with T1D, who rarely lived more than a few years past diagnosis.

In May 1921, working in the laboratory of physiology professor John Macleod, Frederick Banting, a surgeon, and Charles Best, a graduate student, began experimenting with a new strategy for extracting insulin from pancreata of dogs and cattle. By November 1921, the team had used their crude insulin preparations to successfully treat a dog with diabetes for 70 days. This groundbreaking achievement led them to collaborate with James Collip, a biochemist, who was able to isolate a more purified insulin preparation that they hoped would be safe for use in human diabetes patients.

In January 1922, the researchers performed their first clinical test, injecting cow insulin into Leonard Thompson, a 14-year-old boy with T1D. For more than two years, Thompson had been on an extremely strict "starvation" diet — the only treatment available for T1D at the time — but his body had nearly succumbed to the disease. That first insulin injection was only mildly effective, and Thompson had a reaction at the injection site. The team refined their insulin formulation even more and tried again 12 days later. This time, Thompson's blood and urinary glucose dropped to near-normal levels and his symptoms began improving almost immediately. Insulin was quickly tested on other diabetes patients, who also showed dramatic improvements over the next weeks and months.

The resounding success of those first insulin tests transformed diabetes medicine and offered new hope to all those affected by T1D. By the end of 1923, only two and a half years after Banting and Best started their research, Eli Lilly & Co. was manufacturing purified insulin for the widespread treatment of T1D. Banting and Macleod were awarded the 1923 Nobel Prize in Physiology or Medicine for the discovery of insulin (they split their prize with Best and Collip). And now, in 2022, millions of people around the world rely on insulin therapy to lead active, long lives with T1D.

INSULIN THERAPY IS TRANSFORMATIVE, BUT IT IS NOT A CURE FOR T1D

Of course, the discovery of insulin was not the end of the story for T1D. A century of research has given us many paradigm-shifting discoveries and technological developments that continue to improve our understanding of and ability to treat T1D. For example, in 1979, investigators at City of Hope, Arthur Riggs, Ph.D., the Samual Rahbar Distinguished Chair in Diabetes & Drug Discovery, Keiichi Itakura, Ph.D., and their colleagues made another revolutionary advance in diabetes medicine. They developed a synthetic form of human insulin for use in diabetes patients. Their formulation was the first biologic to receive approval from the U.S. Food and Drug Administration and eventually replaced the need to purify insulin from animal organs. Over time, numerous research teams around the world have contributed to improved insulin formulations, continuous glucose monitors, insulin pumps, islet transplantation, insights into genetic and environmental risk factors, knowledge of autoimmunity and the role of the immune system in beta cell destruction and identification of potential biomarkers for early diagnosis, among other exciting and transformative advances.

Yet, there is more to learn: Why does the immune system attack and kill beta cells to begin with? How do some beta cells survive in the autoimmune environment of a diabetic pancreas, sometimes for years after the clinical onset of T1D? Can beta cells be protected from autoimmune destruction

to prevent or reverse T1D? What roles do other pancreatic tissues or cells, such as blood vessels, neurons or exocrine cells, play in the development or progression of T1D? Can parts of the immune system be harnessed to prevent or stop autoimmunity before it can destroy the beta cells? What unique features of beta cells can be targeted to deliver protective or regenerative therapies? What other cells in the body could be engineered to mimic glucose-sensitive insulin secretion and replace beta cell function? What new technologies must be created or applied to accelerate diabetes research and the development of novel therapies?

HIRN ACCELERATES RESEARCH ON T1D

Established in 2014, the HIRN undertakes research to understand how and why beta cells are lost in T1D and to devise safe and effective methods for preserving or replacing beta cell function in people with T1D. The HIRN embraces key principles exemplified by the Canadian team's discovery of insulin. HIRN investigators pursue *innovative science and technology development*, foster an environment of *teamwork and multidisciplinary collaboration* and maintain a *steadfast focus on the ultimate goal of improving the health of those living with or at risk for T1D* through research.

The HIRN is organized into five consortia of research teams centered on common scientific goals:

- The Consortium on Beta Cell Death and Survival (CBDS) investigates mechanisms of beta cell stress or dysfunction, identifies biomarkers of the asymptomatic phase of T1D and develops strategies to stop beta cell destruction.
- The Consortium on Human Islet Biomimetics (CHIB) designs and builds microdevices that support functional human islets and related tissues for research.
- The Consortium on Modeling Autoimmune Interactions (CMAI) models basic aspects of human T1D immune system biology using novel *in vitro* and *in vivo* platforms.
- The Consortium on Targeting and Regeneration (CTAR) studies methods to increase or maintain functional beta cell mass in T1D by targeting islet plasticity or engineering protection of beta cells.

- The Human Pancreas Analysis Consortium (HPAC) and its subset, the Human Pancreas Analysis Program (HPAP), explore the physical and functional organization of the human pancreas environment and develop high value datasets from human pancreata for use by the entire research community.

The HIRN was composed of 33 cooperative and individual research grants in Year 5 and 38 grants in Year 6. More than 175 investigators from institutions across the United States and internationally participated in those grant projects. In addition, the HIRN and the National Institute of Diabetes and Digestive and Kidney Diseases (NIDDK) offered three new funding awards for training and career development of junior investigators embarking on careers in T1D research. Six early-career investigators of exceptional creativity received New Investigator grants in 2018. These scientists were embedded in the HIRN consortia for Years 5 and 6. In 2020, at the end of Year 6, four junior faculty members were named as Gateway Investigators for Collaborative T1D Research. These awardees were assigned to a relevant HIRN consortium and participated in network activities, starting in Year 7. Finally, in Year 6, the Emerging Leaders in T1D Research program to support promising postdoctoral fellows was launched; the first round of applications for this program were due in Year 7.

All HIRN operations are administered and managed by the Human Islet Research Enhancement Center (HIREC) in support of the network's mission and team science. The HIREC provides critical research coordination, facilitation and information technology infrastructure support for the five HIRN consortia, individually and collectively. The HIREC also coordinates communication and resource sharing between HIRN and the entire diabetes research community.

This Executive Summary report highlights examples of scientific progress and technology development made by HIRN investigators during Years 5 and 6, covering the period of October 1, 2018, through September 30, 2020.

SUMMARY OF SIGNIFICANT RESEARCH PROGRESS, YEARS 5 AND 6

This chapter describes some of the major type 1 diabetes (T1D) scientific themes addressed by the Human Islet Research Network (HIRN) consortia in Years 5 and 6. Subsequent chapters dive into more detail on specific research goals and progress made through the career development programs and by each team of HIRN investigators.

CONSORTIUM ON BETA CELL DEATH AND SURVIVAL (CBDS)

Pancreatic beta cells are the only cells in the body that produce the hormone insulin in response to rising levels of glucose (sugar) in the blood. Insulin helps glucose enter cells throughout the body, where it is used as a source of fuel. When beta cells are killed by a person's own immune system — a process known as autoimmunity — insulin production is lost. Glucose, now unable to get into the cells, builds up to dangerous levels in the bloodstream, and the clinical signs and symptoms of T1D arise. The autoimmune process may last for years in some people, and it is silent, meaning that without specialized blood tests there are no obvious warning signs that a person would notice before a critical number of beta cells are destroyed and T1D sets in.

CBDS investigators focus on understanding the biology of beta cells in the earliest stages of autoimmunity in the human pancreas. A key research topic is understanding whether beta cells and/or other cells of the pancreas experience some type of stress or dysfunction that attracts the immune system and triggers autoimmunity. Investigators also map biological pathways that determine whether beta cells are killed by or survive autoimmunity. Some of these studies may reveal biological markers ("biomarkers") associated with the silent phase of autoimmunity that could be harnessed for early detection of T1D. Ultimately, the results of CBDS research will help scientists devise ways to halt beta cell destruction early in the disease process to preserve insulin production and prevent T1D.

In Years 5 and 6, *CBDS research labs developed and applied multiple state-of-the-art imaging technologies designed to probe human pancreas cells in unprecedented molecular detail.* Among the findings, investigators found evidence that a defect in glucagon-producing alpha cells may be involved in T1D progression and disease severity. Another study demonstrated that some pancreatic islets in organ donors with recent-onset T1D have normal numbers of beta cells, while other islets have none. The difference seems to be related to interactions between beta cells and cells of the immune system. A third investigation used high-resolution imaging of lipids (fats) to uncover differences in the fat composition of individual islets that may affect the islets' level of resistance or susceptibility to autoimmunity. Many high-resolution images from CBDS teams have been added to public databases, such as Pancreatlas™ and the NIH Human BioMolecular Atlas Program (HuBMAP), where they can be accessed and further analyzed by the wider research community.

Throughout life, the DNA in our cells accumulates changes ("mutations") that we did not inherit from our parents. Chemical modifications that our cells make to DNA and RNA add yet another layer of noninherited genetic regulation. *A team of CBDS investigators conducted a pioneering investigation of noninherited mutations in the human pancreas* and found a high frequency of these mutations in a section of the human genome associated with the immune system and with T1D risk. Another group discovered a mechanism for RNA modification in beta cells that may be associated with their ability to withstand an immune attack. This pathway may be a novel therapeutic target for strategies to improve beta cell survival.

The autoimmune process cannot be directly imaged in the pancreas of a living person. Therefore, *CBDS teams explored potential biomarkers in the bloodstream that might be useful surrogates for monitoring the development and progression of beta cell autoimmunity*. Cell-free DNA (cfDNA) and a small piece of RNA called miR-204 are released by dying beta cells. Investigators made significant progress by measuring the levels of these two biomarkers under different conditions, such as in individuals with high risk for T1D and those with new-onset or long-term T1D. Validating a bloodborne biomarker of beta cell autoimmunity would fulfill a critical, unmet need for a noninvasive method to detect pre- and early T1D.

CBDS teams took multiple approaches to understanding the role that beta cells themselves play in autoimmunity. One investigator studied how beta cells respond to oxidative damage triggered by molecules released from

the immune system. Identifying small molecules that affect this pathway may point the way to drugs that improve beta cells' chances of surviving an autoimmune attack. Another team worked to detect changes in the protein complement of beta cells under healthy versus stressed conditions. Unusual proteins produced under stress might not be recognized by the body as "self" and could be one explanation for why the immune system targets beta cells.

CONSORTIUM ON HUMAN ISLET BIOMIMETICS (CHIB)

A major challenge in studying the development of T1D in people is that most of the important events involved in autoimmune destruction of beta cells happen during the silent period before the disease is diagnosed. For this reason, the T1D field has long relied on animal models, especially mice, to investigate early events in T1D autoimmunity. Mouse research has been and continues to be invaluable, but it is not a perfect substitute for understanding beta cell biology and autoimmunity in humans. Isolated human islets or beta cells have yielded key insights, but they too have significant limitations. Islets in the body exist in a rich microenvironment that is permanently disrupted during the isolation process to extract the islets. The islet vasculature (the network of blood vessels), nerves, extracellular matrix (ECM), most immune cells and interactions between islets and other parts of the pancreas are all lost during islet isolation. Purification of beta cells from islets leads to additional loss of connections that may exist between the beta cells and other islet cells (e.g., alpha cells).

Multidisciplinary CHIB research teams integrate knowledge of beta cell biology with such fields as microengineering, biofluidics and materials science to create innovative microdevices for research on all cells involved in T1D progression and development. These microdevices are "biomimetic," meaning that they mimic key aspects of the normal biologic environment of the pancreas and enable state-of-the-art T1D research on islets outside of the human body. In Year 6, new or reorganized CHIB research teams that include immunologists were funded to facilitate studies of immune cell-beta cell interactions within the microdevices.

The loss of vasculature during islet isolation rapidly degrades islet structure and function, leaving little time for researchers to study islets or beta cells

as they exist in the body. In Years 5 and 6, *two CHIB teams each designed three-dimensional (3D) microenvironments that replicate physiological islet structure.* After adding human islets, endothelial cells (the type of cell that lines blood vessels) and other support cells to their devices, both teams found that the endothelial cells assembled into networks of blood vessels that improved islet viability and function. Using their system, one team was able to directly observe immune cells killing the beta cells. A different research group showed that islets in their device retained a physiologically normal insulin secretion pattern when exposed to glucose. This finding demonstrated that islets in their microdevice work more like islets in the body than those that are grown in traditional laboratory conditions do. They also saw immune cells exit the blood vessels, infiltrate islets and attack beta cells. These observations represent a huge leap forward in scientists' ability to study T1D autoimmunity and test interventions to block it.

A CHIB group engineered a microdevice for long-term, 3D culture of human islets or beta cells derived from progenitor cells. Using unique microfluidic technologies, researchers can control the flow of liquids above and below the islets or beta cells in this device, enabling them to add different factors that closely replicate the islets' environment under various physiological conditions. This device opens up new possibilities for innovative research on islet physiology and immune cell-beta cell interactions. *Another microfluidic device developed by CHIB researchers provides a robust and sensitive method to evaluate beta cell function.* This device has many potential applications, including the assessment of strategies to generate beta cells from progenitor cells, measurement of islet function before transplantation and screening of small molecules that may aid beta cell function or survival.

Accurate, sensitive measurement of insulin secretion is essential for islet/beta cell research. *A CHIB team designed a new strategy for real-time, low-cost insulin detection* that can be used in conjunction with many of the microdevices developed in other CHIB labs. The successful development and dissemination of this technology, as well as the different human islet microdevices, to multiple labs demonstrate the important benefits of HIRN research for the entire T1D research community.

CONSORTIUM ON MODELING AUTOIMMUNE INTERACTIONS (CMAI)

In the 1970s, about 50 years after the first use of insulin to treat T1D, researchers discovered the autoimmune process that underlies the disease. As it patrols the body, the immune system recognizes cells or proteins as "self" (i.e., belonging to the body) or "nonself" (i.e., pathogens, such as viruses or bacteria). Normally, immune cells ignore the self cells, while killing the nonself invaders. In autoimmune diseases, immune cells mistake some self for nonself and begin to attack and destroy some of the body's cells. In people with or at risk for T1D, the immune system targets the insulin-producing beta cells as nonself. The autoimmune process of beta cell destruction is often gradual, and the body can tolerate some loss of insulin production before any diabetes symptoms appear. This slow timeline and the inability to directly observe the process in pancreatic islets in living people make it challenging to address key questions about T1D autoimmunity.

The primary focus for CMAI investigators is creating new, innovative models that accurately recreate the autoimmune process in pancreatic islets and facilitate research on its underlying causes and mechanisms. These models bring together human beta cells with human immune cells either *in vitro* or in engineered mouse models to shed light on how and why the immune system attacks beta cells. The models can be used to test potential solutions for preventing, blocking or reversing beta cell autoimmunity. CMAI teams have a particular interest in the "T cells" of the immune system. T cells mature in the thymus (a small gland located near the heart), where those that recognize self are normally eliminated by the thymus before they can be released to the rest of the body.

"Autoreactive" T cells recognize self, but have escaped the elimination process in the thymus and found their way into the circulation. CMAI teams isolated autoreactive T cells from pancreata of organ donors who had beta cell autoimmunity but had not yet developed T1D, or from those with diagnosed T1D. *One team built the largest collection of T cell lines to date, creating a unique and invaluable resource for research on islet-autoreactive T cells.* This collection has already generated important new information, such as the identity of multiple beta cell proteins that are targeted by autoreactive T cells. Studies of the T cells in an *in vitro* model allowed the investigators to monitor changes in beta cell gene expression when beta cells were exposed

to T cells that had been isolated from islets. Another CMAI team profiled similar islet-infiltrating T cells and found an unusual subset of T cells that do not recognize known beta cell protein targets. The investigators are following up on this observation as they model the earliest stages of T1D autoimmunity.

A subtype of T cells, known as regulatory T cells or Tregs, work in opposition to autoreactive T cells. Tregs can recognize targets on beta cells and infiltrate islets, but they do not kill the beta cells. *A CMAI team made exciting progress toward the development of Treg-based immunotherapy for T1D.* They designed Tregs that recognize a specific protein on the surface of beta cells and demonstrated that those Tregs could find and localize to transplanted human islets in a mouse model without destroying them.

CMAI teams advanced the use of human progenitor cells to model cell types associated with T1D autoimmunity, including beta cells, specialized immune cell types, endothelial (blood vessel) cells and cells of the thymus. One team made progress on developing a fully isogenic system (different cell types that all have identical genes) to study beta cell-immune cell interactions *in vitro*. This model supports innovative research on genetic regulation of T1D autoimmunity. Another team uncovered factors involved in development of thymus cells and used that knowledge to differentiate progenitor cells into a specific thymus cell type in the lab. This unique model of thymus biology accelerates research on the thymus' role in the development and release of autoreactive T cells that target beta cells.

CMAI teams created humanized mouse models for leading-edge research on the causes and mechanisms of T1D autoimmunity in a living system. The mouse models have fully functional human immune systems comprising all types of immune cells, including T cell lineages. One team extended the features of their model with beta cells derived from progenitor cells that were genetically identical to the cells used to generate the mice's humanized immune systems. They also generated human thymus cells from progenitor cells to facilitate research on autoreactive T cell development. A second team assembled a collection of human progenitor cells from individuals with T1D who had different genetic markers of T1D risk. The team differentiated those genetically diverse progenitor cells into beta cells, immune cell types and thymus cells for incorporation into their humanized

mouse model. The novel mouse models created by the CMAI teams are available to researchers across HIRN, and many have been shared with researchers in the broader community.

CONSORTIUM ON TARGETING AND REGENERATION (CTAR)

In T1D, the absence of insulin production in the pancreas causes glucose to accumulate in the blood to toxic levels, producing both short-term symptoms and long-term damage to the kidneys, nerves, heart, eyes and other organs. Since the first trial of insulin revolutionized T1D treatment in 1922, diabetes management has rapidly evolved. Now, 100 years later, people with T1D can check their blood glucose levels multiple times per day using a fingerstick method or they can use a continuous glucose monitor. Various insulin formulations are available that act rapidly or over long time periods, and some people use insulin pumps to more finely regulate their insulin doses. These major advances have helped people with T1D live long lives with fewer complications. Nonetheless, diabetes management remains arduous, especially for caregivers of children or others with T1D who cannot manage the disease on their own and for people with "brittle" diabetes that is hard to control. All people with T1D must learn to adjust their insulin doses in response to meals, exercise, illness and other common elements of daily life. In short, insulin therapy is essential for those living with T1D, but it is not a cure.

Curing T1D without the need for insulin therapy means either protecting or replacing beta cells that have been targeted or killed by the immune system. CTAR scientists explore both options with the ultimate goal of increasing or maintaining insulin-producing beta cell mass. A key approach is identifying and exploiting unique characteristics (e.g., proteins, molecular pathways) of beta cells to selectively deliver therapeutic or research payloads straight to the islets. Those payloads might come in the form of small molecule drugs, new genetic information or cellular therapies. The consortium also develops strategies to regenerate beta cell function, either within the islets or with cells from other tissues that can be reprogrammed to produce and release insulin in response to glucose.

Small molecules are low molecular weight chemicals that affect biological pathways; some small molecules are the basis of pharmaceutical drugs

and others serve as important research tools. *A CTAR team developed a unique "Beta Cell Informer Set" of small molecules that affect beta cell function, proliferation and survival.* The team established collaborations with several investigators to use this innovative resource, as well as other compound collections, in the search for small molecules that might benefit beta cell research or therapy. Other CTAR teams created novel small molecules to block a mechanism that triggers cell death when beta cells are under a specific type of stress and developed a rationally designed prodrug system to deliver a small molecule that stimulates beta cell proliferation. These research directions represent important steps toward targeted therapies for regenerating beta cell mass in people with T1D.

CTAR investigators took on the challenge of beta cell targeting with several innovative strategies to affect beta cell survival, proliferation or regeneration. One group designed adeno-associated virus vectors to carry new genes directly to beta cells. They demonstrated that the vectors can robustly infect islet cells (without causing disease) and drive strong expression of any gene in those cells. Another CTAR team studied the use of RNA molecules to precisely target and influence beta cell biology. The investigators started with mice that carried transplanted human islets and injected them with a small RNA molecule. The RNA treatment activated an immune-protective protein only in beta cells. *This CTAR study was a major advance in applying a nonviral approach to selective regulation of a beta cell gene in a living organism.* A third CTAR group explored the use of Tregs that were modified with a surface protein that causes the Tregs to selectively localize to beta cells. The investigators designed a new system for culturing human islets to facilitate research on their Tregs that have been engineered to deliver signals for beta cell protection, regeneration or immune modulation.

One option for regenerating beta cell function is to reprogram nonbeta cells so that they make insulin and release it appropriately in response to changing glucose levels. A CTAR team applied this approach to alpha cells — the subset of islet cells that make and release glucagon in response to low glucose levels. *The investigators reported that reprogrammed alpha cells secreted insulin in response to glucose, reversed diabetes in a mouse model and were less vulnerable to being killed by autoreactive T cells than beta cells.* Another CTAR team successfully reprogrammed progenitor

cells found in the lining of the stomach. Their cells also produced and released insulin and reversed diabetes in mice. The feasibility of this team's approach is supported by access to an abundant supply of progenitor cells in stomach tissue discarded during weight loss surgeries.

CTAR scientists investigate basic aspects of beta cell and islet biology with the goal of finding novel beta cell-specific targets for regeneration and other therapies. *A CTAR team discovered that residual beta cells from T1D pancreata had levels of a specific type of DNA modification that were more like those of neonatal beta cells than mature, adult cells*. Understanding the molecular pathways involved in the DNA modification may reveal new targets to improve beta cell health and mass. In a separate study, CTAR researchers characterized neurons associated with islets as a starting point for the development of neuromodulation treatments for T1D.

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

The human pancreas is central to diabetes pathogenesis, but it cannot be safely biopsied or routinely imaged in a living person. Scientists must use organs donated after death to study how and why beta cells and other parts of the human pancreas are affected in diabetes. Few individual laboratories have the resources necessary to identify and obtain rare pancreata and other tissues, such as lymph nodes, from donors who were at risk of or diagnosed with diabetes.

To fill this gap, the Human Pancreas Analysis Program (HPAP, a subset of the Human Pancreas Analysis Consortium [HPAC]), coordinates the identification, acquisition, processing and analysis of organs from individuals who had islet autoantibodies (preclinical T1D), T1D, prediabetes (pre-type 2 diabetes [T2D]) or T2D, along with age-matched, nondiabetic individuals. HPAC/HPAP investigators use these pancreata to address fundamental questions in human islet biology. HPAC/HPAP teams derive many high value datasets from donated human organs and upload them to a publicly accessible database for use by the entire diabetes research community. This process allows investigators to evaluate the data from diverse perspectives, maximizing the knowledge gained from each human pancreas.

In Years 5 and 6, HPAC scientists made groundbreaking advances in the development and use of pancreas slice technology for diabetes research. *An HPAC team developed and validated a method to maintain the viability and function of a large slice of a human pancreas for more than 10 days in a lab, enabling unprecedented research on human islets embedded in their natural pancreatic environment*. Multiple labs within and outside of the HIRN quickly adopted this state-of-the-art technology for their own work. One HPAC team used pancreas slice technology to discover several differences among pancreata of donors with T1D; their findings suggest the potential for multiple molecular pathways that can each lead to T1D. Another team used the technology to conduct a groundbreaking functional analysis of the islet blood vessel network in a nondiabetic pancreas. This work revealed a role for pericytes, specialized cells that wrap around small blood vessels, in regulating the blood flow through islets. Future studies will evaluate how pericyte function is affected before and after onset of diabetes.

Beta cells are not all alike. They all make and secrete insulin in response to glucose — but there are important structural and functional differences. *HPAC researchers applied advanced single-cell analysis technologies to investigate a range of differences among islet cells*. One team looked at how T1D changes cellular activities in the pancreas. They identified 13 distinct cell types in the human pancreas, including immune cells, and future studies of these cells may help clarify why the immune cells attack beta cells during T1D-related autoimmunity. Using an innovative combination of two single-cell techniques, another HPAC team linked functional problems to specific changes in gene expression in alpha and beta cells. These results laid a foundation for a new understanding of defects in insulin and glucagon secretion in people with diabetes.

HPAP's collection and analysis of donated human pancreata and other organs represent an exceptional resource that benefits the broad diabetes research enterprise. For each donated organ, HPAP core laboratories conduct standard tests and collect data on donor characteristics, islet function, immune assays and other measures. All data are deposited into PANC-DB, a public database that can be accessed by researchers around the world. An HPAP team also developed an online image database, named Pancreatlas™, that contains hundreds of images of human pancreata

generated by various state-of-the-art microscopy technologies. These images are extremely rich in data, but difficult to publish in standard venues. Pancreatlas, which was developed with funding from the HIRN and other sponsors, gives all researchers an unprecedented opportunity to explore novel research directions in pancreas biology, regardless of their individual ability to access rare human tissues or expensive imaging technology.

In addition to standard tests, HPAP teams applied their own technologies to donated pancreata and forged new directions for human diabetes research. *One HPAP team made a remarkable finding that challenges the longstanding paradigm in the diabetes field that T1D is a beta cell-specific disease*. They discovered that pancreata from donors with T1D were 45% smaller than those from age-matched, nondiabetic donors. A likely explanation for this result is a decrease in the size of the exocrine pancreas (the part that makes digestive enzymes), which was not known to be involved in T1D pathogenesis. Another HPAP team used a powerful imaging technology to study islet architecture; they observed that T1D donor islets were smaller and more disordered than nondiabetic islets.

Pancreata from T2D donors were acquired by the HPAP for the first time in this reporting period. HPAP investigators led a pioneering study of human T2D pancreata using CODEX, a new imaging modality that captures images of up to 40 different proteins in a 100 mm^2 area of tissue. Researchers can use CODEX to characterize proteins in the cells of diabetic versus nondiabetic islets in extraordinary detail. *An intriguing RNA sequencing study of T2D pancreata by an HPAP team showed that beta cells in people with T2D look more like juvenile beta cells than adult beta cells*; this analysis of T2D-related changes in gene expression patterns may reveal new targets for drug development.

PROMOTING INNOVATION, COMMUNICATION AND RESOURCE DEVELOPMENT

At the heart of the Human Islet Research Network (HIRN) is a commitment to advancing innovative science and technologies that lead to improved understanding of and better treatments for type 1 diabetes (T1D). This mission is enhanced by an environment that fosters robust communication and exchange of ideas among HIRN investigators and between the network and the broader research community. HIRN maximizes its impact on the T1D research community by developing and disseminating leading-edge research tools, technologies and resources that benefit all diabetes investigators.

The Human Islet Research Enhancement Center (HIREC), located at City of Hope, facilitates and coordinates all activities in support of the HIRN consortia and their team science (Figure 1), as described in this chapter. As of Year 6, HIREC consolidates all roles and responsibilities that were previously carried out by the HIRN Coordinating Center (CC) and Bioinformatics Center (BC) in Years 1 through 5.

The HIRN Trans-Network Committee (TNC) includes representatives from each consortium, the HIREC and NIDDK program staff. The TNC facilitates communication and collaboration among researchers across the network, advises on the organization of the HIRN Annual Investigator Meeting and confers on the use of the HIRN's Opportunity Pool funds.

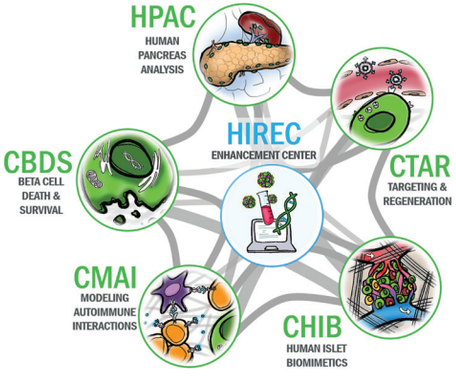


Figure 1. Overview of HIRN. *The HIRN structure allows scientists to focus on unifying themes and obstacles in T1D, while providing a cross-disciplinary collaborative environment for sharing, to advance discoveries and promote therapeutic approaches. The network includes the Human Pancreas and Analysis Consortium (HPAC), the Consortium on Targeting and Regeneration (CTAR), the Consortium on Human Islet Biomimetics (CHIB), the Consortium on Modeling Autoimmune Interactions (CMAI), the Consortium on Beta Cell Death and Survival (CBDS) and the Human Islet Research Network Enhancement Center (HIREC).*

HIRN FUNDING OPPORTUNITIES: A DYNAMIC RESOURCE FOR INNOVATIVE RESEARCH

The HIRN is intentionally designed to be an evolving community of islet/beta cell biology researchers that changes and adapts over time as science and technology advances. HIRN grant awards provide funding for a team of collaborating investigators or a single researcher for a period of two to five years. New initiatives are issued every year (contingent on the availability of funds) to solicit innovative hypotheses, original approaches and state-of-the-art technologies. In addition, the HIRN offers short-term funding opportunities for exploratory research in the form of Opportunity Pool Projects. Since Year 5, the HIRN and NIDDK have funded career development awards for newly independent investigators and senior postdoctoral fellows, who then become integrated into the relevant HIRN consortia.

New Collaborative HIRN Research Teams

In Year 5, the HIRN expanded with the addition of five new research teams:

- Four new teams joined the HPAC to conduct pioneering, high-resolution studies on the human islet tissue environment and its contribution to T1D pathogenesis. These research groups study several key issues in human islet biology: how progenitor cells located in pancreatic ducts can be harnessed to regenerate islet function; what events during the maturation of islets after birth affect those islets' susceptibility to inflammation and autoimmunity; understanding the basis of beta cell heterogeneity and how it is affected by the islet environment (e.g., other islet cells, blood vessels, nerves); and changes in islet cell DNA and RNA related to T1D and what those changes can tell us about the molecular pathways leading to disease.
- One investigator joined the CBDS to continue studies of a potential biomarker of T1D autoimmunity and a novel treatment strategy that were initiated in the CTAR.

In Year 6, the HIRN welcomed 11 new research teams:

- CTAR added four new teams to conduct research related to its goal of developing therapies for preserving or restoring beta cell function in individuals with T1D. The new projects explore: a gene therapy to reprogram alpha cells as a strategy to regenerate beta cell mass; an advanced, high-throughput screening platform to identify small molecules that impact beta cell function or mass; the unfolded protein response to cellular stress as a novel target for T1D therapy; and cell-based strategies to target beta cells and enhance their function, survival and regeneration.
- The CMAI welcomed one new and one renewal research team to work toward establishing experimental models of human T1D pathogenesis. Both teams aim to build humanized mouse models that incorporate the full repertoire of immune cells with human beta cells and thymus cells. These platforms will enable sophisticated studies of the molecular and cellular determinants of T1D-related autoimmunity.
- Three teams joined the CHIB for development of engineered platforms that support the long-term survival, maintenance and function of human islets. The new projects improve on microdevices previously designed by CHIB teams with a novel focus on probing interactions between human beta cells and cells of the immune system.
- Two research teams initiated the HPAP-T2D for the collection and detailed analyses of pancreata and other tissues from human organ donors with T2D or prediabetes. As in the HPAP-T1D, all data collected from these analyses are uploaded to a comprehensive, open access, searchable database (PANC-DB) for use by the entire diabetes research community.

Five new CBDS teams will participate in the HIRN as of Year 7. These teams will explore early disease processes in the human pancreas that contribute to T1D. Progress from these teams will be featured in future biannual Executive Summary reports.

Opportunity Pool Projects

In Years 2 through 4, each HIRN consortium had access to a pool of funds to support short-term, innovative projects related to the cause, prevention or cure of T1D. These Opportunity Pool Projects (OPPs) provided a mechanism for HIRN investigators to test out fresh ideas for research or technology development that, if successful, could open up new scientific directions. In

Years 5 and 6, 14 OPPs (Appendix 1) completed their work and submitted final progress reports. In addition, in Year 5, the CHIB used Opportunity Pool funds for an in-person investigator meeting to facilitate interaction and coordination of technology development within that consortium.

Beginning in Year 5, the Opportunity Pool funds transitioned to support of pilot projects for newly independent, junior investigators and postdoctoral fellows pursuing careers in islet/beta cell research, as detailed below.

The HIREC manages all aspects of the OPP process from receipt and peer review of applications to execution of subawards to awardee institutions and collection of progress reports.

Support of New and Emerging Investigators

Since Year 5, HIRN has offered new programs dedicated to the development of exceptional new investigators and trainees who are establishing careers in diabetes research (Appendix 2). The New Investigator Pilot Awards and Emerging Leaders in T1D Research programs are supported by HIRN Opportunity Pool funds, and applications are evaluated for scientific and technical merit by an external peer review panel. The New Investigator Gateway Awards for Collaborative T1D Research program is a National Institutes of Health (NIH)/National Institute of Diabetes and Digestive and Kidney Diseases (NIDDK)-led initiative that provides R03 grants to successful applicants; each Gateway awardee is affiliated with one of several NIDDK multi-investigator consortia/networks for T1D research — either the HIRN, Type 1 Diabetes TrialNet or The Environmental Determinants of Diabetes in the Young (TEDDY) study.

Details of these career programs, along with descriptions of awardees' research plans, are available in the chapter "Fostering New Talent in the Human Islet/Beta Cell Research Community."

COMMUNICATION AND COLLABORATION WITHIN THE NETWORK

The HIREC provides team science facilitation and organizational support for all network activities, including HIRN annual retreats, teleconferences and other meetings within the consortia.

Annual HIRN Investigator Meetings 2019 and 2020

HIRN investigators and invited stakeholders convene each year for the HIRN Annual Investigator Meeting. The structure and content of these conferences are planned by working groups composed of representatives from each HIRN consortium, the HIREC and NIH program staff (Appendix 3). HIREC staff handle all logistical needs from negotiating and executing vendor contracts to facilitating the submission and review of scientific abstracts. Each year, the Annual Meetings attract a diverse mix of attendees, including investigators, trainees, NIH staff, HIREC staff, scientific advisors and guests from other T1D-related organizations (e.g., the NIDDK Information Exchange Network [dkNET], JDRF, Network for Pancreatic Organ Donors with Diabetes [nPOD] and T1D Exchange). With ample time set aside for one-to-one and small group discussions, these meetings promote a spirit of open communication and collaboration among HIRN investigators with complementary skills and resources.

The HIRN Annual Investigator Meeting 2019 (Year 5) was held from April 28 to May 1 in Washington, D.C., with 232 registered attendees. The meeting featured 39 presentations, including updates on each consortium and introduction of new investigators. Other presenters reported on a range of innovative research directions underway in HIRN labs; for example: immune cell infiltration and targeting, the pacing and timing of beta cell function, miniaturization technologies in islet biology, islet genes and mechanisms, next-generation tools to understand islet biology and protecting islets in T1D. Eight break-out sessions gave participants an opportunity to explore focused topics, such as navigating Pancreatlas™, beta cell fragility in T1D, connections among endocrine cells and the Small Business Innovation Research (SBIR) process. Two poster sessions were held with a total of 95 research posters displayed.

The 2019 Annual Meeting marked the final year of a five-year supplemental grant from The Leona M. and Harry B. Helmsley Charitable Trust for sponsorship of the meetings. Additional financial support was provided through partnerships established between the Coordinating Center and six corporate sponsors. The aim of these partnerships was to broker mutual relationships and foster future interactions between HIRN scientists and the exhibitors. The companies paid vendor fees for the opportunity to serve as exhibitors at the meeting.

The HIRN Annual Investigator Meeting 2020 (Year 6) was held virtually from September 30 to October 2 with 375 registrants convening by teleconference. Despite being unable to gather in person, the HIREC deployed video conferencing technology to successfully and seamlessly simulate an in-person meeting experience, including oral presentations, discussion sessions, poster presentations and break-out, interactive discussions. Meeting attendees took advantage of the virtual format to engage in spirited discussions about scientific advances and future research directions for the network.

The 41 presentations included overviews from each consortium and the slate of new investigators, as well as the breadth of research being pursued by HIRN teams: lessons from gene expression studies, engineering and delivery in T1D, islet-immune interactions, mechanisms of beta cell death, development of technologies for islet research and the role of nonbeta cells in diabetes. Thirteen virtual break-out sessions encouraged discussion on hot-topic issues, such as career development, state-of-the-art imaging approaches, the islet niche, SARS-Co-V-2 and its relationship to diabetes, novel CHIB devices and the HPAP pancreas database, PANC-DB. Two interactive poster sessions were held with 43 virtual posters. The Planning Committee conducted a competitive review of abstracts submitted for the poster session and awarded HIRN 2020 Trainee Scholarships to nine graduate students and postdoctoral fellows.

Other Network Meetings

In addition to the annual investigator meetings, the HIREC facilitated virtual and in-person communications within each consortium and across the network.

In Year 5, 28 meetings were held for HIRN investigators, including scientific retreats and consortium-specific meetings. In addition to virtual meetings, the Coordinating Center organized the CHIB In-Person Investigator Meeting in December 2018 at Harvard University. This meeting was attended by 46 individuals representing each of the four CHIB grant teams, JDRF, NIH/NIDDK and Coordinating Center staff. CHIB provided 12 travel awards to trainees using the consortium's Opportunity Pool funds. Supplemental funding was provided by a JDRF meeting grant to Kevin Parker, Ph.D.

In Year 6, when travel was restricted by the COVID-19 pandemic, the HIREC helped organize and provided administrative support for more than 60 teleconferences for working groups, consortium-specific calls, the Trans-Network Committee and other meetings.

COORDINATION AND COMMUNICATION WITH EXTERNAL RESEARCH ORGANIZATIONS AND THE BROADER T1D COMMUNITY

The HIRN participates in an active community of T1D-related research organizations, patient groups and research funders. In addition, the network makes it a high priority to share news of HIRN activities, accomplishments and research progress with interested stakeholders, including the general public.

Networking With Other T1D Research Organizations

The HIREC serves an important role in establishing ongoing interactions with both NIH and non-NIH supported research organizations to share information and facilitate collaborations with the common goal of accelerating T1D research.

- The HIRN has a relationship with the Integrated Islet Distribution Program (IIDP), a critical partner that supplies human islets to HIRN investigators. The IIDP and HIRN HIREC are both NIH-funded coordinating centers with Joyce Niland, Ph.D., the Estelle & Edward Alexander Chair in Information Sciences, as principal investigator. Staff from the two organizations work collaboratively to take advantage of synergies across the programs.
- The HIRN maintains an active, close relationship with the JDRF nPOD, which is instrumental in obtaining pancreatic tissue from organ donors with T1D. John Kaddis, Ph.D., co-principal investigator of the HIREC, also serves as principal investigator for the nPOD data management core. In HIRN Years 1 through 5, nPOD representatives attended the HIRN Annual Investigator Meetings and manned an information table to introduce HIRN researchers to their project.
- The HIRN works with the NIDDK Information Exchange Network (dkNET) to exchange and register scientific resources, thus enabling HIRN scientists to share bioreagents, technologies, documents and datasets with the broader research community. In Year 6, Kaddis promoted HIRN research to dkNET investigators through their online webinar series.

- In Year 5, the Coordinating Center assisted the staff of the Levine-Riggs Diabetes Research Symposium to identify and implement an online meeting mobile app to facilitate their conference.
- The HIREC has explored opportunities for interactions with the T1D Exchange, an organization that provides T1D education, research, resources and services with The Leona M. and Harry B. Helmsley Charitable Trust, a major funder of T1D research.

The HIRN Website

The HIREC develops and manages the HIRN website (https://hirnetwork.org), the online face of the HIRN community. The website regularly updates public information related to each consortium, profiles of each grant team, working groups, publications, research resources, new funding opportunities and job postings in academia and research funding organizations. A secure section of the website allows HIRN investigators to access meeting agendas, minutes and other internal documents. By the end of Year 5, the HIREC had created 292 secure accounts for investigators, trainees, collaborators, External Scientific Panel members, NIH program officers and HIRN staff.

Public Outreach

The HIREC actively utilizes social media accounts on Twitter, Facebook and LinkedIn to share HIRN-related news with the scientific community at large and the general public. The accounts post regular updates on HIRN publications and events. Since Year 3, the HIREC has created a "Spotlight" series of social media posts that provide detailed narratives of individual HIRN investigators and their research objectives.

In Year 6, a new webinar series was initiated to promote and encourage the use of HIRN resources and scientists; this forum was open to all diabetes researchers both within and outside of the HIRN. The first webinar, held in August 2020, covered the topic "The Development and Use of Adeno-Associated Virus (AAV) Vectors" and was presented by CTAR investigators Hiroyuki Nakai, M.D., Ph.D., and Mark Kay, M.D., Ph.D. All webinar topics and links to archived videos are available at https://hirnetwork.org/webinar_series.

The HIREC develops and releases monthly newsletters with the latest information on HIRN projects, members and research findings, along with general notices, such as job postings, webinar invitations and meeting announcements (https://hirnetwork.org/news-events/newsletters). The newsletters are distributed via email to all HIRN investigators, trainees and staff and are posted on the HIRN website and social media sites for those interested in learning more about HIRN accomplishments. Members of the public can subscribe to receive the newsletter automatically.

DATA, MATERIAL AND RESOURCE SHARING ACROSS THE HIRN AND WITH THE BROADER RESEARCH COMMUNITY

An important purpose of the HIRN is to create datasets, technologies and other innovative resources that expedite T1D research across the network and throughout the scientific community.

Research Publications

To highlight the scientific findings and accomplishments of HIRN investigators, the HIREC maintains a centralized listing of all manuscripts that result from HIRN-supported research (https://hirnetwork.org/all-hirn-publications). New publications are identified via self-reports from authors, NIH notifications or quarterly publication searches, then catalogued in the HIRN publications database. Publications are coded with a unique GrantID and consortium affiliation to ensure real-time display of publications from each grant, consortium and the entire network. HIRN investigators are encouraged to share advance copies of their manuscripts with other members of the network. These prepublications are posted on the secure section of the HIRN website and serve to foster an environment of trust and collaboration within the network.

To help inform HIRN investigators and other members of the T1D scientific community of other, non-HIRN related scientific advances, the HIREC maintains a "Publications of Interest" page on its website (https://hirnetwork.org/publicationsofinterest).

The HIRN Resource Browser

The HIREC oversees a web-based catalog of resources used and/or generated by the network, including bioreagents (antibodies, constructs, differentiated cells, model organisms, other lines, primary cells, stem cells), datasets (epigenomics,

genomics, metabolomics, proteomics, transcriptomics), documents (protocols) and technologies (assays, code/pipeline, devices/equipment, software/database). Originally, Coordinating Center staff worked proactively with HIRN research teams to collect, curate, load and display this information. As of Year 5, resource information is also identified and entered by the HIRN Data Curator. The HIRN Resource Browser is available at https://resourcebrowser.hirnetwork.org.

In Year 6, the browser infrastructure was updated based on input from a focus group of HIRN investigators. Refinements of the data collection fields in the browser were made to ensure that the platform best captures and adapts to new and existing resources. Multiple enhancements were also completed, including development of google analytics to allow storage and reporting of view/unique visitor count metrics per resource. This approach is the beginning of an effort to provide transparency on the interest level for and overall impact level of particular resources. More than 550 new resources were identified, curated and added to the browser in Year 6, for a total nearing 1,000 resources.

The HIREC has found that investigators may be unfamiliar with the Findable, Accessible, Interoperable and Reusable (FAIR) principles, as well as emerging NIH-required standards for rigor and reproducibility of data, resources and reporting thereof. To assist researchers with contemporary data sharing practices and to facilitate data reporting, a series of infographic-like documents were created to illustrate sharing of various resources. These documents were accompanied by a new Help/FAQ section in the HIRN Resource Browser site. In addition, vignettes were developed that illustrate how to programmatically interact with the browser and submit or retrieve resources. Besides simple download/upload, these vignettes demonstrated how to custom sort/filter/analyze data in a manner that is more relevant to the end user, as well as facilitate comparisons of resources to published data (preliminary). These approaches help to ensure the quality of HIRN data inputs and outputs.

New Data Sharing Initiatives

In Year 5, a new effort was initiated to combine several HIRN datasets with data from other T1D networks (e.g., nPOD). Development of this tool, named "DataView," was explored as an approach to integrating donor-connected heterogeneous datasets and making them available for exploration and new

applications. As of Year 6, the DataView application had integrated 15 HIRN datasets and was set to be released in the next grant year. Also in Year 6, the HPAP began a collaboration with the IIDP to allow investigators searching one database to seamlessly link to the other for comparison of islet isolations and tissues.

Standards to Ensure Robust and Unbiased Results

The HIRN takes steps to enhance the rigor and reproducibility of the public reporting and sharing of HIRN resources and scientific research findings. The following actions have been taken:

- The use of data sharing guidelines established by Force11 and the Joint Declaration of Data Citation Principles to inform data collection, storage and sharing efforts. Steps include the assignment of unique identifiers for all HIRN resources using resource-specific nomenclature developed by authoritative communities (e.g., antibody registry for antibodies) and the identification and development of different data access and output formats.
- Implementation of metadata standards (e.g., https://fairsharing.org) and relevant ontologies (e.g., http://bioportal.bioontology.org) for nearly all resource types catalogued by the HIREC
- Registration of HIRN resources with available authoritative public repositories (and through collaboration with dkNET), such as http://antibodyregistry.org and https://web.expasy.org/cellosaurus
- Maintenance of HIRN IT infrastructure backups, including HIRN servers, data, source code, tools and resource data

NETWORK-WIDE INVESTIGATORS AND PERSONNEL

Human Islet Research Enhancement Center Investigators and Other Personnel, Years 5 and 6

Joyce Niland, Ph.D., Co-Principal Investigator, *City of Hope*
John Kaddis, Ph.D., Co-Principal Investigator, *City of Hope*
Kristin Abraham, Ph.D., Project Scientist, *National Institute of Diabetes and Digestive and Kidney Diseases*
Thomas Eggerman, M.D., Ph.D., Project Officer, *National Institute of Diabetes and Digestive and Kidney Diseases*
Layla Rouse, M.S., Program Manager, *City of Hope*

Nelly Berger, Project Assistant, *City of Hope*
David Ko, Senior Technical Lead, *City of Hope*
Anh Nguyet Vu, Scientific Data Curator, *City of Hope*

Trans-Network Committee (TNC) Members, Years 5 and 6 [2]

Bridget Wagner, Ph.D., TNC Chair and CTAR Representative
Kristin Abraham, Ph.D., NIDDK Program Staff
Ashu Agarwal, Ph.D., CHIB Representative
Olivier Blondel, Ph.D., NIDDK Program Staff
Todd Brusko, Ph.D., CMAI Representative
John Kaddis, Ph.D., HIREC Representative
Klaus Kaestner, Ph.D., HPAC Representative
Joyce Niland, Ph.D., HIREC Representative
Audrey Parent, Ph.D., CMAI Representative
Layla Rouse, M.S., Program Manager
Sheryl Sato, Ph.D., NIDDK Program Staff
Anath Shalev, M.D., CBDS Representative
Cherie Stabler, Ph.D., CHIB Representative
Doris Stoffers, M.D., Ph.D., HPAC Representative
Lori Sussel, Ph.D., CBDS Representative

[2]This list includes all investigators who served as TNC members during HIRN Years 5 and 6. Each consortium had one representative on the TNC per year.

FOSTERING NEW TALENT IN THE HUMAN ISLET/BETA CELL RESEARCH COMMUNITY

A key goal for the Human Islet Resource Network (HIRN) is to promote a vibrant, diverse and interactive type 1 diabetes (T1D) research community. To advance this goal, the HIRN and NIDDK developed three career development initiatives aimed at fostering new research talent and supporting early-stage scientists at critical moments of transition to successful research careers in T1D research.

- *New Investigator Pilot Awards* and *New Investigator Gateway Awards for Collaborative T1D Research* focused on newly independent investigators with funding for research projects that could form the basis of competitive first R01 applications to the NIH. New Investigator Pilot Awards were funded in Year 5. The first New Investigator Gateway Awards were made at the end of Year 6, with the awardees beginning their work at the start of Year 7.
- The *Emerging Leaders in T1D Research* initiative targeted experienced postdoctoral fellows who demonstrated a commitment to seeking an independent faculty position in T1D research. The RFA for this program was released in Year 6, with the first awards made in Year 7.

Each New Investigator, Gateway Investigator and Emerging Leader was assigned to a specific HIRN consortium and invited to participate in all activities, including consortium-specific meetings and the HIRN Annual Investigator Meeting. Being an active member of the HIRN consortia offered each career development awardee opportunities to expand their knowledge of T1D research, network with colleagues across the diabetes field and establish unique and potentially long-lasting collaborations that will propel their careers in T1D research.

In addition to interactions within their assigned consortia, the new investigators and trainees meet together monthly to discuss their work, including advances, challenges and obstacles they have faced, and to develop collaborations with each other. Beginning in Year 7, the group will have the opportunity to nominate one individual to serve on and be their voice in the HIRN TNC. This information provides a valuable chance for an up-and-coming investigator to participate in a large network governing body.

NEW INVESTIGATOR PILOT AWARDS

New Investigator Pilot Awards fostered bold and highly innovative research approaches to biological problems under current investigation in the HIRN. The awards funded a small number of early career investigators of exceptional creativity to explore the feasibility of new concepts in support of an eventual R01 application to the NIH. Based on a competitive application process, six New Investigators were selected in 2018. This section describes hypotheses and research plans for each award recipient; examples of current scientific progress made under these awards are available in the individual consortium chapters of this report.

Joana Almaca, Ph.D., University of Miami, Human Pancreas Analysis Consortium

Changes in Human Islet Microvasculature During Type 1 Diabetes

Pancreatic islets are embedded in a network of microvessels — small blood vessels or capillaries — made up of a thin layer of endothelial cells covered by pericytes. While much is known about islet endothelial cells, islet pericytes are understudied and their potential contributions to T1D are unknown. Joana Almaca, Ph.D., hypothesizes that abnormal pericyte coverage of islet capillaries causes dysfunction of the microvessels and contributes to T1D development. Her studies on the function and structure of human islet capillaries in nondiabetic and T1D pancreas donors may reveal new insights into how blood vessels can impact beta cell performance and may challenge the current understanding of T1D pathogenesis.

Sangeeta Dhawan, Ph.D., City of Hope, Consortium on Targeting and Regeneration

Targeting DNA Hydroxymethylation to Promote Human Beta Cell Function

Beta cells begin to secrete insulin in response to changes in glucose levels only at birth. Sangeeta Dhawan, Ph.D., previously found that two small chemical modifications of DNA in beta cells are critical for initiating this process and that perturbing these modifications leads to loss of beta cell function. She proposes to define a role for DNA modification in beta cell physiology, investigate whether and how this process is impaired in T1D and strategize ways to target this pathway to improve beta cell function. The results of Dhawan's research may suggest better approaches to generate replacement beta cells for diabetes therapy, point to new targets for drug development and reveal key steps in T1D pathogenesis.

Dhawan's presentation at the 81st American Diabetes Association (ADA) Scientific Sessions "Epigenetic Regulation of Functional Beta Cell Mass by Cohesin Smc3 (Want et al.)" was chosen as a President's Select Abstract.

Abdelfattah El Ouaamari, Ph.D., Rutgers University, Consortium on Targeting and Regeneration

Neuromodulation for Type 1 Diabetes: Harnessing Sensory Innervation to Promote Regeneration and Function of Insulin-Producing Cells

Neuromodulation, or alteration of nerve activity, is an emerging field in biomedicine for treatment of some chronic conditions. Abdelfattah El Ouaamari, Ph.D., is identifying and characterizing neurons that project into the vicinity of insulin-producing beta cells in pancreatic islets using a variety of technologies, such as trans-synaptic tracing, RNA sequencing and immunofluorescence imaging. This research project represents a first step in exploring the potential of neuromodulation to restore beta cell numbers and/or function. In the long term, El Ouaamari aims to identify neuronal signals that can be leveraged to prevent, delay or treat diabetes.

Eddie James, Ph.D., Benaroya Research Institute, Consortium on Modeling Autoimmune Interactions

HLA Multimer-Based Characterization of Islet-Resident CD4+ T Cells that Target Beta Cell Epitopes and Neo-epitopes

Eddie James, Ph.D., uses several technologies to robustly characterize T cells isolated from the pancreatic islets of autoantibody-positive organ donors who had not yet developed T1D. He anticipates finding two distinct populations of T cells in the islets: autoreactive T cells that promote inflammation and autoimmune destruction of the beta cells and regulatory T cells that counteract autoimmunity. Confirming the presence of either population would be informative. James also plans to define beta cell proteins that are recognized by the islet-infiltrating T cells. He is particularly interested in detecting T cells that recognize modified proteins, which may provide clues to a role for these cells in promoting or regulating beta cell autoimmunity.

Amelia Linnemann, Ph.D., Indiana University, Consortium on Beta Cell Death and Survival

Real-Time in Vivo Analysis of Islet Redox Dynamics

Many factors that contribute to beta cell death in T1D also lead to accumulation of reactive oxygen species (ROS) — unstable molecules containing oxygen that can trigger cellular damage and death. Some beta cells survive this process by initiating an antioxidant response. Amelia Linnemann, Ph.D., proposes that differences among individual islets in their ability to mitigate excessive ROS conditions contribute to beta cell failure and T1D development. To test this concept, Linnemann is developing a new research platform to study antioxidant mechanisms and response to drugs targeting this pathway in human islets. Her research will improve our understanding of human islet adaptation to ROS accumulation *in vivo* and reveal whether small molecule drugs can rescue deficiencies in this process.

Linnemann received a HIRN Gateway Investigator award in 2020.

Holger Russ, Ph.D., University of Colorado Denver, Consortium on Beta Cell Death and Survival

Elucidating the Human Beta Cell Translatome in Health and Disease

One theory for the origin of T1D pathogenesis is that some beta cells produce incorrect or unusual proteins that break down into peptides (small protein pieces) that are not recognized as "self" by the immune system. These "diabetogenic" peptides trigger the autoimmune attack leading to beta cell death. Holger Russ, Ph.D., is exploring this hypothesis by comprehensively defining the beta cell translatome — the complete population of mRNA molecules that are being actively translated into proteins under typical versus stressed conditions. His studies will help define the mechanisms the beta cell uses to produce abnormal peptides and could revolutionize our knowledge of T1D initiation and progression.

NEW INVESTIGATOR GATEWAY AWARDS FOR COLLABORATIVE T1D RESEARCH

Gateway Awards for T1D Research support new and early-stage investigators who are interested in pursuing careers in T1D. The program supports high-quality, innovative and significant research by junior faculty engaged in basic, translational or clinical research that will firmly establish the awardees in a T1D-oriented career path and set the stage for competitive first R01 research grant submissions to the NIH. Four Gateway Investigators were chosen at the end of Year 6, and they began their research in Year 7. Additional Gateway Investigator awards were made later in Year 7, and the initiative will be repeated in the 2022 to 2023 funding cycle.

Rafael Arrojo e Drigo, Ph.D., Vanderbilt University, Consortium on Beta Cell Death and Survival

Mapping the Association of Beta Cell Longevity and Senescence in T1D

Rafael Arrojo e Drigo, Ph.D., studies rare beta cells that persist in the pancreata of individuals with T1D, sometimes years or even decades after the onset of clinical diabetes symptoms. No one knows how these cells survive for so long in the harsh T1D environment. Arrojo e Drigo uses high-resolution transcriptional sequencing and imaging technologies to investigate the longevity and molecular signatures of beta cells in pancreata from humans and mice with T1D. Understanding how these long-lived cells maintain their function and health, despite the ongoing autoimmune process, may lead to new methods to promote beta cell survival in individuals with T1D.

Arrojo e Drigo recently received two grant awards: a Pilot and Feasibility Award from the Vanderbilt Diabetes Center and a dkNET New Investigator Pilot Program in Bioinformatics grant.

Jing Hughes, M.D., Ph.D., Washington University School of Medicine in St. Louis, Human Pancreas Analysis Consortium

Primary Cilia in Human T1D Pancreas

The primary cilium is a hair-like structure that sticks out from the surface of a cell into the surrounding environment. Primary cilia on beta cells are involved in communication with neighboring islet cells and other key beta cell functions, such as insulin secretion. Jing Hughes, M.D., Ph.D., studies the role of primary cilia in T1D. She uses high-resolution microscopy on T1D versus nondiabetic human pancreas tissue to look for differences in the distribution and structure of primary cilia on beta cells. Little is known about how primary cilia contribute to T1D, so this project has the potential to open up new avenues of beta cell research.

Alok Joglekar, Ph.D., University of Pittsburgh, Consortium on Modeling Autoimmune Interactions

Identification of the Cognate Epitopes of Autoreactive T Cells in T1D

An open question in T1D research is which islet proteins (or other molecules) interact with T cells to trigger the autoimmune process that destroys the beta cells. Alok Joglekar, Ph.D., is pioneering a new approach to the discovery of T cell targets, called "Signaling and Antigen-Presenting Bifunctional Receptors (SABRs)." Joglekar is creating a library of beta cell-specific potential targets to determine which of those are recognized by T cells that have infiltrated islets. His work will improve our understanding of the early stages of T1D autoimmunity and may point to new therapeutic approaches to block the T cell attack on islets that leads to T1D.

Amelia Linnemann, Ph.D., Indiana University, Consortium on Beta Cell Death and Survival

Functional and Molecular Characterization of the Human Islet Interferon Alpha Response

Interferon (IFN)-alpha is a signaling protein that participates in the immune system's response to viral infection. Multiple observations from people with genetic risk markers for T1D and from islets obtained from living donors with T1D suggest a critical role for IFN-alpha during the early stages of T1D pathogenesis. Amelia Linnemann, Ph.D., a 2018 HIRN New Investigator Award Recipient, is continuing her research on the role of IFN-alpha in beta cell-immune system interactions that lead to T1D. She expects to define a unique molecular signature that predisposes some beta cells to initiating cell death after IFN-alpha exposure and reactive oxygen species accumulation. Her long-term goal is to identify new targets for drugs that can prevent these early events in T1D development.

In March 2021, Linnemann received her first NIH/NIDDK R01 grant "Autophagy/Antioxidant Response Coupling in Pancreatic Beta Cell Homeostasis Regulation."

HIRN EMERGING LEADERS IN T1D RESEARCH

Emerging Leader awards support experienced senior postdoctoral fellows who intend to establish independent research careers in scientific areas of interest to the HIRN. These awards provide funding and protected time for the fellows to pursue studies that will aid their transition to independence.

All Emerging Leaders pursue research on key issues in the T1D field in the context of career development plans that include mentoring by established investigators, as well as participation in HIRN activities. An RFA was released during Year 6 to solicit applications for this program. Five Emerging Leader award recipients joined the HIRN in Year 7; descriptions of their research plans will be presented in future Executive Summary reports.

CONSORTIUM ON BETA CELL DEATH AND SURVIVAL

Researchers in the Consortium on Beta Cell Death and Survival (CBDS) use human pancreatic tissues to *discover mechanisms of cellular stress or dysfunction* that may contribute to the development of autoimmunity in at-risk individuals, to *identify specific biomarkers of the asymptomatic phase* of type 1 diabetes (T1D) and to develop innovative strategies to *stop beta cell destruction* early in the disease process.

DEVELOPMENT AND APPLICATION OF ADVANCED IMAGING TECHNOLOGIES FOR T1D RESEARCH

Chris Wright, D.Phil., led a team of investigators at Vanderbilt University and the California Institute of Technology that made multiple significant advances in microscopic imaging of pancreatic tissue from human organ donors. For example, team members at Caltech developed a computational pipeline for quantitative understanding of three-dimensional (3D) image datasets. This allowed automated identification of individual islets in large tissue volumes, as well as global characterization of islets (e.g., size, distribution) and quantification of specific cell types within the islets. They also adapted a "neutube" technique to semi-automatically trace and visualize nerve fibers within the human pancreas. Vanderbilt team members used an advanced fluorescent imaging technology, CODEX, to uncover a new molecular mechanism for impaired glucagon secretion by alpha cells in T1D pancreata. Their findings suggest that the disordered response to hypoglycemia (low blood sugar) experienced by many people with T1D may be caused by an intrinsic alpha cell defect — implying that alpha cells, not just beta cells, are central to T1D progression and disease severity. The team's work on developing, optimizing and applying advanced imaging technologies for analysis of the human pancreas maximizes the knowledge that can be gained from every rare autoantibody-positive and T1D organ donor. Much of their data are available to the research community through Pancreatlas™, a curated, accessible and searchable database under development by an HPAP-T1D team.

Mark Atkinson, Ph.D., University of Florida, and his colleagues at the Universities of Geneva and Zurich pioneered a new technology known as Imaging Mass Cytometry (IMC) to perform highly multiplexed imaging of the human pancreas. This technology offers an unprecedented opportunity to identify precise cell subsets and their two-dimensional (2D) interactions in the pancreas. The team validated three panels of 40 markers each that allowed them to localize 120 different proteins in islet or immune cells; in addition, they could image up to 20 mRNA markers simultaneously in these cells. Using this technology, the team probed the dynamics of how beta cells and T cells of the immune system interact during T1D development. They showed that in pancreata from donors with recent-onset T1D, some islets have normal beta cell numbers, while other islets have no remaining beta cells. The difference between these types of islets correlated with infiltration of islets by T cells and with the frequency of beta cell-T cell interactions. Moreover, using pseudotime analysis of islet expression profiles, they defined three pseudostages with progression from pseudostage 1 to 2 characterized by downregulation of beta cell-specific markers, while beta cell death drove progression from pseudostage 2 to 3.

The team also developed new computer algorithms known as the Histology Topography Cytometry Analysis toolbox (histoCAT) to visualize and analyze highly multiplexed 2D IMC images (Figure 2). They plan to further explore this phenomenon by investigating how islet infiltration evolves before T1D diagnosis. Additionally, they have developed histoCAT-3D, which can be used to compile 2D IMC images into 3D renderings of the human pancreas with cell-level resolution. This effort set the stage for the group's entry into the NIH Human BioMolecular Atlas Program (HuBMAP) where they are generating 3D cellular maps of the human lymphatic system (thymus, spleen and lymph nodes). Finally, in addition to their work with IMC, the team established an open-access, online repository for large-scale electron microscopy images collected from human pancreas tissue. Their library of images represents a valuable resource for the T1D research community that has already uncovered new features of T1D pancreas nano-anatomy.

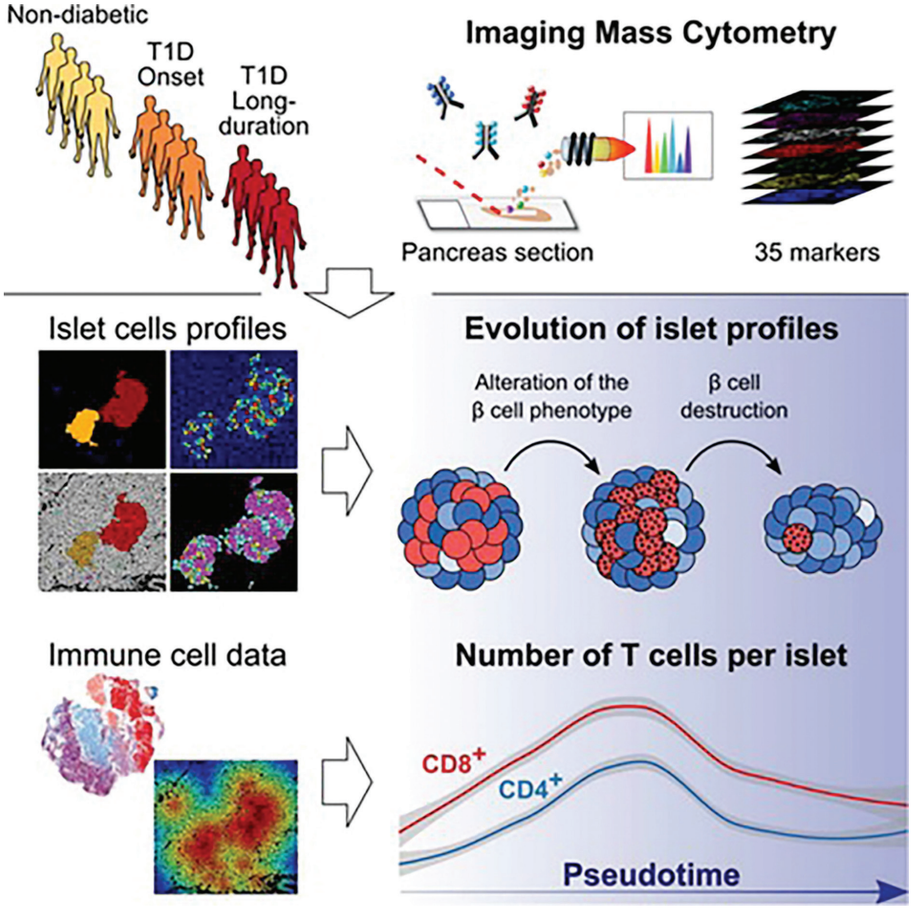
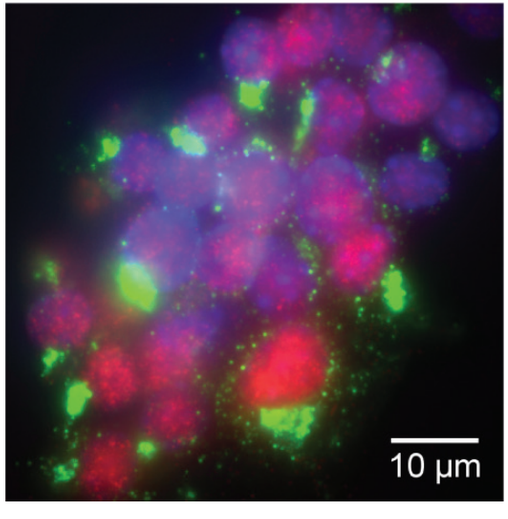


Figure 2. Imaging Mass Cytometry (IMC) in Human Pancreas. *IMC, which involves laser ablation to detect isotope tagged antibodies, was utilized to visualize 35 protein markers with single-cell and two-dimensional (2D) spatial resolution in formalin-fixed paraffin-embedded (FFPE) human pancreas tissue sections from eight donors with T1D, as well as four control donors without diabetes. Islet and immune cell identities were assigned using lineage-defining markers. The evolution of islet and immune cell profiles were then evaluated using pseudotime analysis. (Photo credit: Graphical Abstract from Damond et al., Cell Metabolism, 2019 Mar 5; 29(3): 755-768.e5).*

Islets are heterogeneous in composition, function and response to autoimmune attack. A central goal of the CBDS is the development of technologies that facilitate analysis of single islets or cells to shed light on the molecular reasons for this heterogeneity. Charles Ansong, Ph.D., and his colleagues at the Pacific Northwest National Laboratory and University of Colorado Denver created new techniques that combine high-resolution RNA localization with protein and histone modification localization within individual islet cells. In one application of their methods, the team co-localized a long noncoding RNA (lncRNA) called "Paupar" (Pax6 Upstream Antisense RNA) to the nuclei of glucagon-expressing alpha cells (Figure 3). They discovered that Paupar kicks off a molecular pathway that activates all alpha cell-specific genes. In other ongoing studies, the team adapted and improved nano-DESI technology for high-resolution imaging of lipids (fats) and other molecules to examine heterogeneity in mouse and human islets. They demonstrated that individual islets within a single pancreas have different lipid complements. Their next step will be applying this technology to islets from T1D and nondiabetic organ donors to determine whether specific lipid profiles are associated with different levels of susceptibility or resistance to autoimmune attack.

Figure 3. Single-Cell Technologies for Co-Localization of RNA, Protein, and DNA. *Single molecule RNA fluorescent in situ hybridization (smFISH) images in the alphaTC cell line showing Paupar lncRNA (red) co-localized with antibody staining for glucagon (green) and DAPI staining of nuclei (blue). Scale bar indicates 10 µm. (Photo credit: Dr. Lori Sussel)*



NON-INHERITED MODIFICATION OF DNA AND RNA IN BETA CELL FUNCTION AND SURVIVAL

Somatic mutations are non-inherited changes to the DNA in any cell of the body other than reproductive cells (sperm or ova). Klaus Kaestner, Ph.D., and his colleagues at the University of Pennsylvania and Hebrew University of Jerusalem analyzed the accumulation of somatic mutations in different areas of the human pancreas to determine whether changes in relevant genes contribute to loss of beta cell function or to the autoimmune response that initiates T1D. After looking at 10 T1D and 10 nondiabetic pancreata obtained from the HPAP, the team found a high frequency of mutations in the HLA locus of both T1D and nondiabetic donors (Figure 4). The HLA locus is a stretch of DNA containing numerous genes involved in the immune system that have been implicated in T1D risk. The team hypothesized that somatic mutations in HLA genes may change the way beta cells interact with T cells of the immune system, causing an inappropriate T cell response and, eventually, T1D. Their observations could explain why some people develop T1D despite low inherited (genetic) risk. This study was a pioneer in the analysis of somatic mutations in the human pancreas.

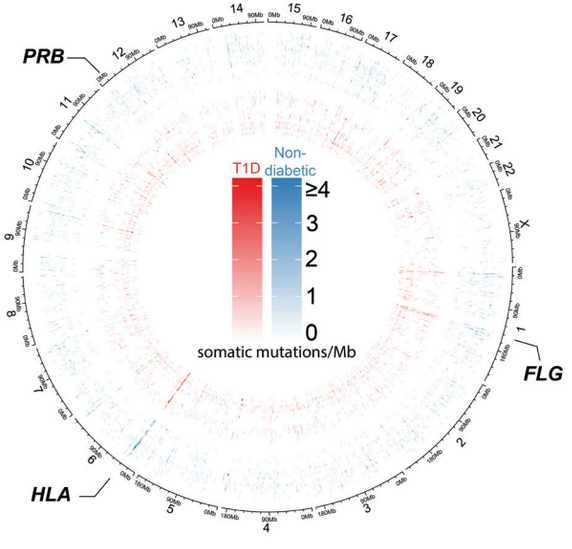


Figure 4. Somatic Mutation Load in the Pancreas of T1D and Nondiabetic Organ Donors.
The genome is displayed by chromosomes on the outer circle. Red and blue dots indicated somatic mutations present in T1D and nondiabetic organ donors, respectively. Labels on the outer circle indicate hotspots for somatic mutations in the human pancreas. (Courtesy of Dr. Klaus Kaestner)

Epitranscriptomics is the study of RNA modification and regulation. Rohit Kulkarni, M.D., Ph.D., Joslin Diabetes Center, and Chuan He, Ph.D., University of Chicago, proposed that epitranscriptomics may be an emerging layer of genetic regulation that affects beta cell function and survival. Specifically, the team looked at the impact of m^{6}A (N6-methyladenosine) modification of RNA in beta cells. They discovered that healthy human islets exposed to signaling molecules released by immune cells (e.g., cytokines called interleukin 1β [IL-1β] or interferon alpha [IFN-alpha]) increased their level of methyltransferase 3 (METTL3), an enzyme that "writes" m^{6}A onto RNA (Figure 5). Levels of m^{6}A-modified RNA also increased in beta cells in response to the signaling molecules, including many m^{6}A-RNAs that are part of molecular pathways associated with combatting the T1D immune attack. Interestingly, the team also showed that the up-regulation of METTL3 and m^{6}A-RNAs is lost in islets in established T1D, possibly resulting in an exacerbated immune response that leads to beta cell death. Together, this body of research reveals a novel layer of beta cell regulation and may point to METTL3 as a therapeutic target to control immunity and promote beta cell survival.

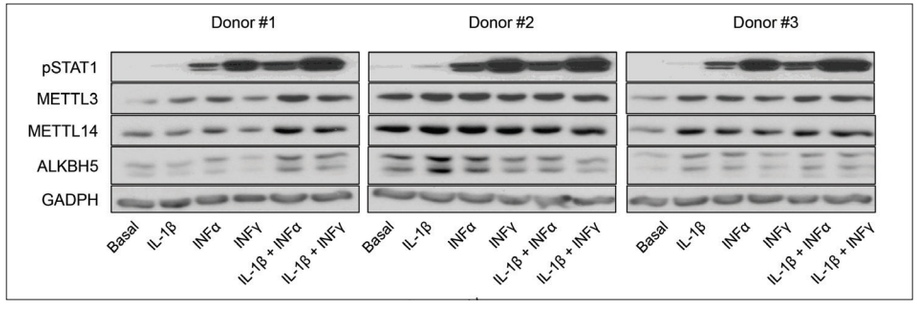


Figure 5. Cytokines Affect Expression of Enzymes Involved in m^{6}A Modification of RNA. *Protein level of pSTAT1, m^{6}A writers (METTL3 and METTL14) and eraser (ALKBH5) in human islets after 24h treatment with represented cytokines. ALKBH5, AlkB Homolog 5, RNA Demethylase; GADPH, glyceraldehyde 3-phosphate dehydrogenase; IL-1β, interleukin 1β; INFα, interferon alpha; INFγ, interferon gamma; METTL3/14, methyltransferase 3/14; STAT1, signal transducer and activator of transcription 1. (Courtesy of Dr. Rohit Kulkarni)*

BIOMARKERS OF T1D DEVELOPMENT AND PROGRESSION IN THE BLOODSTREAM

Desmond Schatz, M.D., University of Florida, and collaborators at Hebrew University and the Benaroya Research Institute developed methods for detection of circulating cell-free DNA (cfDNA), small fragments of DNA that are released into the bloodstream by dying cells. The team first identified methylation markers — or patterns of DNA modification — that are unique to different cell types involved in T1D (beta cells, exocrine cells, pancreatic duct cells and immune cells). Then, they constructed cell type-specific assays to reliably define the levels and tissue sources of cfDNA in plasma of persons with T1D. Their beta cell assay, made from a mixture of six beta cell-specific methylation markers, was highly sensitive and could detect beta cell DNA when it was present in less than 0.1% of a mixture of DNA from other cell types (Figure 6). That assay successfully detected increases in beta cell cfDNA in blood from islet transplant recipients and children with congenital hyperinsulinism (a beta cell disorder), but not from persons with T1D. Next, the team is focused on optimizing the assays to increase their clinical utility in T1D. This research on cfDNA brings the field closer to identifying bloodborne biomarkers for key stages in T1D, such as early beta cell loss, the involvement of exocrine pancreas, changes in immune cells that precede and accompany T1D and even long-term diabetes complications. Furthermore, the team continues to demonstrate that other cell types in and around pancreatic islets display abnormalities that may contribute to disease initiation, including glucagon-secreting alpha cells, as well as cells of the islet vasculature. The investigators continue to learn more from examining exocrine pancreas dynamics during the development of T1D, using DNA methylation markers of pancreatic exocrine cells.

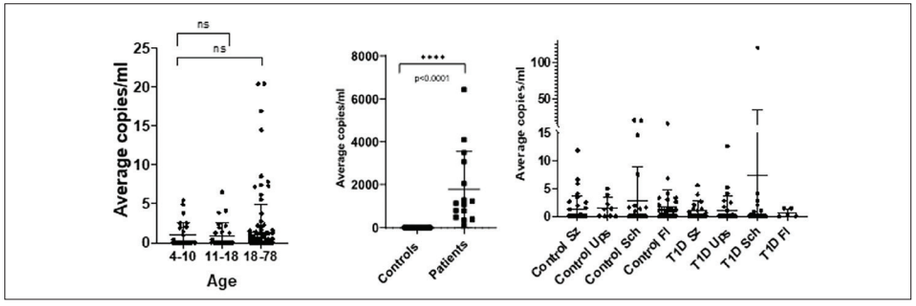


Figure 6. Beta Cell-Specific Circulating Cell-Free DNA (cfDNA) in the Bloodstream. *Levels of beta cell-derived cfDNA in healthy people of different ages (left), in islet transplant recipients (middle), and in recently diagnosed T1D patients from four independent sources (right). (Courtesy of Dr. Desmond Schatz)*

MicroRNAs (miRNAs) are small RNA molecules that do not contain a genetic code needed to make a protein. Anath Shalev, M.D., University of Alabama at Birmingham, identified a specific miRNA, called miR-204, that is present in high levels in human beta cells. Importantly, she discovered that miR-204 was released from dying beta cells and detectable in human blood. The level of miR-204 tracked with the expected pattern of beta cell death during different stages of T1D (Figure 7) — it was high in at-risk, autoantibody-positive individuals and those who had been recently diagnosed with T1D (less than three months), but declined back to nondiabetic levels in people with longstanding T1D (>one year) or those with no detectable C-peptide (a measure of insulin production). Both of the latter groups would have few, if any, beta cells left in the pancreas. MiR-204 levels did not change in people with T2D or rheumatoid arthritis, another autoimmune disease, demonstrating that miR-204 release is specific to the T1D disease process. Measuring miR-204 in the blood may provide a targeted approach to assessing beta cell loss associated with the development of T1D.

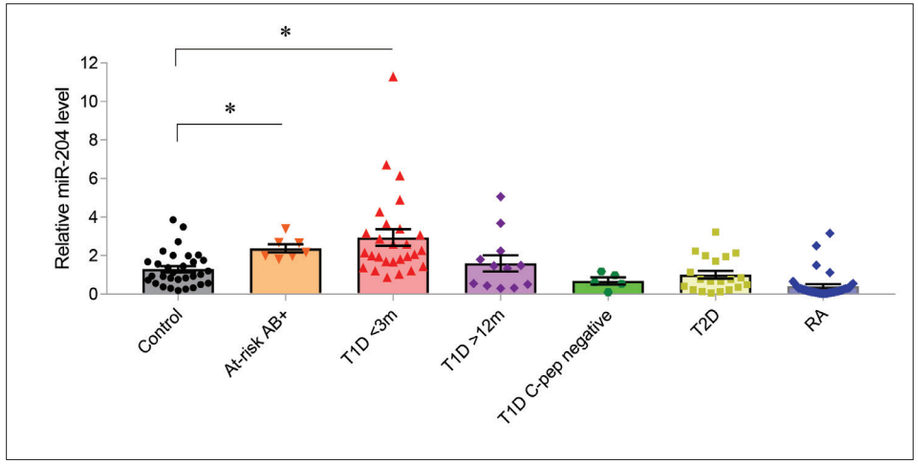


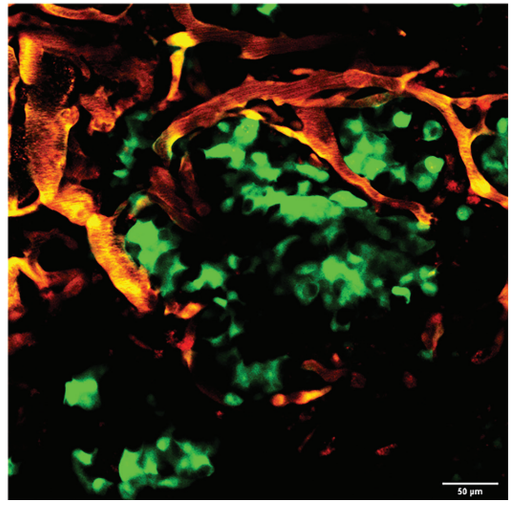
Figure 7. Serum miR-204 Is Elevated in Recent-Onset T1D. *Serum miR-204 in healthy controls (n=31), in at-risk subjects with positive T1D-associated autoantibodies (at-risk AB+) (n=7), in adults with recent-onset T1D (T1D <3m; n=27), with T1D for over one year (T1D >12m; n=12), or with longstanding diabetes who had become C-peptide negative (T1D C-pep negative; n=5), as well as in individuals with type 2 diabetes (T2D; n=20) or with rheumatoid arthritis (RA; n=37) (ANOVA: P < 0.0001; at-risk AB+ and T1D <3m Bonferroni adjusted *P = 0.0288 and *P = 0.0060, respectively, compared to controls. Nonparametric Kruskal-Wallis test: P < 0.0001; at-risk AB+ and T1D <3m Bonferroni adjusted *P = 0.0414 and *P = 0.0006, respectively.) All bars represent means ± SEM. (Courtesy of Dr. Anath Shalev; Xu et al. Am J Physiol Endocrinol Metab. 2019. 317(4):E723-E730. doi: 10.1152/ajpendo.00122.2019 PMID: 31408375)*

MECHANISMS OF BETA CELL DYSFUNCTION IN T1D

Interferon (IFN) alpha is part of the immune system's inflammatory response to infection and may play a part in T1D development. When exposed to IFN-alpha, beta cells accumulate reactive oxygen species (ROS), which cause oxidative damage and, in some cases, cell death. Beta cells can survive this outcome by mounting an antioxidant response. Amelia Linnemann, Ph.D., Indiana University, established a novel platform to study how beta cells respond to IFN-alpha *in vivo*. She transplanted human islets under the kidney capsule in a mouse model and used a fluorescent ROS biosensor "GRX1-roGFP2" developed in her lab to measure how individual beta cells accumulate ROS in response to IFN-alpha (Figure 8). This work led Linnemann to create a new method for single cell analysis in a living organism that accounts for movement during imaging (for example, caused by the animal's breathing). This technique was shared with the Human Islet Research Network (HIRN)

community and has applications for other imaging studies where the sample is not completely still. In addition, Linnemann began a collaboration with Bridget Wagner, Ph.D., (CTAR) to identify small molecules that can modulate the ROS response and tip the balance toward beta cell survival. Linnemann's biosensors, including GRX1-roGFP2, are being shared within the CBDS and other consortia.

Figure 8. A Human Islet Engrafted Under the Kidney Capsule of an NSG Mouse. *Beta cells (green) are expressing a virally transduced vector encoding the reactive oxygen species (ROS) biosensor, GRX1-roGFP2, whereas the vasculature (blood vessels; orange) is labeled using a fluorescent dextran. (Photo credit: Dr. Amelia Linnemann)*



Ribosomes are the cellular machinery that translate mRNA molecules into proteins. A cell's "translatome" is the set of all mRNA molecules that are attached to ribosomes at any particular moment or under a specific physiologic condition. Holger Russ, Ph.D., University of Colorado Denver, worked toward defining the human beta cell translatome in normal and stressed states. In this grant period, he developed a virus-mediated strategy to introduce a gene for a tagged ribosome into human beta cells or progenitor-derived beta cells (Figure 9). The tags on the ribosomes work like molecular hooks and allow Russ to selectively pull out the ribosomes and all of their associated mRNAs from beta cells in healthy or disease states. In this way, he can isolate the human beta cell translatome and determine whether beta cells under stress produce unusual proteins that the body does not recognize as "self." Such nonself proteins could help trigger the immune response that leads to T1D development and may be useful as biomarkers for early detection of disease onset.

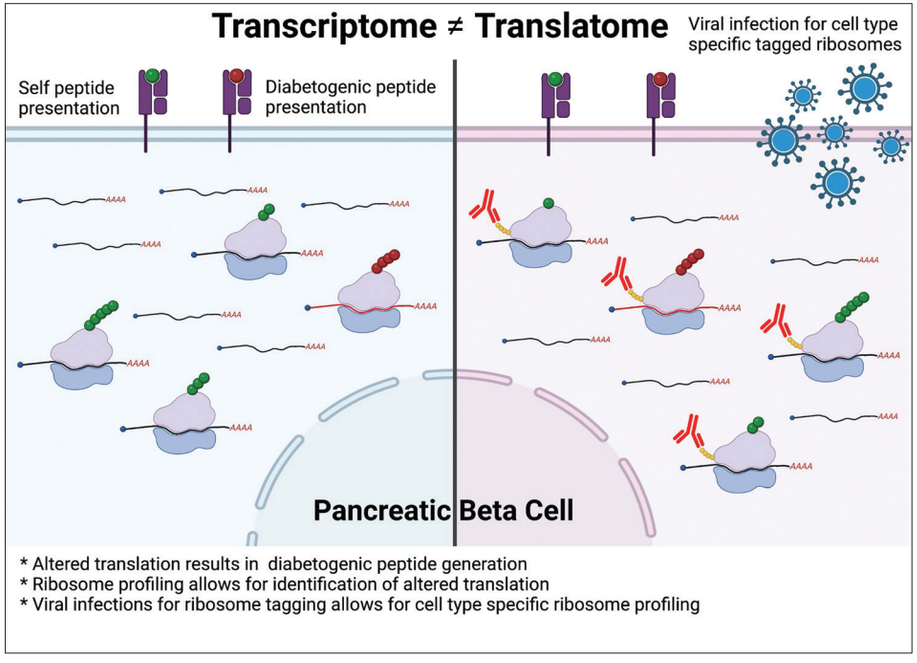


Figure 9. Technique for Ribosome Tagging in Beta Cells. *Viruses are engineered to introduce a "tag" (yellow molecules in right panel) onto beta cell ribosomes. Antibodies (red "Y"-shaped molecules in right panel) can be used to selectively isolate ribosomes and their associated mRNA molecules. By applying this technique to beta cells that come from a healthy pancreas or one in a disease state (e.g., diabetes), investigators can identify differences in protein translation. (Courtesy of Dr. Holger Russ)*

LOOKING TO THE FUTURE

In HIRN Year 7, the CBDS welcomes five investigators or research teams that are working to expand our understanding of early disease processes in T1D.

Peter Arvan, Ph.D., and his co-investigators at the University of Michigan will test their hypothesis that proinflammation triggers of T1D can initiate a vicious cycle of endoplasmic reticulum and mitochondria dysfunction that leads to beta cell failure. The team will also explore strategies to break the cycle and rescue beta cell survival and function, with the ultimate goal of preventing T1D onset and progression.

The integrated stress response (ISR) is a protective mechanism that is activated when a cell encounters environmental stress signals, such as from inflammation. A multidisciplinary, multi-institutional team of investigators led by Raghu Mirmira, M.D., Ph.D., University of Chicago, will explore how the ISR is triggered in beta cells during early T1D and how that process determines beta cell survival or death. The knowledge gained through this research will help the team identify and validate biomarkers that reflect this stressed state in human beta cells.

Alternative RNA splicing is a process that allows for multiple, distinct proteins to be encoded by a single gene. Lori Sussel, Ph.D., University of Colorado Denver, and a team of collaborators at Pacific Northwest National Laboratory propose that alternative splicing events may contribute to beta cell dysfunction in T1D, possibly by forming novel proteins that trigger an autoimmune response. They will use single-cell technologies, advanced proteomics and innovative computational approaches to investigate the functional consequences of beta cell-specific splice variants.

Many genetic differences related to T1D susceptibility have been identified, but the molecular processes that link genetics and the autoimmune process in islets are largely unknown. Golnaz Vahedi, Ph.D., University of Pennsylvania, will generate deep molecular profiles of pathogenic immune cells in pancreata and blood from autoantibody-positive and T1D organ donors collected by HPAP. This line of research may reveal biomarkers in the blood that can diagnose beta cell destruction before diabetes symptoms occur.

Shuibing Chen, Ph.D., Weill Cornell Medical College, and collaborators at the Universities of Michigan and Pennsylvania will undertake an interdisciplinary study of beta cell intrinsic and environmental changes during T1D development. Combining approaches from computational and functional genomics, progenitor cell biology and islet biology, the team will map out the network of biological pathways controlling beta cell dysfunction in autoimmunity and T1D onset. Their findings may point to new drug targets or biomarkers of disease progression.

In addition, the CBDS welcomes two Gateway Investigators who are establishing careers focused on key questions in T1D research: Rafael Arrojo e Drigo, Ph.D., Vanderbilt University, and Amelia Linnemann, Ph.D., Indiana University. Summaries of their research plans, entitled

"Mapping the Association of Beta Cell Longevity and Senescence in T1D" and *"Functional and Molecular Characterization of the Human Islet Interferon Alpha Response,"* respectively, can be found in the chapter "Fostering New Talent in the Human Islet/Beta Cell Research Community."

CBDS INVESTIGATORS, YEARS 5 AND 6

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

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

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*

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

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

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

Anath Shalev, M.D., Investigator, *University of Alabama at Birmingham*
Christopher Wright, D.Phil., 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*
Alvin C. Powers, M.D., Investigator, *Vanderbilt University*
Marcela Brissova, Ph.D., Co-Investigator, *Vanderbilt University*
Jeremy Norris, Ph.D., Co-Investigator, *Vanderbilt University*

**Year 6 only*

New in HIRN Year 7

Rafael Arrojo e Drigo, Ph.D., Gateway Investigator, *Vanderbilt University*

Peter Arvan, Ph.D., Investigator, *University of Michigan*
Leslie Satin, Ph.D., Investigator, *University of Michigan*
Scott Soleimanpour, Ph.D., Investigator, *University of Michigan*

Shuibing Chen, Ph.D., Investigator, *Weill Cornell Medical College*
Steven Parker, Ph.D., Investigator, *University of Michigan*
Chengyang Liu, Ph.D., Co-Investigator, *University of Pennsylvania*

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

Raghu Mirmira, M.D., Ph.D., Investigator, *University of Chicago*
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Scott Oakes, Ph.D., Co-Investigator, *University of Chicago*
Sarah Tersey, Ph.D., Co-Investigator, *University of Chicago*
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Xiaoyong Lei, Ph.D., Co-Investigator, *University of Alabama at Birmingham*

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

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CONSORTIUM ON HUMAN ISLET BIOMIMETICS

In the Consortium on Human Islet Biomimetics (CHIB), scientists combine advances in beta cell biology and stem cell biology with tissue engineering technologies to *develop microdevices that support functional human islets.*

ENGINEERING VASCULARIZED MICROENVIRONMENTS FOR BETA CELL SURVIVAL AND FUNCTION

In the body, pancreatic islets are surrounded by a network of blood vessels, collectively called the "vasculature." As blood flows through the vasculature, nutrients like glucose and oxygen are brought to the islet cells, while insulin, glucagon or other hormones made by the islets are carried away to the rest of the body. CHIB investigators created microdevices that mimic the natural environment of human islets/beta cells with a vasculature and extracellular matrix (ECM) that support extended islet survival and function in the lab.

Human islets rapidly lose function after removal from the body, leaving a very short window for type 1 diabetes (T1D) research. Ben Stanger, M.D., Ph.D., University of Pennsylvania, and a multi-institutional research team have engineered several prototype microdevices that serve as artificial environments supporting human islet viability and function (Figure 10). The investigators added human islets, endothelial cells (cells that line blood vessels) and other support cells (fibroblasts) to a central gel core in the device (Figure 10, Panel A). Over the course of one week, the endothelial cells assembled themselves into blood vessels that surrounded and penetrated the islets (Figure 10, Panels B and C). Channels on either side of the core allowed the investigators to flow substances, such as glucose, chemicals, drugs or cells, through the vascularized islets and observe how islets survive and function under different conditions. The same channels were also used to collect and measure insulin and other hormones released out of the islets. These devices have opened up new possibilities for innovative studies on beta cell health and autoimmunity. For example, Stanger's team is now using their microdevices to model autoimmunity (the immune system's destruction of beta cells that underlies T1D) in the laboratory. Human immune cells added to the device travel through the

vasculature and enter islets. In certain settings, they have directly observed some types of immune cells killing the beta cells. Thus, this set-up presents a novel opportunity to study autoimmunity and test interventions that might halt beta cell destruction in a system that closely mimics human islet physiology.

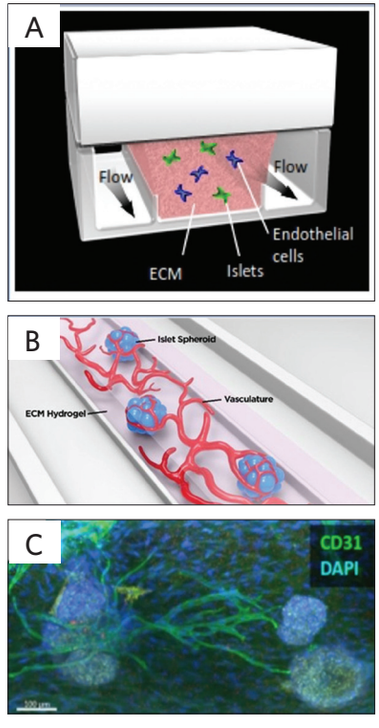

Figure 10. Multi-Channel Biomimetic Device for Three-Dimensional, Vascularized Islet Culture. *Panel A: Schematic of the vascularized islet biomimetic device, containing two side channels and a central gel core containing islets, extracellular matrix (ECM), fibroblasts and endothelial cells. Panel B: Schematic of the interior of the device after 1 week. The endothelial cells added to the device self-assemble into perfusable vascular channels (vasculature/blood vessels), some of which penetrate inside islets. Panel C: Immunofluorescence image showing endothelial vessels (i.e., vasculature; CD31, green) in the device, which also contains islets (round structures) and fibroblasts, identifiable by DAPI (blue). (Photo credit: Dr. Dan Huh)*

Maike Sander, M.D., University of California San Diego, led a team of investigators that designed a three-dimensional vascularized islet micro-organ (VMO-I) platform. This team identified and quantified scaffolding proteins that surround and support islets in the pancreas and optimized the formulation of an ECM gel to mimic this environment in the VMO-I. By adding human islets, endothelial cells and other support cells to their ECM gel, the team was able to re-create the complex vascularized structure of human islets found in the body. The investigators found that when a glucose-rich liquid was added to the VMO-I, it took about 20 to 30 minutes for the glucose to travel through the system, and the islets released insulin shortly after. This insulin secretion pattern is comparable to normal

insulin secretion in human islets following a meal, representing a major improvement over standard beta cell function tests where islets in a lab dish are directly exposed to high levels of glucose and secrete insulin immediately. Another significant advance was made by the team when they modeled immune cell-beta cell interactions using the VMO-I. In this model, the researchers observed in real time as PBMCs exited the blood vessels to infiltrate islets and attack beta cells (Figure 11). (PBMCs, or peripheral blood mononuclear cells, are a mixture of different types of immune cells.) The VMO-I platform will accelerate the field's efforts to model and analyze mechanisms of T1D development.

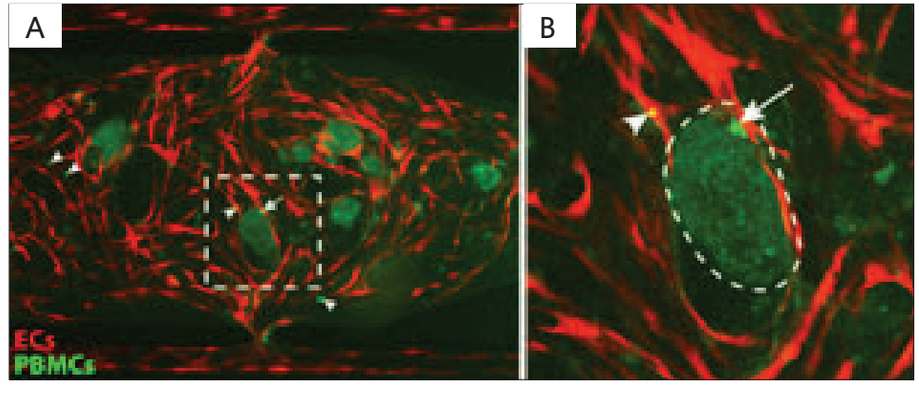

Figure 11. Infiltration of Islets Within the Vascularized Islet Micro-Organ (VMO-I) Platform by Immune Cells. *Panel A: Perfusion of peripheral blood mononuclear cells (PBMCs, green) shows adhesion of some PBMCs to the vessel walls (red, arrowheads). Panel B: Magnification (inset, panel A) shows that a subset of PBMCs invade an islet (dashed outline) after 6 hours of perfusion (arrow). (Photo credit: Dr. Maike Sander)*

BUILDING AN "ISLET-ON-A-CHIP" TO FACILITATE T1D RESEARCH

Cherie Stabler, Ph.D., and researchers from the University of Florida and University of Miami developed a platform to integrate the culture and analysis of cells in an organ-on-a-chip device. Their versatile microphysiological system (MPS) supports the long-term culture of either primary human islets or beta cells derived from progenitor cells, and it can be used to culture and characterize islets within more physiologically relevant environments, such as under fluidic flow and/or within three-dimensional (3D) matrices. The incorporation of fluidic flow into 3D pancreatic islet cultures can not only support the survival of the beta cells but also preserve native 3D features,

such as the richly distributed intra-islet vascular network (Figure 12). In addition, 3D matrices can be easily incorporated into the MPS, which allows for the creation of a supportive 3D niche capable of temporal and spatial interrogation. For example, the team added T cells to islets within these 3D matrices, and the movements and interactions of these immune cells with the beta cells could be tracked in real time. This unique benchtop window allowed for the visualization and quantification of T cell tracking to and killing of beta cells, mimicking the autoimmune attack that leads to T1D. Another unique feature of their newer MPS prototypes is the use of pneumatic microfluidic pumps and automated software that can drive two independent flow rates for liquids above and below the islets or beta cells. This capability provides additional utility to customize flow patterns and incorporate different soluble stimuli that more closely mimic native physiology.

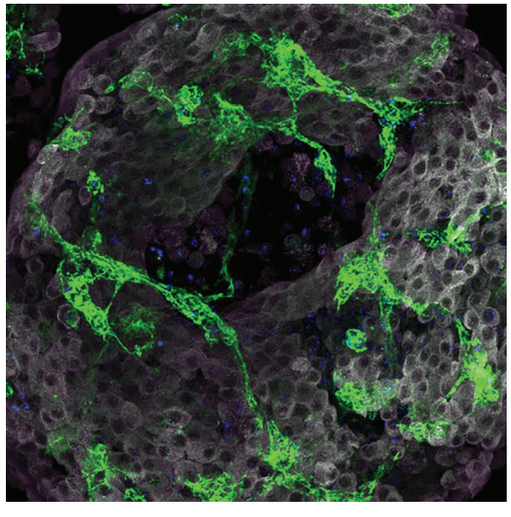

Figure 12. Vascularized Pancreatic Islet. *An endothelial cell network (green) surrounds a primary pancreatic islet (white). (Photo credit: Dr. Cherie Stabler)*

Overall, these innovative platforms enable new avenues of research on long term islet culture, retention of vascular features, tracking of cell homing to the islets (e.g., infiltration by immune cells) and visualization of dynamic interactions between islets or beta cells and other cell types (e.g., endothelial cells, immune cells). The potential impact of the MPS for T1D research is significant — it could aid in the identification of factors that enhance islet health and support islet survival for transplantation, provide an environment that might improve the conversion of progenitor cells to beta cells or serve as a transformative pharmaceutical screening tool to find drugs that enhance beta cell function or suppress autoimmunity. The team actively collaborates with investigators across the Human Islet Research Network, ensuring that their MPS technology is leveraged for a wide range of T1D research.

At Harvard University and Brigham and Women's Hospital, Douglas Melton, Ph.D., and his colleagues designed a microfluidic IsletChip to provide a robust, sensitive and routine technology to assess beta cell function (Figure 13). Solutions of human islets and glucose were loaded into one side of the chip and insulin was measured in the liquid that flowed out from the islets. The team developed the system with scalable manufacturing materials, automated processes for islet loading and insulin sensing and a need for significantly fewer islets than traditional insulin secretion tests. These and other features offer an easy-to-use, yet powerful new technology for islet research that can be adopted in most labs. In a parallel study, the team generated islets from induced pluripotent (iPS) cells made from individuals with T1D, T2D or no diabetes. The investigators can evaluate the function of these islets with the IsletChip, model T1D and T2D physiology and screen drugs that may improve beta cell function or survival. The IsletChip has generated significant interest from islet distribution centers, transplantation clinicians, technology consulting firms and others. The team's vision is that commercialization of this technology will advance the state-of-the-science in islet function testing and reduce the time needed to perform insulin secretion tests in research settings.

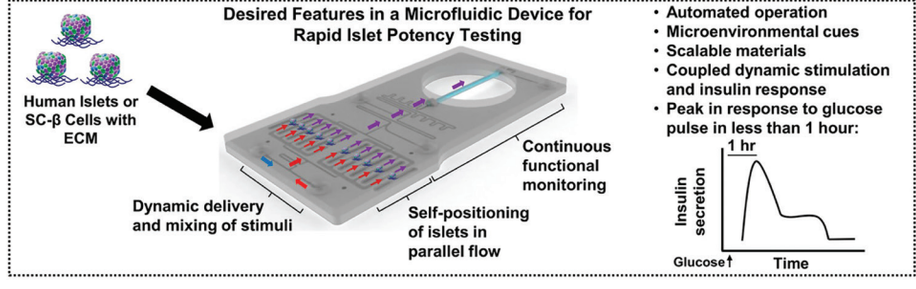

Figure 13. IsletChip. *The Islet-on-a-Chip platform is a microfluidic sensor to measure insulin secretion from cadaveric human beta cells or progenitor cell-derived beta cells. ECM, extracellular matrix. (Photo credit: Glieberman AL, Pope BD, Melton DA, and Parker KK. Diabetes. Feb 2021; 70(2):347–363. doi: 10.2337/db20-0297. PMID: 33472944)*

MEASURING INSULIN SECRETION IN HUMAN ISLET MICROSYSTEMS

Underlying all of the microdevices that support islet function and survival is the need to accurately and sensitively detect insulin secretion in real time and at low cost. Michael Roper, Ph.D., Florida State University, and investigators at the University of California and University of Cincinnati developed an optical approach to measuring insulin using technology known as "fluorescence anisotropy." When islets on microfluidic chips are exposed to glucose, they release insulin into the fluid moving through the chip. That insulin combines with immunoassay reagents causing a mixture of antibody-bound and free insulin that can be detected by fluorescence anisotropy. A key innovation was the team's use of fluids that cannot mix together to carry the immunoassay reagents as "droplets" from the islet chip to the anisotropy detection equipment (Figure 14). This separation of individual droplets offered several advantages. For example, the time course of insulin secretion in response to glucose can be preserved because the droplets, which are produced at regular intervals, cannot mix with each other while they travel the long distance (~5 feet) between the islet microdevice and the detection equipment. The team showed that the fluorescence anisotropy approach can be used in combination with islet microdevices developed by multiple labs, including the Melton team's IsletChip. The system's ease of use and low costs should allow more islet function testing to be performed across the research community, supporting rapid advancement in our knowledge of islet biology.

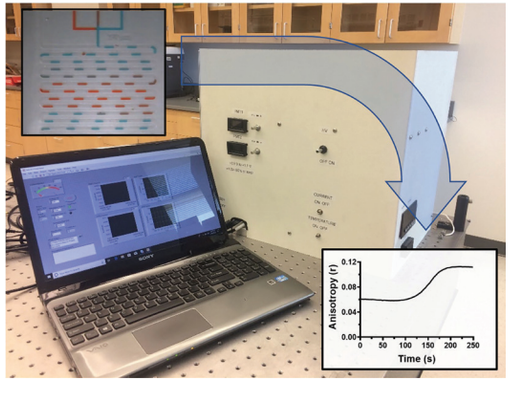

Figure 14. Portable System for Measurement of Insulin Secretion From Islet Microfluidic Devices. *This figure shows the overall scheme of how insulin levels are delivered in droplets from islet mimetic systems to a fluorescence anisotropy detection system. Droplets of immiscible liquids (upper left image) move through the outlet channel of an islet microfluidic device and into anisotropy detection equipment that measures the fluorescence anisotropy in each droplet. The fluorescence anisotropy reflects the amount of insulin produced by the islets in the microfluidic device. The readout (bottom right image) shows an example of how fluorescence levels can change over time. (Photo credit: Dr. Michael Roper)*

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

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Ryan White, Ph.D., Investigator, *University of Cincinnati*

Maike Sander, M.D., Investigator, *University of California San Diego*
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**Year 5 only* *†Year 6 only*

CONSORTIUM ON MODELING AUTOIMMUNE INTERACTIONS

Investigators in the Consortium on Modeling Autoimmune Interactions (CMAI) develop innovative approaches to *model basic aspects of human type 1 diabetes (T1D) immunobiology* using novel *in vivo* and *in vitro* platforms.

DEFINING THE ROLE OF AUTOREACTIVE T CELLS IN T1D AUTOIMMUNITY

T cells belong to the arm of the immune system whose job is to defend the body against infections. For unknown reasons, rogue T cells sometimes attack the body's own cells. Such "autoreactive" T cells are at the center of insulitis, the autoimmune/inflammation process that leads to beta cell death and T1D.

Sally Kent, Ph.D., and a team of researchers at the University of Massachusetts Medical School and the Icahn School of Medicine at Mount Sinai focused on characterizing autoreactive T cells that infiltrate human islets and contribute to insulitis and beta cell dysfunction. The team isolated and grew >600 cell lines derived from T cells found in pancreatic islets of 45 organ donors who were autoantibody-positive ("preclinical T1D") or had recent-onset or long-term T1D, along with 11 nondiabetic donors (not shown) (Figure 15, Panel A). This is the largest cell line bank of its type in existence and represents an unparalleled resource for the study of islet-infiltrating T cells. Already, the T cell bank has produced new insights into the structure and function of these important cells. An example of CD8 T cell islet autoreactivity is shown in Figure 15, Panel B. The investigators discovered that islet-infiltrating T cells recognized a range of different islet proteins, and some of those proteins were modified in donors with T1D. The team identified CD8 T cells in islets of five donors (one is shown) with T1D duration of two to eight years that expressed high levels of the protein CD57 and PD-1 (Figure 16). CD57 protein is associated with T cell exhaustion or senescence and raises intriguing new questions, including the possibility of a window for intervention before clinical onset of T1D. Finally, in an *in vitro* system, the team identified gene expression patterns of beta cells from islets with and without T cell infiltration and saw that beta cells from islets with T cell infiltration express cytokines

A

Islet Donors

6 "pre-clinical" donors

Gender	Age (years) upon demise	Status No T1D diagnosis
M	3	GADA+
F	18	pending
M	19	GADA+
M	20	GADA+
F	24	GADA+
M	30	GADA+

autoantibody to GAD65

16 donors with recent onset T1D

Gender	Age (years) upon demise	Duration of T1D years
M	13	0
F	3.75	0
M	25	0
M	23	0.42
M	25	0.58
F	14	2
M	24	2
F	14	2
M	17	2.5
F	6	3
F	12	3
F	12	3
F	10	4
F	20	4
M	13	6
F	22	6

23 donors with long-term T1D

Gender	Age (years) upon demise	Duration of T1D
M	20	7
F	20	8
M	10	8
M	20	8
M	19	10
M	27	11
F	25	12
M	18	14
M	27	14
F	54	14
M	20	15
F	28	15
M	45	15
M	30	16
M	27	17
F	24	18
M	22	20
M	30	20
M	35	22
M	56	25
F	49	27
M	37	31
F	39	31

B

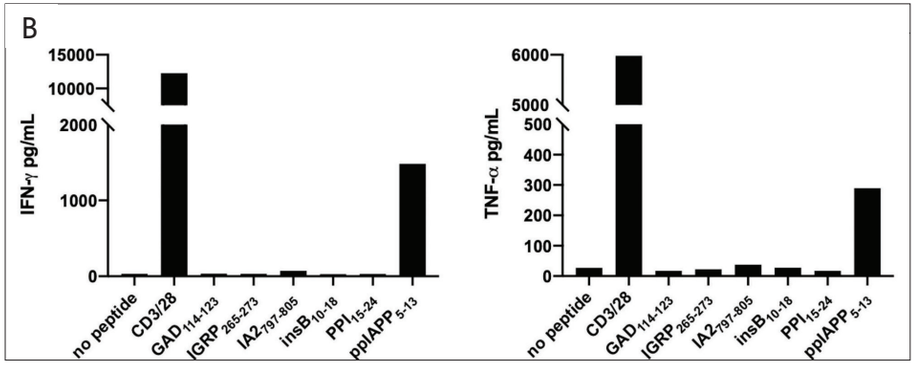


Figure 15. Characterizing Autoreactive T Cells in Human Islets. *Panel A: Islet donors for islet-derived T cell isolation were: "pre-clinical" donors with a single autoantibody (GADA), but without a clinical diagnosis of T1D (source: Dr. Ali Naji, HPAP and nPOD), donors with recent-onset T1D (source: nPOD) and donors with long-term T1D (source: Dr. Al Powers). From individual islets, the team derived more than 600 T cell lines. This resource forms the basis for examination of the characteristics and functions of islet-infiltrating and potentially pathogenic T cells in T1D. Panel B: Islet-infiltrating T cells recognize peptides (small protein pieces) from islet proteins, including GAD, IGRP, IA-2, Ins (insulin), PPI (preproinsulin) and IAPP. A CD8+ T cell line derived from the islets of nPOD 6342 were screened against HLA-A2 restricted peptide epitopes. Cytokine secretion was determined by cytometric bead array (CBA). (Courtesy of Dr. Sally Kent)*

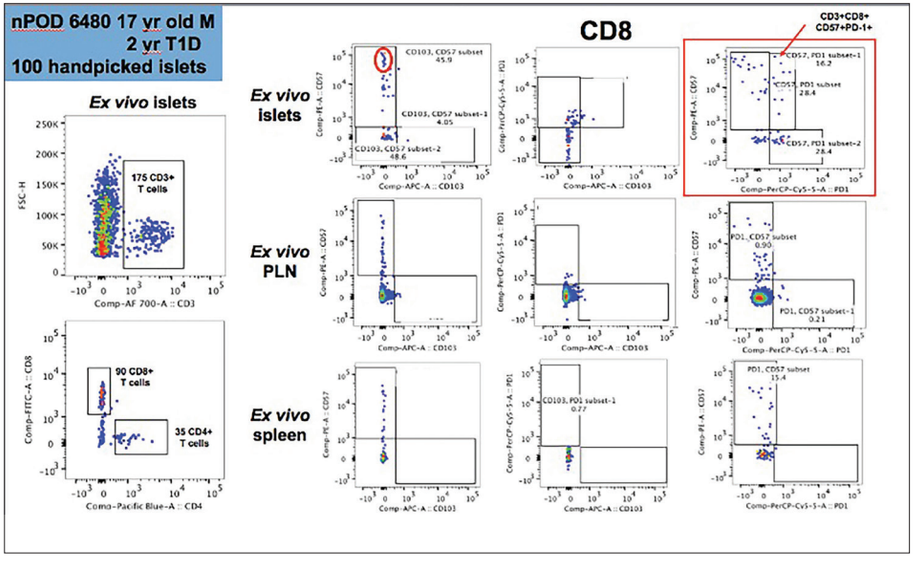


Figure 16. Autoreactive T Cells From Islets Express CD57 and PD-1. *A unique subpopulation of islet-infiltrating CD8+CD57+PD-1+ cells were identified ex vivo from hand-picked islets, but not from autologous spleen or pancreatic draining lymph nodes from a donor with recent onset T1D. This subpopulation may represent the autoreactive cytotoxic effector cells in the islet. (Courtesy of Dr. Sally Kent)*

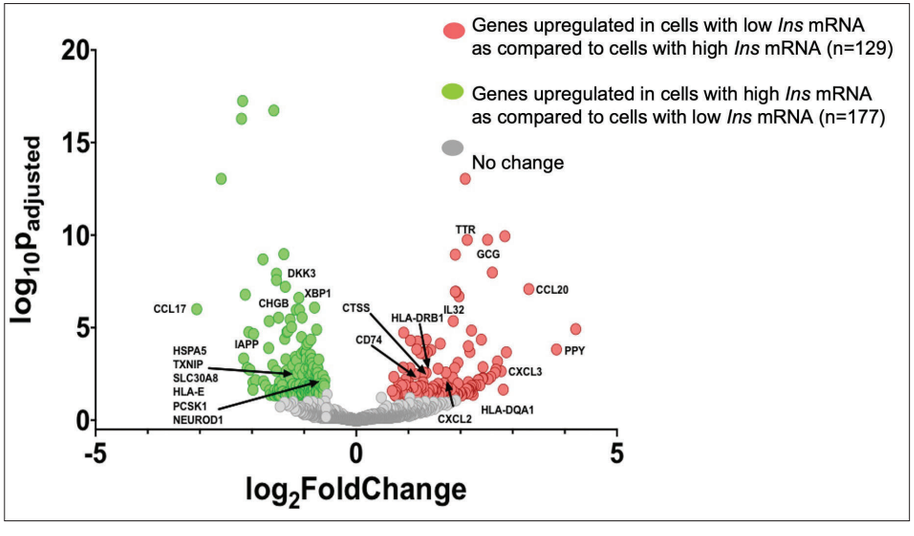
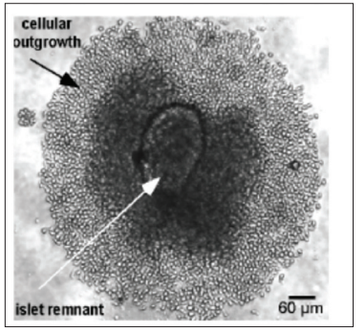


Figure 17. Gene Expression in Beta Cells Varies by Level of T Cell Infiltration of Islets. *Volcano plot of differential gene expression of cells with high (without infiltrate) and low (with infiltrate) INS (insulin) mRNA expression from nPOD 6477 islets. (Courtesy of Dr. Sally Kent)*

and chemokines that are T cell attractants (Figure 17). These data will suggest ways to protect beta cells from the autoimmune attack in T1D.

T cells are called into action when receptors on their surface (T cell receptors, or TCRs) recognize a piece of a virus or other infectious agent and signal the cell to destroy the invader. In the case of autoreactive T cells in T1D, TCRs seem to recognize something in or on the beta cells, but the exact TCR triggers and how they initiate the immune pathway are unknown. Ronald Gill, Ph.D., and his colleagues at the University of Colorado and the National Jewish Medical and Research Center created mouse models carrying TCR genes from human autoreactive T cells. These mice were used to investigate beta cell autoreactivity in a living system and to probe the role of autoreactive TCRs in insulitis. Information from this study may reveal targets for therapeutic intervention in the early stages of T1D autoimmunity.

At Benaroya Research Institute, Eddie James, Ph.D., described the composition and functional profiles of islet-infiltrating T cells that he isolated from the islets of autoantibody-positive organ donors who had not yet developed T1D (Figure 18). An interesting finding from this study was the presence of gamma delta T cells. These are a subset of T cells characterized by a specific type of TCR on their surface; gamma delta T cells are a small and understudied portion of the body's T cell population. The gamma delta T cells from autoantibody-positive donor islets failed to respond to proinsulin and GAD65, which are known to be islet-specific targets of the immune system. This observation raises the possibility that T cells with unexpected specificities may play a role in the earliest stages of T1D or that some islet-infiltrating T cells are not islet-specific but are recruited to the islets due to local inflammation (insulitis).



Donor	Line	CD4+ T cells	CD8+ T cells	gamma delta T cells
HPAP012	HP.AI.1			
	HP.AI.3			
	HP.AI.4			
	HP.AI.5			
	HP.Bulk.1			
	HP.Bulk.2			
	HP.Bulk.3			
	HP.Bulk.4			
HPAP019	nHP.Bulk.1			
	HP.Bulk.3			
	nHP.AI.2			

Figure 18. Islet-Derived T Cells. *To test the hypothesis that the islet-infiltrating repertoire includes clonally expanded T cells that recognize beta cell epitopes and neo-epitopes, we characterized the composition and probed the specificity of T cell lines isolated from donors who were autoantibody positive at the time of their demise. Islets from donor pancreata were cultured in the lab. After several days, cells that grew out of the islet remnant were recovered and sorted to isolate islet-infiltrating T cell lines. The resulting lines consisted of conventional CD4+ and CD8+ T cells and gamma delta T cells. (Courtesy of Dr. Sally Kent and Dr. Eddie James)*

HARNESSING REGULATORY T CELLS FOR T1D THERAPY

Some T cells — "regulatory" T cells or "Tregs" — guard against destruction caused by autoreactive T cells. Tregs have potential as treatments for autoimmune diseases, cancer and transplant rejection.

Jeffrey Bluestone, Ph.D., and his colleagues at the University of California San Francisco worked to build better Tregs that specifically home to human islets and oppose the destruction of beta cells. As an initial step, the investigators tested whether DPP6, a protein found on the outer surface of beta cells, could be used to aim T cells at islets. To do this, CD4+ T cells (a nonregulatory type of T cells) were engineered with a chimeric antigen receptor (CAR) that selectively recognized DPP6. Human islets were transplanted into mice and shown to stabilize blood glucose at normal levels. After 60 days, engineered DPP6 CAR T cells were injected into the mice, and the investigators observed a dramatic increase in blood glucose — a sign that the DPP6 CAR T cells had found the transplanted human islets and initiated immune destruction of the beta cells (Figure 19). CD4+ T cells that were unmodified or engineered with an irrelevant CAR had no effect on the transplanted islets. Next, the team engineered Tregs with the DPP6 CAR and injected them into transplanted mice. The

DPP6 CAR Tregs also accumulated in transplanted human islets, but in this case, blood glucose levels in the mice remained steady. Thus, the DPP6 CAR Tregs found but did not destroy the transplanted human beta cells. This finding represents a significant advance toward the development of Treg-based therapeutics for T1D.

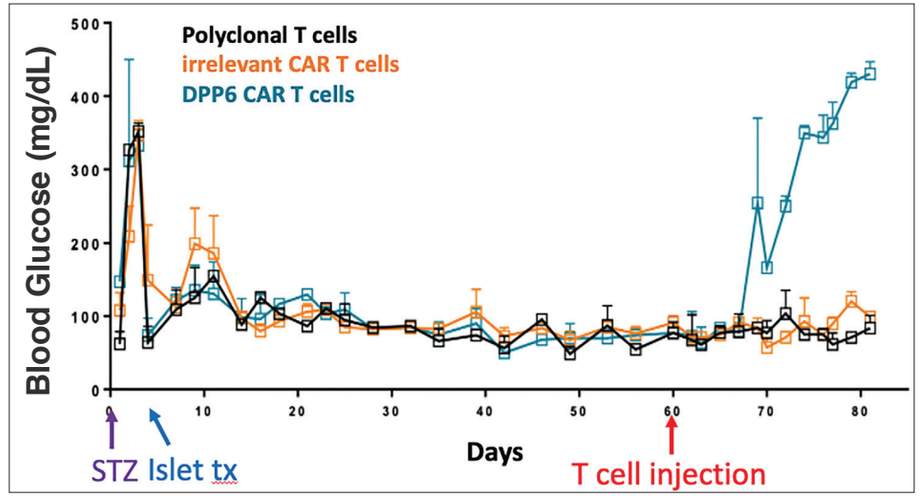


Figure 19. DPP6 CAR T Cell Recognition of Transplanted Human Islets in Mice. *Human CD4+ Tconv were isolated from PBMC (peripheral blood mononuclear cells) of healthy donors and transduced with CARs against DPP6 or against HLA-A2 as an irrelevant control. CAR T cells or unmodified polyclonal CD4+ Tconv cells were injected into NSG mice stably transplanted with human islets from an HLA-A2(-) donor. Blood glucose was monitored for signs of graft rejection. (Courtesy of Dr. Jeffrey Bluestone)*

MODELING T1D-RELEVANT TISSUES WITH PROGENITOR CELLS

At least 60 regions in the human genome are associated with increased risk for developing T1D. One of these regions, the PTPN22 gene, is involved in regulation of autoreactive T cell activity, although exactly how it acts is not known. A group of investigators from the University of Florida and Sanford Research/University of South Dakota led by Clayton Mathews, Ph.D., developed new cellular tools to probe how the T1D-associated variant of PTPN22 promotes inflammation and participates in the immune response against beta cells (Figure 20). The team made significant progress toward creation of a fully isogenic system (different populations of cells that carry identical genes) to study T1D in a dish. They started with induced pluripotent

(iPS) cells, which are cells that have the potential to mature into almost any type of human cell. (IPS cell lines are typically generated by coaxing a donor's skin or blood cells to return to a less specialized, more primitive condition.) First, the team edited the DNA in iPS cells from six unique donors so that the cells contained the T1D risk-associated variant of the PTPN22 gene. They then developed protocols to efficiently differentiate the edited iPS cells into populations of specialized immune cells (monocytes, macrophages and T cells) and endothelial (blood vessel) cells. The team initiated collaborations with Dieter Egli, Ph.D., (CMAI), and Holger Russ, Ph.D., (CBDS), to also differentiate their iPS cells into insulin-producing beta cells. When all genetically identical cell types are assembled, this isogenic system will open up new opportunities for innovative research on the pathogenesis and genetic regulation of T1D.

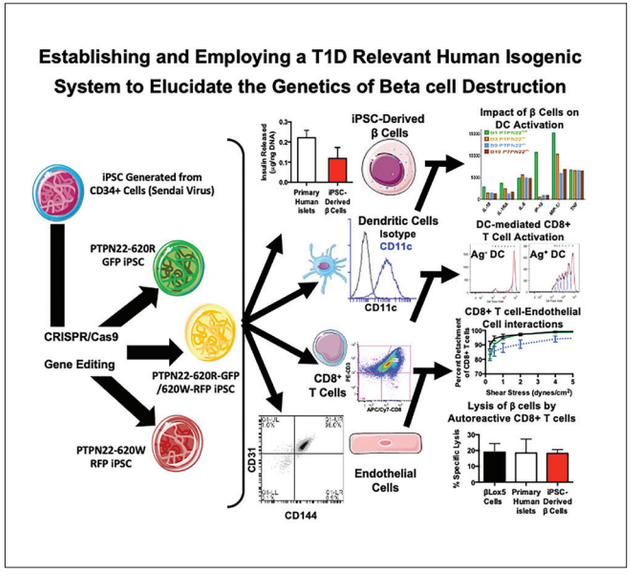


Figure 20. Using Progenitor Cells to Model the Effects of a T1D Susceptibility Gene, PTPN22. *T1D is an autoimmune disease that is regulated by genetic factors, including protein tyrosine phosphatase, non-receptor type 22 (PTPN22). The goal of our project is to identify how PTPN22 participates in the development of T1D using isogenic systems (different populations of cells that carry identical genes or are derived from a single person). PTPN22 regulates signaling pathways in immune cells to cause improper white blood cell responses to self-tissues, specifically the insulin-producing pancreatic beta cells. To achieve this goal, we use induced isogenic patient-derived systems where gene modifications in PTPN22 allow intensive study of the risk and common gene variants using cell-cell interactions. These changes result in distinctive signaling cascades in both lymphoid and myeloid cells when the risk allele of PTPN22 is present. The risk allele modifies how autoreactive T cells are activated, as well as their path into the pancreas. Using gene-edited iPS (induced progenitor) cells differentiated into beta-like cells, dendritic cells, macrophages, endothelial cells, and CD8+ T cells, we have begun to elucidate the panoply of effects regulated by the T1D-risk allotype of PTPN22. (Courtesy of Dr. Clayton Mathews)*

The thymus, a small gland located in front of the heart, helps our bodies build healthy immune systems. New T cells are generated in the bone marrow, then make their way to the thymus where they undergo a selection process to eliminate autoreactive T cells. Mark Anderson, M.D., Ph.D., and his colleagues at the University of California San Francisco began development of a cellular model of the thymus to better understand how and why the selection process goes awry in people with autoimmune diseases like T1D. The team performed single-cell RNA sequencing on human thymus tissue. That study revealed a wide diversity of cell types that reside in the thymus, including thymus epithelial cells (TECs), mesenchyme, endothelium, pericytes, mesothelium and blood/immune cells (Figure 21). With these data, the team created reference maps of the RNA complement within different thymic cell types across multiple stages of life in order to better understand how the thymus microenvironment is established and maintained during aging. These reference maps, in turn, helped the team identify factors involved in TEC development and devise effective protocols to differentiate human pluripotent cells (hPSC) into TECs in a lab setting. In collaboration with Megan Sykes, M.D., (CMAI), the team's hPSC-derived TECs were transplanted into humanized mouse models to study how the cells function in the body. These ongoing experiments should lead to new insights on the role of the thymus in the early stages of T1D autoimmunity.

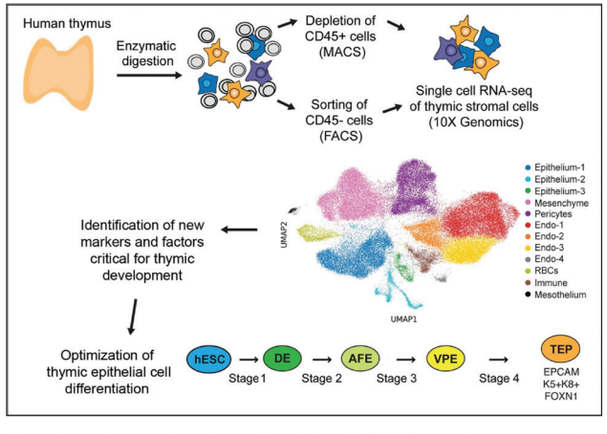


Figure 21. Mapping the Cellular Components of the Human Thymus. *To better understand which cell types are normally present in the human thymus, we performed single-cell RNA sequencing of thymic stromal cells. We identified populations corresponding to epithelium, mesenchyme, endothelium, pericytes, mesothelium and blood/immune cells. Analysis of these datasets led to the identification of new thymic epithelial cell (TEC) markers, as well as some candidate regulators of TEC cell fate commitment and differentiation. These data are critical to efforts to refine TEC differentiation protocols and will guide the choice of stromal cells to be included in thymic grafts.(Courtesy of Dr. Mark Anderson)*

MODELING HUMAN ISLET RESEARCH NETWORK (HIRN) IN MICE

CMAI teams worked in parallel to create humanized mouse models of human T1D. These leading-edge models facilitate studies on the genetic causes and mechanisms of T1D progression that cannot be conducted in humans with or at risk for the disease.

Megan Sykes, M.D., and her co-investigators at Columbia University designed a personalized mouse model that allows synchronized development of a functional human immune system from hematopoietic progenitor cells of T1D patients or healthy controls in immune-deficient mice. They showed that these mice produce all types of immune cells (e.g., T cells, antibody-producing B cells, macrophages) and that cells from the humanized immune system infiltrate human islets transplanted into the mice (Figure 22). Taking it a step further, the team devised an advanced model that incorporates beta cells derived from progenitor cells from the same donor used to construct the humanized immune system. This model permits the researchers to directly observe and analyze human beta cell-immune cell interactions in a living system. Working in collaboration with Mark Anderson, M.D., Ph.D., and Audrey Parent, Ph.D., (CMAI), the team extended their model to incorporate human progenitor cell-derived thymus epithelial cells into a porcine thymic graft that supports human T cell development in humanized mice that have had their own thymuses removed. This humanized mouse model enables innovative research on autoreactive T cell development in the human thymus, a key early step in the development of T1D.

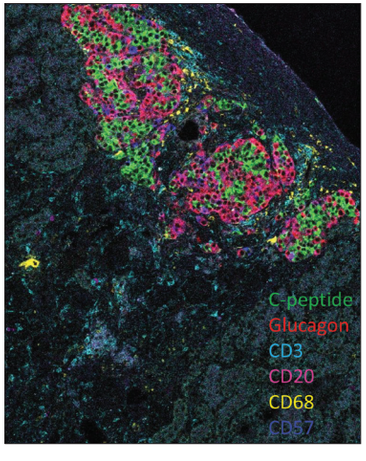


Figure 22. Humanized Immune System in a Mouse Model. *Human immune cell infiltration of human islet allografts transplanted under the kidney capsule of humanized mice. Mass cytometry stain for C-peptide (beta cells), glucagon (alpha cells), CD3 (T cells), CD20 (B cells), CD68 (macrophages) and CD57 (NK and T cells). (Photo credit: Dr. Megan Sykes)*

Dale Greiner, Ph.D., of the University of Massachusetts Medical School and a multi-institutional group of investigators worked toward development of a model that recapitulates the entire human T1D disease process in optimized immune-deficient mice (OPTI-MICE). The team made a bank of human iPS cells derived from individuals with T1D who had different genetic markers associated with T1D risk, as well as from volunteers who did not have autoimmune disease. Some of those iPS cells were differentiated into human insulin-producing beta cells (Figure 23), which were shown to be fully functional and susceptible to immune destruction. The team also made progress using their iPS cells to develop renewable sources of hematopoietic progenitor cells that can be differentiated into all immune cell lineages and thymus epithelial cells that are essential for modeling the development of autoreactive T cells. Each of these differentiated human cell populations can be implanted into the OPTI-MICE to model T1D pathogenesis in real time and identify therapeutic approaches to prevent or cure T1D without putting patients at risk. The unique mouse models generated by these investigators are available to all members of the CMAI and other T1D consortia.

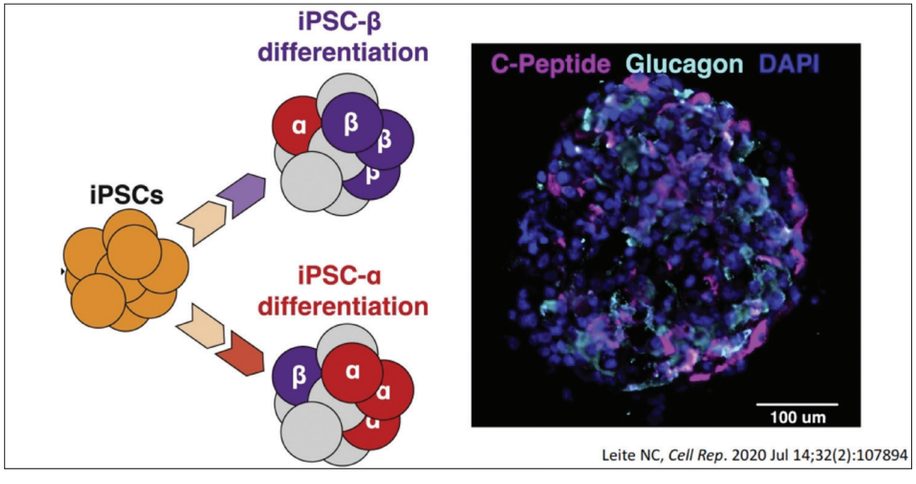


Figure 23. Induced Pluripotent Cell (iPSC)-Derived Human Islets. *Development of islets enriched in beta cells (stained for C-peptide in purple) or alpha cells (stained for glucagon in teal) derived from iPSC cells. (Photo credit: Dr. Dale Greiner)*

LOOKING TO THE FUTURE

In HIRN Year 7, the CMAI welcomes Alok Joglekar, Ph.D., a Gateway Investigator at the University of Pittsburgh. A summary of his research plan, entitled *"Identification of the Cognate Epitopes of Autoreactive T Cells in T1D"* can be found in the chapter "Fostering New Talent in the Human Islet/Beta Cell Research Community." Joglekar's work is an important addition to the consortium because he provides new opportunities to link T cell receptor sequences in the islets to the antigen-specificity of these cells.

CMAI INVESTIGATORS, YEARS 5 AND 6

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*

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

***Ronald Gill, Ph.D., Investigator,** *University of Colorado Denver*
***Peter Gottlieb, M.D., Co-Investigator,** *University of Colorado Denver*
***John Kappler, Ph.D., Co-Investigator,** *National Jewish Medical and Research Center*
***Aaron Michels, M.D., Co-Investigator,** *University of Colorado Denver*
***Maki Nakayama, M.D., Ph.D., Co-Investigator,** *University of Colorado Denver*

Dale Greiner, Ph.D., 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*
Douglas Melton, Ph.D., Investigator, *Harvard University*
Leonard Shultz, Ph.D., Investigator, *The Jackson Laboratory*
David Harlan, Ph.D., Co-Investigator, *University of Massachusetts Medical School*
Sally Kent, Ph.D., Co-Investigator, *University of Massachusetts Medical School*
Rene Maehr, Ph.D., Co-Investigator, *University of Massachusetts Medical School*

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

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*

***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*

Megan Sykes, M.D., Investigator, *Columbia University*
†Mark Anderson, M.D., Ph.D., Investigator, *University of California San Francisco*
†Matthias Hebrok, Ph.D., Investigator, *University of California San Francisco*
†Audrey Parent, Ph.D., Investigator, *University of California San Francisco*
***Xiaojuan Chen, M.D., Ph.D., Co-Investigator,** *Columbia University*
†Remi J. Creusot, Ph.D., Co-Investigator, *Columbia University*
***Nichole Danzl, M.D., Co-Investigator,** *Columbia University*
***Dieter Egli, Ph.D., Co-Investigator,** *Columbia University*
Robin Goland, M.D., Co-Investigator, *Columbia University*
***Hans Snoeck, M.D., Ph.D., Co-Investigator,** *Columbia University*
***Yong-Guang Yang, M.D., Ph.D., Co-Investigator,** *Columbia University*

**Year 5 only*
†Year 6 only

New in HIRN Year 7

Alok Joglekar, Ph.D., Gateway Investigator, *University of Pittsburgh*

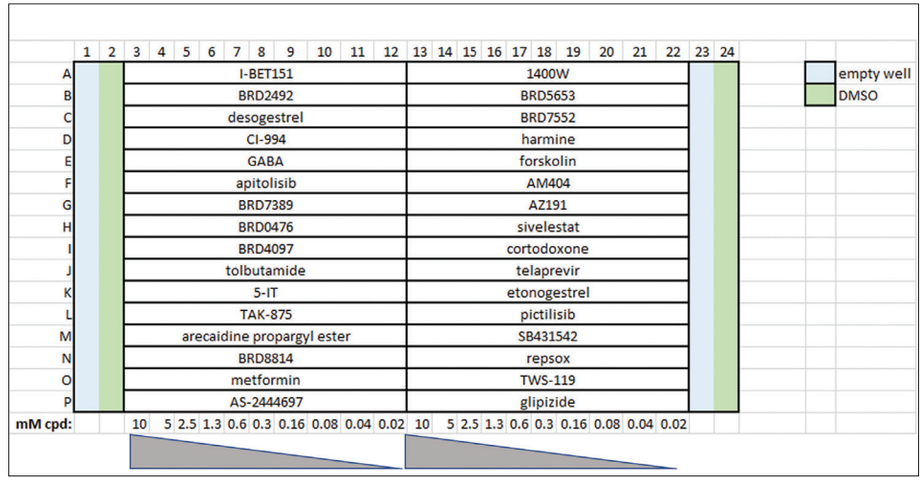
CONSORTIUM ON TARGETING AND REGENERATION

Researchers in the Consortium on Targeting and Regeneration (CTAR) investigate *methods to increase or maintain functional beta cell mass in type 1 diabetes (T1D)* through targeted manipulation of islet plasticity or engineered protection of beta cells from immune-mediated destruction.

TARGETING BETA CELLS WITH SMALL MOLECULES

Small molecules are low molecular weight chemical compounds that influence biological pathways. Many pharmaceutical drugs are small molecules. They also can be used as research tools for imaging, investigating molecular mechanisms and many other applications.

Bridget Wagner, Ph.D., led a team at the Broad Institute and Joslin Diabetes Center that developed a "Beta Cell Informer Set" of small molecules with beneficial effects on beta cells (Figure 24). These compounds include molecules that improve insulin secretion, stimulate cell proliferation, enhance beta cell survival and increase insulin expression in other cell types, such as alpha cells. The team used their informer set to validate assays in human islets and progenitor cell-derived beta cells; these assays, in turn, can be used to find additional small molecules with potential benefits for T1D research and therapy. Already, the investigators identified novel small molecules that stimulate beta cell proliferation in human islets and promote survival when beta cells are exposed to various insults, such as proinflammatory cytokines. These studies revealed a previously unknown role in beta cell survival for casein kinase 2, an enzyme involved in DNA repair and other cellular processes, suggesting a new target for T1D drug development. The Wagner team is sharing their Beta Cell Informer Set widely and adapting assays developed in other HIRN labs to expand their search for beta cell-relevant small molecules.



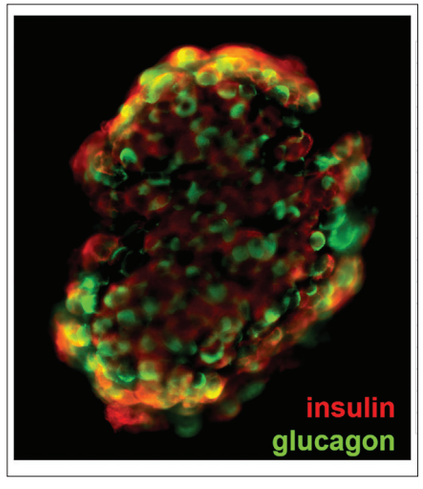


Figure 24. Array of Beta Cell-Targeting Small Molecules. *Bottom panel: A pancreatic islet, showing insulin-producing beta cells (red) and glucagon-producing alpha cells (green). Top panel: The Beta Cell Informer Set for validation of assays in human islets and progenitor cell-derived beta cells. These compounds promote beta cell proliferation, enhance beta cell survival, induce insulin secretion, or increase insulin expression in non-beta cell types. (Photo credit: Dr. Bridget Wagner)*

The endoplasmic reticulum (ER) is the cellular organelle in which secreted proteins, such as insulin, are folded into their proper shapes. Cells have an intracellular signaling pathway with two outputs to handle ER stress, which is caused by the accumulation of too many unfolded proteins: an adaptive unfolded protein response (UPR) that helps the ER better manage the rising tide of unfolded proteins and a terminal UPR that triggers apoptosis (cell death) when ER stress becomes unmanageable. When immune cells infiltrate pancreatic islets, beta cells begin to accumulate unfolded proteins in their ER and exhibit conversion from adaptive to terminal (destructive) UPR. Thus, high/chronic ER stress leading to beta cell death may be an early step in the development of T1D. Feroz Papa, M.D., Ph.D., University of

California San Francisco, and his collaborator, Dustin Maly, Ph.D., University of Washington, created small molecules called "PAIRs" (partial antagonists of IRE1 RNAse) that selectively target UPR outputs (Figure 25). They showed that PAIRs can block the terminal UPR pathway that sets off beta cell death, while maintaining the adaptive UPR that helps beta cells manage ER stress. Their next step is testing PAIRs in a humanized mouse model to assess whether these compounds can help preserve beta cells in the face of a T1D-related immune assault.

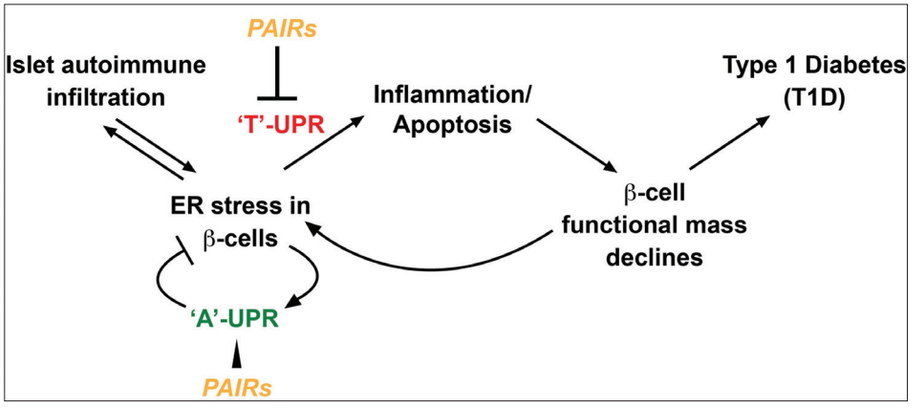


Figure 25. Small Molecules to Modulate the Unfolded Protein Response in Beta Cells. *The small molecule PAIRs (partial antagonists of IRE1 RNAse) truncate the terminal unfolded protein response ('T"-UPR) while permitting an adaptive unfolded protein response ('A'-UPR), thus preventing a continuum of beta cell degenerative changes that can lead to T1D. ER, endoplasmic reticulum. (Courtesy of Dr. Feroz Papa)*

Mitogens are small molecules that instruct cells to divide, thus triggering cell proliferation. Prodrugs are inactive molecules that can be processed by the body to produce a therapeutically useful drug, such as a mitogen. Amit Choudhary, Ph.D., and other investigators from the Broad Institute and Joslin Diabetes Center developed a rationally designed, modular, zinc-based prodrug system to selectively deliver mitogens to beta cells (Figure 26). Using a known mitogen, called "GNF-4877," that effectively stimulates beta cell proliferation, the team constructed a zinc-based prodrug for delivery. Due to its modular nature, the prodrug system can be adapted to deliver other small molecules, such as protective agents developed by CBDS investigators, directly and selectively to beta cells without impacting

other cells in the pancreas. These studies represent a key step toward developing targeted medications to halt or reverse T1D progression.

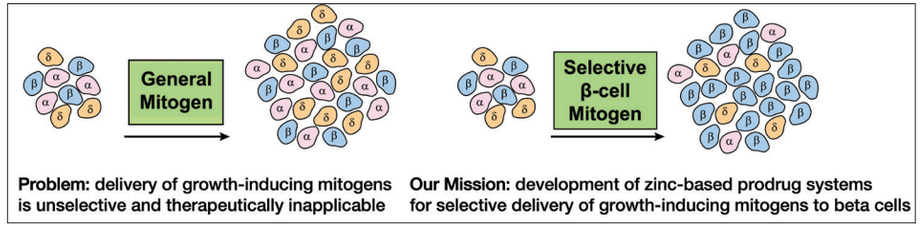


Figure 26. Development of a Zinc-Based Prodrug System. *Left panel: General mitogens stimulate the growth of all or most cell types and are not therapeutically useful for a specific disease. Right panel: Zinc-based prodrug systems for delivery of mitogens that promote selective growth of beta cells, without harmful effects on other cells and organs in the human body, are under development. Restoration of beta cell numbers in the pancreas using mitogens may halt or reverse the progression of T1D. (Courtesy of Dr. Amit Choudhary)*

GENETIC STRATEGIES TO TARGET BETA CELLS

Adeno-associated viruses (AAV) are small viruses that can enter human cells, but do not cause disease. AAV vectors are manmade versions of these viruses that have been modified in the lab to carry a new gene — such as a gene to strengthen beta cells against immune attack or promote beta cell regeneration. Hiroyuki Nakai, M.D., Ph.D., and Markus Grompe, M.D., at Oregon Health & Science University and Mark Kay, M.D., Ph.D., at Stanford University engineered AAV vectors to effectively target pancreatic islet cells. The team led by Nakai established a real-time, image-guided method to inject AAV vectors directly into pancreatic ducts of nonhuman primates and demonstrated the safety of that method. After testing 45 different AAV capsids, the investigators identified two that robustly infect pancreatic endocrine cells (Figure 27). Kay's lab worked on modifying these vectors so that they selectively target only a single cell type (e.g., alpha versus beta cells). And, Grompe and his group discovered a piece of DNA that can drive strong expression of any gene carried by the AAV vectors. Collectively, these results are building proof-of-principle for the development of innovative AAV vector-mediated gene therapy for human diabetes.

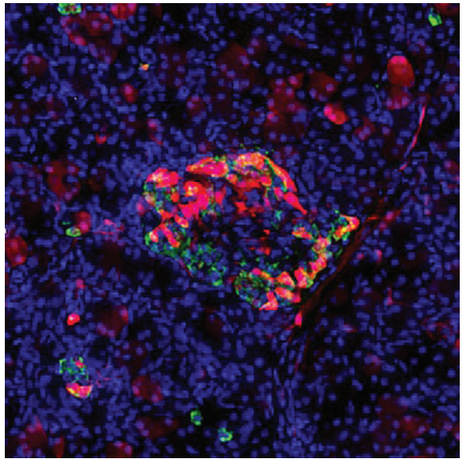


Figure 27. A Novel AAV Vector for Pancreatic Islet Gene Therapy. *Pancreatic islets can be effectively transduced with AAV (adeno-associated virus) vector following vector administration into the pancreatic duct in a rhesus monkey. tdTomato fluorescent marker expression from an AAV vector was assessed four weeks post-injection. Red: tdTomato; green: Insulin; blue: DAPI. (Photo credit: Dr. Craig Dorrel)*

The emerging and exciting field of RNA therapeutics has many potential applications in T1D treatment, including measuring human beta cell mass in living patients, protecting beta cells from immune attack, blocking beta cell death and increasing beta cell proliferation.

At the University of Miami, Paolo Serafini, Ph.D., and his colleagues validated RNA molecules as a strategy to selectively deliver therapeutic payloads to beta cells. In one study, the team used RNA "chimeras" to increase the efficiency of islet transplantation. Chimeras are a fusion of two different types of RNA molecules — an RNA aptamer (a small piece of single-stranded RNA that binds to a specific target, such as a protein, to affect its function) and a small activating RNA (saRNA; a double-stranded piece of RNA that can turn on a specific gene). Human islets were treated with a mixture of two chimeras designed to activate the XIAP (X-linked inhibitor of apoptosis) gene prior to transplantation in immune-deficient, diabetic NOG mice. The investigators observed that nearly all mice that received chimera-treated islets became nondiabetic compared to only about 50% of mice with untreated islets (Figure 28, Panels A and B). Importantly, mice with chimera-treated islets achieved healthy glucose levels about four days earlier than those with untreated islets. Thus, the chimeras reduced early islet loss and improved the efficiency of islet transplantation.

Then, the investigators evaluated whether the chimeras could be used to precisely deliver the therapeutics to the beta cells *in vivo.* In these

experiments, human islets were transplanted in immunodeficient mice. Three weeks after transplant, mice were given intravenously chimeras generated by fusing the RNA aptamers to an saRNA able to activate an immune protective protein called PDL1. The investigators found that the treatment with chimeras upregulated the desired gene only in the beta cells *in vivo* but not in any other organs (Figure 28, Panel C). This study represents a major development in the use of non-viral strategies for the precise, therapeutic modulation of genes in beta cells *in vivo.* Serafini and colleagues are now investigating whether this treatment can protect mice from T1D.

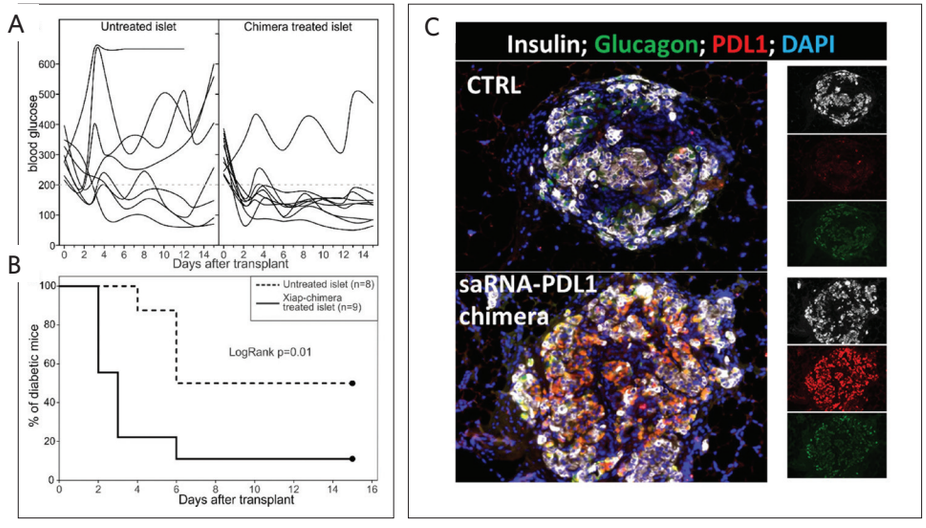


Figure 28. Aptamer-Small Activating RNA Chimeras Improve the Efficacy of Islet Transplantation. *Human islets (500 IEQ) were treated 24h and 48h after isolation with an equimolar mixture of two chimeras (saRNA-751/1-717 and saRNA-751/m12-3773; 420 picomoles each) and immediately transplanted under the kidney capsule of streptozotocin-treated, diabetic, immune-deficient NOG mice. Untreated islets from the same preparation were used as control. Blood glycemia was monitored three times per week. Data are derived from two independent experiments. Blood glucose concentration of the individual mouse (Panel A) and Kaplan Meier curve and log-rank analysis (Panel B) are reported. Panel C: Human islets were transplanted in the epididymal fat pad of immunodeficient mice. Three weeks later, mice were treated intravenously with aptamer chimera specific for PDL1 of control chimera. The grafts and other tissues were evaluated five days later by immune fluorescence microscopy. Data show that treatment with chimeras upregulated PDL1 specifically on the human islets. No activation of this protein was observed in any other tissue. (Courtesy of Dr. Paolo Serafini)*

CELL-BASED STRATEGY FOR BETA CELL TARGETING

The immune system is usually known as the villain in T1D — after all, it is an autoimmune attack on the beta cells that causes the body to lose its only source of insulin and develop disease. However, there is a type of immune cell, the regulatory T cell (Treg), that works to suppress aberrant immune activity. If Tregs could be selectively targeted to beta cells, it might be possible to use them therapeutically to counteract the autoimmune process. Seung Kim, M.D., Ph.D., and co-investigators at Stanford University and Vanderbilt University took a major step toward this goal. They engineered human Tregs to carry a protein on their surface known as a chimeric antigen receptor (CAR); the CAR modification causes Tregs to specifically migrate to human beta cells. These CAR-Tregs can be further engineered to deliver signals for beta cell protection, beta cell regeneration or immune modulation to the islets. To facilitate their research, the team developed a culture system that keeps individual human islets alive in a laboratory setting (Figure 29). The system helps investigators observe and characterize interactions between their modified human CAR-Tregs and a human islet, providing a platform to evaluate CAR-Treg strategies for T1D treatment.

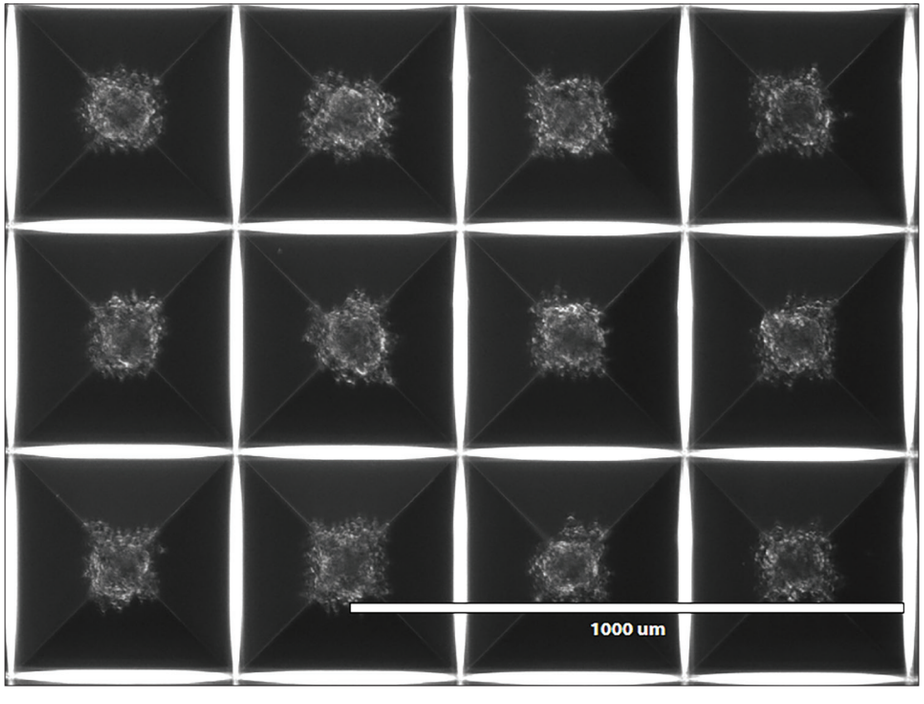


Figure 29. A New Human Islet Culture System. *Pseudoislets develop uniformly in a microwell array, facilitating genetic modulation and functional studies. This culture system can be used to investigate interactions between Tregs and human beta cells. (Photo credit: Drs. Robert Whitener and Seung K. Kim)*

REGENERATING BETA CELL FUNCTION IN OTHER CELL TYPES

A potential strategy for regenerating beta cell function is to stimulate insulin production and secretion in a nonbeta cell type. For this strategy to be viable T1D therapy, such reprogrammed cells would have to make and release insulin at variable levels in response to rising and falling blood glucose levels — a process called glucose-stimulated insulin secretion (GSIS).

Alpha cells are neighbors of beta cells in pancreatic islets and normally make the hormone glucagon. Pedro Herrera, Ph.D., led a team of investigators at the University of Geneva who reprogrammed alpha cells for insulin production and GSIS. The team found that most reprogrammed human alpha cells continued to express glucagon, along with their newly acquired ability to produce insulin. Moreover, the cells displayed appropriate GSIS, releasing insulin in response to increased glucose levels. When transplanted under the kidney capsule of diabetic mice, the reprogrammed cells reversed diabetes and maintained insulin production for at least six months, while ceasing glucagon production (Figure 30). Importantly, the team showed that reprogrammed alpha cells were less vulnerable than beta cells to being killed by autoimmune T cells isolated from patients with recent-onset T1D.

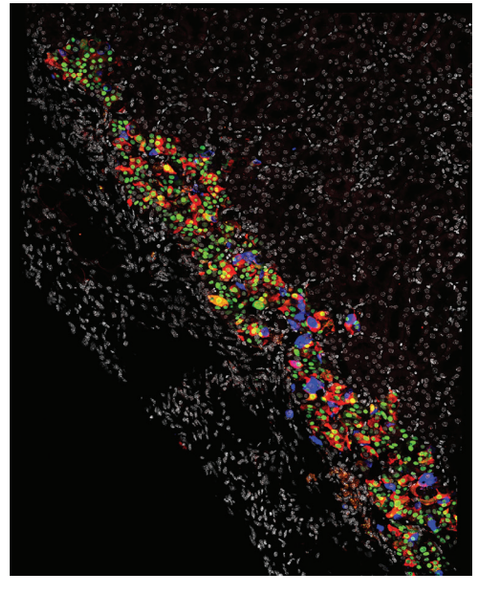


Figure 30. Reprogrammed Insulin-Producing Alpha Cells. *Glucose-sensing and insulin-secreting human alpha cells have a stable functional phenotype, even 6 months after transplantation in mice (renal capsule). Red: INSULIN, blue: GLUCAGON, green: GFP-tracer, white: DAPI. (Photo credit: Dr. Pedro Herrera)*

These findings demonstrate the feasibility of reprogramming human alpha cells as an effective replacement for beta cell function.

Joe Zhou, Ph.D., Harvard University (now at Weill Cornell Medicine), and his co-investigator, Stephan Kissler, Ph.D., of the Joslin Diabetes Center, started with a different cell source for reprogramming — progenitor cells found in the stomach (gastric) lining. The team developed a protocol that successfully redirected the differentiation of the gastric progenitor cells to produce GINS (gastric insulin-secreting) cells by sequentially expressing three proteins, Ngn3, Pdx1 and MafA, associated with beta cell genesis in embryos. Spherical clusters of GINS cells, or GINS organoids, activated insulin expression and GSIS (Figure 31). After transplantation into mice, GINS organoids released human insulin into the bloodstream, reversed

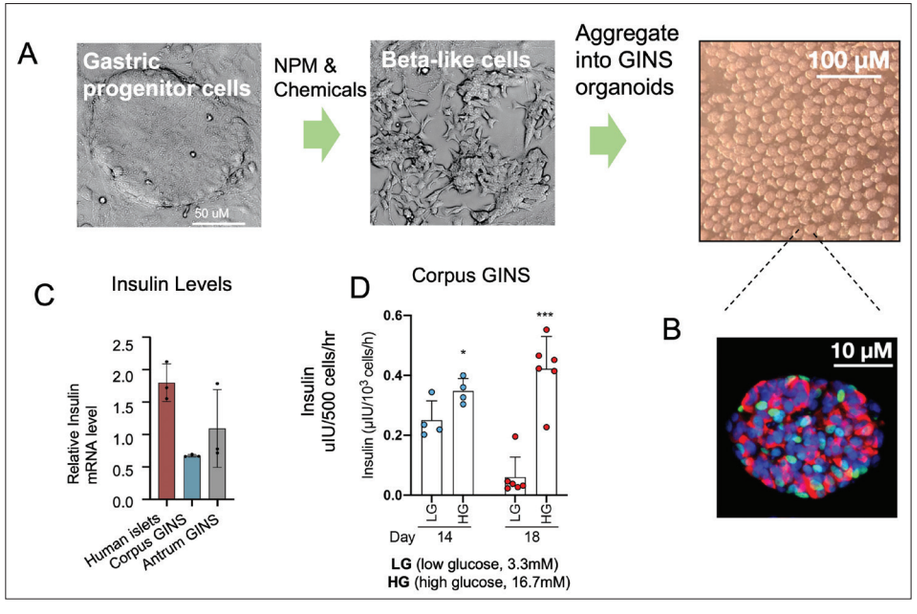
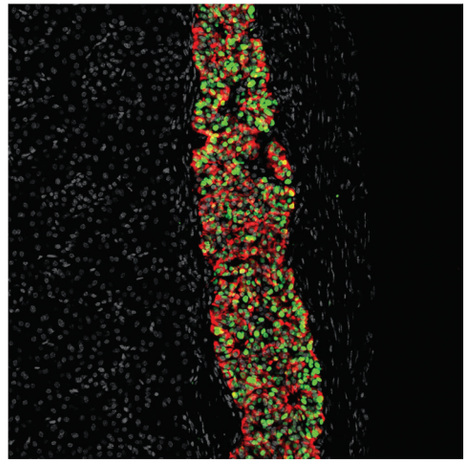


Figure 31. Derivation of Insulin-Producing Cells From Primary Human Gastric Progenitor Cells. *Panel A: The major steps of creating islet-like cell aggregates by reprogramming cultured primary human antral progenitor cells are shown. NPM refers to three proteins (Ngn3, Pdx1 and MafA) that stimulate insulin gene activity. In Aggrewell dishes, the antrum-derived cells form ~50um-in-diameter insulin-positive cell clusters. Panel B: Close-up of an islet-like aggregate derived from reprogrammed human antral progenitor cells. Insulin is shown in green; DNA is stained blue. Panels C and D: The aggregates express insulin and exhibit glucose-stimulated insulin secretion (GSIS) in vitro (six independent experiments). (Courtesy of Dr. Joe Zhou)*

diabetes, and the cells lived for at least three months (Figure 32). The induction method had a success rate of around 20% to 30% at generating beta-like cells in a population of progenitor cells, and the investigators are now evaluating new approaches to increase that rate to over 50%. An advantage of using gastric progenitor cells to reprogram beta cell function is that human stomach tissue discarded from weight loss surgeries provides an abundant supply of this cell type.

Figure 32. Gastric Insulin-Secreting (GINS) Cells Produce Insulin In Vivo. *Section of a kidney graft of corpus GINS (gastric insulin-secreting) cells at 4 month after transplantation. Red: insulin. Green: Mafa. Cell nuclei are stained with DAPI (white). (Photo credit: Dr. Joe Zhou)*



EXPLORING BETA CELL BIOLOGY TO FIND NEW TARGETS FOR REGENERATIVE THERAPY

Sangeeta Dhawan, Ph.D., City of Hope, studied how 5-hydroxymethylcytosine (5hmC), a type of chemical modification of DNA, contributes to beta cell identity and functional development (Figure 33). She found that levels of 5hmC increased as human beta cells mature after birth and that those levels were abnormal in beta cells from individuals with diabetes. Residual beta cells in T1D pancreata had reduced levels of 5hmC, similar to those seen in immature neonatal beta cells. In T2D islets, patterns of 5hmC were disrupted in genes associated with beta cell proliferation, cell movement, hypoxia (oxygen deficiency) and calcium regulation (an important issue for insulin secretion). These studies revealed a novel mechanism of genetic control that is central to beta cell health and function. Working out how this mechanism goes awry in diabetes will help Dhawan search for novel targets within the 5hmC pathway that can be manipulated to promote beta cell function and regeneration.

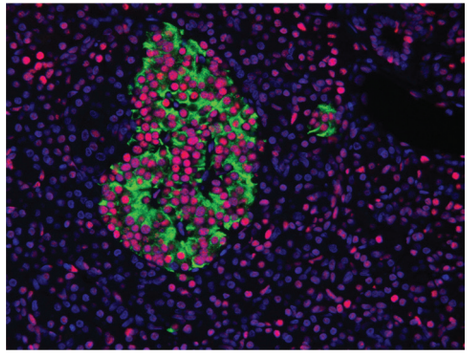


Figure 33. Human Pancreatic Islets.
A snapshot of adult human pancreas, showing an abundance of the DNA modification 5hmC (red) in mature beta cells marked with insulin (green). Blue color marks the nuclei of all the cells in the pancreas. In contrast to islets, most of the non-hormone expressing cells of pancreas have lower levels of 5hmC. (Photo credit: Dr. Sangeeta Dhawan)

Neuromodulation, the alteration of nerve activity at specific sites in the body, is emerging as an exciting treatment for chronic conditions, such as Parkinson's disease, epilepsy and others. Abdelfattah El Ouaamari, Ph.D., Rutgers University, worked toward identifying and characterizing neurons that project into pancreatic islets using a variety of techniques (Figure 34). He uses this information to identify neuronal signals that modulate beta cell functions. For example, he showed that TRVP1 sensory neurons directly control glucose-induced insulin secretion, but only in male mice. Ultimately, El Ouaamari plans to leverage knowledge about neuronal signals to develop neuromodulation approaches to T1D treatment.

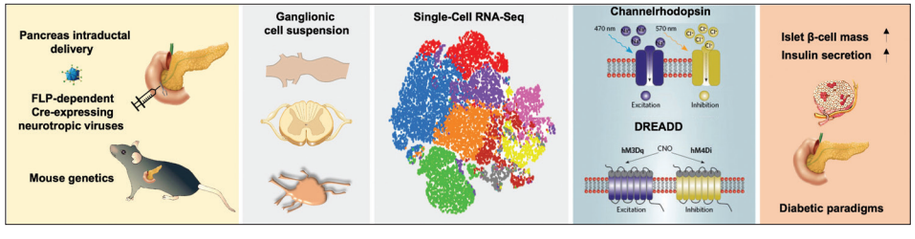


Figure 34. Molecular Profiling of the Pancreas-Projecting Sensory Afferents.
Investigators use a combination of techniques for selective neuromodulation of pancreatic nerves to assess their role in beta cell functions, such as insulin secretion. (Courtesy of Dr. Abdelfattah El Ouaamari)

During the first 10 years of life, pancreatic beta cell mass undergoes a marked expansion, but the ability to proliferate appears to be lost or dormant in adult beta cells. Al Powers, M.D., Vanderbilt University, and a multi-institutional team of investigators conducted an in-depth, interdisciplinary study of juvenile beta cells from human organ donors. Their findings on the

molecular pathways involved in juvenile beta cell proliferation may point to new strategies to stimulate regeneration of mature beta cells in T1D.

Similarly, Klaus Kaestner, Ph.D., University of Pennsylvania, and investigators at the Hadassah-Hebrew University investigated genes associated with beta cell hyperplasia (overgrowth) syndromes, including Focal Hyperinsulinism of Infancy and Beckwith-Wiedemann Syndrome. By understanding how those genes contribute to excessive beta cell proliferation in those syndromes, the team can develop strategies for controlled beta cell growth in the T1D setting.

CTAR INVESTIGATORS, YEARS 5 AND 6

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

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

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

***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*

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***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*

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***Rita Bottino, Ph.D., Co-Investigator,** *Children's Hospital of Pittsburgh*
***Marcela Brissova, M.D., Co-Investigator,** *Vanderbilt University*
***Chunhua Dai, M.D., Co-Investigator,** *Vanderbilt University*
***Peng Wang, Ph.D., Co-Investigator,** *Icahn School of Medicine at 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*
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Joe Zhou, Ph.D., Investigator, *Harvard University*
Stephan Kissler, Ph.D., Co-Investigator, *Joslin Diabetes Center*

**Year 5 only*
†*Year 6 only*

HUMAN PANCREAS ANALYSIS CONSORTIUM

Human Pancreas Analysis Consortium (HPAC) researchers investigate the *physical and functional organization of the human islet* tissue environment, the *cell-cell relationships within the pancreatic tissue* ecosystem and the *contributions of non-endocrine components* (acinar, ductal, vascular, perivascular, neuronal, lymphatic, immune) to islet cell function and dysfunction.

ADVANCES IN HUMAN PANCREAS SLICE TECHNOLOGY ACCELERATE DIABETES RESEARCH

Pancreas slice technology offers a new physiologic model to study human beta cell regeneration — the formation of new beta cells from progenitor cells living in the tissue — with unprecedented accuracy and resolution.

Juan Dominguez-Bendala, Ph.D., and his co-investigators at the University of Miami developed a novel culture system that keeps human pancreas tissue alive and fully functional in the laboratory for more than 10 days. Human pancreas slice technology, which builds on a technique first used with mouse pancreata, is a major advancement in diabetes research technology and dramatically broadens the scope of research that this team and others can pursue. Rather than separate islets from the rest of the pancreas, this technology allows researchers to maintain a large slice or cross-section of a pancreas in a lab dish. In this system, islets remain embedded within the normal pancreas ecosystem in contact with blood vessels and other non-islet cells. Dominguez-Bendala's team collaborated with numerous investigators within HPAC, other Human Islet Research Network (HIRN) consortia and outside of the network, so that human pancreas slice technology — described as a "surrogate window into the human pancreas" — can be widely adopted across the diabetes research community.

The Dominguez-Bendala team described a population of BMP (bone morphogenetic protein)-responsive progenitors that reside within human pancreatic ducts. When sorted and transplanted into immunodeficient mice, these cells not only differentiate along all the adult pancreatic lineages but, remarkably, self-organize into "micro-organs" that resemble anatomically

the native pancreas (Figure 35). These progenitors are preserved in human pancreas slices, where addition of a BMP receptor agonist led to the formation of new beta cells, which could be monitored in real time. Beta cell regeneration from ductal progenitors was seen in pancreas slices from nondiabetic, autoantibody-positive, type 1 diabetes (T1D) and T2D donors. The ability to conduct this line of research in human tissue is an exciting leap forward from mouse studies and may, in the future, facilitate progress of regenerative therapies from lab tests to clinical trials.

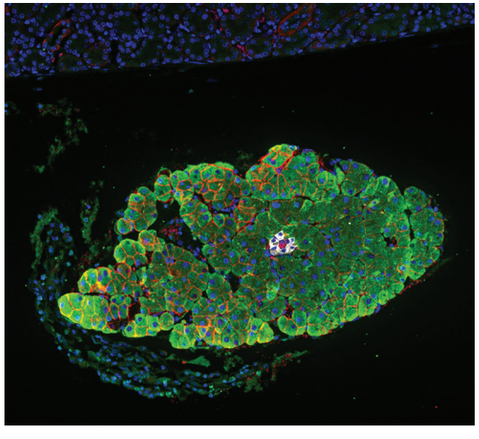
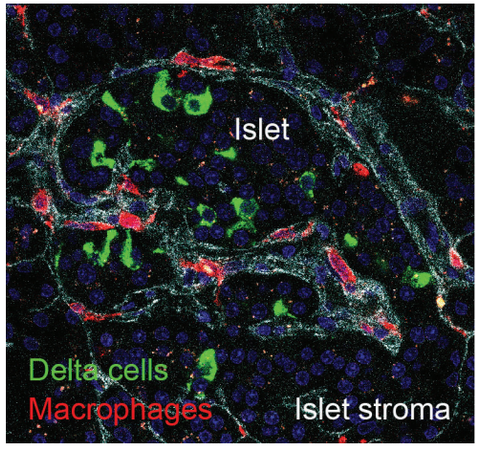


Figure 35. Human Pancreas Progenitor Cells Form All Types of Pancreas Cells When Transplanted in Mice. *Human pancreatic cells sorted for the markers ALK3 (a BMP receptor) and P2RY1 (a surrogate surface marker for PDX1), predicted to be progenitor-like, differentiate into cells of all adult pancreatic lineages upon transplantation in immunodeficient mice. Shown in the picture is an epithelial cluster (E-CAD, red) of well-formed acinar cells (AMY, green) and a small endocrine aggregate (INS, grey) under the kidney capsule of nu/nu mice. While some regions of the graft did not exhibit mature histological patterns, regions such as the one depicted in this figure, with fully developed acini and small endocrine clusters, show a remarkable degree of organization considering that only single cells sorted for ALK3 and P2RY1 (none of which is expressed in human acinar tissue) were transplanted. (Photo credit: Dr. Juan Dominguez-Bendala)*

Alejandro Caicedo, Ph.D., University of Miami, and his collaborators at Vanderbilt University and the Paul Langerhans Institute Dresden used human pancreas slice technology to compare multiple features of islet structure and function among four pancreata from T1D donors and 19 from nondiabetic donors. The team found that beta cell loss, abnormal beta cell function, changes in beta cell physiology and infiltration of islets by immune cells contributed differently to each individual case of T1D. For example, a pancreas from a donor with adult-onset T1D had a fairly typical amount of beta cells compared to nondiabetic donors, but those beta cells were not working correctly. In contrast, two donors with childhood-onset T1D had very few beta cells in their pancreata and nearly complete loss of beta cell

function. These observations suggest that multiple pathways may lead to the same outcome — T1D development — in susceptible individuals. In a related study, the team characterized the anatomy and function of macrophages, immune cells involved in inflammation, that reside in human islets (Figure 36). They discovered that macrophages are controlled by signals sent out from beta cells, opening up a new avenue for research on how inflammation contributes to the development of T1D and T2D.

Figure 36. Macrophages in Human Islets. *Confocal image of a human pancreas section showing macrophages (red) residing in the tissue surrounding an islet (the islet stroma). Somatostatin-producing delta cells are labeled in green. (Photo credit: Dr. Alejandro Caicedo)*



The microvasculature is an extensive network of small blood vessels (capillaries) that weave around and through the islets (Figure 37). These blood vessels serve as entry points into the bloodstream for insulin and other hormones made in the islets; once in the bloodstream, those hormones circulate to tissues and organs throughout the body. Joana Almaca, Ph.D., of the University of Miami studied specialized cells, known as pericytes, that wrap around capillaries and asked whether loss of those pericytes from the islet microvasculature over time contributes to the development of T1D. Using living pancreas slice technology, she carried out a cutting-edge, detailed functional analysis of the human islet microvasculature in nondiabetic pancreata. She discovered that pericytes help regulate the flow of blood through the islet microvasculature. With this new understanding from healthy islets, Almaca can now analyze pancreas tissue from donors at different stages of T1D to determine how pericyte function is disrupted before and during disease onset.

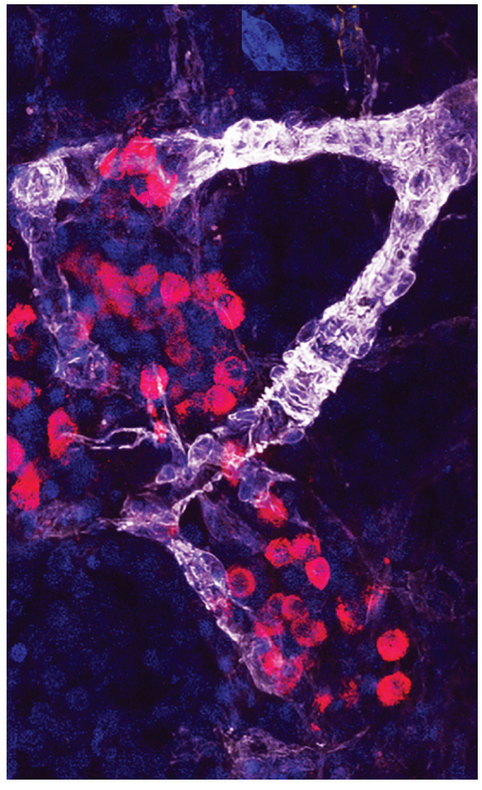


Figure 37. Microvasculature in a Human T1D Islet. *Maximal projection of confocal images were obtained from an islet from a type 1 diabetic individual (disease duration 2.5 years). Delta cells are shown in red (immunostained for somatostatin), and blood vessels are shown in white (stained for alpha smooth muscle actin). (Photo credit: Dr. Joana Almaca)*

SINGLE-CELL TECHNOLOGIES MAY UNCOVER THE MOLECULAR BASIS OF BETA CELL HETEROGENEITY

HPAC researchers applied single-cell technologies to map out functional and structural differences — "heterogeneity" — among beta cells. Their goal was to understand how and why beta cells vary within an islet, across a range of conditions, such as age or the spectrum from glycemic health to autoimmunity and T1D, or among individuals.

Maike Sander, M.D., and her colleagues at the University of California, San Diego and Vanderbilt University used novel single-cell analysis technologies to determine how T1D changes the existence, composition, regulation and connections among pancreatic cell types. By measuring chromatin accessibility and transcriptomic profiles (both are indications of which genes are active in a particular cell), the team identified 13 distinct cell types in human pancreatic tissue (Figure 38), including immune

cells (macrophages and T cells) present in the pancreas. Ongoing study of those rare cells may provide clues about why immune cells attack beta cells during T1D development. After validating the technologies, the team looked at pancreas tissue from nine T1D, four autoantibody-positive and nine nondiabetic donors. One striking finding was that beta cell gene activity changes in autoantibody-positive people who are at high risk for developing T1D, but do not yet have the disease, compared to people without diabetes or T1D-related autoantibodies. Studying those early, pre-diabetes changes can help the team understand how beta cells contribute to their own demise during T1D development.

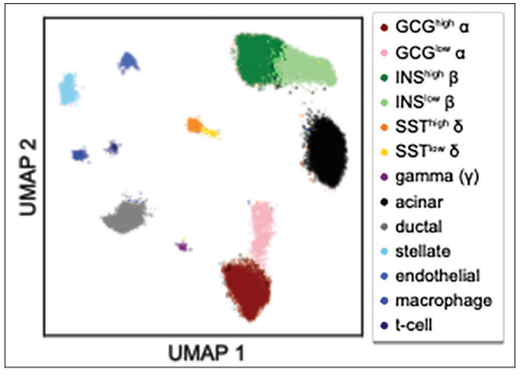


Figure 38. Human Pancreatic Cell Types Identified by Single-Cell Technologies. *Clustering of accessible chromatin profiles from pancreatic cells identifies 13 distinct clusters. Cells are plotted using the first two UMAP components, and clusters are assigned cell type identities based on promoter accessibility of known marker genes for each cell type. UMAP is a method for dimension reduction of complex data. Islet (alpha, beta, delta, gamma), exocrine (acinar), ductal, stellate, blood vessel (endothelial) and immune (macrophage, T cell) cell types are present. (Courtesy of Dr. Maike Sander)*

Patrick MacDonald, Ph.D., University of Alberta, and an international team of collaborators combined two single-cell techniques on more than 1,300 islet cells from 34 pancreas donors with or without diabetes. Patch-clamp electrophysiology measures the electrical properties of a cell, and RNA-sequencing reports which genes are active. Together, the "Patch-seq" technology helped the researchers directly link physiologic dysfunction to changes in gene expression in alpha and beta cells (Figure 39). Among their findings, the team identified key genes and multiple biochemical pathways that are more strongly activated in alpha and beta cells of T1D and T2D versus nondiabetic pancreata. These changes in defined sub-groups of cells might point to the underlying causes of altered insulin and glucagon secretion in diabetes. The team's datasets from this work were made available to the research community for additional analysis.

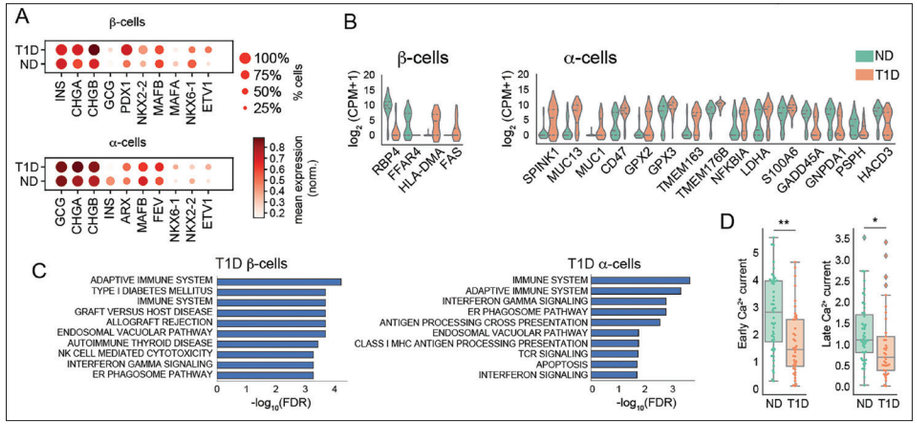


Figure 39. Assessment of Islet Cells From Donors With T1D and No Diabetes (ND). *Islet cells were obtained from the Alberta Diabetes Institute IsletCore cryo-bank (www.isletcore.ca) and analyzed by Dual Patch-Clamp scRNAsequencing. Panel A: Expression of key identity genes on alpha- and beta-cells from T1D and ND matched controls. Panel B: Representative genes obtained in a differential expression analysis between T1D and ND for beta and alpha cells, respectively. Panel C: Pathways enriched in up-regulated genes in T1D alpha and beta cells. Panel D: Distribution of early and late voltage-dependent calcium currents showing statistically significant differences between alpha cells of T1D and ND. (Courtesy of Dr. Patrick MacDonald; Camunas-Soler et al., Cell Metab. 2020 31(5):1017-1031. e4. doi: 10.1016/j.cmet.2020.04.005. PMID: 32302527)*

LOOKING TO THE FUTURE

In Year 7, the HPAC welcomes Jing Hughes, M.D., Ph.D., of Washington University School of Medicine in St. Louis. Hughes is a Gateway Investigator who is establishing a career focused on key questions in islet cilia research. A summary of her research plan, entitled *"Primary Cilia in Human T1D Pancreas,"* can be found in the chapter "Fostering New Talent in the Human Islet/Beta Cell Research Community."

The Human Pancreas Analysis Program (HPAP), a subset of the HPAC, performs deep phenotyping of the human endocrine pancreas and its interaction with the immune system to better *understand the cellular and molecular events that precede and lead to beta cell loss* in T1D and type 2 diabetes (T2D). The primary goal of HPAP is to *accumulate, analyze and distribute high value T1D and T2D datasets* to the diabetes research community.

BUILDING RESOURCES FOR THE HUMAN DIABETES RESEARCH COMMUNITY

HPAP is a highly integrated and collaborative effort to acquire, analyze and distribute data from rare human donor pancreatic tissue for the benefit of the entire diabetes research community (Figure 40). First, the HPAP-T1D team at the University of Florida identifies a pancreas of interest that becomes available through the national organ donor system. Rare pancreata from individuals with recent-onset T1D or who have autoantibodies that indicate a high risk of developing T1D and those with T2D are collected, along with age-matched control organs. Once an appropriate donor is identified, the pancreas and associated immune organs (spleen, lymph nodes, duodenum) are shipped to the University of Pennsylvania for processing and islet

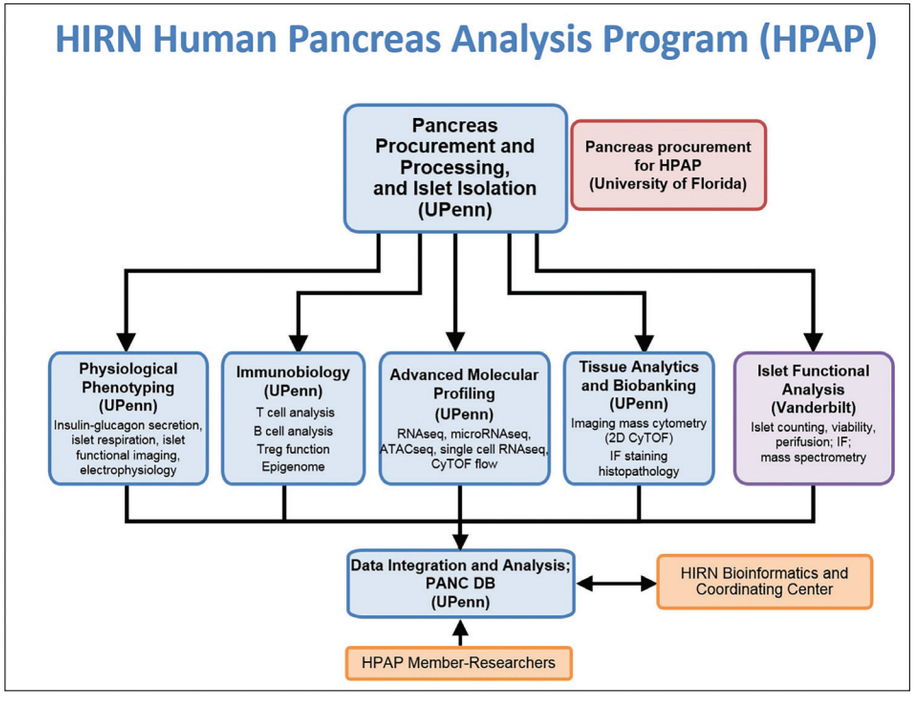


Figure 40. Human Pancreas Analysis Program (HPAP) Workflow. *HPAP is a highly integrated, collaborative effort among the University of Florida (pancreas procurement), the University of Pennsylvania (pancreas procurement, processing, islet isolation, multifaceted data collection and tissue biobanking) and Vanderbilt University (islet function analyses). The resulting data are made available to investigators for research on human T1D, T2D and islet/beta cell biology.*

isolation. Tissues and islets are then sent to investigators at the University of Pennsylvania, Vanderbilt University, Stanford University and the University of Alberta for functional and structural studies. Extensive and detailed datasets are collected and deposited into PANC-DB and Pancreatlas™, as described below, and made available to the research community

HPAP-T1D expanded its outreach efforts during 2019 and 2020. The number of labs screening for organ donors for autoantibodies increased from 10 to 12, and 32 organ procurement organizations tested potential donors for diabetes-related autoantibodies, up from 30 in 2018. The University of Florida team captured an estimated 63% of the U.S. organ donor pool compared to 59% in 2018. In 2019, the autoantibody screening program yielded six cases of pre-T1D donors versus three in 2018. In 2020, operations were impacted by the COVID-19 pandemic; yet, HPAP-T1D was able to find five T1D and two autoantibody-positive cases. Identifying and collecting these rare donor pancreata provides a critical resource for human T1D research that would not be possible without HPAP's highly cooperative organization and teamwork.

New to this reporting period, HPAP expanded to include collection of pancreata from donors with T2D, prediabetes and age-matched controls. Scientifically diverse groups led by Al Powers, M.D., Vanderbilt University, and Klaus Kaestner, Ph.D., University of Pennsylvania, joined HPAP to accomplish this new task. The HPAP-T2D workflow follows the path already established for procurement and analysis of T1D pancreata (Figure 40). Suitable pancreata are shipped to the University of Pennsylvania for processing and islet isolation. Pancreas tissue or islets are then sent to investigators at Vanderbilt University Medical Center, University of Pennsylvania, Stanford University and the University of Alberta for research and data collection. Despite delays due to the COVID-19 pandemic, the teams procured five pancreata from individuals with T2D and eight from matched individuals without diabetes by the end of September 2020, which exceeded their goal of 12 pancreata in the first year of HPAP-T2D operation.

For every organ procured through HPAP, pancreas tissue, immune tissues and isolated islets are distributed to multiple core laboratories that conduct standard tests. HPAP labs continually refine their workflow as new technologies, such as RNA sequencing, epigenetics and measurements

of DNA methylation, emerge. All data, including donor characteristics, islet function analyses, immune assays and other parameters, are deposited into a public database, PANC-DB (https://hpap.pmacs.upenn.edu/about-pancdb; Figure 41). Diabetes investigators from anywhere in the world have immediate access to the data, ensuring that these rare tissues are studied to their fullest potential from a variety of scientific perspectives. HPAP investigators at the University of Pennsylvania develop and maintain PANC-DB, integrating user feedback and newly developed bioinformatics tools.

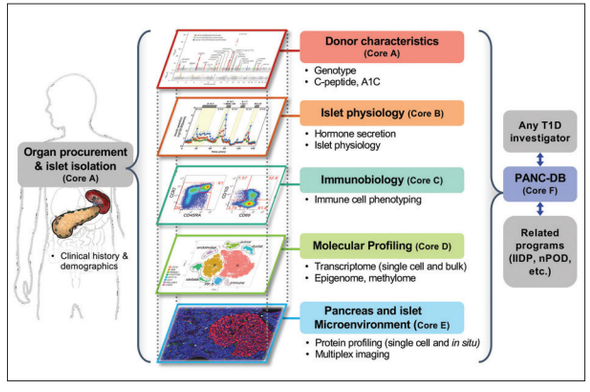


Figure 41. PANC-DB: Co-Registration of Multi-Modal Datasets by HPAP. *PANC-DB (https://hpap.pmacs.upenn.edu/about-pancdb) stores clinical, molecular, cellular, immunologic, imaging and pathology data from all pancreata procured and processed by HPAP. PANC-DB can be accessed by any diabetes investigator, and the database regularly interacts with related programs that investigate human diabetes tissues, such as the Integrated Islet Distribution Program (IIDP) and the Network for Pancreatic Organ Donors with Diabetes (nPOD). (Courtesy of Dr. Ali Naji)*

At Vanderbilt University, a team of bioinformatics specialists, software developers and islet biologists developed an online image database called Pancreatlas™, containing more than 800 images from young, adult and T1D pancreata (www.pancreatlas.org; Figure 42). The development of Pancreatlas™, which was sponsored by the HIRN and other funders, complements PANC-DB and addresses a significant need in the diabetes research community. Deep phenotyping of human tissues, such as the pancreas, generates complex spatial information from a variety of microscopy technologies. Phenotyping is the process of defining the physical, biochemical, developmental, behavioral or other observable characteristics of a cell, tissue or organism. Yet, images created with these technologies must be compressed into small, static figures for publication, and large amounts of valuable data never become available to the community. These images reside in large files that are not easily shared or transferred between labs. Now, Pancreatlas™ allows users to access curated, easy-

to-navigate webpages, drill down to individual images and interact with those images online, without the need to download large files or install specialized software. All images come with annotated data, enabling users to build their own datasets with biological and clinical relevance and ask novel research questions. This exciting new resource greatly expands the impact of the HPAP to diabetes researchers without direct access to rare T1D tissue or expensive, state-of-the-science microscopy technologies.

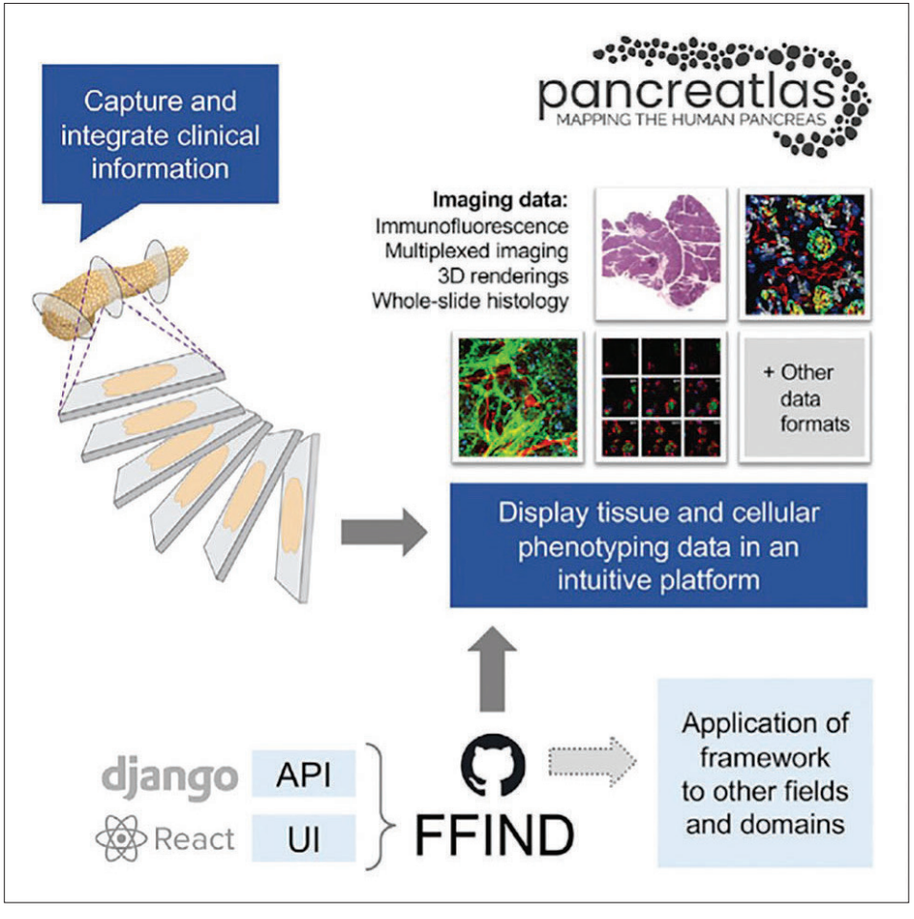


Figure 42. Pancreatlas™. *The Pancreatlas™ (www.pancreatlas.org) is an online image resource that provides access to large, complex imaging data files from young, adult and T1D human pancreata. FFIND, Flexible Framework for Integrating and Navigating Data, is a web application and novel interface that help users access and interact with individual images in the database. (Courtesy of Dr. Alvin C. Powers; Saunders et al. Patterns (N Y). 2020. 1(8):100120. doi: 10.1016/j.patter.2020.100120. PMID: 33294866)*

ACCESS TO HUMAN T1D OR T2D PANCREATA ENABLES PARADIGM-SHIFTING RESEARCH

In addition to creating widely used data resources for human diabetes research, HPAP teams reported findings from their own in-depth analyses of human pancreas tissue.

At the University of Pennsylvania, a group led by Klaus Kaestner, Ph.D., was among the first to use Imaging Mass Cytometry (IMC) to study the human pancreas. IMC is a powerful technique to visualize and quantify more than 30 types of proteins simultaneously and provide a cell-by-cell level view of tissue environments. In a study of T1D versus nondiabetic pancreata, researchers observed significant changes in islet architecture — T1D islets were smaller and more "ragged" than their nondiabetic counterparts. They also found differences in the cellular composition of T1D islets compared to nondiabetic islets and measured an increase in

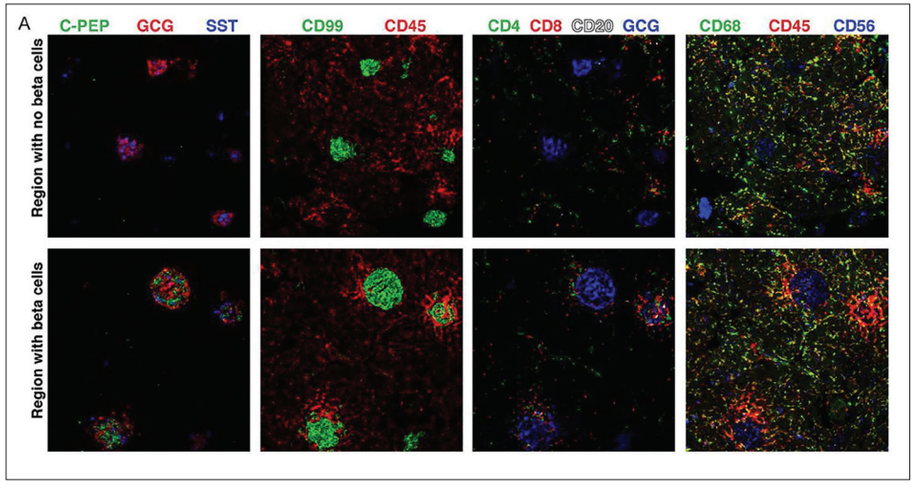


Figure 43. Imaging Mass Cytometry (IMC) Analysis of Human Pancreas Tissue. *Selected channel overlays for human pancreatic regions from a donor with recent-onset T1D, consisting of islets with no beta cells (upper panels) or with beta cells (lower panels). Islets with remnant beta cells exhibit increased peri- and intra-islet accumulation of immune cells. Displayed channels are: left, C-peptide (C-PEP; green), glucagon (GCG; red), and somatostatin (SST; blue); middle-left, CD99 (green), and CD45 (red); middle-right, CD4 (green), CD8 (red), CD20 (white) and GCG (blue); right, CD68 (green), CD45 (red) and CD56 (blue). Apart from being an NK-cell marker, CD56 also labels neurons and islets (right panels). (Courtesy of Dr. Klaus Kaestner; Wang et al. Cell Metab. 2019 Mar 5;29(3):769-783.e4. doi: 10.1016/j.cmet.2019.01.003. Epub 2019 Jan 31. PMID: 30713110)*

immune cells, particularly macrophages, within T1D islets. These studies provided proof-of-principle that IMC can be a valuable tool to investigate the complexity of T1D pathogenesis within intact human islets (Figure 43).

The whole pancreas is smaller in a person with T1D than in a nondiabetic person. The beta cells lost in T1D represent a tiny portion of the pancreas (~3%–4%). Thus, the change in pancreas size is likely due to a decrease in the exocrine pancreas, the part that makes and releases digestive enzymes. Investigators led by Al Powers, M.D., studied pancreata from adults with T1D and age-matched, nondiabetic donors. They found that T1D pancreata were 45% smaller, regardless of diabetes duration or age of onset. Islets in the T1D pancreata had fewer beta cells, as expected, but a typical number of alpha and delta cells. T1D organs had more fibrosis in the exocrine tissue (Figure 44), and the difference in overall pancreas size could be mostly explained by fewer numbers of acinar cells (the ones that make digestive enzymes), rather than smaller acinar cells, compared to nondiabetic pancreata. These changes seem to happen early in T1D development. The team's findings challenge the long-standing paradigm that T1D is a beta cell-specific disease; more research is needed to understand what this means for T1D pathogenesis.

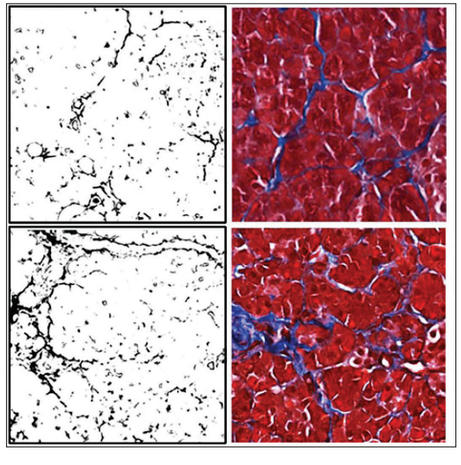
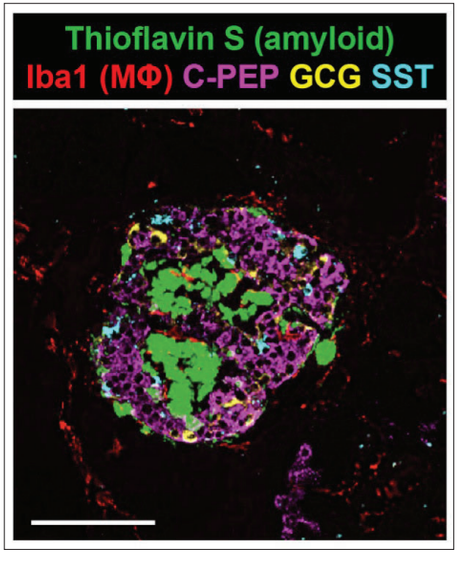


Figure 44. Altered Acinar Cell Number and Extracellular Matrix in Donors With T1D Pancreata. *Pancreas fibrosis was measured after Masson's trichrome stain in T1D and nondiabetic pancreases, showing analysis markup (left side) and high-magnification inset (right side). (Photo credit: Dr. Alvin C. Powers; Wright et al. Diabetologia. 2020. 63(7):1418-1423. doi: 10.1007/s00125-020-05155-y. PMID: 32388592)*

Groups led by Al Powers, M.D., and Seung Kim, M.D., Ph.D., Stanford University, applied CODEX technology to study the human pancreas (Figure 45). CODEX is a powerful new technology that can capture images

of more than 40 different proteins in a 100 mm^2 area of tissue (less than one-third the size of a penny). This size is big enough to include an entire islet, and it allows researchers to characterize the protein complement of islet cells in detail and to document the location of rarer cell types, such as immune cells (e.g., macrophages or T cells), within the islet. The teams worked through several technical challenges, including optimizing image resolution and quality for human pancreas tissue. In addition, they validated key antibodies that can be used to measure the amount of specific proteins in T2D pancreata versus nondiabetic pancreata. This ongoing HPAP-T2D study is a leader in the use of CODEX with human T2D pancreas tissue.

Figure 45. CODEX Panel Validation in Control (Nondiabetic) Tissue. *Co-registration of amyloid stain (Thioflavin S; green) with CODEX antibodies that mark specific cell types in a pancreatic islet (Iba1, macrophages; C-peptide [C-PEP], beta cells; glucagon [GCG], alpha cells; somatostatin [SST], delta cells). With CODEX technology, pictures of the islet are taken for each antibody; those pictures are then co-registered or integrated into a single image that reveals where different cell types reside in the islet. (Photo credit: Dr. Alvin C. Powers)*



Klaus Kaestner, Ph.D., and his colleagues investigated individual islets from nondiabetic individuals of all ages and from people with T2D using single cell RNA sequencing. They found that the developmental path of glucagon-producing alpha cells differs from that of the insulin-producing beta cells. In addition, they discovered that beta cells from people with T2D resemble those of juvenile beta cells rather than mature, adult cells (Figure 46). These findings are consistent with the maturation of beta cells over the first few years of life and with the loss of beta cell function that happens in T2D. Knowing how beta cell gene expression patterns change during T2D may point to new targets for therapeutic intervention.

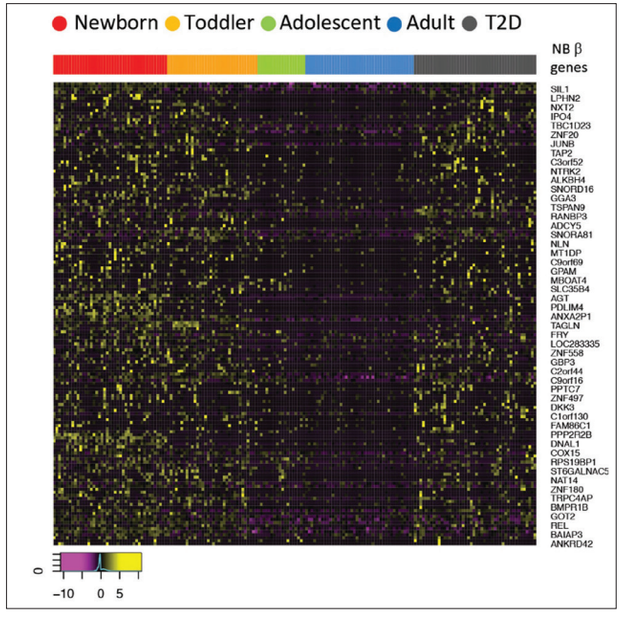


Figure 46. Gene Expression in T2D Beta Cells Revert From Adult to Neonatal Patterns. *Heatmap of single cell RNAseq data from beta cells of organ donors ranging in age from 18 days to adulthood and those with T2D demonstrates that T2D beta cells revert to a neonatal gene expression profile. Gene names are listed on the right side of the figure. Magenta is low, black is intermediate, and yellow is high expression. (Courtesy of Dr. Klaus Kaestner; Avrahami et al. Mol Metab. 2020. 42:101057. doi: 10.1016/j.molmet.2020.101057. PMID: 32739450)*

HPAC INVESTIGATORS, YEARS 5 AND 6

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

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

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, Ph.D., Investigator, *Stanford University*
Seung Kim, M.D., Ph.D., Co-Investigator, *Stanford University*
Linford Briant, Ph.D., Collaborator, *University of Oxford*

Patrik Rorsman, Ph.D., Collaborator, *University of Oxford*
Marjan Slak Rupnik, Ph.D., Collaborator, *Medical University of Vienna*
Andraz Stozer, M.D., Ph.D., Collaborator, *University of Maribor*

Maike Sander, M.D., Investigator, *University of California, San Diego*
Kyle Jeffrie Gaulton, Ph.D., 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*
Sebastian Preissl, Ph.D., Co-Investigator, *University of California, San Diego*

HPAP-T1D

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*

HPAP-T2D

***Klaus Kaestner, Ph.D., Investigator,** *University of Pennsylvania*
***Ben Voight, Ph.D., Investigator,** *University of Pennsylvania*
***Michael Feldman, M.D., Ph.D., Co-Investigator,** *University of Pennsylvania*
***Jason Moore, Ph.D., Co-Investigator,** *University of Pennsylvania*
***Robert Faryabi, 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., Investigator,** *Vanderbilt University*
***Chunhua Dai, M.D., Co-Investigator,** *Vanderbilt University*
***Anna Golyn, Ph.D., Investigator,** *Stanford University*

***Dirk Homann, M.D., Investigator,** *Icahn School of Medicine at Mount Sinai*
***Seung Kim, M.D., Ph.D., Investigator,** *Stanford University*
***Patrick MacDonald, Ph.D., Investigator,** *University of Alberta*

**Year 6 only*

New in HIRN Year 7

Jing Hughes, M.D., Ph.D., Gateway Investigator, *Washington University School of Medicine in St. Louis*

ACKNOWLEDGMENTS

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APPENDIX 1
SUMMARY OF OPPORTUNITY POOL PROJECTS

	PROJECT TITLE	PRINCIPAL INVESTIGATOR	INSTITUTION
Completed Projects — Year 6			
CTAR	New Immunodeficient Mouse Model with Stable Hyperglycemia for the Study of Human Beta Cells	Klaus Kaestner	University of Pennsylvania
Completed Projects — Year 5			
CBDS CTAR	Mass Spectrometry-Based Proteome Maps for Human Islet Cells	Wei-Jun Qian	Pacific Northwest National Laboratory
		Klaus Kaestner	University of Pennsylvania
CMAI	Characterization of In Silico Reconstruction of TCRs for Modeling Autoreactive T cells in T1D	Todd Brusko	University of Florida
		Sally Kent	University of Massachusetts Medical School
		Maki Nakayama	University of Colorado Denver
CTAR CBDS	Antibodies for Beta Cell Subtype Identification by Immunohistochemistry	Markus Grompe	Oregon Health & Science University
CHIB	Quantitative Mass Spectrometry Analysis of Human Islet and Pancreas ECM	Karen Christman	University of California San Diego
CBDS CHIB	Workshop for Continued Harmonization of Beta Cell Death Assays	Carmella Evans-Molina	Indiana University
CHIB	Real-time Detection of Insulin Surrogate Markers Within Physiomimetic Islet Microsystems	Ashu Agarwal	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
CMAI 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	Dirk Homann	Icahn School of Medicine at Mount Sinai
		Andrew Stewart	

	PROJECT TITLE	PRINCIPAL INVESTIGATOR	INSTITUTION
Completed Projects — Year 5			
CHIB	Functional Testing of Candidate HSC-Derived Islet Cells	Maike Sander	University of California, San Diego
CMAI	Islet-Reactive TCR Clones in Humanized Mice Generated With Type 1 Diabetes Patient vs. Healthy Control Hematopoetic Progenitor Cells	Megan Sykes	Columbia University
		Todd Brusko	University of Florida
CTAR	The Role of Beta Cell Senescence in the Pathogenesis of Diabetes	Benjamin Glaser	Hadassah-Hebrew University
CBDS CTAR	Can Genomic 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

Opportunity Pool funds were issued for CHIB In-Person Meetings annually in 2015–2018. In this reporting period, a meeting was held at Harvard University in Cambridge, MA, on December 6–7, 2018. Funds were used to support travel awards for 12 trainees. In addition, Opportunity Pool funds were used to award HIRN 2020 Trainee Scholarships to nine graduate students and postdoctoral fellows who submitted abstracts for the Human Islet Research Network Annual Investigator Meeting 2020. The Meeting Planning Committee chose the scholarship awardees based on a competitive review of all submitted abstracts.

APPENDIX 2
LIST OF NEW INVESTIGATOR PROGRAM AWARDEES[3]

	PROJECT TITLE	RECIPIENT	INSTITUTION
New Investigator Pilot Awards			
CBDS	Real-Time in Vivo Analysis of Islet Redox Dynamics	Amelia Linnemann	Indiana University
CBDS	Elucidating the Human Beta Cell Translatome in Health and Disease	Holger Russ	University of Colorado, Denver
CMAI	HLA Multimer Based Characterization of Islet Resident CD4+ T Cells that Target Beta Cell Epitopes and Neo-epitopes	Eddie James	Benaroya Research Institute
CTAR	Targeting DNA Hydroxymethylation to Promote Human Beta Cell Function	Sangeeta Dhawan	City of Hope
CTAR	Neuromodulation for T1D: Harnessing Sensory Innervation to Promote Regeneration and Function of Insulin-Producing Cells	Abdelfattah El Ouaamari	Rutgers University
HPAC	Changes in Human Islet Microvasculature During Type 1 Diabetes	Joana Almaca	University of Miami
HPAC	Changes in Human Islet Micro-vascularization During Type 1 Diabetes*	Joana Almaca	University of Miami
New Investigator Gateway Awards for Collaborative T1D Research			
CBDS	Mapping the Association of Beta Cell Longevity and Senescence in T1D	Rafael Arrojo e Drigo	Vanderbilt University
CBDS	Functional and Molecular Characterization of the Human Islet Interferon Alpha Response	Amelia Linnemann	Indiana University

	PROJECT TITLE	RECIPIENT	INSTITUTION
New Investigator Gateway Awards for Collaborative T1D Research			
CMAI	Identification of the Cognate Epitopes of Autoreactive T Cells in T1D	Alok Joglekar	University of Pittsburgh
HPAC	Primary Cilia in Human T1D Pancreas	Jing Hughes	Washington University School of Medicine in St. Louis

[3] The New Investigator Pilot Award program was funded by Human Islet Research Network Opportunity Pool funds. The New Investigator Gateway Awards for Collaborative T1D Research were funded directly by the National Institute of Diabetes and Digestive and Kidney Diseases. All awards were based on competitive review of applications.

APPENDIX 3
WORKING GROUPS

2019 ANNUAL MEETING PLANNING COMMITTEE (YEAR 5)

Doris Stoffers (Co-chair), HPAC, *University of Pennsylvania*
Bridget Wagner (Co-chair), CTAR, *Broad Institute*
Chris Wright (Co-chair), CBDS, *Vanderbilt University*
Charles Ansong, CBDS, *Pacific Northwest National Laboratory*
Annie Bowles, CHIB, *University of Miami*
Mike Roper, CHIB, *Florida State University*
Audrey Parent, CMAI, *University of California San Francisco*
Eddie James, CMAI, *Benaroya Research Institute*
Aaron Michels, CMAI, *University of Colorado Denver*
Abdel El Ouaamari, CTAR, *Rutgers University*
Seung Kim, CTAR, *Stanford University*
Marcela Brissova, HPAC, *Vanderbilt University*
Joyce Niland, CC, *City of Hope*
Nelly Berger, CC, *City of Hope*
Layla Rouse, CC, *City of Hope*
John Kaddis, BC, *City of Hope*
Alvin C. Powers, TNC, *Vanderbilt University*
Kristin Abraham, *NIH NIDDK*
Olivier Blondel, *NIH NIDDK*
Sheryl Sato, *NIH NIDDK*

2020 ANNUAL MEETING PLANNING COMMITTEE (YEAR 6)

Todd Brusko (Co-chair), CHIB, *University of Florida*
Doris Stoffers (Co-chair), HPAC, *University of Pennsylvania*
Bridget Wagner (Co-chair), CTAR, *Broad Institute*
Klaus Kaestner, CBDS, *University of Pennsylvania*
Amelia Linnemann, CBDS, *Indiana University*
Holger Russ, CBDS, *University of Colorado Denver*
Paul Gadue, CHIB, *Children's Hospital of Philadelphia*
Ed Phelps, CHIB, *University of Florida*
Rhonda Bacher, CHIB, *University of Florida*
Sally Kent, CMAI, *University of Massachusetts Medical School*
Qizhi Tang, CMAI, *University of California San Francisco*
Nichole Danzl, CMAI, *Columbia University*
Hiro Nakai, CTAR, *Oregon Health and Science University*
Midhat Abdulreda, CTAR, *University of Miami*
Sangeeta Dhawan, CTAR, *City of Hope*
Stephan Speier, HPAC, *Paul Langerhans Institute Dresden*
Rafael Arrojo e Drigo, HPAC, *Salk Institute*
Diane Saunders, HPAC, *Vanderbilt University*
Joyce Niland, HIREC, *City of Hope*
John Kaddis, HIREC, *City of Hope*
Nelly Berger, HIREC, *City of Hope*
Layla Rouse, HIREC, *City of Hope*
Kristin Abraham, *NIH NIDDK*
Olivier Blondel, *NIH NIDDK*
Sheryl Sato, *NIH NIDDK*

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