Optimizing Stage 2 T1DM Management: Assessing the Impact of GLP-1Ra on Metabolic Outcomes in Patients Receiving Teplizumab

Justin M. Gregory, M.D., M.S.C.I.
Assistant Professor of Pediatrics
Ian M. Burr Division of Pediatric Endocrinology and Diabetes, Department of Pediatrics
Vanderbilt University Medical Center

1500 21st Avenue South, Suite 1514
Nashville, TN 37212-3157

Table of Contents:

1.0 Background

Type 1 Diabetes Mellitus (T1DM) is a chronic autoimmune disorder affecting millions of individuals worldwide. It is characterized by the destruction of insulin-producing pancreatic beta cells, leading to absolute insulin deficiency. Despite its global impact, management of T1DM remains a complex task, with challenges arising from the intricate factors influencing glycemic control and disease progression.

T1DM is often categorized into different stages to facilitate diagnosis and management. Stage 1 T1DM is defined by the presence of multiple diabetes-related autoantibodies with normal blood glucose levels, indicating an early phase of autoimmune beta cell destruction but no clinical symptoms yet. Stage 2 T1DM, also known as dysglycemia, is marked by the presence of autoantibodies and abnormal blood glucose levels. In this stage, patients start to show signs of impaired glucose metabolism but may not have overt diabetes symptoms. Stage 3 T1DM is the stage where clinical symptoms of diabetes, such as increased thirst and frequent urination, become apparent due to significant insulin deficiency.

Our study primarily focuses on individuals with stage 2 T1DM, who show signs of impaired glucose metabolism but are not yet fully symptomatic. In this group, the autoimmune process has started, and the beta cells are under attack, but the body still produces enough insulin to prevent overt symptoms of diabetes.

A significant breakthrough in the field is the use of teplizumab (TZIELD®), an immunotherapeutic monoclonal antibody. Recent investigations have shown this drug delays the progression from stage 2 to stage 3 T1DM in high-risk individuals by modulating the immune response against pancreatic beta cells. However, even with TZIELD® treatment, these individuals eventually progress to insulin dependency, highlighting the ongoing challenges in managing T1DM.

Alongside, these patients often face cardiometabolic challenges such as postprandial hyperglycemia, impaired glucose disposal, and endothelial dysfunction. These factors contribute to long-term complications of diabetes, presenting an urgent need for additional therapeutic interventions.

In this context, we propose to investigate the potential benefits of GLP-1 receptor agonists (GLP-1Ra) in individuals with stage 2 T1DM undergoing TZIELD® treatment. GLP-1Ra have demonstrated metabolic advantages in Type 2 Diabetes Mellitus (T2DM), including improvements in postprandial glycemia, insulin sensitivity, and endothelial function. However, their role in T1DM, particularly in conjunction with TZIELD® therapy, remains unexplored.

Unraveling the potential metabolic benefits of GLP-1Ra in this patient population could significantly enhance our understanding of T1DM pathophysiology and open new avenues for therapeutic interventions. This could improve patient outcomes, enriching the quality of life and life expectancy for individuals with T1DM.

2.0 Rationale and Specific Aims

The monoclonal antibody TZIELD® demonstrates potential for preserving β-cell function in early-stage type 1 diabetes mellitus (T1DM). As mass screening programs identify

more patients in the early stages of T1DM, endocrinologists will confront Dual Derangements: diminishing insulin secretion and detrimental hyperglucagonemia. TZIELD® alone, however, does not provide long-term relief from these challenges, especially for patients with certain HLA haplotypes. Glucagon-like peptide-1 receptor agonists (GLP-1Ra) hold promise for enhancing immunotherapy by addressing these Dual Derangements due to their insulinotropic and glucagonostatic properties. Despite this potential, clinical and mechanistic data for GLP-1Ra as a disease-modifying therapy in early-stage T1DM remain limited. This pilot and feasibility grant proposal investigates the innovative combination of GLP-1Ra and immunotherapy in stage 2 T1DM patients, setting the foundation for a larger, definitive trial to determine this novel therapeutic approach's impact on dysmetabolism.

Our long-term goal is to determine whether repurposing GLP-1Ra for stage 2 T1DM in combination with immunotherapy can modify the disease course, reducing the need for exogenous insulin therapy and leading to improved cardiometabolic outcomes and quality of life. The immediate objective is to investigate the impact of GLP-1Ra's insulinotropic and glucagonostatic effects on dysmetabolism in stage 2 T1DM patients treated with TZIELD®. We hypothesize that these effects will each delay the need for exogenous insulin by improving three key aspects of dysmetabolism: 1) postprandial glycemia, 2) disposition index (i.e., the ability of the islet cells to compensate for a given insulin sensitivity), and 3) endothelial function. The rationale for this hypothesis is based on two observations: first, GLP-1Ra combined with immunomodulatory therapy sustains endogenous secretion in response to an MMTT during the first year of stage 3; and second, GLP-1Ras mitigate postprandial hyperglucagonemia in longer-duration T1DM. To test our hypothesis, we will conduct studies in individuals with stage 2 T1DM treated with TZIELD® using a crossover design (figure 1) structured around the following specific aims:

Aim 1: Investigate the impact of GLP-1Ra on postprandial glycemia in a pilot study. We will measure postprandial glycemia during an MMTT before TZIELD® treatment. After TZIELD® we will compare the effects of placebo versus semaglutide (Rybelsus®),a GLP-1Ra.

Aim 2: Study the impact of GLP-1Ra on the disposition index (DI) in a pilot study. We will use the oral glucose minimal model to measure DI during an MMTT before and after TZIELD® treatment, comparing the effects of placebo versus Rybelsus®. As an exploratory outcome, we will quantify β-cell endoplasmic reticulum dysfunction by measuring the proinsulin-to-C-peptide ratio during the MMTT.

Aim 3: Determine the impact of GLP-1Ra on endothelial function in a pilot study. We will use B-mode ultrasound to measure flow mediated vasodilation (FMD), a bioassay of endothelial function, during each MMTT. Because endothelial cells are often among the first affected by hyperglycemia and insulin resistance, we aim to illuminate how GLP-1Ra may mitigate early vascular disease progression.

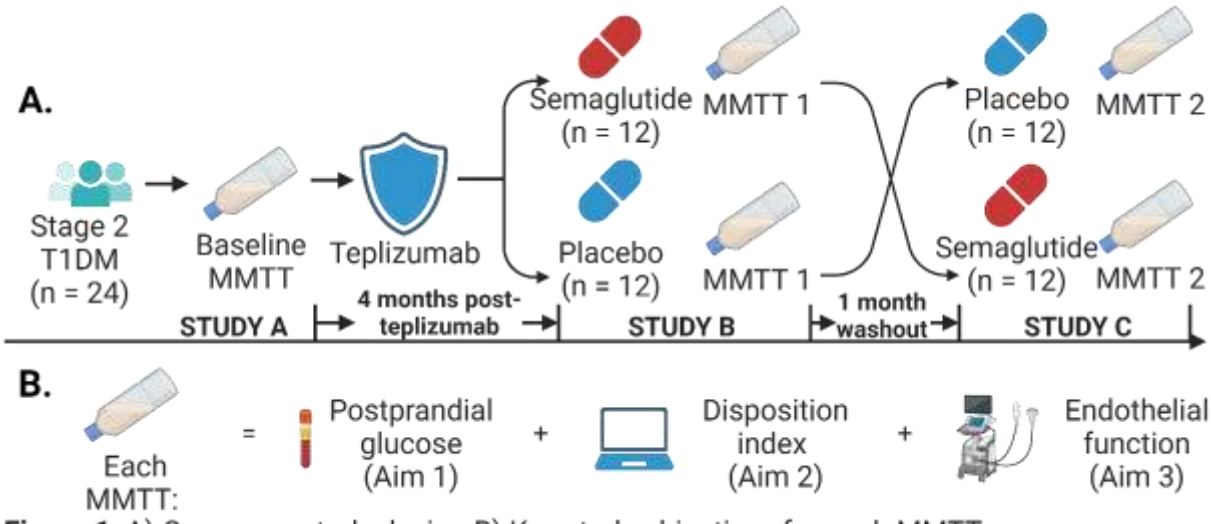

Figure 1: A) Crossover study design B) Key study objectives for each MMTT.

To enhance our understanding of GLP-1Ra's mechanistic effects in stage 2 T1DM, we will perform bivariate analyses examining the relationship between GLP-1Ra's insulinotropic and glucagonostatic effects and each aim's primary outcome. These analyses will inform the design of a fully powered multivariate linear regression analysis in a subsequent larger study.

This pilot and feasibility trial will generate vital preliminary data on combining GLP-1Ra with TZIELD® in stage 2 T1DM patients. Our specific aims focus on collecting early data on dysmetabolism's essential aspects, addressing feasibility concerns, and guiding the design of a larger, fully powered R01-funded clinical trial. Achieving these aims will advance our understanding of GLP-1Ra's therapeutic potential in reducing the need for exogenous insulin in early-stage T1DM, paving the way for future studies aiming to improve patient outcomes and clinical practice.

3.0 Animal Studies and Previous Human Studies

Doctors face **Dual Derangements** in treating early-stage T1DM: **diminishing insulin secretion** and **detrimental hyperglucagonemia**.

T1DM is a chronic autoimmune disorder characterized by the destruction of insulin-producing β-cells in the pancreas, leading to lifelong dependence on exogenous insulin(1). Interventions during the critical period of early-stage T1DM, particularly stage 2, could potentially slow β-cell decline and delay the need for exogenous insulin therapy, improving long-term outcomes. TZIELD®, a monoclonal antibody, shows potential to extend β-cell function in stage 2(2-5) and early stage 3 T1DM(6), spurring interest in additional therapeutic strategies to target the ***Dual Derangements*** of ***diminishing insulin secretion***(1, 7) and ***detrimental hyperglucagonemia***(8-10) which are hallmarks of early-stage T1DM.

Patients treated with TZIELD® during early-stage T1DM may particularly benefit from the insulinotropic and glucagonostatic properties of GLP-1Ra in confronting the Dual Dilemmas.

GLP-1Ra, a class of medications used in T2DM, lower blood glucose by enhancing glucose-dependent insulin secretion, suppressing glucagon release, and slowing gastric emptying (11-13). Studies in T2DM(14, 15) and longstanding T1DM(16) suggest GLP-1Ra improve endothelial function, reduce inflammation, and potentially preserve β-cell function. In longer-duration T1DM, exenatide, a GLP-1Ra, mitigates postprandial hyperglucagonemia and improves glycemic control(17-19). Combining GLP-1Ra with immunomodulatory therapy (liraglutide and anti-interleukin-21 antibody) sustained endogenous insulin secretion in response to a mixed meal tolerance test (MMTT) during the first year of ***stage 3*** T1DM(20). This evidence suggests GLP-1Ra, particularly when combined with TZIELD®, may hold promise for ***stage 2*** T1DM patients.

In summary, while TZIELD® offers a promising avenue for preserving β-cell function in early-stage T1DM, its potential may be further enhanced when combined with GLP-1Ra. Previous studies indicate that GLP-1Ra addresses the Dual Derangements characteristic of early-stage T1DM and has been beneficial in T2DM and longer-duration T1DM. Given this background, our study aims to explore the complementary effects of combining TZIELD® with GLP-1Ra in stage 2 T1DM patients, potentially ushering in a novel therapeutic strategy.

4.0 Inclusion/Exclusion Criteria

Inclusion Criteria
- Age: 12-50 years
- BMI: 18-31 kg/m^2 (adults) or 5-95th %ile (pediatric)
- Stage 2 T1DM (i.e., ≥ 2 islet auto-antibodies and:
    - fasting glucose ≥ 100 mg/dL and < 126 mg/dL OR
    - 2-hr OGTT /MMTT ≥ 140 mg/dL and < 200 mg/dL OR
    - During an OGTT having a glucose of > 199 mg/dL at 30, 60, or 90 minutes)

Exclusion Criteria
- Comorbidities:
    - SBP > 140 mmHg and DBP > 100 mmHg
    - eGFR by MDRD equation of < 60 mL/min/1.73m^2
    - AST or ALT > 2.5 times ULN
    - Family history of medullary thyroid carcinoma
    - Diagnosis of pancreatitis or gastroparesis within the past 3 years
- Medications: Any diabetes medication, any antioxidant vitamin supplement (<2 weeks before a study), any systemic glucocorticoid, antipsychotic, atenolol, metoprolol, propranolol, niacin, any thiazide diuretic, any OCP with > 35 mcg ethinyl estradiol, growth hormone, any immunosuppressant, antihypertensive, any antihyperlipidemic
- Other: pregnancy, peri- or post-menopausal women, active smoker

5.0 Enrollment/Randomization

We have chosen a randomized, double-blind, placebo-controlled, crossover study design. Volunteers will participate in three MMTT studies as depicted in Figure 2. We will

recruit many participants from Vanderbilt's T1DM Immunotherapy Clinic, but in some cases will recruit participants receiving TZIELD® from other institutions.

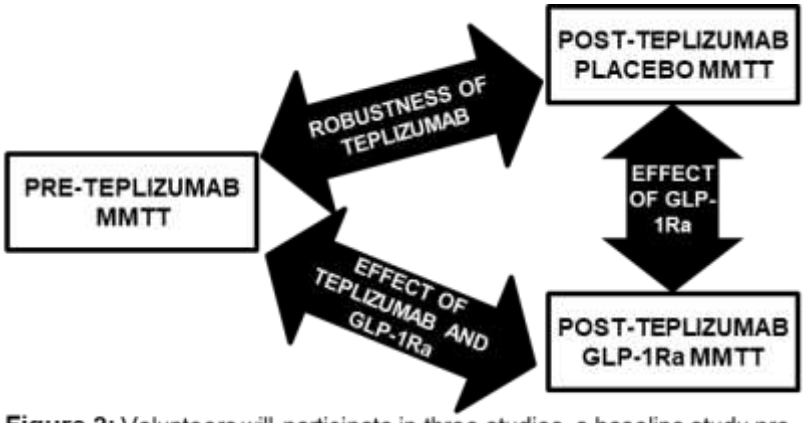

Figure 2: Volunteers will participate in three studies, a baseline study pre-teplizumab, then in two studies 3-5 months post-teplizumab in random order.

Randomization: The first study visit will occur before participants receive TZIELD®. We will schedule the first of two post-TZIELD® studies between three and five months after completing TZIELD® treatment. The study team will randomize the sequence of the two studies into one of two treatment orders using random permuted blocks of four.

Enrollment: Enrollment will occur at the beginning of the first MMTT study.

Enrollment will take place in the Vanderbilt Clinical Research Center (CRC). The Vanderbilt CRC is located on the 2rd floor of Medical Center North on the Vanderbilt University Medical Center (VUMC) campus (1211 Medical Center Dr, Nashville, TN 37232; telephone number: 615-322-2312).

6.0 Study Procedures

6.1 Overview

In this randomized, double-blind, placebo-controlled, crossover study, we aim to evaluate the metabolic benefits of GLP-1Ra for individuals with stage 2 T1DM receiving TZIELD® treatment. Participants undergo three Mixed Meal Tolerance Test (MMTT) studies to quantify postprandial hyperglycemia, disposition index (DI), and nitric oxide mediated endothelial function.

6.2 Timeline

The first study visit will occur before participants receive TZIELD® (pre-TZIELD® MMTT in figure 2). We will schedule the first of two post-TZIELD® studies (post-TZIELD® placebo MMTT and post-TZIELD® GLP-1Ra MMTT in figure 2) between three and five months after completing TZIELD® treatment. The study team will randomize the sequence of the two post-TZIELD® studies into one of two treatment orders (e.g., GLP-1Ra then placebo or placebo then GLP-1Ra). Participants will complete these two studies over a maximum span of two months, with a minimum washout period of one week between studies to minimize carryover effects.

In certain circumstances, a potential participant may have already undergone TZIELD® treatment prior to joining the study. This could be because they received TZIELD® at a different institution or due to scheduling constraints that prevent them from participating in the pre-TZIELD® MMTT before receiving TZIELD®. In such cases, where the participant is otherwise eligible for the study, the PI may elect to skip the pre-TZIELD® MMTT and proceed directly to the two post-TZIELD® MMTTs.

6.3 Study preparation

To promote consistency across participants and limit potential external influences, we have established several guidelines. To limit potential confounding factors that could impact metabolic responses to GLP-1Ra, we will ask subjects to refrain from vigorous exercise and adhere to an isocaloric diet with a standardized macronutrient composition three days prior to each study. Participants will maintain a food diary to monitor adherence. Our team will provide a standardized, isocaloric diet for consumption during the day before each experiment. Participants will begin fasting at 10 PM the night before the study. For female participants, we will aim to align study visits with the follicular phase of their menstrual cycle (days 2-10) to minimize potential confounding factors related to insulin sensitivity(21).

To ensure an accurate measurement of key study outcomes with minimal confounding, participants will take the following precautions before each STUDY visit:

- Hold vitamin supplementation for 72 hours
- Avoid NSAIDs and aspirin for 24 and 72 hours, respectively, if feasible
- Refrain from smoking and avoid secondhand smoke exposure for 12 hours
- Consume no caffeine for 12 hours
- Abstain from exercise for 12 hours
- Consume a standardized 2,000 kcal/day diet on the day preceding each STUDY visit.
- Consume the standardized dinner between 1700 and 2200 on the evening prior to STUDY visit. Thereafter, the participant will begin fasting except for drinking sugar free liquids, except if hypoglycemia occurs.

6.4 Protocol overview

Figure 3 illustrates the experimental protocol for each study.

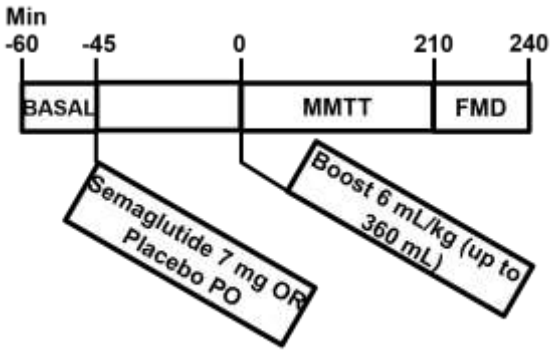

Figure 3: Schematic for MMTT visits. We will study participants with Stage 2 T1DM just prior to teplizumab therapy and two times during the six months following teplizumab therapy. In the two post-teplizumab studies, participants will receive a GLP-1Ra (semaglutide) or placebo in random order prior to consuming a standardized oral nutrient load. We will serially measure plasma levels of glucose, insulin, and C-peptide throughout the experiment. Primary outcomes include mean plasma glucose (Aim 1), disposition index (DI) obtained from the oral glucose minimal model (Aim 2). During the last 30 mins of the MMTT we will use high resolution B-mode ultrasound to measure endothelium-mediated vasodilation to quantify nitric oxide bioavailability (Aim 3).

6.5 Consent, parental consent, and adolescent assent

The PI or designated KSP will obtain consent from all participants in the the clinic or before the first visit. In the case of participants ages 12-17 the PI or KSP will obtain adolescent and consent from both parents, consistent with guidance from 45 CFR 46 Subpart D § 46.406406Consent will be obtained in a private room in the CRC, in the Eskind Diabetes Center, in the pediatric endocrinology clinic in the Doctors' Office Tower, or in some cases in a telephone call prior to beginning study procedures. The consent and assent form will be provided to the subject and family (if applicable) for review prior to the visit. The PI or designated Key Study Personnel will review the consent forms with the participant in detail and provide time for discussing any questions. The study team will provide a copy of the consent form to the participant. The participant and parents will sign the consent or assent either using an e-Consent document securely stored in Redcap or a paper copy, which the study team will store in a study folder kept in a locked drawer.

6.6 History and Physical Exam

The PI or designee will review each subject's clinical history, perform a physical exam, measure vital signs, and make anthropometric measurements.

6.7 Urine pregnancy test

Before each MMTT, female participants will provide a urine sample to exclude pregnancy to ensure safety and prevent potential harm to an unborn child.

6.8 Intravenous (IV) angiocatheter placement

Upon arrival, CRC staff will establish IV catheter access. This will facilitate serial blood sampling.

6.9 Basal measurements

A study team member will draw three baseline blood samples (at -60, -52.5, and -45 minutes in figure 3) as listed in Table 1.

Table 1: Blood samples drawn during study. Normal font indicates sample drawn serially. Italics indicates sample drawn at baseline only. CATS = catecholamines

Hormones	Metabolites	Cytokines	Other
Insulin Proinsulin C-peptide Glucagon Cortisol CATS *Estradiol*	Glucose NEFAs Glycerol Lactate Alanine β-OH-butyrate	TNF-α IL-1 and 6 VEGF sICAM sVCAM E-selectin Endothelin-1	*HLA-haplotype* Acetaminophen

6.10 Study drug administration

Forty-five minutes before receiving a liquid mixed meal, participants will receive either Rybelsus® or placebo as depicted in figure 3. For the pre-TZIELD® MMTT, participants will receive placebo. In the two post-TZIELD® studies participants will receive Rybelsus® or placebo in a random, double-blinded fashion. We chose this timing to align with the pharmacokinetics of Rybelsus®, maximizing its potential effect during the postprandial period.

The Vanderbilt Investigational Drug Service will store the study drugs and manage the blinding procedure.

Rationale for drug dose and timing: We pragmatically selected a drug dose and timing based on existing literature and approved uses in T2DM. Semaglutide is a long-acting GLP-1Ra with a half-life that allows for once-weekly dosing, when injected subcutaneously. However, in this study, we will use the oral form of semaglutide (Rybelsus®). This oral version requires daily administration and reaches peak plasma concentrations in approximately 1 hour(22). Therefore, administration 45 minutes before the MMTT should maximize its postprandial effect. As our participants will be naïve to this drug class, we aim to minimize adverse drug effects, especially nausea.

6.11 Mixed meal tolerance test (MMTT)

At time zero (figure 3) participants will consume a BOOST® High Protein shake (at 6 mL/kg up to 360 mL, consistent with other T1DM studies(18, 23-29)). We will label this shake with 500 mg of liquid acetaminophen.

The addition of acetaminophen serves as a marker for gastric emptying, allowing us to measure its levels later to assess the rate at which the stomach empties. We will collect plasma samples at -30, -15, 0, 10, 20, 30, 60, 90, 120, 150, 180, and 240 min for measurement of compounds listed in table 1.

6.12 Flow mediated dilation (FMD)

At 210 minutes, we will determine FMD. Originally developed in 1992, the FMD technique has become the most commonly utilized non-invasive method for assessing vascular endothelial dysfunction.(30) The study team will use the Phillips EPIQ 7C ultrasound machine with an L12-3 transducer. Ms. JoAnn Gottlieb, an ultrasonographer with extensive experience using this technique, will assist in these studies.

Baseline Measurements: The baseline radial artery diameter and velocity will be measured. The brachial artery will be scanned beginning at the insertion of the biceps and proceeding proximally. Color flow imaging will verify the brachial artery and to locate collateral vessels to serve as landmarks in sequent studies. Once a suitable position is found, brachial artery diameter will be measured over at least 10 cardiac cycles. Velocity will be averaged over at least a 10-20 second period.

Vascular occlusion: A pillow will support each participant's arm with the palm facing forward. We will position an appropriately sized blood pressure cuff 5 cm distal to the elbow. A rapid cuff inflator will occlude radial artery blood flow. The cuff will be inflated to at least 25-50 mm Hg above systolic blood pressure for five minutes to elicit a reactive hyperemic stimulus for endothelium mediated vasodilation.

Reactive Hyperemia (Post-cuff release) Measurements: Post-cuff measurements of arterial diameters and blood velocities will be initiated ten seconds prior to cuff release and continued for three minutes following cuff release.

FMD Analysis: The primary outcome of the FMD study is %FMD, defined as:

$$\%FMD = \frac{diameter_{hyperemia} - diameter_{baseline}}{diameter_{baseline}}$$

Endothelium independent vasodilation: After the participant rests for another 10 min, we will measure nitroglycerin-induced vasodilation as an index of endothelium-independent vasodilation. We will administer a 400 µg sublingual dose of nitroglycerin and assess the brachial artery response by imaging the artery continuously for 3 min. We will quantify

endothelium-independent vasodilation as the percent increase in brachial artery diameter 3 min after the nitroglycerin dose.

6.13 Study completion

Upon completion of the study, we will stop the insulin infusion and allow the study subject to eat, then the CRC staff will discharge the patient to home.

6.14 Rescheduling studies after a technical issue

It is possible once a clamp visit has begun a technical issue will prevent the successful completion of the study. For example, IV access may be lost during the study. In the event such a technical issue occurs, the PI may elect to reschedule a new, repeat study visit provided he deems the technical issue poses no serious danger to the participant were it to occur again and the participant wishes to repeat the study.

7.0 Risks

The safety and wellbeing of study participants is paramount. All potential risks will be communicated transparently during the consent/assent process and included in the relevant documentation.

7.1 Perceived low risks:

- Venipuncture and Intravenous Angiocatheter Placement: Risks include potential hematoma, site infection, nausea, and vasovagal syncope. Mitigation: All procedures will be performed with participants seated. Sites will be cleaned with appropriate antiseptic before any skin incision.
- Liquid Meal Consumption: Some participants may experience gastrointestinal symptoms like bloating or nausea post-consumption. Mitigation: An anti-emetic may be provided under the discretion of the PI.

7.2 Other considered risks:

- Nitroglycerin administration: Used in flow-mediated dilation studies, nitroglycerin might cause transient headaches, a dip in blood pressure, or symptoms of hypotension. Nitroglycerin will not be given to participants with a systolic blood pressure ≤100 mmHg. After administration, participants will remain supine for 10 minutes for the medication to clear.

Rybelsus® use: Rybelsus®, as with any medication, carries potential risks and side effects. Some of the most commonly reported side effects include nausea, vomiting, and diarrhea. Acute pancreatitis, though rare, is a serious side effect that has been reported with exenatide use (another medicine in the GLP-1Ra class). To minimize these risks, we will carefully screen all participants prior to enrollment in the study. Our exclusion criteria exclude individuals with a history of pancreatitis and gastroparesis within the past 3 years. We have chosen a conservative (7 mg) single dose of Rybelsus® for the study to minimize potential side effects. If appropriate in the judgement of the PI, the study team may provide an anti-emetic.

7.3 Discussion of Risks as Part of the Consent Process

We will transparently discuss risks, benefits, and all procedures with potential study participants during the consent process. We will always use an IRB-approved consent and assent document. Understanding that consent is an ongoing process and not a one-time event, participants retain the right to withdraw their consent at any stage. Unforeseen adverse effects might emerge, and we will continuously monitor and, if necessary, adjust the protocol for patient safety. Any changes in study procedures, risks, or benefits will be communicated to participants after IRB approval. A revised written consent will be obtained when necessary.

8.0 Reporting of Adverse Events or Unanticipated Problems involving Risk to Participants or Others

Adverse Events

An AE will be considered to be any untoward medical occurrence in a subject, not necessarily having a causal relationship with the study. The PI will continually monitor for any AEs.

AEs will be graded according to following scale:

0 = No adverse events

1 = Mild: no limitation of usual activities, did not require treatment

2 = Moderate: some limitation of usual activities, resolved with or without treatment,

3 = Severe: inability to carry out usual activities, requiring medical intervention

4 = Life-threatening or disabling

5 = Death

Serious Adverse Events

Defining Serious Adverse Events

Consistent with FDA guidelines, serious adverse events (SAEs) will be defined as any untoward medical occurrence that:

- requires inpatient hospitalization
- results in persistent or significant disability
- is suspected to cause a congenital anomaly or birth defect in a subject's unborn child
- is life-threatening
- results in death
- is considered to be an important medical event based on appropriate medical judgement (e.g., bronchospasms requiring

emergency department referral, seizures that might not result in hospitalization).

Assessing relationship between a SAE and relationship to study procedures

An SAE's relationship to the study procedures will be assessed and graded as either: not related, unlikely, possible, probable, or definite.

Assessing whether an AE is an anticipated problem

Any AE will be assessed for whether or not it was an anticipated problem. In accordance with Department of Health and Human Services guidance and consistent with 45 CFR part 46, an "unanticipated problem" will include any incident, experience, or outcome that meets all the following criteria:

1. unexpected (in terms of nature, severity, or frequency) given
    a. the research procedures that are described in the protocol-related documents, including the IRB-approved research protocol and informed consent document; and
    b. the characteristics of the subject population being studied; related or possibly related to participation in the research; and
2. suggests that the research places subjects or others at a greater risk of harm.
3. related or possibly related to participation in the research;
4. suggests that the research places subjects or others at a greater risk of harm than was previously known or recognized.

Unanticipated, non-serious AEs

All unanticipated, non-serious AEs and the study team’s response to the non-serious AE will be included in a report at the time of annual continuing review. The PI will review the AEs and notify the Data Safety Monitor (DSM) and the IRB of any changes needed to the protocol. If needed, appropriate changes will be made to the consent form.

Unanticipated SAEs

In accordance with IRB policy, any unanticipated SAE that is considered possibly related to participation in the study will be reported within 7 calendar days of the PI's notification of the event to the IRB and DSM. The study team will continue to follow or obtain documentation of the resolution of any SAE.

Adverse Event Reporting

The annual summary of all unanticipated adverse events and any audit reports will be sent to the IRB at the time of continuing review. A copy of this report will also be sent to the NIH who will fund the PI on a K23 grant with the Research Performance Progress Report.

Data and safety monitoring activities for this study will continue until all subjects have completed their participation and until a sufficient amount of time has passed beyond which any study-related AEs are unlikely.

This protocol will be reviewed annually (at a minimum) by the Vanderbilt IRB. The goal of this process is to determine the risks and benefits of the study in the actual experience of subjects and that measures taken to minimize risks are adequate.

9.0 Study Withdrawal/Discontinuation

Subjects will be free to withdraw from the study at any time, which will be made clear at enrollment. Subjects will be withdrawn from the study if:

- Pregnancy is detected
- The PI's (or designated MD, NP, or PA KSP) medical judgement is that participation places the subject at risk for harm

10.0 Statistical Considerations

A goal of this pilot study is to generate preliminary data to support a future, fully powered future study. This future study will determine how the insulinotropic and glucagonostatic components of GLP-1Ra therapy will improve metabolic outcomes in patients with stage 2 T1DM treated with TZIELD®. We will employ a crossover study design to quantify the metabolic effect of the GLP-1Ra, Rybelsus®, versus placebo delivered 45 minutes before a mixed-meal tolerance test (MMTT). We will analyze this metabolic effect for each aims primary outcome: 1) postprandial glycemia, 2) disposition index, and 3) endothelial function.

We plan to enroll twenty-four participants with stage 2 T1DM. Our plan allows for as much as a 20% drop-out rate and a final analysis of twenty participants. To address potential missing data due to dropouts, we will employ multiple imputation techniques. In determining each group's sample size, we intend to obtain a statistical point estimate of the effect size of the two differing conditions (Rybelsus® versus placebo) on each aim's primary outcome.

We will also carry out bivariate linear regression analyses to estimate the individual contributions of the insulinotropic and glucagonostatic components of GLP-1Ra therapy on metabolic outcomes. This will be treated as a secondary analysis and hence, it will not directly influence our primary power analysis. However, the results from this analysis will provide valuable insights for planning the fully powered future study.

Primary outcome for Aim 1: Difference in postprandial hyperglycemia between GLP-1Ra and placebo studies, as measured by the incremental increase above basal in the plasma glucose concentration from 0 to 120 minutes, divided by the 120-minute time interval (i.e., $iAUC_{glucose}$/120 min = $AUC_{glucose0\rightarrow120}$/120 min – $AUC_{glucose\ -30\rightarrow\ -15}$/15 min).

- Expected effect size (Δ): We expect a mean within-participant difference of approximately 12% or a that GLP-1Ra will effect a reduction in glucose AUC/120 mg/dL from 165 to 145 mg/dL, based on other studies of postprandial glucose in early-stage T1DM(4, 20, 31).
- Expected variance (σ): DeFronzo et al. found the SD of the within-participant difference in the 2-hour postprandial glucose between exenatide (another GLP-1Ra), and placebo was 6 mg/dL*(32)*. Because our impression is that this variance for an

OGTT seems small, we have set an expectation that our data will show a σ of twice this amount, or 12 mg/dL.

- Minimum detectable difference (MDD): We have considered the minimum detectable difference for this pilot study as part of overall planning for larger, fully powdered future studies of GLP-1Ra in early stage T1DM. Based on n = 20, σ = 6 mg/d, α = 0.05, and 1- β = 0.80, the minimum detectable difference is -8 to 8 mg/dL.
- Minimum clinically important difference (MCID): Based on our clinical practice and studies of the renal glycosuric threshold,(33, 34) we consider a 15 mg/dL difference the MCID because this reduction in glucose is sufficiently large to forestall the initiation of intensive insulin therapy in early-stage T1DM. Because the MDD and the MCID are similar in an absolute sense, we plan to use the observed Δ and σ from this study to inform planning the sample size of the fully powered follow up study testing the same outcome.

Primary outcome for Aim 2: Difference in disposition index (DI) between GLP-1Ra and placebo studies, as calculated by a set of differential equations encompassed by the oral glucose minimal model is that describe the glucose-insulin dynamics during an MMTT*(35)*.

- Expected effect size (Δ): We expect GLP-1Ra will increase DI 21% based on studies of DI in stage 1 T1DM and the insulin-sensitizing and insulinotropic effect of GLP-1Ra in healthy volunteers and individuals with T2DM(36, 37). This amount would correspond to a 20.6 x 10^{-12} dL/kg/min^2 per pmol/L increase in DI.
- Expected variance (σ): Literature reporting the within-participant variance in DI across an intervention in early-stage T1DM is very limited, which is a factor prompting the present pilot study. We estimated the within-group variance in DI in a study of stage 1 T1DM as SD ≈ 38.7 × 10^{-12} dL/kg/min^2 per pmol/L*(37)*. Assuming the standard deviation of DI would be similar for both exenatide and placebo and that the correlation coefficient, ρ, between the two treatment*s for* DI was 0.5, we can estimate the expected standard deviation of the within-participant difference using the formula $\sqrt{(SD_{GLP\text{-}1Ra}^2 + SD_{placebo}^2 - 2\rho \cdot SD_{GLP\text{-}1Ra} \cdot SD_{placebo})} \approx 27.4 \times 10^{-12}$ dL/kg/min^2 per pmol/L.
- Minimum detectable difference (MDD): Based on n = 20, σ = 27.4 × 10^{-12} dL/kg/min^2 per pmol/L, α = 0.05, and 1- β = 0.80, the minimum detectable difference is -18 to 18 dL/kg/min^2 per pmol/L.
- Minimum clinically important difference (MCID): Because DI highly influences glycemia in patients with stage 2 T1DM, we consider a 10% increase in DI this study's MCID. Based on Galderisi et al., this amount would correspond to 9.8 x 10^{-12} dL/kg/min^2 per pmol/L increase*(37)*. Although the MDD is less than the MCID, pre-existing data informing our estimate of expected variance is scant, underscoring the importance of measuring this parameter in the pilot framework of this proposal.

Primary outcome for Aim 3: Difference in brachial artery flow mediated dilation (%FMD) between GLP-1Ra and placebo studies, as measured by B-mode ultrasound calculated by the equation FMD (%) = [(Post-deflation diameter - Baseline diameter) / Baseline diameter] × 100.

- Expected effect size (Δ): We expect GLP-1Ra will increase FMD by 2% based on a study of young adults with T1DM who received a GLP-1 infusion during hyperglycemia(16).

- Expected variance (σ): In our unpublished, crossover trial of two nutritional interventions in T1DM, the standard deviation of the difference in %FMD is 1.7%. We expect a similar variance in this study.
- Minimum detectable difference (MDD): We have considered the minimum detectable difference for this pilot study as part of overall planning for larger, fully powdered future studies of GLP-1Ra in stage 2 T1DM. Based on n = 20, σ = 1.7%, α = 0.05, and 1- β = 0.8, the minimum detectable difference is -1.1 to 1.1%.
- Minimum clinically important difference (MCID): A large meta-analysis of patients with cardiovascular risk factors suggests that a 1% increase in FMD corresponds to a 10% decrease in cardiovascular disease risk. Accordingly, we consider the MCID to be 1% in this study(38). Because the MDD and the MCID are similar, we plan to use the observed Δ and σ from this study to inform planning the sample size of the fully powered follow up study testing the same outcome.

11.0 Privacy/Confidentiality Issues

A database will be designed for this study using REDCap (Research Electronic Data Capture) tools. REDCap is a secure, web-based application designed to support data capture for research studies, providing validated data entry, audit trails, seamless data downloads to common statistical packages, and mechanisms for importing data from external sources. It will reside on a secure server with access provided exclusively to the research personnel. Subjects will be identified with a study identification number. A key to the subject identification number will be kept in a separate locked file drawer to which only the Principal Investigator and research coordinators have access. Reports will thereby be generated without Protected Health Information (PHI) data, and access will be restricted so that statisticians, etc. do not have access to all data.

Risk of leakage of PHI is minimized by keeping paper records in a locked cabinet and maintaining computerized records in the password protected REDCap data base. The principal investigator and the research staff are trained in HIPAA privacy regulations. The participant's identification is concealed, and a number is used as the identifier instead of the subject's name. Only the principal investigator or members of the research team will have the list of study patient's names as the correlate with the study number.

12.0 Follow-up and Record Retention

The study is anticipated to last for up to four years. The study results will be maintained indefinitely for research purposes.

Works Cited

1. Eisenbarth GS. Type I diabetes mellitus. A chronic autoimmune disease. The New England journal of medicine. 1986;314(21):1360-8. Epub 1986/05/22. doi: 10.1056/nejm198605223142106. PubMed PMID: 3517648.
2. Herold KC, Bundy BN, Long SA, Bluestone JA, DiMeglio LA, Dufort MJ, Gitelman SE, Gottlieb PA, Krischer JP, Linsley PS, Marks JB, Moore W, Moran A, Rodriguez H, Russell WE, Schatz D, Skyler JS, Tsalikian E, Wherrett DK, Ziegler AG, Greenbaum CJ. An Anti-CD3 Antibody, Teplizumab, in Relatives at Risk for Type 1 Diabetes. The New England journal of medicine. 2019;381(7):603-13. Epub 2019/06/11. doi: 10.1056/NEJMoa1902226. PubMed PMID: 31180194; PMCID: PMC6776880.
3. Perdigoto AL, Preston-Hurlburt P, Clark P, Long SA, Linsley PS, Harris KM, Gitelman SE, Greenbaum CJ, Gottlieb PA, Hagopian W, Woodwyk A, Dziura J, Herold KC. Treatment of type 1 diabetes with teplizumab: clinical and immunological follow-up after 7 years from diagnosis. Diabetologia. 2019;62(4):655-64. Epub 2018/12/21. doi: 10.1007/s00125-018-4786-9. PubMed PMID: 30569273; PMCID: PMC6402971.
4. Sims EK, Bundy BN, Stier K, Serti E, Lim N, Long SA, Geyer SM, Moran A, Greenbaum CJ, Evans-Molina C, Herold KC. Teplizumab improves and stabilizes beta cell function in antibody-positive high-risk individuals. Sci Transl Med. 2021;13(583). Epub 2021/03/05. doi: 10.1126/scitranslmed.abc8980. PubMed PMID: 33658358; PMCID: PMC8610022.
5. Sims EK, Cuthbertson D, Herold KC, Sosenko JM. The Deterrence of Rapid Metabolic Decline Within 3 Months After Teplizumab Treatment in Individuals at High Risk for Type 1 Diabetes. Diabetes. 2021;70(12):2922-31. Epub 2021/09/24. doi: 10.2337/db21-0519. PubMed PMID: 34551936; PMCID: PMC8660991.
6. Sherry N, Hagopian W, Ludvigsson J, Jain SM, Wahlen J, Ferry RJ, Jr., Bode B, Aronoff S, Holland C, Carlin D, King KL, Wilder RL, Pillemer S, Bonvini E, Johnson S, Stein KE, Koenig S, Herold KC, Daifotis AG. Teplizumab for treatment of type 1 diabetes (Protégé study): 1-year results from a randomised, placebo-controlled trial. Lancet. 2011;378(9790):487-97. Epub 2011/07/02. doi: 10.1016/s0140-6736(11)60931-8. PubMed PMID: 21719095; PMCID: PMC3191495.
7. Eisenbarth G, Wilson P, Ward F, Lebovitz HE. HLA type and occurrence of disease in familial polyglandular failure. The New England journal of medicine. 1978;298(2):92-4. Epub 1978/01/12. doi: 10.1056/nejm197801122980209. PubMed PMID: 619238.
8. Greenbaum CJ, Prigeon RL, D'Alessio DA. Impaired beta-cell function, incretin effect, and glucagon suppression in patients with type 1 diabetes who have normal fasting glucose. Diabetes. 2002;51(4):951-7. Epub 2002/03/28. doi: 10.2337/diabetes.51.4.951. PubMed PMID: 11916912.
9. Brown RJ, Sinaii N, Rother KI. Too much glucagon, too little insulin: time course of pancreatic islet dysfunction in new-onset type 1 diabetes. Diabetes Care. 2008;31(7):1403-4. Epub 2008/07/03. doi: 10.2337/dc08-0575. PubMed PMID: 18594062; PMCID: PMC2453684.
10. Sherr J, Tsalikian E, Fox L, Buckingham B, Weinzimer S, Tamborlane WV, White NH, Arbelaez AM, Kollman C, Ruedy KJ, Cheng P, Beck RW. Evolution of abnormal plasma glucagon responses to mixed-meal feedings in youth with type 1 diabetes during the first 2 years after diagnosis. Diabetes Care. 2014;37(6):1741-4. Epub 2014/04/04. doi: 10.2337/dc13-2612. PubMed PMID: 24696460; PMCID: PMC4030093.

11. Drucker DJ, Nauck MA. The incretin system: glucagon-like peptide-1 receptor agonists and dipeptidyl peptidase-4 inhibitors in type 2 diabetes. Lancet. 2006;368(9548):1696-705. Epub 2006/11/14. doi: 10.1016/s0140-6736(06)69705-5. PubMed PMID: 17098089.
12. Holst JJ. The physiology of glucagon-like peptide 1. Physiol Rev. 2007;87(4):1409-39. Epub 2007/10/12. doi: 10.1152/physrev.00034.2006. PubMed PMID: 17928588.
13. Nauck MA, Meier JJ. Incretin hormones: Their role in health and disease. Diabetes, obesity & metabolism. 2018;20 Suppl 1:5-21. Epub 2018/01/25. doi: 10.1111/dom.13129. PubMed PMID: 29364588.
14. Lovshin J, Cherney D. GLP-1R Agonists and Endothelial Dysfunction: More Than Just Glucose Lowering? Diabetes. 2015;64(7):2319-21. Epub 2015/06/25. doi: 10.2337/db15-0366. PubMed PMID: 26106189.
15. Drucker DJ. The role of gut hormones in glucose homeostasis. The Journal of clinical investigation. 2007;117(1):24-32. Epub 2007/01/04. doi: 10.1172/jci30076. PubMed PMID: 17200703; PMCID: PMC1716213.
16. Ceriello A, Novials A, Ortega E, Canivell S, La Sala L, Pujadas G, Esposito K, Giugliano D, Genovese S. Glucagon-like peptide 1 reduces endothelial dysfunction, inflammation, and oxidative stress induced by both hyperglycemia and hypoglycemia in type 1 diabetes. Diabetes Care. 2013;36(8):2346-50. Epub 2013/04/09. doi: 10.2337/dc12-2469. PubMed PMID: 23564922; PMCID: PMC3714509.
17. Dupré J, Behme MT, McDonald TJ. Exendin-4 normalized postcibal glycemic excursions in type 1 diabetes. The Journal of clinical endocrinology and metabolism. 2004;89(7):3469-73. Epub 2004/07/09. doi: 10.1210/jc.2003-032001. PubMed PMID: 15240633.
18. Ghazi T, Rink L, Sherr JL, Herold KC. Acute metabolic effects of exenatide in patients with type 1 diabetes with and without residual insulin to oral and intravenous glucose challenges. Diabetes Care. 2014;37(1):210-6. Epub 2013/08/14. doi: 10.2337/dc13-1169. PubMed PMID: 23939544; PMCID: PMC3968447.
19. Raman VS, Mason KJ, Rodriguez LM, Hassan K, Yu X, Bomgaars L, Heptulla RA. The role of adjunctive exenatide therapy in pediatric type 1 diabetes. Diabetes Care. 2010;33(6):1294-6. Epub 2010/03/25. doi: 10.2337/dc09-1959. PubMed PMID: 20332358; PMCID: PMC2875441.
20. von Herrath M, Bain SC, Bode B, Clausen JO, Coppieters K, Gaysina L, Gumprecht J, Hansen TK, Mathieu C, Morales C, Mosenzon O, Segel S, Tsoukas G, Pieber TR. Anti-interleukin-21 antibody and liraglutide for the preservation of β-cell function in adults with recent-onset type 1 diabetes: a randomised, double-blind, placebo-controlled, phase 2 trial. Lancet Diabetes Endocrinol. 2021;9(4):212-24. Epub 2021/03/05. doi: 10.1016/s2213-8587(21)00019-x. PubMed PMID: 33662334.
21. Hashimoto M, Akishita M, Eto M, Ishikawa M, Kozaki K, Toba K, Sagara Y, Taketani Y, Orimo H, Ouchi Y. Modulation of Endothelium-Dependent Flow-Mediated Dilatation of the Brachial Artery by Sex and Menstrual Cycle. Circulation. 1995;92(12):3431-5. doi: doi:10.1161/01.CIR.92.12.3431.
22. Baekdal TA, Donsmark M, Hartoft-Nielsen ML, Søndergaard FL, Connor A. Relationship Between Oral Semaglutide Tablet Erosion and Pharmacokinetics: A Pharmacoscintigraphic Study. Clin Pharmacol Drug Dev. 2021;10(5):453-62. Epub 2021/03/23. doi: 10.1002/cpdd.938. PubMed PMID: 33750044; PMCID: PMC8251533.

23. Fredheim S, Andersen ML, Pörksen S, Nielsen LB, Pipper C, Hansen L, Holst JJ, Thomsen J, Johannesen J, Mortensen HB, Svensson J. The influence of glucagon on postprandial hyperglycaemia in children 5 years after onset of type 1 diabetes. Diabetologia. 2015;58(4):828-34. Epub 2014/12/30. doi: 10.1007/s00125-014-3486-3. PubMed PMID: 25541633.
24. Rickels MR, Evans-Molina C, Bahnson HT, Ylescupidez A, Nadeau KJ, Hao W, Clements MA, Sherr JL, Pratley RE, Hannon TS, Shah VN, Miller KM, Greenbaum CJ. High residual C-peptide likely contributes to glycemic control in type 1 diabetes. The Journal of clinical investigation. 2020;130(4):1850-62. Epub 2020/01/03. doi: 10.1172/jci134057. PubMed PMID: 31895699; PMCID: PMC7108933.
25. Bellin MD, Beilman GJ, Dunn TB, Pruett TL, Sutherland DE, Chinnakotla S, Hodges JS, Lane A, Ptacek P, Berry KL, Hering BJ, Moran A. Sitagliptin Treatment After Total Pancreatectomy With Islet Autotransplantation: A Randomized, Placebo-Controlled Study. Am J Transplant. 2017;17(2):443-50. Epub 2016/07/28. doi: 10.1111/ajt.13979. PubMed PMID: 27459721; PMCID: PMC5266635.
26. Andersen ML, Rasmussen MA, Pörksen S, Svensson J, Vikre-Jørgensen J, Thomsen J, Hertel NT, Johannesen J, Pociot F, Petersen JS, Hansen L, Mortensen HB, Nielsen LB. Complex multi-block analysis identifies new immunologic and genetic disease progression patterns associated with the residual β-cell function 1 year after diagnosis of type 1 diabetes. PLoS One. 2013;8(6):e64632. Epub 2013/06/12. doi: 10.1371/journal.pone.0064632. PubMed PMID: 23755131; PMCID: PMC3674006
Petersen is employed by Novo Nordisk A/S. Novo Nordisk A/S partially funded this study. This does not alter the authors' adherence to all the PLOS ONE policies on sharing data and materials.
27. Ruan Y, Willemsen RH, Wilinska ME, Tauschmann M, Dunger DB, Hovorka R. Mixed-meal tolerance test to assess residual beta-cell secretion: Beyond the area-under-curve of plasma C-peptide concentration. Pediatr Diabetes. 2019;20(3):282-5. Epub 2019/01/18. doi: 10.1111/pedi.12816. PubMed PMID: 30652426; PMCID: PMC6487945.
28. Haller MJ, Gitelman SE, Gottlieb PA, Michels AW, Rosenthal SM, Shuster JJ, Zou B, Brusko TM, Hulme MA, Wasserfall CH, Mathews CE, Atkinson MA, Schatz DA. Anti-thymocyte globulin/G-CSF treatment preserves β cell function in patients with established type 1 diabetes. The Journal of clinical investigation. 2015;125(1):448-55. Epub 2014/12/17. doi: 10.1172/jci78492. PubMed PMID: 25500887; PMCID: PMC4382237.
29. Moran A, Bundy B, Becker DJ, DiMeglio LA, Gitelman SE, Goland R, Greenbaum CJ, Herold KC, Marks JB, Raskin P, Sanda S, Schatz D, Wherrett DK, Wilson DM, Krischer JP, Skyler JS, Pickersgill L, de Koning E, Ziegler AG, Böehm B, Badenhoop K, Schloot N, Bak JF, Pozzilli P, Mauricio D, Donath MY, Castaño L, Wägner A, Lervang HH, Perrild H, Mandrup-Poulsen T. Interleukin-1 antagonism in type 1 diabetes of recent onset: two multicentre, randomised, double-blind, placebo-controlled trials. Lancet. 2013;381(9881):1905-15. Epub 2013/04/09. doi: 10.1016/s0140-6736(13)60023-9. PubMed PMID: 23562090; PMCID: PMC3827771.
30. Harris RA, Nishiyama SK, Wray DW, Richardson RS. ULTRASOUND ASSESSMENT OF FLOW-MEDIATED DILATION: A TUTORIAL. Hypertension. 2010;55(5):1075-85. doi: 10.1161/HYPERTENSIONAHA.110.150821. PubMed PMID: PMC2878744.
31. Sosenko JM, Skyler JS, Herold KC, Palmer JP. The metabolic progression to type 1 diabetes as indicated by serial oral glucose tolerance testing in the Diabetes

Prevention Trial-type 1. Diabetes. 2012;61(6):1331-7. Epub 2012/05/24. doi: 10.2337/db11-1660. PubMed PMID: 22618768; PMCID: PMC3357303.
32. DeFronzo RA, Okerson T, Viswanathan P, Guan X, Holcombe JH, MacConell L. Effects of exenatide versus sitagliptin on postprandial glucose, insulin and glucagon secretion, gastric emptying, and caloric intake: a randomized, cross-over study. Curr Med Res Opin. 2008;24(10):2943-52. Epub 2008/09/13. doi: 10.1185/03007990802418851. PubMed PMID: 18786299.
33. ElSayed NA, Aleppo G, Aroda VR, Bannuru RR, Brown FM, Bruemmer D, Collins BS, Hilliard ME, Isaacs D, Johnson EL, Kahan S, Khunti K, Leon J, Lyons SK, Perry ML, Prahalad P, Pratley RE, Seley JJ, Stanton RC, Gabbay RA, Association obotAD. 6. Glycemic Targets: Standards of Care in Diabetes—2023. Diabetes Care. 2022;46(Supplement_1):S97-S110. doi: 10.2337/dc23-S006.
34. DeFronzo RA, Hompesch M, Kasichayanula S, Liu X, Hong Y, Pfister M, Morrow LA, Leslie BR, Boulton DW, Ching A, LaCreta FP, Griffen SC. Characterization of renal glucose reabsorption in response to dapagliflozin in healthy subjects and subjects with type 2 diabetes. Diabetes Care. 2013;36(10):3169-76. Epub 2013/06/06. doi: 10.2337/dc13-0387. PubMed PMID: 23735727; PMCID: PMC3781504.
35. Cobelli C, Dalla Man C, Toffolo G, Basu R, Vella A, Rizza R. The oral minimal model method. Diabetes. 2014;63(4):1203-13. Epub 2014/03/22. doi: 10.2337/db13-1198. PubMed PMID: 24651807; PMCID: PMC4179313.
36. Mager DE, Abernethy DR, Egan JM, Elahi D. Exendin-4 pharmacodynamics: insights from the hyperglycemic clamp technique. J Pharmacol Exp Ther. 2004;311(2):830-5. Epub 2004/06/17. doi: 10.1124/jpet.104.069765. PubMed PMID: 15199095; PMCID: PMC9973385.
37. Galderisi A, Moran A, Evans-Molina C, Martino M, Santoro N, Caprio S, Cobelli C. Early Impairment of Insulin Sensitivity, β-Cell Responsiveness, and Insulin Clearance in Youth with Stage 1 Type 1 Diabetes. The Journal of clinical endocrinology and metabolism. 2021;106(9):2660-9. Epub 2021/05/18. doi: 10.1210/clinem/dgab344. PubMed PMID: 34000022; PMCID: PMC8372628.
38. Ras RT, Streppel MT, Draijer R, Zock PL. Flow-mediated dilation and cardiovascular risk prediction: a systematic review with meta-analysis. Int J Cardiol. 2013;168(1):344-51. Epub 2012/10/09. doi: 10.1016/j.ijcard.2012.09.047. PubMed PMID: 23041097.