A randomized, placebo-controlled, double-blinded cross-over study of the pharmacologic action of a GPR119 agonist on glucagon counter-regulation during insulin-induced hypoglycemia in Type 1 diabetes mellitus.

Short title: GPR119 agonist for hypoglycemia in T1D

Protocol Number: IRBNet #1552172

National Clinical Trial (NCT) Identified Number: NCT04432090

Principal Investigator: Richard E. Pratley, MD

Senior Investigator and Diabetes program lead
Translational Research Institute – Advent Health
301 E. Princeton Street
Orlando, Florida, 32804
Phone 407-303-2519
email: Richard.Pratley.MD@AdventHealth.com

IND Sponsor: AdventHealth

IND # 151908

Funded by: The Helmsley Charitable Trust

Version Number: v.10.0

21Nov2022

Table of Contents

STATEMENT OF COMPLIANCE

This trial will be conducted in compliance with the International Council for Harmonisation (ICH) E6(R2) guideline for Good Clinical Practice (GCP), and the applicable regulatory requirements from the United States Code of Federal Regulations (CFR), including 45 CFR 46 (Human Subjects Protection); 21 CFR 312 (Investigational New Drug); 21 CFR 50 (Informed Consent), and 21 CFR 56 (Institutional Review Board [IRB]).

All individuals who are responsible for the conduct, management, or oversight of this study have completed Human Subjects Protection and ICH GCP Training.

The protocol, informed consent form(s), recruitment materials, and all participant materials will be submitted to the Institutional Review Board (IRB) for review and approval. Approval of both the protocol and the consent form must be obtained before any participant is enrolled. Any amendment to the protocol will require review and approval by the IRB before the changes are implemented to the study. In addition, all changes to the consent form will be IRB-approved; a determination will be made regarding whether a new consent needs to be obtained from participants who provided consent, using a previously approved consent form.

SITE PRINCIPAL INVESTIGATOR STATEMENT

I have read the protocol, including all appendices, and the package insert/product label, and I agree that the protocol contains all necessary details for my staff and me to conduct this study as described. I will personally oversee the conduct of this study as outlined herein and will make a reasonable effort to complete the study within the time designated. I agree to make all reasonable efforts to adhere to the attached protocol.

I will provide all study personnel under my supervision with copies of the protocol and access to all information provided by the sponsor or the sponsor's representative. I will discuss this material with study personnel to ensure that they are fully informed about the efficacy and safety parameters and the conduct of the study in general. I am aware that, before beginning this study, the Institutional Review Board (IRB), or equivalent oversite entity must approve this protocol in the clinical facility where it will be conducted.

I agree to obtain informed consent from participants, as required by the IRB of record and according to government regulations and ICH guidelines. I further agree to ensure the study is conducted in accordance with the provisions as stated and will comply with the prevailing local laws and customs.

_____

Principal Investigator Name (Print)

_____ _____

Principal Investigator Signature Date

ABBREVIATIONS

↑	Increased
↓	Decreased
A/G ratio	Albumin Globulin Ratio
AE	Adverse event
AGAP	Anion Gap
ALT	Alanine Aminotransferase
ANOVA	Analysis of variance
Anti GAD Ab	Antibodies to Glutamic Acid Decarboxylase
Anti IA-2 Ab	Antibodies to Thyrosinephosphatase IA-2
Anti ZnT8 Ab	Antibodies to Zinc Transporter 8
APA	Action potential amplitude
APD	Action potential duration
API	Active pharmaceutical ingredient
AST	Aspartate Aminotransferase
AUC	Area under the time-plasma concentration curve
AUC0-24h	The area under the concentration-time curve from 0 to 24 h
BUN	Blood Urea Nitrogen
cAMP	Cyclic Adenosine Monophosphate
CAP	Cellulose acetate phthalate
CGM	Continuous Glucose Monitoring
CHO	Chinese hamster ovary
CKD-EPI	Chronic Kidney Disease – Epidemiology Collaboration
Cmax	Maximum plasma concentration
CMC	Carboxymethylcellulose
CYP	Cytochrome P450 isozyme
DIA	Diastolic arterial pressure
DMSO	Dimethylsulfoxide
DPP-4	Dipeptidyl peptidase-4
EC50	Concentration that produced 50% of the maximum possible response
ECG	Electrocardiogram
eGFR	Estimated Glomerular Filtration Rate
FBG	Fasting blood glucose
FDA	Food and Drug Administration
FPG	Fasting plasma glucose
FT3	Free thyroxine
FT4	Free triiodothyronine
GH	Growth Hormone
GIP	Gastric inhibitory peptide
GLP	Good laboratory practice
GLP-1	Glucagon-like peptide-1
GPR	G-protein coupled receptor
Gαs	Trimeric G protein α
h	Hour
HCD	20% Hydroxycyclodextrin/Water

HCl	Hydrochloride
HDL	High Density Lipoprotein
HEENT	Head, Eye, Ear, Nose and Throat exam
HEK-293	Human embryonic kidney cells
hERG	Human ether-a-go-go-related gene
HFD	High- fat diet
HGP	Hepatic glucose production
HIV	Human immunodeficiency virus
HOMA-B	Homeostasis model assessment of beta cell function
HOMA-IR	Homeostasis model assessment of insulin resistance
HR	Heart rate
Hz	Hertz
IB	Investigator's Brochure
IC50	Concentration that causes 50% inhibition
ICH	International Conference on Harmonisation
IFG	Impaired fasting glucose
IKr	Cardiac delayed rectifier repolarizing current
IND	Investigational New Drug
IRB	Institutional Review Board
IV	Intravenous
kg	Kilogram
LC-MS/MS	Liquid chromatography coupled with tandem mass spectrometry
LDL	Low Density Lipoprotein
LLOQ	Low limit of quantitation
MAD	Multiple ascending dose
MAP	Mean arterial pressure
MCH	Mean Corpuscular Hemoglobin
MCHC	Mean Corpuscular Hemoglobin Concentration
MCV	Mean Corpuscular Volume
mg	Milligram
mL	Milliliter
MMTT	Mixed meal tolerance test
mRNA	messenger Ribonucleic Acid
NA	Not applicable
NADPH	Reduced nicotinamide adenine dinucleotide phosphate
ng	Nanogram
NOAEL	No observed adverse effect level
NOEL	No observed effect level
OGTT	Oral glucose tolerance test
PD	Pharmacodynamics
PEG400	Polyethylene glycol 400
PK	Pharmacokinetics
POC	Point of care
PoP	Proof of Pharmacology
PP	Pulse pressure
PR	Measurement from beginning of P wave to beginning of QRS complex

QBS	Qualified Bilingual Staff
QRS	Measurement of depolarization of the ventricles; from beginning of Q to end of S wave
QT	Measurement from beginning of QRS complex to the end of the T Wave
QTc	QT interval corrected by heart rate
RH	Relative humidity
RMP	Resting membrane potential
RR	Measurement from one R wave to the following R wave
S9	Metabolic activation with rat hepatic A9 microsomal fraction
SAE	Serious adverse event
SD	Sprague Dawley
SDD	Spray Dried Dispersion
SYS	Systolic arterial pressure
t½	Half-life
T1DM	Type 1 Diabetes Mellitus
T2DM	Type 2 Diabetes Mellitus
tmax	Time at which maximum plasma concentration occurs
TPGS	D-alpha-tocopheryl polyethylene glycol 1000 succinate
TSH	Thyroid stimulating hormone
VLDL	Very Low Density Lipoprotein
Vmax	Maximum rate of depolarization
Vss	Volume of distribution at steady state
X	Fold (e.g. 10X = 10-fold)
ZDF	Zucker diabetic fatty rat
µg	Microgram
µM	Micromolar

1 PROTOCOL SUMMARY

1.1 SYNOPSIS

Title:

A randomized, placebo-controlled, double-blinded cross-over study of the pharmacologic action of a GPR119 agonist on glucagon counter-regulation during insulin-induced hypoglycemia in Type 1 diabetes mellitus.

Study Description:

This is a placebo-controlled, double-blinded, within-subject, cross-over Phase 2a study to test the hypothesis that short term (14-day) administration of MBX-2982, a G-protein coupled receptor-119 (GPR119) small molecule agonist, will significantly increase the glucagon counter-regulatory response to hypoglycemia in individuals with Type 1 Diabetes Mellitus (T1DM).

Objectives:

Primary objective:

- To compare (relative to placebo treatment) the effect of MBX-2982, a GPR119 agonist, on glucagon counter-regulatory responses to insulin-induced hypoglycemia in subjects with T1DM.

Secondary objectives:

- To determine whether MBX-2982, through its effect upon glucagon counter-regulation, increases hepatic glucose production (HGP) during insulin-induced hypoglycemia and during recovery to euglycemia in subjects with T1D relative to placebo treatment.
- To determine whether MBX-2982 reduces time of recovery from hypoglycemia to euglycemia in T1DM relative to placebo treatment.
- To determine whether there is any effect of MBX-2982 upon other key counter-regulatory hormones in T1D relative to placebo treatment.
- To compare the glucagon counter-regulatory response in healthy (non-diabetic) volunteers during insulin-induced hypoglycemia to that of subjects with T1DM treated with MBX-2982 and placebo.
- To compare time to recovery to euglycemia from insulin-induced hypoglycemia in healthy (non-diabetic) volunteers to that of subjects with T1DM treated with MBX-2982 and placebo.
- To compare the hepatic glucose production response to insulin-induced hypoglycemia in healthy (non-diabetic) volunteers to that of subjects with T1DM treated with MBX-2982 and placebo.

Exploratory objectives:

- To evaluate the effects of MBX-2982 upon fasting and postprandial glucagon, GLP-1 and GIP concentrations in subjects with T1DM relative to placebo treatment.
- To evaluate the effects of MBX-2982 on patterns of glycemia determined by continuous glucose monitoring in subjects with T1DM relative to placebo.
- To evaluate the pharmacokinetic profile of MBX-2982 in subjects with T1DM.

Endpoints:

Primary Endpoint:

- The glucagon response to hypoglycemia defined as:
  - Maximal glucagon concentration during hypoglycemia
  - Total area under the curve (AUC) for glucagon during hypoglycemia.
  - Incremental AUC for glucagon during hypoglycemia (above baseline levels during euglycemia).

Measured in subjects with T1D treated with MBX-2982 relative to placebo.

Secondary and Exploratory Endpoints:

- The glucagon response to hypoglycemia defined as:
  - Maximal glucagon concentration during hypoglycemia
  - Total area under the curve (AUC) for glucagon during hypoglycemia.
  - Incremental AUC for glucagon during hypoglycemia (above baseline levels during euglycemia).

Measured in subjects with T1D treated with MBX-2982 or placebo relative to healthy controls.

- HGP during insulin-induced hypoglycemia
- HGP during recovery from insulin-induced hypoglycemia to euglycemia
- Time of recovery from hypoglycemia (time to reach 85 mg/dL)
- Plasma levels of counter regulatory hormones during hypoglycemia including epinephrine, cortisol, GH.
- Fasting and post-prandial levels of glucagon, GLP-1 and GIP.

All measured in subjects with T1D treated with MBX-2982 relative to placebo and relative to healthy controls.

- Continuous glucose monitoring (CGM) indices of glycemic control including hypoglycemia (percent of time < 70 mg/dL, < 54 mg/dL), hypoglycemic events, mean glucose, percent of time 70-180 mg/dL, percent of time > 180 mg/dL, percent of time > 250 mg/dL, glycemic variability.
- MBX-2982 plasma levels

Measured in subjects with T1D treated with MBX-2982 relative to placebo.

Study Population:

The study will recruit 20 participants with T1DM fulfilling the following criteria:

- Age 20-60 years
- Diagnosis of T1DM according to American Diabetes Association (ADA) criteria continuously requiring insulin for survival
- Diabetes diagnosis performed more than 5 years before enrollment
- Fasting C-peptide levels < 0.7 ng/mL with a concurrent plasma glucose concentration > 90 mg/dL (Labs may need to be repeated if FBG $\leq$ 90)

- For female participants: agrees not to become pregnant during the study and for at least 2 weeks after the last dose of the study medication.
- For male participants: agrees not to donate sperm and to avoid getting a woman pregnant during the study and for at least 2 weeks after the last dose of the study medication.

A cohort of 9 healthy volunteers will also be recruited, fulfilling the following criteria:

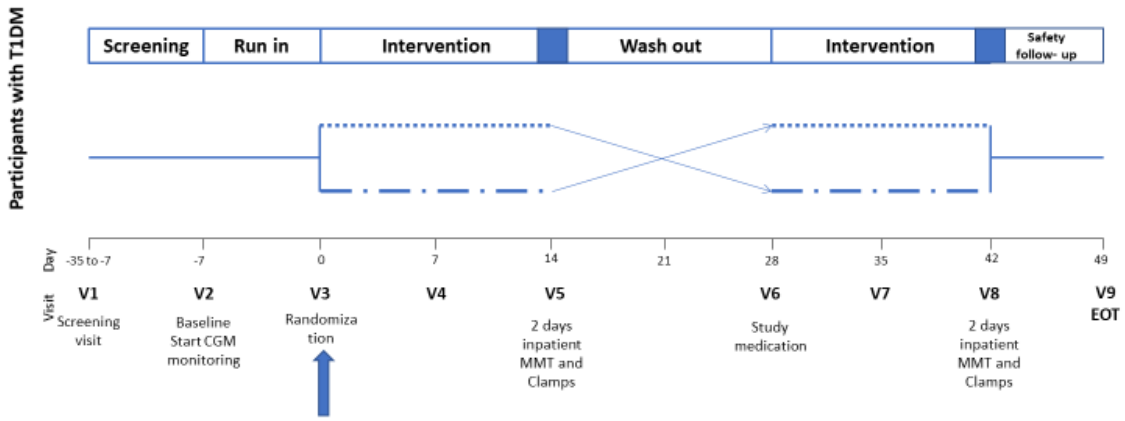
- Age 20 -60 years
- General good health
- Creatinine clearance >80 mL/min based on CKD-EPI equation
- Fasting blood glucose (FBG) >70 mg/dL and <100 mg/dL
- No history of diabetes
- For female participants: agrees not to become pregnant during the study

T1DM subjects must be generally healthy, without clinical evidence of impaired adrenal, pituitary, or thyroid function, and without evidence of symptomatic autonomic neuropathy. The subjects should not have:

1. Evidence of moderate or severe end-organ diabetic complications of retinopathy, nephropathy or neuropathy; proliferative retinopathy. Non-proliferative retinopathy will be allowed as is microalbuminuria but subjects must have a GFR > 60 mL/min/m^2 by CKD-EPI.
2. Evidence by history, ECG or exam of clinically significant cardiovascular disease. Subjects must have a QTcF <450 ms for males and < 470 ms for females.
3. Body mass index outside the range of 18.5 – 30 kg/m^2 for females and 20-30 kg/m^2 for males. Weight must have been stable (±3 kg) for at least the preceding 3 months.
4. Subjects can have stable hyperlipidemia and hypertension treated with no more than one lipid lowering medication or two antihypertensive medications. For each medication the dose should be stable for the past 3 months. Beta-adrenergic blocking agents will not be allowed during the study.
5. Hypoglycemia unawareness, as assessed by GOLD score at screening (1, 2). For this study, subjects with severe hypoglycemic episodes associated with seizure or coma within three months of screening or diabetic ketoacidosis within six months of screening will be excluded.
6. Predictive low blood glucose suspend mode on an insulin pump, or a hybrid closed loop algorithm for insulin delivery
7. Hba1c >9%
8. One or more DKA episodes in the past 3 months
9. Insulin dose less than 0.3 U/kg or on a low carbohydrate diet

Phase:	2a
Description of Sites/Facilities Enrolling Participants:	The study will be conducted at the AdventHealth Translational Research Institute (TRI) in Orlando, Florida, USA and ProSciento, Chula Vista, California, USA. Assays will be performed at the TRI, ProSciento laboratories, Scripps Mercy Hospital Laboratory, LabCorp and at Covance Laboratory Inc. (pharmacokinetic measures).
Description of Study Intervention:	Overview of the study design: • In randomized order, (Latin square, randomly assigned to placebo-active and active-placebo periods) and in a double-blinded manner, T1DM subjects will receive 14 days of daily dosing with a MBX-2982 (or placebo), taken at the same time each day after breakfast, except on Day 14 and Day 42. The last dose of treatment/placebo will be given in the morning (before the tracer) before the euglycemic/ hypoglycemic glucose clamp is started. T1DM subjects will undergo two Euglycemic - hypoglycemic clamps (induction of controlled hypoglycemia by an insulin infusion), using a within-subject cross-over design, with the two clamps separated by approximately four weeks, that is, two weeks of drug washout followed by two weeks of treatment with the alternative therapy. • After completion of the clamp, participants will not receive any study medication for two weeks (washout phase) and will then begin 14 days of the other arm medication (placebo or MBX-2982) in a double-blinded manner, followed by a repeat Euglycemic -hypoglycemic clamp study. During the study blinded CGM will be used to assess daily and nocturnal patterns of glycemia. • On the day preceding a clamp study, while admitted to the research unit, a standardized meal test will be used to assess fasting and postprandial glucagon, GLP-1 and GIP secretion. Regarding the clamp procedures, there will be an initial euglycemic run-in phase of the clamp (~85 mg/dL), followed by induction and maintenance of hypoglycemia (target 50 mg/dL), and then a phase of recovery from hypoglycemia. Throughout, glucagon, other counter-regulatory hormones, and hepatic glucose production will be measured. Healthy, non-diabetic control subjects will be studied on a single occasion and will not receive pre-treatment medication or placebo; they are being studied to establish “normal physiological responses” for comparison to drug and placebo treated responses in T1DM.
Study Duration:	The study will last 12 months.
Participant Duration:	Each T1DM participant will be enrolled for 84±2 days and each healthy participant will be enrolled for up to 28 days.

1.2 SCHEMA


Participants with T1DM
Screening
Run in
Intervention
Wash out
Intervention
Safety follow- up
Day
-35 to -7
-7
0
7
14
21
28
35
42
49
Visit
V1
Screening visit
V2
Baseline Start CGM monitoring
V3
Randomization
V4
V5
2 days inpatient MMT and Clamps
V6
Study medication
V7
V8
2 days inpatient MMT and Clamps
V9
EOT

1.3 SCHEDULE OF ACTIVITIES (SOA)

Visits	SV1	V2	V3	V4	V5	Week 4	V6	V7	V8	V9
Days	-35 up to -7	-7	0	7	13-14	21	28	35	41-42	49
Windows		±2	±2	±2	+5	±2	±2	±2	+5	±2
Cohort completing visit	T1DM Healthy	T1DM Healthy	T1DM only	T1DM only	T1DM Healthy	T1DM only	T1DM only	T1DM only	T1DM only	T1DM only
Informed Consent	X									
Inclusion/Exclusion	X									
Demography	X									
Medical History/Prior Medications	X									
Anthropometry	X				X		X		X	X
Vital signs	X				X		X		X	X
ECG	X			X	X			X	X	
Screening labs (CBC, CMP, HbA1c, lipid panel, C-peptide, islet autoAb, hepatitis/HIV, βHCG)	X									
CBC, CMP										X
TSH, FT4	X				X				X	
Follow up labs (CBC, CMP, HbA1c, lipid panel, thyroid function)					X				X	
Archive blood			X		X				X	
Drug screening	X									
GPR119 agonist plasma level					X*				X	
Urine POC Pregnancy test			X		X		X		X	
Urinalysis	X									
Archive urine			X		X				X	
Adverse Events		X	X	X	X	X	X	X	X	X
Concomitant Medications	X	X	X	X	X	X	X	X	X	X
CGM training and placement		X								
CGM wear time for T1DM		X**								
CGM data review			X	X	X	X	X	X	X	
Glucose and insulin log review			X	X	X*	X	X	X	X	
Study medication log review				X	X*			X	X	
Randomization			X							
Drug distribution			X				X			
Adherence to study drug					X*				X	
CRU admission					X				X	
Physical exam with fundoscopy	X				X		X		X	X
Standard mixed meal challenge					X				X	
Overnight IV insulin					X*				X	
Euglycemic-hypoglycemic clamp					X				X	

*On Visit 5 – these study activities are not completed by the Healthy cohort. See for further details in section 8.1 Schedule of events.

** Healthy volunteers will wear the CGM for the 10 days preceding and including the clamps.

2 INTRODUCTION

2.1 STUDY RATIONALE

GPR119 is a X-linked, class A (rhodopsin-like) Gαs receptor expressed on enteroendocrine cells and in pancreatic islets for which lysophospholipids and other lipid metabolites have been identified as endogenous ligands. Activation of GPR119 stimulates GLP-1 and GIP secretion from enteroendocrine cells and insulin secretion by β-cells in a hyperglycemia-dependent manner (3-5). More recently it has been reported that agonism of GPR119 augments the glucagon counter-regulatory response of α-cells to hypoglycemia (6), also in a glucose dependent manner as it did not stimulate glucagon secretion at euglycemia or hyperglycemia. Of relevance, recently reported single cell transcriptional analyses reveal that GPR119 is more highly expressed in islet α-cells than on β-cells (7). These emerging novel insights into GPR119 biology (and pharmacology) could fundamentally shift perceptions about the therapeutic potential of this target. Though the effects of GPR119 small molecule agonists on glucose-dependent insulin secretion and incretin secretion have been demonstrated preclinically and clinically, the effect to bolster the glucagon counter-regulatory response to hypoglycemia has only been demonstrated in rodents and has not yet been tested clinically.

A spike of increased glucagon secretion as plasma glucose levels descend to hypoglycemic thresholds (approximately 60-65 mg/dl) is considered a crucial counter-regulatory response. Yet, a severely impaired glucagon secretion in the face of insulin-induced hypoglycemia is commonly found in individuals with T1DM and is widespread by the time patients have had the disease for more than 5 years (8-10). This impairment in glucagon counter-regulation raises risk for hypoglycemia and its severity in T1DM. Though the attainment of tight glycemic control in T1DM has been demonstrated to lessen the risk of end-organ diabetic complications and of cardiovascular events, intensified glycemic control is commonly associated with an increased risk of hypoglycemic events (11). There is considerable morbidity caused by hypoglycemia in T1DM and nocturnal hypoglycemia is of a special concern as is hypoglycemia occurring in patients with “hypoglycemia unawareness”. More broadly, fear of hypoglycemia is regarded as a major barrier to patients achieving tight glycemic control and thereby improving diabetes outcomes (12). Similar concerns about hypoglycemia (and impaired glucagon counter-regulation) apply to many patients with type 2 diabetes (T2DM) who are reliant upon exogenous insulin and have severely depleted β-cell function (12, 13), however, at this juncture our re-evaluation of the therapeutic potential of GPR119 agonism to enhance hypoglycemic counter-regulation will focus upon T1DM.

The failure of glucagon counter-regulation in T1DM was initially reported by Gerich and colleagues nearly 50 years ago (8) and has since been confirmed repeatedly. Extensive clinical research through use of an insulin-infusion hypoglycemic clamp has been conducted on hypoglycemic counter-regulation in healthy individuals, those with T1DM and those with T2DM (10, 14, 15); we propose to use this procedure in the current study. An adaptation of the hypoglycemic clamp (from humans to rodents) was used to demonstrate the effect of various GPR119 agonists on glucagon secretion during hypoglycemia in rodents (6).

There are no currently approved therapeutics to address the defect in glucagon secretion during severe hypoglycemia, apart from the administration of exogenous glucagon which is used as an emergency treatment of severe hypoglycemia. Rectifying impaired glucagon secretion could lessen the frequency and severity of hypoglycemia seen in T1DM. Several years ago, a salutary effect to bolster glucagon secretion was reported using a somatostatin receptor antagonist in a rat model of T1DM (16,

17), however clinical translation of this effect has not been reported. As earlier cited, recent research at Merck Research Laboratories and in collaboration with investigators at Yale University, reported that in rodents, structurally diverse small molecule GPR119 agonists significantly increased glucagon counter-regulatory response to hypoglycemia, including in a STZ diabetic rat model of severely impaired glucagon counter-regulation (6). The goal of this proof of pharmacology (PoP) study is to test the clinical translation of this pharmacologic effect, from rodents to humans, and more specifically to those with T1DM.

In considering how a GPR119 agonist might be used by patients with T1DM, we envision that GPR119 agonist administration would not be used as an emergency treatment (as is the case for glucagon injection or its nasal inhalation), but instead could be taken daily as an adjunct to insulin therapy as prophylaxis to reduce the frequency and severity of hypoglycemia. Ultimately, pivotal clinical trials will be needed using a GPR119 agonist to test whether this adjunctive approach to insulin therapy reduces hypoglycemic events, especially severe episodes. The proof of pharmacology study outlined in this proposal would serve as a segue in support of undertaking the larger and longer duration clinical trials that measure hypoglycemic events. In this context, we also propose in the current study to collect continuous glucose monitoring (CGM) to assess whether GPR119 agonist treatment reduces the time spent with glucose values < 70 mg/dl, and < 54 mg/dl; these CGM data could help inform the design of a subsequent proof of concept clinical trial.

Initial interest in the GPR119 pathway was as a novel target for treating type 2 diabetes (T2DM), leveraging its effects on glucose-dependent insulin secretion and incretin secretion and thereby on glycemic control; the effect on glucagon was at that time not yet appreciated. Medicinal chemistry efforts led to the creation of numerous small-molecule GPR119 agonists, a number of which entered clinical trials. Unfortunately, only a modest effect in reducing hyperglycemia was observed in T2DM (17, 18) and this effect was not competitive with existing oral therapies. Consequently, further development of GPR119 agonists as treatment for T2DM was mostly halted. It is noteworthy that GPR119 agonists have been generally well-tolerated and found to be safe in the various short-term clinical studies, several of which were of 4 weeks duration. Also, it is noteworthy that GPR119 agonist effects on glucose-dependent insulin secretion and incretin secretion were demonstrated to translate from rodents to humans. However, the effect to mitigate hypoglycemia, and specifically, to augment glucagon counter-regulation was not examined in prior trials. In addition to direct effects on α-cell glucagon secretion, GPR119 agonism increases secretion of GIP which has been shown to further enhance the glucagon response to hypoglycemia (19, 20).

We propose to evaluate the effects of MBX-2982, a GPR119 agonist, on glucagon counter-regulatory responses during hypoglycemia in a placebo-controlled, randomized, cross-over design clinical trial in subjects with T1DM. To our knowledge, GPR119 agonists have not been previously given to subjects with T1DM. While our primary goal will be to examine an effect on glucagon counter-regulation in T1DM, we also propose to examine any effects on fasting and postprandial glucagon, GLP-1 and GIP.

Success in a proof of pharmacology study with a GPR119 agonist, such as the one that we are proposing, would demonstrate to the biomedical community that the GPR119 agonists may have therapeutic value to mitigate hypoglycemia in T1DM. Thus, a successful outcome would set the stage for subsequent clinical trials of longer duration dosing of a GPR119 agonist (adjunctive to insulin) to assess its efficacy to reduce hypoglycemic events and impact upon daily patterns of glucose (e.g. time below 70 mg/dl and effect on glycemic variation) as measured by CGM.

Also, if the findings from the current proposal are favorable, further uses of the hypoglycemic clamp might be envisioned. For example, might GPR119 agonism alter the glycemic threshold at which

glucagon counter-regulation is triggered? Use of a so-called "stepwise" hypoglycemic clamp platform to delineate glycemic threshold could be undertaken. This would provide additional insights into mechanisms by which GPR119 agonism might reduce risk of hypoglycemia. Another example is to conduct studies in insulin-treated T2DM who have impaired glucagon counter-regulation and vulnerability to hypoglycemia.

The capacity of GPR119 agonism to augment the glucagon counter-regulatory response to hypoglycemia in individuals with T1DM is the focus of this research protocol, a placebo-controlled proof of pharmacology study.

2.2 BACKGROUND

MBX–2982 is an agonist of the G-protein coupled receptor 119 (GPR119) that was initially studied as a novel therapeutic agent for the treatment of T2DM, evaluating its dual mechanisms of direct and incretin-mediated effects on glucose-dependent insulin secretion. As previously described in the Introduction, GPR-119 is an X-linked, class A (rhodopsin-like) Gαs receptor expressed on entero-endocrine cells and in pancreatic islets, for which lysophospholipids and other lipid metabolites are endogenous ligands. Activation of GPR-119 stimulates GLP-1 and GIP secretion from enteroendocrine cells and insulin secretion by β-cells in a hyperglycemic-dependent manner (3-5). Clinical studies have demonstrated translation of these actions from preclinical models into humans, including those with T2DM (See section on Human Experience). Recently it has been reported that agonism of GPR119 augments the glucagon counter-regulatory response of pancreatic islet alpha-cells (α-cells) (6) to hypoglycemia and does so in a glucose dependent manner as it did not stimulate glucagon secretion during hyperglycemia or euglycemia. In addition, recently reported single cell transcription analysis revealed that GPR-119 is more highly expressed in islet α-cells than on β-cells (7). These emerging and novel insights into GPR119 biology and its effects on pharmacodynamics could fundamentally shift the therapeutic potential of this receptor, re-focusing upon glucagon mediated hypoglycemic counter-regulation. Multiple and structurally diverse GPR-119 receptor agonists have demonstrated effects to bolster the glucagon counter regulatory response to hypoglycemia in rodents, but this pharmacology has not been tested clinically.

2.2.1 PHYSICAL, CHEMICAL AND PHARMACEUTICAL PROPERTIES

MBX-2982 was synthesized and screened as a GPR119 agonist. It was found to be capable of activation of endogenous GPR119 in a cell line overexpressing the receptor. MBX-2982 also increased incretin hormone levels in animals which may contribute to its glucose lowering effects in a hyperglycemic state. MBX-2982 is 5-ethyl-2-{4-[4-(4-tetrazol-1-yl-phenoxymethyl)-thiazol-2-yl]-piperidin-1-yl}-pyrimidine. It has a molecular formula of C22H24N8OS, and a molecular weight of 448.55 Daltons. MBX-2982 will be used as the HCl salt MBX-2982 in this study.

2.2.1.1 SALT FORMULATION PHARMACOKINETIC PROFILE

An HCl salt formulation of MBX-2982 (MBX-2982A) has been identified. Similar to the spray dried dispersion (SDD) formulation, MBX-2982A has increased aqueous solubility relative the MBX-2982 microcrystalline free base formulation. This enhancement of solubility by the SDD and the HCl salt formulation, relative to the microcrystalline free base formulation, is thought to provide supersaturated drug levels for increased absorption in the intestinal tract.

The pharmacokinetics of MBX-2982A have been assessed following single oral dosing of suspensions to rats and dogs. Direct comparisons of the oral exposure of the MBX-2982A and SDD formulations were

made in order to select the range of MBX-2982A doses for the clinic. Single oral doses of MBX-2982A suspension at 200 mg/kg in male rats were rapidly absorbed with a mean plasma Cmax value that was 80% of that for the SDD formulation at the same dose level. The mean plasma AUC values ranged from 57% (AUC0-48h) to 87% (AUC0-24h) compared to equivalent doses of the SDD suspension.

A cross-over study comparing SDD and MBX-2982A suspensions as single oral doses was conducted in male Beagle dogs at 200 mg/kg. Relative to the SDD suspension, MBX-2982A suspensions achieved 67% of the Cmax and 56% (AUC0-24h) to 89% (AUC0-∞) exposure parameters. Thus, the single dose oral exposure of the MBX-2982A is comparable to the SDD material in both species at 200 mg/kg.

2.2.1.2 SINGLE DOSE PHARMACOKINETICS

MBX-2982 was absorbed with oral bioavailability of 36% and 39% in mice and rat following single oral doses of 5 and 10 mg/kg, respectively. Absorption appeared to be limited in rats and dogs at the higher doses used in the single dose pharmacokinetic and multiple dose pharmacokinetic and toxicology studies. No sex differences were noted in dogs; however, the toxicokinetic data from the 4-week repeat oral dose study in the rats indicated that exposures in female rats were approximately 2- to 3-fold higher than in male rats. Following single dose IV administration, the half-life (t1/2) ranged from 2.4 to 3.1 hours, and clearance from 120-156 mL/h/kg for mice and rats, respectively.

2.2.2 REPEAT DOSE PHARMACOKINETICS

In repeat dose pharmacokinetic studies in rats, dogs and monkeys, MBX-2982 exhibited non-dose proportional, non-linear pharmacokinetics. Systemic exposure to MBX-2982, as measured by Cmax and AUC, increased with increasing dose, although the increase appeared to be less than proportional to dose in both rats and dogs, suggesting limitation of absorption at higher doses. No marked accumulation of MBX-2982 was observed in dog plasma after repeated oral dosing, and no gender differences were noted. Exposure in rats was 3-fold higher in females than in males. Minimal accumulation was observed after multiple doses in rats.

2.2.3 PHARMACOLOGIC PROFILE

MBX-2982 is a GPR119 agonist that binds and modulates the GαS activity of the GPR on pancreatic β-cells and enteroendocrine cells. As earlier noted, the original intent in developing GPR119 agonists was to exploit its potential to induce glucose-dependent insulin secretion for the treatment of T2DM; the pharmacology studies described below are pertinent toward this objective. Studies have demonstrated that MBX-2982 effectively reduces plasma glucose excursion during an Oral Glucose Tolerance Test (OGTT) in normal mice and rats. Several lines of evidence support a model in rodents in which MBX- 2982 exerts its actions directly at the pancreas (insulin secretion from β-cells) and in the GI tract (incretin secretion from enteroendocrine cells). The pancreatic effects of MBX-2982 were demonstrated by its enhancement of glucose-dependent insulin secretion from isolated rat and human pancreatic islets and by its dose-dependent stimulation of insulin secretion and glucose utilization in a rat hyperglycemic clamp model. Intestinal effects were documented by enteroendocrine-derived increases in active GLP-1 and total GIP in normal mice. This effect was enhanced when MBX-2982 was given in combination with the DPP-4 inhibitor sitagliptin, when compared to sitagliptin alone. As expected, the increases in incretins were accompanied by increases in insulin and by decreases in glucose excursion in response to an oral glucose challenge. These latter effects could presumably be due to the combined effects of incretins on their receptors and agonism of the GPR119 receptor.

From a therapeutic perspective, a follow-up study in mice showed that MBX-2982 and sitagliptin are additive in lowering the glucose excursion during an OGTT. These combined studies suggest that there may be dual or multiple mechanisms responsible for the glucose-lowering effects of MBX-2982, including but not limited to direct insulin secretion by islet cells and the stimulation of incretin secretion from enteroendocrine cells. Furthermore, the ability of MBX-2982 to lower glucose may be enhanced by a DPP-4 inhibitor and supports the role of an incretin signal in the mechanism of action of MBX-2982. Female leptin-deficient Zucker diabetic fatty (ZDF) rats are obese and insulin-resistant but develop diabetes mellitus only if fed a high-fat diet (HFD). In this pre-diabetic model, daily MBX-2982 for five weeks delayed the onset of post-prandial hyperglycemia and prevented the development of hyperinsulinemia and subsequent decline in insulin levels. It also blunted the increase in fasting glucose levels that generally occurs after a period of HFD, in conjunction with increasing fasting insulin levels. These effects translated to an increase in HOMA-B and a decrease in HOMA-IR in MBX-2982 treated animals on HFD, suggesting improved β-cell function and insulin sensitivity, respectively. There was no observed increase in pancreatic insulin content or changes in islet cell architecture.

2.2.4 TOXICOLOGY PROFILE

The results from in vivo animal toxicity and toxicokinetic studies indicate that MBX-2982 has a very low order of toxicity, with a no-observed-adverse-effect level (NOAEL) at doses up to 2000 mg/kg in single-dose studies and 1000 mg/kg in repeat-dose studies (top doses studied). The single and repeat dose toxicity studies described below were performed with the microcrystalline free base formulation.

2.2.4.1 ACUTE TOXICITY

Single dose toxicity at doses of 250, 750 and 2000 mg/kg MBX-2982 was evaluated after oral administration in mice and rats. No test article-related mortality or clinical observations were noted within the 14-day observation period. In rats, slight increases (up to 1.6-fold) in cholesterol levels were noted in female rats orally administered MBX-2982; these increases were not considered adverse and were not observed in mice. Histopathologic examination of tissues from mice and rats administered 2000 mg/kg indicated no test article-related changes. The NOAEL in mice and rats following a single oral dose is >2000 mg/kg.

2.2.4.2 REPEAT DOSE TOXICITY

In repeat dose toxicity studies of 1-month duration in both rats and dogs, there were no apparent effects of microcrystalline form of MBX-2982 up to doses of 1000 mg/kg/day (NOAEL > 1000 mg/kg/day). Specifically, there were no toxicologically meaningful changes in body weight, food consumption, necropsy parameters, histopathologic parameters, or laboratory measurements in either species. The maximum dose of 1000 mg/kg/day was based on the single dose and multiple dose pharmacokinetic and toxicokinetic data which demonstrated that at dosages above 300 mg/kg in rats and dogs, the total amount absorbed did not increase markedly with increasing dose, suggesting limitation of absorption at higher doses.

Additional repeat dose toxicity studies of 1-month duration in both rats and dogs were conducted with the SDD formulation to explore higher exposures. Despite achieving higher exposure than with the microcrystalline form of MBX-2982 in both species, no treatment related organ toxicity was identified. The only noted adverse effect for either study was decreased body weight gain that was observed in male rats at the top dose investigated (230 mg/kg/day) that was associated with an AUC0-24h of 698 µg*h/mL. The NOAELs were 75 mg/kg/day in male rats and 115 mg/kg/day, the top dose evaluated in female rats.

These represent approximate 1.8-fold and 2.4-fold increases in the respective previous NOAEL exposures (AUC0-24h) from the microcrystalline free base formulation. In dogs, the NOAEL was the top dose evaluated, 400 mg/kg/day of the SDD formulation, in both males and females. The associated plasma exposures were increased 3.1-fold and 3.3-fold, respectively, over the microcrystalline free base formulation NOAEL exposures. While not considered adverse, there was increased emesis and non-formed feces observed in the MBX-2982 treated dogs.

2.2.5 SAFETY PHARMACOLOGY

During safety pharmacology studies with the microcrystalline free base formulation, no neurobehavioral effects were observed in male rats treated with MBX-2982. No changes in QT interval were observed in an oral safety pharmacology study designed to assess cardiovascular effects in conscious telemeterized dogs or in a general toxicology study conducted in dogs. During the conscious telemeterized dog study, minimal hemodynamic changes, considered physiologically insignificant, were observed at the highest dosages (300 and 1000 mg/kg).

To further enable human studies with the anticipated improvements in exposure due to the SDD formulation of MBX-2982, an additional oral safety pharmacology study was conducted with the SDD formulation to assess cardiovascular effects in conscious telemeterized dogs with greater exposure to MBX-2982. No test article-related changes in electrophysiology parameters, including QT interval, were observed in this second study at the top dose (100 mg/kg), which was associated with a Cmax of 13.2 µg/mL. This represents an approximate 5.1-fold increase over the previous no effect Cmax achieved at 1000 mg/kg with the microcrystalline free base formulation in the first dog cardiovascular safety study. Except for minor and transient higher heart rates compared to control (but not to baseline), there were no hemodynamic changes in the oral safety pharmacology study with the SDD formulation.

In vitro assays were performed to evaluate the effects of MBX-2982 on cardiac ion channel and action potential durations (APD). In the first assay, effects of MBX-2982 concentrations on the IKr (cardiac delayed rectifier repolarizing current) channel were assessed. In this study, the IKr channel was inhibited by MBX-2982 in a concentration-dependent manner with a 50% inhibitory concentration (IC50) of 1.16 µM (0.52 µg/mL). In the APD assay, MBX-2982 produced no effect on the action potential at a normal stimulation rate. At the highest concentration, 20 µM (9.0 µg/mL), a slight but statistically significant APD90 prolongation occurred under stimulation rates corresponding to bradycardia and tachycardia. As noted above, no in vivo cardiac effects or QT prolongation were observed in the telemetrized dog studies or during the 4-week repeat dose dog study. MBX-2982 was not genotoxic in any of the systems evaluated

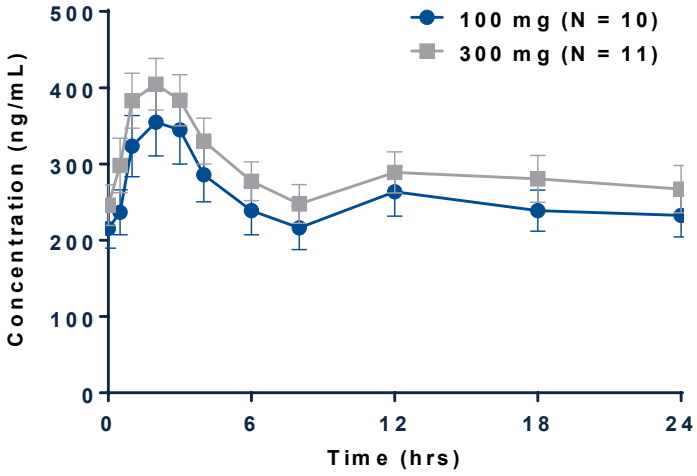
2.2.6 HUMAN EXPERIENCE

Phase 1 and Phase 2 Studies in Patients

In early human studies, as earlier noted, undertaken with the original intent of exploring a potential for treating T2DM, MBX-2982 consistently lowered fasting plasma glucose (FPG) and glucose excursion following a mixed meal tolerance test (MMTT) and an oral glucose tolerance test (OGTT). During a graded glucose infusion, MBX-2982 attenuated hyperglycemia and enhanced glucose-sensitive insulin secretion. Across the three completed Phase 1 studies, MBX-2982 had variable effects on other biomarkers such as GLP-1 and glucagon during an MMTT or OGTT. Four Phase l studies and a 28-day Phase 2 human study have been completed and there has been no safety, pharmacokinetic (PK) or tolerability concerns with MBX–2982 dosing. A Phase 2 study evaluated sitagliptin as a comparator to MBX-2982. These data

suggested that there may be additional benefit on glucose homeostasis if MBX-2982 is combined with sitagliptin.

MBX-2982 was studied in 3 formulations in the clinic. A microcrystalline free base formulation (MBX-2982), a spray dried dispersion (MBX-2982 SDD) formulation and an HCl salt formulation (MBX-2982A). The HCl salt, which had an exposure of 54,600 ng*ml/hr., was deemed the formulation best suited to take forward in development. Administration of MBX-2982 HCl salt formulation in single and multiple doses of 100 mg and 300 mg capsules have been safe and well tolerated.



	100 mg (Day 4)	300 mg (Day 4)	1000 mg* (Day 1)
C_{max} (ng/mL)	365	437	818
T_{max}	4.1	1.7	3.0
$t_{1/2}$	19.2	29.1	18.4
AUC_{0-24} (ng*h/mL)	6098	6998	7924

Table 1 and Figure 1: Data from the first-in-human study (*Protocol M2982-10712)*

Micronized free base formulation

2.2.6.1 MBX-2982 COMPLETED CLINICAL STUDIES

Phase	Protocol Number	Description	Formulation(s) Studied	Number on MBX-2982
1	M2982-10712	Single ascending dose study	Micronized free base 10, 30, 100, 300, 600, 1000 mg	48
1	M2982-10813	Multiple ascending dose study – 4 days – in IFG patients	Micronized free base 100 and 300 mg	23
1	M2982-10918	5-day repeat dose PK study in IFG or IGT	SDD 25, 100, 300, 600 mg	33
1	M2982-11021	Single dose PK in fed and fasted subjects	SDD 300 mg HCl salt 50, 200, 600 mg	32
2	M2982-20920	4-week study in T2D patients	SDD: 25, 100, 300 mg Sitagliptin: 100 mg	68

Table 2: Human studies

2.2.7 HUMAN SAFETY AND TOLERABILITY PROFILE

Overall, there were good non-clinical and clinical safety and tolerability profiles with wide safety margins against 6-month toxicity studies. PK of the salt formulation was found to be excellent and exposure increased in a dose related manner. There was a significant increase in exposure with food relative to fasting therefore dosing in this study will be in the fed condition.

2.2.7.1 SAFETY EVALUATIONS

In both Phase 1 and Phase 2 studies with MBX-2982 there were no AE or SAE trends, and most AEs were mild and few possibly related drug AEs and 1 related. One subject did withdraw consent due to AEs. There were no group or individual adverse treatment effects in either safety labs or ECGs. Unblinded individual ECG data did not show an imbalance of QTc prolongation and no patient met severe QTc threshold. In the Phase 2a study there was no dose-limiting safety or toxicity up to 300 mg for 28 days.

2.2.7.2 ANALYSIS OF ADVERSE EVENTS

There were no significant differences between MBX-2982 and placebo in the number of subjects with AEs overall or the number of subjects with AEs that were judged to be possibly or probably related to study drug. Two subjects in the 300 mg cohort experienced symptoms consistent with possible hypoglycemia, although in one case the blood glucose was normal and in the other case the blood glucose value was mildly decreased towards the end of the OGTT but no different than the mildly low value observed at the baseline pre-dosing OGTT. This suggested the possibility of a delayed insulin response to a glucose load, as is well described in this population. Nevertheless, these events were captured as AEs.

Constipation was the most common possibly treatment-related AE overall (7 subjects [16%]). The percentage of subjects experiencing constipation in the MBX-2982 treated groups (6 subjects [18%]) was

only modestly greater than the percentage in the placebo treated group (1 subject [9%]) and the incidence was generally not dose-dependent. An overview of AEs and the incidence of the most frequently occurring treatment-related AEs, by treatment, can be found in the Investigator's Brochure (IB).

No life-threatening AEs, serious AEs, or deaths were reported. One subject in the 300 mg MBX-2982 SDD group withdrew due to AEs. The majority of AEs reported were mild. There were no severe AEs (See the Investigator Brochure [IB] for detailed information).

2.2.7.3 LISTING OF DEATHS, OTHER SERIOUS ADVERSE EVENTS, AND OTHER SIGNIFICANT ADVERSE EVENTS

No life-threatening AEs, SAEs, or deaths were reported (see IB for detailed information).

2.2.8 SUMMARY OF NON-CLINICAL AND CLINICAL INVESTIGATIONS WITH MBX-2982

Non-clinical and clinical studies showed that MBX-2982 has desirable effects on blood glucose levels. Nonclinical studies and clinical studies to date including safety pharmacology studies and repeat dose toxicokinetic studies, failed to show any significant safety or toxicological concerns.
Please see the IB for additional details of the nonclinical studies conducted with MBX-2982.

2.2.9 OVERALL SAFETY CONCLUSIONS

MBX-2982 dosed daily was generally safe and well tolerated. No clinically significant changes or findings were noted in clinical laboratory evaluations, vital sign measurements, physical examinations, or 12-lead ECGs for any of the subjects studied or treatment groups.

A total of six subjects (4 at 100 mg and 2 at 300 mg) experienced mild, subclinical increases in TSH (< 10 µIU/mL) without changes in FT4 or FT3 in one study. The changes in TSH in this study were not observed in the subsequent Phase 1c study which produced significantly higher drug exposures. Based upon the absence of significant changes in FT4 or FT3, as well as the absence of a thyroid signal in 4-week animal toxicology studies, these findings are not felt to be clinically or toxicologically meaningful and may represent a spurious measurement.

2.3 RISK/BENEFIT ASSESSMENT

2.3.1 KNOWN POTENTIAL RISKS

For potential risks please refer to the IB. Some of these potential risks are due to taking the investigational drug MBX-2982, and some may be due to the underlying disease or being on placebo. Although no significant safety concerns have arisen from the animal or human studies with MBX-2982 to date, the compound remains in the early phases of human testing and therefore carries the risk associated with a compound with limited human experience.

2.3.1.1 RISKS ASSOCIATED WITH THE STUDY PROCEDURES

Intravenous lines/blood draws (lab samples) – there is a risk of pain, vasovagal syncope, hematomas, and/or infection at IV insertion/blood draw site (low risk of serious AEs).

CGM placement
There is a low risk for developing a local skin infection at the site of the sensor needle placement. Itchiness, redness, bleeding, and bruising at the insertion site may occur, as well as local tape allergies.

Standardized meal test
Participants may experience increase of their blood glucose.

Insulin infusion and hypoglycemic clamp
The administration of insulin intravenously during the euglycemic-hypoglycemic clamp may lead to a greater degree of hypoglycemia than expected. Hypoglycemia could be associated with adrenergic and neuroglycopenic symptoms, that could rarely cause seizure. The stable isotope of glucose infused during the euglycemic-hypoglycemic clamp carries no additional risks. Intravenous infusion of glucose may cause venous irritation (phlebitis) or less commonly infiltration of the surrounding tissues, both which may cause discomfort.

2.3.1.2 POTENTIAL RISKS ASSOCIATED WITH THE INVESTIGATIONAL MEDICATION

Human Safety and Tolerability Profile
Overall, there were good non-clinical and clinical safety and tolerability profiles with wide safety margins against 6-month toxicity studies. The PK of the salt formulation was found to be excellent and exposure increased in a dose related manner. There was a significant increase in exposure with food relative to fasting therefore dosing in this study will be given in the fed condition.

Analysis of Adverse Events
There were no significant differences between MBX-2982 and placebo in the number of subjects with AEs overall or the number of subjects with AEs that were judged to be possibly or probably related to study drug. Two subjects in the 300 mg cohort experienced symptoms consistent with possible hypoglycemia, although in one case the blood glucose was normal and in the other case the blood glucose value was mildly decreased towards the end of the OGTT but no different than the mildly low value observed at the baseline pre-dosing OGTT. This suggested the possibility of a delayed insulin response to a glucose load, as is well described in the T2DM population. Nevertheless, these events were captured as AEs. Constipation was the most common possibly treatment-related AE overall (7 subjects [16%]). The percentage of subjects experiencing constipation in the MBX-2982 treated groups (6 subjects [18%]) was only modestly greater than the percentage in the placebo treated group (1 subject [9%]) and the incidence was generally not dose-dependent. An overview of AEs and the incidence of the most frequently occurring treatment-related AEs, by treatment, can be found in the IB.
No life-threatening AEs, serious AEs, or deaths were reported. One subject in the 300 mg MBX-2982 SDD group withdrew due to AEs. The majority of AEs reported were mild. There were no severe AEs. (See the IB for detailed information)

Listing of Deaths, Other Serious Adverse Events, and Other Significant Adverse Events
No life-threatening AEs, SAEs, or deaths were reported (see IB for detailed information).

Unknown safety areas
To date, no in vivo human or animal drug interaction studies have been performed with MBX-2982. Based on in vitro data and the anticipated maximum human concentrations, no significant drug-drug interactions are expected.

Based upon safety and tolerability monitoring from the 4 phase 1 and 1 phase 2 studies completed to date, there are no apparent adverse central nervous system effects of MBX-2982 in humans. No neurobehavioral effects were observed in rats.

MBX-2982 was not genotoxic in any of the systems evaluated, but no data are available regarding animal or human carcinogenicity. No specific animal or human studies have been performed to determine the reproductive and developmental toxicity of MBX-2982. In human studies to date, there have been no reported cases of pregnancy in subjects receiving MBX-2982 or in partners of subjects receiving MBX-2982. There is no information about the effect of MBX-2982 on sperm or its production in the body, nor is there information about effects on the development of the fetus.

2.3.2 KNOWN POTENTIAL BENEFITS

The study being proposed is a placebo-controlled, double-blind, cross-over study design to test the hypothesis that short term (14 day +/- 2 days drug administration) of GPR119 will significantly increase the glucagon counter-regulatory response to hypoglycemia in subjects with T1DM.

Participation in this study will not result in any direct benefits to participants, however participation in the screening may identify previously unrecognized medical conditions, which may provide a general health risk assessment. Subjects will also receive some general medical information, including laboratory testing and medical examination.

2.3.3 ASSESSMENT OF POTENTIAL RISKS AND BENEFITS

2.3.3.1 MITIGATION OF RISKS ASSOCIATED WITH THE STUDY PROCEDURES

Intravenous lines/blood draws (lab samples)
All venipuncture will be conducted by qualified staff using aseptic techniques.

Standardized meal test
Suggestion for appropriate insulin dosing will be provided to correct abnormal glucose excursion.

CGM placement
The site of CGM insertion will be periodically monitored and strategies to prevent local skin reaction will be suggested such as, but not only, site rotation, liquid adhesive or additional tape.

Insulin infusion and hypoglycemic clamp
During the clamp studies, a qualified team member (either MD/PA/ARNP and RN) will be present at bedside to mitigate any risks of hyper- and hypoglycemia. Hypoglycemia will be closely monitored, kept within the planned range and severe effects will be rapidly corrected with intravenous glucose. At the end of each visit, the participant will receive guidance for glucose monitoring, food intake, and insulin dosing as needed.

2.3.3.2 MITIGATION OF POTENTIAL RISKS ASSOCIATED WITH THE INVESTIGATIONAL DRUG

The drug was well tolerated and had a very low order of toxicity at the proposed concentration in previous human studies and at higher concentrations in animal models.

It is important that women of childbearing potential receiving MBX-2982 in early investigational development do not become pregnant for at least 2 weeks after the last dose. As a precaution, women of childbearing potential receiving MBX-2982 should use at least one medically accepted method of contraception with >99% effectiveness when used consistently and correctly. As the induction potential of MBX-2982 on CYP enzymes is not clear. Given that the effectiveness of hormonal contraceptives can be reduced by CYP enzyme inducers, we recommend that study subjects who are women of childbearing potential use a barrier method during the study to prevent unintended pregnancy. In addition, it is important that partners of male subjects receiving MBX-2982 should not become pregnant during the study. As a precaution, male subjects receiving MBX-2982 should use dual method of contraceptive use: medically accepted contraception (e.g., condoms w/spermicide), or their partner should also use a medically accepted form of contraception along with condoms during this period.

Although the studies in humans did not show clinically relevant ECG alterations, in vitro studies showed potential for QT prolongation. For this reason, ECGs will be performed on Visits 4, 5, 7, 8 during investigational medication treatment, in addition to the screening Visit 1. At visits 5 and 8, the ECG will be performed during the meal test at the time of predicted drug concentration peak 180 min after taking the medication. The QTcF will be manually calculated for each ECG.

Elevated TSH levels were reported in one of the human studies but not confirmed in others. TSH and FT4 have been added to the safety measures at visit 5 and visit 8 during study treatment.

MBX-2982 could potentially affect plasma glucose levels, causing either a reduction via incretin stimulation or an increase via glucagon stimulation. The participants will be instructed to check their blood glucose with a glucometer before meals and at bedtime. The participants will be instructed on signs and symptoms of hyper- or hypo-glycemia, and on appropriate treatment. To prevent the risk of DKA caused by a decrease of insulin due to incretin reduction of blood glucose and glucagon potentiation, subjects requiring less than 0.3 U/Kg/day of insulin, on a low carb diet or with an episode of DKA in the past three months will not be enrolled. Written instruction on sick day management and on management of unusual symptoms associated with alcohol intake, strenuous or prolonged activity will be provided. Urine ketone checks will be recommended when blood glucose is >250 mg/dl and whenever the participant is not feeling well. If small to moderate amounts of ketones are measured (small to moderate in the urine or blood β-hydroxybutyrate 0.6-1.5 mmol/L) the participants will be instructed to contact the research team, administer rapid-acting insulin, consume 15-30g of carbohydrate, hydrate with 300-500 ml of fluid hourly, check self-monitored blood glucose frequently and ketones every 3-4 hours. If trace ketones are present on more than 3 separate days in a 7-day period, the participant will be instructed to contact the study team, measure fasting ketone daily and hold the study medication.

Further mitigation of glucose excursions will be facilitated through the weekly review of the CGM patterns and adjustment of insulin doses. As much as possible, the basal insulin dose will be maintained at baseline levels to prevent DKA

Due to the unknown profile in elderly and children, these categories of participants will not be enrolled. Since no specific studies have been performed in patients with renal dysfunction or renal failure, only subjects with a glomerular filtration rate >60 ml/min will be enrolled.

3 OBJECTIVES AND ENDPOINTS

Primary Objectives and Endpoints	
Objective	**Endpoint**
To compare (relative to placebo treatment) the effect of MBX-2982, a GPR119 agonist, on glucagon counter-regulatory responses to insulin-induced hypoglycemia in subjects with T1DM.	Maximal glucagon concentration during hypoglycemia. Total area under the curve (AUC) for glucagon during hypoglycemia. Incremental AUC for glucagon during hypoglycemia (above baseline levels during euglycemia)

Secondary Objectives and Endpoints	
Objectives	**Endpoint**
To determine whether MBX-2982, through its effect upon glucagon counter-regulation, increases hepatic glucose production (HGP) during insulin-induced hypoglycemia and during recovery to euglycemia in subjects with T1D relative to placebo treatment.	HGP during insulin-induced hypoglycemia. HGP during recovery from insulin-induced hypoglycemia to euglycemia.
To determine whether MBX-2982 reduces time of recovery from hypoglycemia to euglycemia in T1DM relative to placebo treatment	Time of recovery from hypoglycemia (time to reach 85 mg/dL).
To determine whether there is any effect of MBX-2982 upon other key counter-regulatory hormones in T1D relative to placebo treatment	Plasma levels of counter regulatory hormones during hypoglycemia including epinephrine, cortisol, GH.
To compare the glucagon counter-regulatory response in healthy (non-diabetic) volunteers during insulin-induced hypoglycemia to that of subjects with T1DM treated with MBX-2982 and placebo.	Total area under the curve (AUC) for glucagon during hypoglycemia Incremental AUC for glucagon during hypoglycemia (above baseline levels during euglycemia) Plasma levels of counter regulatory hormones during hypoglycemia including epinephrine, cortisol, GH.
To compare time to recovery to euglycemia from insulin-induced hypoglycemia in healthy (non-diabetic) volunteers to that of subjects with T1DM treated with MBX-2982 and placebo	Time of recovery from hypoglycemia.
To compare the hepatic glucose production response to insulin-induced hypoglycemia in healthy (non-diabetic) volunteers to that of subjects with T1DM treated with MBX-2982 and placebo	HGP during insulin-induced hypoglycemia. HGP during recovery from insulin-induced hypoglycemia to euglycemia.

Exploratory Objectives and Endpoints	
Objective	**Endpoint**
To evaluate the effects of MBX-2982 upon fasting and postprandial glucagon, GLP-1 and GIP concentrations in subjects with T1D relative to placebo treatment.	Fasting and post-prandial levels of glucagon, GLP-1 and GIP
To evaluate the effects of MBX-2982 on patterns of glycemia determined by continuous glucose monitoring in subjects with T1DM relative to placebo.	Continuous glucose monitoring (CGM) indices of glycemic control including hypoglycemia (percent of time < 70 mg/dL, < 54 mg/dL), hypoglycemic events, mean glucose, percent of time 70-180 mg/dL, percent of time > 180 mg/dL, percent of time > 250 mg/dL, glycemic variability
To evaluate the pharmacokinetic profile of MBX-2982 in subjects with T1DM.	MBX-2982 plasma levels.

4 STUDY DESIGN

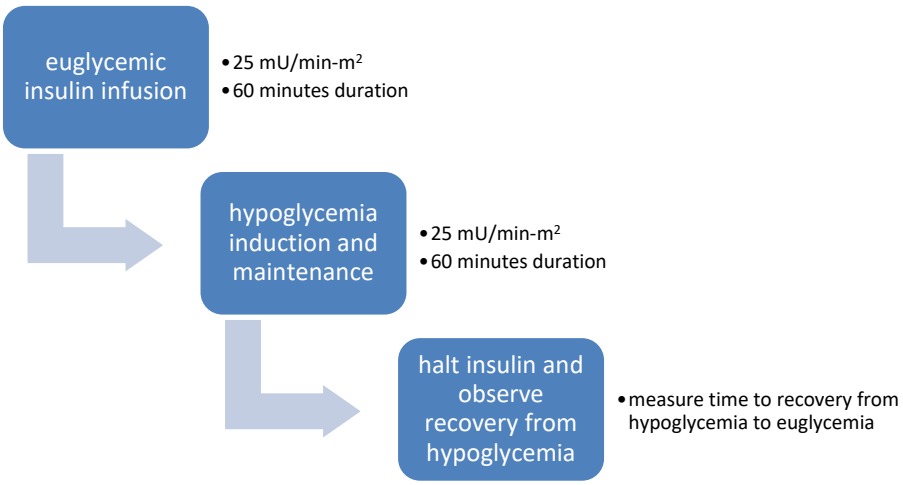
4.1 OVERALL DESIGN

This research will be carried out as a placebo-controlled, double-blinded, within-subject, cross-over phase 2a study.
Overview of the study design:

- In randomized order, (Latin square, randomly assigned to placebo-active and active-placebo periods) and in a double-blinded manner, the T1DM subjects will receive 14 days of daily dosing with MBX-2982 (or placebo), taken at the same time each day after breakfast. The last dose of treatment/placebo will be given when the glucose tracer infusion for the euglycemic/hypoglycemic-glucose clamp is started.
- T1DM subjects will undergo two euglycemic-hypoglycemic clamps (induction of controlled hypoglycemia by an insulin infusion), using a within-subject cross-over design, with the two clamps separated by approximately four weeks, that is, two weeks of drug washout followed by two weeks of treatment with the alternative therapy.
- After completion of the first clamp study, participants will not receive any study medication for two weeks (washout phase) and then begin 14 days of the other arm (placebo or MBX-2982) in a double-blinded manner, followed by a repeat euglycemic-hypoglycemic clamp study. During treatment on each arm and during wash out phase, a blinded CGM will be used to assess daily and nocturnal patterns of glycemia.
- On the day preceding a clamp study, while admitted to the research unit, a standardized meal test will be used to assess fasting and postprandial glucagon, GLP-1 and GIP secretion.

Overview of the hypoglycemic clamp: Regarding the procedures of the clamp, there will be an initial euglycemic run-in phase of the clamp, (~85 mg/dL), followed by induction and maintenance of hypoglycemia (target 50 mg/dL), and then a phase of recovery from hypoglycemia. Throughout, glucagon, other counter-regulatory hormones, and hepatic glucose production will be measured. Healthy, non-diabetic control subjects will be studied on a single occasion and will not receive pre-treatment

medication or placebo; they will be studied to establish “normal physiological responses” for comparison to drug and placebo treated responses in T1DM.



4.2 SCIENTIFIC RATIONALE FOR STUDY DESIGN

The study design and procedures were chosen to assess the effect of 14 days of treatment on glucagon response to insulin-induced hypoglycemia. The study design allows an ample wash out time to minimize the risk of carry-over effects before crossing over the two groups.

A hypoglycemic clamp has been chosen to evaluate the response to hypoglycemia during treatment.
It is acknowledged by the field that a hypoglycemic clamp does not fully recapitulate all aspects of clinical episodes of hypoglycemia occurring under conditions of daily life (such as effects of stress, physical activity, altered food intake or variation in insulin dose and its subcutaneous absorption). However, it is a controlled, reproducible platform enabling delineation of the counter-regulatory hormonal response and lends itself as particularly suitable for paired, within-subject evaluations as we propose to do in evaluating the pharmacology of a GPR119 agonist on glucagon counter-regulation.

While our primary goal will be to examine an effect on glucagon counter-regulation in T1DM, we also propose to examine any effects on fasting and postprandial glucagon, as well as upon incretin secretion (GLP-1 and GIP). We acknowledge that it is improbable much effect upon hyperglycemia will be observed in T1DM, given the severity of the deficiency in insulin secretion in T1DM, and given that the effect in T2DM was only modest. Yet, the testing is important for several reasons. Firstly, if a GPR119 agonist were to elevate glucagon in T1DM under fasting and postprandial conditions, this would be quite unfavorable and thus important to know early in its evaluation. Increases in incretin secretion in T1DM (as have been observed with DPP-4 inhibitors) could also be potentially favorable, perhaps especially for increased GIP which can mitigate risk of hypoglycemia by bolstering glucagon counter-regulation to hypoglycemia; this might be most relevant to mitigation of risk for postprandial episodes hypoglycemia in T1DM.

4.3 JUSTIFICATION FOR DOSE

The dose proposed for this study is 600 mg of MBX-2982 as the HCl salt formulation. Though the dose-response relationship for MBX-2982 and stimulation of insulin and incretin secretion has been described,

it is uncertain whether this pertains equally to a stimulatory effect on glucagon counter-regulation during hypoglycemia. In consideration that the current proposal will be the first study of potential clinical translation of this effect previously observed in rodent and the safety of the medication, a dose that achieves high exposure of MBX-2982 was chosen to ensure a high level of GPR119 target engagement. If positive findings are obtained, consideration will be given to a supplemental study to delineate if doses lower (and higher) than 600 mg daily achieve similar or accentuated stimulatory effect on glucagon counter-regulation during hypoglycemia.

4.4 END OF STUDY DEFINITION

A participant is considered to have completed the study if he or she has completed all phases of the study including the end of trial visit seven days after the last dose of the study medication as shown in the Schedule of Activities (SoA).

4.5 STUDY SITE(S)/LOCATION(S) AND NUMBER OF SUBJECTS

The study will be conducted at two centers with AdventHealth Orlando, Translational Research Institute serving as the principal site. The participating centers are as follows:

- AdventHealth Translational Research Institute (TRI) in Orlando, Florida
- ProSciento, Chula Vista, California

Estimated number of subjects at both sites combined: 20 T1DM and 9 Healthy
Estimated number of subjects at AdventHealth TRI site: 15 T1DM and 4 Healthy
Estimated number of subjects at ProSciento site: 5 T1DM and 5 Healthy

4.6 MULTI-SITE RESEARCH LOGISTICS/COMMUNICATION PLAN

AdventHealth Orlando Translational Research Institute will be the coordinating center and Dr. Richard Pratley will be the lead investigator for this study. As this is a multi-site study, the following processes will be put in place with all the participating centers:

- All sites will have the most current version of the protocol and consent document
- All required approvals will be obtained at each site (including approval by the site's IRB of record).
- All modifications will be communicated to sites and approved (including approval by the site's IRB of record) before the modification is implemented.
- All engaged participating sites will safeguard data as required by local information security policies.
- All local site investigators conduct the study appropriately.
- All non-compliance with the study protocol or applicable requirements will be reported in accordance with local policy.

Emails, Teams Meetings, and telephone calls will be used to communicate with all participating sites to address any study-related issues and to inform the sites on closure of the study. Interim analysis will not be performed.

5 STUDY POPULATION

5.1 INCLUSION CRITERIA

Type 1 diabetes cohort

1. Age 20-60 years
2. Diagnosis of T1DM according to American Diabetes Association (ADA) criteria continuously requiring insulin for survival
3. Diabetes diagnosis performed more than 5 years before enrollment
4. Fasting C-peptide levels < 0.7 ng/mL with a concurrent plasma glucose concentration > 90 mg/dL (Labs may need to be repeated if the Plasma glucose is $\leq$ 90 mg/dL)
5. For female participants: must be > 6 months post-partum and not lactating and agrees not to become pregnant during the study and for at least 2 weeks after the last dose of the study medication. For male participants: agrees not to donate sperm or not to get a woman pregnant during the study and for at least 2 weeks after the last dose of the study medication.

Healthy subject cohort

1. Age 20-60 years
2. General good health
3. Creatinine clearance >80 mL/min based on CKD-EPI equation
4. Fasting blood glucose (FBG) >70 mg/dL and <100 mg/dL
5. No history of diabetes
6. For female participants: must be > 6 months post-partum and not lactating and agrees not to become pregnant during the study

5.2 EXCLUSION CRITERIA

An individual who meets any of the following criteria will be excluded from participation in this study:

1. BMI >35 kg/m^2 and <18.5 kg/m^2 for females and BMI >35 kg/m^2 and <20 kg/m^2 for males.
2. Increase or decrease body weight greater than 3kg in the 3 months before enrollment.
3. Evidence by history, ECG or exams of clinically significant cardiovascular disease (unstable angina, myocardial infarction or coronary revascularization within 6 months, clinically significant abnormalities on ECG, presence of cardiac pacemaker, implanted cardiac defibrillator)
4. Evidence of autonomic neuropathy
5. Liver disease (AST or ALT >2.5 times the upper limit of normal)
6. Kidney disease (creatinine >1.6 mg/dl or estimated GFR <60 ml/min).
7. Dyslipidemia, including triglycerides >500 mg/dl, LDL >200 mg/dl or unstable hyperlipidemia. Treatment with a single lipid lowering agents is allowed if stable within the previous 3 months.
8. Anemia (hemoglobin <12 g/dl in men, <11 g/dl in women)

9. Thyroid dysfunction (suppressed TSH, elevated TSH <10 µIU/ml if symptomatic or elevated TSH >10 µIU/ml if asymptomatic)
10. Uncontrolled hypertension (BP >160 mmHg systolic or >100 mmHg diastolic) or treatment with more than 2 antihypertensive medications.
11. Current use of beta-adrenergic blocking agents or their use was stopped less than one month before recruitment
12. History of cancer within the last 5 years (skin cancers, with the exception of melanoma, may be acceptable)
13. History of organ transplant
14. History of HIV, active Hepatitis B or C, or Tuberculosis
15. Pregnancy, lactation or 6 months postpartum from the scheduled date of screening lab collection
16. Females of childbearing potential (any female except those with tubal ligation, hysterectomy, or absence of menses >2 years) unwilling to use an approved method of contraception (one medically accepted method of contraception with ≥99% effectiveness when used consistently and correctly). Male participants: he or he and his partner unwilling to use an approved method of contraception with ≥99% effectiveness when used consistently and correctly
17. History of Major Depression in the last 5 years
18. History of an eating disorder
19. History of bariatric surgery
20. History of drug or alcohol abuse (≥ 3 drinks per day) within the last 5 years
21. Self-report of marijuana use ≥3 days/week in any form
22. Psychiatric disease prohibiting adherence to study protocol
23. Current use of oral or injectable anti-hyperglycemic agents: metformin, sulfonylureas, DPP IV inhibitors, SGLT-2 inhibitors, thiazolidinediones, acarbose, GLP-1 analogs
24. Initiation or change in hormone replacement therapy within the past 3 months (including, but not limited to thyroid hormone or estrogen replacement therapy). Hormone based contraception is acceptable.
25. Use of any medications known to influence glucose, fat and/or energy metabolism (e.g., growth hormone therapy, glucocorticoids [steroids], prescribed medications for weight loss, etc.). Patients on medications with acute effects on glucose metabolism used for other indications (certain antidepressants, ADHD and antiepileptic medications) may be enrolled if they have been on chronic, stable doses (≥6 months)
26. Uncontrolled seizure disorder
27. Current night shift worker
28. Presence of any condition that, in the opinion of the Investigator, compromises participant safety or data integrity or the participant's ability to complete study visits
29. Unwilling and/or unable to follow and comply with scheduled visits and protocol requirements

Additional exclusion Criteria for the type 1 diabetes cohort

1. HbA1c >9%
2. Insulin dose less than 0.3 U/kg or low carbohydrate diet
3. History of T2DM or any form of diabetes other than T1DM
4. Hypoglycemia unawareness as assessed using the GOLD score
5. Using a predictive low blood glucose suspend mode on an insulin pump or a hybrid closed loop algorithm for insulin delivery. For those applying these strategies for everyday

management of blood glucose and willing to participate, the algorithm will be stopped at enrollment.

6. Two or more episodes of severe hypoglycemia (Hypoglycemia requiring help from a third party) per month in the past six months
7. One or more DKA episodes in the past 3 months
8. QTcF >450 msec for males and >470 msec for females
9. Using non-insulin agents to control blood glucose levels
10. History or evidence of moderate or severe end-organ diabetic complications of retinopathy, nephropathy or neuropathy. Proliferative diabetic retinopathy. Non-proliferative retinopathy and microalbuminuria will be allowed.

Additional exclusion Criteria for the healthy cohort

1. Insulin treatment

5.3 SCREEN FAILURES

Screen failures are defined as participants who consent to participate in the clinical trial but are not subsequently randomly assigned to the study intervention or entered in the study. A minimal set of screen failure information is required to ensure transparent reporting. Minimal information includes demography, screen failure details, eligibility criteria, and any serious adverse event (SAE).

Individuals who do not meet the criteria for participation in this trial (screen failure) because of a modifiable factor may be rescreened. Rescreened participants will be assigned a new study number.

5.4 STRATEGIES FOR RECRUITMENT AND RETENTION

5.4.1 SUBJECT RECRUITMENT

Recruitment methods utilized may include, but will not be limited to, the following: recruitment from within the AdventHealth TRI or ProSciento patient population, electronic medical records and database searches (including third party recruitment vendors); advertising in multiple media such as print ads, flyers, brochures, posters; radio ads; television spots; and internet/social media advertising. All advertising materials will be submitted to the AdventHealth Orlando IRB or ProSciento's IRB of record for review prior to using or publishing them. Recruitment efforts will follow AdventHealth Orlando or ProSciento recruitment SOPs for research.
Advertising material, phone screening script and worksheet will be approved by the IRB before use.

5.4.2 VULNERABLE POPULATIONS

Cognitively Impaired Adults: N/A
Children: N/A
Pregnant Women: N/A
Neonates of non-viable or uncertain viability: N/A
Prisoners: N/A
Students: N/A

Employees: AdventHealth Employees: Recruitment efforts will follow AdventHealth recruitment Standard Operating Procedures (SOPs) for research. AdventHealth employees will not be individually targeted nor excluded from study participation based on employment. AdventHealth employees who engage the AdventHealth Translational Research Institute asking to participate in the study will be processed per standard consent procedures for participants. In addition, during the consent process, the study staff will review standard consent language stating that an employee's participation or lack of participation in the study will not affect their employment status or relationship with AdventHealth.

5.4.3 SUBJECT STIPENDS OR PAYMENTS

After the participant's completion of all study visits, a total amount for the study will be $3075.00 (T1DM group) and $1100.00 (Heathy group). A payment Card will be processed with the Dollar amount per visits completed as indicated in the table below. Payments may take up to 3 business days to be processed, once requested. In the event the participant is unable to complete all study visits the payment will be prorated. Participants who agree to take part in this study will be paid for completed study visits according to the following schedule:

Visit	Type 1 Diabetes Amount	Heathy participant Amount
Screening	$150	$150
Visit 2 – Baseline	$150	$150
Visit 3 - Randomization	$150	
Visit 4	$150	
Visit 5	$1000	$800
Payment # 1 (after Visit 5)	**$1600**	**$1100**
Week 4 Visit	$25	
Visit 6	$150	
Visit 7	$150	
Visit 8	$1000	
Visit 9 - ETO	$150	
Payment # 2 (after Visit 9)	**$1475**	
Total Amount	**$3075**	**$1100**

6 STUDY INTERVENTION

6.1 STUDY INTERVENTION(S) ADMINISTRATION

6.1.1 STUDY INTERVENTION DESCRIPTION

Study drug is defined as the investigational drug(s), and placebo intended to be administered to a study participant according to the study protocol.
The intervention will be done by administering MBX-2982, a G-protein coupled receptor-119 (GPR119) agonist and placebo. The study is investigator initiated and AdventHealth will be the holder of the IND.

6.1.2 MBX-2982

6.1.2.1 FORMULATION, APPEARANCE, PACKAGING, AND LABELING

MBX-2982 HCl capsules and matching placebo are provided by Cymabay. The investigational drug product has been produced as a formulation in capsules that are white in color. Each capsule contains 150 mg active drug.
Study drug will be packaged and labeled in a masked manner and in compliance with regulatory requirements.

6.1.2.2 PRODUCT STORAGE AND STABILITY

MBX-2982 will be stored at 25ºC with excursions from 15-30°C permitted. Temperatures will be monitored daily.

6.1.2.3 PREPARATION AND DRUG DISPENSING

After randomization, a quantity of study drug sufficient for 14+5 days will be dispensed by authorized pharmacy personnel for the participant with T1DM at Visit 3 (Day 0).
At Visit 6 (Day 28), subjects with T1DM will receive study medication, the opposite treatment arm, in a double-blinded manner.

6.1.2.4 DOSING AND ADMINISTRATION

Study drug will be supplied as 150 mg capsules. Participants will receive a 600 mg daily dose (4 capsules) to be taken daily after the morning meal. All study drug doses will be oral self-administrations.

6.1.2.5 RATIONALE FOR SELECTION OF DOSE

The rationale for the proposed dose is described above with the scope of ensuring a high level of GPR119 target engagement (see section 4.3).

6.1.3 PLACEBO

6.1.3.1 FORMULATION, APPEARANCE, PACKAGING, AND LABELING

Placebo will be provided in a similar appearance as the study drug and be packaged and labeled in a masked manner in compliance with regulatory requirements. Inactive capsule ingredients are starch, lactose monohydrate, sodium starch glycolate, colloidal silicon dioxide, sodium lauryl sulfate, stearic acid and gelatin.

6.1.3.2 PRODUCT STORAGE AND STABILITY

Placebo should be stored at room temperature (15°C - 30°C).

6.1.3.3 PREPARATION AND DRUG DISPENSING

After randomization, a quantity of study drug sufficient for 14+5 days will be dispensed by qualified pharmacy personnel for the participant with T1DM at Visit 3 (Day 0).
At Visit 6 (Day 28), subjects with T1DM will receive study medication, the opposite treatment arm, in a double-blinded manner.

6.1.3.4 DOSING AND ADMINISTRATION

All placebo doses will be oral self-administrations. Participant will receive 4 capsules to be taken daily after the morning meal.

6.1.3.5 RATIONALE FOR SELECTION OF DOSE

N/A

6.2 PREPARATION/HANDLING/STORAGE/ACCOUNTABILITY

6.2.1 ACQUISITION AND ACCOUNTABILITY

All study drugs will be received, stored and dispensed according to regulatory requirements and will be used only on subjects and be used only by authorized investigators.

6.3 MEASURES TO MINIMIZE BIAS: RANDOMIZATION AND BLINDING

Individuals with T1DM will be randomized in a blinded manner in a 1:1 ratio to receive either placebo or MBX-2982 as the treatment for Period 1. Randomization will be conducted by a statistician. This information will only be released as the patients are enrolled. A master list of the patient names and intervention group will be stored in a limited access, confidential file.

6.4 STUDY INTERVENTION COMPLIANCE

Use of study drug will be monitored at Visit 5 (Day 14) and Visit 8 (Day 42). Adherence to study drug as provided will be assumed unless reported otherwise.

6.5 CONCOMITANT THERAPY

Medication use will be permitted if not conflicting with the exclusion criteria. Concomitant prescription medications, over-the-counter medications and supplements will be reported in the Case Report Form (CRF).
Use of beta-adrenergic blocking agents and hypoglycemic agents other than insulin is not permitted. Their use will be assessed at enrollment.

6.5.1 RESCUE MEDICINE

No experiments have been performed to determine a specific antidote to MBX-2982. General supportive measures should be taken as appropriate.
Glucagon can be used as a rescue medication for severe hypoglycemia.

7 STUDY INTERVENTION DISCONTINUATION AND PARTICIPANT DISCONTINUATION/WITHDRAWAL

7.1 DISCONTINUATION OF STUDY INTERVENTION

The study will be stopped if one of the events leading to participant discontinuation occurs in more than 15% (n=3) of the planned number of 20 enrolled participants with Type 1 diabetes.

7.2 PARTICIPANT DISCONTINUATION/WITHDRAWAL FROM THE STUDY

7.2.1 INVESTIGATOR WITHDRAWAL OF SUBJECTS

The participation in this study may be stopped at any time by the study PI without the participant's consent because:

- The study Medical investigator thinks it necessary for subject's health or safety;
- Participant has not followed study instructions;
- The AdventHealth Translational Research Institute has stopped the study; or
- Administrative reasons require the participant's withdrawal.

A participant will be discontinued/withdrawn if the following occur during study treatment:

- Any episode of Level 3 hypoglycemia according to the ADA classification
- Any episode of Diabetes Ketoacidosis (DKA) defined by serum or urine ketones greater than the upper limit of normal range and serum bicarbonate <15 mmol/l or blood pH <7.3 without a satisfactory alternative cause for anion-gap acidosis
- Persistent glycemia aberration defined as:
    - Hypoglycemia: ≥ 3 episodes per week of level 2 hypoglycemia (blood glucose ≤ 54 mg/dL, [3.0 mmol/L]) not prevented by appropriate insulin dose adjustments.
    - Hyperglycemia: Level 2 fasting hyperglycemia (blood glucose ≥ 250 mg/dL [13.9 mmol/L]) in 4/7 days not prevented by appropriate insulin dose adjustments
- Increase in QTcF to >500 ms or >60 ms over baseline confirmed in at least two ECGs

7.2.2 SUBJECT REQUEST FOR WITHDRAWAL FROM STUDY

Participation in this study is voluntary. Participants may decide not to participate in this study or may withdraw from this study at any time without penalty or loss of benefits. If a participant leaves the study before the final regularly scheduled visit, she/he may be asked by the study doctor to make a final visit for some 'end-of–study' procedures. This is to make sure that there are no safety concerns.

7.2.3 DATA COLLECTION AND FOLLOW-UP FOR WITHDRAWN SUBJECTS

Participants who request withdrawal or who are withdrawn by the PI from the study will have their data maintained in the research database up to the point of withdrawal. The available data will be included in subsequent analysis because a participant may have withdrawn due to possible drug side effects (if applicable) and keeping these participants in the analysis is essential for study validity.

7.3 LOST TO FOLLOW-UP

A participant will be considered lost to follow-up if he or she fails to return for one scheduled visit and is unable to be contacted by the study site staff.

The following actions must be taken if a participant fails to return to the clinic for a required study visit:

- The site will attempt to contact the participant and reschedule the missed visit and counsel the participant on the importance of maintaining the assigned visit schedule and ascertain if the participant wishes to and/or should continue in the study.
- Before a participant is deemed lost to follow-up, the investigator or designee will make every effort to regain contact with the participant. These contact attempts will be documented in the participant's study file.
- Should the participant continue to be unreachable, he or she will be considered to have withdrawn from the study with a primary reason of lost to follow-up.

8 STUDY ASSESSMENTS AND PROCEDURES

8.1 SCHEDULE OF EVENTS

Each participant will be enrolled for up to 84±2 days.
The intervention study will take up to 19 + 19 days.
The total length of the study is expected to be 1 year.

8.1.1 CONSENT PROCESS

We attest that all study staff delegated the authority to obtain informed consent will follow The AdventHealth institutional SOP: 401.116 Informed Consent Process and Written Documentation of Informed Consent or ProSciento's consent process. If applicable due to participant preferences or circumstances that would require the consent to be obtained at an earlier or same day via remote consent, the institute will follow the HRP-831 Investigator Guidance: Remote Informed Consent Process in Non-Exempt Research.

8.1.1.1 SUBJECTS WHO ARE NOT YET ADULTS (INFANTS, CHILDREN, TEENAGERS)

N/A

8.1.1.2 COGNITIVELY IMPAIRED ADULTS

N/A

8.1.1.3 ADULTS UNABLE TO CONSENT

N/A

8.1.1.4 DOCUMENTATION OF INFORMED CONSENT PROCESS

Documentation of the informed consent process is required to establish that the subject was accurately and adequately informed and that no study-related procedures were initiated prior to obtaining informed consent. A research team member will note in the source documentation the consent process, date consent was obtained and that consent was obtained prior to initiating any research procedures.

8.1.1.5 WAIVER OF WRITTEN DOCUMENTATION OF CONSENT OR WAIVER OF CONSENT

N/A

8.1.1.6 NON-ENGLISH SPEAKING SUBJECTS

The participant population is primarily English speaking. In the unlikely event that a non-English speaking participant meets criteria for enrollment, a Qualified Medical Interpreter or Video Remote Interpretation will be utilized when there is a need of sight translation. A Qualified Bilingual Staff (QBS), or if not available, a Qualified Medical Interpreter or Video Remote Interpretation will be utilized if only interpreting for another staff member is required. We will use an Institutional approved short form (HRP-804 Investigator Guidance: Short-Form Consent Process in Research) when needed. The plan is not to translate the consent, study questionnaires or instructions.

8.1.2 VISIT 1- SCREENING VISIT/ DAY -35 UP TO -7 (OUTPATIENT, 2-3 HOURS)

Subjects will take part in a screening visit in the TRI or ProSciento.
After the participant has read and signed the study informed consent and agreed to study participation, the following procedures will be completed for both healthy participants and participants with T1DM:

- Assessment of medical history and demographics
- Physical exam
- Height and weight measurement
- BMI calculation
- Vital signs (Heart rate x 2, blood pressure x 2, respirations, temperature)
- Con-med Assessment
- ECG
- Screening blood (fasting for at least 8 hours, water is permitted): CBC, CMP, HbA1c, thyroid function tests, lipid panel, C-peptide, islet autoantibody panel, hepatitis/HIV
- Urinalysis and urine drug screening
- Serum beta HCG for pregnancy in female subjects of childbearing potential (all women are considered to be of childbearing potential unless they have undergone a hysterectomy, tubal ligation, or have had an absence of menses for > 2 years)

Inclusion and exclusion criteria will be verified. Recruited candidates meeting all enrollment criteria will be scheduled for visit 2.

8.1.3 VISIT 2 – BASELINE VISIT/ DAY -7±2 (T1DM COHORT) OR 8±2 DAYS BEFORE V5 (HEALTHY COHORT) (OUTPATIENT, 1 HOUR):

The visit will be done at the TRI or ProSciento.

The following procedures will be completed for patients with T1DM and healthy volunteers:

- CGM placement (Healthy volunteers will wear the CGM for the 10 days preceding and including the clamps).

- Training on sensor insertion and use for continuous glucose monitoring (CGM). Sites provided CGM will be blinded and subjects with T1DM should continue their normal finger stick glucose and/or personal CGM monitoring.
- AE/Con-med Assessment
- Instruction on insulin dose adjustments, ketone testing and sick day management, hypoglycemia symptoms and treatment of participants with T1DM

8.1.4 VISIT 3 – RANDOMIZATION VISIT/ DAY 0±2 (OUTPATIENT, 1 HOUR)

The visit will be done at the TRI or ProSciento.

The following procedures will be completed for participants with T1DM:

- CGM management and data review
- Review knowledge of insulin dose adjustments, ketone testing and sick day management, hypoglycemia symptoms and treatment
- Fingerstick blood glucose/personal CGM data, and insulin log review
- AE/Con-med Assessment
- Urine pregnancy test
- Blood draw for archive (fasting for at least 10 hours)
- Urine for archive
- Randomization
- Drug distribution for Period 1

8.1.5 VISIT 4 – FOLLOW UP VISIT/ DAY 7±2 (OUTPATIENT, 1-1.5 HOURS)

The visit will be done at the TRI or ProSciento.

The following procedures will be completed for participants with T1DM:

- CGM management and data review
- Review knowledge of insulin dose adjustments, ketone testing and sick day management, hypoglycemia symptoms and treatment
- Fingerstick blood glucose/personal CGM data, insulin and study medication log review
- AE/Con-med Assessment
- ECG

Data from the CGM will be analyzed.

8.1.6 VISIT 5 – PERIOD 1/ DAY 13-14+5 (T1DM COHORT) (INPATIENT, 2 DAYS) OR WITHIN 28 DAYS AFTER SCREENING (HEALTHY COHORT)

The visit will be done at the TRI or ProSciento.
The scheduled day of intervention is valid for participants with T1DM.

Healthy participants will undergo the same procedures of this visit any time between 7 and 28 days after screening
Because moderate and severe episodes of hypoglycemia can have a transient effect of at least several days to dampen counter-regulatory responses to a subsequent episode of hypoglycemia (12, 15, 21) the clamp procedure may need to be re-scheduled so that a window of at least 5 days without moderate or severe episodes of hypoglycemia (blood glucose <55 mg/dl) precedes the clamp study (and supplemental study medication can be provided under such circumstances). Subjects will be admitted in the fasting state (10hours) to the Research Center in the morning the day prior to the clamp study (which will be conducted on the following morning).
The following procedures will be completed in participants with T1DM and in healthy participants:

- Physical exam
- Height and weight measurement
- BMI calculation
- Vital signs (Heart rate x 2, blood pressure x 2, respirations, temperature) on each day of CRU staying
- ECG during the meal test
- AE/Con-med Assessment
- Follow up labs (fasting for at least 10 hours, water is permitted): CBC, CMP, HbA1c, lipid panel, thyroid function.
- Urine pregnancy test
- Standard meal challenge with pre- and post-meal blood draw to measure glucose, glucagon, total GLP-1, total GIP, and blood study medication levels (GRP119 PK)
- Overnight IV infusion of regular insulin (T1DM participants only)
- Euglycemic and hypoglycemic clamp
- CGM management and data review
- Fingerstick blood glucose/personal CGM data, ketone, insulin and study medication log review *(T1DM participants only)*
- Adherence to study drug will be evaluated (T1DM participants only)
- Blood for archive
- Urine for archive

On the morning of admission, subjects with T1DM will be instructed to withhold long-acting insulin injections, and not to eat breakfast (which will be served at the research center after admission). A T1DM subject will be asked to monitor blood glucose every four hours (or more frequently) and administer short-acting insulin to control hyperglycemia. The study physician and nurse will help guide subjects in these dose selections. Subjects who are on insulin pump therapy will continue their usual basal rates and prandial dosing of insulin delivery since this will clear rapidly once discontinued.

Upon admission to the Research Center, the participant will have an intravenous catheter inserted and afterwards will receive a standard mixed meal challenge of consistent composition and calorie amount on both admissions, preceded by a pre-prandial dose of insulin. The intervention drug will be taken 30 minutes, ±5 minutes window, after beginning the meal. At 30-minute intervals, preceding and during the meal, for the next 3.5 hours, blood samples will be obtained according to Table 2. The key objective of this meal study is to examine fasting and prandial glucagon responses while on GPR119 agonist treatment. After the mixed meal challenge, the IV catheter will be maintained. Subjects will receive a weight maintaining, controlled carbohydrate lunch and dinner on the unit. After dinner, research subjects will be kept NPO (other than water) and will remain fasting in the morning and until completion of the clamp study.

For T1DM subjects, a second catheter might be placed for an overnight infusion of low-dose regular insulin to control plasma glucose within a range of 80 to 120 mg/dl. A POC and/or CGM instrument will be used for bed-side glucose determinations. To monitor plasma glucose overnight, every 1 to 2 hours (more frequently if necessary). Blood glucose sampling/evaluation via CGM/POC will be done to inform adjustments of the insulin infusion rate using a previously established algorithm. No further subcutaneous insulin use, by injection or via pump delivery, will be used until subjects resume their usual therapy after the clamp study. The last medication dose will be taken before the beginning of the clamp.

At the end of the clamp procedure, subjects will be served a meal and observed for two to three hours afterwards to monitor blood glucose and for adverse effects of the study. Subjects may receive a pre-prandial dose of short acting insulin and will receive instructions from the study physician on re-starting their usual insulin regimen and any adjustment or changes for that day. Blood glucose levels will be monitored every 15-30 minutes until stable or in case of hypoglycemia symptoms.

The study medications will be stopped. The ppt will be wearing a CGM during the wash out phase, additional supplies will be provided if needed. In the scenario the participant is not comfortable with placing new CGM at home a TRI site visit will be scheduled for a study staff member to assist with CGM insertion.

The study stops here for healthy participants.

8.1.7 WEEKS 4 DAYS 21±2 (OUTPATIENT, 15 TO 30 MIN)

The visit will be done at the TRI or ProSciento.

The following will be done:

- Fingerstick blood glucose/personal CGM data and insulin log review
- AE/Con-med Assessment
- AE/Con-med Assessment

During the first 14 days after discharge, subjects will not receive any study medication; this will be a “drug washout” phase. The research team member will be in contact with the subject to monitor for adverse effects.

8.1.8 VISIT 6 – DRUG START FOR PERIOD 2/ DAY 28±2 (OUTPATIENT, 1 HOUR)

The visit will be done at the TRI or ProSciento.

The following procedures will be completed for participants with T1DM:

- Physical exam
- Height and weight measurement
- BMI calculation
- Vital signs (Heart rate x 2, blood pressure x 2, respirations, temperature)
- CGM management and data review
- Review knowledge of insulin dose adjustments, ketone testing and sick day management, hypoglycemia symptoms and treatment
- Fingerstick blood glucose/personal CGM data, and insulin log review
- AE/Con-med Assessment

- Urinary pregnancy test
- Drug distribution for Period 2

During the following 14 days, subjects with T1DM will receive study medication, the opposite treatment arm, in a double-blinded manner.

8.1.9 VISIT 7 – FOLLOW UP VISIT/ DAY 35±2 (OUTPATIENT, 1-1.5 HOURS):

The visit will be done at the TRI or ProSciento.

The following procedures will be completed for participants with T1DM:

- CGM management and data review
- Review knowledge of insulin dose adjustments, ketone testing and sick day management, hypoglycemia symptoms and treatment
- Fingerstick blood glucose/personal CGM data, insulin and study medication log review
- ECG
- AE/Con-med Assessment

Data from the CGM will be analyzed primarily for the period commencing 7 days of dosing, and assuming steady-state drug concentrations that are within the desired therapeutic target range are achieved.

8.1.10 VISIT 8 – PERIOD 2/ DAY 41-42+5 (INPATIENT, 2 DAYS):

The visit will be done at the TRI or ProSciento.

Because moderate and severe episodes of hypoglycemia can have a transient effect of at least several days to dampen counter-regulatory responses to a subsequent episode of hypoglycemia (12, 15, 21) the 2nd clamp procedure may need to be re-scheduled so that a window of at least 5 days without moderate or severe episodes of hypoglycemia (blood glucose <55 mg/dl) precedes the clamp study (and supplemental study medication can be provided under such circumstances).

Subjects will be admitted to the TRI or ProSciento in the morning, the day prior to the clamp study (which will be conducted on the following morning).

The following procedures will be completed in participants with T1DM:

- Physical exam
- Height and weight measurement
- BMI calculation
- Vital signs (Heart rate x 2, blood pressure x 2, respirations, temperature) on each day of CRU staying
- ECG during the meal test
- AE/Con-med Assessment
- Follow up labs (fasting for at least 10 hours, water is permitted): CBC, CMP, HbA1c, lipid panel, thyroid function.
- Urine pregnancy test
- Urine for archive
- Standard meal challenge with pre- and post-meal blood draw to measure glucose, glucagon, total GLP-1, total GIP, and blood study medication levels (GRP119 PK).
- Overnight IV infusion of regular insulin

- Euglycemic and hypoglycemic clamp
- CGM management and data review
- Fingerstick blood glucose/personal CGM data, ketone, insulin and study medication log review
- Adherence to study drug

The study visit will be identical to those described for visit 5.

At the end of the visit the TRI or ProSciento provided CGM will be removed.

8.1.11 VISIT 9 EOT – SAFETY VISIT/ DAY 49±2 (OUTPATIENT, 1-1.5 HOURS):

The visit will be done at the TRI or ProSciento.

The following procedures will be completed for patients with T1DM:

- Vital signs
- Physical exam
- Height and weight measurement
- BMI calculation
- AE/Con-med Assessment
- Blood collection (fasting for at least 10 hours, water is permitted) for CBC, and CMP.

8.2 EFFICACY ASSESSMENTS

Continuous Glucose Monitoring (CGM)
Starting at Visit 2, participants with T1DM will wear a blinded continuous glucose monitor (CGM) for the duration of the study as a safety measure for glucose levels.
Healthy volunteers will wear the CGM for the 10 days preceding and including the clamps.

The CGM will be placed by a member of the study team (unless the participants is familiar and prefers to do it on their own), participants will be instructed on the use and removal of the device, as well as reinsertion of the device as needed. During CGM wear, participants will be instructed to perform standard fingerstick glucose measurements and/or continue with personal CGM to manage their blood glucose. Participants will be encouraged to document their blood glucose levels and insulin coverage on a home Glucose/Insulin log, for review by the medical study staff. The FDA-approved system (Dexcom) includes a sensor and transmitter. It measures interstitial fluid glucose levels in the range of 40 mg/dl to 400 mg/dl every 5 minutes.

Standardized meal test
Upon admission to the TRI or ProSciento, the participants will have an intravenous catheter inserted and afterwards will receive a standard mixed meal challenge of consistent composition and calorie amount on both admissions. The meal will comprise 25% of daily calorie requirement with a 48-50% Kcal from carbohydrate, 35% from fat and 15-17%% from proteins. The meal will be preceded by a pre-prandial dose of insulin calculated based on the participant insulin to carbohydrate ratio. 15 minutes prior to the start of the meal, a baseline sample will be drawn. From the start of meal, for the next 3.5 hours, at 30-minute intervals, blood samples will be obtained for glucose, glucagon, total GLP-1 and total GIP, and the study drug plasma levels (PK). The key objective of this meal study is to examine fasting and prandial glucagon responses while on GPR119 agonist treatment.

ECG will be performed during the meal test at the time of predicted drug concentration peak, 180 minutes, ±5minutes window, after taking the medication.

Euglycemic and hypoglycemic clamp procedures

At approximately 6 a.m., a primed, continuous infusion of [6,6] di-deuterated glucose will be started to enable determination of hepatic glucose production (HGP) and systemic glucose utilization via the glucose isotope dilution method; at least 2.5 hours will be allowed for isotope equilibration. The principal target of glucagon action is the liver, and HGP more specifically, and assessing this parameter will give physiological context to any observed changes in glucagon secretion during induced hypoglycemia.

At about 6 a.m. T1DM subjects will take the final dose of the study medication. A catheter will be placed in a vein on contralateral hand or arm and the arm warmed (via heating box or warming pad) to achieve "arterialization" of blood samples. Around 8:30am, shortly before the clamp insulin infusion start, the overnight insulin infusion will be discontinued. The euglycemic portion of the clamp will commence with insulin infusion at a constant rate of 25 mU/min·m^2. Blood / plasma glucose will be measured every 5 minutes and allowed to decrease until it reaches a value of approximately 90 mg/dl, at which point a 20% dextrose (D20) spiked with [6,6] di-deuterated glucose will be given (glucose infusion rate; GINF), at an adjustable rate, in order to maintain blood glucose at approximately 85 mg/dl. At approximately 9:30 a.m. (or following at least 15 minutes of stable euglycemia, operationally defined as 85 ± 10 mg/dl, 3 sets of blood samples at 10 min intervals will be obtained for glucagon, insulin, catecholamines, cortisol, growth hormone, GLP-1 and GIP, and glucose enrichment.

Subsequent to the 3rd baseline sample, the GINF will be stopped, the 25 mU/min-m^2 insulin infusion will be continued, and the hypoglycemic portion of the clamp study will begin. Blood glucose will be allowed to decrease over ~30 min until values of 50 to 55mg/dl are observed and then will be maintained at this level for approximately 30 minutes by infusion of D20. Mild fluctuation of blood glucose values during the hypoglycemic portion of the clamp can be expected. As the blood glucose values approach the target level of hypoglycemia, an adjustable rate GINF infusion can be started at a low rate to prevent overshoot into more severe hypoglycemia and GINF adjusted as needed to maintain the target level of hypoglycemia. Blood sampling for glucose will occur every 5 minutes and at every 10 minutes for a panel of hormones and metabolite determinations (as described above). A questionnaire will be administered during the study to quantitate autonomic symptoms as the sum of scores ranging from 0 (none) to 5 (severe) for each of the following symptoms: anxiety, palpitations, sweating, tremor, hunger, and tingling.

After 30 minutes of sustained hypoglycemia, the insulin infusion will be discontinued, and the recovery phase of the clamp will start. Including the time needed to reach hypoglycemia (induction), it is estimated that this hypoglycemic phase of the clamp will be approximately 60 minutes. The time to recover to euglycemia (operationally defined as a target blood glucose of 85+/- 5 mg/dL) will be ascertained. Once insulin infusion has been stopped, to prevent potential further decline in plasma glucose, GINF will initially be continued, at least until plasma glucose has risen above 70 mg/dl, at which point GINF will be rapidly tapered and discontinued over the next 10 minutes. GINF will not be re-instituted unless plasma glucose declines again into the range of 50 mg/dl. In situations where blood glucose values decrease remain less than 50 mg/dl, at any time during the recovery phase, additional glucose will be given as a safety measure. Blood samples for glucagon, counter-regulatory hormones and glucose enrichment will be obtained every 10 minutes for 30 minutes, then every 30 minutes during the recovery period. The recovery phase of the study will continue until one hour into recovery, at which point a meal will be provided, and blood glucose values monitored until euglycemic values are attained.

Samples for PK studies will also be obtained.

Efficacy laboratory measures

During the standardized meal test the following will be measured:

Mixed Meal Tolerance (MMT)									
Time (min)	**-15**	**-1**	**30**	**60**	**90**	**120**	**150**	**180**	**210**
Glucose	x	x	x	x	x	x	x	x	x
Glucagon	x	x	x	x	x	x	x	x	
GLP-1	x	x	x	x	x	x	x	x	
GIP	x	x	x	x	x	x	x	x	
GPR119PK		x	x	x	x		x		x

Table 2

During the clamp studies, samples for blood / plasma glucose will be measured with an automated glucose analyzer and recorded on the study flow sheet, along with the rate of GINF at 5-minute intervals. All the other samples, per the flow sheet, will be collected, placed on ice at the time of collection and after centrifugation by study staff, aliquots will be frozen at -80° until analyses.

	Hyperinsulinemic euglycemic and hypoglycemic glucose clamp																				
	Baseline	**Prior to Insulin infusion**				**Euglycemic phase**					**Euglycemic Steady state** *(maintenance)*			**Transition from Euglycemic to Hypoglycemic phase**			**Hypoglycemic phase steady state** *(maintenance)*			**Recovery phase**	
Sample nomenclature	Baseline Lab Draws	-150	-20	-10	0	10	20	30	40	50	E10 min	E20 min	E30 min		T15 min		H10 min	H20 min	H30 min	R30 min	R60 min
Time (min)*	**Prior to -150**	**-150 Start Glucose Test**	**-20**	**-10**	**0**	**10**	**20**	**30**	**40**	**50**	**60**	**70**	**80**		**100**		**120**	**130**	**140**	**170**	**200**
Glucagon			x	x	x	x	x	x	x	x	x	x	x		x		x	x	x	x	x
Insulin			x	x	x	x	x	x	x	x	x	x	x		x		x	x	x	x	x
Glucose enrichment	x		x	x	x						x	x	x				x	x	x	x	x
Catecholamines			x		x			x			x	x	x				x	x	x	x	x
Cortisol			x		x			x			x						x	x	x	x	x
Growth Hormone			x		x			x			x						x	x	x	x	x
GLP-1, GIP			x		x			x			x	x	x				x		x	x	x
GPR119 PK	x				x						x						x				x
Exosome					x						x								x		x
FFA			x		x			x			x	x	x		x		x	x	x	x	x
Archive					x						x								x		x

Table 3

*Time 0 corresponds to the CLAMP insulin 25 infusion start

Glucagon and other hormone assays will be done using the paired samples from the two studies performed in each T1DM subject to reduce inter-assay variations. Because it has long been recognized that glucagon assays can be difficult, and can have relatively high levels of non-specific background, particular care will be made to select the most accurate available glucagon assay(s), and depending upon sample requirements, to run these in duplicate or triplicate.

PK determinations of study drug concentrations will be done by the pharmaceutical partner.

Additional plasma samples obtained during the clamp (baseline, end of euglycemic clamp, end of hypoglycemic phase, end of recovery) will be archived.

9 SAFETY AND OTHER ASSESSMENTS

Medical History and Physical Exam
A Health History will be obtained. A standard physical examination will be performed by a study physician, physician assistant, or nurse practitioner.
Physical exam will include general status, skin, HEENT (including fundoscopic examination), neck, cardiovascular, lungs, abdomen, upper and lower extremities, neurological, musculoskeletal and psychiatric systems.

Anthropometric Measures
Body weight (calibrated scale), height, will be obtained while in a gown, without shoes. BMI will be calculated.

Screening Labs
The laboratory assessments will be used to confirm study eligibility (inclusion/exclusion criteria), as well as overall health status:

- complete blood count (CBC) white blood count, red blood count, Hemoglobin, Hematocrit, MCV, MCH, MCHC, RDW, Platelet Count, MPV, Neutrophils, Lymphocytes, Monocytes, Eosinophils, Basophils, Abs Neutrophil Count, Abs Lymphocyte Count, Abs Monocyte Count, Abs Eosinophil Count, Abs Basophil Count
- comprehensive metabolic panel (CMP) (Anion Gap, Albumin, Globulin, A/G Ratio, Alkaline Phosphatase, ALT, AST, Bilirubin Total, BUN, Creatinine, eGFR by CKD-EPI, Calcium, Chloride, CO_2, Creatinine, Glucose, Potassium, Sodium, Total Protein)
- HbA1C
- lipid panel (Total Cholesterol, HDL, LDL, Chol/HDL ratio, LDL/HDL Ratio, Non-HDL Cholesterol, Triglycerides, VLDL, CHD Risk Assessment)
- thyroid function test: thyroid stimulating hormone (TSH) and free T4
- C-peptide (requires a blood glucose $\geq$ 90mg/dL to be accurate)
- islet autoantibody panel (anti GAD Ab, anti IA-2 Ab, Anti ZnT8 Ab)
- Hepatitis B and Hepatitis C antibody tests [Hepatitis B Surface Antigen (HBsAg), Hepatitis B Surface Antibody (HBsAb), Hepatitis C Surface Anitbody (HCsAB)]
- HIV 1 &2 Ag/Ab Screening
- urinalysis
- Urine toxicology screening test (Amphetamine, Barbiturate, Benzodiazepine, Cocaine, Methadone, Opiate, THC, Tricyclics, Oxycodone

- Serum Beta HCG for pregnancy in female subjects of childbearing potential

Screening labs can be repeated per PI discretion.

Follow up labs

- Complete blood count (CBC) white blood count, red blood count, Hemoglobin, Hematocrit, MCV, MCH, MCHC, RDW, Platelet Count, MPV, Neutrophils, Lymphocytes, Monocytes, Eosinophils, Basophils, Abs Neutrophil Count, Abs Lymphocyte Count, Abs Monocyte Count, Abs Eosinophil Count, Abs Basophil Count
- Comprehensive metabolic panel (CMP) (Anion Gap, Albumin, Globulin, A/G Ratio, Alkaline Phosphatase, ALT, AST, Bilirubin Total, BUN, Creatinine, eGFR by CKD-EPI, Calcium, Chloride, CO_2, Creatinine, Glucose, Potassium, Sodium, Total Protein)
- HbA1C
- lipid panel (Total Cholesterol, HDL, LDL, Chol/HDL ratio, LDL/HDL Ratio, Non-HDL Cholesterol, Triglycerides, VLDL, CHD Risk Assessment)
- Thyroid function test: thyroid stimulating hormone (TSH) and free T4• urine pregnancy test, for female subjects of childbearing potential

The **total amount of blood** required for the study will be about 547.1 ml during the whole study.

Vital Sign Measurements
Measurement of vital signs will include heart rate (HR), blood pressure (BP), respirations and temperature will be measured sitting for at least 5 min.

Electrocardiograms (ECGs): ECG will be done at screening and during intervention periods at visits 4 and 5, and visits 7 and 8. At visits 5 and 8, the ECG will be performed during the meal test at the time of predicted drug concentration peak 180 min after taking the medication. The QTcF will be manually calculated for each ECG.

Fingerstick glucose and ketone measures
Fingerstick glucose and/or monitoring of personal CGM will be encouraged to measure/monitor at home by the participant before meals and at bedtime.
Participants will be instructed to check urinary ketones and in case of symptoms indicative of ketoacidosis or blood glucose >250 mg/dl.

Counseling procedures and Insulin dose adjustments
The participants will be instructed to check their blood glucose with a glucometer/personal CGM device before each meal and at bedtime. The participants may be instructed on insulin dose adjustments, ketone testing and sick day management, hypoglycemia symptoms and treatment.

Assessment of adverse events. See below.

9.1 ADVERSE EVENTS AND SERIOUS ADVERSE EVENTS

9.1.1 DEFINITION OF ADVERSE EVENTS (AE)

Adverse event means any untoward medical occurrence associated with the use of an intervention in humans, whether or not considered intervention-related (21 CFR 312.32 (a)).

9.1.2 DEFINITION OF SERIOUS ADVERSE EVENTS (SAE)

An adverse event (AE) or suspected adverse reaction is considered "serious" if, in the view of either the investigator or sponsor, it results in any of the following outcomes: death, a life-threatening adverse event, inpatient hospitalization or prolongation of existing hospitalization, a persistent or significant incapacity or substantial disruption of the ability to conduct normal life functions, or a congenital anomaly/birth defect. Important medical events that may not result in death, be life-threatening, or require hospitalization may be considered serious when, based upon appropriate medical judgment, they may jeopardize the participant and may require medical or surgical intervention to prevent one of the outcomes listed in this definition.

9.1.3 CLASSIFICATION OF AN ADVERSE EVENT

9.1.3.1 SEVERITY OF EVENT

Adverse event severity will be graded as follows:

- **Mild** – Events require minimal or no treatment and do not interfere with the participant's daily activities.
- **Moderate** – Events result in a low level of inconvenience or concern with the therapeutic measures. Moderate events may cause some interference with functioning.
- **Severe** – Events interrupt a participant's usual daily activity and may require systemic drug therapy or other treatment. Severe events are usually potentially life-threatening or incapacitating. Of note, the term "severe" does not necessarily equate to "serious".

9.1.3.2 RELATIONSHIP TO STUDY INTERVENTION

All adverse events (AEs) will have their relationship to study intervention assessed by the clinician who examines and evaluates the participant based on temporal relationship and his/her clinical judgment. The degree of certainty about causality will be graded using the categories below. In a clinical trial, the study product must always be suspect.

- **Definitely Related** – There is clear evidence to suggest a causal relationship, and other possible contributing factors can be ruled out. The clinical event, including an abnormal laboratory test result, occurs in a plausible time relationship to study intervention administration and cannot be explained by concurrent disease or other drugs or chemicals. The response to withdrawal of the study intervention (de-challenge) should be clinically plausible. The event must be pharmacologically or phenomenologically definitive, with use of a satisfactory re-challenge procedure if necessary.
- **Probably Related** – There is evidence to suggest a causal relationship, and the influence of other factors is unlikely. The clinical event, including an abnormal laboratory test result, occurs within a reasonable time after administration of the study intervention, is unlikely to be attributed to concurrent disease or other drugs or chemicals, and follows a clinically reasonable response on withdrawal (de-challenge). Re-challenge information is not required to fulfill this definition.
- **Potentially Related** – There is some evidence to suggest a causal relationship (e.g., the event occurred within a reasonable time after administration of the trial medication). However, other factors may have contributed to the event (e.g., the participant's clinical condition, other concomitant events). Although an AE may rate only as "possibly related" soon after discovery, it

can be flagged as requiring more information and later be upgraded to “probably related” or “definitely related”, as appropriate.

- **Unlikely to be related** – A clinical event, including an abnormal laboratory test result, whose temporal relationship to study intervention administration makes a causal relationship improbable (e.g., the event did not occur within a reasonable time after administration of the study intervention) and in which other drugs or chemicals or underlying disease provides plausible explanations (e.g., the participant’s clinical condition, other concomitant treatments).
- **Not Related** – The AE is completely independent of study intervention administration, and/or evidence exists that the event is definitely related to another etiology. There must be an alternative, definitive etiology documented by the clinician.

9.1.3.3 EXPECTEDNESS

The PI and medical investigator will be responsible for determining whether an adverse event (AE) is expected or unexpected. An AE will be considered unexpected if the nature, severity, or frequency of the event is not consistent with the risk information previously described for the study intervention as reported in the IB.

9.1.4 TIME PERIOD AND FREQUENCY FOR EVENT ASSESSMENT AND FOLLOW-UP

The occurrence of an adverse event (AE) or serious adverse event (SAE) may come to the attention of study personnel during study visits, and interviews of a study participant presenting for medical care, or upon review by a study monitor.

All AEs including local and systemic reactions not meeting the criteria for SAEs will be captured on the appropriate case report form (CRF). Information to be collected includes event description, time of onset, clinician’s assessment of severity, relationship to study product (assessed only by those with the training and authority to make a diagnosis), and time of resolution/stabilization of the event. All AEs occurring after signing the informed consent must be documented appropriately regardless of relationship. All AEs will be followed to adequate resolution.

Changes in the severity of an AE will be documented to allow an assessment of the duration of the event at each level of severity to be performed. AEs characterized as intermittent require documentation of onset and duration of each episode.

A study team member will record all reportable events with start dates occurring any time after informed consent is obtained until 7 days after the last day of administration of the medication. At each study visit, a research team member will be responsible for notifying the Food and Drug Administration (FDA) of any unexpected fatal or life-threatening suspected adverse reaction as soon as possible, but in no case later than 7 calendar days after the initial acquisition of the information.

9.1.5 REPORTING EVENTS TO PARTICIPANTS

N/A

9.1.6 EVENTS OF SPECIAL INTEREST

High and low glycemic events will be recorded by CGM and through the participant's history and glucose log at each visit. Hypoglycemia will be defined and recorded according to the American Diabetes Association guidelines (Level 1 hypoglycemia is defined as a measurable glucose concentration <70 mg/dL (3.9 mmol/L) but ≥54 mg/dL (3.0 mmol/L). Level 2 hypoglycemia (defined as a blood glucose concentration <54 mg/dL [3.0 mmol/L]) is the threshold at which neuroglycopenic symptoms begin to occur and requires immediate action to resolve the hypoglycemic event. Level 3 hypoglycemia is defined as a severe event characterized by altered mental and/or physical functioning that requires assistance from another person for recovery. Hypoglycemic events are among the endpoints measured to evaluate the effects of MBX-2982 on patterns of glycemia as described in section 3.

9.1.7 REPORTING OF PREGNANCY

Pregnancy in a study participant will be reported among adverse events and reported to the IRB and regulatory agencies.
The study medication will be discontinued, and no additional study tests will be done, while continuing safety follow up.

9.2 UNANTICIPATED PROBLEMS

9.2.1 DEFINITION OF UNANTICIPATED PROBLEMS (UP)

The Office for Human Research Protections (OHRP) considers unanticipated problems involving risks to participants or others to include, in general, any incident, experience, or outcome that meets all of the following criteria:

- Unexpected in terms of nature, severity, or frequency given (a) the research procedures that are described in the protocol-related documents, such as the Institutional Review Board (IRB)-approved research protocol and informed consent document; and (b) the characteristics of the participant population being studied;
- Related or possibly related to participation in the research ("possibly related" means there is a reasonable possibility that the incident, experience, or outcome may have been caused by the procedures involved in the research); and
- Suggests that the research places participants or others at a greater risk of harm (including physical, psychological, economic, or social harm) than was previously known or recognized.

9.2.2 UNANTICIPATED PROBLEM REPORTING

The investigator will report unanticipated problems (UPs) to the reviewing Institutional Review Board (IRB). The UP report will include the following information:

- Protocol identifying information: protocol title and number, PI's name, and the IRB project number;
- A detailed description of the event, incident, experience, or outcome;
- An explanation of the basis for determining that the event, incident, experience, or outcome represents an UP;

- A description of any changes to the protocol or other corrective actions that have been taken or are proposed in response to the UP.

To satisfy the requirement for prompt reporting, UPs will be reported using the following timeline:

- UPs that are serious adverse events (SAEs) will be reported as described above (see SAE reporting).
- Any other UP will be reported to the IRB within 10 days of the investigator becoming aware of the problem.
- All UPs should be reported to appropriate institutional and regulatory officials (as required by an institution's written reporting procedures), the supporting agency head (or designee), and the Office for Human Research Protections (OHRP) within 10 business days, of the IRB's receipt of the report of the problem from the investigator per the guidelines per AH policy.

The TRI has a standing committee that meets monthly to review all adverse events in our clinical trials and will additionally be charged with review of the study. ProSciento will report adverse events to TRI monthly. SAE/Unanticipated/Pregnancy events will be reported via email/call during working hours. After hours ProSciento will contact our on call line 407-303-7100 or Dr. Richard Pratley via cell.

9.2.3 REPORTING UNANTICIPATED PROBLEMS TO PARTICIPANTS

N/A

10 STATISTICAL CONSIDERATIONS

10.1 STATISTICAL HYPOTHESES

For the primary endpoints, we hypothesize that the medication (14-day administration of MBX-2982) will increase the measured glucose counter-regulation to hypoglycemia expressed as the maximal glucagon concentration, AUC, and Incremental AUC (iAUC) for glucagon during hypoglycemia compared to placebo. For the secondary endpoints, we hypothesize that the medication:

- will increase hepatic glucose production during insulin-induced hypoglycemia and during a subsequent recovery to euglycemia;
- will reduce the time of recovery from hypoglycemia to euglycemia;
- will increase other counter-regulatory hormones;
- the above-mentioned effects will approach the counter-regulatory response in healthy (non-diabetic) volunteers during insulin-induced hypoglycemia;

Exploratory analysis will:

- evaluate for any effects of a GPR119 agonist upon fasting and/or postprandial glucagon concentrations in T1DM, together with potential effects on fasting and postprandial, GLP-1 and GIP;
- collect data patterns of glycemia of participants with T1DM while are receiving daily therapy with a GPR119 agonist (or placebo);
- evaluate the pharmacokinetic profile of MBX-2982 in participants with T1DM.

10.1.1.1 PRIMARY EFFICACY ENDPOINT(S):

- Maximal glucagon concentration during hypoglycemia
- Total area under the curve (AUC) for glucagon during hypoglycemia
- Incremental AUC for glucagon during hypoglycemia above baseline levels during euglycemia

10.1.1.2 SECONDARY EFFICACY ENDPOINT(S):

- HGP during insulin-induced hypoglycemia
- HGP during recovery form insulin-induced hypoglycemia to euglycemia
- Time of recovery from hypoglycemia (time to reach 85 mg/dL
- Plasma levels of counter regulatory hormones during hypoglycemia: epinephrine, cortisol, GH

10.1.1.3 EXPLORATORY ENDPOINT(S):

- Levels of GLP-1, GIP in fasting and post-prandial conditions
- Continuous glucose monitoring (CGM) indices of glycemic control including hypoglycemia (percent of time < 70 mg/dL, < 54 mg/dL), hypoglycemic events, mean glucose, percent of time 70-180 mg/dL, percent of time > 180 mg/dL, percent of time > 250 mg/dL, glycemic variability.
- MBX-2982 plasma levels

10.2 SAMPLE SIZE DETERMINATION

It is not precisely known how much of an improvement in glucagon counter-regulation is needed to be clinically significant for mitigating risk of hypoglycemia. Restoring glucagon counter-regulation to levels observed in people without diabetes would meet and quite likely exceed expectations, this may be too high a bar by which to judge success or failure in this initial short-term study. We estimate that a lower boundary of meaningful therapeutic effect would be at least a 30% increase in glucagon counter-regulation and have used this criterion to estimate sample size for the current study.

According to a published study that measured glucagon counter-regulation to hypoglycemia in T1DM (19), of the 28 T1DM subjects, the maximal glucagon concentration in placebo treated T1DM group during hypoglycemia was 23 pmol/l, while the standard deviation was 10.4 pmol/l. To apply these statistics in the current crossover design, 16 subjects are required to detect a 30% increase (6.9 pmol/l) in the GPR119 agonist treated group compared to a placebo treated group, this will have an 0.8 power, assuming a 0.5 correlation coefficient between two treatments within the same subjects, and a one-sided paired t-test at 0.05 significant level. Accounting for a potential (up-to) 25% dropout rate, 20 T1DM subjects are expected to be recruited to the current study. A sample size of 9 Healthy subjects should be adequate to establish the normative (descriptive) data using the same assays and clamp procedures as being used for T1DM.

10.3 RANDOMIZATION

Randomization schedule based on the two-treatment, two-period, two-sequence cross-over design for 20 patients will be generated by a biostatistician using SAS (9.4). The treatment assignments will be coded as A or B. This information will only be released as the patients are enrolled. The message communicating group assignment will be saved in the subject's study file.

10.4 BLINDING

The randomization schedule will be delivered to the pharmacist, who is the only unblinded person of the study. The TRI pharmacist will provide the randomization schedule to the ProSciento pharmacist. Then the pharmacist will determine the treatment assignments (placebo or GPR119) based on the treatment codes (A or B). The unblinded pharmacist will prepare the study drug, and then give to the designated staff member for administration. Allocation concealment, unexpected and/or severe adverse events will be reported to the AdventHealth Orlando IRB or ProSciento's IRB of record.

10.4.1 INSTITUTIONAL REVIEW BOARD

Prior to study initiation the protocol and the informed consent documents will be reviewed and approved by the Institutional Review Board (IRB) of record. Any amendment to the protocol or consent materials must also be approved by the IRB before they are implemented.

The study will be reviewed and overseen by the AdventHealth Orlando Institutional Review Board for the Translational Research Institute The study will be reviewed and overseen by ProSciento's IRB of record for ProSciento.

10.4.2 DATA AND SAFETY MONITORING BOARD (DSMB) OR EQUIVALENT

Not required.

10.4.3 MONITORING PLAN

Clinical trial monitoring is conducted to ensure that the rights and well-being of trial participants are protected. Reported trial data are accurate, complete and verifiable.

Clinical trial monitoring will be conducted by AdventHealth Office of Research Integrity monitoring team to ensure compliance with all applicable federal and state regulations, ICH GCP E6 (R2) standards and approved protocol. This study will be monitored using a risk-based approached. A monitoring plan will be created to ensure patient safety, data quality and integrity is ongoing throughout the life of the study. The frequency and elements of monitoring will be outlined in the monitoring plan. The monitor will ensure study is conducted, recorded and reported as required by federal regulations and IRB of record. Monitoring will be conducted on-site and/or remote. After completion of site monitoring, a report will be provided to PI and study team. The PI and study team are required to review the report and work on significant findings or discrepancies to resolution. If any findings are required to be reported to the IRB, the PI will be made aware and prompted to self-report. All serious and continuing non-compliance is shared with the Research Oversight Committee who may require appropriate actions.

10.4.4 QUALITY ASSURANCE AND QUALITY CONTROL

We attest that all AdventHealth Translational Research Institute faculty and staff and ProSciento staff will be trained, and this training will be documented as described in AdventHealth Translational Research Institute Work Instruction 031.100.015 Documentation of Protocol Training and ProSciento.

We will implement regular, ongoing discussions between the PI and coordinator as per the AdventHealth Translational Research Institute SOP 030.000.002 Oversight of Research Studies at the Translational Research Institute. The coordinator will review source and communicate with all applicable study team members involved in the study on a regular basis regarding reportable new information, implementing amendments, study progress, and quality assurance/control.

We will implement regular, ongoing discussions between the ProSciento PI and ProSciento coordinator. The ProSciento coordinator will review source and communicate with all applicable study team members involved in the study on a regular basis regarding reportable new information, implementing amendments, study progress, and quality assurance/control.

The AdventHealth Translational Research Institute facilities and ProSciento have state of the art and have within each building all required resources and staff to execute the study. The TRI has a Medical Oversight team, Medical Oversight Committee, as well as a Quality Committee to appropriately monitor and address adverse events.

The AdventHealth Translational Research Institute and research team assigned to this protocol will meet prior to initiation to assure that the team members' roles and the required timelines are clear. Protocol specific training will be carried out with the research team members designated in the Delegation of Authority Log. Re-training will occur at any time IRB reviewed and approved amendments or revisions to this protocol are made. Documentation of all training will be kept in the regulatory binder.

10.5 MATERIALS OF HUMAN ORIGIN: COLLECTION, PREPARATION, HANDLING AND SHIPPING

The study will collect and archive biological specimens (blood, urine) from the study participants to achieve the study endpoints and for safety purposes. Any diagnostic testing will be performed at a CLIA accredited lab.

Biospecimens collected for study-related endpoints, will be analyzed/tested: both at AdventHealth Orlando and outside laboratories/institutions. Assays for pharmacokinetic endpoints on de-identified samples will be performed at Covance Laboratory Inc.

Additionally, remaining biospecimens will be archived for any additional hypothesis-related experimentation or testing for this study, which cannot be predicted at the time the protocol is developed. Furthermore, if there are any left-over biospecimens after completing the endpoints described above, any left-over biospecimens may be archived for other research (not for this study), of any type (without limitation to disease, process, or research methods). This other research can take place at AdventHealth Orlando or at outside institutions.

Lastly, a predetermined amount of biospecimen samples will be collected specifically for archiving for future use, such as other research (not for this study), of any type (without limitation to disease, process, or research methods). This other research can take place at AdventHealth Orlando or outside institutions.

The biospecimens collected for this study will be separated into biospecimen samples that will be used for the study and biospecimen samples that were collected to be archived for future use. After study aims have been achieved and study related endpoints have been measured and analyzed, any remaining biospecimens will be stored at the TRI Biorepository and will also be considered as "archived biospecimens." Archived biospecimens will be used for any additional hypothesis-related experimentation or testing for the purposes of this study, consistent with the original aims, which cannot be predicted at the time the protocol is developed due to the evolving nature of scientific exploration.

Additionally, archived biospecimen samples may be stored indefinitely for future research. Archived biospecimens could be used for separate research by both AdventHealth Orlando scientists and scientists outside of AdventHealth Orlando. This would be allowed for research of any type (without limitation to disease, process, or research methods) if it has scientific merit as determined by the TRI Scientific Review Committee. For research outside of AdventHealth Orlando, a Material Transfer Agreement will be obtained, which will govern the transfer and chain of custody of the biospecimens outside of ADVENTHEALTH ORLANDO. Some of the endpoint testing will be conducted at outside laboratories/institutions. To perform these analyses/testing/etc. and to interpret results, certain data elements will need to be shared along with the biospecimen samples. Data Use Agreements (DUAs) will be obtained, which will identify the specific data elements to be shared and will govern the sharing of data related to this study. Data will be de-identified, but a link/code is managed within an electronic research management system and maintained by the research team.

Should archived biospecimens be needed for research outside of AdventHealth Orlando and certain data elements that are connected to the archived biospecimen samples are needed to conduct the research, then Data Use Agreement(s) will be obtained. The Data Use Agreement(s) will identify the purpose for data sharing, the specific data elements to be shared, and will govern the sharing of data related to this study. Data will be de-identified, but a link/code is managed within an electronic research management system and maintained by the research team.

10.6 DATA HANDLING AND RECORD KEEPING

10.6.1 DATA COLLECTION AND MANAGEMENT RESPONSIBILITIES

The AdventHealth Translational Research Institute will act as the coordinating center throughout the study. Data collection will be performed via case report forms (CRF) at each site. Data from the CRFs will then be transferred into an electronic database. The AH-REDCap (Research Electronic Data Capture) will be used as the central location for data processing and management. All study activities will be conducted in coordination with the study PI/study coordinator and the clinical sites. Each site investigator is responsible for complete data ascertainment at their site and entry into the database.

Data quality control will occur according to our SOPs on Data Entry, Quality Control Procedures and Query Management. All data will be entered into an electronic data capture (EDC) system and checked against the paper source for accuracy by a second party (Data Entry SOP) and errors resolved through the Query

Management SOP. Ten percent of the data points will be routinely checked at the beginning, middle, and close of a study for quality control (Quality Control SOP). Finally, all critical endpoints (as determined by the PI or Sub-I) will be assessed using quality control analyses. The data will be loaded into the clinical research database. Data in the warehouse will also be routinely monitored over time.

10.6.2 STUDY RECORDS RETENTION

AdventHealth Translational Research Institute retention policy is maintained in the Records Management Policy. Electronic de-identified data will be kept indefinitely in our data warehouse.
Per the institutional policy, investigator records must be kept for a minimum of 7 years after completion of discontinuation of the study, or for longer if required by applicable regulations.

ProSciento will maintain records on site until after study closure. After study closure records will be stored in a secured offsite facility according to ProSciento's policy.

10.6.3 DATA SHARING

Each site will have appropriate Data Use Agreements in place prior to sharing of any data.

10.6.4 PROTOCOL DEVIATIONS

A protocol deviation is any noncompliance with the clinical trial protocol or International Conference on Harmonization Good Clinical Practice (ICH GCP) requirements. The noncompliance may be either on the part of the participant, the investigator, or the study site staff. As a result of deviations, corrective actions are to be developed by the site and implemented promptly.

These practices are consistent with ICH GCP:

- 4.5 Compliance with Protocol, sections 4.5.1, 4.5.2, and 4.5.3
- 5.1 Quality Assurance and Quality Control, section 5.1.1
- 5.20 Noncompliance, sections 5.20.1, and 5.20.2.

It is the responsibility of the Sponsor to use continuous vigilance to identify and report deviations. All deviations must be addressed in study source documents. ProSciento will report all deviations to the IRB of Record per policy and to TRI. All deviations will be reported to AH ORI and regulatory team.

10.6.5 PUBLICATION AND DATA SHARING POLICY

Sharing of anonymized data with other organizations would be allowed, as defined in respective Data Use Agreements, for research of any type if the research has scientific merit as determined by the TRI Scientific Review Committee.

Some of the endpoint testing will be conducted at outside laboratories/institutions. To perform these analyses/testing/etc. and to interpret results, certain data elements will need to be shared along with the biospecimen samples. Data Use Agreements will be obtained, which will identify the specific data elements to be shared and will govern the sharing of data related to this study. Data will be de-identified, but a link/code is managed within an electronic research management system and maintained by a study coordinator.

Should archived biospecimens be needed for research outside AdventHealth Orlando and certain data elements that are connected to the archived biospecimen samples are needed to conduct the research, then Data Use Agreement(s) will be obtained. The Data Use Agreement(s) will identify the purpose for data sharing, the specific data elements to be shared, and will govern the sharing of data related to this study. Data will be de-identified, but a link/code is managed within an electronic research management system and maintained by a study coordinator.

TRI will publicly disclose data. Whenever possible, data dissemination will occur through presentation at major scientific conferences and/or publication in peer-reviewed journals, and will be complete, accurate, balanced, and timely. De-identified data will be made available to qualified investigators upon the completion of the study.
The study will be published on the ClinicalTrials.gov website.

10.6.6 CONFLICT OF INTEREST POLICY

The study leadership and AdventHealth has established policies and procedures for all study group members to disclose all conflicts of interest and will establish a mechanism for the management of all reported dualities of interest.

10.7 ADDITIONAL CONSIDERATIONS

N/A

11 PROTOCOL AMENDMENT HISTORY

The table below is intended to capture changes of IRB-approved versions of the protocol, including a description of the change and rationale. A Summary of Changes table for the current amendment is located in the Protocol Title Page.

Version	Date	Description of Change	Brief Rationale
V9.0	29Sep2022	**Screening visit, pg. 36:** definition of non-childbearing potential women updated. **Table 3, pg. 42-43:** The glucose tracer time would be the (-150) timepoint. The Baseline lab draw will be performed shortly before (-150) the glucose tracer timepoint.	It was updated to align with exclusion # 16.
V8.0	08Aug2022	**Exclusion # 20:** marijuana use ≥3 days/week in any form **Exclusion # 24 & 25:** Allowing stable use of the antiepilectic drugs, however, uncontrolled seizure is exclusionary. **Exclusion # 29:** Added: **"***Unwilling and/or unable to follow and comply with scheduled visits and protocol requirements*" **Screening:** Added "up to"	"Evidence for whether cannabis use has beneficial or adverse effects in diabetes patients remains inconclusive." Furthermore, there is increasing use of cannabis recreationally, regardless of state laws, that is becoming more widely accepted socially. This increase has been seen broadly across different socio-economic and demographic groups. Given the numbers of diabetics, including younger type 1 diabetics, that are consuming cannabis, it is important to understand and observe the prevalence of marijuana use among this population and to capture data that may provoke new questions or suggest some mechanisms for its impact, whether beneficial or adverse, on glucose and metabolic regulation. To ensure ppts compliance with the protocol requirements and study visits Clarification regarding the screening window

		Glucose Clamp: given the meal at the end of the clamp procedure not requiring the blood sugar to be at 85; due to some time points the ECG, IP, blood draws were happening at the same times, it was added a window **ConMeds:** When we originally wrote the protocol, we excluded ppts on drugs that could impact weight or metabolism.	In completing 10 ppts with T1D and 2 HNV, we have come up with several small changes to our glucose clamp procedures to improve flow and data acquisition. We have had several ppts who have been on such medications for several years. In this case, we think it is highly unlikely that chronic use of these drugs would materially affect weight or metabolic responses.
V7.0	28Feb2022	**ConMeds:** When we originally wrote the protocol, we excluded ppts on drugs that could impact weight or metabolism. **Glucose Clamp procedure:** given the meal at the end of the clamp procedure; shorten the timeframe of the required stable euglycemia to have 15 min before starting euglycemic steady state blood collection and widen the range of 85 mg/dl (+/- 10) for their blood sugar level; T1D and healthy ppts euglycemia level changed to 70 mg/dl; in the recovery phase the blood glucose levels will be monitored every 15-30 minutes until stable **Exclusion # 1:** BMI >35 kg/m^2	We have had several ppts who have been on such medications for several years. In this case, we think it is highly unlikely that chronic use of these drugs would materially affect weight or metabolic responses. In completing 3 ppts with T1D and 2 HNV, we have come up with several small changes to our glucose clamp procedures to improve flow and data acquisition. Per NIH the T1DM population has a BMI significantly higher than the mean BMI general population
V5.0	19Jul2021	**Exclusion criteria # 4** (Labs may need to be repeated if FBG $\leq$ 90) **Section "Description and Study Interventions and Section 4.1** The last dose of treatment/placebo will be given in the morning (before the tracer) before the euglycemic/	Clarifications to ensure that C-peptide value is accurate, which requires a FBG $\geq$ 90 As the clamp timing is hard to predict, changed the verbiage and timing for more consistency and clearance.

		hypoglycemic glucose clamp is started. Regarding the clamp procedures, there will be an initial euglycemic run-in phase of the clamp **(~85 mg/dL)** –it was changed from 95 to 85 **Section: Schedule of Events and section 4.1, 8.1.3:** Added a line for total CGM wear time and foot note ** Healthy volunteers will wear the CGM for the 10 days preceding and including the clamps. **Section 2.3.3.2 and Exclusion # 16** Rewording/ clarifying methods of barrier methods **Section 8.1.4** Blood draw for archive – added **(fasting for at least 10 hours)** **Section 8.1.6** Overnight Blood glucose parameters changed to 80-120 CGM/**POC** (added POC) Statement added during wash out period for the CGM wear time **Section 8.2** Added: Around 8:30am, shortly before the clamp insulin infusion start, the overnight insulin infusion will be discontinued. **Table 3:** Nomenclature and times modified	Oversight – to keep the protocol BG goals consistent Added for clarification about CGM wear times. Clarifications to reflect the acceptable methods of pregnancy prevention Clarifications that blood sample is fasting. Modified to keep the ppt at a more normal BG level. Added POC to allow flexibility if CGM reading not available Added for clarification Change the stop overnight insulin time to right before the start of clamp insulin time to reduce the time frame insulin Is turned off and the participant blood glucose could increase. Modifications made to distinguish the different clamp intervals and corresponding time frames/sample collections.
V4.0	01Apr2021	**Section 5.2** Exclusion: Additional exclusion Criteria for the type 1 diabetes cohort	This allows participants to continue to monitor and manage their Diabetes more efficient.

		-Removed Exclusion criteria # 6: "Not willing/able to stop the use of their own CGM." **Section 8.1.3** -Added "TRI provided" CGM -Added "and/or personal CMG" -Removed the statement " *If subjects normally wear their own CGM, that should be removed."* **Section 8.1.4, 8.1.5, 8.1.6, 8.1.7, 8.1.8, 8.1.9, and 8.1.10** -Added "*personal CGM data*" **Section 8.1.6** -Added "(*Type 1 diabetes only*)" **Section 8.1.10** -Added "*TRI provided*" **Section 8.2** -Added "*and/or continue with personal CGM"* **Section 9** **Fingerstick glucose and ketone measures** -added "*and/or monitoring of personal CGM will be encouraged to measure/monitor"* at home by the participant before meals and at bedtime. **Counseling procedures and Insulin dose adjustments** -added "*personal CGM device*"	Clarifications made for which CGM is being referred too. Removed to reflect Exclusion #6 removal. Clarifications made for which CGM is being referred too Clarifications made for which Cohort will undergo the procedure/test/task Clarifications made for which CGM is being referred too Clarification to incorporate CMG monitoring for Blood glucose checks and diabetes management. Clarification to incorporate CMG monitoring for Blood glucose checks and diabetes management.
V3.0	10Aug2020	Description of Study Intervention: Schedule of Activities (SOA) *Section 5.2* Exclusion criteria 8 was split to Exclusion # 8 and Exclusion #9 for clarification. *Section 6.2.1*	Clarifications on the days when not to take study medication Added which cohort will be completing the visit.

		Sentence "will be used only on subjects and be used only by authorized investigators." Was blue and changed to black ink. *Section 8.1.1* Added optional remote consent language *Section 8.1.6* Removed specific test and added reference to table. *Section 8.2* -Added partial sentence: "(unless the participants is familiar and prefers to do it on their own)" *section Standardized meat test:* Removed "exosome" test. Section: Euglycemic and hypoglycemic clamp procedures: -Changed 3hrs to 2.5 hours -removed 7:00 am -MMT (table 2) - Endpoint samples added and removed -Clamp (table 3) - Endpoint samples added and removed *Section 9.0* -Total blood volume increased from 514ml to 547.1 ml - removed statement "suggestions will be provided to adjust the insulin treatment before and during the study. "	Added in the event remote consenting needs to be utilized. Added in the scenario the ppt would like to complete CGM insertion by themselves 3 hrs are not needed, 2.5 is sufficient Allows more time flexibility

12 REFERENCES

1. Gold AE, Macleod KM, Frier BM. Frequency of Severe Hypoglycemia in Patients With Type I Diabetes With Impaired Awareness of Hypoglycemia. Diabetes Care. 1994;17(7):697-703.

2. Geddes J, Wright RJ, Zammitt NN, Deary IJ, Frier BM. An Evaluation of Methods of Assessing Impaired Awareness of Hypoglycemia in Type 1 Diabetes. Diabetes Care. 2007;30(7):1868-70.

3. Lauffer LM IR, Brubaker PL. GPR119 is essential for oleoylethanolamide-induced glucagon-like peptide-1 secretion from the intestinal enteroendocrine L-cell. Diabetes. 2009;58:1058-66.

4. Flock G HD, Seino Y, Drucker DJ. GPR119 regulates murine glucose homeostasis through incretin responses receptor-dependent and independent mechanisms. Endocrinology. 2011;152:374-83.

5. Ekberg JH HM, Kristensen LV, Madsen AN, Engelstoft MS, Husted A-S, Sichlau R, Egerod KL, Timshel P, Kowalski TH, Gribble FM, Reiman F, Hansen HS, Howard AD, Holst B, Schwartz TW. GPR119, a major enteroendocrine sensor of dietary triglyceride metabolites coacting in synergy with FFA1 (GPR40). Endocrinology. 2016;157:4561-6.

6. Li NX BS, Kowalski T, Yang L, Wu M, Dai G, Petrov A, Woods HB, Wang L, Sherwin R, Kelley DE. . GPR119 agonism increases glucagon secretion during insulin-induced hypoglycemia. . Diabetes. 2018;67:1401-13.

7. Segerstolpe A PA, Eliasson P, Andersson E-M, Andreasson A-C, Sun X, Picelli S, Sabirsh A, Calusen M, Bjursell MK, Smith DM, Kasper M, Ammala C, Sandberg R. Single-cell transcriptome profiling of human pancreatic islets in health and type 2 diabetes. Cell Metabolism. 2016;24:593-607.

8. Gerich J LM, Noacco C, Karam J, Forsham P. Lack of glucagon response to hypoglycemia in diabetes: evidence for an intrinsic pancreatic alpha-cell defect. Science. 1973;182:171-3.

9. Boli G CG, De Feo P, Compagnucci P, Zega G, Angeletti G, Cartechini MG, Santeusanio G, Brunetti P. Lack of glucagon response in glucose counter-regulation in type 1 diabetics: absence of recovery after prolonged optimal insulin therapy. Diabetologia. 1982;22:100-5.

10. Gerich JE. Glucose counterregulation and its impact on diabetes mellitus. Diabetes. 1988;37:1608-17.

11. Group TDCaCTR. Hypoglycemia in the Diabetes Control and Complications Trial. Diabetes. 1997;46:271-86.

12. Frier BM. Hypoglycaemia in diabetes mellitus: epidemiology and clinical implications. Nature Reviews Endocrinology. 2014;10:711-22.

13. Group UHS. Risk of hypoglycaemia in types 1 and 2 diabetes: effect of treatment modalities and their duration. Diabetologia. 2007;50:1140-7.

14. Rizza RA CP, Gerich JE. Role of glucagon, catecholamines, and growth hormone in human glucose counter-regulation. Journal of Clinical Investigation. 1979;64:62-71.

15. PE C. Mechanisms of hypoglycemia-associated autonomic failure in diabetes. New England Journal of Medicine. 2013;369:362-72.

16. Yue JTY BE, Coy DH, Giacca A, Efendic S, Vranic M. Somatostatin receptor type 2 antagonism improves glucagon and corticosterone counterregulatory responses to hypoglycemia in streptozotocin-induced diabetic rats. Diabetes. 2012;61:197-207.

17. He YL DL, Bhad P, Amer A, Colin L, Yan J-H, Harris S, Harris, Taylor A. LEZ763, a novel GPR119 agonist, increases GLP-1, GIP, PYY, and glucagon, but has minimal effects on glucose in patients with type 2 diabetes. . Diabetes. 2015;(Supplement).

18. Szewcyzk JW AJ, Adams AD, Chcchi G, Freeman S, Howard AD, Huang Y, Li C, Mosely R, Murphy E, Samuel R, Santini C, Yang M, Zhang Y, Zhao K, Wood HB. Design of potent and selective GPR119 agonists for type II diabetes. Bioorganic and Mecicinal Chemistry Letters. 2011;21:2665-9.

19. Christensen M CS, Sparre-Ulrich AH, Kristensen PL, Rosenkilde MM, Faber J, Purrello F, van Hall G, Holst JJ, Visboll T, Knop FK. Glucose-dependent insulinotropic polypeptide augments glucagon responses to hypoglycemia in type 1 diabetes. Diabetes Care. 2015;64:72-8.

20. Farngren J, Persson, M., Schweizer, A., Foley, J. E., Ahrén, B. . Vildagliptin reduces glucagon during hyperglycemia and sustains glucagon counterregulation during hypoglycemia in type 1 diabetes. . J Clin Endocrinol Metab. 2012;97:3799–806

21. Davis SN MS, Briscoe VJ, Ertl AC, Tate DB. Effects of intensive therapy and antecedent hypoglycemia on counterregulatory responses to hypoglycemia in type 2 diabetes. . Diabetes. 2009;58:701-9.