Challenges and Opportunities for Understanding the Pathogenesis of Type 1 Diabetes: An Endocrine Society Scientific Statement

Aaron W. Michels[1], Todd M. Brusko[2], Carmella Evans-Molina[3], Dirk Homann[4], Sarah J. Richardson[5], Alvin C. Powers[6,7,8]

[1]University of Colorado School of Medicine, Anschutz Campus, Denver, CO
[2]Departments of Pathology and Pediatrics, Diabetes Institute, College of Medicine, University of Florida, Gainesville, FL
[3]Indiana University School of Medicine, Indianapolis, IN and the Roudebush VA Medical Center, Indianapolis IN
[4]Mt Sinai School of Medicine, New York NY
[5]Islet Biology Exeter (IBEx), Department of Clinical and Biomedical Sciences, University of Exeter Medical School, Exeter, UK
[6]Division of Diabetes, Endocrinology and Metabolism, Department of Medicine, Vanderbilt University Medical Center, Nashville, Tennessee, USA
[7]Department of Molecular Physiology and Biophysics, Vanderbilt University School of Medicine, Nashville, Tennessee, USA
[8]VA Tennessee Valley Healthcare System, Nashville, Tennessee, USA

Correspondence:
Alvin C. Powers
al.powers@vumc.org
and
Aaron W. Michels
aaron.michels@cuanschutz.edu

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ORCID:
0000-0003-1941-5786 (ACP)
0000-0003-2878-9296 (TMB)
0000-0001-7764-8663 (CEM)
0000-0002-7622-5754 (DH)
0000-0002-1160-6062(SJR)
0000-0003-3766-5244 (AM)

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ABSTRACT

The discovery of insulin transformed type 1 diabetes (T1D) from a lethal disease to a chronic health condition where individuals can lead long and productive lives. However, T1D is still associated with considerable morbidity and mortality, underscoring the need for disease-modifying therapies to delay clinical onset and preserve residual pancreatic β cell function in those newly diagnosed with T1D. Notably, the first disease-modifying therapy (teplizumab, a monoclonal antibody targeted to CD3 on T lymphocytes) was approved by the US Food and Drug Administration in November of 2022 to delay the clinical onset of T1D, thus opening new avenues to treat T1D as an immunologic disease rather than simply as a metabolic disease with lifelong insulin administration. In this Scientific Statement, we will integrate and summarize information about the pathogenesis of T1D, highlight gaps in current knowledge, and propose future activities that may lead to additional approaches to treat the underlying autoimmunity and β cell defects in diabetes. Hopefully these efforts, when combined with other rapidly improving T1D therapeutics including automated insulin delivery and cell replacement therapy, will lead to better long-term outcomes for those living with T1D.

Rationale for a Scientific Statement on the Pathogenesis of T1D

The centennial celebration of the discovery of insulin in 2021 provided a reminder that this breakthrough has saved millions of lives, especially those with type 1 diabetes mellitus (T1D)[1]. At the time of insulin's discovery, T1D was a death sentence with individuals surviving only a few days, weeks, or months after receiving the diagnosis. While long-term outcomes for individuals with T1D have improved dramatically, there remains a great need to prevent and further reduce the daily management and comorbidities associated with T1D[2,3].

T1D therapeutics are rapidly developing and currently fall into three broad categories: 1) delivery of exogenous insulin (e.g., insulin analogs, continuous glucose monitoring, automated insulin delivery devices), 2) disease-modifying therapies to treat the underlying β cell-directed autoimmunity (e.g., teplizumab, B or T cell-directed interventions, anti-cytokine therapies, antigen-specific immunotherapies), and 3) therapies that seek to increase or amplify endogenous insulin production and secretion (e.g., transplantation of insulin-producing cells or islets, protection or expansion of remaining endogenous β cells, transdifferentiation of other cell types into insulin-producing cells). Each of these approaches or combination of approaches has the potential to transform the treatment of T1D in the coming years, with the expectation that T1D therapies will evolve and compete based on real-world effectiveness, durability, and therapy-related side effects. Additionally, efforts to delay clinical T1D onset by identifying individuals at early stages of T1D development and intervening before the onset of hyperglycemia and clinical symptoms have shown promise (e.g., treating individuals with Stage 2 T1D with teplizumab to delay the clinical onset of Stage 3 T1D[4,5]).

In this Scientific Statement, we seek to provide an overview of scientific challenges and highlight opportunities for improving our understanding of the pathogenesis of T1D. This Statement is not a comprehensive review of T1D etiology, molecular pathways, or clinical trials, but instead seeks to integrate emerging information to identify opportunities for future research related to the pathogenesis of T1D. Additionally, this statement does not discuss in detail all

immunomodulatory therapies to treat the underlying autoimmunity in T1D, current T1D treatments, or T1D-related complications. This statement focuses on human T1D; animal models of T1D are not discussed. We will address selected areas where our knowledge base is insufficient, and we will highlight scientific opportunities that could lead to new approaches to modify the T1D disease course.

Outline of Scientific Statement on T1D Pathogenesis

While certain aspects of T1D pathogenesis are generally accepted, it is clear there is much we do not know and often our conclusions about processes we thought we understood have been shown to be incorrect or inaccurate. The widely cited model proposed by George Eisenbarth more than 30 years ago has been modified, amplified, and criticized[6], highlighting our evolving understanding of T1D progression and pathology. This Scientific Statement will use a revised version of this schematic (**Figure 1**) because it provides a useful framework for discussion, especially regarding the concept of disease stages and β cell loss. A major advance in the classification of T1D is the concept of "Stages", which highlights how T1D occurs over a period of months to years. This staging paradigm has led to considerable effort focused on identifying individuals at high risk of developing T1D so that the disease course can be modified, and clinical onset can be delayed[7]. However, such efforts are still hindered by T1D heterogeneity (discussed below), leading to imprecision in predicting when T1D will develop or evaluating the effectiveness of interventions. Over the past decade, the Eisenbarth model has been refined to delineate three stages:

- In Stage 1, there is evidence of β cell-directed autoimmunity as reflected by two or more autoantibodies against glutamic acid decarboxylase (GAD), insulin, insulinoma antigen-2 (IA- 2A), and/or the zinc transporter 8. Glucose tolerance is normal.
- In Stage 2, multiple islet autoantibodies are now accompanied by dysglycemia, but glucose intolerance does not meet the criteria for a diagnosis of diabetes.

- In Stage 3, hyperglycemia is present along with β cell-directed autoimmunity and glucose criteria are met for diabetes.

The schematic in figure 1 depicts β cell mass that is initially in the normal range but progressively (and smoothly) declines from Stage 1 to Stage 3. However, it is currently not possible to measure β cell mass in living humans and post-mortem assessment of β cell mass translates into a single cross-sectional measurement for one individual. Therefore, the longitudinal changes and patterns of loss of β cell mass shown in the schematic are mostly conjecture. For example, some versions of this model show β cell mass declining irregularly and possibly stopping and then restarting,[8] likely correlating to the relapsing and remitting nature of other autoimmune disorders. An alternate hypothesis is that there is a tipping point where β cell mass and function rapidly decline one to two years prior to Stage 3 T1D[9]. An addition to this schematic is the concept of "Stage 0" meaning the time before the development of immune abnormalities or β cell alterations. Little is known about diabetes-specific immune cells and β cells during Stage 0; however, events during this period are likely critical for the ultimate development of T1D and may provide a time window to prevent progression through the remaining stages of T1D.

This Scientific Statement is based on features of T1D pathogenesis for which there is general consensus (**Box 1**), and we aim to highlight gaps in our understanding that need to be addressed to ultimately prevent and cure T1D. We focus on the following areas relevant to an improved understanding of T1D pathogenesis:

A. Genetics
B. Heterogeneity
C. Pathology of the pancreas
D. Assessment of β cell function and mass
E. Immunologic biomarkers in peripheral blood

F. Exocrine pancreas in T1D

G. Screening to identify individuals at-risk for T1D

For each of these topics, we provide a summary of the current understanding followed by a discussion of knowledge gaps and suggestions for areas of future efforts. Some suggestions are conceptual in nature, others relate to needed experiments, and others relate to research infrastructure and collaborations across disciplines.

A. Genetics Related to T1D

A1. Summary

The genetic contribution to T1D is clear and initially was based primarily on observations of increased familial risk and higher T1D concordance in monozygotic versus dizygotic twins and non-twin siblings[10]. The major driver of genetic risk for T1D resides in the human leukocyte antigen (HLA) locus within the class II region (e.g., HLA-DR and HLA-DQ)[11,12]. Alleles such as HLA-DQ8, DQ2, DR4, and DR3 confer significant risk for developing T1D, while HLA-DQ6 (*DQB1*06:02*) provides nearly dominant protection from disease development[13-15]. All of these risk and protective alleles are frequent in European Americans, approximately 10-20%, and the dichotomy of risk strongly implicates their role in T1D pathogenesis. However, T1D is a polygenic disorder, and genome wide-association studies (GWAS) revealing over 75 minor loci throughout the genome that contribute to the overall genetic risk for disease, with many loci projected to influence β cells, immune cell function, or possibly both[16-19]. The vast majority of identified variants are not located in protein-coding regions of the genome, raising questions as to how they pattern disease risk. Quantitatively accumulating the relative odds ratio (OR) for various disease-associated loci into a polygenic risk score (PRS), also commonly referred to as a genetic risk score (GRS), in certain settings, may help identify individuals at-risk of developing T1D[20,21]. Now used in research settings, this type of approach has led to the adoption of risk scoring for broad population screening efforts to identify individuals at elevated risk for disease

development who may benefit from subsequent islet autoantibody testing[21]. Notable findings related to T1D GRS and T1D-associated loci are: 1) GRS that include HLA and non-HLA loci better identify individuals of younger age who are at risk but are less useful in identifying individuals older than 35 years, whereas non-HLA loci such as *INS* and those associated with type 2 diabetes (T2D) are more strongly associated with adult-onset autoimmune diabetes; 2) most loci associated with more rapidly progressing T1D diagnosed in younger individuals appear to target immune-related genes; and 3) no current combination of genetic loci predicts T1D development completely, with approximately half of individuals with high genetic risk not developing clinical T1D.

A2. Knowledge Gaps

- Are there genetic drivers of β cell mass, function, and rate of β cell loss during T1D development?
- How do T1D-associated genetic loci affect immune cell and/or β cell function?
- What are the epigenetics, chromatin structure, and transcriptional pathways leading to islet autoimmunity and progression through stages of T1D development?

A3. Suggested Future Efforts

- Design studies to investigate the functional genetics of immune response genes and genes implicated in β cell function across the stages of T1D development.
- As whole genome sequencing becomes more widely available and integrated into health systems, develop strategies to identify at birth or early in life those who are at high genetic risk for developing T1D.
- Utilize whole genome sequencing data to identify novel rare variants that confer risk or protection from T1D development.

- Continue refinement of T1D GRS for their potential use in population-based genetic screening, including the development of risk scores for individuals from diverse ancestries[22-24].

B. Heterogeneity of T1D

B1. Summary

T1D was initially thought to be more easily defined than T2D; however, it is now recognized that there is considerable heterogeneity in T1D on several levels (**Table 1**)[25,26]. Age of onset, immunologic variability, and rates and degree of β cell loss differ among individuals who have features of clinical T1D (e.g., glycemic lability, propensity for diabetic ketoacidosis, insulin deficiency, islet autoimmunity)[27,28]. Furthermore, T1D can occur over a broad age range in children, adolescents, and adults[29]. Studies of the human pancreas show histologic evidence of differences based upon age of T1D onset, with insulitis (e.g., islet immune cell infiltration) in children having a greater absolute number of CD45+ immune cells in and around islets, higher CD20+ B lymphocytes, and a more rapid loss of β cells (**Figure 2**)[30]. Adult-onset T1D has similarities and differences compared with T1D with onset in childhood or adolescence, including variations in genetic risk, severity of hyperglycemia, intensity of the cellular immune response directed against β cells, presence of obesity/insulin resistance, and the rate of β cell loss following diagnosis (summarized in **Figure 3**). Currently, adult-onset T1D poses a diagnostic challenge in clinical practice, as components of autoimmunity and insulin resistance can be present in the same individual, leading to frequent misclassification or misdiagnosis, and likely necessitating the treatment of both underlying mechanisms of diabetes development[31].

The concept of disease heterogeneity also has practical implications when considering immune and β cell-specific interventions to modify the disease course. For example, heterogeneity among clinical trial participants creates challenges for assessing the efficacy of therapeutics to preserve endogenous insulin production, as evidenced by children having a more rapid loss of β cell function following diagnosis compared to adults[32]. Thus, prototypical

new-onset intervention trials need to include individuals within a similar age range when assessing β cell decline after clinical T1D diagnosis. Additionally, certain immunotherapies have shown more efficacy in younger children and adolescents. Thus, a strong rationale exists to dissect disease heterogeneity across the stages of T1D development to identify pathways that can be therapeutically targeted to intercept and modify the T1D disease course in specific populations.

B2. Knowledge Gaps

- Is childhood-onset T1D the result of different pathogenic processes compared to adult-onset T1D?
- What is the natural history of adult-onset T1D? When do islet autoantibodies initially develop? What is the underlying pancreatic islet pathology in adult-onset T1D?
- Does defining T1D heterogeneity improve the testing of disease-modifying therapies? It is known that there are treatment responders and non-responders in T1D immunotherapy clinical trials. Could selecting a more homogenous clinical trial population improve response rates when testing immune and β cell-specific interventions?
- Are there age-specific treatments that should be identified and tested?

B3. Suggested Future Efforts

- Establish subtypes of T1D considering age, genetics, islet autoantibody profiles (e.g. number and specificities), clinical criteria such as BMI and degree of insulin resistance, and immune/β cell biomarkers.
- Determine whether there are T1D subtypes that can be used to discover pathogenic mechanisms or respond to specific immunotherapy intervention in clinical trials.
- Actively seek organ donors with Stage 1 or 2 T1D, marked by the presence of two or more biochemical islet autoantibodies but no overt hyperglycemia, so very few of these donors

are available globally. Very few such donors are available globally so this will require coordinated efforts among organ procurement organizations, funding organizations, and laboratory-based scientists across disciplines and institutions.

- Identify organ donors that were diagnosed with T1D in adulthood to study pancreatic pathology.
- Study the natural history of adult-onset T1D in terms of genetics (e.g., influence of immune, β cell, insulin resistance), timeline of islet autoantibody development, and progression through the early Stages of T1D (e.g., Stage 1 and 2 T1D). This could be accomplished by following participants from existing birth cohort studies (e.g., TEDDY, DAISY, BABYDIAB) or enrolling new cohorts focused on adults.

C. Pathology of the Pancreas in T1D

C1. Summary

The specific pathologic and molecular changes that occur in the human pancreas during T1D are incompletely defined. This lack of understanding is due, in part, to the difficulty in safely obtaining biopsy samples from the pancreas of patients; although, pancreatic biopsy samples have been attempted and obtained in several recent onset T1D patients in the past[33]. However, prior to 2000, the majority of pancreas studies were mostly performed following pancreas removal at autopsy, and the most extensive collection of these samples is held in the Exeter Archival Diabetes Biobank (EADB)[34]. Despite the issues associated with autopsy samples including the rapid autolysis of the pancreas following death, time to harvest the pancreas and fixatives used to preserve the pancreas, these early approaches led to critical discoveries and new concepts. These include the finding that a modest number of intra islet inflammatory cells (insulitis) are present in the early stages of T1D, although the degree of insulitis and the proportion of islets affected appears to be impacted by age at T1D onset[35,36]. These studies also

demonstrated that there is lobular loss of β cells and hallmark hyperexpression of HLA class I within residual insulin-containing islets of individuals with T1D[37-39].

These pioneering studies using autopsy samples were followed by considerable efforts to procure pancreata soon after death from individuals with recent-onset T1D using protocols that would allow for more detailed cellular and molecular analyses. These efforts, led by the Network for Pancreatic Organ Donors with Diabetes (nPOD), have led to gains in fundamental knowledge about human islet architecture, islet composition, and immune infiltration through the procurement and study of pancreata from organ donors[40]. The study of these organs with ever-advancing technologies and the continued efforts to make these tissue available for study by investigators from around the world is rapidly increasing our understanding of the human pancreas in health and disease. It is now appreciated that other mechanisms of β cell loss or dysfunction, in addition to autoimmune-mediated destruction, may contribute to T1D. These mechanisms include dedifferentiation (β cells reverting to precursors), transdifferentiation (β cells transitioning to other endocrine cell types), and stress-induced senescence. Subsequent efforts by the Human Islet Research Network (HIRN) and the Human Pancreas Analysis Program (HPAP) have further advanced our understanding of the human pancreatic islet and the immune system during T1D[41]. Fortunately, few individuals die at the onset of T1D or in the autoimmunity phase. This explains the limited number of organs or cells available for study at these critical timepoints. The insights from early autopsy studies, nPOD, HPAP, and a number of additional investigations are summarized in **Figure 2**.

C2. Knowledge Gaps

- What is baseline β cell mass at early ages in genetically at-risk individuals who subsequently develop T1D? What are the determinants of β cell mass?
- What are the earliest lesions in the islet in T1D, and are these pathologies the same in all individuals with T1D? Are disease processes the same in individuals across ethnicities?

- Is β cell loss during T1D development sustained or does it wax and wane, mimicking the relapsing and remitting nature of other autoimmune disorders?
- Why do some β cells survive and why are some islets not infiltrated with immune cells? Can the remaining β cells regenerate, and if not, why not?
- What are the relative contributions of dedifferentiation, transdifferentiation, and stress-induced senescence to β cell dysfunction and loss in T1D? Are these processes driven by common or differing factors? Are their contributions different in various individuals? Can these processes be targeted therapeutically?
- How can we best model the interface of immune cells and β cells in a realistic microenvironment that includes other endocrine cells, the extracellular matrix, and support cells?
- Do β cell or islet cell abnormalities precede β cell autoimmunity marked by the presence of islet autoantibodies?

C3. Suggested Future Efforts

- Greater efforts to procure and study human pancreas and islets from donors who have high genetic risk compared to those at low risk of developing T1D.
- Enhance efforts to procure human pancreas and islets from donors with recent-onset T1D and those in early stages of islet-directed autoimmunity to allow for evaluation of tissue and islet cells with state-of-the-art single-cell technologies, including transcriptomic, epigenomic, proteomic, and metabolomic profiling.
- Expand pancreas bioresources from individuals from different ethnicities and consider creating an open-access registry to collate the location of pancreas resources worldwide to help facilitate the collaborative and meaningful study of rare donor groups.
- Improve the understanding of normal pancreatic development from birth to adulthood and relate this knowledge to preclinical and clinical T1D at different ages. For example, improve

our understanding of islet basement membrane development, islet restructuring, and the recruitment of support cells including mesenchymal stem cells.

- Develop processes and policies to increase the amount of deidentified clinical information that can be correlated with studies of pancreatic tissue and islets.
- Improve how the field connects molecular alterations detected during the study of the pancreas and islets (e.g., single cell transcriptome analyses) with clinical information from longitudinal clinical trials such as those in Type 1 Diabetes TrialNet and TEDDY.

D. Assessment of β Cell Function and Mass in T1D

D1. Summary

Indirect assessment of β cell function in living individuals is performed by measuring insulin and C-peptide secretion following administration of oral or intravenous glucose or in response to a mixed-meal nutrient stimulation[42,43]. Longitudinal assessment of β cell function in individuals at risk of developing diabetes has revealed several key metabolic checkpoints during disease progression. Following the development of islet autoantibodies (i.e., seroconversion), there is loss of early C-peptide responses and measurable impairments in total C-peptide secretion during an oral glucose tolerance test (OGTT) that can be detected as early as six years prior to diagnosis in older children and adolescents developing T1D. During a second phase that continues until approximately two years before diagnosis, OGTT C-peptide measures are relatively stable. Beginning about two years prior to diabetes onset, there is evidence of rising glycemia and declining C-peptide secretion. Rapid metabolic deterioration begins one year to six months prior to Stage 3 T1D onset, with marked declines in β cell glucose sensitivity, rate sensitivity, and potentiation, coupled with decreased insulin sensitivity and markedly rising blood glucose levels[9,44,45].

Because measurement of β cell mass in humans has been historically performed by morphometric analysis of insulin-positive cells in tissue sections from autopsy specimens[46], less

is known about the longitudinal dynamics of β cell mass in living individuals during disease progression. Provocative tests, such as glucose-potentiated arginine administration, achieve maximal stimulation of β cell insulin release and have thus been proposed to provide insight into functional β cell mass[47-49]. Studies in living cohorts comparing responses to IVGTT and glucose-potentiated arginine administration indicate that these measures may not be well correlated in some at-risk individuals, suggesting a potential disconnect between β cell mass and function in the early stages of T1D development[50]. A similar notion has emerged from histologic studies of pancreata of organ donors with recent-onset T1D[37]. There is great interest in developing methods to quantitate β cell mass in living individuals. Several current efforts have focused on the development of positron emission tomography (PET) imaging techniques that utilize radiotracers conjugated to GLP-1 analogues[51].

D2. Knowledge Gaps

- A recent study showed that blood glucose levels rise in children prior to the development of islet autoantibodies[52]. However, the majority of natural history studies documenting changes in β cell function during disease progression are based on individuals who are already autoantibody positive. Therefore, there is limited information about how C-peptide changes in individuals prior to and during the development of islet autoantibodies.
- It is not well understood whether patterns of C-peptide decline are modified by underlying genetic factors, other proteins, or factors such as non-coding RNA.
- Whereas measurement of C-peptide is the gold standard for assessment of β cell function and secretory capacity, validated biomarkers that provide additional insight into β cell function, mass, and stress during disease progression are needed.
- Non-invasive methods to quantify β cell mass in living humans have not been widely tested in populations developing T1D.

- Some data suggest β cell function and mass do not correlate in different T1D stages. This is likely because studies to assess β cell function in living humans cannot be correlated with β cell mass measurements in pancreatic specimens. Could these findings imply that a portion of β cells may be “sleeping” or recoverable at advanced disease stages?

D3. Suggested Future Efforts

- Focus on the development and validation of methods to monitor β cell function and mass in combination in longitudinal cohorts. Combine molecular phenotyping with physiologic approaches to provide deeper insight into the health status of the β cell during different stages of disease progression.
- Leverage the knowledge of metabolic progression of T1D, incorporating measures of both β cell mass and function, to optimally time the administration of disease-modifying therapies.
- Reach consensus on the most meaningful and practical clinical assessment of β cell function across the stages of T1D development, which is especially important for clinical testing of disease-modifying therapies.
- Continue development of clinically safe β cell tracers for *in vivo* imaging in humans.

E. Immunological Biomarkers in Peripheral Blood

E1. Summary

Islet autoantibodies are robust and validated markers of autoimmune diabetes, thus allowing for the differentiation of T1D from other forms of diabetes[53]. The presence of two or more autoantibodies directed against GAD, insulin, insulinoma antigen-2 (IA- 2A), and/or the zinc transporter 8 make T1D a predictable disease as their number and levels predict, on a population level, time to progression to Stage 3 T1D[54]. However, predictions with autoantibodies at the individual level do not reliably predict progression and timing of clinical T1D onset (e.g., hyperglycemia). For example, the time to clinical disease onset can span months to years after

387 the development of islet autoantibodies. The rates of progression through the stages of T1D
388 vary and are likely modified by age, family history, environmental factors, genetics, antigens
389 targeted by the immune system, and β cell-intrinsic factors (e.g., endoplasmic reticulum stress,
390 proinsulin misfolding, and cell-surface overexpression of HLA class I molecules).

391 The autoimmune processes underlying T1D pathogenesis are reflected in both acute and
392 prolonged alterations of immune cell abundance, phenotype, and functionality and the presence
393 of immune cell infiltrate within the pancreatic islets. However, beyond the established utility of
394 several robust soluble biomarkers (islet autoantibody profiles and C-peptide levels), the search
395 for reliable serum or plasma biomarkers of T1D progression and prospective treatment
396 outcomes has been challenging. In wide-ranging research efforts, reported changes in the
397 composition of numerous serum metabolites, proteins, nucleic acids (including microRNAs),
398 extracellular vesicles, and other analytes have repeatedly generated tantalizing hypotheses and
399 mechanistic speculations, yet there is inconsistent reproduction of these results across different
400 studies. Similar considerations also apply to peripheral blood cellular biomarkers including β
401 cell-antigen specific T cells, follicular helper and regulatory T cells, NK cells, B cells, antigen-
402 presenting cells, and neutrophils. From these studies, it is becoming apparent that any single
403 metric will not capture all aspects pertinent to the prediction of disease progression and
404 therapeutic responsiveness. Rather, combinatorial biomarker profiles may eventually be
405 leveraged to dissect disease heterogeneity to allow for better classification of T1D subtypes,
406 improved disease staging, and the development of individual progression rates to clinical
407 disease.

408 Notable progress on developing biomarkers has been made from the direct interrogation of
409 human pancreata over the last two decades through the efforts of nPOD, HPAP, and HIRN. It is
410 established that pancreata from organ donors with T1D show immune cell infiltrates within
411 residual pancreatic islets in a lobular distribution, increased B cell infiltration especially among
412 younger age of onset donors, and HLA class I hyperexpression by residual insulin-containing

islets. Additionally, there are more CD4 and CD8 positive T cells within the islets of T1D organ donors compared to controls, and many efforts have defined the antigen specificity of these individual T cell clones and described unique T cell receptors from the residual inflamed pancreatic islets of organ donors[55-59]. There is strong interest directed at sequencing the adaptive immune receptor repertoire (AIRR) to identify these pancreatic islet-derived T cell receptor sequences from peripheral blood DNA or RNA, given that these sequences could provide knowledge on prior and ongoing T cell clonal expansion to antigens[60]. Remarkably, a number of these pancreatic islet-derived T cell receptor sequences are shared among patients and these cells circulate in the peripheral blood[61-64]. Efforts are now underway to test the utility of T cell receptor sequences as a molecular biomarker of disease activity during disease progression and as a therapeutic response indicator following interventional trials[65]. This may prove to be of crucial importance as some immune interventions might only be effective during active phases of the T1D disease process.

E2. Knowledge Gaps

- How do the composition and properties of immune cell subsets evolve across stages of T1D in the pancreas, draining lymph nodes, and peripheral blood?
- How are features of the autoimmune process different across stages of T1D development in both the pancreas and peripheral blood?
- What are the primary antigens involved in T1D initiation, and what is the pathogenetic relevance of additional antigens throughout subsequent stages of T1D? How are these parameters shaped by HLA haplotypes (e.g., DR4/DQ8 predisposes to insulin autoimmunity while DR3/DQ2 confers GAD autoimmunity in young children[66]), other genetic risk factors, and potential environmental determinants (e.g., viral infections)?
- Which antigen(s) drive the disease from islet autoimmunity initiation to clinical onset, and is this process same in everyone?

- What are the properties that reliably distinguish islet antigen-specific T cells throughout T1D stages from those found in healthy individuals?

E3. Suggested Future Efforts

- Expanded implementation of high-dimensional imaging and other single-cell technologies to better deconvolute immune cell subset identities, properties, and distribution across tissues (e.g., pancreas, pancreatic lymph nodes, and blood).
- Identify and validate biomarkers of disease activity that can be reliably measured in the blood to determine when autoimmune diabetes is active and if there is a loss of β cells during pre-clinical disease. These biomarkers could be immunologic markers, β cell-intrinsic factors, or a combination of both.
- Leverage samples collected from longitudinal studies (e.g., TEDDY, Type 1 Diabetes TrialNet Pathway to Prevention) to better understand the temporal changes in immune and other peripheral biomarkers.
- Standardize assays among laboratories and validate candidate biomarkers across ages, ethnicities, and stages of T1D.

F. Exocrine Pancreas T1D

F1. Summary

Studies in humans show a reduction in total pancreas size and the presence of inflammatory infiltrates in pancreatic exocrine tissue in donors with T1D but these individuals rarely have overt pancreatitis. These findings raise the possibility that the pathogenic process of T1D involves both the endocrine and exocrine compartments of the pancreas. In fact, endocrine and exocrine cells of the pancreas share a common embryologic heritage and blood supply, and there are clear connections between some forms of diabetes and exocrine disease (e.g., cystic fibrosis, pancreatitis). Because the pancreatic endocrine compartment makes up only 1-2% of

pancreatic mass or volume, the long-noted smaller pancreas size in donors with longstanding T1D must reflect changes in the ductal and/or exocrine compartment. More recently, post-mortem studies have shown a decrease in pancreas size in the early years after a T1D diagnosis and in autoantibody-positive individuals at Stage 1 or 2[67,68]. Pancreatic imaging studies (e.g., MRI, CT, ultrasound), which allowed the study of a larger number of individuals, have mostly supported the concept of a smaller pancreas in long-standing T1D, and recent MRI studies have found that pancreas volume is reduced at onset of T1D (e.g., < 3 months) in children, adolescents, and adults[69]. Additionally, individuals with autoantibodies at Stage 1 or Stage 2 also had a smaller pancreas compared with age-matched controls, with the MRI-defined pancreas volume being intermediate between that of controls and those with new-onset hyperglycemia[70]. Surprisingly, autoantibody-negative first-degree relatives of individuals with T1D also appear to have a slightly smaller pancreas volume[71]. Interestingly, a recent study showed that individuals with a monogenic form of diabetes that results from insulin deficiency in the absence of autoimmunity have a significantly smaller pancreas than their healthy family members without the mutation and similar to individuals with T1D[72]. Insulin is a known trophic factor for acinar cells, and this study suggests that insulin deficiency, even in the absence of islet-directed autoimmunity, is sufficient to reduce exocrine pancreas size.

Post-mortem assessment of pancreas size is cross-sectional in nature and, thus far, most MRI and CT studies have likewise been cross-sectional, leading to somewhat conflicting results regarding pancreas volume or size in T1D and the relationship of pancreas size to other parameters such as diabetes duration or BMI. Cross-sectional assessments are greatly confounded by the observation that pancreas volume or size varies considerably (two- to four-fold range) in the non-diabetic population, even when corrected for body weight, body surface area, or BMI. A similar variation in pancreas size has also been noted in populations with T1D, leading to overlap in pancreas size between controls and those with T1D. However, because imaging studies can be repeated in the same individual, this technology allows comparison of

pancreatic size over time and is beginning to provide clarification on pancreas size throughout the stages of T1D. For example, one study used serial MRI scans in individuals with recent-onset T1D to show that pancreas volume was reduced at the onset of hyperglycemia and continued to decline in the first five years after T1D diagnosis[69].

Histologic analysis of pancreata from donors with T1D shows that the mass of α cells and other islet endocrine cells is not changed in T1D, reflecting the β cell-specific loss in T1D. However, more recently, studies of the pancreas during T1D have expanded beyond investigation of alterations in the islet (e.g., insulitis, changes in gene expression) to include studies of the exocrine compartment in both recent-onset and long-standing T1D[73,74]. In long-standing T1D, there are fewer acinar cells and increased fibrosis (intralobular rather than interlobular) in the pancreas, which does not appear to correlate with diabetes duration, age of T1D onset, or glycemic control, and some reports have noted vascular changes and acinar cell atrophy. Several studies have reported a greater number of CD45+, CD4+ and CD8+ T cells in the exocrine tissue in long-standing T1D and stage 2 T1D[75]. Autoreactive CD8+ T cells, some of which target antigens such as pre-proinsulin, are found in T1D islets, implying that such cells are involved in β cell destruction[55,76]. Surprisingly, pre-proinsulin CD8+ T cells are also found in the pancreas from non-diabetic, autoantibody-negative donors, with the frequency of such cells being much greater in T1D[77].

F2. Knowledge Gaps

- Are changes in the pancreatic exocrine compartment part of the primary pathologic process of T1D or are these changes to the exocrine occurring secondary to the T1D-induced alterations in the islet compartment and β cells?
- What is the cause of reduced overall pancreas mass in T1D?

- Is pancreas mass or volume related to insulin secretion and/or islet/β cell mass? If pancreas mass is a surrogate for β cell mass, could measures of pancreas mass predict and determine the timing in which individuals develop Stage 3 T1D?
- What is the time course for the reduction in pancreas mass? Do individuals destined to develop T1D have a smaller pancreas early in life? When in the developmental process is pancreas volume/size determined and what are the molecular determinants?

F3. Suggested Future Efforts

- The determinants of pancreas mass should be investigated to define critical time periods (e.g., in utero, and in early life) and events (e.g., environmental, dietary) that influence the wide range of normal pancreas mass. Likewise, the role of genetic influences should be investigated by pairing assessment of pancreas mass with genetic studies to identify genes or combination of genes that influence pancreas mass.
- Standardized, longitudinal pancreatic imaging approaches paired with assessment of β cell function across the stages of T1D are needed to potentially link pancreas size to β cell function.
- Investigations to determine the reason for reduced pancreas mass in T1D should use pancreatic tissue biorepositories that are linked to single-cell analysis, multi-plex imaging, and clinical information.

G. Screening to Identify Individuals at-risk for T1D

G1. Summary

Current strategies to identify individuals who are likely to develop T1D in the future have been primarily based on a family history of T1D (e.g., first-degree relatives). This screening approach has been quite successful[25,78]. However, over up to 90% of people who develop T1D do not have a family member with T1D. Therefore, early identification of at-risk individuals

cannot solely rely on family history-based screening, and general population screening for T1D will be necessary and underway[79]. Several studies, including the Fr1da Study in Bavaria[80], the Autoimmunity Screening for Kids (ASK) in the USA[81], and the T1Early in the UK[82] support the feasibility of broad population-based screening for T1D-associated autoantibodies in individuals without a family history of T1D. However, concerns remain related to the financial cost associated with implementing such programs on a national scale, the frequency of monitoring that would be required, and the best methods for communicating risk without causing harm or distress. Genetic screening at birth for HLA haplotype or polygenic risk scores for T1D could identify individuals with an increased likelihood of developing islet autoantibodies and possibly provide insight into time to seroconversion to inform screening time points. These approaches would reasonably narrow the population that would require longitudinal screening for islet autoantibodies, thereby reducing the burden on the healthcare system. Additionally, population-based T1D screening would decrease the likelihood of life-threatening diabetic ketoacidosis and identify individuals with early-stage T1D who can be offered disease-modifying therapy to delay clinical disease onset[83,84], thus further reducing the personal and economic burden of T1D. One approach to T1D screening is shown in **Figure 4**.

A GRS for T1D provides a simple continuous variable that researchers and clinicians can utilize to understand the overall genetic burden. When coupled with autoantibody testing, these screening approaches can improve disease prediction and differential diagnoses, with the goal of guiding rational interventions in individuals at varying degrees of risk for developing T1D. A recent example of this type of approach involved a broad population screen in infants across multiple European sites as part of the Global Platform for the Prevention of Autoimmune Diabetes (GPPAD-02) study[85]. Those with high genetic risk were monitored for subsequent autoantibody development and offered enrollment in a clinical trial of oral insulin to potentially delay or prevent T1D onset. These studies are promising; however, much of this research was conducted in White/Caucasian study participants of European genetic ancestry, and the original

T1D GRS (commonly known as GRS1) demonstrated poor performance in individuals of Black and African ancestry. To address this clear shortcoming, a distinct African American T1D GRS (AA-GRS) has also been developed[23]. Ultimately, however, the successful implementation of a wide-spread T1D GRS in large populations will require a demonstration of improved patient outcomes and successful interventions to modify the disease course. In addition to teplizumab (anti-CD3 monoclonal antibody)[4,5], precision medicine-based selection of therapies may also include use of antigen-specific immunotherapies for individuals with HLA-DR3 and autoreactivity to GAD or those with HLA-DR4-DQ8 who display early immune responses to proinsulin[66].

G2. Knowledge Gaps

- How do we transition T1D screening from research to clinical practice and the general population?
- What is the best approach for screening? Genetic risk determination at birth or early life, followed by islet autoantibody measurements in the high-risk population or measurement of islet autoantibodies in all children throughout childhood?
- When is the best time to screen for islet autoantibodies? How do we identify adults who are at-risk for developing T1D?
- How will the screening information be relayed to patients and families, in what format, and what additional support should be available?
- Should screening be cost-effective or should efforts for screening be cost-agnostic?
- Development of T1D registries to track at-risk individuals.

G3. Suggested Future Efforts

- Monitor outcomes of studies that screen for T1D risk, including TEDDY, Fr1da, ASK, T1Early, ELSA, T1DRA and others elsewhere in the world, for feasibility and acceptability.
- Consider whether it is feasible to test approaches and acceptability for screening through randomized controlled trials.

Concluding Comments, Future Efforts, and Unresolved Questions Related to the Pathogenesis of T1D

By applying new approaches and technologies to integrate studies of human tissue and cells with clinical investigation of individuals across the stages of T1D development, the field is poised to improve the understanding of T1D pathogenesis and pathophysiology. However, a comprehensive understanding of T1D will require interdisciplinary efforts to address knowledge gaps at multiple levels. Most likely, T1D pathogenesis is a bi-directional process in which both the immune system and the pancreas/islet are active participants in a progression that leads to reduced β cell mass and inadequate insulin secretion. Although immune and β cell changes have been noted in Stages 1-3 of T1D, it is unclear if there are distinct alterations at Stage 0. Also, it is uncertain whether there are changes in these processes during the transition from one stage to the next stage of T1D development. To promote open dialogue and discussion around these knowledge gaps, we pose these conceptual, experimental, and organizational topics for consideration.

Conceptual:

- The increasingly recognized clinical heterogeneity of T1D likely has correlates at the molecular level. How does the field connect these processes in a bi-directional manner so that advances in molecular understanding will drive new clinical approaches and new findings in clinical investigations will drive novel experiments in the laboratory? Examples of existing resources related to T1D pathogenesis are included in **Table 2**.

Experimental:

- How do we better define islet cell composition, architecture, and microenvironment and immune cell repertoire in T1D? How does the field study and better define the interaction between β cells and immune cells in T1D?

- Can advances in transcriptomics, proteomics, lipidomics, and metabolomics provide insight into the molecular pathogenic processes and better define the stages of T1D , leading to the identification of new biomarkers that can be adapted to clinical trials?
- How do we ensure that data collection is not skewed towards select groups of individuals with or at-risk of T1D (ethnicity, age, duration, etc.)?
- How do we ensure access to appropriate materials from age-matched individuals without diabetes to control for the impact of age on pancreas development and immune cell repertoire?

Organizational:

- How do we integrate information from studies of isolated islets and pancreatic tissue with clinical studies in individuals at-risk for T1D?
- What types of interdisciplinary teams are needed to conduct these types of studies, and how will individual and team efforts be encouraged and recognized?

Over 100 years after the discovery of insulin, the field is poised to again make rapid progress in the understanding, prevention, and treatment of T1D. Access to human pancreatic tissue, novel molecular and imaging techniques, and exciting new disease-modifying therapies can be harnessed to greatly improve the quality of life for those impacted by T1D.

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Box 1. Summary of selected features of T1D pathogenesis*

- T1D is the result of an autoimmune process in which β cells are destroyed by T cells, with preservation of other islet cell types (e.g., alpha cells, delta cells).
- Some, but not all, individuals with certain HLA-DR-DQ genotypes develop T1D, and individuals with specific HLA-DR-DQ haplotypes are dominantly protected from developing T1D. These risk and protective HLA genotypes vary across ethnicities.
- The onset of hyperglycemia with a clinical T1D diagnosis can occur over a wide age range (< 5 years to more than 70 years).
- β cell loss occurs at a different rate in different individuals and may or may not be complete. The pancreatic islet and its β cells are likely not innocent bystanders in the autoimmune disease process.
- Autoantibodies directed against insulin and specific intracellular β cell proteins (GAD65, IA-2, and ZnT8) are markers of T1D-related autoimmunity and predict, on a population level, subsequent development of dysglycemia. These autoantibodies can appear very early in life in some genetically susceptible individuals and target intracellular molecules and thus are not cytotoxic. It is not known when autoantibodies develop in individuals who are diagnosed later in life with adult-onset T1D.
- A number of cellular immune alterations have been noted in Stage 1, 2, and 3 T1D. Presumably, β cell loss is a cell-mediated and cytokine-mediated process, but direct evidence of this in humans is inconclusive.
- Targeting anti-CD3 on the surface of T cells in Stage 2 T1D with teplizumab delays the progression to Stage 3 (new-onset T1D) by 2-3 years.
- A number of immunotherapies can slow the loss of residual β cell function (as measured by stimulated C-peptide secretion) at Stage 3 T1D, presumably reflecting modification of the autoimmune process directed at β cells.

675 **Table 1. Examples of heterogeneity relevant to the pathogenesis of T1D***

Clinical	β cell-directed autoimmunity and hyperglycemia onset across a wide age spectrum (from 9 months to adult)
Genetic	High risk HLA DR3/DR4-DQ8 haplotypes in some, but not all, individuals with T1D
Immunologic	Autoantibody frequency, profile, and target epitopes Type 1 interferon, innate immunity, and T cell signatures
Metabolic	Variable rates of decline in β cell function (as assessed by C-peptide) in stage 2 and 3; residual C-peptide production variable after T1D onset
Pathologic	Degree of insulitis differs between individuals with T1D and is variable between islets in the same individual CD20+ B lymphocytes more frequent in younger onset T1D Degree of β cell loss is variable (relatively understudied)

676 *Adapted from Battaglia et al., Diabetologia, Powers, JCI, and references therein.

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Table 2. Summary of resources related to the pathogenesis of T1D.

Data Platforms	**Hyperlink**
cellxgene	https://faryabi16.pmacs.upenn.edu/view/T1D_T2D_20220428.h5ad
CMDGA	https://cmdga.org/matrix/?type=Experiment&award.project=HPAP
dbGaP	https://www.ncbi.nlm.nih.gov/gap
PANC-DB	https://hpap.pmacs.upenn.edu
Pancreatlas	https://www.pancreatlas.org
T1D TCR/BCR Repository	https://gateway.ireceptor.org/login
Organizations and Resources	
Alberta Diabetes Institute IsletCore	http://www.isletcore.ca
Exeter Archival Diabetes Biobank	https://pancreatlas.org/datasets/960/overview)
Global Platform for the Prevention of Autoimmune Diabetes	https://www.gppad.org/de-en/
GTEx	https://gtexportal.org
HIRN	https://www.hirnetwork.org
HumanIslets	https://www.humanislets.com/#/
IIDP	https://iidp.coh.org
INNODIA	https://www.innodia.eu/
Islet Regulome Browser	http://www.isletregulome.com
Network for Pancreatic Organ Donors with Diabetes (nPOD)	https://www.jdrfnpod.org
PanKbase	https://pankbase.org/
RHAPSODY	https://imi-rhapsody.eu

The Environmental Determinants of Diabetes in the Young (TEDDY)	https://teddy.epi.usf.edu/index.htm
TIGER	http://tiger.bsc.es
TrialNet	https://www.trialnet.org/
Type 1 Diabetes Research Networks	https://scicrunch.org/T1D/about/t1d_networks

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Figure Legends

Figure 1. Schematic diagram of the stages of T1D development. Graph shows functional β cell mass on the y-axis and time on the x-axis, which could be months to many years. Both axes are quantitatively ambiguous as there is likely to be marked individual variation in both β cell mass and time of progression through T1D stages. Critical questions and unknowns related to T1D pathogenesis are shown with an arrow and accompanying text.

Figure 2. Changes to the pancreas, islets, and immune cells in the pancreas during T1D development. The pancreas with islets undergoing immune cell infiltration (e.g., insulitis) is shown on the left, with progressive loss of insulin-producing β cells. The upper portion schematically shows a progressive reduction in pancreas size as T1D develops. Alterations and abnormalities in T1D are indicated in the surrounding text.

Figure 3. Examples of heterogeneity in T1D related to age at onset. Factors that influence T1D development include genetic risk scores (GRS), severity of hyperglycemia at diabetes onset, intensity of the autoimmune response, additional contributors such as obesity and insulin resistance, and the rate of β cell loss.

Figure 4. Schematic of a potential screening effort to identify those in the general population likely to develop T1D and intervene with disease-modifying therapies. Depicted in the diagram is initial screening for genetic risk, followed by measuring biochemical islet autoantibodies (IAA, GADA, IA-2A, and ZnT8), staging T1D, and offering disease-modifying therapy to delay clinical diabetes onset at the appropriate stage of T1D.

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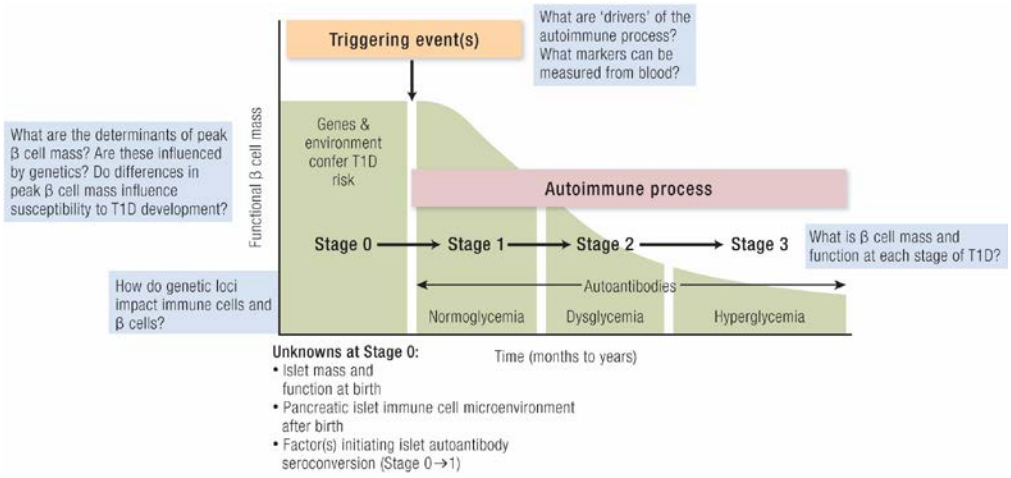
Fig. 1



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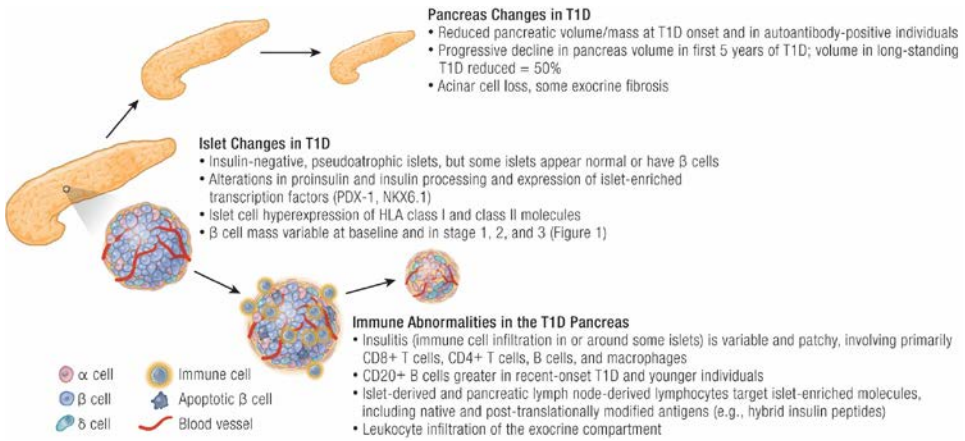

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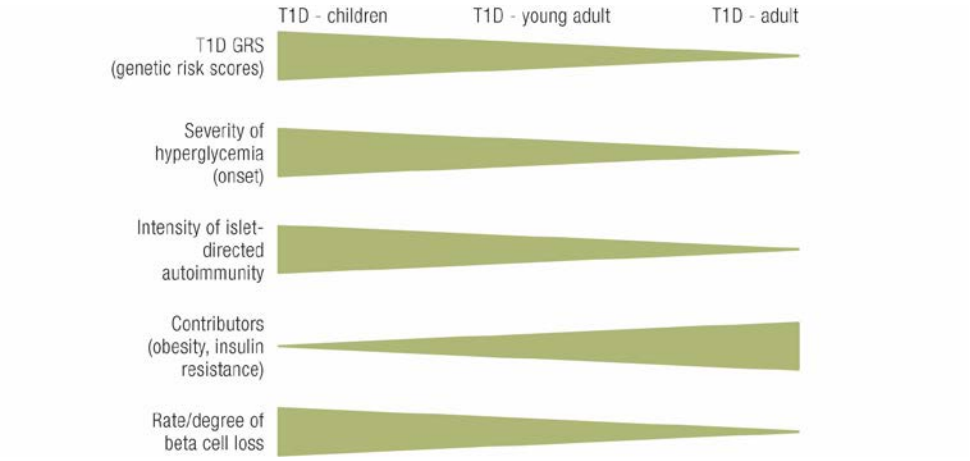

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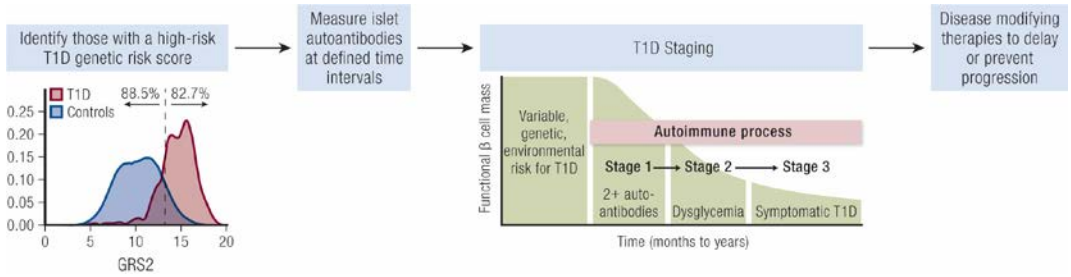

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