Combination Immunotherapy for Type 1 Diabetes

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Abstract

Purpose of Review: Type 1 Diabetes (T1D) is an autoimmune disease marked by β-cell destruction. Immunotherapies for T1D have been investigated since the 1980s and have focused on restoration of tolerance, T-cell or B-cell inhibition, regulatory T-cell (Treg) induction, suppression of innate immunity and inflammation, immune system reset, and islet transplantation. The purpose of this review is to provide an overview and lessons learned from single immunotherapy trials, describe recent and ongoing combination immunotherapy trials, and

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provide perspectives on strategies for future combination clinical interventions aimed at preserving insulin secretion in T1D.

Recent Findings: Combination immunotherapies have had mixed results in improving short-term glycemic control and insulin secretion in recent-onset T1D.

Summary: A handful of studies have successfully reached their primary end-point of improved insulin secretion in recent-onset T1D. However, long-term improvements glycemic control and the restoration of insulin independence remain elusive. Future interventions should focus on strategies that combine immunomodulation with efforts to alleviate β-cell stress and address the formation of antigens that activate autoimmunity.

Introduction

Type 1 diabetes (T1D) is characterized by absolute insulin deficiency secondary to autoimmune-mediated ablation of pancreatic islet β cells (1). Hallmarks of T1D are the development of circulating autoantibodies against β-cell antigens (2), the presence of immune cell infiltrates within pancreatic islets (3), and a progressive decline in insulin secretion that eventually culminates in clinically significant hyperglycemia and metabolic instability. Once a terminal disease, T1D is now manageable with exogenous insulin administration. However, insulin therapy is not a cure, and persons with T1D remain susceptible to labile blood glucose levels and the development of microvascular and macrovascular diabetic complications (4, 5).

The first clinical trial that tested an immunological intervention in T1D was the French Cyclosporine Diabetes Study (6). Cyclosporine A (CSA) interferes with T-cell receptor-mediated signal transduction thereby inhibiting T-cell activation and helper T-cell IL-2 production (7). Two studies showed a significant decrease in the need for exogenous insulin

following CSA treatment for over one year (6, 8), however, after CSA withdrawal, blood glucose control worsened and autoantibody levels rebounded (9). Furthermore, CSA treatment had the potential for renal and β-cell toxicity (9). Despite this lack of a lasting impact and potential toxicity, these trials ushered in a new clinical era focused on immunomodulatory strategies to delay or prevent T1D. To date, a number of additional interventions have been tested, including parenteral insulin administration, dietary exposures, broad spectrum immunosuppressants, anti-inflammatory drugs, and T- or B-cell targeted immunosuppressants. While a handful of trials have shown moderate benefits, true remission, as defined by insulin independence, remains elusive. The goal of this review is to provide an overview of lessons learned from early single target immunotherapy regimens and to describe more recent efforts focused on combination immunotherapies for T1D treatment and prevention.

The Pathogenesis of T1D

Multiple different cell types contribute to the pathogenesis of T1D, which involves a complex interaction between the β cell and components of both the innate (non-specific) and adaptive (specific) immune responses. While the focus of this review will be immunotherapies, a basic understanding of the mechanisms of T1D development is integral and will be summarized here (for additional detail see reviews by Wållberg and Cooke (10) or Lehuen and associates (11)). The precipitating trigger of the autoimmune attack on the β cell remains unclear. However, it is thought to result from the complex interplay between genetic predisposition and environmental influences (12). The strongest contributor to genetic predisposition (~60%) is the human leukocyte antigen (HLA) class II, which encodes for components of the class II major histocompatibility complex present on antigen presenting cells

(APCs) (13). HLA class and other major genetic predisposition contributors (e.g. *INS*, *CTLA4*, *PTPN22*, and *IL2RA*) persist for life and progression to T1D is usually preceded by years of autoantibody expression against β-cell autoantigens (13). Emerging opinions suggest β-cell autoantigens may be generated by posttranslational modifications in which newly generated "foreign" β-cell proteins are not present during thymic selection leading to autoantibody production (14). In the initial phases of disease, islet resident APCs (e.g. macrophages and dendritic cells) take up autoantigens and migrate to pancreatic lymph nodes (15). Within the lymph nodes, autoantigens are presented by APCs resulting in the activation of circulating naïve autoreactive T cells (15). Activation of these T cells allows them to migrate through tissues and into the islet, where they encounter β-cell autoantigens, resulting in T-cell reactivation and the initiation of islet inflammation and insulitis. (15). These islet infiltrates typically contain a mixture of cytotoxic $CD8^+$ T cells, helper $CD4^+$ T cells, B cells, dendritic cells, and macrophages, and each of these cell types plays a role in autoimmune-mediated β-cell death (11, 16). In addition to antigen presentation, islet-associated macrophages secrete pro-inflammatory cytokines that promote T-cell responses and the production of cytotoxic free radical species, which contribute to β-cell death (17). Dendritic cells have been implicated in the development of regulatory T cells (Tregs) that promote immune cell tolerance and prevent autoimmunity (18). However, dendritic cell populations are diminished in at-risk individuals and in recent-onset T1D (19). B cells also serve as APCs, and following $CD4^+$ T-cell-mediated activation, produce autoantibodies against islet autoantigens (20) and secrete TNFα contributing to inflammation (21). Pro-inflammatory $CD4^+$ T-cells do not cause β-cell death through direct contact, but rather $CD4^+$ T cells secrete pro-inflammatory cytokines to promote recruitment of other immune cells (22). In contrast, $CD8^+$ T cells lead to β-cell death through direct contact with β cells (23, 24),

predominately utilizing the perforin/granzyme B apoptotic death pathway (25), but they may also utilize the Fas/FasL apoptotic death pathway (24). Pro-inflammatory cytokines secreted from T cells and macrophages, such as IFNγ, IL-1β, and TNFα, also promote β-cell apoptosis, exacerbating islet loss during T1D development (26, 27).

Single immunotherapies

Immune-mediated reactions against the β cell encompass several different cell types and multiple pathways of autoimmune-mediated death, providing ample targets for immunotherapies aimed at treating or preventing T1D. Since the French Cyclosporine Diabetes study, a number of therapies have been tested. To date, the majority of these initial studies have undertaken a single intervention approach. A focus of many trials has been on the induction of self-tolerance to prevent autoimmunity. The Diabetes Prevention Trial-Type 1 Diabetes (DPT-1) consisted of two studies aimed at defining whether oral or parenteral insulin could prevent or delay T1D development in first- or second-degree relatives of a person with T1D. In the first DPT-1 study, participants with a high risk of T1D development (>50%) administered twice daily subcutaneous doses of insulin (0.25 U/kg body weight/day) plus annual insulin infusions (28), while in the second DPT-1 study, participants with an elevated risk of T1D development (26-50%) consumed oral insulin capsules daily (7.5 mg/day) (29). Subcutaneous insulin did not delay or prevent T1D (28). Similarly, oral insulin did not alter T1D incidence, however, in a subgroup with higher insulin autoantibody, the incidence rate was improved (29).

Following the DPT-1 oral insulin study, The Type 1 Diabetes TrialNet Study initiated a second prevention trial in relatives of persons with T1D. TrialNet Oral Insulin participants were confirmed IAA positive with at least one other autoantibody and then randomized to receive a once daily insulin capsule (7.5 mg) or placebo. At follow-up, participants will have glycemic control and autoantibody status recorded (https://clinicaltrials.gov/ct2/show/NCT00419562), and results from this trial are due to be reported soon. Another ongoing study centered on restoring tolerance to insulin is the Fr1da Insulin Intervention study. While TrialNet Oral Insulin participants had a relative with T1D, Fr1da participants are not required to have a relative with T1D and could be identified by population-based screening. Additionally, Fr1da treatment boosted the oral insulin dose from 7.5 mg/day to 67.5 mg/day after the first three months of the study. Fr1da participants are extensively screened for presence of islet autoantibodies (GADA, IA2A, and ZnT8) and then randomized to receive oral insulin capsules or placebo (30). At follow-up, participants will be screened for changes in islet autoantibodies, $CD4^+$ T-cell response to insulin, and changes to the number of circulating Tregs (https://clinicaltrials.gov/ct2/show/NCT02620072) (30).

Multiple studies have also focused on intranasal insulin delivery to delay or prevent T1D. The Type 1 Diabetes Prediction and Prevention (DIPP) study screened for T1D HLA susceptibility alleles in infants and in siblings of individuals with T1D. Those with high-risk HLA alleles were eligible to receive daily doses of intranasal insulin (1 U/kg/day); however, the rate of progression to T1D was unchanged in either cohort (31). The Intranasal Insulin Trial (INIT I) treated autoantibody-positive participants with intranasal insulin (1.6 mg/day) and similarly showed that intranasal insulin did not prevent or accelerate T1D incidence. In this trial, intranasal insulin was associated with increased antibody and decreased T-cell responses to

insulin (32). The INIT II study is ongoing and will expand the number of subjects from 38 to 300 and will further investigate autoantibody level changes in addition to glycemic control (https://clinicaltrials.gov/show/NCT00336674).

Early prevention studies have also focused on neonatal dietary interventions. A study of infants with a first-degree relative with T1D found that infants receiving hydrolyzed casein-based formula in place of breast milk were less likely to be positive for two or more autoantibodies, versus infants receiving conventional formula. At study end, no difference in autoantibodies or diabetes incidence was evident seven years post intervention (33, 34). The FINDIA pilot study found that removal of bovine insulin from formula resulted in blunted progression of additional islet autoantibodies three years after intervention compared to conventional cow's milk formula, supporting the idea of restoration of tolerance. However no long term follow-up has been reported from this study (35). Other dietary intervention including delayed gluten exposure (36), omega-3 fatty acid supplementation (37), and nicotinamide (38) have not significantly prevented or delayed T1D onset.

While the above studies focused on intervention prior to clinical onset of T1D, interventions after clinical onset of T1D have tested a number of immunosuppressive drugs to prevent or reverse T1D development. This strategy has produced limited long-term success or detrimental side effects that precluded therapeutic outcomes. The Cyclosporine trials provided an impetus for targeting T-cells, and several antibodies against the Fc receptor of T-cells preventing complement binding have been tested. While the mechanism of CD3 inhibition is not well understood, T-cell apoptosis, altered T-cell trafficking, antigenic immunomodulation of the T-cell receptor, and Treg induction have been observed pre-clinically following anti-CD3 therapy (39). Given these effects, the anti-CD3 antibody teplizumab was administered to

individuals with recent-onset T1D. Unfortunately, one year after initiation, participants in placebo, full-dose, and low-dose teplizumab were not insulin independent (40). After two-year follow-up, post hoc analysis revealed that teplizumab improved C-peptide and HbA1c levels in responders with higher baseline glycemic control or altered memory T-cell populations (41, 42). Otelixizumab, another anti-CD3 antibody, led to an improvement in C-peptide levels, but only in participants whose β-cell function was in the top 50th percentile at baseline (43). TrialNet is currently testing tepluzimab for prevention or delay of T1D in high-risk relatives of persons with T1D (https://clinicaltrials.gov/ct2/show/NCT01030861).

CTLA4-Ig is a co-stimulatory modulator that prevents T-cell activation by binding to CD80 and CD86, preventing subsequent APC binding and downstream signaling (44). In recent-onset T1D, abatacept administration delayed C-peptide decline and decreased the need for exogenous insulin over the first twelve months (44). However, protection was lost by twenty-four months (44), and blockade of CD80 and CD86 drastically reduced Tregs and exacerbated autoimmunity (45). Prevention of T1D with abatacept is currently being tested in autoantibody positive relatives of persons with T1D (https://clinicaltrials.gov/ct2/show/NCT01773707).

Since Tregs have been shown to be reduced in T1D, efforts to restore functional Tregs to reverse autoimmunity and preserve remaining β-cell mass are underway. Marek-Trzonkowska and associates (46) and Bluestone and associates (47) recently reported on respective phase I trials to assess safety of using Treg adoptive immunotherapy in T1D. Participants with T1D, either within two months of diagnosis or ranging from 14-104 weeks post diagnosis, had their own Tregs isolated from peripheral blood, expanded ex vivo with anti-CD3 and anti-CD28 plus IL-2, and varying numbers of cells were adoptively transferred back into the donor (46, 47). Bluestone found a population of transferred Tregs that were long-lived and still in circulation one

year post transfer (47). Marek-Trzonkowska study participants exhibited an increase in C-peptide levels and lower exogenous insulin requirement (46). Bluestone study participants exhibited no decline in C-peptide levels and no worsening in HbA1c over 1 year post transfer (47). Bluestone and associates are currently investigating the combined use of adoptively transferred Tregs plus IL-2 administration (https://clinicaltrials.gov/ct2/show/NCT02772679). Taken together, these early data suggest that Treg therapy may be beneficial for preserving β-cell mass and possibly reversing T1D.

At least one trial has focused on the B cell using Rituximab, which targets the B-cell μ immunoglobulin chain. In recent onset T1D, Rituximab was found to significantly lower HbA1c levels, increase C-peptide levels, and reduce exogenous insulin demand (48). However, $CD19^+$ B cells steadily rebounded over the following twelve months as tolerance was not established with rituximab (48). Two-year post-intervention follow-up reported rituximab delayed the decrease in C-peptide levels, but did not appear to alter $CD19^+$ B cells or antibody production (49). Interestingly, Rituximab has yet to be tested in the pre-clinical phase of T1D.

Another avenue of intervention has been to target inflammation and innate immunity. Imatinib is an inhibitor of protein tyrosine kinases, specifically c-Abl, c-Arg, PDGFR, and c-Kit (50). Imatinib also has anti-inflammatory effects, including decreasing production of TNFα by macrophages. In mouse models, imatinib has been shown to protect β cells against cytokine and chemical agent induced apoptosis and protect against autoimmune-mediated and chemical agent-induced T1D (51, 52). Currently, imatinib is being used in a phase II study in recent-onset T1D (https://clinicaltrials.gov/ct2/show/NCT01781975). Another inhibitor of TNFα activity is entanercept, which is a soluble recombinant TNFα receptor fusion protein that binds to TNFα to inhibit activity (53). In participants with recent-onset T1D, entanercept improved HbA1c and C-

peptide levels (53). IL-1 has also been a target for intervention in two studies. Anakinra is an IL-1 receptor agonist and has also been used for rheumatoid arthritis therapy (54). Anakinra was administered to recent-onset T1D participants. Unfortunately, this agent did not alter C-peptide levels (55). Canakinumab is a monocolonal antibody against IL-1β, which was investigated in recent-onset T1D concurrently with anakinra (55). In similar fashion, canakinumab did not improve C-peptide levels (55). Innate immunity modulation is also being investigated with the use of the Bacillus Calmette-Guérin (BCG) vaccine. BCG is an FDA approved vaccine primarily used for tuberculosis prevention, which also induces production of TNF (56). TNF destroys insulin-reactive T cells and may also induce Treg production, but does not destroy healthy T cells (56). Over twenty years ago, an initial clinical trial with low dose BCG induced remission of T1D in some participants (57). Unfortunately, remission was not observed in an expanded trial. More recently, a small proof of concept trial in participants with long-standing T1D resulted in improved C-peptide levels, fewer circulating autoreactive T cells, reduced GAD autoantibody levels, and Treg induction (56). Currently, BCG is being investigated in a larger clinical trial in participants with long standing T1D in effort to repeat the pilot trial's results (https://clinicaltrials.gov/ct2/show/NCT02081326).

Combination Immunotherapies

Whereas trials of single agent immunotherapeutic regimens have elucidated important insights into T1D pathogenesis, long-term insulin independence remains an aspirational outcome. The majority of single-agent studies have focused on recent-onset diabetes, when the autoimmune reaction against β cells has been occurring for a number of years and substantial loss of β-cell mass has already occurred (58). To address this, several drugs are now being tested

as preventive therapies in autoantibody positive at-risk individuals, including GAD-alum, oral insulin, Tregs, abatacept, and tepluzimab. A second approach has been to develop multifaceted combination approaches that target different arms of T1D pathology. Preclinical studies in animal models (see reviews by Shoda and associates (59) and Reed and Herold (60)), insights from other autoimmune diseases, and experience from the islet transplantation field provide justification for this approach.

Since the publication of the Edmonton Protocol (61), the islet transplantation field has tested a number of combination immunotherapy approaches to prevent nonspecific inflammatory reactions against the islet graft and to prevent recurrent autoimmunity. These include: daclizumab or basiliximab (62, 63), anti-thymocyte globulin with entanercept (64), anti-CD3 antibodies with TNFα inhibition (65), alemtuzumab (63), and anakinra with etanercept (66, 67). These strategies have led to improved glycemic control following islet transplant and have provided insight into modulating the immune system and promoting β-cell survival.

One of the first combination trials tested mycophenolate mofetil (MMF) alone or in combination with daclizumab (DZB) in recent onset T1D. MMF is an immunosuppressant used during organ transplantation, that when hydrolyzed becomes mycophenolic acid (MPA). MPA is an inhibitor of inosine monophosphate dehydrogenase, which controls guanine monophosphate production during purine synthesis required for T- and B-cell proliferation (68). DZB binds to the α subunit (CD25) of IL-2 receptor expressed on activated T and B cells (69). The combination of MMF and DZB proved successful in delaying or preventing diabetes in rats (70). However, in the human trial, MMF/DZB or MMF alone was unsuccessful in preventing loss of C-peptide or the need for exogenous insulin over two years (71). Additionally, despite an initial drop in HbA1c at three months post treatment, HbA1c levels gradually rose to baseline levels

over two years (71). Furthermore, a number of adverse effects were reported during the study, including neutropenia and leukopenia (71). Mechanistic follow-up also suggested that MMF/DZB was likely ineffective because levels of $CD4^+CD25^+$ Tregs, essential regulators of self-tolerance in T1D, were reduced by the intervention (72).

A phase I trial focused on use of rapamycin and IL-2 in an effort to boost Treg function in recent-onset T1D, and the use of this combination was based on strong preclinical data suggesting modulation of multiple aspects of T1D pathogenesis in mouse studies (73, 74). Rapamycin is routinely used during organ transplantation and blocks the mammalian target of rapamycin complex 1 (mTORc1), which is an important regulator of cell cycle progression (75). Rapamycin inhibits proliferation of pro-inflammatory Th1 and Th17 T-cells, but has a weaker effect on Tregs, which do not require mTORc1 for cell growth (76, 77). Furthermore, low dose rapamycin had been shown to enhance Treg function (78). IL-2 acts on multiple cell types expressing the IL-2 receptor and has been shown to prevent or reverse hyperglycemia in NOD mice through activation and expansion of Tregs (79, 80). Moreover, Rapamycin/IL-2 prevented diabetes in NOD mice (74) . Surprisingly, this combination led to a marked decrease of β-cell function, as measured by C-peptide, in participants with T1D duration between 4 and 48 months (81). However, rapamycin/IL-2 treatment was successful in boosting the number of Tregs and participants maintained an enhanced response to IL-2, however, no differences were found in $CD4^+/CD8^+$ T-cell ratio and participants exhibited increased eosinophilia and acute TGF-β and soluble IL-2 receptor elevations (81). The investigators who conducted the study concluded, combined with published reports, that IL-2 therapy may be beneficial in enhancing Tregs in T1D subjects, but in combination with rapamycin, a suspected β-cell toxicant (82), led to impaired β-cell function (81).

Recently, combination therapy with low-dose anti-thymocyte globulin (ATG) and pegylated granulocyte CSF (G-CSF) has shown promising results. While other efforts to preserve functional β-cell mass largely focused on recent-onset intervention, within 100 days of clinical diagnosis, ATG/G-CSF administration was focused on patients with established T1D of at least four months, but less than two years duration (83). ATG has previously used as acute anti-rejection therapy during organ transplantation, and the main mechanism of this agent is T-cell depletion in the circulation and peripheral lymphoid tissues through complement-dependent lysis and T-cell activation and subsequent apoptosis (84). Additionally, ATG has diverse effects on other immune system components, including: altered cell-surface moieties that mediate leukocyte interactions, B-cell apoptosis induction, dendritic cell inhibition, and stimulation of Tregs and natural killer T cells (84). G-CSF, or granulocyte colony stimulating factor, also has diverse functions. G-CSF maintains circulating neutrophils in a steady state, inhibits TLR-induced pro-inflammatory cytokine production in macrophages and neutrophils, enhances IL-4 and IL-10 production from T cells, and decreases pro-inflammatory Th-17 cell populations (85). In this Phase IIa clinical trial, participants received a low-dose ATG/G-CSF regimen and β-cell function tended to maintained at 12 months in the treated group, as measured by the 4 hour area under the curve of the C-peptide response to mixed meal tolerance stimulation. HbA1c levels also tended to be lower at 6 months in those who received ATG/G-CSF (83). A two-year follow-up revealed no difference in C-peptide levels 24 months post-intervention (86). However, this follow-up study found subjects receiving ATG/G-CSF had reduced $CD4^+$ T-cells and $CD4^+/CD8^+$ T-cell ratio and increased natural killer cells, memory T-cells, and neutrophils (86). Additionally, Tregs were elevated after 6, 12, and 18 months, but not after 24 months (86). Taken together, these results suggest that ATG/G-CSF therapy leads to prolonged

immunomodulatory effects and a larger clinical trial in recent-onset T1D is underway within the TrialNet Clinical Network (https://clinicaltrials.gov/ct2/show/NCT02215200).

A recently reported study tested intralymphatic injection of GAD65 in an aluminum hydroxide formulated vaccine (GAD-alum) in combination with oral vitamin D in recent onset T1D (87). L-glutamic acid decarboxylase (GAD) is an autoantigen found in ~80% of recent-onset T1D (88). In a phase II clinical trial, GAD-alum alone preserved C-peptide in recent-onset T1D (89) and participants exhibited increased Tregs (90, 91), however, a subsequent phase III trial showed no significantly beneficial effect in glycemic control (92). In a separate TrialNet study, two- or three-doses of GAD-alum did not improve C-peptide level, HbA1c levels, or insulin requirement (93). In mouse studies, vitamin D3 has been shown to reduce insulitis and diabetes (94) and modulate dendritic cell maturation (95). In clinical trials, however, vitamin D3 has failed to significantly improve C-peptide, HbA1c, or exogenous insulin requirements (96, 97). Intralymphatic GAD-alum injection resulted in stable C-peptide levels, improved HbA1c levels, and reduced insulin requirement and led to up-regulation of anti-inflammatory Th2 T-cells and decreased pro-inflammatory Th1 T-cell cytokines (87). Additional GAD-alum combination studies are ongoing, including combined with: vitamin D and the anti-inflammatory ibuprofen (https://clinicaltrials.gov/ct2/show/NCT01785108), the anti-inflammatory agent GABA, (https://clinicaltrials.gov/ct2/show/NCT02002130), etanercept and vitamin D (https://clinicaltrials.gov/ct2/show/NCT02464033), and alone with vitamin D for T1D prevention in high-risk subjects (https://www.clinicaltrials.gov/ct2/show/NCT02387164). These studies should yield insight into whether GAD-alum is more effective in combination with other immunomodulatory agents versus GAD-alum alone.

Autologous hematopoietic stem cell transplantation (AHSCT) is currently being investigated as therapy for T1D. AHSCT are thought to "reset" immune tolerance system by ablating all immune cells (98). Following peripheral blood hematopoietic stem cells mobilization from the bone marrow with cyclophosphamide/G-CSF, they are collected by leukapheresis and frozen (99). Shortly thereafter, high dose immunosuppression with cyclophosphamide/ATG is administered to ablate the immune system and the previously collected stem cells are reconstituted and injected intravenously (99). Following AHSCT, participants with recent-onset T1D had improved C-peptide levels, with many participants found to be insulin independent beyond one year (99-101). Other studies have shown varying degrees of improved C-peptide levels and exogenous insulin independence, however, risk of adverse effects due to immune system ablation are high and success of AHSCT is predicated by the participant's glycemic control history (102-104). Additionally, an ongoing clinical trial in multiple autoantibody positive participants is investigating the feasibility of infusing cryopreserved core blood to prevent T1D development (https://www.anzctr.org.au/Trial/Registration/TrialReview.aspx?ACTRN=12613000186752), an approach that may prove applicable to future AHSCT or tolerance restoration studies.

Concluding Remarks and Future Perspectives

The discovery of insulin in the 1920s was essential for transforming a once fatal disease into a manageable disease. Exogenous insulin therapy, however, is not an outright cure and persons utilizing exogenous insulin are unable to manage the minute-to-minute fluctuations in blood glucose and are still subject to the development of significant co-morbidities, including micro and macrovascular complications and severe hypoglycemia. Closed-loop artificial

pancreas systems (105, 106) are a step in the right direction, but do not address the underlying causes of T1D. Since the identification of T1D as an autoimmune disease in the 1970s (2, 3), efforts to reverse or prevent the autoimmune insult have focused solely on the immune system. As summarized in this review, multiple strategies have been utilized in an effort to cure T1D and active immunotherapy clinical trials are summarized in Table 1. Single target immunotherapies have shown success in achieving their predetermined endpoints, however, they have largely been unsuccessful in maintaining long-term glycemic control and significantly preserving insulin secretion. Refinement and combinations of these immunotherapies have the potential to lengthen the duration of glycemic control, but as of yet, combination immunotherapies have not completely reversed T1D. Continued refinement of intervention doses, more rigorous investigation of intervention responders, and/or combinations of minimally successful single target immunotherapies should continue to be investigated in a clinical setting.

The majority of interventions reviewed here were implemented in recent-onset T1D. Since 60-90% of β-cell mass is dysfunctional or destroyed by the time of clinical onset, intervention may be more beneficial prior to onset of T1D. Prevention studies mentioned above used autoantibodies as biomarkers for T1D. Other potential biomarkers of T1D development include: genetic predisposition (13, 58, 107-109), unmethylated preproinsulin (110, 111), proinsulin-to-C-peptide ratios (112, 113), and microRNA species (114). In addition, β-cell derived neo-antigens offer another potential biomarker of T1D, but also a target for T1D prevention (115, 116). Alleviation of inherent β-cell stress has emerged recently as an important avenue to consider in future therapies. ER stress has been shown to precede T1D development and lead to β-cell death and formation of neo-antigens (117-119). A clinical trial is underway using TUDCA, a chemical chaperone that alleviates ER stress, in recent-onset T1D

(https://clinicaltrials.gov/ct2/show/NCT02218619). Furthermore, imatinib has been found to suppress β-cell ER stress mediated through IRE1α signaling (120). This finding supports the concept of utilizing interventions to target not only the immune system, but also the β cell. In addition, lessons on preventing β-cell death and promoting β-cell regeneration may be discerned from therapies used to treat type 2 diabetes (reviewed in (121, 122)) in combination with immunotherapies and agents focused on alleviating β-cell stress.

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Compliance with Ethics Guidelines

Conflict of Interest

Robert N. Bone and Carmella Evans-Molina declare that they have no conflict of interest.

Human and Animal Rights and Informed Consent

Carmella Evans-Molina is a coauthor on three references cited that utilized human or animal subjects; these studies complied with all relevant human and animal subject Ethical Guidelines.

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•• Of major importance

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Table 1. Active Type 1 Diabetes Immunotherapy Clinical Trials				
Single Immunotherapies				
Title	Intervention	Primary Outcome(s)	Secondary Outcome(s)	Registry Link
Oral Insulin for Prevention of Diabetes in Relatives at Risk for Type 1 Diabetes Mellitus	Oral Insulin	Glycemic control Autoantibody status	Metabolic status	https://clinicaltrials.gov/ct2/show/NCT00419562
Fr1da Insulin Intervention	Oral Insulin	Activation of Immune Response Efficacy of Immune Response	FOXP3/IFNG gene expression IgG-binding to Insulin Circulating Insulin-tetramer positive T cells Progression to Diabetes	https://clinicaltrials.gov/ct2/show/NCT02620072
Trial of Intranasal Insulin in Children and Young Adults at Risk of Type 1 Diabetes (INITII)	Intranasal Insulin	Proportion of subjects diagnosed with type 1 diabetes	B cell function Insulin Action Circulating autoantibodies GAD-65 and IA2 T cell response	https://clinicaltrials.gov/show/NCT00336674
Teplizumab for Prevention of Type 1 Diabetes In Relatives "At-Risk"	Teplizumab	Proportion of subjects diagnosed with type 1 diabetes	Adverse effects of teplizumab	https://clinicaltrials.gov/ct2/show/NCT01030861
CTLA4-Ig (Abatacept)for Prevention of Abnormal Glucose Tolerance and Diabetes in Relatives At - Risk for Type 1	CTLA4-Ig (abatacept)	Change from normal glucose tolerance to abnormal glucose tolerance	Change in C-peptide to oral glucose tolerance test	https://clinicaltrials.gov/ct2/show/NCT01773707
Imatinib Treatment in Recent Onset Type 1 Diabetes Mellitus	Imatinib Mesylate	Change in baseline to 12 month 2 hour area under the curve in residual β cell function (C-peptide)	HbA1c levels C-peptide response Exogenous insulin use Number of severe hypoglycemic events Adverse effects	https://clinicaltrials.gov/ct2/show/NCT01781975

Tauroursodeoxycholic Acid (TUDCA) in New-Onset Type 1 Diabetes	Tauroursodeoxycholic Acid (TUDCA)	Change in baseline to 6, 12, & 18 month 2 hour area under the curve in residual β cell function (C-peptide)	Endoplasmic reticulum stress Liver function	https://clinicaltrials.gov/ct2/show/NCT02218619
Repeat BCG Vaccinations for the Treatment of Established Type 1 Diabetes	Bacillus Calmett-Guérin (BCG)	Improvement in HbA1c levels	Change in immune response C-peptide levels	https://clinicaltrials.gov/ct2/show/NCT02081326
Combination Immunotherapies				
Title	Intervention	Primary Outcome	Secondary Outcome(s)	Registry Link
T1DM Immunotherapy Using Polyclonal Tregs + IL-2 (TILT)	Tregs + IL-2	Adverse effects Survival of Tregs	C-peptide response Exogenous insulin use HbA1c levels Number of severe hypoglycemic events IL-2 effect on Treg kinetics β-cell death Circulating autoantibodies GAD-65, IA2, and ICA Circulating Insulin-tetramer positive T cells General immune response	https://clinicaltrials.gov/ct2/show/NCT02772679
ATG-GCSF in New Onset Type 1 Diabetes (ATG-GCSF)	Anti-tymocyte globulin (ATG) Granulocyte colony stimulating factor (GCSF)	Change in baseline to 12 month 2 hour area under the curve in residual β cell function (C-peptide)	Effect of treatment on surrogate markers for immunologic and metabolic outcomes	https://clinicaltrials.gov/ct2/show/NCT02215200
DIABGAD - Trial to Preserve Insulin Secretion in Type 1 Diabetes Using GAD-Alum (Diamyd) in	Glutamic Acid Decarboxylase in alum formulation (GAD-alum)	Change in baseline to 6, 15, and 30 month 2 hour area under the curve and 90 minute	Maximum C-peptide level HbA1c Exogenous insulin	https://clinicaltrials.gov/ct2/show/NCT01785108

Combination With Vitamin D and Ibuprofen	Vitamin D Ibuprofen	value in residual β cell function (C-peptide)	dose Th-2 cell-mediated immune response Circulating inflammatory markers Fasting C-peptide	
The Use of Glutamic Acid Decarboxylase (GAD) and Gamma-Amino Butyric Acid (GABA) in the Treatment of Type I Diabetes (GABA)	Maltodextrin Glutamic Acid Decarboxylase in alum formulation (GAD-alum) Gamma-Aminobutyric Acid (GABA)	Change in baseline to 12 month total daily insulin dose requirement Change in baseline to 12 month 2 hour area under the curve residual β cell function (C-peptide)	Circulating autoantibodies GAD-65, IA2, and ICA	https://clinicaltrials.gov/ct2/show/NCT02002130
EDCR Study - Etanercept Diamyd Combination Regimen -Open Trial to Evaluate Safety in Children With Type 1 Diabetes	Glutamic Acid Decarboxylase in alum formulation (GAD-alum) Vitamin D Etanercept	Tolerability of combination therapy (injection site, incidence of infection, number of adverse effects, number of serious adverse effects, neurological assessments) Serum calcium and vitamin D Circulating autoantibody (GAD-65)	Change in immune system markers from baseline to 6 months (inflammatory markers, Th2 cell-mediated immune response, Tregs Change in baseline to 6, 9, 15, 30 month 2 hour area under the curve residual β cell function (C-peptide) Maximum C-peptide Exogenous insulin dose Fasting C-peptide	https://clinicaltrials.gov/ct2/show/NCT02464033
Prevention Trial: Immune-tolerance With Alum-GAD (Diamyd) and Vitamin D3 to Children With Multiple Islet Autoantibodies (DiAPREV-	Glutamic Acid Decarboxylase in alum formulation (GAD-alum) Vitamin D3	Proportion of subjects diagnosed with type 1 diabetes	Change from baseline to 5 years in glucose metabolism Occurrence of	https://www.clinicaltrials.gov/ct2/show/NCT02387164

IT2)			adverse effects	