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The Role of Beta Cell Dysfunction in Early Type 1 Diabetes

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Abstract

Purpose of Review: Emerging data have suggested that β-cell dysfunction may exacerbate the development and progression of type 1 diabetes (T1D). In this review, we highlight clinical and preclinical studies suggesting a role for β-cell dysfunction during the evolution of T1D and suggest agents that may promote β-cell health in T1D.

Recent Findings: Metabolic abnormalities exist years before development of hyperglycemia and exhibit a reproducible pattern reflecting progressive deterioration of β-cell function and increases in β-cell stress and death. Preclinical studies indicate that T1D may be prevented by modification of pathways impacting intrinsic β-cell stress and antigen presentation. Recent findings suggest that differences in metabolic phenotypes and β-cell stress may reflect differing endotypes of T1D. Multiple pathways representing potential drug targets have been identified, but most remain to be tested in human populations with pre-clinical disease.

Summary: This cumulative body of work shows clear evidence that β-cell stress, dysfunction, and death are harbingers of impending T1D and likely contribute to progression of disease and insulin deficiency. Treatment with agents targeting β-cell health could augment interventions with immunomodulatory therapies but will need to be tested in intervention studies with endpoints carefully designed to capture changes in β-cell function and health.

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Keywords

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Introduction

Type 1 diabetes (T1D) is defined classically as insulin deficiency arising from autoimmune destruction of the pancreatic β cells (1). However, recent studies in both preclinical model systems and humans have reinforced the idea that the β cell actively contributes to disease pathogenesis as well as its own demise in T1D (2, 3), indicating a need to refine classic paradigms of T1D pathogenesis. Moreover, trials of therapies focused solely on modulating the immune system have had limited success in normalizing insulin secretion (1), suggesting that efforts for disease modification may benefit by combining immune therapies with agents that improve β-cell health. Below, we highlight studies identifying a role for β-cell dysfunction during T1D progression, including metabolic studies in humans and data from preclinical models. We also highlight potential biomarkers capable of reflecting β-cell health, and identify next steps for application of potential therapeutics targeting the β cell in T1D.

β-cell Dysfunction During T1D Development in Humans

The majority of our knowledge about the metabolic progression of T1D comes from longitudinal cohort studies of individuals with high genetic risk, where β-cell function has been measured using oral or IV glucose tolerance tests (OGTTs or IVGTTs, respectively) (4-6). Results from these studies suggest a framework for declining β-cell function that encompasses at least three metabolic phases prior to the onset of clinical, or Stage 3 T1D (7).

The first appreciable defect observed in at-risk individuals appears to be a loss of early C-peptide secretion that can be documented at the time of seroconversion to islet autoantibody positivity (Aab+). The Type I Diabetes Prediction and Prevention (DIPP) study followed Finnish children with high-risk HLA alleles pre and post-seroconversion. IVGTTs performed at the time of seroconversion showed that 42% of 52 children with newly identified islet cell autoantibodies had first phase insulin responses (FPIR) below the 5^{th} percentile. Within this cohort, progressors to T1D had significantly lower FPIR documented as early as 4-6 years prior to T1D diagnosis (8). Similar results were seen in Aab+ relatives monitored in the TrialNet Pathway to Prevention (PTP) Cohort for at least 5 years before diabetes onset. While PTP participants are identified as Aab+ in a cross-sectional manner, early C-peptide responses were reduced in progressors at study entry by 40%, as compared to autoantibody negative relatives, on average 6.5 years before diagnosis (9). Consistent with this observation, alterations in early C-peptide secretion have been used to stratify Aab+ individuals with the highest risk of T1D. In the European Nicotinamide Diabetes Intervention Trial (ENDIT) reduced FPIR was predictive for diabetes development over a median 4.95 years of follow-up (10).

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These initial defects appear to be followed by a second phase of metabolic progression that typically lasts until about 2 years before T1D onset. During this period, overall glycemia worsens gradually, whereas average OGTT C-peptide responses can appear largely stable. However, there are important changes in the architecture of C-peptide responses during this period. Analysis of placebo-treated subjects in the Diabetes Prevention Trial-Type 1 (DPT-1) and in PTP participants demonstrate reciprocal increases in late C-peptide responses during the OGTT that follow earlier losses of first phase C-peptide responses (9, 11, 12). Whether these changes reflect dysfunctional insulin secretion or an attempt at compensation by the by the β cell is not clear.

Finally, there is evidence for a third metabolic phase that begins around two years before the onset of Stage 3 T1D. This phase is characterized by significant reductions in C-peptide secretion and rising glycemia, with accelerated metabolic decompensation in the peri-onset period (defined as 6 months prior to diagnosis). Analysis of data from DPT-1 and PTP cohorts indicates there are reductions in both early and late C-peptide responses as well as evidence of reduced β-cell glucose sensitivity, followed by significantly rising blood glucose levels culminating in the need for exogenous insulin therapy (9, 13, 14).

Pancreas Imaging

A longstanding question is whether these metabolic changes primarily reflect reductions in β-cell mass or β-cell function. Comparisons between physiologic studies aimed at quantifying β-cell function in living subjects and histologic studies in organ donors with T1D highlight an important disconnect between β-cell mass and function in Aab+ individuals as well as in early stage and established T1D (16-19). In contrast to physiologic assessment of function, efforts to measure β-cell mass have proven challenging in humans. However, two groups recently reported reduced pancreas volume on magnetic resonance imaging relative in persons with T1D and nondiabetic Aab+ individuals compared to nondiabetic controls (20, 21). While these changes could be acquired, autoantibody negative first-degree relatives of individuals with diabetes also showed reduced pancreas volume relative to controls, suggesting inherited differences in whole-pancreas physiology (22). Along these lines, several studies have suggested that defects in β-cell function may be present in some relatives without detectable islet autoimmunity (13, 23, 24). Taken together, these findings indicate there could be differences in β-cell reserve that are independent of changes in autoimmunity in some individuals. Investigations defining the mechanistic link between pancreatic exocrine and β-cell mass and function and longitudinal studies of pancreas volume in at-risk populations are needed to better understand the relevance of these findings.

Preclinical Studies Suggesting Mechanistic Contributions of β-cell Dysfunction to T1D Development

The non-obese diabetic (NOD) mouse model has been a gold standard preclinical model to study the immunobiology of T1D for more than 30 years (25). These animals typically develop T1D after 12 weeks of age, but show signs of insulitis as early as the first 3-4 weeks after birth that intensifies gradually with age (26). Detailed analyses of β-cell function

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during the prediabetic phase have revealed patterns that parallel human data, including evidence that loss of β-cell function precedes loss of mass and that changes in mass and function are not always linked (26-28).

The molecular mechanisms giving rise to loss of β-cell function in prediabetic NOD mice provide important insight into potential disease-modifying approaches to preventing T1D. Because β cells are the only significant source of circulating insulin, they depend heavily upon the endoplasmic reticulum (ER) to ensure that proteins, particularly insulin, are produced and folded efficiently. As such, even minor perturbations that alter homeostasis in calcium handling, oxidation-reduction balance, or insulin demand can impose stresses that lead to ER decompensation or stress. The consequence of ER stress is a failure to efficiently produce and process relevant proteins (29). During the pre-diabetic phase in NOD mice, there is increasing development of ER stress in islets as evidenced by elevation in genes corresponding to the unfolded protein response (UPR) (*Hspa5, Xbp1s, and Ddit3*), an increase in activation of the three molecular arms of the UPR (ATF6, IRE1α, PERK), a suppression of mRNA translation initiation, and a striking increase in unprocessed proinsulin in the circulation (27, 30). The administration of a protein folding chaperone, taurine-conjugated ursodeoxycholic acid (TUDCA), to NOD mice alleviated islet ER stress, enhanced insulin secretion, and reduced T1D incidence (31). More recently, it was shown that β-cell-specific deletion of one of the UPR arms, IRE1α, strikingly reduced insulitis and prevented the onset of T1D in NOD mice (30), thereby implicating directly a role for the β-cell UPR in propagating the autoimmune response and progression to frank diabetes. The nature of the trigger for ER stress in β cells has remained elusive, although it is plausible that an early type 1 interferon response (e.g. from viral infections) or proinflammatory cytokines (IL-1β, IFN-γ, TNF-α) from early invading innate immune cells might serve as potential sources (32, 33).

Despite these observations, it remains unclear how activation of ER stress and the UPR might directly initiate or propagate autoimmunity. Recent studies have begun to elucidate a model, wherein unfolded or improperly processed proteins that are expressed or released by β cells have the potential to serve as neo-antigens that trigger an adaptive immune response. In one study, it was demonstrated that calcium-mediated activation of tissue transglutaminase 2 (tTG2) in human islets by ER stress led to the generation of deamidated proteins that served as neoepitopes (34). Using a peptidomics/transcriptomics strategy, islet stress elicited by proinflammatory cytokines led to association of altered peptides from a host of β-cell proteins and identified recognition of these altered peptides by CD8 cytotoxic T cells (35).

Biomarkers of β-cell Health in Type 1 Diabetes

Collectively, findings from preclinical and human studies highlight a prominent role for the β cells in T1D pathogenesis and raise the possibility that interventions targeting β cells could have therapeutic utility in diabetes prevention strategies. However, given limitations in our ability to image the β cell and challenges associated with the widespread application of OGTT and IVGTT, there is a need for reliable circulating biomarkers that provide a readout of β-cell stress and mass (reviewed in detail in (36)). Below we highlight examples of

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potential biomarkers, with a summary of markers assayed in human populations with or at-risk for T1D in Table 1.

Circulating Proinsulin

As noted above, under circumstances of β-cell stress where whole body insulin demand exceeds β-cell secretory capability, the machinery for proinsulin processing can become overwhelmed. Increased expression of proinsulin relative to insulin has been shown in islets from donors with autoantibody positivity and recent onset and longstanding T1D (37-39). These changes can be reflected in the circulation as increased secretion of proinsulin relative to mature insulin or c-peptide (proinsulin:C-peptide ratios or PI:C) (27, 40). Elevated serum PI:C levels were associated with increased progression to T1D in Aab+ relatives, suggesting this biomarker may also complement risk prediction (41-43). At the time of T1D diagnosis, PI:C is very elevated relative to nondiabetic controls (44, 45). Despite improved C-peptide secretion, ratios in children remain elevated during the honeymoon or clinical remission, and these increases often persist into longstanding T1D, suggesting persistent β-cell stress (45-47). Older studies have shown that PI:C may also be increased in some autoantibody negative relatives of individuals with T1D (48-50).

PI:C ratios may be also useful in dissecting T1D endotypes, with more pronounced aberrations in processing (43, 46). A high PI:C ratio was most strongly associated with progression to diabetes in younger (≤10 YO) Aab+ children, and individuals with longstanding T1D diagnosed with T1D at younger ages (<7 YO) are more likely to have high PI:C ratios compared to those diagnosed in later childhood or adulthood (43, 46). Interestingly, pancreas sections from young pediatric donors with T1D also show a strong association between islet insulitis profiles exhibiting increased B lymphocytes and aberrant proinsulin processing. Islets exhibiting these phenotypes were most common in samples from younger pediatric donors (46). Finally, ratios may also be helpful in identifying treatment responders, as older work showed that an elevated PI:C was linked to a more pronounced remission after cyclosporin treatment (44).

Cell free preproinsulin DNA

Because β-cells demethylate CpG sites on the gene encoding preproinsulin (*INS*) to increase expression of its mRNA, and dying cells release their genetic material extracellularly, β-cell death results in increases in cell free unmethylated *INS* DNA (51-53). Multiple groups have developed assays showing that circulating *INS* DNA increases acutely with β-cell death in preclinical models and after islet transplants. Clinical data suggest that circulating unmethylated *INS* DNA is increased at T1D diagnosis but decreases to near normal during clinical remission, followed by a slow increase to milder elevations in longstanding T1D (54-57). Cross-sectional comparisons between Aab+ progressors and nonprogressors were less drastic; although very high risk Aab+ positive dysglycemic individuals have clearer elevations (55). Longitudinal analysis of Aab+ individuals suggests that increases in unmethylated INS DNA over time are associated with younger age of T1D onset (58).

Several limitations have made implementation of this biomarker challenging. Firstly, *INS* DNA in circulation has a relatively short (~2.2 hour) half-life, therefore, cross-sectional

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analyses may miss transient elevations if β-cell death exhibits a relapsing/remitting pattern as T1D progresses (59). Sensitivity and variability of signal to detect small increases may be compromised with smaller sample volumes (60). Additionally, analyses have shown that other islet cells exhibit unmethylated CpG sites around the *INS* gene locus, and even after pancreatectomy, low-level background signal exists, suggesting that a subset of other cell types also exhibit unmethylation of the *INS* gene locus (59, 61, 62). Despite these limitations, current assays appear to be able to discriminate between differences in high levels of acute β-cell death or turnover. Important steps moving forward will be to quantify cumulative increases in *INS* DNA over time, validate assays using additional differentially methylated genes to improve signal specificity, and determine if circulating levels can be useful as alternative endpoints in intervention trials.

Extracellular noncoding RNAs

Numerous groups have described alterations in extracellular noncoding RNAs, most commonly circulating microRNAs (miRNAs), in clinical populations relevant to T1D (63-73). Several reports have specifically described increased serum or plasma levels of miRNAs highly expressed or enriched in the β cell as reflecting β-cell loss or reductions in β-cell mass (63, 73). However, reports often vary between groups (63-72). This is likely related in part to methodologic differences in sample collection and storage, miRNA isolation and quantification, and normalization strategies. Another key limitation is the lack of truly β-cell-specific noncoding RNAs, so that even RNAs consistently released by the β cell in the microenvironment of T1D can be obscured by varying background signal from other cell types. One potential solution could be interrogation of RNA cargo within circulating extracellular vesicles or attached to binding proteins that are derived from β cells or islets (74, 75). However, to date, this has not been successfully reported using native islets.

Interventions targeting β-cell health and function in humans with or at-risk for T1D

Although a sustainable impact on the natural history of T1D will likely require immunomodulatory therapy, a multi-drug regimen that includes an agent that targets β-cell health could feasibly improve outcomes of treatment with immunomodulatory therapies alone. However, several challenges remain to be addressed. The first involves identification of an optimal agent to target β-cell stress pathways. Although several drugs increase insulin secretion in T2D, therapies working solely as secretagogues (such as sulfonylureas) may have negative impacts on long-term β-cell survival in T1D (76). Agents specifically targeting the β cell are lacking, although multiple drugs may nonspecifically impact β-cell health or β-cell stress pathways (Table 2). Conjugation to ligands for receptors enriched on β-cell surfaces, such as the glucagon-like peptide-1 receptor, is an alternative approach of great interest, which would allow for targeted drug delivery while minimizing extra-islet effects (77). Another important question to address, is the optimal endotypes for treatment and optimal timing of intervention, as treatment after irremediable progression of β-cell dysfunction may show little benefit. Alternatively, β-cell-targeted treatment of individuals without signs of β-cell stress or dysfunction may not be needed. Finally, proof-of-principle

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trials utilizing targets aimed at β-cell health should be designed to optimally quantify informative metabolic endpoints, such as insulin resistance, first-phase insulin secretion, and functional β-cell mass, all which require more invasive testing than the standard 2-hour OGTT, as well as the application of carefully curated biomarkers.

Conclusions

Studies in humans and pre-clinical models show clear evidence that β-cell stress, dysfunction, and death are harbingers of impending T1D and likely contribute to disease progression. These data suggest that treatment with agents targeting β-cell health could augment interventions with immunomodulatory therapies. Important next steps in the field will involve intervention studies using therapeutic agents that target β cells, with endpoints carefully designed to capture changes in β-cell function and health. Successful implementation of such studies could ultimately lead to combination regimens for T1D prevention and treatment, which are better able to promote long-term β-cell survival in T1D.

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Key Bullet Points:

- Both pre-clinical and clinical studies show clear evidence that β-cell stress, dysfunction, and death are harbingers of impending T1D and likely contribute to disease progression.
- Circulating biomarkers that provide a readout of β-cell stress and mass can provide important information between the relationship of β-cell health with T1D progression.
- Potential candidate agents targeting β-cell health are highlighted; such agents could augment interventions with immunomodulatory therapies.
- Careful study design to include assessments of β-cell health and function will be necessary to understand impacts of interventions targeting these pathways.

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Table 1:

Biomarkers of β-cell Health that Have Been Tested in Clinical Samples from population with or at-risk for T1D

Biomarker	Proposed β-cell Intramolecular Process Represented by Increases in Circulation	Clinical Findings
Proinsulin/C-peptide or Insulin Ratio	• Altered prohormone processing reflecting β-cell stress	• Altered processing associated with islet inflammatory stress as well as altered insulitis profiles in recent onset T1D (CD20 high/increased B cell profile) (39, 46). • Increased ratios associated with increased progression (41-43). • Ratios higher in younger children before and after T1D onset (43, 46). • Ratios increased during honeymoon in children, but reduced during honeymoon period in adults (44, 45, 78). • Persistent detectable proinsulin in over half of individuals with longstanding T1D, even in absence of detectable C-peptide, suggesting ongoing β-cell stress (43, 46).
Pro-Islet Amyloid Polypeptide (ProIAPP)/Mature IAPP	• Altered prohormone processing reflecting β-cell stress	• Increased in recent onset T1D (79).
Cell free unmethylated *preproinsulin* DNA	• Extracellular release of cellular DNA reflecting β-cell death/ turnover	• Increased in Aab+ progressors relative to Aab− controls. Very increased in Aab+ dysglycemic individuals. Higher levels associated with younger age of T1D onset (55, 58). • Increased at the time of T1D diagnosis (54-56). • Levels normalize during clinical remission (54). • Lower elevations may be present in longstanding T1D (57).
Cell free unmethylated amylin DNA	• Extracellular release of cellular DNA reflecting β-cell death/ turnover	• Increased in recent onset T1D (80).
miR-375	• Extracellular nucleic acid release reflecting β-cell death/ turnover	• Varying data regarding increased levels in humans with new, recent onset, and longstanding T1D compared to controls (64, 65, 67, 68). • Not increased in Aab+ individuals (70).
miR-204	• Extracellular nucleic acid release reflecting β-cell death/ turnover	• Increased in Aab+ individuals and recent onset T1D (73).
miR-21	• miR-21 transcription increased during islet inflammatory stress • miR-21-5p with increased release in beta cell extracellular vesicles (EVs) under conditions of islet inflammatory stress	• Serum EV miR-21-5p increased in new onset T1D (66). • Cell-free serum miR-21-5p increased in longstanding T1D (68, 69). • Cell-free serum miR-21-3p increased in AAb+ progressors and new-onset T1D (66, 70).

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Biomarker	**Proposed β-cell Intramolecular Process Represented by Increases in Circulation**	**Clinical Findings**
Insulin-like Growth Factor 1 (IGF1) and IGF2	• Effects of insulin action	• Decreased in Aab+ individuals (81, 82). • IGF1 decreases over time in multiple Aab+ individuals and after T1D diagnoses (81).
Glutamate decarboxylase 65 kDA (GAD65)	• Extracellular release reflecting β-cell death/turnover • Identified in exosomes isolated from human islets treated with cytokines (83)	• Early increases after islet transplants in GAD- individuals with T1D associated with poor graft outcomes (84, 85).

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Table 2:

Potential Candidate Agents for Interventions Targeting β-cell Health

Therapeutic Class	Mechanism of Action on β-cell Health	Considerations
Thiazolidinediones (86)	Act on peroxisome proliferator-activated receptors (PPAR) receptors to improve insulin sensitivity and decrease functional demand on β-cells; Directly increase β-cell antioxidant expression, enhance unfolded protein response and improve ER calcium levels in preclinical models (87-89).	• Rosiglitazone or rosiglitazone+insulin treatment improved β-cell function compared to sulfonylureas or insulin alone in 54 latent autoimmune diabetes patients (90). • Potential side effects including weight gain, edema and heart failure, decreased bone mineral density in women, possible increased risk of bladder cancer with pioglitazone, increased cardiovascular events with rosiglitazone. • Development of newer agents targeting PPAR receptors may allow for effects with decreased side effect profile.
Glucagon-like peptide-1-based therapies (GLP-1 agonists and dipeptidyl peptidase 4 inhibitors (91)	Potentiate insulin secretion, delay gastric emptying to decrease glycemia. Directly increase β-cell regeneration, differentiation, and proliferation in preclinical models (92, 93).	• Exendin-4 administration reduced meal-associated glycemic excursions in 8 adults with T1D (94). • Acute potentiation of insulin secretion may not be optimal for long-term β-cell health. • Side effects including nausea, weight loss, possible association with pancreatitis and/or pancreatic cancer, association with thyroid C cell tumors in rats.
Dual sodium glucose cotransporter (SGLT2) Inhibitors (95)	Decrease functional demand on β cells by improving glycemia.	• Treatment associated with improved glycemic control and reduced insulin doses in meta-analysis of randomized control trials (RCTs) in 3238 adults with T1D (96). • Side effects including increased risk of diabetic ketoacidosis, and increased risk of genital infections which may be exacerbated by concurrent immunotherapy.
Intensive insulin therapy	Allow for period of β cell rest and potential recovery by obviating need for intrinsic insulin secretion	• Supported by idea that patients undergo clinical remission/ honeymoon period after starting insulin (97). • Diabetes Prevention Trial-Type 1 showed no effect of twice daily subcutaneous ultralente insulin and yearly IV insulin infusion on diabetes progression in 339 Aab+ positive relatives (98). • DirecNet RCT showed no effect of ~3 days inpatient hybrid closed loop therapy followed by sensor augmented pump therapy (vs. usual care) in 68 children with new-onset T1D (99). • Multicenter study underway testing impact of longer-term use of more modern hybrid closed loop systems to optimize dosing and time-in-range (CLVer mulli-center study NCT04233034).
Pharmacologic inhibition of insulin secretion (diazoxide and somatostatin analogs)(100)	Allows for period of β-cell rest and potential recovery by inhibiting insulin secretion through binding of K_{ATP} channels or somatostatin receptors.	• RCT showed 3-month diazoxide treatment of 20 Swedish adults with new-onset T1D resulted in increased fasting C-peptide at 12 and 18 months of follow-up (101). • RCT showed 3-month diazoxide treatment of 56 Swedish children with new-onset T1D resulted in increased meal stimulated C-peptide at 6-12 months of follow-up, but improvement was lost by 24 months of follow-up (102). • RCT showed no β-cell function effects of 6 months of lower-dose intermittent diazoxide treatment in 41 adults with recent onset T1D (103). • Side effects of temporarily worsened β-cell function, diazoxide with severe side effects including hypertrichosis and risk of pulmonary hypertension. Octreotide with nonspecific somatostatin receptor activity.
Verapamil (104)	Reduces β-cell calcium entry, reduces transcription of	• Favorable side effect profile.

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Therapeutic Class	Mechanism of Action on β-cell Health	Considerations
	Thioredoxin-Interacting Protein (TXNIP) to attenuate β-cell oxidative stress in preclinical models (105).	• 12-month treatment associated with increased mixed meal C-peptide AUC in recent onset T1D in 32 adult RCT (104). • Larger follow up study underway in children in multiple arm trial with hybrid closed loop therapy (CLVer mullti-center study NCT04233034).
Tyrosine kinase inhibitors	In addition to effects on immune cell tolerance, may have direct impact to inhibit β-cell platelet-derived growth factor receptor intrinsic inflammatory response signaling and reduce β-cell endoplasmic reticulum stress (106, 107).	• Imatinib mesylate RCT in 67 adults with recent onset T1D (NCT01781975) reportedly with increased MMTT C-peptide AUC after 12 months of treatment.
Molecular chaperone proteins	Chemical mitigation of β-cell endoplasmic stress via improved mediation of unfolded protein response in preclinical models of T1D, leading to improved function and survival (31)	• Favorable side effect profile. • Tauroursodeoxycholic Acid (TUDCA) RCT underway (Columbia) in 20 adults with recent-onset T1D (NCT02218619). • May need to be administered before Stage 3 T1D for efficacy based on preclinical data.
Polyamine synthesis inhibitors	β-cell polyamine depletion inhibited islet inflammation, preserved β-cell area and function, and delayed T1D onset in preclinical models (108)	• Multicenter dose ranging RCT of Difluoromethylornithine (DFMO) underway in 41 children with recent-onset T1D (NCT02384889). • May need to be administered before Stage 3 T1D for efficacy based on preclinical data.
Tyrosine Kinase 2 (TYK2) Inhibitors and Janus Kinase (JAK) Inhibitors	Inhibitor treatment reduces interferon I intracellular signaling, decreasing cytokine-induced β-cell MHC Class I expression and chemokine secretion. JAK inhibitors have been shown to prevent and reverse diabetes in preclinical models (33, 109, 110).	• SNPs in TYK2 locus leading to reduced function are associated with decreased T1D susceptibility (111). • JAK Inhibition may reduce cytokine-induced β-cell PDL1 expression (112).
Small-molecule inhibitors of dual-specificity tyrosine-regulated kinase 1A (DYRK1A) + GLP1R) agonists	Act synergistically to increase human β-cell replication without loss of differentiation in preclinical models (113).	• Despite targeting with GLP1R agonist, still concern for nonspecific effects on proliferation. • Not tested in humans in-vivo.