

Study Title:

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

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

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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.

Aims 1-3: Stage 2 T1DM and teplizumab

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.

Aim 4: Early Stage 3 T1DM and GLP-1 Receptor Agonists

Despite the promise of immunotherapy, there remains a critical need for additional interventions, particularly for patients who progress into early stage 3 T1DM. It's important to recognize that the distinction between stage 2 and stage 3 T1DM is somewhat arbitrary, as the progression from one stage to the next is continuous rather than discrete. Late stage 2 and early stage 3 patients often exhibit similar clinical characteristics, such as mildly elevated HbA1c levels, reflecting a gradual decline in β -cell function. This continuum underscores that there's not a substantial difference

between patients at these stages, particularly those we're recruiting with mildly elevated HbA1c.

In this context, our study will include patients with early stage 3 T1DM to assess the effectiveness of GLP-1Ra therapy. This class of medication is uniquely suited to this population, as it addresses not only insulin secretion deficits but also metabolic abnormalities like postprandial hyperglucagonemia—two factors that exacerbate postprandial hyperglycemia.

A recent open-label, uncontrolled clinical study suggested that semaglutide provides benefits in improving glycemic outcomes in early stage 3 T1DM¹. Building upon these findings, our study will rigorously investigate the cardiometabolic effects of semaglutide in early stage 3 T1DM. Furthermore, the insights gained will lay the groundwork for future combination trials that incorporate immunomodulation (teplizumab) alongside metabolic therapy (GLP-1Ra) in early stage 3 T1DM, potentially offering a comprehensive approach to preserving β -cell function.

The transition from stage 2 to stage 3 represents a critical window where metabolic and immunological interventions might synergistically delay the complete β -cell destruction characteristic of established T1DM. Given the minimal differences between late stage 2 and early stage 3 patients, studying early stage 3 T1DM allows us to evaluate whether oral GLP-1Ra alone can stabilize or improve β -cell function at this crucial phase. By acknowledging the continuum of disease progression, our approach provides an additional therapeutic tool for managing T1DM progression and may improve outcomes for patients at this pivotal stage.

Unraveling the potential metabolic benefits of GLP-1Ra in these patient populations 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 study is designed to investigate the innovative combination of GLP-1Ra and immunotherapy in stage 2 T1DM patients, while also expanding to examine the effects of GLP-1Ra in early stage 3 T1DM. This approach aims to set the foundation for larger, definitive trials that could determine the impact of this novel therapeutic approach on dysmetabolism in early T1DM.

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. For early stage 3 T1DM, we aim to determine whether GLP-1Ra can offer similar protective effects in preserving β -cell function and stabilizing glycemic control.

The immediate objective is to investigate the impact of GLP-1Ra's insulinotropic and glucagonostatic effects on dysmetabolism in stage 2 and early stage 3 T1DM. 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.

Aims 1-3: Stage 2 T1DM and TZIELD® with GLP-1Ra

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.

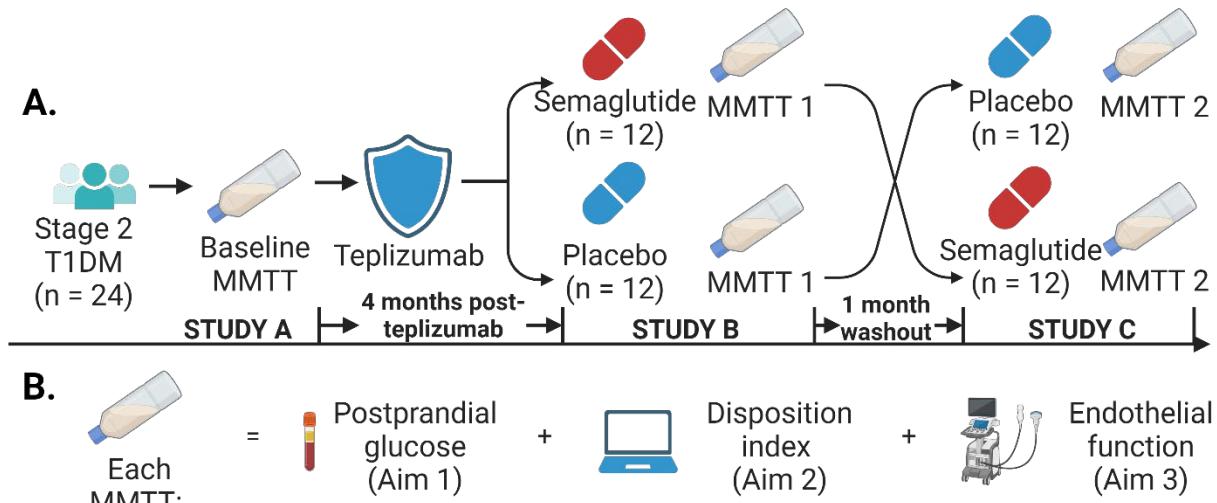


Figure 1: A) Crossover study design B) Key study objectives for each MMTT.

Aim 4: Determine how much GLP-1Ra monotherapy therapy changes the disposition index (DI) in a pilot study of early stage 3 T1DM.

Despite the promise of TZIELD®, the medication is not currently approved for patients with stage 3 T1DM. Thus, individuals who progress into stage 3 T1DM continue to face significant challenges, including loss of β -cell function and the need for exogenous insulin. As patients move into stage 3, there is an opportunity for metabolic interventions that may preserve residual insulin production and mitigate the long-term impact of hyperglycemia. Accordingly, we will conduct a randomized, double-blind, placebo-controlled crossover study involving patients with early stage 3 T1DM. Each participant will undergo four mixed meal tolerance tests (MMTTs): two at baseline shortly after diagnosis and two after a six-month interval. During each MMTT, participants will receive either a single dose of GLP-1Ra (oral semaglutide) or placebo in a randomized order. This design allows us to assess the independent effects of GLP-1Ra on the disposition index (DI) by comparing the results between the GLP-1Ra and placebo conditions at both time points. By evaluating changes in DI and other metabolic parameters over time, we aim to determine how GLP-1Ra monotherapy influences β -cell function and insulin sensitivity in early stage 3 T1DM.

To enhance our understanding of GLP-1Ra's mechanistic effects in both stage 2 and early stage 3 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.

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

insulin². 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 2³⁻⁶ and early stage 3 T1DM⁷, spurring interest in additional therapeutic strategies to target the ***Dual Derangements*** of ***diminishing insulin secretion***^{2, 8} and ***detrimental hyperglucagonemia***⁹⁻¹¹ 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 emptying¹²⁻¹⁴. Studies in T2DM^{15, 16} and longstanding T1DM¹⁷ 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 control¹⁸⁻²⁰. 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 T1DM²¹. 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

Aims 1-3: Stage 2 T1DM with TZIELD® and GLP-1Ra

Inclusion Criteria

- Age: 12-50 years
- BMI: 18-31 kg/m² (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/1.73m²
 - 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

Aim 4: Early Stage 3 T1DM with GLP-1Ra Monotherapy

Inclusion Criteria

- Age: 12-50 years
- BMI: 18-31 kg/m² (adults) or 5-95th %ile (pediatric)
- Early stage 3 T1DM with either
 - HbA1c 6.5% to 8.0% at diagnosis OR
 - HbA1c 5.7% to 6.4% with oral glucose test meeting ADA criteria for stage 3 T1DM within the past three months prior to or during screening visit
- Time of stage 3 diagnosis: within eight weeks of first study visit

Exclusion Criteria

- DKA history: history of diabetic ketoacidosis requiring hospital admission
- Comorbidities:
 - 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

Aims 1-3: Stage 2 T1DM with TZIELD® and GLP-1Ra

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.

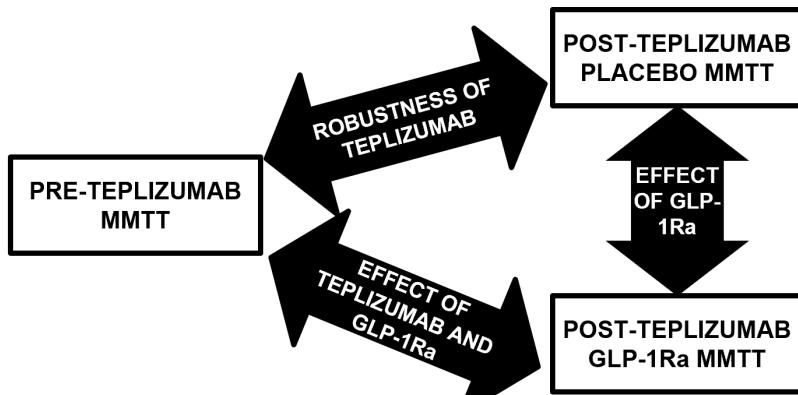


Figure 2: Volunteers will participate in three studies, a baseline study pre-teplizumab, then in two studies 2-7 months post-teplizumab in random order.

Randomization: The first study visit will occur before participants receive TZIELD®. We will schedule the first of two post-TZIELD® studies between two to seven 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 2nd 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).

Aim 4: Early Stage 3 T1DM with GLP-1Ra Monotherapy

We will employ a randomized, double-blind, placebo-controlled crossover design. Participants with early stage 3 T1DM will undergo four Mixed Meal Tolerance Test (MMTT) studies: two at baseline shortly after diagnosis and two after a six-month interval (figure 3). During each pair of studies, participants will receive a single dose of GLP-1Ra (oral semaglutide, Rybelsus®) in one study and placebo in the other, with the order randomized to minimize potential sequencing effects. This approach allows us to assess the effects of GLP-1Ra on metabolic parameters and β-cell function at two critical time points in disease progression.

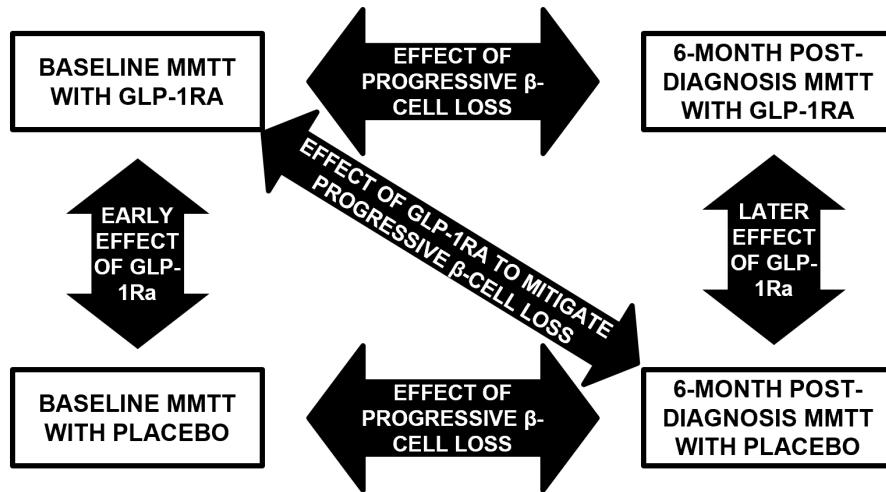


Figure 3: Volunteers will participate in four studies. Two studies will occur soon after the initial diagnosis of stage T1DM. Two studies will occur six months after the initial diagnosis.

Randomization: The study team will randomize participants to receive either GLP-1Ra (oral semaglutide, Rybelsus®) or placebo at the first visit.

Enrollment: enrollment will occur at the initial at the first visit. Eligibility will be confirmed based on clinical HbA1c or OGTT results obtained prior to the first visit, as well as a brief medical history and physical examination conducted at the start of the visit.

Enrollment will take place in the Vanderbilt Clinical Research Center (CRC). The Vanderbilt CRC is located on the 2nd 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

Aims 1-3: Stage 2 T1DM with TZIELD® and GLP-1Ra

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 two to seven 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 seven 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 prior to each study.. Our team will instruct participants to consume a diet consisting of at least 150 grams of carbohydrates 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 sensitivity²².

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

6.4 Protocol overview

Figure 3 illustrates the experimental protocol for each study.

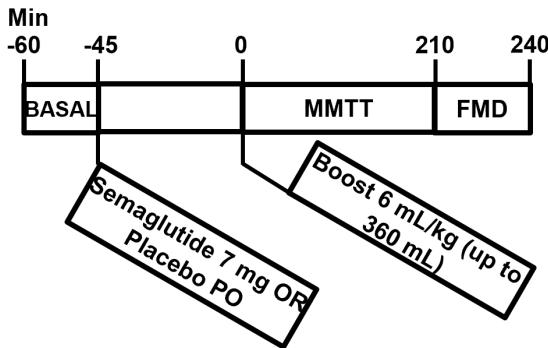


Figure 3: Schematic for MMTT visits. We will study participants with Stage 2 T1DM just prior to teplizumab therapy and two times during the seven months following teplizumab therapy. In the two post-teplizumab studies, participants will receive a GLP-1Ra (semaglutide) or placebo in random order prior 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 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 Eskin 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	Glucose	TNF- α	<i>HLA-haplotype</i>
Proinsulin	NEFAs	IL-1 and 6	Acetaminophen
C-peptide	Glycerol	VEGF	
Glucagon	Lactate	sICAM	
Cortisol	Alanine	sVCAM	
CATS	β -OH-butyrate	E-selectin	
<i>Estradiol</i>		Endothelin-1	

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 hour²³. 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^{19, 24-30}). 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.³¹ 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 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.

Aim 4: Early Stage 3 T1DM with GLP-1Ra Monotherapy

6.15 **Overview**

In this section, we outline the study procedures for Aim 4, which focuses on evaluating the metabolic and beta-cell function effects of GLP-1 receptor agonist (GLP-1Ra) therapy in participants with early stage 3 Type 1 Diabetes Mellitus (T1DM). This study will utilize a randomized, double-blind, placebo-controlled crossover design, where participants will undergo four Mixed Meal Tolerance Test (MMTT) visits. Participants will receive oral semaglutide (GLP-1Ra) during two visits and placebo during the other two visits, with the order randomized and counterbalanced.

All participants will continue their background insulin therapy as prescribed by their primary endocrinologist throughout the study. The trial is designed to assess the acute effects of GLP-1Ra on metabolic outcomes at two critical time points: shortly after diagnosis and after a six-month interval.

The primary goal of Aim 4 is to assess the potential for GLP-1Ra to improve the disposition index (DI), reflecting the preservation of beta-cell function and enhancement of insulin sensitivity in early stage 3 T1DM. By comparing the effects of GLP-1Ra and placebo within the same participants at two time points, we aim to determine how GLP-1Ra monotherapy influences metabolic outcomes independent of other therapies.

6.16 **Timeline and Visits**

Participants will undergo four study visits for data collection:

- Visit 1 and Visit 2 (Baseline Visits): Shortly after enrollment, participants will undergo two MMTT study visits, each separated by at least three days to allow for washout of the study drug. In one visit, participants will receive oral semaglutide (GLP-1Ra), and in the other, they will receive placebo. The order of GLP-1Ra and placebo administration will be randomized and double-blinded.

- Visit 3 and Visit 4 (Follow-up Visits): Approximately six months after the baseline visits, participants will undergo two additional MMTT study visits, again separated by at least one week. They will receive GLP-1Ra and placebo in randomized order.

6.17 Consent, parental consent, and adolescent assent

The PI or designated KSP will obtain consent from all participants in the clinic or at the beginning of the first study 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 § 46.406406. Consent will be obtained in a private room in the CRC, in the Eskin 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.

6.18 Study Preparation

6.18.1 Standardization to minimize confounding of key outcomes

To promote consistency across participants and limit potential external influences, we have established several guidelines. We ask subjects to refrain from vigorous exercise and adhere to a standardized diet prior to each study visit to limit potential confounding factors that could impact metabolic responses to GLP-1Ra. Participants will be instructed to consume a consistent diet, maintaining their usual caloric intake and macronutrient composition for three days prior to each study. Our team will provide dietary guidelines to ensure consistency. 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 sensitivity²².

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 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.

6.18.2 Dietary guidelines before study visits

Participants should not restrict carbohydrates from the diet before the test. A general guideline is that pre-adolescents should consume at least 25 kcal (6.25 g/kg/day) in carbohydrates and adolescents and adults should consume at least 15 kcal (3.75 g)/kg/day in carbohydrates for 3 days before the MMTT. These are minimum amounts of carbohydrates; most diets will include more carbohydrates. Participants will not need to alter their diets unless they are consuming a carbohydrate-restricted diet.

6.18.3 Insulin administration and glucose management

Participants should maintain routine monitoring of blood glucose on the evening preceding and the morning of the MMTT.

- Rapid-Acting Insulin: Participants may administer boluses of rapid-acting insulin analogs (e.g., lispro, aspart) up until two hours before beginning the MMTT experiment.
- Continuous Subcutaneous Insulin Infusion: Participants using insulin pumps may continue their basal insulin infusion up until the beginning of the basal sampling period of the MMTT.
- Long-Acting Basal Insulin: Participants taking long-acting basal insulin (e.g., glargine, degludec) may continue taking their insulin per their current prescription.

Hypoglycemia Management:

- If participants have blood glucose levels between 54-70 mg/dL on the night prior to the test, they should break their fast to consume 4-16 grams of glucose using glucose tablets, wait 15 minutes, and repeat a glucose measurement. If the glucose rises above 100 mg/dL, they should resume fasting. If the glucose remains below 100 mg/dL, they should consume another 4-16 grams of glucose and repeat the cycle until glucose rises above 100 mg/dL.
- If glucose levels fall below 54 mg/dL on the night prior to the study, research staff will reschedule the MMTT study.

6.19 Study protocol overview

Figure 4 illustrates the experimental protocol for each study.

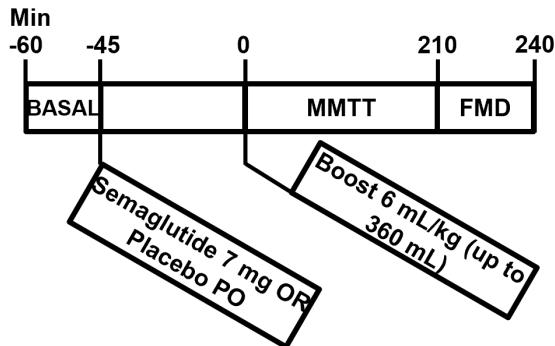


Figure 4: Schematic for MMTT visits. We will study participants with Stage 3 T1DM twice at baseline within eight weeks of their diagnosis. Participants will receive a GLP-1Ra (semaglutide) or placebo in random order prior to consuming a standardized oral nutrient load. We will serially measure plasma levels of glucose, insulin, and C-peptide throughout the experiment. We will then conduct a second set of identical paired experiments approximately six months post-diagnosis. During the final 30 minutes of each experiment, we will use high resolution B-mode ultrasound to measure endothelium-mediated vasodilation to quantify nitric oxide bioavailability.

6.20 Arrival and preparation

Upon arrival at the CRC, the PI or his MD, NP, or PA designate will conduct a brief history and physical exam. Then, clinical staff will establish intravenous catheter access to facilitate serial blood sampling throughout the study visit. Study personnel will use proper aseptic techniques to minimize the risk of infection.

6.21 Basal measurements

A study team member will draw three baseline blood samples at -60, -52.5, and -45 minutes before the MMTT begins (figure 4). These samples will serve as baseline measurements for glucose, insulin, C-peptide, and other metabolites 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	Glucose	TNF- α	<i>HLA-haplotype</i>
Proinsulin	NEFAs	IL-1 and 6	Acetaminophen
C-peptide	Glycerol	VEGF	
Glucagon	Lactate	sICAM	
Cortisol	Alanine	sVCAM	
CATS	β -OH-butyrate	E-selectin	
<i>Estradiol</i>		Endothelin-1	

6.22 Study drug administration

Forty-five minutes before receiving the mixed meal, participants will receive either Rybelsus® (oral semaglutide) or placebo, according to their randomized treatment sequence. The study drug will be administered with up to 120 mL of water in a fasting state, and participants will be instructed not to eat, drink, or take any other oral medications for at least 30 minutes after administration.

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 hour²³. 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. For this reason, we are using a single conservative 7 mg dose, rather than the full 14 mg dose.

6.23 Mixed meal tolerance test (MMTT)

At time zero (figure 4) participants will consume a BOOST® High Protein shake (at 6 mL/kg up to 360 mL, consistent with other T1DM studies^{19, 24-30}). 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.

6.24 Flow mediated dilation (FMD)

We will conduct FMD assessments during each MMTT visit to evaluate endothelial function, a key indicator of vascular health.

Procedure:

- Timing: FMD measurements will be performed at approximately 210 minutes after the mixed meal ingestion.
- Equipment: The Phillips EPIQ 7C ultrasound machine with an L12-3 transducer will be used.
- Personnel: An experienced ultrasonographer will conduct the FMD studies.

Steps:

1. Baseline Measurements:

- The participant rests in a supine position for at least 10 minutes.
- Baseline brachial artery diameter and blood flow velocity are measured over at least 10 cardiac cycles.

2. Vascular Occlusion:

- A blood pressure cuff is placed on the forearm, 5 cm distal to the elbow.
- The cuff is inflated to at least 50 mm Hg above systolic blood pressure for five minutes to induce ischemia.

3. Post-Cuff Release Measurements:

- The cuff is rapidly deflated.
- Arterial diameter and flow velocity measurements are taken immediately and continued for three minutes to capture reactive hyperemia.

4. Data Analysis:

- FMD is calculated using the formula:

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

5. Endothelium-Independent Vasodilation:

- After a 10-minute rest, 400 µg of sublingual nitroglycerin is administered.
- Brachial artery diameter is measured continuously for three minutes.
- This assesses the vascular smooth muscle response, independent of endothelial function.

Safety Considerations:

- Nitroglycerin administration
 - o Participants with systolic blood pressure ≤100 mmHg will not receive nitroglycerin.
 - o Participants will remain supine for at least 10 minutes after nitroglycerin administration to monitor for hypotension.

6.25 Study completion

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

6.26 Rescheduling studies after a technical issue

If a technical issue (e.g., loss of IV access) prevents the successful completion of a study visit, the PI may elect to reschedule the visit, provided there is no risk to the participant and they are willing 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).

Gastrointestinal side effects are a relatively commonly adverse event associated with GLP-1Ra therapy. In the pivotal PIONEER 6 study, gastrointestinal adverse events occurred more commonly in participants receiving oral semaglutide (Rybelsus®). Gastrointestinal side effects occurred in 108 of 1591 patients (6.8%) in the oral semaglutide group vs. in 26 of 1592 (1.6%) in the placebo group. These gastrointestinal side effects were primarily nausea (in 46 of 1591 patients [2.9%] vs. in 8 of 1592 [0.5%]), vomiting (in 24 of 1591 patients [1.5%]

vs. in 4 of 1592 [0.3%]), and diarrhea (in 22 of 1591 patients [1.4%] vs. in 6 of 1592 [0.4%]), mostly nonserious. However, serious adverse events led to permanent discontinuation of oral semaglutide in 41 of 1591 patients (2.6%) and of placebo in 48 of 1592 patients (3.0%)³².

Mitigation: To minimize these risks, we will carefully screen all participants prior to beginning 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

Aims 1-3

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., $i\text{AUC}_{\text{glucose}}/120 \text{ min} = \text{AUC}_{\text{glucose}0 \rightarrow 120} / 120 \text{ min} - \text{AUC}_{\text{glucose} -30 \rightarrow -15} / 15 \text{ min}$).

- 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^{5, 21, 33}.
- 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/dL³⁴. 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 powered future studies of GLP-1Ra in early stage T1DM. Based on $n = 20$, $\sigma = 6 \text{ mg/dL}$, $\alpha = 0.05$, and $1 - \beta = 0.80$, 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,^{35, 36} 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 MMTT³⁷.

- 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^{38, 39}. This amount would correspond to a $20.6 \times 10^{-12} \text{ dL/kg/min}^2$ 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 $SD \approx 38.7 \times 10^{-12} \text{ dL/kg/min}^2$ per pmol/L³⁹. Assuming the standard deviation of DI would be similar for both exenatide and placebo and that the correlation coefficient, ρ , between the two treatments for DI was 0.5, we can estimate the expected standard deviation of the within-participant difference using the formula $\sqrt{(SD_{\text{GLP-1Ra}}^2 + SD_{\text{placebo}}^2 - 2\rho \cdot SD_{\text{GLP-1Ra}} \cdot SD_{\text{placebo}})} \approx 27.4 \times 10^{-12} \text{ dL/kg/min}^2$ per pmol/L.
- Minimum detectable difference (MDD): Based on $n = 20$, $\sigma = 27.4 \times 10^{-12} \text{ dL/kg/min}^2$ per pmol/L, $\alpha = 0.05$, and $1 - \beta = 0.80$, 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 9.8×10^{-12} dL/kg/min² per pmol/L increase³⁹. 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 $FMD (\%) = [(Post\text{-}deflation\ diameter - Baseline\ diameter) / Baseline\ diameter] \times 100$.

- 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 hyperglycemia¹⁷.
- 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 $n = 20$, $\sigma = 1.7\%$, $\alpha = 0.05$, and $1 - \beta = 0.8$, 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 study⁴⁰. 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.

Aim 4

Our primary objective in this pilot study is to evaluate the independent effects of GLP-1Ra (oral semaglutide) on metabolic outcomes in patients with early stage 3 Type 1 Diabetes Mellitus (T1DM) using a randomized, double-blind, placebo-controlled crossover design. Each participant will undergo four Mixed Meal Tolerance Test (MMTT) visits: two at baseline and two after a six-month interval. During each pair of visits, participants will receive GLP-1Ra during one visit and placebo during the other, with the order randomized.

We will analyze the metabolic effects of GLP-1Ra versus placebo for our primary outcome: the disposition index (DI). Secondary outcomes include postprandial glycemia and endothelial function as measured by flow-mediated dilation (FMD).

We plan to enroll twelve participants with early stage 3 T1DM. Our plan allows for up to a 20% dropout rate, aiming for a final analysis of at least ten participants. Given the pilot nature of this study, our primary goal is to estimate effect sizes and variances to inform future, fully powered studies.

We will employ statistical methods appropriate for a crossover design with repeated measures. Specifically, we will use linear mixed-effects models to analyze within-subject differences between GLP-1Ra and placebo conditions, accounting for potential period,

treatment, and carryover effects. The model will include fixed effects for treatment, period, and sequence, and a random effect for participants.

- Expected effect size (Δ): Given the early-stage 3 T1DM participants and the effect seen in similar populations, we anticipate GLP-1Ra will increase DI by approximately 15%. This would correspond to an increase of approximately 14.7×10^{-12} dL/kg/min² per pmol/L.
- Expected variance (σ): Since this is a pilot study with limited data on DI in Stage 3 T1DM, we have assumed a within-participant variance similar to previous studies, estimated at $SD \approx 38.7 \times 10^{-12}$ dL/kg/min² per pmol/L. Using this variance for the sample size of 12 participants, we will calculate confidence intervals for our estimates rather than aiming for a fully powered hypothesis test.
- Minimum detectable difference (MDD): Based on $n = 12$, $\sigma = 38.7 \times 10^{-12}$ dL/kg/min² per pmol/L, $\alpha = 0.05$, and $1 - \beta = 0.80$, the minimum detectable difference is estimated to be approximately -22 to 22 dL/kg/min² per pmol/L. Due to the small sample size, results will primarily be used to determine the feasibility of DI measurements and estimate effect sizes for future studies.
- Minimum clinically important difference (MCID): Because DI highly influences glycemia in patients with stage 2 T1DM, we consider a 10% increase in DI at the 12-month MMTT as this study's MCID. Based on Galderisi et al., this amount would correspond to 9.8×10^{-12} dL/kg/min² per pmol/L increase³⁹. 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.

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.

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