Immune Intervention and Preservation of Pancreatic Beta Cell Function in Type 1 Diabetes

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Abstract

Type 1 diabetes (T1D) results from the immune-mediated destruction of insulin-producing β cells located within the pancreatic islets of Langerhans. The autoimmune process leads to a deficiency in insulin production and resultant hyperglycemia requiring lifelong treatment with insulin administration. T1D continues to dramatically increase in incidence, especially in young children. Substantial knowledge surrounding human disease pathogenesis exists, such that T1D is now predictable with the measurement of antibodies in the peripheral blood directed against insulin and other β cell proteins. With the ability to predict, it naturally follows that T1D should be preventable. As such, over the last two decades, numerous well-controlled clinical trials have been completed attempting to prevent diabetes onset or maintain residual β cell function after clinical onset, all providing relatively disappointing results. Here, we review the T1D prevention efforts, the current landscape of clinical therapies, and end with a discussion regarding the future outlook for preventing T1D.

Keywords

Diabetes; Type 1; Autoimmunity; Prevention; Intervention; Clinical trials

Introduction

Type 1 diabetes (T1D), the immune-mediated form of diabetes, continues to dramatically increase in incidence and is one of the most common chronic diseases in children [1••]. Although treatment with intensive insulin therapy and blood glucose monitoring has improved dramatically in the last two decades, the majority of T1D patients do not meet recommended glycemic goals [2]. These individuals are at an increased risk of developing microvascular (retinopathy, nephropathy, and neuropathy) and macrovascular complications

associated with diabetes, severe hypoglycemia, and depression [3–6]. In order to decrease T1D burden, preventing or delaying T1D onset is critical such that patients can live with the disease for fewer years and more easily meet glycemic goals with residual β cell function. Developing safe, specific, and effective treatments and optimally timing those therapies during T1D development requires that the natural history and pathogenesis of T1D be well defined. It is well appreciated that T1D is a chronic T lymphocyte-mediated autoimmune disorder leading to specific pancreatic β cell destruction that evolves over time defined by stages (Fig. 1) [7]. Several prospective cohort studies following at-risk children have aided understanding the natural history of T1D. The Diabetes Autoimmunity Study in the Young (DAISY) in Colorado, The Environmental Determinants of Diabetes in the Young (TEDDY) multicenter international collaboration, the Type 1 Diabetes Prediction and Prevention Study (DIPP) in Finland, and BABYDIAB studies in Germany have followed genetically at-risk children from birth and have been instrumental in outlining the development of islet autoimmunity (determined by measuring antibodies in the peripheral blood directed against insulin, glutamic acid decarboxylase, islet antigen 2, and zinc transporter 8) and progression to clinical T1D, which is marked by hyperglycemia [8–12]. Importantly, once an individual develops two or more islet autoantibodies (iAb), the risk of developing T1D is >85 % within 15 years and nearly 100 % over time [13••]. DIPP also determined that of the children who developed T1D before puberty, 65 % had measureable iAb before the age of 2 years and 95 % before the age of 5 years [14]. Therefore, T1D is not only predictable but can also be identified at a very young age.

A recent scientific statement from the Juvenile Diabetes Research Foundation, Endocrine Society, and American Diabetes Association divides T1D into three distinct stages (Fig. 1) [15•]. Stage 1 is defined by the presence of islet autoimmunity with normal blood glucose and no symptoms. In stage 2, islet autoimmunity and dysglycemia are present, but there are still no clinical symptoms. Stage 3 is clinically diagnosable T1D with iAb, hyperglycemia, and the presence of polyuria, polydipsia, and weight loss. Being genetically at risk, which is predominantly conferred by human leukocyte antigen (HLA) genes, is classified as pre-stage 1. As a person progresses through the stages of T1D, it is believed that there is a steady decline in functional β cells. Intervening early in the disease course when a substantial amount of β cell mass exists is important as preserving β cell function improves metabolic control, decreases severe hypoglycemia, and protects from long-term microvascular changes seen in T1D [16]. Now that T1D is predictable, prevention trials should routinely be offered earlier in the disease course. Intervening early has many potential benefits including increased efficacy and the ability to use fewer therapies, thereby reducing drug side effects. We provide an overview of completed prevention trials moving through the stages of disease development, with a particular focus on prevention trials before clinical diagnosis (stages 1 and 2). We will also discuss those agents that showed mild efficacy in new-onset cohorts (stage 3) and the future challenges and outlook for making T1D a preventable disease.

Primary Prevention Trials in Genetically At-Risk Individuals (Pre-Stage 1)

Islet autoantibodies are used to establish T1D risk; however, antibodies can develop at any age, as evidenced from studying T1D development in identical twins [17], which necessitates repeated iAb measurements over time. Prior to the presence of iAb, family

history with a first-degree relative and genes provides risk assessment. Genes within the HLA complex confer over 50 % of the genetic risk for T1D development. Specifically, HLA class II genes account for most of this risk [18], and these genes are expressed on antigen-presenting cells (B cells, dendritic cells, macrophages) functioning to present processed protein fragments to CD4 T cells, also referred to as helper T cells [19]. Importantly, approximately 90 % of all individuals with T1D have specific HLA genes termed DR4-DQ8 and/or DR3-DQ2 [20]. However, the vast majority of individuals with diabetes-susceptible HLA class II genes do not develop the disease such that only 5–10 % of those with high-risk HLA genes develop T1D [21, 22]. These observations strongly implicate environmental factors in the presence of disease-susceptible HLA genes leading to the development of islet autoimmunity and eventual clinical T1D.

Most primary prevention trials have focused on very safe interventions, such as diet and supplements, as these studies are conducted in very young children (Table 1). One well-studied intervention is the composition of infant milk formulas. The hypothesis tested was that formulas composed of digested milk proteins (i.e., Nutramigen) may provide less of an immune stimulus than conventional cow milk formula (CMF). The Trial to Reduce Incidence of Diabetes in Genetically at Risk (TRIGR) in Finland randomized 230 infants (high-risk HLA and first-degree relatives) to either CMF or a casein hydrosylate formula (Nutramigen) within the first 8 months of life at the time the infants began to require breastfeeding supplementation [23]. Infants who received the casein hydrosylate formula had a decreased incidence of iAb development over the subsequent 10 years. A larger confirmatory trial enrolled 2159 infants from 78 centers in 15 countries within the first week of life. Infants were randomized to casein hydrosylate formula ($n = 1078$) or CMF ($n = 1081$) in addition to breastfeeding as often as desired. Unfortunately, the trial did not decrease the risk for developing multiple iAb, and the children are being followed to clinical T1D development [24].

In the Finnish Dietary Intervention Trial for the Prevention of Type 1 Diabetes (FINDIA) trial, 1133 infants with high-risk HLA were randomized to CMF, whey hydrosylate formula, or a whey-based formula free of bovine insulin in the first 6 months of life. Within the first 3 years of the study, infants receiving the insulin-free formula were less likely to develop islet autoimmunity than those receiving CMF [25]. These patients need to be followed over a longer time period to determine the effect of formula composition on the development of islet autoimmunity and clinical T1D.

Dietary omega-3 fatty acids are thought to have anti-inflammatory properties and have retrospectively been shown to reduce the risk of islet autoimmunity in genetically at-risk children [26]. Based on this premise, the TrialNet Nutritional Intervention to Prevent T1D (NIP) study was a randomized, double-blinded pilot trial that supplemented infants who had high genetic risk DR3 and/or DR4 genes with docosahexaenoic acid (DHA). The two arms of the study consisted of mothers supplemented with DHA in the third trimester ($n = 41$) and supplementation beginning in early infancy ($n = 57$). After following the infants for 3 years, the levels of DHA increased significantly in red blood cells, but peripheral blood inflammatory cytokines (IL-1β, TNFα, and IL-12p40) were not reduced [27].

Looking beyond dietary interventions, Pre-POINT is a recent pilot study completed in children aged 2–7 years ($n = 25$) determined to be at high risk for T1D development by HLA genes and family history of T1D [28]. Oral insulin, given in escalating doses up to 67.5 mg daily, resulted in protective immune responses to insulin. The ability of oral insulin to induce an immune response in pre-stage 1 of diabetes argues for a larger clinical trial to assess the progression to islet autoimmunity and dysglycemia in these patients. Oral insulin has also been used extensively in T1D secondary prevention trials as outlined in following section.

Secondary Prevention Trials in Autoantibody-Positive Individuals (Stage 1 and 2 T1D)

Many well-controlled randomized clinical trials have been conducted in individuals with iAb but no overt hyperglycemia. Most of these trials have utilized antigen-specific therapies with a focus on insulin preparations administered subcutaneously, orally, or intranasally (Table 2). Antigen-specific therapy aims to restore self-tolerance to insulin and β cell proteins without having to administer immunosuppressive agents [29]. Insulin is an important autoantigen for both T and B cells in T1D as it is often the first iAb to appear in young children, a higher titer correlates with a faster time to onset of T1D, and Tcell responses to insulin can be detected in the peripheral blood [30, 31••]. The goal of insulin administration in iAb individuals is to enhance regulatory T cell function that will then traffic to pancreatic islets and ideally help to prevent immune-mediated destruction of insulin-producing β cells by dampening the immune responses to insulin and other β cell proteins [32].

In the Diabetes Prevention Trial, Type 1 (DPT-1), sponsored by the National Institutes of Health, two insulin therapies were evaluated simultaneously to prevent T1D in at-risk relatives [33]. Relatives with iAb positivity and dysglycemia, thereby having a higher risk of progression to T1D, were randomized to placebo or low doses of ultralente insulin daily along with continuous insulin infusions for 4 days at the beginning of the study and annually thereafter ($n = 339$). The second cohort enrolled relatives with a lower T1D risk having iAb positivity (insulin autoantibodies present) and no metabolic abnormalities to daily oral insulin or placebo ($n = 372$). Unfortunately, after 6 years of follow-up, neither treatment delayed progression to T1D. Notably, a post hoc analysis of the oral insulin participants showed that patients with high insulin autoantibody levels (80 U/ml) had a delayed onset to T1D of approximately 5 years ($n = 263$) [34]. This finding prompted a repeat trial through TrialNet (), which is set to report results in 2017. In addition to these studies and the recent Pre-POINTstudy in primary prevention, TrialNet is currently conducting a secondary prevention study administering higher doses of oral insulin, to individuals with the same two iAb criteria for the current oral insulin trial, to help elucidate the mechanism of oral insulin's immunologic effect ().

The Belgian T1D Registry also conducted a prospective, nonrandomized study to determine if parenteral insulin could delay progression to T1D. Patients with IA-2 autoantibodies were given two injections of short-acting insulin for 36 months. Similar to the DPT-1 trial, the treated patients developed T1D at the same rate as the control population [35].

Intranasal insulin has also been used to prevent T1D and induce tolerance. The Intranasal Insulin Trial (INIT)-1 was a randomized, double-blinded, crossover pilot study conducted in Australia ($n = 38$) that indicated that intranasal insulin was safe, tolerable, and decreased T cell responses to insulin. These results prompted a larger study, INIT-2, which has completed enrollment and is following patients for 10 years to evaluate for T1D development () [36]. Another study utilized the DIPP cohort in Finland and randomized iAb-positive individuals to intranasal insulin or placebo ($n = 264$). An interim analysis showed no benefit of intranasal insulin in delaying progression to T1D, which resulted in stopping the trial [37].

Other therapies that have been used for secondary prevention include glutamic acid decarboxylase 65 formulated with the adjuvant aluminum hydroxide (GAD-alum) and nicotinamide (vitamin B6). The DIA-PREVIT trial is an ongoing study administering a GAD-alum vaccine to children with one or more iAb, one of which is an autoantibody to GAD. Participants are being followed for T1D development over 5 years. Nicotinamide increases nicotinamide adenine dinucleotide (NAD) and can potentially inhibit free radical formation, resulting in reduced β cell inflammation. Pilot studies had promising results [38–40]; however, two large randomized, double-blinded, placebo-controlled trials failed to delay progression to T1D [41, 42]. Other agents have been utilized in pilot trials including bacillus Calmette-Guerin (BCG), ketotifen (histamine antagonist), and cyclosporine [43–45]. Oral cyclosporine administration resulted in a slight delay in the time to T1D onset but was not able to prevent T1D onset.

The National Institutes of Health (NIH)-sponsored DPT-1 has continued as Type 1 Diabetes TrialNet with the goals of understanding disease pathogenesis, screening at-risk relatives for iAb and conducting clinical intervention trials to prevent T1D [46, 47]. Two therapies currently being used in TrialNet studies to delay T1D onset include an anti-CD3 monoclonal antibody (teplizumab) and CTLA-4 Ig (abatacept), which both had a mild effect on preserving β cell function (i.e., extending the "honeymoon period") in new-onset T1D patients. TrialNet is currently enrolling children and adults with multiple iAb positivity into trials utilizing these agents to assess if there is a change from normal to abnormal glucose tolerance with the use of abatacept (). Teplizumab is being used in iAb-positive individuals with impaired glucose tolerance (stage 2 in Fig. 1), which represents a period closer to clinical T1D, to delay T1D onset ().

New-Onset T1D Trials (Stage 3 T1D)

A large number of clinical trials have been completed in new-onset T1D, which is a population with limited β cell function. New-onset or tertiary prevention trials have aimed to preserve residual β cell function by targeting inflammation, modulating the adaptive immune response, inducing tolerance with antigen-specific therapies, and infusing cell-based therapies. The most widely accepted method to assess efficacy in these trials is to monitor β cell function by measuring stimulated C-peptide, which is cleaved 1:1 from insulin, over a 2–4-h period in response to a mixed meal tolerance test (MMTT) [48]. The most successful trial to date required an aggressive course of therapies including autologous nonmyeloblative stem cell transplantation (AHSCT) with granulocyte colony stimulation

factor (G-CSF) for cell mobilization, and cyclophosphamide plus anti-thymocyte globulin (ATG) for induction therapy to remove activated immune cells [49–52]. The protocol aimed to induce peripheral tolerance through hematopoietic stem cell immune regulation. In the initial trial, 20 of 23 patients were free from insulin use (12 continuously, 8 transiently) for a mean of 31 months [51, 52]. Of the three patients who did not have a time of insulin independence, they either had diabetic ketoacidosis or received glucocorticoids prior to the treatment regimen. It is likely that approaches requiring this degree of medical intervention provide more risk than benefit, especially in a pediatric population, given that good glycemic control is possible with current medical therapy. However, the results do provide a proof of concept that the autoimmune destruction of pancreatic β cells can be halted for a period of time late in the disease course. In fact, a recent randomized, single-blinded, placebo-controlled pilot trial using ATG and G-CSF preserved residual β cell function in recent-onset T1D patients for the first year after therapy [53]. A larger trial is now enrolling new-onset adolescents and adults (). Interestingly, ATG alone [54, 55] and G-CSF [56] alone do not maintain endogenous insulin production, indicating that certain combination therapies are capable of providing synergy to improve efficacy.

Although no new-onset study to date has permanently stopped β cell destruction, several studies have shown transient preservation of β cell function in phase 2/3 efficacy studies. A number of studies utilized a humanized monoclonal antibody to CD3 (teplizumab or otelixizumab), a cell surface marker found on mature T cells, in an attempt to target pathogenic immune cells [57–62]. With initial signals of efficacy in phase 2 trials, a large phase 3 clinical trial using teplizumab did not meet its primary endpoint to reduce daily insulin use and improve hemoglobin A1c values compared to placebo-treated controls. A post hoc analysis identified a distinct subset of responders that were young (ages 8–17 years), received drug within 6 weeks of diagnosis, and started treatment with good metabolic control (A1C <7.5 %, C-peptide mean AUC >0.2 nmol/l) [57, 61].

Other immune-modulating agents that have shown variable degrees of success include rituximab (anti-CD20) [63, 64], abatacept (CTLA-4 Ig) [65, 66], and alefacept (anti-CD2) [67]. Binding of rituximab to the CD20 transmembrane protein on B cells essentially eliminates mature B cells, which may be important antigen-presenting cells to activate self-reactive T cells. New-onset T1D patients treated with a course of rituximab had an approximate 8-month delay in C-peptide decline compared to placebo-treated controls. Similarly, patients receiving abatacept, which blocks T cell costimulatory receptor CTLA4, had an approximate 10-month delay in C-peptide decline in response to a 2-h MMTT and also had a lower HbA1c than the control group. Another recent multicenter, randomized, double-blinded, placebo-controlled trial (TIDAL) administered alefacept ($n = 33$) or placebo ($n = 16$) to new-onset T1D patients in two 12-week courses over 9 months. Alefacept targets memory T cells by binding to CD2, which is predominately expressed on the CD4+ and CD8+ effector T cells that are thought to play a key role in β cell destruction. Administration of alefacept maintained endogenous β cell function after 1 year, and treated patients also had lower daily insulin requirements and less hypoglycemic events [67].

Other agents that can modulate the immune response have been investigated and have not preserved residual β cell function. Two randomized, multicenter, placebo-controlled trials

administered agents directed against interleukin-1, an inflammatory cytokine [68]. In the first trial, patients with new-onset T1D were given subcutaneous injections of human monoclonal anti-interleukin-1 antibody, canakinumab, monthly for a year. The second trial gave anakinra, a human interleukin-1 receptor antagonist, by mouth daily for 9 months. Using an intention to treat analysis, neither agent delayed the loss of C-peptide in response to a MMTT. Several new-onset clinical trials utilizing antigen-specific therapies have been completed with the administration of GAD-alum, various forms of insulin and proinsulin, or heat-shock protein peptide (DiaPep277) [69–74]. Unfortunately, none of these trials preserved residual β cell function. As antigen-specific therapies move forward, the peptide, dose, route, and timing within T1D development need to be optimized [75].

Finally, cell-based therapies, including dendritic cells and regulatory T cells, have been safely administered in phase 1 trials [76, 77]. A recent trial isolated and expanded regulatory T cells from patients and then re-infused them into a total of 14 adult patients with new-onset T1D divided into four dosing cohorts. There were no infusion reactions or severe adverse events. Interestingly, there were several patients who had persistence of C-peptide in response to a MMTT at 1 year, and a phase 2 trial is planned to examine the effect of this therapy on maintaining β cell function after T1D diagnosis. Although not cell-based, low-dose interleukin-2 is currently being evaluated to specifically induce regulatory T cells to preserve β cell function [78, 79].

At the current time, despite T1D being a well-studied autoimmune disorder, only insulin preparations and amylin are approved for use by the US Food and Drug Administration to treat the disorder. No therapies to modulate or stop the immune destruction of β cells are used to treat T1D in clinical practice.

Challenges and Future Outlook

Identify Individuals at Risk for T1D

In order to eventually prevent T1D, two avenues need to be simultaneously pursued: (1) At-risk individuals need to be identified and (2) safe, specific, and personalized therapies to interdict the autoimmune response need to be evaluated in the preclinical T1D setting. First, the vast majority of screening efforts have focused on at-risk relatives of individuals with T1D. The American Diabetes Association currently recommends screening for iAb in relatives of patients with T1D through available clinical research studies, which is most often the Type 1 Diabetes TrialNet Pathway to Prevention Study. However, over 85 % of individuals who develop T1D do not have a family history [80]. With the incidence of diabetes increasing and diabetic ketoacidosis (DKA) being a significant comorbidity, it is our view that the general population should be screened for T1D risk by measuring iAb, especially in children. This would allow earlier diagnosis and opportunities for clinical prevention trials and lessen the morbidity and mortality of DKA [81, 82]. Screening for iAb currently requires venipuncture, which can be difficult in young children. Therefore, the need exists for a more widely adaptable and cost-effective method for screening iAb in the general population [83]. One such method is to measure iAb from dried blood spots (DBS) on filter paper. TrialNet recently conducted a study that showed decent correlation between serum and DBS measurements for GAD, IA-2, and ZnT8 [84]. Insulin autoantibodies have

historically been difficult to measure from DBS, but they are often the first iAb to develop and levels correlate with time to disease onset [9, 30]. We recently developed a method for measuring all four conventional iAb, including insulin autoantibodies, from DBS which may help facilitate large-scale general population screening (unpublished data).

Personalized Therapies

Over the last two decades, many well-controlled clinical trials have been enrolled and completed in an attempt to prevent T1D. Despite the disappointing results, T1D is now predictable and the infrastructure is in place to evaluate therapies. The development of more personalized therapies with specific mechanisms of action holds the promise to preserve β cell function. As previously mentioned, T1D genetic risk is predominantly conferred through specific HLA class II genes, such that ~60 % of all T1D patients have the DQ8 and DR4 genes, which are closely linked on chromosome 6. Studies using small "drug-like" molecules targeting the HLA-DQ8 molecule on antigen-presenting cells can block resultant peptide presentation to T cells [85]. One such molecule, methyldopa (Aldomet), is being evaluated in a phase 1b proof of concept trial in adults with recently diagnosed T1D having the DQ8 gene (). Methyldopa is a clinically well-established anti-hypertensive medication being used for more than 50 years; it is currently used clinically for the treatment of pregnancy-induced hypertension. With such a safe and well-studied medication, methyldopa provides safety, specificity, and personalization (targeting HLA-DQ8) for use in delaying or preventing T1D.

Antigen-specific therapy is also being directed to specific HLA genes. A peptide of proinsulin (C19-A3) is able to elicit T cell responses in T1D patients having HLA-DR4. A phase 1 trial treated T1D patients with intradermal injection of the peptide, showing safety and tolerability along with signals of immune efficacy. The low-dose peptide group had the induction of C19-A3-specific T cells producing interleukin-10, an anti-inflammatory cytokine associated with regulatory T cells [86]. Peptide immunotherapy personalized to a specific HLA gene is currently being evaluated in a recent-onset T1D trial ().

The combination of HLA class II molecules, self-peptide (insulin peptide) and T cell receptor form a trimolecular complex to active self-reactive T cells. The anti-insulin trimolecular complex (HLA-peptide-T cell receptor) provides components to target with specific therapies [87]. As such, there are therapies under preclinical development in animal models of autoimmune diabetes with monoclonal antibodies that can specifically block insulin/HLA complexes, thus inhibiting T cell activation [88]. There are also antibodies directed against a component of a disease-relevant T cell receptor to specifically deplete T cells involved in the autoimmune process [89]. Finally, a self-peptide that activates T cells toward islet autoimmunity has been well studied in the mouse model of spontaneous autoimmune diabetes. A specific fragment of insulin, B chain amino acids 9–23 (B:9–23), is an essential autoantigen driving T1D development [90], which may very well be the case in human disease [31••, 91]. How this peptide is presented by specific HLA-DQ molecules appears to be important in directing T cell responses [92, 93]. Attempts at mutating this peptide to improve insulin antigen-specific therapy by inducing protective T cell responses (i.e., insulin-specific regulatory T cells) have been encouraging in animal models, including

humanized mice with human HLA-DQ8 [94, 95]. All of these approaches represent specific immunologic therapies with a defined target. Islet autoimmunity is extremely specific and directed toward insulin-producing β cells, and as such, we believe that therapies having these same qualities will lead to improved T1D prevention efforts.

Conclusions

Type 1 diabetes is a chronic T cell-mediated autoimmune disorder with the natural history divided into stages. These stages allow for understanding disease pathogenesis as well as providing a blueprint for clinical prevention trials. Numerous primary, secondary, and new-onset clinical trials have been completed; however, β cell function cannot be sustainably preserved in those at risk or with newly diagnosed T1D. On the horizon, there are therapies with defined molecular targets involved in disease pathogenesis. Once these therapies are validated in clinical trials and combined based upon mechanism of action, we believe that T1D will be prevented and a foundation will be in place to cure the disease.

Acknowledgments

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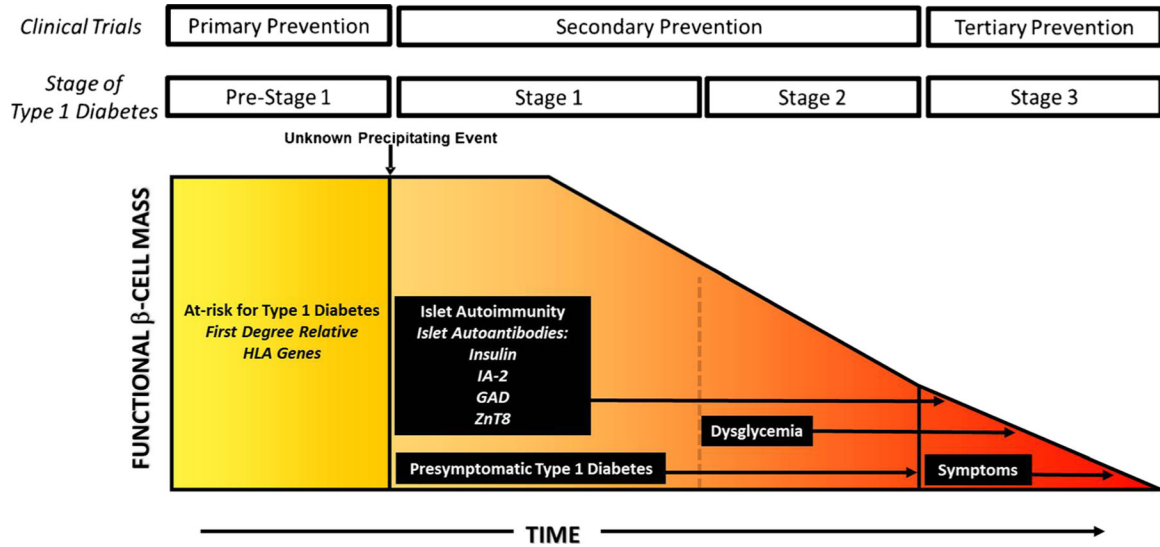
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Fig. 1.

Stages in the development of type 1 diabetes as functional β cell mass declines over time. Genes and currently unknown environmental factors initiate β cell autoimmunity. In stage 1, islet autoimmunity, marked by the presence of serum islet autoantibodies (insulin, GAD, IA-2, ZnT8), develops. Glucose homeostasis is normal, and patients are asymptomatic. As β cell function wanes, dysglycemia occurs, but not overt hyperglycemia (stage 2). Finally, overt type 1 diabetes (stage 3) develops with loss of significant insulin release and resultant hyperglycemia with clinical symptoms (polyuria, polydipsia, weight loss). Staging type 1 diabetes allows for prevention strategies in an attempt to preserve residual β cell function based upon the stage of disease such that primary prevention occurs in pre-stage 1, secondary prevention in stages 1 and 2, and tertiary prevention (or new-onset intervention) at stage 3

Table 1

Primary prevention trials in type 1 diabetes (pre-stage 1)

Intervention (trial)	Number	Age (years)	Primary endpoint	Status	Clinicaltrials.gov identifier
Antigen-specific therapies Oral insulin (Pre-POINT)	25	2–7	Hypoglycemia	Completed, safe and signals of efficacy for protective immune response to insulin	
Dietary exposure therapies Casein hydrosylate vs. cow's milk formula	230	Newborn infants	Decreased development of islet autoantibodies	Completed	
Casein hydrosylate formula vs. cow's milk formula (TRIGR)	2159	Newborn infants	Decreased development of islet autoantibodies	Completed enrollment, in follow-up	
Cow's milk formula vs. whey-based hydrosylate vs. whey-based hydrosylate free from bovine insulin (FINDIA)	1133	Newborn infants	Decreased development of islet autoantibodies	Completed enrollment, in follow-up	
Omega-3 and docosahexaenoic acid (DHA) (NIP)	98	Prenatal and newborn infants	Decreased inflammatory cytokines in peripheral blood	Completed	

Table 2

Secondary prevention trials in type 1 diabetes (stages 1 and 2)

Intervention (trial)	Age (years)	Primary endpoint	Status	Clinicaltrials.gov identifier
Antigen-specific therapies				
Parenteral insulin (DPT-1)	4–45	T1D onset	Completed	
Parenteral insulin (Belgian Diabetes Registry)	10–23	T1D onset	Completed	
Oral insulin (DPT-1)	3–45	T1D onset	Completed; post hoc analysis showed delay in progression to T1D in relatives with insulin autoantibodies 80 U/ml	
Oral insulin	3–45	T1D onset	Follow-up	TrialNet
Oral insulin	3–45	Change in insulin immune function	Recruiting; open-label dosing and immune mechanism of action study	TrialNet
Intranasal insulin (DIPP birth cohort)	1–5	T1D onset	Completed; trial stopped after interim analysis showed no effect on T1D progression	
Intranasal insulin (DIPP sibling cohort)	4–11	T1D onset	Completed; trial stopped after interim analysis showed no effect on T1D progression	
Intranasal insulin (INIT-2)	4–20	T1D onset	Completed; data collection and analysis	
GAD-alum (DiAPREV-IT)	4–18	T1D onset	Follow-up	
GAD-alum and vitamin D3 (DiAPREV-IT2)	4–18	T1D onset	Recruiting	
Anti-inflammatory therapies				
Nicotinamide (DENIS)	3–12	T1D onset	Completed	DENIS [42]
Nicotinamide (ENDIT)	5–40	T1D onset	Completed	ENDIT [41]
Immune-modulating therapies				
CTLA-4 Ig, abatacept	6–45	Abnormal glucose tolerance by OGTT	Enrolling	TrialNet
Anti-CD3 mAb, teplizumab	8–45	T1D onset	Enrolling	TrialNet

To date, β cell-specific or cell-based therapies have not been used in secondary type 1 diabetes clinical prevention trials