From Pancreatic β-Cell Gene Networks to Novel Therapies for Type 1 Diabetes

Decio L. Eizirik,[1,2] Florian Szymczak,[2] Maria Inês Alvelos,[2] and Frank Martin[3]

Diabetes 2021;70:1915–1925 | https://doi.org/10.2337/dbi20-0046

Completion of the Human Genome Project enabled a novel systems- and network-level understanding of biology, but this remains to be applied for understanding the pathogenesis of type 1 diabetes (T1D). We propose that defining the key gene regulatory networks that drive β-cell dysfunction and death in T1D might enable the design of therapies that target the core disease mechanism, namely, the progressive loss of pancreatic β-cells. Indeed, many successful drugs do not directly target individual disease genes but, rather, modulate the consequences of defective steps, targeting proteins located one or two steps downstream. If we transpose this to the T1D situation, it makes sense to target the pathways that modulate the β-cell responses to the immune assault—in relation to signals that may stimulate the immune response (e.g., HLA class I and chemokine overexpression and/or neoantigen expression) or inhibit the invading immune cells (e.g., PDL1 and HLA-E expression)—instead of targeting only the immune system, as it is usually proposed. Here we discuss the importance of a focus on β-cells in T1D, lessons learned from other autoimmune diseases, the "alternative splicing connection," data mining, and drug repurposing to protect β-cells in T1D and then some of the initial candidates under testing for β-cell protection.

The Human Genome Project opened the door for a systems- and network-level understanding of biology (1). This remains, however, to be fully applied to understand the pathogenesis of type 1 diabetes (T1D) and, based on this knowledge, identify new therapies for the disease. We will discuss the hypothesis that defining the key gene regulatory networks that drive β-cell dysfunction and death in T1D might enable the design of therapies that target the ultimate causative factor of T1D, namely, the progressive loss of functional β-cell mass, instead of only managing hyperglycemia or attempting to inhibit the immune system to delay disease. This approach has already been attempted for other human diseases, such as heart valve diseases (2). Importantly, early ("stage 1") T1D may now be detected years ahead of clinical outbreak (3), indicating the potential for effective β-cell–protective therapies that may modify the natural history of diabetes.

Many successful drugs do not directly target individual disease genes; instead, they modulate the consequences of defective steps, targeting proteins located one or two steps downstream (4). For instance, during the ongoing coronavirus disease 2019 (COVID-19) pandemic the use of artificial intelligence to search for drugs to be repurposed for the treatment of the disease found that only 1 of 77 candidates directly targeted the virus, while the 76 other drugs that reduced viral infections relied on network-based cellular responses to the virus (5). If we transpose this to the T1D situation, instead of only targeting the immune system as is usually proposed (6), it would make sense to also target the pathways that modulate the β-cell responses to the immune assault—intrinsic β-cell signals that may stimulate the immune response (e.g., HLA class I and chemokine overexpression or neoantigen generation [7,8]) or inhibit the invading immune cells (e.g., PDL1 and HLA-E expression) (9,10).

Why Focus on the β-Cells in T1D?

We can learn from the Buddha's advice as outlined in the *Kalama Sutta*: "Do not go upon what has been acquired by repeated hearing; nor upon tradition . . . nor upon an axiom . . . nor upon a bias towards a notion that has been pondered over. . . . " In other words, let's leave aside for a moment the "axiomatic and many times pondered" view that prevention of T1D equals inhibiting or modulating

[1]Indiana Biosciences Research Institute, Indianapolis, IN
[2]ULB Center for Diabetes Research and Welbio, Medical Faculty, Université Libre de Bruxelles, Brussels, Belgium
[3]JDRF, New York, NY

Corresponding author: Decio L. Eizirik, deizirik@indianabiosciences.org

Received 21 April 2021 and accepted 10 June 2021

the immune system in T1D (an approach that—even when implemented in the prediabetes state—at most delays but has not yet been shown to completely prevent disease [11]) and focus instead on the key role for β-cells in the disease. This has been the subject of recent reviews by us and others (7,12,13), and we will here only emphasize key points suggesting the relevance of the β-cells in T1D. Priority is on data obtained with human islets/β-cells and histology of the pancreas from individuals affected by T1D. We are aware that the role for β-cells as a key agent in T1D pathogenesis is coming into increasing focus (7,12,13) and that the notion that new interventions in T1D should also target β-cell protection is gaining traction; we hope that this article will further support this welcome trend.

Some individuals at increased risk for T1D, due to strong family history, show a decrease in their first-phase glucose-stimulated C-peptide release and an increase in proinsulin-to-insulin ratios in the circulation even in the absence of autoantibodies against β-cells, indicating that disturbed β-cell function may precede in some cases the autoimmune assault (14).

There is histological evidence that many patients—including those who died of ketoacidosis close to diagnosis—retain up to 40% of their normal β-cell mass during the first year of T1D (15), and islets isolated from T1D patients are dysfunctional but regain at least part of their ability to release insulin in response to glucose after 5–6 days in culture at 5.6 mmol/L glucose (16,17). These observations suggest that there remains a population of "sleeping" β-cells after clinical disease onset of T1D, still alive but unable to release insulin. Some patients with long-term T1D and with undetectable circulating C-peptide still release proinsulin (18), suggesting that a fraction of β-cells may survive for >3 years but are not able to convert proinsulin into mature insulin. HLA class I hyperexpression (7), markers of endoplasmic reticulum (ER) stress (19), chemokines, and an interferon (IFN) signature (10,20) are present in these remaining insulin-containing islets from T1D patients, suggesting that they are engaged in a cross talk with the invading immune cells. This cross talk may have negative or potentially positive consequences. Thus, triggering of ER stress (which by itself may lead to apoptosis) and changes in alternative splicing (AS) by proinflammatory mediators, together with upregulation of HLA class I and production of chemokines by β-cells, can lead to increased presentation of neoantigens to the immune cells homing into the islets (7,8,21), amplifying the immune assault. This suggests that acting at the β-cell level may be relevant not only to prevention of immune-mediated β-cell death but also to modulation of the components of β-cell dysfunction that contribute to autoimmunity in the first place—an area where much remains to be learned.

On the other hand, cytokines such as IFNα and IFNγ induce the expression of potentially protective molecules in human islets, namely, PDL1 and HLA-E (7,10), a finding confirmed by histology of insulin-containing islets from patients affected by T1D (9,10). PDL1 is a ligand of PD1, a protein expressed in T cells, and PDL1-PD1 binding inhibits T cell function: for instance, overexpression of PDL1 in β-cells protects mice against immune-mediated diabetes, while inhibiting PD1-PDL1 in the context of immune checkpoint therapy for metastatic tumors triggers immune-mediated endocrine diseases, including T1D (22). HLA-E is a potential inhibitor of both natural killer and T cells (7). A challenge that remains to be solved is how to dissociate the cytokine-induced harmful effects (i.e., induction of HLA class I, ER stress, and chemokines) from the potentially beneficial ones (i.e., induction of PDL1 and HLA-E).

Learning From the Target Tissues in Other Autoimmune Diseases

There is conclusive evidence of increased incidence of other autoimmune diseases in patients affected by T1D, and T1D and other autoimmune diseases have many candidate genes in common (23). We recently compared the gene networks expressed in β-cells isolated from patients affected by T1D against the target tissues of three other autoimmune diseases, namely, kidney cells in systemic lupus erythematosus, optic chiasma in multiple sclerosis (MS), and joint tissue in rheumatoid arthritis (RA) (24). While the target tissues of these diseases are different, they share genetic risk loci and have in common local inflammation (with a role for innate immunity), ER stress (24), and the generation of reactive oxygen species and proinflammatory cytokines as causes of tissue damage. Available RNA sequencing (RNA-seq) data for these different tissues (25–28) were downloaded and processed by a common pipeline (24), and comparisons between diseases were done with the rank-rank hypergeometric overlap analysis (RRHO) (29), a genome-wide approach that enables comparisons between two equally ranked data sets. There were confluent signatures mostly among the upregulated genes, many of them related to types I and II interferon signaling. In agreement with these observations, there was an enrichment of binding motifs for IFN-induced transcription factors in expressed genes at the target tissue level in all four diseases. On the other hand, downregulated pathways were mostly disease specific, indicating dysfunction at the level of the target tissues. For example, β-cells obtained from patients affected by T1D had downregulation of pathways involved in energy metabolism (required for insulin release) and in key transcription factors for the maintenance of the differentiated β-cell phenotype, including PDX1 and MAFA (24).

Of particular relevance, in all four diseases >80% of known candidate genes were expressed at the target tissue level (24), which is in line with previous observations that candidate genes for T1D regulate β-cell responses to potential "danger signals," such as viral infections or endogenous nuclear acid fragments and type 1 IFN

signaling (30,31). T1D-associated variants have been described as "enriched for immune cell types" but not or only marginally present in pancreatic islets regulatory elements under basal conditions (32). This apparent discrepancy was clarified by recent findings showing that human islet regulatory elements responsive to the cytokines IL1β and IFNγ are enriched for T1D risk variants (33), indicating that T1D risk variants indeed impact β-cells but this only becomes detectable upon exposure of these cells to relevant immune stimuli, such as proinflammatory cytokines (7,33).

Interestingly, one of the few candidate genes expressed in the target tissues of the four autoimmune diseases studied is *TYK2*, a key kinase activated during the early steps of the signal transduction of type I IFNs. Genetic variants that partially decrease TYK2 activity are protective against T1D diabetes (31), and a 50% inhibition of *TYK2* by specific siRNAs or chemical inhibitors protects human β-cells against death caused by exposure to the viral by-product double-stranded RNA or the proinflammatory cytokine IFNα (34,35) without sensitizing β-cells to infections by the potential diabetogenic enteroviruses Coxsackie B virus types 1 and 5 (35). TYK inhibitors are being tested as therapeutic agents for psoriasis (phase 2 trial) (36) and RA and inflammatory bowel disease (preclinical studies) (37).

The studies described above involved comparisons between an autoimmune endocrine disease, i.e., T1D, against three other autoimmune diseases that do not target primarily endocrine glands. We recently gained access to RNA-seq of thyroid tissue obtained from patients affected by Hashimoto's thyroiditis (HT) as compared with normal thyroid tissue (38). These data sets were analyzed with our pipeline (24) and compared by RRHO to RNA-seq data from β-cells from T1D patients as compared with respective control subjects (25) (Fig. 1). As an external comparison, we also included the RRHO analysis of β-cells from T1D patients versus optic chiasma from patients affected by MS (27) (Fig. 1*B*), the nonendocrine autoimmune disease with the closest similarity to T1D regarding changes in global gene expression (24). There is clearly a more marked similarity between the upregulated genes in the target tissues of the two endocrine diseases as compared with optic chiasma in MS (Fig. 1*B*). Enrichment analysis of the upregulated pathways in common between target tissues of T1D and HT identified neutrophil degranulation, signaling downstream of types I and II IFNs, cytokine-cytokine receptor interactions, and cell adhesion molecules, among others (Fig. 1*C* and *D*). These novel observations reinforce the role for IFNs in autoimmune endocrine diseases and point to novel therapeutic possibilities (see below).

The AS Connection

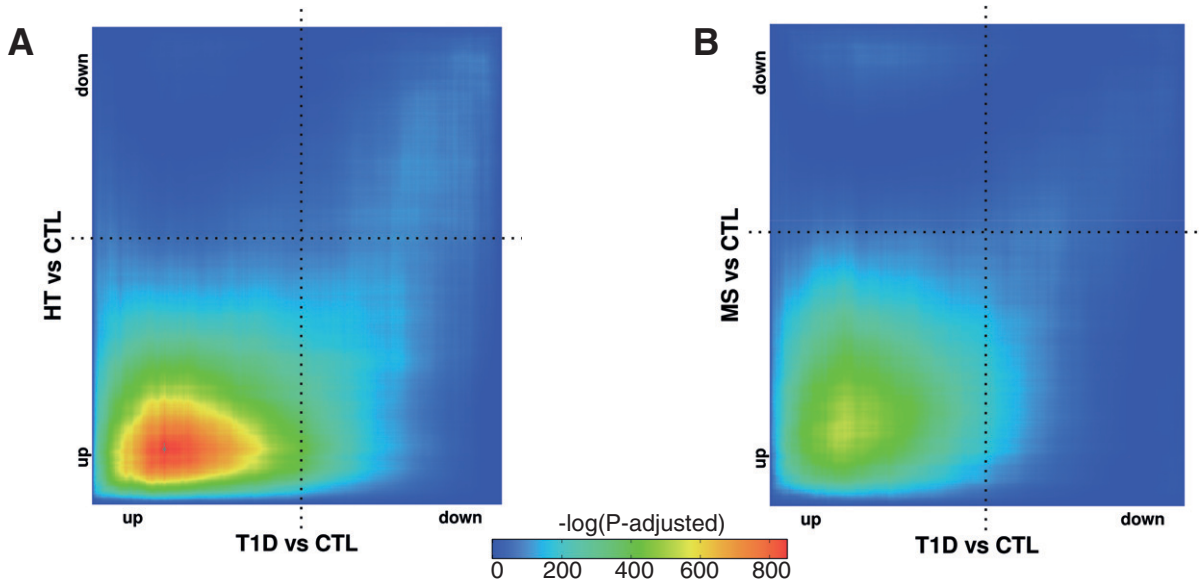
AS is a major generator of transcriptome and proteome diversity. Pre-mRNA splicing is a highly regulated process that involves the interaction between specific *cis*-acting pre-mRNA elements and different *trans*-acting factors (RNA-binding proteins [RBPs]), which together form the so-called "splicing code" and shape splicing decisions. AS outcome is influenced by modifications at the level of *cis*-regulatory regions (e.g., mutations, methylation) or the expression of *trans*-acting factors (39,40). Proinflammatory cytokines such as IFNα or IL1β plus IFNγ and diabetes candidate genes such as *GLIS3* modulate the AS repertoire of human pancreatic β-cells (10,41–43). Proinflammatory cytokines modify the expression of >30 RPBs in β-cells (40,43). The effects of cytokines in AS seem to be specific, as they induce particular AS patterns that are different from those induced by the metabolic stressor palmitate (39). Inhibition of the candidate gene *GLIS3* has a proapoptotic effect on human β-cells, which is mediated at least in part via downregulation of the splicing factor SRSF6 (also known as SRp55). *SRSF6* inhibition impairs directly or indirectly the splicing of pre-mRNAs encoding key proteins for β-cell function (e.g., INSR, SNAP25), and survival (e.g., BIM-Small, BAX-β) (41,44).

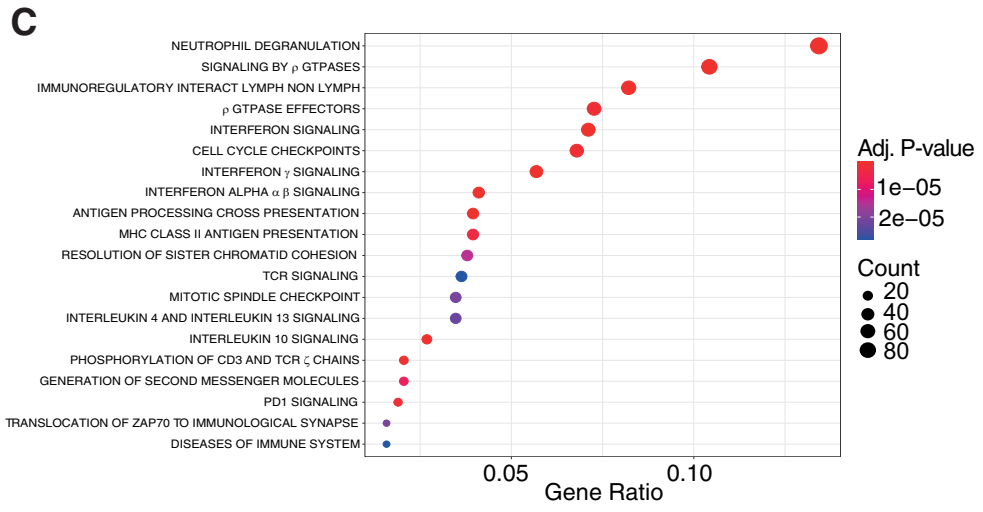
Many of the cytokine-induced changes in gene expression in human islets are similar to the ones observed in human β-cells isolated from patients affected by T1D (7,10) and are also present in the target tissues of other autoimmune diseases (24) (Fig. 1), suggesting that the putative "dialog" between β-cells and the immune system may be valid for other autoimmune diseases. This may also extend to changes in the regulation of AS. As such, human islets exposed to IL1β plus IFNγ share a large number of modified RBPs in common with joint tissues of patients affected by RA (Fig. 2).

Most T1D risk loci are located in intronic regions, suggesting that they are involved in β-cell genomic regulation rather than protein function (7). T1D-associated risk single nucleotides polymorphisms may disrupt splicing, altering the transcription of T1D candidate genes. This, and the impact of inflammatory mediators on β-cell splicing, suggests that antisense oligonucleotide (ASO)-mediated splicing modulation could be a tractable therapeutic approach in T1D. This approach is already being tested in the clinic for other diseases caused by AS defects, such as in Duchenne muscular dystrophy and spinal muscle atrophy (45).

Data Mining and Drug Repurposing to Protect β-Cells in T1D—An Unmet Need

In silico correlation between disease-related pathways and drugs that induce an opposite gene signature ("perturbagens") may reveal new therapies and/or agents to be repurposed between autoimmune diseases (46). We compared a panel of drug-modified data sets (46) against the gene pathways induced by exposure of human β-cells to relevant proinflammatory cytokines (7,10) or against the pathways present in β-cells obtained from patients affected by T1D (24,25). This identified interesting candidates, including bile acid–derived molecules (that may modulate ER stress),





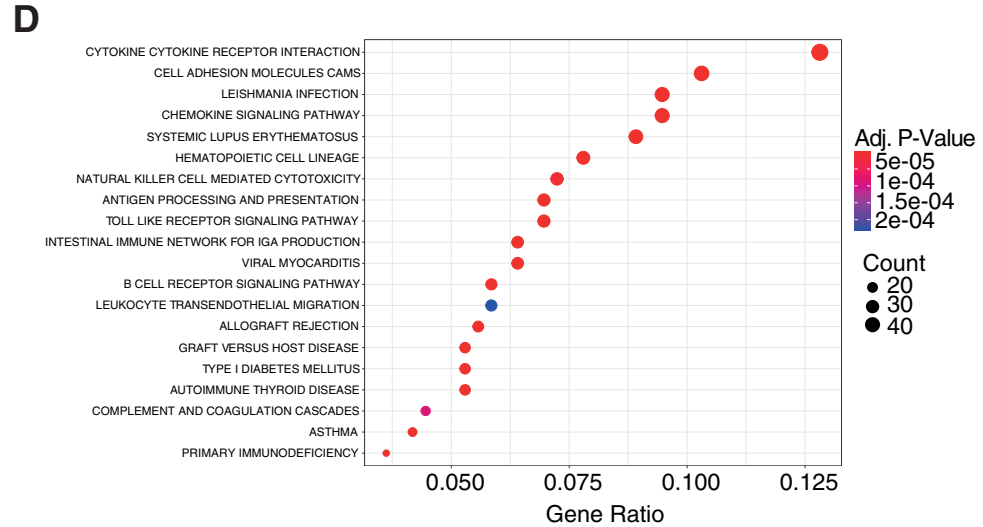


Figure 1—RRHO analysis comparing the gene expression signatures of FACS-purified β-cells in T1D, thyroid tissue samples in HT, and optic chiasm samples in MS reveals similarities in upregulated genes and enriched pathways. *A* and *B*: After a differential expression analysis between the target tissues in T1D, MS, HT, and their healthy controls (CTL) tissues as previously described (24), genes were ranked by their fold change from the most down- to the most upregulated ones and submitted to the RRHO algorithm. The level map colors indicate the adjusted log *P* values of the overlap (Benjamini and Yekutieli correction) between genes upregulated in both data sets (bottom-

bromodomain inhibitors, and TYK2 inhibitors (10,24) (see below). This approach is, however, limited by two main factors: *1*) the use of surrogate cell lines that are not directly related to the disease under study and *2*) the fact that surrogate cells are exposed to the tested agents under basal conditions, which is different from the disease situation. For instance, in our previous search for perturbagens, we correlated human β-cells exposed to cytokines or isolated from the pancreas of patients affected by T1D (10,24) against a database containing thousands of standard cell lines—none of them insulin producing—exposed to a panoply of test agents under basal conditions (46). Disease biology is in most cases cell type specific, and pancreatic β-cells have particular characteristics; for instance, to sense nutrients and release insulin, β-cells are unique by increasing, instead of decreasing, mitochondrial ATP production in face of rising glucose concentrations. Furthermore, adult human β-cells have very low proliferative capability (47), which is not the case for the cell lines used in the global screening for drug-modifying signatures (46). Thus, comparing stressed β-cells with cell lines derived from other tissues and exposed to putative therapeutic agents under nonstressed conditions has clear limitations.

A better approach would be to map the dysregulated networks in the relevant cell type, i.e., human β-cells in the case of T1D. This is difficult due to the limitations in obtaining sufficient amounts of human islets and their resistance to genetic modifications. An alternative is to use induced pluripotent stem cell (iPSC)-derived β-cells from patients with type 1 diabetes with relevant disease-predisposing polymorphisms or to introduce these polymorphisms by CRISPR/Cas9 and then expose the cells to relevant stimuli, such as proinflammatory cytokines (7,48) or $CD8^+$ T cells (49), ahead of RNA-seq and proteomics to define the key hubs that drive β-cell dysfunction and death. The next step would be interfering with these disease-related hubs to restore physiology; for instance, the kinase JAK1 is a key hub for the deleterious effects of IFNα on human β-cells, and decreasing JAK1 activity using specific inhibitors preserves β-cell function and survival in the presence of IFNα (10) (see below). Such a global approach has been used with success to identify therapeutic candidates for heart valve disease (2).

Initial Candidates for β-Cell Protection

Most β-cell–targeting therapies are still at the preclinical stage or undergoing clinical trials at phase I (Table 1). To exemplify this approach, we list below potential therapies to increase β-cell resistance to IFNα (Fig. 3). This cytokine and its footprints are present in islets from T1D patients, and it may play a crucial role in the early phases of insulitis and β-cell destruction (reviewed in 7). Some of the downstream consequences of IFNα exposure (Fig. 3), such as ER stress, are probably a common end point for diverse β-cell stresses (7,50,51), while some of the proposed approaches, such as exercise, may have benefits that go well beyond protection against β-cell ER stress (52).

The first step of type 1 IFN signaling is binding to its receptors (IFNαR1 and IFNαR2) and activation of the kinases JAK1 and TYK2. This is followed by phosphorylation and activation of the transcription factors STAT1/STAT2, which then dimerize and move into the cell nucleus, leading to a series of effects that, in pancreatic β-cells, may trigger ER stress and upregulation of HLA class I and chemokines (consequently increasing antigen presentation and the attraction of immune cells) but also the upregulation of the potentially protective proteins PDL1 and HLA-E (7,34) (Fig. 3). Potential therapies targeting *TYK2*, a candidate gene for T1D, discussed above, provide a promising strategy for an early therapy for T1D (35). Currently approved JAK inhibitors usually block the activity of JAK1 and JAK2, affecting both IFNα/β and IFNγ. These inhibitors, including baricitinib, have been approved as a therapy for RA (53) and are being tested in phase 2 trials for systemic lupus erythematosus (54). JAK inhibitors prevent the deleterious effects of IFNα on human β-cells, decreasing HLA class I and CXCL10 expression and IFNα plus IL1β–induced apoptosis (10), and reverse diabetes in newly diagnosed diabetic NOD mice (55). A clinical trial based on the use of baricitinib to prevent progressive β-cell death in T1D is starting now (Table 1) (clinical trial reg. no. NCT04774224, ClinicalTrials.gov).

The fact that both TYK2 (36) and JAK inhibitors (53,54) are already in use, or in advanced stages of clinical tests, increases their potential as a T1D therapy. Indeed, repurposing drugs whose toxicology, pharmacodynamics, and pharmacokinetic profiles are already characterized facilitates the bench-to-bedside transition (56). While systemic inhibition of this family of kinases has been linked to some rare safety concerns, newer generations of these inhibitors with increased specificity against single JAK family members are posed to address these.

left quadrant), downregulated in both (top-right quadrant), upregulated in the left-hand pathology and downregulated in the bottom part (top-left quadrant), and downregulated in the left-hand pathology and upregulated in the bottom part (bottom-right quadrant). The color palette was scaled up to the highest −log(*P* value adjusted) to allow the comparison between the two results. Pairwise analysis between FACS-purified β-cells in T1D and thyroid tissue samples in HT (*A*) and optic chiasm samples in MS (*B*). *C* and *D*: Overlapping genes between T1D and HT in the upregulated quadrant were submitted to a hypergeometric test using clusterProfiler to highlight common enriched pathways between the target tissues of the two diseases and then analyzed using the Reactome (*C*), and Kyoto Encyclopedia of Genes and Genomes (KEGG) (*D*) pathway databases. We downloaded data for T1D (from 25), HT (27), and MS (38), and they were reanalyzed as previously described (24). Adj., adjusted. IMMUNOREGULATORY INTERACT LYMPH NON LYMPH, immunoregulatory interactions between a lymphoid and a non-lymphoid cell.

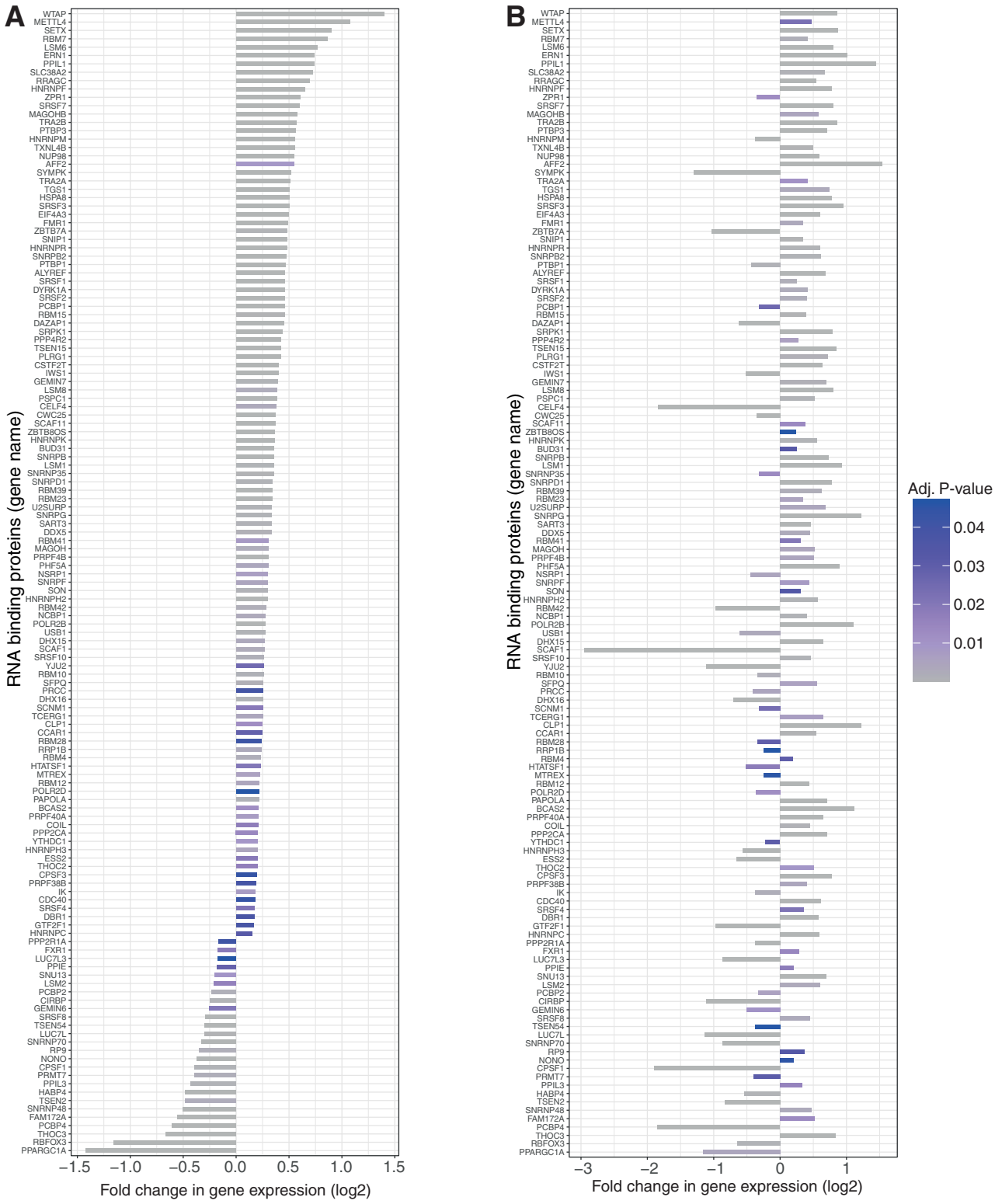


Figure 2—Similar modifications on the mRNA expression of RBPs in human islets exposed to proinflammatory cytokines (IL1β plus IFNγ) and in the target tissue of RA. *A* and *B*: Bar charts depict log2-transformed fold change of the 137 differentially expressed RBP genes (adjusted *P* value <0.05) in human islets (*A*) exposed to IL1β plus IFNγ and in the target tissue of RA (*B*) as compared with their respective controls. We downloaded data for human islets (from 33) and RA (28), and they were reanalyzed as previously described (24). Adj., adjusted.

Table 1—Registered clinical trials aiming, at least in part, to directly protect β-cells in T1D

Clinical trial reg. no. (ClinicalTrials.gov)	Title	Target population	Location	Main outcomes	Current phase of clinical trial
NCT04233034	Hybrid Closed Loop Therapy and Verapamil for Beta Cell Preservation in New Onset Type 1 Diabetes (CLVer)	T1D (age 7–17 years)	U.S.	C-peptide, CGM, etc.	3
NCT04545151	Verapamil SR in Adults with Type 1 Diabetes (Ver-A-T1D)	T1D (age 18–45 years)	Europe	C-peptide, proinsulin/insulin, CGM, etc.	2
NCT02218619	Tauroursodeoxycholic Acid (TUDCA) in New-Onset Type 1 Diabetes	T1D (age 18–45 years)	U.S.	C-peptide	2
NCT02407899	Protective Effects of Saxagliptin (And Vitamin D3) on β Cell Function in Adult-onset Latent Autoimmune Diabetes	LADA (age 18–70 years)	China	C-peptide	4
NCT02820558	Neuropeptide Therapy of Recent Onset Type 1 Diabetes	T1D (age 10–18 years)	Canada	C-peptide	1
NCT04774224	Baricitinib in New-onset Type 1 Diabetes (BANDIT)	T1D (age 12–30 years)	Australia	C-peptide	2
NCT02384889	DFMO in Children with Type 1 Diabetes	T1D (age 12–40 years)	U.S.	C-peptide, proinsulin/insulin, etc.	1
NCT03635437	Evaluation of Safety and Diabetes Status Upon Oral Treatment With GABA in Patients With Longstanding Type-1 Diabetes	T1D (age 18–50 years)	Sweden	C-peptide, safety of oral GABA	1/2
NCT04729296	Anti-TNFα to Delay or Prevent Progression to Stage 3 T1D	T1D (age 3–46 years)	U.S.	Not provided	2

All trials listed above are intervention trials, aiming to preserve/restore β-cell function in patients with early or, in one case (γ-aminobutyric acid [GABA]) long-standing, clinical T1D or, in one trial, adult-onset latent diabetes mellitus (LADA). CGM, continuous glucose monitoring.

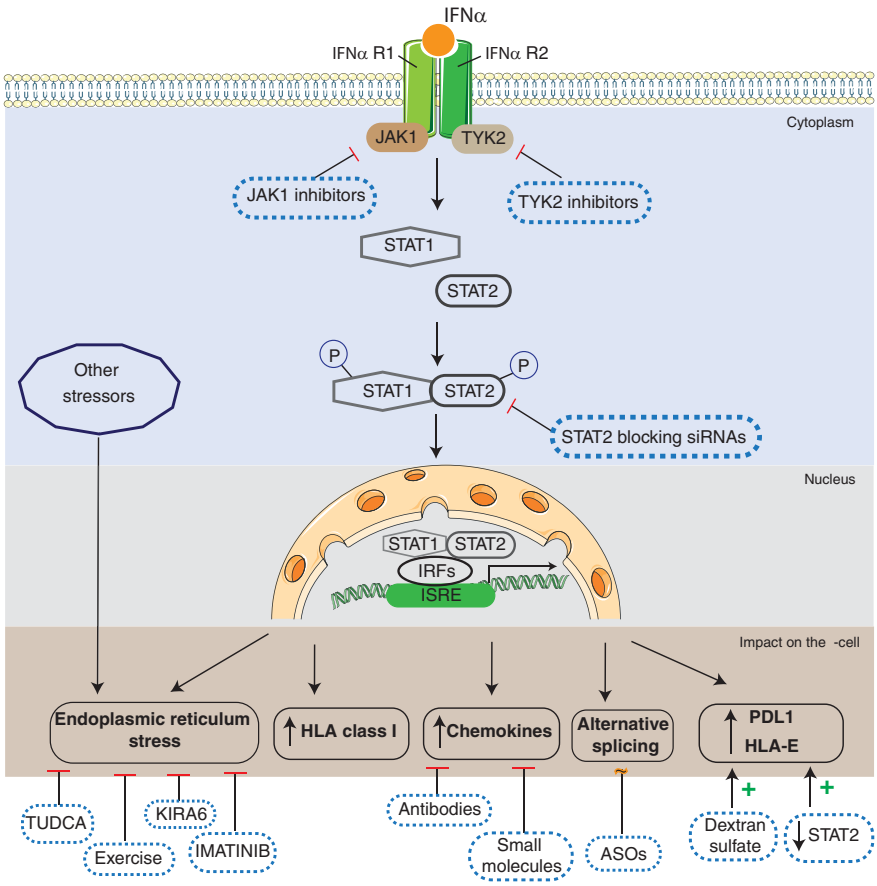


Figure 3—Downstream signaling pathways induced by IFNα in pancreatic β-cells and putative therapeutic intervention points. INFα binds and activates β-cell type I interferon surface receptors—the IFNα receptors R1 and R2—leading to the activation of the receptor-associated tyrosine kinases Janus kinase 1 (JAK1) and tyrosine kinase 2 (TYK2). Both JAK1 and TYK2 can be blocked by specific inhibitors (see text for details). JAK1 and TYK2 phosphorylate the cytoplasmic transcription factors signal transducer and activator of transcription 1 (STAT1) and STAT2, promoting their dimerization and translocation to the nucleus. STAT2 can be inhibited by STAT2 blocking siRNAs and thus preventing the heterodimers' formation. STAT1/STAT2 assemble with members of interferon regulatory factor (IRFs) and form a trimolecular complex that moves into the nucleus and regulates the expression of target genes by binding to interferon-stimulated responsive elements (ISRE) present in their promoter regions. The cellular response to IFNα involves ER stress, which is also triggered by additional stressors and can be prevented and/or ameliorated by different compounds (e.g., TUDCA, KIRA6, imatinib) and also by regular physical exercise; increased expression of HLA class I at the β-cell surface; increased secretion of chemokines such as CXCL10, which can be blocked with use of specific antibodies and small molecules; and modifies the β-cell AS pattern. The targeted delivery of ASOs to the β-cell might lead to modulation of the splicing of harmful isoforms and thus prevent β-cell dysfunction and death. Additionally, IFNα increases expression of protective molecules, such as programmed death-ligand 1 (PDL1) and HLA-E. The use of dextran sulfate or the decreased expression of STAT2 may act as a positive regulator of these β-cell–protective pathways.

A key issue in interfering with IFN signal transduction is dissociation of the beneficial from the deleterious effects of the cytokine at the β-cell level. Knocking down the transcription factor *STAT2* induces these effects, preventing HLA class I expression while upregulating PDL1 (9). Furthermore, repeated short-term exposures to IFNγ also dissociates these two pathways in iPSC-induced islet cells, likely via epigenetic mechanisms, and restricts activation of T cells cocultured with iPSC-derived human islets (57). IFNs have, however, multiple stimulatory effects on the immune system, and it is difficult to envisage translation of this approach to the early stages of T1D.

Many of the approaches discussed here will not act at the β-cells only. For example, targeting JAKS and TYK2 will affect many pathways in the immune system, while imatinib may interfere with T cell function. Furthermore, many of the gene networks addressed here are not unique to β-cells, although the way human β-cells respond to their activation may indeed be specific due to unique characteristics of the β-cells, including high insulin synthesis, low proliferative capacity, and a particular susceptibility to viral- and metabolic-induced stress (12,31).

Localized drug delivery of inhibitors with improved specificity to key nodes in this signaling cascade could obviate this issue and address potential safety concerns. For example, blocking of *STAT2*, either by small molecules or via in vivo use of cell-targeted siRNAs, is an interesting approach, particularly if targeted to the β-cells. RNA-based therapies are being developed for multiple oncology

indications, rare/orphan diseases, and severe hypercholesterolemia (58). In the case of T1D, siRNA-mediated *STAT2* blockade could be achieved by targeting the siRNA to proteins specifically expressed at the β-cell surface, as has recently been done with another cargo with use of a GLP1 analog coupled to an ASO (59). Targeting moieties for β-cell delivery of therapeutic siRNA can be readily developed from the ligands validated in human pancreatic imaging studies such as exendin 4, nanobodies against dipeptidyl peptidase like 6 (DPP6), and others (60). Another interesting approach in this context is the use of sulfated semisynthetic polysaccharide dextran sulfate, which upregulates PDL1 expression in mouse islets (among other anti-inflammatory effects) and reverses diabetes in NOD mice (61).

Once the dimer STAT1-STAT2 moves to the nucleus, often after forming a trimer with members of the IRF1 family, it binds to interferon-stimulated responsive elements and triggers expression of several pathways that will lead to ER stress (which may also be caused by other cytokines such as IL1β plus IFNγ and metabolic stress), upregulation of HLA class I, production of chemokines, and, on the positive side, upregulation of PDL1 and HLA-E (7). Several of these pathways can be targeted for β-cell protection (Table 1 and Fig. 3).

Exposure of β-cells to the proinflammatory environment prevailing during insulitis, including the exposure to the cytokines IFNα, IL1β, TNFα, and IFNγ, triggers ER stress (7,19). As discussed above, this may contribute to both the presentation of neoantigens and, if unresolved, to β-cell death. The key pathway for cytokine-induced human β-cell ER stress and consequent apoptosis is activation of IRE1α and, downstream of it, degradation of relevant mRNAs (including insulin, a target of IRE1α RNAse activity) and JNK phosphorylation (7). The facts that β-cells have endogenous protection mechanisms against excessive induction of IRE1α, namely, cytokine induction of N-MYC interactor and ubiquitin D (proteins that provide a specific negative feedback against JNK activation) (7), and that ablation of IRE1α in β-cells of NOD mice decreases diabetes incidence (62) indicate the importance of this pathway. Two drugs have been shown to prevent excessive IRE1α activation and revert diabetes in NOD mice, namely, ATP-competitive IRE1α kinase–inhibiting RNase attenuator 6 (KIRA6) and imatinib (63–65) (KIRA6 also prevents diabetes in the Akita mouse, a monogenic model of diabetes where ER stress is caused by insulin misfolding [64]). Imatinib has been approved by the U.S. Food and Drug Administration as an anticancer inhibitor of tyrosine kinase and may prevent destruction of β-cells in people when administered early after T1D diagnosis. Another broader inhibitor of the deleterious effects of ER stress is the chemical chaperone tauroursodeoxycholic acid (TUDCA); it partially protects human β-cells from cytokine-induced apoptosis (66) and delays (but apparently does not revert) diabetes in mouse models of T1D (67). TUDCA is used for the therapy of liver diseases, and there is an ongoing clinical trial with use of TUDCA to delay β-cell loss after the onset of T1D (Table 1) (clinical trial reg. no. NCT02218619, ClinicalTrials.gov). Other interesting strategies include verapamil, an antihypertensive agent that acts as a TXNIP inhibitor and has shown promising results in a small-scale clinical trial (68); blockers of TNF, which were validated by a recent clinical trial in patients with early T1D and may both directly protect β-cells and modulate the immune assault (69); and exercise, which protects β-cells against ER stress probably via the release of protective myokines (70).

One of the key chemokines released by β-cells is CXCL10. While CXCL10 expression is increased in the islets affected by insulitis, infiltrating lymphocytes express the corresponding receptor CXCR3, illustrating the "dialogue" between β-cells and the invading immune cells (7). Small molecules are being developed to block CXCL10 or CXCR3 in autoimmune thyroiditis and other diseases (71), and they may eventually be considered as an adjuvant therapy in T1D.

In conclusion, therapies for β-cell protection in T1D seem to face a situation similar to that of green energy, usually criticized for not being economically viable, which is probably due to the fact that, during decennia, very limited public investments were done to make it economically viable. In the case of therapies for β-cell protection, since most interest is focused on the immune system, there are very few clinical trials aiming to protect β-cells. Worse, these rare trials usually disregard the fundamental issue that once the immune system starts recognizing β-cells as "foreign" and attacks them, β-cell protection alone will not suffice. Indeed, the immune system is resourceful and persistent, and β-cell–protective therapies *must* be combined with therapies aiming to reduce the immune assault. By not taking this into consideration, we may be wasting useful agents that could be used either very early in the disease process or as supportive/combination therapies. There is an increasing interest in other autoimmune diseases, such as MS and inflammatory bowel disease, to develop adjunctive therapies to protect target tissues from inflammation-mediated damage (72), and it is our hope that this trend will also gain further traction for T1D.

Acknowledgments. The authors are grateful to Dr. Maikel Colli (ULB Center for Diabetes Research and Welbio, Medical Faculty, Universitè Libre de Bruxelles [ULB]) for helpful discussions regarding the comparison between different RNA-seq data (Fig. 1). Due to restrictions in the number of references, the authors cite preferentially reviews. The authors acknowledge and apologize to all authors whose relevant original studies were not cited.

Funding. D.L.E. acknowledges the support of grants from the Welbio-FNRS (Fonds National de la Recherche Scientifique) (WELBIO-CR-2019C-04), Belgium; the Dutch Diabetes Research Fundation (Innovate2CureType1), Holland; the JDRF (2-SRA-2019-834-S-B); the National Institutes of Health Human Islet Research Network Consortium on Beta Cell Death & Survival

[HIRN-CBDS]) (grant U01 DK127786); start up-funds from the Indiana Biosciences Research Institute; and the Innovative Medicines Initiative 2 Joint Undertaking under grant agreements 115797 (INNODIA) and 945268 (INNODIA HARVEST), supported by the European Union's Horizon 2020 research and innovation programme. These joint undertakings receive support from the European Union's Horizon 2020 research and innovation programme and European Federation of Pharmaceutical Industries and Associations (EFPIA), JDRF, and the Leona M. and Harry B. Helmsley Charitable Trust.

Duality of Interest. D.L.E. receives grant support from Eli Lilly and Co., Indianapolis, IN, for research on new approaches to protect pancreatic β-cells in T1D. No other potential conflicts of interest relevant to this article were reported.

References

1. Gates AJ, Gysi DM, Kellis M, Barabási AL. A wealth of discovery built on the Human Genome Project - by the numbers. Nature 2021;590:212–215
2. Theodoris CV, Zhou P, Liu L, et al. Network-based screen in iPSC-derived cells reveals therapeutic candidate for heart valve disease. Science 2021;371:371
3. Insel RA, Dunne JL, Atkinson MA, et al. Staging presymptomatic type 1 diabetes: a scientific statement of JDRF, the Endocrine Society, and the American Diabetes Association. Diabetes Care 2015;38:1964–1974
4. Yildirim MA, Goh KI, Cusick ME, Barabási AL, Vidal M. Drug-target network. Nat Biotechnol 2007;25:1119–1126
5. Morselli Gysi D, do Valle I, Zitnik M, et al. Network medicine framework for identifying drug-repurposing opportunities for COVID-19. Proc Natl Acad Sci U S A 2021;118:e2025581118
6. Linsley PS, Greenbaum CJ, Nepom GT. Uncovering pathways to personalized therapies in type 1 diabetes. Diabetes 2021;70:831–841
7. Eizirik DL, Pasquali L, Cnop M. Pancreatic β-cells in type 1 and type 2 diabetes mellitus: different pathways to failure. Nat Rev Endocrinol 2020;16:349–362
8. Piganelli JD, Mamula MJ, James EA. The role of β cell stress and neo-epitopes in the immunopathology of type 1 diabetes. Front Endocrinol (Lausanne) 2021;11:624590
9. Colli ML, Hill JLE, Marroquí L, et al. PDL1 is expressed in the islets of people with type 1 diabetes and is up-regulated by interferons-α and-γ via IRF1 induction. EBioMedicine 2018;36:367–375
10. Colli ML, Ramos-Rodríguez M, Nakayasu ES, et al. An integrated multi-omics approach identifies the landscape of interferon-α-mediated responses of human pancreatic beta cells. Nat Commun 2020;11:2584
11. Herold KC, Bundy BN, Long SA, et al.; Type 1 Diabetes TrialNet Study Group. An anti-CD3 antibody, Teplizumab, in relatives at risk for type 1 diabetes. N Engl J Med 2019;381:603–613
12. Mallone R, Eizirik DL. Presumption of innocence for beta cells: why are they vulnerable autoimmune targets in type 1 diabetes? Diabetologia 2020;63:1999–2006
13. Roep BO, Thomaidou S, van Tienhoven R, Zaldumbide A. Type 1 diabetes mellitus as a disease of the β-cell (do not blame the immune system?). Nat Rev Endocrinol 2021;17:150–161
14. Sims EK, DiMeglio LA. Cause or effect? A review of clinical data demonstrating beta cell dysfunction prior to the clinical onset of type 1 diabetes. Mol Metab 2019;27S:S129–S138
15. Oram RA, Sims EK, Evans-Molina C. Beta cells in type 1 diabetes: mass and function; sleeping or dead? Diabetologia 2019;62:567–577
16. Marchetti P, Dotta F, Ling Z, et al. Function of pancreatic islets isolated from a type 1 diabetic patient. Diabetes Care 2000;23:701–703
17. Krogvold L, Skog O, Sundström G, et al. Function of isolated pancreatic islets from patients at onset of type 1 diabetes: insulin secretion can be restored after some days in a nondiabetogenic environment in vitro: results from the DiViD study. Diabetes 2015;64:2506–2512
18. Sims EK, Bahnson HT, Nyalwidhe J, et al.; T1D Exchange Residual C-peptide Study Group. Proinsulin secretion is a persistent feature of type 1 diabetes. Diabetes Care 2019;42:258–264
19. Marhfour I, Lopez XM, Lefkaditis D, et al. Expression of endoplasmic reticulum stress markers in the islets of patients with type 1 diabetes. Diabetologia 2012;55:2417–2420
20. Lundberg M, Krogvold L, Kuric E, Dahl-Jørgensen K, Skog O. Expression of interferon-stimulated genes in insulitic pancreatic islets of patients recently diagnosed with type 1 diabetes. Diabetes 2016;65:3104–3110
21. Gonzalez-Duque S, Azoury ME, Colli ML, et al. Conventional and neo-antigenic peptides presented by β cells are targeted by circulating naïve CD8+ T cells in type 1 diabetic and healthy donors. Cell Metab 2018;28:946–960.e6
22. Martinov T, Fife BT. Type 1 diabetes pathogenesis and the role of inhibitory receptors in islet tolerance. Ann N Y Acad Sci 2020;1461:73–103
23. Farh KK, Marson A, Zhu J, et al. Genetic and epigenetic fine mapping of causal autoimmune disease variants. Nature 2015;518:337–343
24. Szymczak F, Colli ML, Mamula MJ, Evans-Molina C, Eizirik DL. Gene expression signatures of target tissues in type 1 diabetes, lupus erythematosus, multiple sclerosis, and rheumatoid arthritis. Sci Adv 2021;7:eabd7600
25. Russell MA, Redick SD, Blodgett DM, et al. HLA class II antigen processing and presentation pathway components demonstrated by transcriptome and protein analyses of islet β-cells from donors with type 1 diabetes. Diabetes 2019;68:988–1001
26. Liao Z, Ye Z, Xue Z, et al. Identification of renal long non-coding RNA RP11-2B6.2 as a positive Regulator of type I interferon signaling pathway in lupus nephritis. Front Immunol 2019;10:975
27. Itoh N, Itoh Y, Tassoni A, et al. Cell-specific and region-specific transcriptomics in the multiple sclerosis model: focus on astrocytes. Proc Natl Acad Sci U S A 2018;115:E302–E309
28. Guo Y, Walsh AM, Fearon U, et al. CD40L-dependent pathway is active at various stages of rheumatoid arthritis disease progression. J Immunol 2017;198:4490–4501
29. Plaisier SB, Taschereau R, Wong JA, Graeber TG. Rank-rank hypergeometric overlap: identification of statistically significant overlap between gene-expression signatures. Nucleic Acids Res 2010;38:e169
30. Santin I, Eizirik DL. Candidate genes for type 1 diabetes modulate pancreatic islet inflammation and β-cell apoptosis. Diabetes Obes Metab 2013;15(Suppl. 3):71–81
31. Op de Beeck A, Eizirik DL. Viral infections in type 1 diabetes mellitus–why the β cells? Nat Rev Endocrinol 2016;12:263–273
32. Onengut-Gumuscu S, Chen WM, Burren O, et al.; Type 1 Diabetes Genetics Consortium. Fine mapping of type 1 diabetes susceptibility loci and evidence for colocalization of causal variants with lymphoid gene enhancers. Nat Genet 2015;47:381–386
33. Ramos-Rodríguez M, Raurell-Vila H, Colli ML, et al. The impact of proinflammatory cytokines on the β-cell regulatory landscape provides insights into the genetics of type 1 diabetes. Nat Genet 2019;51:1588–1595
34. Marroqui L, Dos Santos RS, Op de Beeck A, et al. Interferon-α mediates human beta cell HLA class I overexpression, endoplasmic reticulum stress and apoptosis, three hallmarks of early human type 1 diabetes. Diabetologia 2017;60:656–667
35. Coomans de Brachène A, Castela A, Op de Beeck A, et al. Preclinical evaluation of tyrosine kinase 2 inhibitors for human beta-cell protection in type 1 diabetes. Diabetes Obes Metab 2020;22:1827–1836
36. Papp K, Gordon K, Thaçi D, et al. Phase 2 trial of selective tyrosine kinase 2 inhibition in psoriasis. N Engl J Med 2018;379:1313–1321
37. Burke JR, Cheng L, Gillooly KM, et al. Autoimmune pathways in mice and humans are blocked by pharmacological stabilization of the TYK2 pseudokinase domain. Sci Transl Med 2019;11:eaaw173631341059
38. Martínez-Hernández R, Serrano-Somavilla A, Ramos-Leví A, et al. Integrated miRNA and mRNA expression profiling identifies novel targets and

pathological mechanisms in autoimmune thyroid diseases. EBioMedicine 2019;50:329–342
39. Juan-Mateu J, Villate O, Eizirik DL. Mechanisms in endocrinology: alternative splicing: the new frontier in diabetes research. Eur J Endocrinol 2016;174:R225–R238
40. Alvelos MI, Juan-Mateu J, Colli ML, Turatsinze JV, Eizirik DL. When one becomes many-alternative splicing in β-cell function and failure. Diabetes Obes Metab 2018;20(Suppl. 2):77–87
41. Juan-Mateu J, Alvelos MI, Turatsinze JV, et al. SRp55 regulates a splicing network that controls human pancreatic β-cell function and survival. Diabetes 2018;67:423–436
42. Nogueira TC, Paula FM, Villate O, et al. GLIS3, a susceptibility gene for type 1 and type 2 diabetes, modulates pancreatic beta cell apoptosis via regulation of a splice variant of the BH3-only protein Bim. PLoS Genet 2013;9:e1003532
43. Eizirik DL, Sammeth M, Bouckenooghe T, et al. The human pancreatic islet transcriptome: expression of candidate genes for type 1 diabetes and the impact of pro-inflammatory cytokines. PLoS Genet 2012;8:e1002552
44. Alvelos MI, Brüggemann M, Sutandy FR, et al. The RNA-binding profile of the splicing factor SRSF6 in immortalized human pancreatic β-cells. Life Sci Alliance 2020;4:e202000825
45. Aartsma-Rus A. FDA approval of nusinersen for spinal muscular atrophy makes 2016 the year of splice modulating oligonucleotides. Nucleic Acid Ther 2017;27:67–69
46. Subramanian A, Narayan R, Corsello SM, et al. A next generation Connectivity Map: L1000 platform and the first 1,000,000 profiles. Cell 2017;171:1437–1452.e17
47. Cnop M, Igoillo-Esteve M, Hughes SJ, Walker JN, Cnop I, Clark A. Longevity of human islet α- and β-cells. Diabetes Obes Metab 2011;13(Suppl. 1):39–46
48. Demine S, Schiavo AA, Marín-Cañas S, Marchetti P, Cnop M, Eizirik DL. Pro-inflammatory cytokines induce cell death, inflammatory responses, and endoplasmic reticulum stress in human iPSC-derived beta cells. Stem Cell Res Ther 2020;11:7
49. Carré A, Richardson SJ, Larger E, Mallone R. Presumption of guilt for T cells in type 1 diabetes: lead culprits or partners in crime depending on age of onset? Diabetologia 2021;64:15–25
50. Eizirik DL, Miani M, Cardozo AK. Signalling danger: endoplasmic reticulum stress and the unfolded protein response in pancreatic islet inflammation. Diabetologia 2013;56:234–241
51.Clark AL, Urano F. Endoplasmic reticulum stress in beta cells and autoimmune diabetes. Curr Opin Immunol 2016;43:60–66
52. Narendran P, Solomon TP, Kennedy A, Chimen M, Andrews RC. The time has come to test the beta cell preserving effects of exercise in patients with new onset type 1 diabetes. Diabetologia 2015;58:10–18
53. Taylor PC. Clinical efficacy of launched JAK inhibitors in rheumatoid arthritis. Rheumatology (Oxford) 2019;58(Suppl. 1):i17–i26
54. Wallace DJ, Furie RA, Tanaka Y, et al. Baricitinib for systemic lupus erythematosus: a double-blind, randomised, placebo-controlled, phase 2 trial. Lancet 2018;392:222–231
55. Trivedi PM, Graham KL, Scott NA, et al. Repurposed JAK1/JAK2 inhibitor reverses established autoimmune insulitis in NOD mice. Diabetes 2017;66:1650–1660
56. Ashburn TT, Thor KB. Drug repositioning: identifying and developing new uses for existing drugs. Nat Rev Drug Discov 2004;3:673–683
57. Yoshihara E, O'Connor C, Gasser E, et al. Immune-evasive human islet-like organoids ameliorate diabetes. Nature 2020;586:606–611
58. Zhang MM, Bahal R, Rasmussen TP, Manautou JE, Zhong XB. The growth of siRNA-based therapeutics: updated clinical studies. Biochem Pharmacol 2021;189:114432
59. Knerr L, Prakash TP, Lee R, et al. Glucagon like peptide 1 receptor agonists for targeted delivery of antisense oligonucleotides to pancreatic beta cell. J Am Chem Soc 2021;143:3416–3429
60. Demine S, Schulte ML, Territo PR, Eizirik DL. Beta cell imaging-from pre-clinical validation to first in man testing. Int J Mol Sci 2020;21:7274
61. Lu G, Rausell-Palamos F, Zhang J, et al. Dextran sulfate protects pancreatic β-cells, reduces autoimmunity, and ameliorates type 1 diabetes. Diabetes 2020;69:1692–1707
62. Lee H, Lee YS, Harenda Q, et al. Beta cell dedifferentiation induced by IRE1α deletion prevents type 1 diabetes. Cell Metab 2020;31:822–836.e5
63. Morita S, Villalta SA, Feldman HC, et al. Targeting ABL-IRE1α signaling spares ER-stressed pancreatic β cells to reverse autoimmune diabetes. Cell Metab 2017;25:883–897.e8
64. Ghosh R, Wang L, Wang ES, et al. Allosteric inhibition of the IRE1α RNase preserves cell viability and function during endoplasmic reticulum stress. Cell 2014;158:534–548
65. Hägerkvist R, Sandler S, Mokhtari D, Welsh N. Amelioration of diabetes by imatinib mesylate (Gleevec): role of beta-cell NF-kappaB activation and anti-apoptotic preconditioning. FASEB J 2007;21:618–628
66. Brozzi F, Nardelli TR, Lopes M, et al. Cytokines induce endoplasmic reticulum stress in human, rat and mouse beta cells via different mechanisms. Diabetologia 2015;58:2307–2316
67. Engin F, Yermalovich A, Nguyen T, et al. Restoration of the unfolded protein response in pancreatic β cells protects mice against type 1 diabetes. Sci Transl Med 2013;5:211ra156
68. Ovalle F, Grimes T, Xu G, et al. Verapamil and beta cell function in adults with recent-onset type 1 diabetes. Nat Med 2018;24:1108–1112
69. Quattrin T, Haller MJ, Steck AK, et al.; T1GER Study Investigators. Golimumab and beta-cell function in youth with new-onset type 1 diabetes. N Engl J Med 2020;383:2007–2017
70. Paula FMM, Leite NC, Borck PC, et al. Exercise training protects human and rodent β cells against endoplasmic reticulum stress and apoptosis. FASEB J 2018;32:1524–1536
71. Ferrari SM, Fallahi P, Elia G, et al. Novel therapies for thyroid autoimmune diseases: An update. Best Pract Res Clin Endocrinol Metab 2020;34:101366
72. Fugger L, Jensen LT, Rossjohn J. Challenges, progress, and prospects of developing therapies to treat autoimmune diseases. Cell 2020;181:63–80