Insulin immunotherapy for pretype 1 diabetes

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Purpose of review
Loss of tolerance to insulin likely contributes to the immunopathogenesis of type 1 diabetes (T1D). Several large clinical trials and smaller mechanistic studies have failed to demonstrate the efficacy of insulin antigen therapy. The growing awareness of the heterogeneity of T1D likely affects the response to various immune therapies including insulin. Identification of biomarkers of clinical response will provide further insight into mechanisms leading to the disease and classify responders in the quest for personalized therapy.

Recent findings
Several biomarkers have identified subpopulations in posthoc analyses that showed benefit from oral insulin even though the placebo-controlled study was as a whole unsuccessful. High insulin autoantibody titer, low first phase insulin response, and high Diabetes Prevention Trial-Type 1 Risk Score identify at-risk relatives more likely to benefit from oral insulin. Future incorporation of human leukocyte antigen and the variable number of tandem repeats polymorphism located in the insulin gene promoter (*INS* VNTR) is of interest for both primary and secondary prevention studies.

Summary
Although primary and secondary prevention trials using oral insulin are ongoing, those completed have been largely unsuccessful. However, we believe that oral insulin should be considered in future trials as part of combination therapies as prerandomization biomarker testing is refined.

Keywords
antigen therapy, insulin, prevention, type 1 diabetes

INTRODUCTION

The loss of self-antigen recognition or the release of posttranslationally modified peptides into circulation is thought to predispose to the development of islet autoimmunity [1,2]. Islet autoreactivity against the insulin peptide is considered a major driver of type 1 diabetes (T1D) pathogenesis. T cell activation via major histocompatibility complex (MHC) class I and II recognition of multiple insulin epitopes has been identified in the peripheral blood of living subjects with T1D as well as the pancreatic lymph nodes and islets of organ donors [3–5]. The predisposition to inferior self-tolerance is likely multifactorial and includes 1) suboptimal presentation of insulin autoantigens by human leukocyte antigen (HLA) in the thymus, 2) polymorphisms in the *INS* variable nucleotide tandem repeat (VNTR) sequence, and 3) epigenetic modulation of insulin self-epitopes [6,7]. In response B, cells produce autoantibodies directed against insulin which can be detected many years before the onset of stage 3 T1D [8–10].

Insulin antigen-specific immunotherapy initially demonstrated efficacy in preventing progression to diabetes in an autoimmune mouse model. Nonobese diabetic (NOD) mice administered subcutaneous or intranasal insulin (peptide B:9–23) did not develop diabetes [11,12]. Various insulin peptides (altered and unaltered) and carriers have been studied in mice and humans for the purpose of blocking the specific immune response to insulin: B:9–23 [11–14], proinsulin (C19-A3) [15], proinsulin DNA plasmid (NCT03895437, NCT04279613), and autologous dendritic cells with proinsulin (PIpepTolDC NCT04590872). These have been studied in the new onset period, after T1D diagnosis, and prior to disease onset. In this review, we will focus on the latter.

Although the precise mechanism of action remains to be determined, it was initially hypothesized that parenteral preparations of human insulin

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KEY POINTS

- Insulin-specific autoreactive T cells contribute to the pathogenesis of type 1 diabetes.
- Insulin immunotherapy has been studied in primary and secondary prevention of type 1 diabetes with little success.
- Subpopulations of responders from DPT-1, TN-07, Pre-POInT, and Pre-POInT-early can be identified by immune and metabolic biomarkers.
- Although large definitive primary prevention studies are ongoing, secondary prevention trials must shift to combination therapy and could follow an induction/maintenance model where a more potent nonantigen therapy is given first, followed by oral insulin in the maintenance phase.
- Participants for that combination should have at least 1 biomarker present at baseline enrollment (high IAA titer, low FPIR, high DPTRS) or other biomarkers that require further study in this population (HLA, *INS* genotype, microbiome diversity changes, TCR diversity, etc.).

preserved insulin secretion by inducing beta cell rest [16]. However, when insulin encounters the mucosa (oral or nasal) it has been found to activate Th2-like responses which can induce peripheral tolerance spreading this effect to other antigens in close proximity (i.e., bystanders) [17].

CLINICAL TRIALS

The efficacy of insulin immunotherapies in pre-T1D has been largely unsuccessful (Table 1). Most trials have focused on secondary prevention, or the prevention of T1D after the development of autoimmunity (Stage 1 or 2 T1D [9]) utilizing oral, intranasal, or parenteral (IV or subcutaneous) insulin. These trials include the Diabetes Prevention Trial-Type 1 (DPT-1) [18,19], Intranasal Insulin Trial-I (INIT-I) [20], European Prediabetes Prevention Subcutaneous Insulin Trial (EPPSCIT) [21], Belgian Diabetes Registry [22], Type 1 Diabetes Prediction and Prevention (DIPP) study [23], and the Type 1 Diabetes TrialNet Oral Insulin Study (TN07) [24]. Insulin has also been administered orally in two small primary prevention trials prior to the appearance of islet autoantibodies, and presumably autoreactive T cell development. These are the Pre-

Table 1. Primary and secondary prevention trials of insulin immunotherapy in pre-type 1 diabetes

Trial	Route	Population	Primary outcome	Outcome achieved	Refs.
Primary prevention					
Pre-POInT	Oral	Relative, HLA risk, AAb-, 3–7y	Ab and T cell responses	Completed/successful[a]	[25]
Pre-POInT-early	Oral	Relative, HLA risk, AAb-, 6m-2y	Ab and T cell responses	Completed/unsuccessful[a]	[26▪▪]
POInT	Oral	Relative, HLA risk, AAb-, 4–7m	Islet autoimmunity	Ongoing	[42▪]
Secondary prevention					
DPT-1	IV/SC	Relative, ICA+, IAA+, FPIR below threshold, 3–45y	T1D	Completed/unsuccessful[a]	[19]
DPT-1	Oral	Relative, ICA+, IAA+, FPIR above threshold, 3–45y	T1D	Completed/unsuccessful[a]	[18]
DIPP	Intranasal	HLA risk, ≥2 AAb+ 1, 1–15y	T1D	Completed/unsuccessful	[23]
INIT-I	Intranasal	Relative, ≥1 Ab, normal FPIR, 4–32y	FPIR change	Completed/unsuccessful	[20]
INIT-II	Intranasal	Relative, Stage 1, FPIR above threshold, 4–30y	T1D	Ongoing	
Belgian Registry	SC	Relative, IA-2A+, 5–40y	T1D	Completed/unsuccessful	[22]
EPPSCIT	SC	Relative, ≥2 AAb, 7–14y	T1D	Completed/unsuccessful	[21]
TN-07	Oral	Relative, Stage 1 (IAA+ required), 3–45y	T1D	Completed/unsuccessful[a]	[24]
TN-20	Oral	Relative, Stage 1 and 2 (IAA+ required), 3–45y	IAA & GADA titer change	Ongoing	
Fr1da	Oral	Stage 1, 2–12y	Immune responders then Stage 2/3	Ongoing	

[a]Post-hoc subpopulation response.
AAb, autoantibody; FPIR, first-phase insulin response; HLA, human leukocyte antigen; IV, intravenous; m, months; SC, subcutaneous; y, years.
Stage 1=multiple AAb-positive with normal glucose tolerance (via OGTT); Stage 2=multiple AAb-positive with abnormal glucose tolerance; Stage 3=clinical diagnosis of T1D [9].

Primary Oral Insulin Trial (POInT) and Pre-POInT-early studies [25,26▪▪].

Over 690 children and adults at risk for T1D have been treated with oral or intranasal insulin and over 200 with parenteral insulin. There have been no significant safety concerns which is a feat for any single therapy. Importantly, there were no cases of hypoglycemia in the use of oral/intranasal insulin.

CLINICAL RESPONDERS TO ORAL INSULIN

Although primary endpoints have not been achieved in these studies, secondary analyses have suggested potential efficacy. Responders were identified within treated groups. This has been shown in other immunotherapy trials as well [27–31] in those at-risk and those newly diagnosed. Given the heterogeneity of the disease this is not unexpected, and identification of these populations shows us who could benefit from the continued role of insulin immunotherapy in the prevention of T1D.

Diabetes prevention trial-type 1 subjects with high insulin autoantibody titer responded to oral insulin

The DPT-1 study enrolled first and second-degree relatives ($n = 372$) with ICA and IAA positivity, normal glucose tolerance, adequate first-phase insulin response (FPIR; $\geq$60 μU/mL for parents and relatives <8 years or $\geq$100 μU/mL if $\geq$8 years), and the absence of protective HLA haplotype DQA1*0102/DQB1*0602 [18]. An increased rate of progression was suggested by the data in individuals with a confirmed IAA titer $\geq$80 nU/mL (>5 standard deviations [SD] above the mean for the population) at baseline. In a posthoc analysis, this subpopulation ($n = 106$ with IAA $\geq$80 nU/mL based on original trial enrollment criteria) who were treated with oral insulin (7.5 mg/day) had a reduction in their risk of progression to T1D compared to the placebo group. In the oral insulin group, 6.4% per year developed diabetes compared to 11.3% per year in the placebo group (hazard ratio [HR] 0.539, 95% CI 0.30–0.98, $P = 0.035$). Median survival times projected this as a 4.5-year delay in diabetes. This effect persisted at long-term follow-up (median 9.1 years [IQR 8.7–10.1]) but is based on continued therapy administration [32] as well as medication adherence over the course of the trial [33].

TN-07 subjects with low first phase insulin response responded to oral insulin

To confirm or negate these findings, TrialNet embarked on an appropriately powered similar prevention study. Participants in the TN-07 oral insulin trial were similar to those enrolled in DPT-1 and received 7.5 mg/day oral insulin versus placebo [24]. The only differences noted are the use of the micro-insulin autoantibody (mIAA) assay and initial testing for biochemical autoantibodies followed by ICA testing if positive. The primary outcome of the study was determined by the rate of diabetes in participants in the primary stratum (n = 389) with normal FPIR. However, there were two other strata determined *a priori*. Secondary stratum 1 (SS1; $n = 55$) included individuals with a low FPIR (based on DPT-1 thresholds described above). The final group, secondary strata 2/3, included lower risk autoantibody profiles and either normal or low FPIR ($n = 116$). Significance in the primary outcome and the entire cohort was not met. However, individuals in SS1, while having a high overall rate of T1D, demonstrated a reduced risk of progression (HR 0.45, 95% CI 0–0.82, $P = 0.006$) with a delay of 31.0 months in the treated group. In addition, exploratory analyses of individuals in the primary stratum with excellent medication compliance (>85% over 24 months) had delayed progression to diabetes compared to placebo (HR 0.35, 95% CI 0–0.86, $P = 0.016$) [24].

The DIPP intranasal insulin study conducted posthoc analyses of several high-risk groups including high IAA titer (>5 SD above the mean for Finnish children), low FPIR (<38 μU/mL), and the presence of 3–4 autoantibodies at baseline [23]. However, intranasal insulin did not affect the rate of progression to T1D. No increase in insulin antibody titers *following* treatment was seen in the DIPP study (intranasal insulin) or Belgian Registry (subcutaneous insulin). This was not reported for DPT-1 and TN-07. In contrast, higher baseline IAA titers in INIT-I participants were correlated with increased insulin antibody responses to intranasal insulin (Spearman correlation coefficient $r = 0.79$, $P = 0.0001$) [20].

Combined metabolic measures identify responders to oral insulin

Although IAA titers and FPIR may identify populations of clinical responders to oral insulin, other metabolic measures that combine glucose and C-peptide that predict risk of subsequent T1D may also identify potential responders [34,35]. Sosenko *et al.* used such a risk score to identify participants at higher metabolic risk for diabetes in both the DPT-1 and TN-07 (primary stratum) trials [36▪▪]. The Diabetes Prevention Trial-Type 1 Risk Score (DPTRS) was derived from DPT-1 data and includes age, BMI (log), the glucose sum of 30-, 60-, 90-, and 120-min values divided by 100, the C-peptide sum of 30-, 60-, 90-, and 120-min values divided by 10, and log fasting C-

peptide. In those with high metabolic risk for T1D (DPTRS $\geq$6.75), the oral insulin group had a significantly higher AUC C-peptide/AUC glucose ratio (combined glucose and C-peptide outcome measure) at 1 year compared to placebo with adjustment for age and baseline DPTRS value. Among those with a DPTRS <6.75, the AUC C-peptide/AUC glucose ratio did not differ between the oral insulin and placebo groups. Metabolic progression was delayed with oral insulin in these trials. Additionally, measures other than the rate of diabetes development may be beneficial in prevention clinical trials and could result in shorter trial duration.

IMMUNE RESPONSES FOLLOWING INSULIN IMMUNOTHERAPY

Although the prevention of T1D or islet autoimmunity has yet to be achieved there is further evidence of immune modulation following oral insulin administration in humans.

Mucosal immune changes and alterations in the gut microbiome

Intestinal permeability and duodenal mucosa changes may separate individuals with T1D from healthy control subjects as well as from individuals with celiac disease [37,38]. In the small mechanistic Pre-POInT study, children treated with 67.5 mg/day of oral insulin had positive immunomodulatory effects including mucosal immune changes [25]. In this pilot study, children were 3–7 years old with high-genetic risk, negative islet autoantibodies, and a first-degree relative with T1D. Salivary IgA antibodies to insulin were more likely to be present in those treated with oral insulin compared to control subjects [25]. Variability between participants with small numbers limit generalizability.

The study of mucosal antigen therapy remains timely as we learn more about the potential role of the gut microbiome in T1D pathogenesis [39]. Results of the more expansive Pre-POInT-early study ($n = 44$) were published recently [26▪▪]. Young children (6 months-2 years) treated with escalating doses of oral insulin for 12 months demonstrated no adverse effects related to therapy. However, in contrast to the Pre-POInT study, immune responses to insulin were not different between treated and placebo subjects ($P = 0.54$). However, exploratory analyses revealed enhanced antibody responses to insulin in those children with the T1D-susceptible *INS* genotype (VNTR *A/A*). Stool studies from those with the *INS* risk genotype who received oral insulin also demonstrated an increase in the relative abundance of bacterial species (Shannon diversity) as well as specific bacterial species differences between *AA* and *AT/TT* genotypes. Such modifications in the gut microbiome could represent a shift toward increased diversity as seen in healthy controls. However, age is an important factor when considering microbiome diversity so this will need confirmation in those older than 2 years and from various geographical regions as well.

Adaptive immune system remodeling

A complex interplay exists between the adaptive immune system and the gut microbiome. A proinsulin deficient transgenic NOD mouse model with high levels of insulin B15–23-reactive $CD8^+$ T cells demonstrated accelerated progression to diabetes when healthy gut microbiota were removed with a broad-spectrum antibiotic [40]. In addition to expansion of autoreactive $CD8^+$ T cells, a greater proportion of cells produced proinflammatory cytokine INFγ than those not treated with the antibiotic. Peptide-MHC binding may be altered as T cell receptor (TCR)-β chain repertoires are influenced by gut microbiota [40]. All children enrolled in the Pre-POInT studies had the HLA DR4-DQ8 haplotype. The HLA DR-DQ locus which contributes up to 50% of T1D genetic risk [41], has demonstrated preferential insulin autoimmunity in individuals with the HLA DR4 haplotype, most notably in children followed in The Environmental Determinants of Diabetes in the Young (TEDDY) study [8].

T cell proliferation was found to be altered by intranasal administration of insulin in INIT-1 where the stimulation index of T cells decreased in the presence of antigen (denatured human insulin) within-arm crossover comparisons [20]. This reduction in effector cells may tip the scales toward a more regulatory immune phenotype. Indeed, in the Pre-POInT and Pre-POInT-early studies, insulin- and proinsulin-responsive $FOXP3^+CD127^-$Tregs were increased in those randomized to insulin immunotherapy. $CD4^+$ T cell stimulatory responses to insulin were similar overall albeit lower in those treated at 12 months [25,26▪▪].

ON THE HORIZON FOR ORAL INSULIN

What could we learn in the coming years and where should we go from here?

Ongoing oral insulin studies in pretype 1 diabetes

Ongoing trials utilizing insulin antigen therapy in pre-T1D are included in Table 1. The Primary Oral Insulin Trial (POInT) in 5 European countries is set to be the largest placebo-controlled trial in very

young children [42▪]. This will include autoantibody-negative children 4–7 months old who are treated with oral insulin (dose escalation up to 67.5 mg/day) until age 3 years (NCT03364868). This primary prevention trial is part of the Global Platform for the Prevention of Autoimmune Diabetes (GPPAD), screening children from the general population for genetic risk of T1D.

An ongoing secondary prevention trial in Australia and New Zealand will utilize intranasal insulin aiming to prevent progression to diabetes (INIT-II; NCT00336674). Additionally, the Fr1da (NCT02620072) and Immune Effects of Oral Insulin in Relatives at Risk for Type 1 Diabetes Mellitus (TN-20; NCT02580877) studies aim to address mechanistic questions. Fr1da will focus on immune changes (insulin-specific salivary IgA, stimulation of $CD4^+$ T cells, insulin-tetramer positive Tregs) and if at least one of those is present then those 'immune responders' will be compared to all other participants in terms of rate of progression to dysglycemia and/or T1D. Within the TrialNet umbrella, TN-20 has assessed autoantibody titers and T cell responses following 67.5 mg/day oral insulin compared to 500 mg every other week. These data should help answer whether higher doses are effective and necessary for immune activation.

Role of insulin immunotherapy in pretype 1 diabetes moving forward

The use of insulin antigen monotherapy in secondary prevention has been well studied. We posit that further large studies in heterogeneous populations would be of little benefit. However, as shown in Fig. 1, high IAA titers (>5 SD above the population mean) and more severe metabolic dysfunction (low FPIR or elevated DPTRS) identify a population that may benefit especially if considered as part of a combinatorial approach. Further exploration of

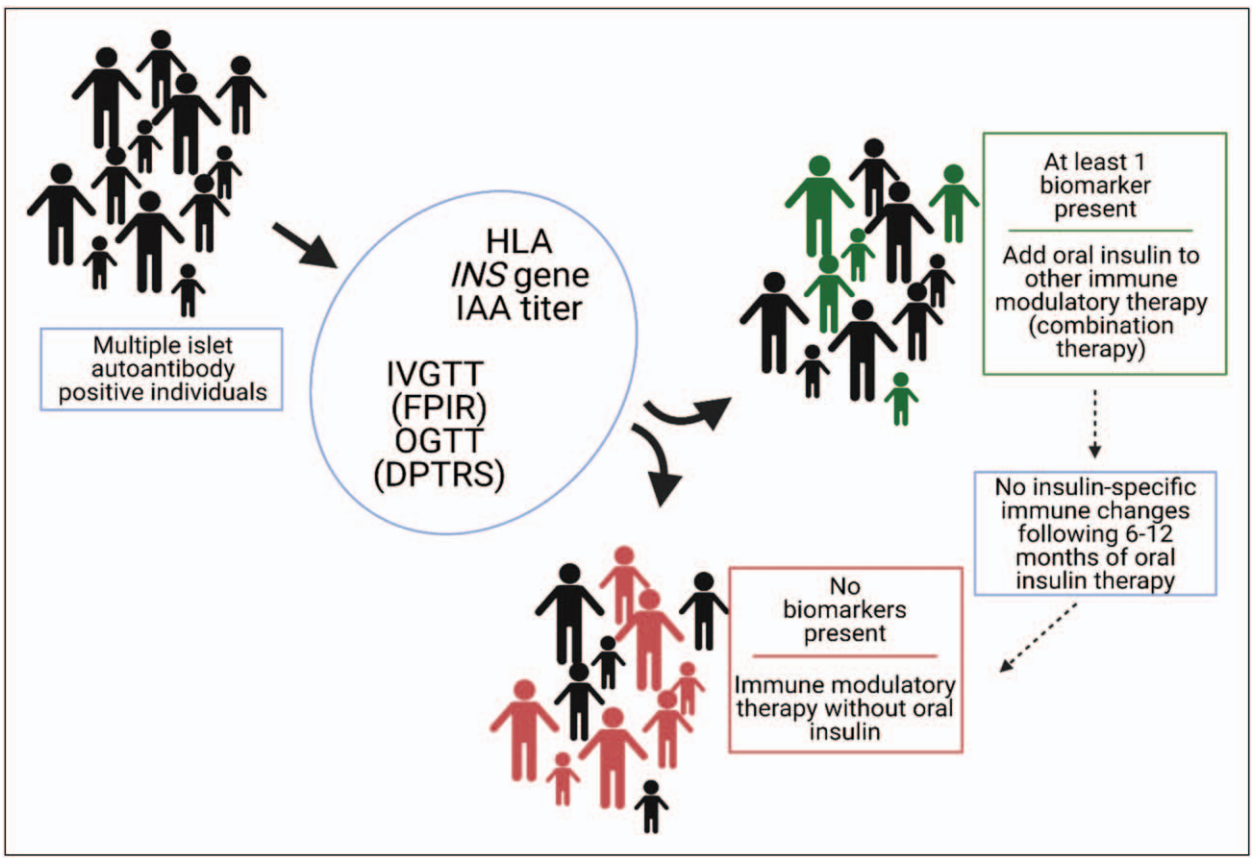

FIGURE 1. Schematic for determining whether individuals at high risk of progressing to type 1 diabetes (multiple autoantibody positive individuals) should go into a single nonantigen specific immune therapy arm (red box) or into a combination immune therapy arm (green box). The combination would include oral insulin after individuals were tested for the biomarkers (in the circle) that have a high likelihood of predicting those most likely to have success from oral insulin. Intermediate immune efficacy endpoints could be assessed 6–12 months after oral insulin initiation if excellent medication adherence present. If no insulin-specific immune effects are found, then that subject may elect to go to the nonantigen specific immune monotherapy arm to decrease participant burden.

the *INS* risk allele and HLA haplotype may further predict responders to oral insulin prior to the development of islet autoimmunity but further study is needed and ongoing.

Ideal timing of immunomodulatory therapy in pre-T1D is not yet known. Until the completion of the POInT study we do not know if oral insulin may actually prevent the induction of islet autoimmunity. Furthermore, intermediate endpoints are essential to further prevention efforts. More than a decade may elapse before a significant number of clinical trial participants with autoantibodies progress to T1D. More extensive analyses of metabolic data from DPT-1 and TN-07, for example, could determine if measures such as Index60, AUC C-peptide/AUC glucose, and glucose C-peptide response curves (GCRCs) could serve as biomarker endpoints should they be shown to correlate with disease progression.

Finally, important concepts to consider in the future use of oral insulin in secondary prevention (Fig. 1): 1) the remarkable safety profile promotes its use in combination therapy following biomarker-directed participant enrollment; 2) oral insulin therapy in combination should be continued for at least 6–12 months or until the diagnosis of diabetes based on the presence of insulin-specific immune changes; and 3) protocols to address adherence are implemented early as they are vital to successful continuation of this maintenance therapy.

CONCLUSION

Large prevention (both primary and secondary) studies have been unsuccessful in the prevention of T1D. However, small subpopulations have shown benefit from this very innocuous, easy to administer therapy. Although the role of insulin immunotherapy is still unclear in primary prevention, we believe it should be strongly considered as part of combination immunotherapy particularly in the pursuit of precision medicine following multiplexed testing for 'responder' features. Vigorous pursuit of precision or personalized medicine must occur as we unravel disease heterogeneity. Although the use of oral insulin monotherapy will not result in the prevention of T1D, its role in interdicting in this disease course may still be substantial.

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Conflicts of interest

There are no conflicts of interest.

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- ■ of special interest
- ■■ of outstanding interest

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