Clinical Study Protocol

A Phase 2 Open Label Study to Evaluate the Safety, Tolerability, and Efficacy of ARD-101 in Subjects At Least One Year After Bariatric Surgery

Sponsor:	Aardvark Therapeutics 4747 Executive Drive, Suite 1020 San Diego, CA 92121
Sponsor Study Number:	AARD-202
IND Number:	142288
IMP Name:	ARD-101
Development Phase:	Phase 2
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Amendment 2	3.0 (October 21, 2021)
Amendment 3	4.0 (January 25, 2022)
Amendment 4	5.0 (March 10, 2022)
Amendment 5	6.0 (May 09, 2022)

This clinical study will be conducted in accordance with the International Council for Harmonization Tripartite Guideline for Good Clinical Practice (GCP) E6(R2), the protocol and with other applicable regulatory requirements.

Confidentiality Statement

SIGNATURE PAGE

Declaration of Sponsor

Protocol Title: A Phase 2 Open Label Study to Evaluate the Safety, Tolerability, and Efficacy of ARD-101 in Subjects At Least One Year After Bariatric Surgery

This clinical study protocol was subjected to critical review. The information it contains is consistent with current knowledge of the risks and benefits of the investigational medicinal product (IMP), as well as with the moral, ethical and scientific principles governing clinical research as set out in the guidelines on GCP applicable to this clinical study.

Sponsor Signatory

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May 12, 2022

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Declaration of Sponsor

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Sponsor Signatory

12MAY2022

Andreas Niethammer, MD, PhD
Chief Medical Officer
Aardvark Therapeutics, Inc.

Date

SIGNATURE PAGE

Declaration of the Principal Investigator

Protocol Title: A Phase 2 Open Label Study to Evaluate the Safety, Tolerability, and Efficacy of ARD-101 in Subjects At Least One Year After Bariatric Surgery

This clinical study protocol was subjected to critical review and has been released by the Sponsor. The information it contains is consistent with current risk and benefit evaluation of the IMP, as well as with the moral, ethical and scientific principles governing clinical research as set out in the guidelines on GCP applicable to this clinical study.

Principal Investigator

Principal Investigator	Signature
Date	Site Number

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LIST OF ABBREVIATIONS AND DEFINITION OF TERMS

Abbreviation	Definition
AE	Adverse event
ALT	Alanine aminotransferase
AST	Aspartate transaminase
AUC	Area under the curve
$AUC_{0-\tau}$	Area under the plasma concentration versus time curve from time zero to time τ
b.i.d.	Twice a day
BLQ	Below the quantitation limit
BMI	Body mass index
bpm	Beats per minute
CBC	Complete blood count
CCK	Cholecystokinin
CFR	Code of Federal Regulations
CGM	Continuous glucose monitoring
CHO	Carbohydrate
CL	Systemic clearance
C_{max}	Maximum observed concentration
CNS	Central nervous system
CoEQ	Control of Eating Questionnaire
CRO	Contract research organization
CRP	C-reactive protein
CSR	Clinical study report
C-SSRS	Columbia-Suicide Severity Rating Scale
CYP 450	Cytochrome P450
ECG	Electrocardiogram
eGFR	Estimated glomerular filtration rate
EOS	End of Study
FIH	First-in-Human
F%	Oral bioavailability
FSH	Follicle stimulating hormone

Abbreviation	Definition
GCP	Good Clinical Practice
GI	Gastrointestinal
GIP	Gastric inhibitory polypeptide
GLP	Good Laboratory Practice
GLP-1	Glucagon-like peptide-1
HbA1c	Glycated hemoglobin
hERG	Human ether-a-go-go-related gene
HOMA-IR	Homeostatic model assessment for insulin resistance
HPBL	Human peripheral blood lymphocytes
ICF	Informed consent form
ICH	International Council on Harmonization
IC_{50}	Half maximal Inhibitory Concentration
IEC	Independent ethic committee
i.g.	Intragastric
IgE	Immunoglobulin E
IMP	Investigational medicinal product
IL	Interleukin
IP	Intraperitoneal
IND	Investigational new drug
IRB	Institutional review board
i.v.	Intravenous
MATE	Multidrug and toxic compound extrusion
MDRI	Multi-drug resistance-1
MedDRA	Medical Dictionary for Regulatory Activities
MMTT	Mixed Meal Tolerance Test
MRI-PDFF	Magnetic resonance imaging-derived proton density fat fraction
NASH	Nonalcoholic steatohepatitis
NOAEL	No observable adverse effect level
OAT3	Organic anion transporter 3
OCT2	Organic cation transporter 2
OGTT	Oral glucose tolerance test

Abbreviation	Definition
OTC	Over-the-counter
PK	Pharmacokinetics
p.o.	*per. os* (orally)
PYY	Peptide YY
QTcB	QT interval corrected for heart rate using Bazett's correction
QTcF	QT interval corrected for heart rate using Fridericia's correction
SAE	Serious adverse event
SD	Standard deviation
SMC	Safety Monitoring Committee
SOC	System organ class
SOP	Standard operating procedure
SUSAR	Suspected unexpected serious adverse reaction
$t_{1/2}$	Terminal elimination half-life calculated as: ln2/λz
t_{max}	Time of the maximum observed concentration
TEAEs	Treatment-emergent adverse events
TNF	Tumor necrosis factor
TSH	Thyroid-stimulating hormone
ULN	Upper limit of normal
US	United States
V_d	Volume of distribution
V_{ss}	Volume of distribution at steady state
WHO	World Health Organization
WHO-DD	WHO Drug Dictionary

1. PROTOCOL SUMMARY

1.1. Protocol Synopsis

Protocol Title	A Phase 2 Open Label Study to Evaluate the Safety, Tolerability, and Efficacy of ARD-101 in Subjects At Least One Year After Bariatric Surgery
Study Number	AARD-202
Development Phase	Phase 2
Sponsor	Aardvark Therapeutics, Inc.
Study Center	The study will be conducted at a single center in the United States (US)
Study Endpoints	**Primary Endpoint:** • The percent total weight change at the end of treatment from baseline **Secondary Endpoints**: • Safety of ARD-101 by assessment of the incidence of Treatment-Emergent Adverse Events (TEAE) • The change in blood lipid concentrations (total cholesterol, triglyceride, high density lipoprotein cholesterol, and low-density lipoprotein cholesterol) at the end of treatment from the baseline • The change in waist circumference at the end of treatment from the baseline as well as 14 days after end of treatment • The change in glycated hemoglobin (HbA1c) at the end of treatment from the baseline **Exploratory Endpoints:** • Categorical weight loss. Proportion of subjects who lose < 5% and ≥ 5% of initial weight • Fasting circulating levels of glucagon-like peptide (GLP)-1 (total and active), cholecystokinin (CCK), peptide YY (PYY), amylin, glucose-dependent insulinotropic polypeptide (GIP) (total and active), ghrelin, leptin, and adiponectin before the first dosing, and at 1h and 2h after the first dosing on day 1 • Changes in response to mixed-meal tolerance test (MMTT), including: ▪ Serum levels of glucose, insulin, and C-peptide prior to (negative timepoints) and post (positive timepoints) the Ensure meal given for MMTT at baseline (run-in visit) and on day 28 ▪ Serum level of free fatty acids (FFA) prior to (negative timepoints) and post (positive timepoints) the Ensure meal given for MMTT at baseline (run-in visit) and on day 28 ▪ Circulating levels of GLP-1 (total and active), CCK, PYY, amylin, GIP (total and active), glucagon, and ghrelin prior to (negative timepoints) and post (positive timepoints) the Ensure meal given for MMTT at baseline (run-in visit) and on day 28 ▪ Circulating cytokines and inflammatory markers such as C-reactive protein (CRP) at run-in visit and end of treatment (day 28) performed during the MMTT

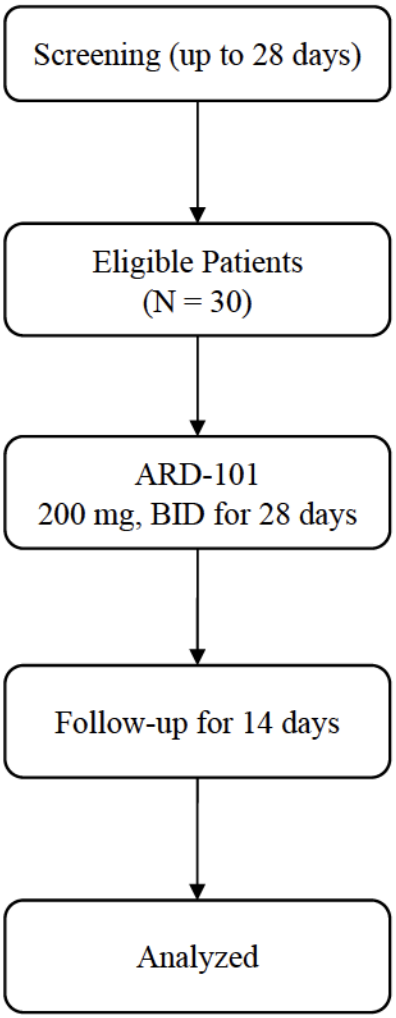
	▪ Circulating levels of bile acids, ferritin, leptin, and adiponectin at run-in visit and end of treatment (day 28) performed during the MMTT • Body composition as measured by bioelectrical impedance scale • Body composition and hepatic steatosis by magnetic resonance imaging-derived proton density fat fraction (MRI-PDFF) + full body composition scan pretreatment and end of treatment • The change in homeostatic model assessment for insulin resistance (HOMA-IR) at the end of treatment from the baseline • The change in fasting blood glucose at the end of treatment from the baseline • Hunger and control of eating as measured by Likert and visual analogue scales, respectively • Change in indirect calorimetry • The fecal samples will be collected during the run-in visit and on day 28 for microbiome analysis to evaluate potential effects of the study medication on fecal microbiome profile (optional for subjects) • Phenotypic bitter taste test (using commercially available test strips/kis) during the run-in visit of the study (optional for subjects)
Study Design	This is a Phase 2, open label study of ARD-101 in subjects with relevant weight regain at least one year after bariatric surgery comparing intrapatient metabolic dynamics. A total of approximately 30 subjects (15 post sleeve gastrectomy and 15 post gastric bypass surgery) will be enrolled in the clinical study. A screening process will be initiated upon completion of the informed consent process. Subjects will be assessed for eligibility through screening tests conducted within 28 days prior to enrollment. Following completion of screening tests and confirmation of eligibility, subjects will be enrolled to receive ARD-101. All available safety and tolerability data will be evaluated throughout study conduct. Pharmacodynamic data will be analyzed after study completion. Subjects will be receiving treatment in an outpatient setting and will be instructed to visit the clinical center periodically as scheduled in Table 1 for safety and efficacy assessments. The treatment may be discontinued due to intolerable toxicity, withdrawal of informed consent, discretion of principal investigator or end of the study. All subjects will be followed up for approximately 14 days after the last dose of study drug.
Test Product, Dose, and Mode of Administration	ARD-101 at 200 mg BID administered orally for 28 days
Inclusion Criteria	Subjects must meet all the following criteria to be eligible for participation in this study: 1. Male and female subjects, 18-75 years of age

	2. Willing and able to provide signed and dated informed consent prior to any study-related procedures and willing and able to comply with all study procedures 3. Body Mass Index (BMI) of ≥ 35 - 60 kg/m^2 during the Screening Period 4. Status of at least 1-year post sleeve gastrectomy or gastric bypass surgery 5. One of the following: a. Weight loss of at least 50% of their excess weight at their nadir, and weight regain at least 20% of their nadir weight loss. Excess weight is defined as maximum preoperative weight – weight corresponding to BMI 25 kg/m^2. b. Excess weight loss less than 50% at the subject's nadir weight 6. Subjects with rescue surgery (e.g. gastric band to sleeve gastrectomy or gastric bypass; sleeve gastrectomy to gastric bypass) will be allowed, with time since second surgery of at least 12 months. 7. Subjects with a history of revision procedures will be allowed if at least 6 months have passed since the procedure and weight has not deviated more than 5% in the 3 months prior to enrollment 8. No abnormal findings or abnormalities of clinical significance in vital signs, physical examination, clinical laboratory tests (complete blood count (CBC), urinalysis, blood biochemistry, coagulation, pregnancy test (females), urine drug test, nicotine test, etc.), 12-lead electrocardiogram (ECG) during the Screening Period 9. Serum creatinine, alkaline phosphatase, hepatic enzymes (aspartate aminotransferase, alanine aminotransferase) and total bilirubin (unless the subject has documented Gilbert syndrome) not exceeding 1.5-fold the upper laboratory norm and eGFR >30 mL/min 10. Standard 12-lead electrocardiogram (ECG) parameters after 10minutes resting in supine position in the following ranges; 120 ms <PR <220 ms, QRS <120 ms, QTc <= 430 ms if male, <= 450 ms if female and normal ECG tracing unless the Investigator considers an ECG abnormality within described limits to be not clinically relevant 11. Stable or well controlled blood pressure per Investigator's judgement during the Screening Period. Specifically: Vital signs after 10 minutes sitting in a chair (feet on floor, back supported): i. 95 mmHg <systolic blood pressure (SBP) <160 mmHg, ii. 45 mm Hg <diastolic blood pressure (DBP) <100 mm Hg, iii. 40 bpm <heart rate (HR) <100 bpm. 12. HbA1c < 9.0% at screening 13. Patients with type 2 diabetes treated with metformin may be enrolled. However, patients with type 2 diabetes on any other therapy will be excluded. 14. Female subjects must have negative pregnancy test and must not be lactating. For females able to bear children, a hormonal (i.e., oral, implantable, or injectable) and single barrier method (i.e., sponge), or a double-barrier method of birth control (i.e., condom with spermicide) or abstinence must be used/practiced throughout the

	study and for 90 days following last dose of study medication; for effective form of birth control. 15. Females of non-childbearing potential, defined as surgically sterile (status post hysterectomy, bilateral oophorectomy, bilateral tubal ligation, bilateral salpingectomy, or bilateral tubal occlusion) or post-menopausal for at least 12 months (may be confirmed with a screening follicle stimulating hormone (FSH) level in the post-menopausal lab range), do not require contraception during the study. 16. Males with female partners of childbearing potential must agree to a double-barrier method if they become sexually active during the study and for 90 days following the last dose of the study medication. Male subjects must not donate sperm for 90 days following their participation in the study.
Exclusion Criteria	Subjects who meet **any** of the following criteria will be excluded from study participation: 1. Any relevant gastrointestinal (GI) surgery (excluding the gastric bypass or sleeve gastrectomy) per Investigator judgement 2. History of significant drug hypersensitivity or anaphylaxis 3. Participation in a weight loss program or clinical trial for weight loss within the 3 months prior to enrollment 4. Received any experimental drugs or devices or have participated in a clinical study within 30 days prior to enrollment 5. Diabetes treatment (unless metformin as outlined), or chronic oral steroids, or treatment with immune modulators, anti-obesity drugs, chronic opiate therapy, or antipsychotic medications 6. Currently receiving any drug-based therapy for weight management 7. Thyroid-stimulating hormone (TSH) level is outside of normal limit during the Screening Period 8. The presence of diseases with abnormal clinical manifestations that need to be excluded based on their possible contribution to weight loss or weight gain, including but not limited to nervous, cardiovascular, blood and lymphatic system, immune, renal, hepatic, gastrointestinal, respiratory, metabolic and skeletal diseases during the Screening Period 9. History of myocardial infarction, unstable angina, arterial revascularization, stroke, New York Heart Association Functional Class II-IV heart failure, or transient ischemic attack within 6 months prior to Visit 1 10. Any malignancy not considered cured (except focal, treated basal cell carcinoma and squamous cell carcinoma of the skin); a participant is considered cured if there has been no evidence of cancer recurrence in the previous 5 years 11. History of major depressive disorder or history of other severe psychiatric disorders (e.g., schizophrenia or bipolar disorder) within the last 2 years. 12. Donated ≥200 mL of blood (blood components) or had massive blood loss, received blood transfusion or blood products within 3 months prior to enrollment 13. Planned sperm/egg donation within 6 months post enrollment 14. Positive urine drug test (morphine, methamphetamine, ketamine, ecstasy, and cannabis) during the Screening Period 15. History of consuming more than 14 units of alcoholic beverages per week or of alcoholism or drug/chemical/substance abuse within past

	2 years prior to enrollment (Note: one unit = 12 ounces of beer, 4 ounces of wine or 1 ounce of spirits) 16. Smoking any amount within 3 months prior to enrollment 17. Excessive consumption of tea, coffee, and/or caffeinated beverages (more than 8 cups, 250 mL for each cup) every day within 3 months prior to enrollment 18. Symptomatic viral, bacterial (including upper respiratory infection), or fungal (non-cutaneous) infection within 1 week prior to enrollment 19. History of human immunodeficiency virus antibody, hepatitis C antibody or hepatitis B virus surface antigen 20. A history of psychiatric and psychological condition that, in the judgment of the investigator, may interfere with the planned treatment and follow-up, affect subject compliance or place the subject at high risk from treatment-related complications 21. Poor venous access or inability to tolerate venipuncture 22. Any condition or active drug treatment that the investigator or primary physician believes may not be appropriate for participating in the study
Duration of Study	The duration of participation for each subject will be approximately 84 days (12 weeks) • Screening Period: Up to 28 days (4 weeks) • Run-in Visit: Up to 14 days (2 weeks) • Treatment Period: 28 days on ARD-101 (4 weeks) • Follow-up Period: 14 days (2 weeks). The End of Study (EOS) Visit will occur within14 days after receiving the dose of ARD-101
Pharmacodynamic Evaluation	All subjects will be required to provide blood samples at defined time points and at the end of treatment visit. These blood samples will be used to explore the parameters described in the endpoint setting. The Control of Eating Questionnaire (CoEQ) will be administered at the timepoints indicated in Table 1.
Safety Evaluations	Adverse events (AE) will be collected from the time of informed consent until 14 days after the last administration of study drug. Safety laboratory assessments, including CBC, chemistries, coagulation tests, urinalysis, and thyroid function, along with ECGs, , and vital signs will be obtained. All concomitant medications will be recorded. All adverse events will be followed to resolution or stabilization. Only TEAE will be summarized.
Efficacy Evaluations	The efficacy assessments include changes from baseline in body weight, BMI, waist circumference, insulin resistance, body fat percentage, HbA1c, serum glucose, lipids, ambulatory blood pressure and defined hormone concentrations.
Statistical Analysis	All safety, and efficacy data will be summarized and presented overall for the study, using descriptive statistics. Mean, median, standard deviation, minimum, and maximum will be presented for continuous variables and frequencies and percentages for discrete variables.

1.2. Schema


Screening (up to 28 days)
Eligible Patients
(N = 30)
ARD-101
200 mg, BID for 28 days
Follow-up for 14 days
Analyzed

1.3. Schedule of Assessments, Time Points, and Window Allowance

Details on procedures and timing of assessments are presented in Table 1.

Table 1 Schedule of Assessments

Period	Screening	Run-in Visit	Treatment Period (±3 days)			Follow Up/End of Study
Study Days	**-42 to -15**	**14 days prior to Day 1**	**1 (predose)**	**15**	**28**	**14**
Informed Consent	X					
Inclusion/Exclusion Criteria	X					
Demographics	X					
Medical History	X					
Physical Examination[a]	X		X	X	X	X
Vital Signs	X		X	X	X	X
12-lead ECG	X		X		X	
Hematology	X		X[b]			X
Comprehensive Serum Chemistry	X		X[b]	X	X	X
Urinalysis	X		X[b]	X	X	X
Urine Toxicology	X					
Coagulation Parameters	X					
Pregnancy Test[c]	X		X[b]			X
TSH	X					
Weight/BMI	X	X	X	X	X	X
Height	X					
Fasting Glucose		X				X
HbA1c	X				X	
Waist Circumference			X	X	X	X
Fasting Blood Lipids		X			X	
Fasting Circulating Hormones[d]			X			
Bioimpedance			X	X	X	

Period	Screening	Run-in Visit	Treatment Period (±3 days)			Follow Up/End of Study
Study Days	**-42 to -15**	**14 days prior to Day 1**	**1 (predose)**	**15**	**28**	**14**
MRI PDFF + Full Body Composition Scan		X			X	
Mixed Meal Tolerance Test (MMTT)		X			X	
Study Drug Dispensation			X[e]			
Concomitant Medication	X		X	X	X	
Indirect Calorimetry		X			X	
Adverse Event Monitoring	X		X	X	X	X
CGM Placement		X		X		
CGM Removal and Download			X		X	X
Fecal Sample Collection[f]		X			X	
Phenotypic Bitter Taste Test[g]		X				
Control of Eating Questionnaire (CoEQ)	X		X	X	X	X

a. Complete physical examination at screening, and brief physical examination for all follow-up visits
b. This procedure is not needed if performed within 3 days
c. For women of child-bearing potential only
d. GLP-1 (total and active), CCK, PYY, amylin, GIP (total and active), ghrelin, leptin, and adiponectin in all subjects at 3 time points (before the first dosing and 1h as well as 2h after the first dosing)
e. A single oral dose of oral ARD-101 will be administered to subjects in the morning of day 1
f. Fecal sample to be collected during run-in visit and on day 28. Optional for subjects
g. Phenotypic bitter taste test is optional for subjects

2. INTRODUCTION

2.1. Background

Obesity

Obesity is a chronic disease with a multifactorial etiology including behavioral, environmental, sociocultural, physiological and genetic factors. obesity and its consequences are an increasing burden globally. Obese, defined as a BMI>30 kg/m^2, is an established risk factor for a number of diseases such as hypertension, cardiovascular disease, cancers, diabetes type 2, and many others (Guh DP et al. 2009). Currently, there are an estimated 600 million obese people worldwide (Collaborators GBDO et al. 2017). BMI data shows that about 40% of American adults are obese (Hruby A and Hu FB 2015), while worldwide, about 3.4 million annual deaths are directly attributed to obesity (Collaborators GBDO et al. 2017).

The treatment of obesity involves lifestyle changes, bariatric surgery and pharmacotherapy (Bray GA et al. 2016). Lifestyle change, including diet and exercise, requires considerable patient commitment, and has poor compliance. Surgery is expensive and invasive. Pharmacotherapy, therefore, is the primary option for the majority of patients. A variety of drugs are designed to prevent food absorption, decrease appetite, or increase metabolism. Compared to controls, most drugs cause weight loss of <4 kg with substantial severe adverse events (Rosa-Goncalves P and Majerowicz D 2019). Some of the adverse events have caused withdrawal of a number of drugs from the market in several countries (Rodgers RJ 2017). As a result, a significant portion of obese patients have no treatment options due to adverse events and limited weight loss. Taken together, obesity remains an unmet medical need and development of new compounds that are safe and effective is paramount. There are currently no drug-based treatments available specifically for subjects regaining weight after bariatric surgery.

Denatonium

Denatonium is one of the bitterest tastants among tens of thousands of natural or synthesized bitter compounds, with a bitterness detection threshold of 0.05 ppm. To date, one of the denatonium salts, denatonium benzoate has been approved as an additive to household products to prevent the ingestion of potentially harmful substances through taste aversion (Sibert JR and Frude N 1991). Importantly, the US Consumer Product Safety Commission has judged denatonium benzoate as generally safe. Like other bitter compounds, denatonium can strongly activate up to 25 G-protein coupled receptors expressed in a variety of tissues and organs including GI tract, pancreas, fat, trachea, and central nervous system. Bitter taste receptors are thought to be evolutionarily

conserved for the detection of potentially toxic compounds. Activation of bitter receptors in the mouth elicits a familiar aversive sensation, while activation of bitter receptors in the gut and chemoreceptor trigger zone located in the medulla leads to reduction of appetite and delayed gastric emptying as well as other adaptations. In a number of animal studies, denatonium benzoate has been shown to inhibit ongoing ingestive behavior, suppress food intake, and inhibit gastric emptying (Janssen S et al. 2011; Schier LA et al. 2011). Moreover, denatonium benzoate can stimulate the release of GLP-1 and CCK that are known to cause satiety and satiation (Chen MC et al. 2006; Kim KS et al. 2014). In human studies, the intragastric administration of denatonium benzoate after a liquid meal has been reported to inhibit relaxation of the proximal stomach. While in an oral nutrient tolerance test, denatonium benzoate was shown to increase satiation (Avau B et al. 2015). In a clinical trial conducted in healthy female volunteers, intragastric administration of denatonium benzoate is shown to decrease both antral motility and hunger ratings during the fasting state, probably via inhibiting motilin release. More importantly, no adverse reactions have been reported in all these human studies (Deloose E et al. 2017). Taken together, intragastric administration of denatonium salts is safe and can lead to weight loss by changing the appetite and eating behaviors and therefore, are promising agents for the treatment of obesity.

Rationale of the Study

ARD-101 is an encapsulated oral formulation of denatonium acetate salt that is designed to bypass the oral taste receptors when administered orally. Aardvark Therapeutics has demonstrated the efficacy and safety of ARD-101 in multiple preclinical animal models of metabolic disorders and weight management. The safety as well as pharmacokinetics have been confirmed in a human trial.

In addition, ARD-101 is minimally absorbed into the circulation through the GI epithelium cells provided the very low cellular penetration rate as demonstrated by the Caco-2 cell monolayer permeability assay (Table 2).

Table 2 Caco-2 cell Monolayer Permeability of ARD-101, Warfarin, Ranitidine and Talinolol

Compounds	Papp (10^{-6} cm/s)		Efflux ratio
	A→B	B→A	
ARD-101 (10 µM)	0.378	4.65	12.3
Ranitidine	0.355	0.812	2.29
Warfarin	34	31	0.913
Talinolol	0.337	5.03	14.9
Talinolol + Verapamil	0.737	1.51	2.05

Taken together, ARD-101 has demonstrated promising activity and a manageable toxicity profile. Subjects with weight regain or otherwise unsatisfactory weight management after bariatric surgery present an increasing population with no specifically approved treatment.

For details of the preclinical results, please refer to the Investigator Brochure.

2.2. Risk-benefit Assessment

2.2.1. Known Potential Risks

ARD-101 has shown a well-tolerated safety profile with no significant organ toxicities up to 200 mg/kg/day in a GLP-compliant pivotal toxicology study with cynomolgus monkeys. As with any new drug, rare or unexpected side effects could occur. The ARD-101 related toxicities may be mild to severe, including adverse hypersensitivities, or adverse reaction in GI system.

Additionally, comprehensive non-clinical toxicology studies have been performed to establish the toxicity profile of ARD-101. The Cynomolgus monkey was identified as the pharmacologically relevant species for assessing human risk of ARD-101. ARD-101 was tolerated in a GLP-compliant, 28-day repeat-dose toxicology study in Cynomolgus monkey where systemic exposure was confirmed in all animals.

No denatonium salt has been approved for treatment in subjects for any indication. Denatonium benzoate is currently the only denatonium salt that has been tested in humans. The potential risks that may be associated with ARD-101 are considered to be similar to denatonium benzoate, which have been shown to be well-tolerated when administered *p.o.* (orally) with no reported adverse reaction (Deloose E et al. 2017). Taken together, denatonium salt is safe and well-tolerated when administered *p.o.* in humans.

ARD-101 has been studied for its safety and pharmacokinetics in humans with the results in detail summarized below. In short, ARD-101 has been found to be safe at a dose level of up to 240 mg BID for 14 days and the PK has confirmed minimal systemic uptake and no accumulation after 11 days.

2.2.2. Known Potential Benefits

Preclinically, ARD-101 was shown to reduce food and water consumption within 2 hours after dosing in SD rats. ARD-101 exhibited significant attenuation of weight gain (compared to control mice) in high fat diet-induced obese mice model after 56 days of treatment. ARD-101 also exhibited nominal attenuation of weight gain in ob/ob mice models.

Further, previous studies, *i.g.* (intragastric) administration of denatonium helped in mitigation of chronic and acute inflammation in multiple preclinical models, characterized by improved pathology, downregulation of inflammatory cytokines (such as IL-6, TNF, IL-1β, etc.). Benefits were also observed with regards to cholesterol levels, HbA1c and other metabolic markers.

In a 48-week study of diet-induced NASH in mice, dosing with ARD-101 largely abrogated development of liver fibrosis over control based on blinded histopathologic review.

2.3. Summary of Findings from Non-clinical, in vitro Studies with Potential Clinical Relevance

An in vitro secondary pharmacology study was performed to evaluate the binding, enzyme, and uptake activity of 10 μM ARD-101 and assess the specificity of ARD-101 activity. The study tested ARD-101 binding to 27 receptors (including subtypes of monoaminergic, cannabinoid, histaminic, nicotinic, opioid, and glucocorticoid receptors), 3 transporters, 8 ion channels, and 6 enzymes. Results from 2 assays, α1A adrenergic receptor and hERG potassium ion channel assays, revealed an inhibition or stimulation higher than 50%, denoting a significant effect of ARD-101 in these two targets. The remaining assays did not show significant ARD-101 activity.

2.3.1. Non-clinical Pharmacology

Effect of ARD-101 to Support Proposed Indication

The key data to support ARD-101 for the treatment of obesity and metabolic syndrome stems from preclinical experiments in multiple rodent models demonstrating benefit of oral dosing with ARD-101 with regards to mitigation of weight gain as well as improvement in pathologies such as hypercholesterolemia, prediabetes and chronic inflammation.

2.3.2. Non-clinical Pharmacokinetics

The PK profile of ARD-101 was characterized in a panel of *in vitro* and *in vivo* studies, including plasma protein binding assays, permeability assays, metabolic stability and metabolite identification assessments, Cytochrome 450 (CYP450) phenotype assays, *in vitro* drug-drug interaction studies on CYP450s, and serum/plasma toxicokinetic studies in Cynomolgus monkeys.

In the Caco-2 cell monolayer permeability assay, ARD-101 showed a low permeability through intestinal epithelia cell, the P_{app} value of ARD-101 in the apical to basolateral direction was 0.378×10^{-6} cm/s. In addition, ARD-101 exhibited efflux effect with an efflux ratio of about 12.3, indicating ARD-101 is a substrate of certain efflux transporters.

To investigate the PK of ARD-101 including systemic exposure, oral bioavailability, and PK linearity, a non-GLP PK study was conducted in Cynomolgus monkeys. Three male Cynomolgus monkeys were assigned to 3 dose groups and a single dose of ARD-101 was administrated *p.o.* at 0.49, 1.62, or 4.85 mg/kg (denatonium base weight). After a 14-day washout period, an intravenous (*i.v.*) administration of ARD-101 was given to the same animal at the same dose level. Blood samples were collected from all animals on Day 1 and Day 15 at 0.25, 0.5, 1, 2, 4, 8, 24, 32, and 48 h post-dose.

Following a single *i.v.* dose of ARD-101 at 0.49, 1.62, and 4.85 mg/kg to Cynomolgus monkeys, the C_{max} and $AUC_{0\text{-}48h}$ were 129, 803, 2270 ng/mL and 270, 1460, 4720 ng*h/mL, respectively. The values of CL and V_d were 2150, 1320, 1240 mL/h/kg and 32500, 25800, 17500 mL/kg, respectively. The elimination of ARD-101 in monkeys was slow, as indicated by the mean $t_{1/2}$ of 11.25 h. Within the dose range of 0.49-4.85 mg/kg, AUC increased higher than dose proportionally (Table 3).

Following a single oral dose of ARD-101 at 0.49, 1.62, and 4.85 mg/kg, the $AUC_{0\text{-}48h}$ was insignificant and C_{max} were 0, 1.98, 4.55 ng/mL, respectively, indicating an extremely low systemic exposure of ARD-101. The oral bioavailability (F%) in monkeys was estimated to be 0% at 0.49, 1.62, and 4.85 mg/kg, respectively. No clinical abnormal signs were noticed given very low absorption and negligible systemic exposure (Table 3).

In summary, the bioavailability of ARD-101 in monkey is negligible, no systemic exposure reached after 4.85 mg/kg *p.o.* administration.

Table 3 The Pharmacokinetic Parameters of ARD-101 After Single Intravenous or Intragastric Administration to Cynomolgus Monkeys

Route	Dose (mg/kg)	C_{max} ng/mL	$AUC_{0\text{-}t}$ ng*h/mL	$t_{1/2}$ hours	T_{max} hours	CL mL/min/kg	V_{SS} L/kg	F%
i.v.	0.49	129	270	105	0.25	2150	32.5	
	1.62	803	1460	13.5	0.25	1320	25.8	
	4.85	2270	4720	9.74	0.25	1240	17.5	
p.o.	0.49	BLQ	NA	NA	NA	NA	NA	0
	1.62	1.98	NC	NC	2	NA	NA	0
	4.85	4.55	NC	NC	1	NA	NA	0

Abbreviations: BLQ: below the quantitation limit; F: fraction (%) of an administered drug that reaches the systemic circulation [oral bioavailability]; *i.v.*: intravenous administration; *p.o.*: orally administration; NA: not applicable; NC: not calculable.

Pharmacokinetic parameters after repeated oral doses were obtained from the toxicokinetic study following *b.i.d. i.g.* administration of 15, 30, or 100 mg/kg/dose of ARD-101 (denatonium base weight) to Cynomolgus monkeys for 28 days. After the first dose, the mean plasma AUC and C_{max} values were similar between male and female animals, indicating a lack of sex-related differences in systemic exposure. There was a trend toward a greater than dose-proportional increase in C_{max} and $AUC_{0\text{-}24h}$. At Day 28, steady state mean $AUC_{0\text{-}24h}$ values were 26.3, 40.6, and 1210 ng*h/mL for female monkeys and 40.1, 48.0, and 1400 ng*h/mL for male monkeys at 15, 30, and 100 mg/kg/doses, respectively. No accumulation of ARD-101 in monkeys was observed after 4 weeks of *b.i.d.* administration.

ARD-101 exhibited low binding (< 50%) to human and rat plasma proteins, and a relatively high binding to monkey plasma proteins (50% to 90%). An *in vitro* liver microsome stability study showed that ARD-101 was quickly metabolized by rat liver microsomes, moderately by monkey microsomes, and almost no metabolism in human liver microsomes. Cytochrome P450s (CYPs) were not the major enzymes involved in ARD-101 metabolism. An *in vitro* metabolite identification study showed that the N-dealkylated metabolite (M1, lidocaine) was only seen in the human S9 samples at trace levels, accounting for only 0.05% of total peak areas. The oxidation product M2 was the major metabolite, accounting for 18.88%, 5.82%, and 0.31% of total peak areas in rat, monkey and humans, respectively.

A probe substrate-based inhibition study in human liver microsomes showed that ARD-101 was a moderate inhibitor of CYP2D6 with an IC_{50} value of 0.233 µM. The CYPs induction study in human hepatocytes showed that ARD-101 was not a potential inducer of CYP1A2, CYP2B6, and CYP3A4. An *in vitro* transporter interaction assay showed that ARD-101 is a substrate of MDRI, MATE1, MATE2-K, OAT3, and OCT2 transporters. ARD-101 is an *in vitro* inhibitor of the MATE1, MATE2-K, and OCT-2 transporters, with IC_{50} values of 21.07, 57.11, and 9.47 µM, respectively.

2.3.3. Non-Clinical Safety Pharmacology and Toxicology

The toxicology program was designed to support clinical studies in accordance with ICH Guidance M3(R2). A non-GLP single dose acute toxicology study (*i.v.* and *p.o.*) and a pivotal GLP compliant general toxicology study with a 28-day treatment (*i.g.*) and a 14-day recovery period have been conducted in Cynomolgus monkeys. *In vitro* studies including bacterial reverse mutation assay

and mammalian cell chromosome aberration test have been conducted to evaluate genotoxicity of ARD-101.

ARD-101 showed inhibition of hERG current with an IC_{50} of 99.2 µM (38143 ng/mL) *in vitro*. Cardiovascular toxicity was observed in a single Cynomolgus monkey after *i.v.* administration of ARD-101 at 4.85 mg/kg (in the single dose toxicology/PK study). The animal showed repetitive arrhythmia immediately following injection, the serum concentration was 2270 ng/mL at the first sampling time point (15 mins post-dose), and this adverse effect recovered by 8 hours post- dose. The tolerated dose for an *i.v.* route is 1.62 mg/kg with a systemic exposure of 1460 ng*h/mL and a peak serum concentration (C_{max}) of 803 ng/mL.

In the 28-day toxicity study in Cynomolgus monkeys, 100 mg/kg *b.i.d. i.g.* administration of ARD-101 (human equivalent dose is 64.5 mg/kg, *b.i.d.*) was well-tolerated in all animals, which is 37.33-fold of the proposed starting dose in a first-in-human (FIH) clinical study (40 mg *b.i.d* for a 70 kg adult). There was no mortality or moribundity found in ARD-101-treated animals. ARD-101 showed no local toxicity in the GI organs (stomach, duodenum, ileum, jejunum, cecum, colon, and rectum) based on gross pathology and histological microscopic examinations. Only transient decreases in body weights in animals administered with 100 mg/kg dose were observed, which may be related to the pharmacological effect of ARD-101. The no-observed-adverse-effect level (NOAEL) was considered to be 100 mg/kg/dose (200 mg/kg/day) with a systemic exposure $AUC_{0\text{-}24h}$ of 1210 ng*mL/h and 1400 ng*mL/h for female and male monkeys, respectively.

ARD-101 did not show mutagenic activity in the bacterial reverse mutation assay. The *in vitro* chromosomal aberration assay showed that ARD-101 was considered negative for inducing structural aberrations of human peripheral blood lymphocytes (HPBL) in the 3-h treatment with metabolic activation and the 22-hour treatment without activation. ARD-101 was positive for inducing structural aberrations of HPBL in the 3-hour treatment without metabolic activation. No precipitate, pH changes or cytotoxicity was observed at any dose level during the study.

Further details can be found in the Investigator's Brochure.

2.4. Summary of Findings from Previous Clinical Studies

A FIH clinical study with the study drug has been performed in healthy volunteers to interrogate safety and pharmacokinetics of multiple dose levels.

The study consisted of 2 parts: A single ascending dose part (SAD) and a multiple ascending dose part (MAD), dosing subjects for 14 days BID. Each part tested three dose levels (40 mg, 100 mg

and 240 mg) consecutively in a randomized, double-blinded manner. A total of 43 subjects were dosed in total, 23 females and 20 males of which 31 received ARD-101.

The results are summarized below:

2.4.1. Overview of Clinical Pharmacology

2.4.1.1. Single Ascending Dose

The SAD study evaluated PK parameters following administration of a single oral dose of ARD-101 at 40 mg, 100 mg, or 240 mg in healthy adult subjects. Eligible subjects were 18-55 years of age, with a body mass index between 18-30 kg/m^2 and a minimum weight of 50 kg. Subjects were orally dosed in 3 escalating cohorts of 40, 100, and 240 mg of ARD-101 or matching placebo capsules. For each dose cohort, 6 subjects received ARD-101 and 2 subjects received placebo. The study medication was administered to eligible subjects after a minimum of 8 h of fasting.

Group PK analyses have been conducted for all dose cohorts in Part 1 of the study. PK parameters for all doses are presented in Table 4. Based on the area under the curves (AUCs) and maximum serum concentration (C_{max}) between-subject variability (Geometric CV%) was high, ranging from 45.8 to 53.7%. CV% for $t_{1/2}$ values increased with increases in ARD-101 dose level.

Table 4 Geometric Mean (%CV) PK Parameters of ARD-101 Following Single Oral Dose Administration of ARD-101

Parameter (Units)	40 mg	100 mg	240 mg
AUC_{last} (h*ng/mL)	4.81 (281.4)	15.9 (61.1)	53.1 (45.8)
AUC_{inf} (h*ng/mL)	12.4 (106.8)	17.5 (57.6)	55.8 (45.2)
C_{max} (ng/mL)	0.999 (259.4)	3.04 (76.2)	12.4 (53.7)
t_{max}[a] (h)	1.33 (0.667 – 4.00)	1.21 (0.667 – 2.00)	1.08 (0.667 – 1.33)
$t_{1/2}$ (h)	8.27 (14.4)	11.1 (39.0)	18.4 (39.0)[b]
CL/F (L/h)	3220 (106.8)	5710 (57.6)	4300 (45.2)
Vz/F (L)	38400 (114.0)	91700 (30.1)	114000 (56.9)

[a] Median (min-max) presented; [b] Half-life estimates were calculated over a period of less than 2 half-lives for 3 subjects in the 240 mg dose group.

Following administration of a single 240 mg ARD-101 dose, quantifiable concentrations for all subjects were observed at 48 hours post dose, with 3 of 6 subjects having quantifiable concentrations at 72 hours post dose. There was a 2.4-fold increase in dose level from 100 to 240 mg ARD-101. This resulted in increases in C_{max}, AUC_{last}, and AUC_{inf} (4.08-, 3.34-, and 3.18-fold, respectively).

The geometric mean and highest peak plasma concentrations (C_{max}) following single oral administration at these doses were 0.999 and 9.59 ng/mL (40 mg), 3.04 and 10.4 ng/mL (100 mg), and 12.4 and 29.7 ng/mL (240 mg) respectively, which represented 0.12% and 1.20%, 0.380% and 1.30%, and 1.55% and 3.71% of the PK stopping criteria (800 ng/mL) for this Phase I study.

2.4.1.2. Multiple Ascending Dose

The MAD study evaluated PK parameters following repeated administration to date of oral doses of ARD-101 at 40 mg, 100 mg and 240 mg BID, in healthy adult subjects. Eligible subjects were 18-55 years of age, with a body mass index between 18-30 kg/m^2 and a minimum weight of 50 kg. Subjects were orally dosed BID, for 14 days, or matching placebo capsules. 4 subjects received ARD-101 and 2 subjects received placebo in each dose level. From Day 1 to Day 14, the study medication was administered twice daily (every 12 h) with approximately 12 h between doses.

PK results are listed in Table 5.

The geometric mean (on Day 14) and highest individual systemic exposure (on Day 1), based on C_{max}, represented 1.14% and 2.55% of PK stopping criteria, respectively, at the 240 mg BID dose level.

Accumulation after BID dosing for 14 days was 1.20-fold for C_{max} and 1.68-fold for AUC_{tau} at 240 mg BID. Steady state appears to have been reached by Day 11 at all tested dose levels.

Table 5 Geometric mean (%CV) PK parameters of ARD-101 BID oral dose administration of ARD-101 for 14 days

	40 mg BID		100 mg BID		240 mg BID	
Parameter (Units)	Day 1	Day 14	Day 1	Day 14	Day 1	Day 14
AUC_{tau} (h*ng/mL)	2.31 (187.9)	5.51 (83.5)	8.48 (63.0)	16.4 (55.3)	27.6 (80.3)	46.3 (46.7)
$DAUC_{tau}$ (h*ng/mL/mg)	0.0577 (187.9)	0.138 (83.5)	0.0848 (63.0)	0.164 (55.3)	0.115 (80.3)	0.193 (46.7)
C_{max} (ng/mL)	0.584 (235.9)	1.27 (141.3)	1.85 (85.9)	3.02 (86.1)	7.64 (135.9)	9.13 (59.6)
DC_{max} (ng/mL/mg)	0.0146 (235.9)	0.0317 (141.3)	0.0185 (85.9)	0.0302 (86.1)	0.0318 (135.9)	0.0380 (59.6)
t_{max}[a] (h)	1.50 (0.50 – 4.00)	1.75 (1.08 – 4.00)	1.50 (1.08 – 2.00)	1.08 (0.50 – 4.00)	1.75 (1.15 – 3.00)	1.08 (0.50 – 2.00)
C_{trough} (ng/mL)	NC	0.147 (51.5)	0.183 (69.3)	0.481 (37.9)	0.415 (76.0)	1.21 (25.8)
C_{min} (ng/mL)	NA	0.119 (33.9)	NA	0.419 (25.1)	NA	1.21 (26.1)
C_{avg} (ng/mL)	NA	0.459 (83.5)	NA	1.37 (55.3)	NA	3.86 (46.7)
CL_{ss}/F (L/h)	NA	7260 (83.5)	NA	6090 (55.3)	NA	5180 (46.7)
R_{acc}, C_{max}	NA	2.17 (495.5)	NA	1.66 (129.3)	NA	1.20 (50.1)
R_{acc}, AUC_{tau}	NA	2.39 (69.2)	NA	2.02 (61.5)	NA	1.68 (26.9)
R_{acc}, C_{trough}	NA	2.04 (21.5)	NA	2.89 (51.9)	NA	2.91 (45.9)

[a] Median (min-max) presented; NC, not calculated

2.4.2. Overview of Clinical Efficacy

At the time of the submission of this IND, no clinical study has been conducted to investigate the efficacy of ARD-101.

2.4.3. Overview of Clinical Safety

Clinical safety is based on, three dose cohorts of the SAD part and three dose cohorts of the MAD part.

In the SAD part of the study, ARD-101 was well-tolerated by subjects after single administration of the three assigned doses (40, 100, and 240 mg). No adverse effects (AEs) were reported for any subject in the three dosing cohorts.

ARD-101 was also well-tolerated by subjects for 14-day repeated administration at 40 mg, 100 mg and 240 mg BID. Treatment-emergent AEs that were judged as treatment related by the investigator were restricted to Grade 1 or 2, and were experienced by a total of 6 subjects out of 13 dosed with ARD-101 (46.2%) (Table 6). These consisted of gastrointestinal disorders (3 cases), nervous system disorders (3 cases), skin and subcutaneous disorders (2 cases), and cardiac disorders (1 case) (Table 7). Two cases of skin restricted hypersensitivity (urticaria) occurred at

100 mg BID and 240 mg BID, respectively. The latter hypersensitivity was successfully addressed with premedication of oral antihistamine dosing as per protocol; while the subject experiencing Grade 1 hypersensitivity at the intermediate 100 mg BID dose was discontinued based on investigator's decision and replaced. Gastrointestinal symptoms usually occurred, if so, early on and were self-limiting. One case of palpitations was reported lasting for about 3h. An ECG performed during that episode showed normal sinus rhythm at 83 bpm with no changes from baseline.

Table 6 Summary of Treatment-emergent Adverse Events - MAD Part

	Placebo (BID) (N = 6) nS (%) [nE]	40 mg ARD-101 (BID) (N = 4) nS (%) [nE]	100 mg ARD-101 (BID) (N = 5) nS (%) [nE]	240 mg ARD-101 (BID) (N = 4) nS (%) [nE]	Overall (N = 19) nS (%) [nE]
			TEAEs		
Overall	3 (50.0%) [5]	1 (25.0%) [5]	3 (60.0%) [5]	3 (75.0%) [6]	10 (52.6%) [21]
Serious	---	---	---	---	---
Leading to Discontinuation	---	---	1 (20.0%) [1]	---	1 (5.3%) [1]
Leading to Death	---	---	---	---	---
Severity	---	---	---	---	---
Grade 1	3 (50.0%) [5]	1 (25.0%) [5]	2 (40.0%) [3]	2 (50.0%) [5]	8 (42.1%) [18]
Grade 2	---	---	2 (40.0%) [2]	1 (25.0%) [1]	3 (15.8%) [3]
Grade 3	---	---	---	---	---
Grade 4	---	---	---	---	---
			Treatment-related TEAEs		
Overall	1 (16.7%) [2]	1 (25.0%) [4]	2 (40.0%) [4]	3 (75.0%) [6]	7 (36.8%) [16]
Serious	---	---	---	---	---
Leading to Discontinuation	---	---	1 (20.0%) [1]	---	1 (5.3%) [1]
Leading to Death	---	---	---	---	---
Severity	---	---	---	---	---
Grade 1	1 (16.7%) [2]	1 (25.0%) [4]	1 (20.0%) [2]	2 (50.0%) [5]	5 (26.3%) [13]
Grade 2	---	---	2 (40.0%) [2]	1 (25.0%) [1]	3 (15.8%) [3]
Grade 3	---	---	---	---	---
Grade 4	---	---	---	---	---

BID = twice daily; nE = number of adverse events; nS = number of subjects with an adverse event; N = number of subjects; % = percentage of subjects with an adverse event (nS/N×100)
Severity grades: 1 = mild; 2 = moderate; 3 = severe; 4 = potentially life-threatening
Adverse events were assigned severity grade using the Toxicity Grading Scale for Healthy Adult and Adolescent Volunteers Enrolled in Preventive Vaccine Clinical Trials Version September 2007.
A treatment-emergent adverse event (TEAE) was defined as an adverse event that started during or after the first dose, or started prior to the first dose and increased in severity after the first dose.
A treatment-related TEAE was defined as a TEAE with a relationship of related to the study treatment, as determined by the investigator.
Where a subject experienced multiple TEAEs with the same preferred term for the same treatment, this was counted as 1 TEAE for that treatment under the maximum severity recorded.

Table 7 Summary of Treatment-related Treatment-emergent Adverse Evens by System Organ Class and Preferred Term - MAD Part

System Organ Class Preferred Term	Placebo (BID) (N = 6) nS (%)	40 mg ARD-101 (BID) (N = 4) nS (%)	100 mg ARD-101 (BID) (N = 5) nS (%)	240 mg ARD-101 (BID) (N = 4) nS (%)	Overall (N = 19) nS (%)
Overall	1 (16.7%)	1 (25.0%)	2 (40.0%)	3 (75.0%)	7 (36.8%)
Gastrointestinal disorders	1 (16.7%)	1 (25.0%)	1 (20.0%)	1 (25.0%)	4 (21.1%)
Diarrhoea	---	---	1 (20.0%)	1 (25.0%)	2 (10.5%)
Dyspepsia	---	1 (25.0%)	---	1 (25.0%)	2 (10.5%)
Nausea	---	1 (25.0%)	1 (20.0%)	---	2 (10.5%)
Abdominal distension	---	1 (25.0%)	---	---	1 (5.3%)
Abdominal pain	---	---	1 (20.0%)	---	1 (5.3%)
Flatulence	1 (16.7%)	---	---	---	1 (5.3%)
Nervous system disorders	1 (16.7%)	1 (25.0%)	---	2 (50.0%)	4 (21.1%)
Headache	1 (16.7%)	1 (25.0%)	---	---	2 (10.5%)
Dizziness	---	---	---	1 (25.0%)	1 (5.3%)
Dysgeusia	---	---	---	1 (25.0%)	1 (5.3%)
Skin and subcutaneous tissue disorders	---	---	1 (20.0%)	1 (25.0%)	2 (10.5%)
Urticaria	---	---	1 (20.0%)	1 (25.0%)	2 (10.5%)
Cardiac disorders	---	---	---	1 (25.0%)	1 (5.3%)
Palpitations	---	---	---	1 (25.0%)	1 (5.3%)

BID = twice daily; nS = number of subjects with an adverse event; N = number of subjects; % = percentage of subjects with an adverse event (nS/N×100)
Adverse events were coded using the Medical Dictionary for Regulatory Activities (MedDRA) Version 23.0.
A treatment-
emergent adverse event (TEAE) was defined as an adverse event that started during or after the first dose, or started prior to the first dose and incr eased in severity after the first dose.
A treatment-related TEAE was defined as a TEAE with a relationship of related to the study treatment, as determined by the investigator.

2.4.4. Benefits and Risks Conclusions

According to our nonclinical studies, clinical data, and reported clinical studies on denatonium benzoate, ARD-101 has demonstrated a well-tolerated safety profile and potential clinical benefit in the treatment of obesity. This safety profile supports the use of the dose and dosing regimen to be used in the Phase 2 trial in overweight patients with weight regain or otherwise unsatisfactory weight management after sleeve-gastrectomy or bypass surgery.

3. STUDY ENDPOINTS

Primary Endpoint:

3.1. Primary Endpoint

- The percent total weight change at the end of treatment from baseline

3.2. Secondary Endpoints:

- Safety of ARD-101 by assessment of the incidence of TEAE
- The change in blood lipid concentrations (total cholesterol, triglyceride, high density lipoprotein cholesterol, and low-density lipoprotein cholesterol) at the end of treatment from the baseline
- The change in waist circumference at the end of treatment from the baseline as well as 14 days after end of treatment
- The change in HbA1c at the end of treatment from the baseline

3.3. Exploratory Endpoints:

- Categorical weight loss. Proportion of subjects who lose $< 5\%$ and $\geq 5\%$ of initial weight
- Fasting circulating levels of GLP-1 (total and active), CCK, PYY, amylin, GIP (total and active), ghrelin, leptin, and adiponectin before the first dosing, and at 1h and 2h after the first dosing on day 1
- Changes in response to the MMTT, including:
  - Serum levels of glucose, insulin, and C-peptide prior to (negative timepoints) and post (positive timepoints) the Ensure meal given for MMTT at baseline (run-in visit) and on day 28
  - Serum level of FFA prior to (negative timepoints) and post (positive timepoints) the Ensure meal given for MMTT at baseline (run-in visit) and on day 28
  - Circulating levels of GLP-1 (total and active), CCK, PYY, amylin, GIP (total and active), glucagon, and ghrelin prior to (negative timepoints) and post (positive timepoints) the Ensure meal given for MMTT at baseline (run-in visit) and on day 28
  - Circulating cytokines and inflammatory markers such as CRP at run-in visit and end of treatment (day 28) performed during the MMTT
  - Circulating levels of bile acids, ferritin, leptin, and adiponectin at run-in visit and end of treatment (day 28) performed during the MMTT

- Body composition as measured by bioelectrical impedance scale
- Body composition and hepatic steatosis by MRI-PDFF + full body composition scan pretreatment and end of treatment
- The change in HOMA-IR at the end of treatment from the baseline
- The change in fasting blood glucose at the end of treatment from the baseline
- Hunger and control of eating as measured by Likert and visual analogue scales, respectively
- Change in indirect calorimetry
- The fecal samples will be collected during the run-in visit and on day 28 for microbiome analysis to evaluate potential effects of the study medication on fecal microbiome profile (optional for subjects)
- Phenotypic bitter taste test (using commercially available test strips/kis) during the run-in visit of the study (optional for subjects)

Already collected blood and fecal samples may also be stored and used for possible retrospective exploratory analysis which may include, but will not be limited to, understanding mechanisms of response to treatment (where response is defined broadly to include biomarker change, tolerability or safety) and identification of other mechanisms of therapy.

4. STUDY DESIGN

4.1. Overview

This is a Phase 2, open label study of ARD-101 in subjects with unsatisfactory weight management at least one year after bariatric surgery (sleeve gastrectomy or gastric bypass).

This study has a planned enrollment of 30 subjects (approximately 15 for each preceding surgical procedure) and will be conducted in a single center in the United States.

The study will consist of a Screening Period (up to 28 days), a Run-in Visit Period (up to 14 days), a Treatment Period (28 days), and a Follow-up Period (EOS Visit within 14 days after receiving the last dose of ARD-101). The screening procedures will be initiated upon completion of the informed consent process. Following completion of screening procedures and confirmation of eligibility, subjects will be enrolled to receive ARD-101.

ARD-101 will be administered orally under fasting conditions (approximately 60 minutes **before** breakfast and approximately 60 minutes **before** dinner) at 200 mg BID for 28 days. Subjects will receive treatment in an outpatient setting and will be instructed to visit the clinical center periodically as scheduled in Table 1 for safety and efficacy assessments. The treatment may be discontinued due to intolerable toxicity, withdrawal of informed consent, discretion of principal investigator or end of the study.

All available safety and tolerability data will be evaluated throughout study conduct. Pharmacodynamic data will be analyzed after study completion.

4.2. Justification for Dose

The dose of 200 mg BID was chosen based on the available nonclinical studies and clinical data. As described above, this dose has been found safe in the first-in-human trial and the pharmacokinetics support the prescribed dose level and duration.

4.3. Study Duration

The duration of participation for each subject will be up to approximately 84 days (12 weeks).

- Screening Period: Up to 28 days (4 weeks)
- Run-in Visit: Up to 14 days (2 weeks)
- Treatment Period: 28 days on ARD-101

- Follow-up Period: 14 days (2 weeks). The EOS Visit will occur within 14 days after receiving the dose of ARD-101

4.4. Study Completion

For the entire study, study completion is defined as the last visit of the last subject for any protocol related activity (last subject, last visit). For individual subjects, study completion is defined as the time of the subject's last data collection.

4.5. Early Termination

If a subject withdraws prematurely after dosing, all data normally collected should be collected at the time of premature discontinuation. If deemed necessary by the Principal Investigator, the subject will be asked to return at the regularly scheduled EOS Visit.

5. STUDY POPULATION

The study population will consist of male and female subjects at least one year after sleeve gastrectomy or gastric bypass surgery). Subjects must be able to provide written informed consent and meet all the inclusion criteria and none of the exclusion criteria.

5.1. Number of Subjects

A total of approximately 30 subjects will be enrolled in the clinical study.

If any assessments for inclusion or exclusion criteria are out of permissible ranges during the Screening Period, repeat assessments may be allowed for those subjects at Principal Investigator's discretion.

5.2. Inclusion Criteria

Subjects must meet all the following criteria to be eligible for participation in this study:

1. Male and female subjects, 18-75 years of age
2. Willing and able to provide signed and dated informed consent prior to any study-related procedures and willing and able to comply with all study procedures
3. Body Mass Index (BMI) of ≥ 35 - 60 kg/m2 during the Screening Period
4. Status of at least 1-year post sleeve gastrectomy or gastric bypass surgery
5. One of the following:
    - Weight loss of at least 50% of their excess weight at their nadir, and weight regain at least 20% of their nadir weight loss. Excess weight is defined as maximum preoperative weight – weight corresponding to BMI 25 kg/m2
    - Excess weight loss less than 50% at the subject's nadir weight
6. Subjects with rescue surgery (e.g. gastric band to sleeve gastrectomy or gastric bypass; sleeve gastrectomy to gastric bypass) will be allowed, with time since second surgery of at least 12 months
7. Subjects with a history of revision procedures will be allowed if at least 6 months have passed since the procedure and weight has not deviated more than 5% in the 3 months prior to enrollment
8. No abnormal findings or abnormalities of clinical significance in vital signs, physical examination, clinical laboratory tests (CBC, urinalysis, blood biochemistry, coagulation,

pregnancy test (females), urine drug test, nicotine test, etc.), 12-lead electrocardiogram (ECG) during the Screening Period

9. Serum creatinine, alkaline phosphatase, hepatic enzymes (aspartate aminotransferase, alanine aminotransferase) and total bilirubin (unless the subject has documented Gilbert syndrome) not exceeding 1.5-fold the upper laboratory norm and eGFR >30 mL/min
10. Standard 12-lead ECG parameters after 10minutes resting in supine position in the following ranges; 120 ms <PR <220 ms, QRS <120 ms, QTc <= 430 ms if male, <= 450 ms if female and normal ECG tracing unless the Investigator considers an ECG abnormality within described limits to be not clinically relevant
11. Stable or well controlled blood pressure per Investigator's judgement during the Screening Period. Specifically: Vital signs after 10 minutes sitting in a chair (feet on floor, back supported):

    i. 95 mmHg < SBP <160 mmHg,

    ii. 45 mm Hg < DBP <100 mm Hg,

    iii. 40 bpm < HR <100 bpm.
12. HbA1c < 9.0% at screening.
13. Patients with type 2 diabetes treated with metformin may be enrolled. However, patients with type 2 diabetes on any other therapy will be excluded.
14. Female subjects must have negative pregnancy test and must not be lactating. For females able to bear children, a hormonal (i.e., oral, implantable, or injectable) and single barrier method (i.e., sponge), or a double-barrier method of birth control (i.e., condom with spermicide) or abstinence must be used/practiced throughout the study and for 90 days following last dose of study medication; for effective form of birth control.
15. Females of non-childbearing potential, defined as surgically sterile (status post hysterectomy, bilateral oophorectomy, bilateral tubal ligation, bilateral salpingectomy, or bilateral tubal occlusion) or post-menopausal for at least 12 months (may be confirmed with a screening FSH level in the post-menopausal lab range), do not require contraception during the study.
16. Males with female partners of childbearing potential must agree to a double-barrier method if they become sexually active during the study and for 90 days following the last dose of the study medication. Male subjects must not donate sperm for 90 days following their participation in the study.

5.3. Exclusion Criteria

Subjects who meet any of the following criteria will be excluded from study participation:

1. Any relevant gastrointestinal (GI) surgery (excluding the gastric bypass or sleeve gastrectomy) per Investigator judgement
2. History of significant drug hypersensitivity or anaphylaxis
3. Participation in a weight loss program or clinical trial for weight loss within the 3 months prior to enrollment
4. Received any experimental drugs or devices or have participated in a clinical study within 30 days prior to enrollment
5. Diabetes treatment (unless metformin as outlined), or chronic oral steroids, or treatment with immune modulators, anti-obesity drugs, chronic opiate therapy, or antipsychotic medications
6. Currently receiving any drug-based therapy for weight management
7. Thyroid-stimulating hormone (TSH) level is outside of normal limit during the Screening Period
8. The presence of diseases with abnormal clinical manifestations that need to be excluded based on their possible contribution to weight loss or weight gain, including but not limited to nervous, cardiovascular, blood and lymphatic system, immune, renal, hepatic, gastrointestinal, respiratory, metabolic and skeletal diseases during the Screening Period
9. History of myocardial infarction, unstable angina, arterial revascularization, stroke, New York Heart Association Functional Class II-IV heart failure, or transient ischemic attack within 6 months prior to Visit 1
10. Any malignancy not considered cured (except focal, treated basal cell carcinoma and squamous cell carcinoma of the skin); a participant is considered cured if there has been no evidence of cancer recurrence in the previous 5 years
11. History of major depressive disorder or history of other severe psychiatric disorders (e.g., schizophrenia or bipolar disorder) within the last 2 years.
12. Donated ≥200 mL of blood (blood components) or had massive blood loss, received blood transfusion or blood products within 3 months prior to enrollment
13. Planned sperm/egg donation within 6 months post enrollment

14. Positive urine drug test (morphine, methamphetamine, ketamine, ecstasy, and cannabis) during the Screening Period
15. History of consuming more than 14 units of alcoholic beverages per week or of alcoholism or drug/chemical/substance abuse within past 2 years prior to enrollment (Note: one unit = 12 ounces of beer, 4 ounces of wine or 1 ounce of spirits)
16. Smoking any amount within 3 months prior to enrollment
17. Excessive consumption of tea, coffee, and/or caffeinated beverages (more than 8 cups, 250 mL for each cup) every day within 3 months prior to enrollment
18. Symptomatic viral, bacterial (including upper respiratory infection), or fungal (non-cutaneous) infection within 1 week prior to enrollment
19. History of human immunodeficiency virus antibody, hepatitis C antibody or hepatitis B virus surface antigen
20. A history of psychiatric and psychological condition that, in the judgment of the investigator, may interfere with the planned treatment and follow-up, affect subject compliance or place the subject at high risk from treatment-related complications
21. Poor venous access or inability to tolerate venipuncture
22. Any condition or active drug treatment that the investigator or primary physician believes may not be appropriate for participating in the study

5.4. Restrictions

5.4.1. Dietary and Fluid Restrictions

Caffeine

Subjects should not consume caffeine containing beverages exceeding 500 mg caffeine per day (5 cups of coffee) during the study.

Alcohol

Subjects should not consume more than 3 units (males) or 2 units (females) per day during the study (1 unit is equal to approximately ½ pint [284 mL] of beer, one small glass [125 mL] of wine, or one measure [25 mL] of spirits).

5.4.2. Lifestyle Considerations

Drugs of abuse

Subjects must refrain from use of recreational drugs including THC containing products for the duration of the study.

Nicotine

No smoking or use of other nicotine-containing products (snuff, chewing tobacco, cigars, pipes or nicotine-replacement products such as nicotine chewing gum and nicotine plasters) will be allowed during the study.

6. INVESTIGATIONAL MEDICINAL PRODUCT

6.1. Premedication

No pre-medication is required prior to administration of study medication. For subjects who experience Grade 2 acute allergic reactions and are able to continue on the treatment, premedication with an H1 antihistamine should be given.

6.2. Identity of the Investigational Medicinal Products

ARD-101 drug product is an encapsulated oral formulation of denatonium acetate monohydrate. The ARD-101 drug product will be supplied as white opaque body and white opaque cap hard gelatin capsules. The ARD-101 drug product contains 23.61 mg or 59.03 mg of denatonium acetate monohydrate (equivalent as 20 mg or 50 mg of denatonium base) together with povidone, sugar spheres, hypromellose, and talc as inactive ingredients.

6.3. Supply, Packaging, Labeling and Storage

Study drug will be supplied by Sponsor. The study medication will be packaged and labeled according to applicable local and regulatory requirements.

All supplies of study medication must be stored in accordance with the manufacturer's instructions. The ARD-101 will be stored at room temperature in a securely locked area, accessible to authorized persons only, until dispensation.

Further details will be provided in the Pharmacy Manual.

6.4. Drug Accountability, Dispensing and Destruction

The Principal Investigator or designee is responsible for maintaining accurate accountability records of the study drug throughout the clinical study. Products returned will be stored under the same conditions as products not yet dispensed.

All dispensing and accountability records will be available for Sponsor review. The Study Monitor will perform drug accountability at regular intervals.

Study medication will be dispensed for each subject according to the protocol and Pharmacy Manual.

After receiving Sponsor approval in writing, all unused or partially used study medication will be returned to the Sponsor or designated third party or for preparing the study medication for destruction via incineration.

6.5. Dose Modification

The dose level may not be reduced. If any ≥ Grade 3 toxicities or any intolerable Grade 2 toxicity occurs, treatment should be discontinued permanently. Intolerable toxicity is defined as, in the opinion of Principal Investigator and Medical Monitor, any toxicity that poses a significant health risk to the subject. Treatment may continue on any Grade 1 toxic reactions or Grade 2 anorexia or Grade 2 weight loss. For any other Grade 2 toxicities, dosing may be interrupted for up to 3 days and may be resumed at the medical judgment of Investigator once the toxicity has resolved to Grade 0-1. A missed dose should not be made up. In the event of multiple toxicities, dosing management should be based on the worst toxicity observed. Subjects will be instructed to notify Investigators at the first occurrence of any adverse symptom.

6.6. Stopping Rules

At any time during the study, the subject must discontinue study drug treatment, if one of the following events occur:

- An increase of AST and/or ALT (> 3x ULN) are observed in the presence of increased (> 2x ULN) alkaline phosphatase and/or total bilirubin.
- A sustained AST or ALT increase, defined as three consecutive values of AST and/or ALT greater than 5x ULN within a period of 14 days.
- A treatment-related Grade 3 or greater adverse event (other than AST or ALT increase as described above) is noted by the Investigator.
- A relevant decrease of eGFR as per investigator

If any of the above stopping rules are met the following action(s) should be taken:

1. Stop dosing and convene a Safety Review Committee meeting;
2. Review AE for evidence of relationship to treatment;
3. May resume enrollment and continue the study if recommended by the Safety Review Committee.

6.7. Subject Identification

6.7.1. Screening Numbers

All screened subjects are assigned a unique screening number. The screening numbers are numbers that identify subjects from time of screening until time of enrollment.

6.7.2. Enrollment Numbers

Eligible subjects will be assigned an enrollment number. The enrollment number will include 6-digit site number and subject numbers starting with 201 as below.

- Subject 100-201

Once an enrollment number has been allocated to one subject, it may not be assigned to another subject.

6.8. Compliance

Dosing will be performed by trained, qualified personnel designated by the Principal Investigator.

6.9. Special Precautions and Management of AEs

6.9.1. Eating disorders

ARD-101 may decrease appetite and subsequently change a subject's eating behavior by delaying gastric emptying and inducing the glucagon-like peptide-1 and cholecystokinin secretion into the circulation that targets the central nervous system (CNS) to cause satiety/hunger sensation. Subjects who develop any eating disorders during study treatment should be permanently discontinued from study drug.

6.9.2. Allergic reactions

One case of allergic reaction has been reported in a female subject receiving denatonium benzoate, a compound in the same class as ARD-101 (Deloose E et al. 2017). Drug allergic reactions can be either acute or delayed. The acute allergic reactions that usually occur within 1 h after drug exposure are typically IgE mediated while those that usually occur 2 to 6 h after drug exposure have more complicated pathophysiological mechanisms. Subjects with a localized Grade 2 acute allergic reaction might continue on the treatment with premedication of H1 antihistamine. Subjects with recurrent Grade 2 acute allergic reactions with premedication, Grade 2 delayed allergic reactions, or Grade 3 to 4 acute allergic reactions should permanently discontinue the study drug.

6.9.3. Management of gastrointestinal disorders

Early recognition and management of potential GI disorders may mitigate more severe and subsequent toxicities.

6.9.4. Embryo-fetal Toxicity

The effect of ARD-101 on embryo-fetal development has not been tested. Thus, a highly effective form of contraception must be used, and enrolled subjects should receive instructions regarding this during the entire study from enrollment to 90 days after last dose administration.

6.10. Permitted Medications/Procedures

Any medicinal product, prescribed or OTC, including vitamins, natural, and herbal remedies, taken by a subject other than the study medication, is considered concomitant medication.

Any concomitant treatment will be given only if deemed strictly necessary by the Principal Investigator or designee. Use of concomitant medication will be recorded and reported. Particularly, as described in Section 2.3.2, ARD-101 is a substrate of the MDR1, MATE1, MATE2-K, OAT3 and OCT2 transporters. Coadministration of these transporter inhibitors have the potential to alter the pharmacokinetics of ARD-101. Therefore, before placing a subject on a specific medication/therapy, it is the responsibility of the investigator to check on potential drug-drug interactions between that medication/therapy and ARD-101 based on the Drug Development and Drug Interactions: Table of Substrates, Inhibitors and Inducers (https://www.fda.gov/drugs/drug-interactions-labeling/drug-development-and-drug-interactions-table-substrates-inhibitors-and-inducers), and Drug-Drug Interaction Database (https://www.fda.gov/drugs/drug-interactions-labeling/databases) of FDA.

6.10.1. Permitted Medications/Procedures

Subjects are permitted to use medications consistent with those permitted, including the use of contraceptive medications and hydrocortisone cream. All concomitant medications are subject to approval by the principal investigator.

No concomitant use of approved obesity drugs or diabetes treatment (excluding metformin), MDR1, MATE1, MATE2-K, OAT3, or OCT2 inhibitors, or any drug deemed incompatible with trial participation as per investigator judgement.

7. DISCONTINUATION

7.1. Subject Withdrawal and Replacement

Subject participation may be terminated prior to completing the study and the reason recorded as follows:

1. Adverse event
2. Protocol violation
3. Lost to follow-up
4. Subject withdrew consent at own request
5. Other

A genuine effort must be made to determine the reason(s) why a subject fails to return for the necessary visits or is discontinued from the study. If the subject is unreachable by telephone, a registered letter, at the minimum, should be sent to the subject requesting him/her to contact the clinic.

Withdrawn subjects may be replaced until the full cohort has completed the study.

8. STUDY ASSESSMENTS AND PROCEDURES

For timing of assessments, refer to the Schedule of Assessments Table 1.

8.1. Eligibility Screening

All subjects must sign an informed consent form (ICF) prior to the conduct of any screening procedures. Screening assessments will occur within 28 days prior to the first dose of study medication. The subject's relevant medical history and demographic data will be obtained. Retesting of abnormal screening values that lead to exclusion are allowed during the Screening Period (to reassess eligibility). The last result obtained prior to the first dose of study medication will be used to determine eligibility.

8.2. Safety Variables

8.2.1. Adverse Events

Adverse event reporting will begin for each subject from the date the ICF is signed and will continue until the EOS Visit.

8.2.1.1. Definitions

8.2.1.1.1. Definition of Adverse Event

Any untoward medical occurrence in a patient or clinical investigation subject administered a pharmaceutical product and which does not necessarily have a causal relationship with this treatment. An AE can therefore be any unfavorable and unintended sign (including an abnormal laboratory finding), symptom, or disease temporally associated with the use of a medicinal (investigational) product, whether or not related to the medicinal (investigational) product.

Other untoward events occurring in the framework of a clinical study will be recorded as AEs, e.g. those occurring during treatment-free periods (including screening or post-treatment follow-up periods), in association with study-related procedures and assessments, or under placebo. For study medication, lack of efficacy may be an expected potential outcome and should not be reported as an AE unless the event is unusual in some way, e.g., greater in severity.

Concomitant illnesses, which existed prior to entry into the clinical study, will not be considered AEs unless they worsen during the Treatment Period. Pre-existing conditions will be recorded as part of the subject's medical history.

8.2.1.1.2. Definition of Serious Adverse Event

An SAE is defined as any untoward medical occurrence that at any dose:

- Results in death
- Is life-threatening; this means that the subject was at risk of death at the time of the event; it does not mean that the event hypothetically might have caused death if it were more severe
- Requires inpatient hospitalization or prolongation in existing hospitalization
- Results in persistent or significant disability/incapacity or substantial disruption of the ability to conduct normal life functions
- Is a congenital anomaly/birth defect, or
- Is another important medical event (see below)

Important medical events that do not result in death, are not life-threatening or do not require hospitalization may be considered SAEs when, based on appropriate medical judgment, they may jeopardize the subject and may require medical or surgical intervention to prevent one of the outcomes listed above. Examples of such medical events include allergic bronchospasm requiring intensive treatment in an emergency room or in a physician's office, blood dyscrasias or seizures that do not result in in-patient hospitalization, and the development of drug dependency or drug abuse.

A distinction should be drawn between serious and severe AEs. Severity is a measure of intensity whereas seriousness is defined by the criteria above. For example, a mild degree of gastrointestinal bleeding requiring an overnight hospitalization for monitoring purposes would be considered an SAE but is not necessarily severe. Similarly, an AE that is severe in intensity is not necessarily an SAE. For example, alopecia may be assessed as severe in intensity but would not be considered an SAE.

Medical and scientific judgment should be exercised in deciding if an AE is serious and if expedited reporting is appropriate.

8.2.1.2. Recording of Adverse Events

Adverse events should be collected and recorded for each subject from the date the ICF is signed until the end of their participation in the study, i.e., the subject has discontinued or completed the study.

Adverse events may be volunteered spontaneously by the subject, or discovered by study staff during physical examinations or by asking an open, non-leading question such as 'How have you been feeling since you were last asked?' All AEs and any required remedial action will be recorded. The nature of AE, date (and time, if known) of AE onset, date (and time, if known) of AE outcome to date, severity and action taken of the AE will be documented together with the Principal Investigator's assessment of the seriousness of the AE and causal relationship to study drug and/or study procedure.

All AEs should be recorded individually in the subject's own words (verbatim) unless, in the opinion of the Principal Investigator, the AEs constitute components of a recognized condition, disease or syndrome. In the latter case, the condition, disease or syndrome should be named rather than each individual symptom. The AEs will subsequently be coded using the Medical Dictionary for Regulatory Activities (MedDRA). Severity of AEs will be graded using the Toxicity Grading Scale for Healthy Adult and Adolescent Volunteers Enrolled in Preventive Vaccine Clinical Trials (September, 2007).

8.2.1.3. Assessment of Adverse Events

Each AE will be assessed by the Principal Investigator as per the categories discussed in the following sections.

8.2.1.3.1. Intensity

The Principal Investigator will assess all AEs for severity in accordance with the following standard ratings.

- Mild (Grade 1): Ordinarily transient symptoms, does not influence performance of subject's daily activities. Treatment is not ordinarily indicated.
- Moderate (Grade 2): Marked symptoms, sufficient to make the subject uncomfortable. Moderate influence on performance of subject's daily activities. Treatment may be necessary.
- Severe (Grade 3): Symptoms cause considerable discomfort. Substantial influence on subject's daily activities. May be unable to continue in the study and treatment may be necessary.
- Potentially Life Threatening (Grade 4): Symptoms require emergency room (ER) visit or hospitalization.

When changes in the intensity of an AE occur more frequently than once a day, the maximum intensity for the event should be noted for that day. Any change in severity of signs and symptoms

over a number of days will be captured by recording a new AE, with the amended severity grade, and the date (and time, if known) of the change.

8.2.1.3.2. Causality

The Principal Investigator will assess the causality/relationship between the study medication and the AE. The Principal Investigator is responsible for assessing the relationship to study medication using clinical judgment and the following considerations:

- No: Evidence exists that the AE has an etiology other than the study drug. For SAEs, an alternative causality must be provided (e.g., pre-existing condition, concomitant medication)
- Yes: There is reasonable possibility that the event may have been caused by the study medication.

8.2.1.4. Reporting of Serious Adverse Events

The Principal Investigator will review each SAE and evaluate the intensity and the causal relationship of the event to study drug. All SAEs will be recorded from signing of the ICF until the EOS Visit. Serious AEs occurring after the EOS Visit and coming to the attention of the Principal Investigator must be reported only if there is (in the opinion of the Principal Investigator) reasonable causal relationship with the study drug.

The Principal Investigator is responsible for providing notification to Sponsor of any SAE, whether deemed study medication-related or not, that a subject experiences during their participation in study within 24 h of becoming aware of the event.

Initial reports of SAEs must be followed later with detailed descriptions, including clear photocopies of other documents as necessary (e.g., hospital reports, consultant reports, autopsy reports), with the subject's personal identifiers removed.

Information on SUSARs will be collected and reported to the regulatory authority and the IEC as required per guidelines.

If the SUSAR is fatal or life-threatening, associated with the use of the study medication, and unexpected, the regulatory authority and the IEC will be notified per guidelines after being made aware of the case. Additional follow-up (cause of death, autopsy report, hospital report) information should be reported per guidelines. SUSARs which are not fatal and not life-threatening are to be reported per guidelines.

The Sponsor will notify the Investigators of relevant information about SUSARs that could adversely affect the safety of subjects in a timely fashion. Follow-up information may be submitted if necessary. The Sponsor will also provide annual safety reports for submission to the regulatory authority and the IEC responsible for the clinical study. These updates will include information on SUSARs and other relevant safety findings.

8.2.1.5. Follow-up of Adverse Events

All AEs experienced by a subject, irrespective of the suspected causality, will be monitored until the event has resolved, until any abnormal laboratory values have returned to baseline or stabilized at a level acceptable to the Principal Investigator and Medical Monitor, until there is a satisfactory explanation for the changes observed or until the subject is lost to follow-up.

8.2.1.6. Pregnancy

The Sponsor has a responsibility to monitor the outcome of all pregnancies reported during the clinical study.

Pregnancy alone is not regarded as an AE unless there is a suspicion that the study drug may have interfered with the effectiveness of a contraceptive medication. Elective abortions without complications should not be regarded as AEs, unless they were therapeutic abortions (see below). Hospitalization for normal delivery of a healthy newborn should not be considered an SAE.

Each pregnancy must be reported by the Principal Investigator to the Sponsor within **2 days** after becoming aware of the pregnancy. The Principal Investigator must follow-up and document the course and the outcome of all pregnancies even if the subject was withdrawn from the clinical study or if the clinical study has finished.

All outcomes of pregnancy must be reported by the Principal Investigator to the Sponsor on the pregnancy outcome report form within **2 days** after he/she has gained knowledge of the normal delivery or elective abortion.

Any SAE that occurs during pregnancy must be recorded on the SAE Report Form (e.g., maternal serious complications, therapeutic abortion, ectopic pregnancy, stillbirth, neonatal death, congenital anomaly, birth defect) and reported within 24 h in accordance with the procedure for reporting SAEs.

8.2.2. Clinical Laboratory Assessments

Samples for clinical laboratory assessments will be collected at the time points detailed in the Schedule of Assessments (Table 1). Samples will be collected in appropriate tubes and handled according to standard procedures of the applicable laboratory.

Any value outside the normal range will be flagged for the attention of the Principal Investigator or designee at the site. The Principal Investigator or designee will indicate whether the value is of clinical significance. Additional testing during the study may be done if medically indicated. If a clinically significant abnormality is found in the samples taken after dosing, during the study, and/or at the EOS Visit, it should be recorded as an AE and the subject will be followed until the test(s) has (have) normalized or stabilized, at the discretion of the Principal Investigator.

8.2.3. Vital Signs

Vital signs will be assessed at the time points detailed in the Schedule of Assessments Table 1. The following vital signs will be measured:

- Blood pressure (systolic and diastolic [mmHg])
- Pulse (bpm)
- Body temperature (°C)
- Respiratory rate (breaths per minute)

8.2.4. Standard 12-lead Electrocardiograms

Standard safety 12-lead ECGs will be performed at the time points detailed in the Schedule of Assessments (Table 1).

The ECG will include all 12 standard leads.

All ECGs must be evaluated by a qualified physician for the presence of abnormalities. If a clinically significant abnormality is found, it should be recorded as an AE and the subject will be followed until the test(s) has (have) normalized or stabilized, at the discretion of the Principal Investigator.

8.2.5. Bioimpedance

Body composition will be assessed via scale measuring bioimpedance at the time points detailed in the Schedule of Assessments (Table 1).

8.2.6. MRI PDFF and Full Body Composition Scan

In order to better assess overall body composition including quantification of lean body mass, liver fat content and other related parameters, MRI scan will be performed at the time points detailed at the time points detailed in the Schedule of Assessments (Table 1).

8.2.7. Mixed Meal Tolerance Test (MMTT)

The MMTT permits measurement of intestinal (incretin secretion), β-cell (insulin secretion), hepatic (insulin extraction), muscle, and adipose tissue responses to ingesting calories. The mixed meal, containing glucose, fat and protein (2x Ensure 8 oz. for a total of 16 oz., 66 grams carbohydrate (CHO), 440 calories), is more physiologic than the glucose only challenge in an oral glucose tolerance test (OGTT). The MMTT will be performed at the time points detailed in the Schedule of Assessments (Table 1) and as described in the Appendix. (Table 1).

8.2.8. Indirect Calorimetry

Continuous indirect calorimetry using a commercial instrument (Vmax Encore VS 29N, CareFusion, Yorba Linda, CA, USA) will be performed during basal and postprandial periods on the days when the MMTT are performed.

At -80 and 195 minutes during the MMTT, the canopy will be placed over the subject's head to collect data from 10 continuous minutes at steady-state (no longer than 30 minutes) during each interval for determination of the substrate oxidation rates at basal condition, and during the MMTT ass outlined in the Schedule of Assessments (Table 1).

8.2.9. Physical Examinations

Physical examinations will be performed at the time points detailed in the Schedule of Assessments (Table 1).

Full physical examination:

An assessment of general appearance and a review of systems (dermatologic, head, eyes, ears, nose, mouth/throat/neck, thyroid, lymph nodes, respiratory, cardiovascular, gastrointestinal, extremities, musculoskeletal, neurologic and psychiatric systems).

Brief physical examination:

An assessment of the general appearance, skin, cardiovascular system, respiratory system and abdomen.

The brief physical examination may be extended to a full physical examination if considered necessary by the Principal Investigator. Other evaluations may be performed as deemed necessary by the Principal Investigator.

8.2.10. Continuous Glucose Monitoring

Assessments will be performed at the time points detailed in the Schedule of Assessments (Table 1).

Glucose levels will be monitored using the Continuous Glucose Monitoring (CGM) device. The CGM device will be used to track blood sugar levels day and night, and it will collect readings automatically every 5 minutes. The CGM sensor is a round disk with the circumference equal to that of a quarter, with the thickness of 2 quarters. It has a small filament that is inserted subcutaneously via a small needle that is then retracted and removed, leaving just the filament. The CGM sensor adheres to the skin leaving the filament in place for 14 days and stores the recorded glucose readings until downloaded by a study team member.

During the Run-in Period, the CGM sensor will be applied to the subject and they will be required to wear it for a minimum duration of two weeks prior to Treatment Period Day 1. Data from the CGM will be downloaded and a report will be generated using the software provided by the device. The report outlines typical measures of glycemic control: mean glucose, standard deviation, time in range and daily patterns. In addition, a raw data file is available with each time point of collection and glucose reading."

8.2.11. Circulating Hormones

To investigate the effect of the study medication on circulating gut hormone levels, all subjects of the study will undergo a fasting blood draw before the first dose, and 1h and 2h after the first dose on day 1 for measuring fasting circulating levels of the following gut hormnones: GLP-1 (total and active), CCK, PYY, amylin, GIP (total and active), ghrelin, leptin, and adiponectin (Table 1).

8.2.12. Fecal Microbiome Analysis

All subjects of the study will optionally provide their fecal samples during the run-in visit and on day 28 for microbiome analysis to evaluate potential effects of the study medication on fecal microbiome profile (Table 1).

8.2.13. Non-invasive Phenotypic Bitter Taste Test

All subjects of the study will optionally undergo a non-invasive phenotypic bitter taste test using commercialized phenylthiocarbamide (PTC) and 6-n-propylthiouracil (PROP) taste strips, and a commercialized Allegro Bitrex® Fit Test Kit during the run-in visit of the study (Table 1). This data can be used to retroactively assess a correlation of bitter taste sensitivity of subjects with the efficacy of the study medication.

9. STATISTICAL CONSIDERATIONS

9.1. Study Population

9.1.1. Protocol Deviations

Important protocol deviations will be listed by subject.

9.2. General Considerations

Continuous data will be summarized using descriptive statistics (number, mean, standard deviation [SD], minimum, median and maximum). Categorical data will be summarized using frequency tables (number and percentage).

9.3. Subject Disposition

Subjects excluded from the safety analysis sets will be listed including the reason for exclusion. Subject disposition will be summarized and will include the following information: number of subjects dosed, number and percentage of subjects completing the study and the number and percentage of subjects who were withdrawn (including reasons for withdrawal). Disposition data will be presented based on all.

Subject discontinuations will be listed including the date of study exit, duration of treatment and reason for discontinuation. A listing of informed consent response will also be presented.

9.4. Demographic and Anthropometric Information and Baseline Characteristics

Demographic and anthropometric variables (age, sex, ethnicity, race, height, body weight and BMI) will be listed by subject. Demographic characteristics (age, sex, ethnicity and race) and anthropometric characteristics (height, body weight, and BMI) will be summarized for all subjects in the safety analysis set. The denominator for percentages will be the number of subjects in the safety analysis set for each treatment or for all subjects as applicable.

Medical history data will be listed by subject including visit, description of the disease/procedure, MedDRA system organ class (SOC), MedDRA preferred term (PT), start date, and stop date (or ongoing if applicable).

9.5. Prior and Concomitant Medication and Drug Administration

Prior medications are those that started and stopped prior to the first dose of study medication. Concomitant medications are those taken after first dosing (including medications that started prior to dosing and continued after).

Prior and concomitant medication will be listed by subject and will include the following information: reported name, PT, the route of administration, dose, frequency, start date/time, duration and indication.

Prior and concomitant medication will be coded according to the World Health Organization Drug Dictionary (WHO-DD) latest version.

Drug administration dates and times will be listed for each subject.

9.6. Exposure

A listing of drug administration will be created and will include the date and time of administration.

9.7. Safety Analyses

Where applicable, pre-dose measurements on Day 1 are considered baseline values for safety variables.

9.7.1. Adverse Events

All AEs will be listed. The number and percent of subjects experiencing an event will be tabulated for each SOC and PT. The AEs will also be tabulated according to intensity and causality. A TEAE is defined as an AE with a start date on or after the first dose of study drug, or that worsened following first administration of study drug. For purposes of data analysis, the end of the TEAE period will be the last time point when a subject was evaluated for AEs.

The AEs (TEAEs) will be tabulated according to intensity and causality, as applicable.

Non-TEAEs will be listed, as applicable.

Serious AEs will be listed separately.

9.7.2. Clinical Laboratory Tests

Individual data listings of laboratory results will be presented for each subject. Flags will be attached to values outside of the laboratory's reference limits along with the Principal

Investigator's assessment or designee. Clinically significant laboratory test abnormalities that were considered AEs by the Principal Investigator will be presented in the AE listings.

Clinical laboratory tests (observed values) will be summarized descriptively in tabular format. Shift tables will be presented for select laboratory parameters.

9.7.3. Vital Signs

Individual data listings of vital signs (observed and change from baseline) will be presented for each subject. Individual clinically significant vital signs findings that were considered AEs by the Principal Investigator will be presented in the AE listings.

Observed values as well as change from baseline data will be summarized descriptively in tabular format.

9.7.4. Standard 12-lead Electrocardiogram

Standard 12-lead ECG data (observed and change from baseline) will be listed for each subject and time point. Observed values will be summarized descriptively in tabular format. Change from baseline will be summarized descriptively for QTc data. A categorical QTc analysis will also be performed.

9.7.5. Physical Examination

Abnormal physical examination findings will be listed.

9.8. Exploratory analysis

Exploratory analysis may be presented separately from the main clinical study report (CSR). Further details on exploratory analyses will be provided in the SAP.

9.9. Determination of Sample Size

The determination of sample size is not based on statistical considerations of power but 25 subjects is considered sufficient to meet the study objectives.

10. ETHICAL, LEGAL AND ADMINISTRATIVE ASPECTS

10.1. Data Quality Assurance

The Sponsor or designee will conduct a study initiation visit to verify the qualifications of the Principal Investigator, inspect the facilities and inform the Principal Investigator of responsibilities and procedures for ensuring adequate and correct documentation.

The Principal Investigator must prepare and maintain adequate and accurate records of all observations and other data pertinent to the clinical study for each study participant. Frequent communication between the clinical site and the Sponsor is essential to ensure that the safety of the study is monitored adequately. The Principal Investigator will make all appropriate safety assessments on an ongoing basis. The Medical Monitor may review safety information as it becomes available throughout the study.

All aspects of the study will be carefully monitored with respect to Good Clinical Practice (GCP) and SOPs for compliance with applicable government regulations. The Study Monitor will be an authorized individual designated by the Sponsor. The Study Monitor will have access to all records necessary to ensure integrity of the data and will periodically review the progress of the study with the Principal Investigator.

10.2. Data Collection and Access to Source Data/Documents

The Principal Investigator will ensure the accuracy, completeness and timeliness of the data reported to the Sponsor. The Investigator or designee will cooperate with the Sponsor's representative(s) for the periodic review of study documents to ensure the accuracy and completeness of the data at each scheduled monitoring visit. Data clarification requests will be provided to the study team by means of electronic or manual queries.

The Investigator will allow Sponsor representatives, contract designees, authorized regulatory authority inspectors and the IEC to have direct access to all electronic records pertaining to the study.

All data should be recorded, handled and stored in a way that allows its accurate reporting, interpretation and verification.

10.3. Archiving Study Documents

All source documents generated in connection with the study will be retained in the limited access file storage area, respecting the privacy and confidentiality of all records that could identify the

subjects. Direct access is allowed only for authorized people for monitoring and auditing purposes. Source documents will be handled, stored and archived according to in-house procedures.

The Investigator's Site File will be archived by the CRO for at least 2 years after completion of the study or longer if agreed upon by the Sponsor.

10.4. Good Clinical Practice

The procedures set out in this clinical study protocol are designed to ensure that the Sponsor and the Principal Investigator abide by the principles of the ICH guidelines on GCP. The clinical study also will be carried out in keeping with national and local legal requirements (in accordance with United States investigational new drug [IND] regulations [21 CFR 56]).

10.5. Informed Consent

Eligible subjects may only be included in the study after providing IRB/IEC approved informed consent.

Informed consent must be obtained from the subject before conducting any study-specific procedure.

As part of the informed consent procedure, the Principal Investigator or designee must explain orally and in writing the nature, duration and purpose of the study and the action of the drug in such a manner that the subject is aware of the potential risks, inconveniences or AEs that may occur. The subject should be informed that he/she is free to withdraw from the study at any time. Subjects will receive all information that is required by federal regulations and ICH guidelines. The ICF must be signed and dated; one copy will be handed to the subject, and the Principal Investigator will retain a copy as part of the clinical study records. The Principal Investigator will not undertake any investigation specifically required for the clinical study until written consent has been obtained. The terms of the consent and when it was obtained must be documented in the subject source documents.

The Sponsor will review the Investigator-proposed ICF to ensure it complies with the ICH GCP guideline (including the ethical principles that have their origins in the Declaration of Helsinki) and regulatory requirements and is considered appropriate for this study. The Principal Investigator or designee will provide the Sponsor with a copy of the IRB/IEC approved ICF prior to the start of the study.

If a protocol amendment is required, then the ICF may need to be revised to reflect the changes to the protocol. If the ICF is revised, it must be reviewed and approved by the responsible IRB/IEC and signed by all subjects subsequently enrolled in the clinical study, as well as those currently enrolled in the clinical study as applicable.

10.6. Insurance and Compensation for Injury

The Sponsor has covered this clinical study by means of an insurance of the clinical study according to national requirements. The name and address of the relevant insurance company, the certificate of insurance, the policy number and the sum insured are provided in the Investigator's Site File.

10.7. Protocol Approval and Amendment(s)

Before the start of the clinical study, the clinical study protocol and other relevant documents will be approved by the IRB/IEC, in accordance with local legal requirements. The Sponsor must ensure that all ethical and legal requirements have been met before the first subject is enrolled in the clinical study.

This protocol is to be followed exactly. To alter the protocol, amendments must be written, which must be released by the responsible staff and receive IRB/IEC approval prior to implementation (as appropriate).

Administrative changes may be made without the need for a formal amendment but will also be mentioned in the integrated CSR. All amendments will be distributed to all study protocol recipients, with appropriate instructions.

10.8. Confidentiality Data Protection

All clinical study findings and documents will be regarded as confidential. Study documents (protocols, IBs and other material) will be stored appropriately to ensure their confidentiality. The Principal Investigator and members of his/her research team (including the IRB/IEC) must not disclose such information without prior written approval from the Sponsor, except to the extent necessary to obtain informed consent from subjects who wish to participate in the study or to comply with regulatory requirements.

The anonymity of participating subjects must be maintained. Subjects will be specified on study documents by their subject number, initial or birth date, not by name. Documents that identify the subject (e.g., the signed ICF) must be maintained in confidence by the Principal Investigator.

10.9. Publication Policy

By signing the clinical study protocol, the Principal Investigator agrees with the use of results of the clinical study for the purposes of national and international registration, publication and information for medical and pharmaceutical professionals. If necessary, the regulatory authorities will be notified of the Principal Investigator's name, address, qualifications and extent of involvement.

A Principal Investigator shall not publish any data (poster, abstract, paper, etc.) without having consulted with the Sponsor in advance.

11. REFERENCE LIST

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12. APPENDIX

Protocol: AARD-201
IRB# 210210
PI: Jeremy Pettus, MD

MMTT

Date: __ __ / __ __ __ / __ __ __ __

Subject #: _____

Subject Initials: ___ ___ ___

Body Composition
Time: _____
Height: _____ cm Weight: _____ kg BMI: _____ Body Fat Percentage: _____ %
Study Staff Initials and Date: _____

Vital Signs
BP and Heart Rate will be measured in the sitting position. The subject MUST be rested for at least 5 min. Time Subject Starts Resting: _____:_____ **Arm (circle): LEFT RIGHT** Time: _____ Blood Pressure: _____ / _____ mmHg Heart Rate: _____ bpm **Any repeat measurements of both the BP and heart rate must be taken <u>at least 2 min</u>. apart and recorded.** Time: _____ Blood Pressure: _____ / _____ mmHg Heart Rate: _____ bpm
Respiratory Rate: _____ breaths/minute **Body Temperature**: _____ °C
Study Staff Initials and Date: _____

AE/SAEs & Concomitant Medications	
Did subject have any changes to their current medications? **If yes, note changes on the concomitant medication log.*	☐ YES* ☐ NO
Did subject report any adverse events or study related problems? **If yes, note changes on the AE/SAE log.*	☐ YES* ☐ NO
Study Staff Initials and Date: _____	

Baseline	
Did subject fast 10 hours as instructed? Date of last meal: _____/_____/_____ Time of last meal: __ __ : __ __	☐ YES ☐ NO
Insert IV draw line into a peripheral arm vein approximately one hour before first draw.	
Study Staff Initials and Date: _____	

Mixed Meal Tolerance Test (MMTT)					
SCHEDULED Timepoint	**Target time (hh:mm)**	**ACTUAL TIME (hh:mm)**	**Sample(s) collected/ Tasks**	**YSI Glucose reading** (at each time point) **YSI#_____**	**Nurse initials**
-120 min			**Instruct subject to empty bladder.**		
-80 min			**Start IDC #1:** _____		
-50 min			**Stop IDC #1:** _____		
-30 min			**Study Drug Given IF SECOND MMTT** **Time:** _____		
-15 min			◯ Glucose ◯ FFA ◯ CCK ◯ GIP ◯ Insulin ◯ Glucagon ◯ PYY ◯ GLP-1 ◯ C-Peptide ◯ Ghrelin ◯ Amylin		
-5 min			◯ Glucose ◯ Insulin ◯ C-Peptide ◯ Cytokine panel ◯ Bile Acids/Ferritin/CRP ◯ Leptin/Adiponectin ◯ 6 ml Serum storage ◯ 6 ml Plasma storage		
0 min			**Give Ensure liquid meal** **(2x Ensure)** **Consume within 5 minutes** **Start Time:** _____ **Stop Time:** _____		

15 min			◯ Glucose ◯ Insulin ◯ C-Peptide ◯ 4 ml Serum storage ◯ 4 ml Plasma storage		
30 min			◯ Glucose ◯ Insulin ◯ C-Peptide ◯ 4 ml Serum storage ◯ 4 ml Plasma storage		
60 min			◯ Glucose ◯ Insulin ◯ C-Peptide ◯ FFA ◯ 4 ml Serum storage ◯ 4 ml Plasma storage		
SCHEDULED Timepoint	**Target time (hh:mm)**	**ACTUAL TIME (hh:mm)**	**Sample(s) collected/ Tasks**	**YSI Glucose reading (at each time point)** **YSI#____**	**Nurse initials**
90 min			◯ Glucose ◯ Insulin ◯ C-Peptide ◯ 4 ml Serum storage ◯ 4 ml Plasma storage		
120 min			◯ Glucose ◯ FFA ◯ CCK ◯ GIP ◯ Insulin ◯ Glucagon ◯ PYY ◯ GLP-1 ◯ C-Peptide ◯ Ghrelin ◯ Amylin ◯ 6 ml Serum storage ◯ 6 ml Plasma storage		
150 min			◯ Glucose ◯ Insulin ◯ C-Peptide ◯ 4 ml Serum storage ◯ 4 ml Plasma storage		
180 min			◯ Glucose ◯ FFA ◯ Insulin ◯ C-Peptide ◯ 4 ml Serum storage ◯ 4 ml Plasma storage		
195 min			**Start IDC #2:** _____		
210 min			◯ Glucose ◯ Insulin ◯ C-Peptide ◯ 4 ml Serum storage ◯ 4 ml Plasma storage		
225 min			**Stop IDC #2:** _____		

240 min			○ **Glucose** ○ **FFA** ○ **CCK** ○ **GIP** ○ **Insulin** ○ **Glucagon** ○ **PYY** ○ **GLP-1** ○ **C-Peptide** ○ **Ghrelin** ○ **Amylin** ○ **4 ml Serum storage** ○ **4 ml Plasma storage**		
AFTER final blood draw, remove IV.					
Nurse completing procedure Signature and Date: _____					
Attending Physician (PI/Sub-I) Signature and Date: _____					

Post-Procedure

Post procedure meal eaten? ☐ **YES** ☐ **NO** **Time:** _____

Post-Procedure Glucose Check? ☐ **YES** ☐ **NO** **Time:** _____ **BG:** _____ **mg/dL**

Time: _____ **BG:** _____ **mg/dL**

Any Adverse Events to report during or after study procedure? ☐ YES* ☐ NO

**If yes, please describe below:*

Study Staff Initials and Date: _____

Post-procedure Vital Signs

BP and Heart Rate will be measured in the sitting position. The subject MUST be rested for at least 5 min.

Time Subject Starts Resting: _____:_____

Arm (circle): LEFT RIGHT

Time: _____ Blood Pressure: _____ / _____ mmHg Heart Rate: _____ bpm

Any repeat measurements of both the BP and heart rate must be taken at least 2 min. apart and recorded.

Time: _____ Blood Pressure: _____ / _____ mmHg Heart Rate: _____ bpm

Respiratory Rate: _____ breaths/minute **Body Temperature**: _____ °C

Study Staff Initials and Date: _____

Next Visit

NEXT VISIT: _____/_____/_____ @ _____:_____

Was subject given instructions for next visit?	☐ YES ☐ NO

Study Staff Initials and Date: _____

Notes

Study Staff Initials and Date: _____